Chromatin Regulatory Mechanisms in Pluripotency

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epigenetics, chromatin remodeling, BAF complexes, stem cells, lineage specificity

Abstract

Stem cells of all types are characterized by a stable, heritable state permissive of multiple developmental pathways. The past five years have seen remarkable advances in understanding these heritable states and the ways that they are initiated or terminated. Transcription factors that bind directly to DNA and have sufficiency roles have been most easy to investigate and, perhaps for this reason, are most solidly implicated in pluripotency. In addition, large complexes of ATP-dependent chromatin-remodeling and histone-modification enzymes that have specialized functions have also been implicated by genetic studies in initiating and/or maintaining pluripotency or multipotency. Several of these ATP-dependent remodeling complexes play non-redundant roles, and the esBAF complex facilitates reprogramming of induced pluripotent stem cells. The recent finding that virtually all histone modifications can be rapidly reversed and are often highly dynamic has raised new questions about how histone modifications come to play a role in the steady state of pluripotency. Another surprise from genetic studies has been the frequency with which the global effects of mutations in chromatin regulators can be largely reversed by a single target gene. These genetic studies help define the arena for future mechanistic studies that might be helpful to harness pluripotency for therapeutic goals.

Contents

GENERAL FEATURES OF CHROMATIN IN STEM CELLS

In eukaryotic cells, 146 base pairs (bp) of DNA wrap an octamer of core histones to form the nucleosome, the basic unit of chromatin (Kornberg 1974). In addition to conventional histones (H2A, H2B, H3, and H4), the incorporation of "variant histones" promotes nucleosome diversity and influences overall chromatin structure (Ahmad & Henikoff 2001). Throughout the genome, nucleosomes occur as repeating arrays, separated by linker DNA associated with a fifth histone, H1, which initiates higher-order chromatin structures. Local chromatin structure is specified by the positioning of nucleosomes, which are progressively folded into poorly characterized higher-order heterochromatin that shows visible differences between cell types and between closely related species (Le Douarin & Teillet 1974). In addition, heterochromatin occurs at sites of repetitive DNA and specific chromosomal regions such as centromeres.

Investigators have long suspected that stem cells maintain their stable, heritable state by epigenetic regulatory mechanisms. Only recently have some of the genes and mechanisms become defined. Embryonic stem (ES) cells, derived from the inner cell mass (ICM) of the blastocyst, possess self-renewal potential as well as the ability to generate all cell types other than the placenta within the body (pluripotency). These characteristics of ES cells, which distinguish them from tissue stem cells with more limited self-renewal and developmental potential (generally termed multipotent), are conferred by unique transcriptional regulation due in part to the specialized and dynamic nature of their chromatin. First, fewer and more diffuse transcriptionally inactive heterochromatic foci are detected in ES cell nuclei compared with their differentiated progeny (Meshorer & Misteli 2006, Meshorer et al. 2006). Upon differentiation, condensation of ES cell chromatin into a more repressive state is associated with increased global incorporation of specific histone variants (microH2A) and concentration of heterochromatin proteins (such as HP1) at discrete foci (Dai & Rasmussen 2007, Meshorer et al. 2006). Fluorescent recovery after photobleaching (FRAP) experiments revealed an increased fraction of loosely bound or soluble structural chromatin proteins in pluripotent ES cells, which become more stably associated with chromatin upon differentiation. Accordingly, the exchange of linker histone H1 by a more tightly chromatin-bound version inhibited ES cell differentiation, whereas replacement in chromatin of core histone H3 by its variant H3.3, a marker of active transcription, accelerated their differentiation (Meshorer et al. 2006). This suggests that reorganization of chromatin structure (more compact and repressive) during lineage specification is achieved, at least in part, through the dynamic exchange of structural proteins.

The status of histone modifications further indicates that the chromatin in ES cells is more transcriptionally permissive than in differentiated cells. Pluripotent chromatin displays properties of euchromatin, such as high levels of acetylated histones and increased nuclease accessibility. Lineage specification and differentiation is accompanied by a decrease in global levels of active histone marks (such as acetylated

histone H3 and H4, including H3K4me3) and an increase in repressive histone marks (such as histone H3 lysine 9 methylation) (Azuara et al. 2006, Lee et al. 2004, Meshorer et al. 2006). However, proper histone methylation at H3K27 does not appear to be essential to maintain pluripotency, as loss of function of Polycomb repressive complex 2 (PRC2) components responsible for making the H3K27me3 repressive histone mark in ES cells does not abolish their self-renewal or their ability to produce all three germ layers (Montgomery et al. 2005, Pasini et al. 2007) but rather gives rise to later specific defects in the allocation and migration of mesoderm.

Consistent with the observation that the pluripotent chromatin is in an open conformation, ES cell chromatin is generally more permissive to the transcriptional machinery than that of differentiated cells, and tissue-specific genes that are expected to be silent in undifferentiated cells may be in a semipermissive transcriptional state in ES cells (Levings et al. 2006, Szutorisz et al. 2005). The proteosome is thought to be involved in this process by regulating the rapid turnover of transcription factors and Pol II binding at the promoters of developmentally regulated genes to restrict permissive transcriptional activity while keeping the genes in a potentiated state for later activation (Szutorisz et al. 2006). Altogether, these observations suggest that restriction of developmental potential is associated with a marked decrease in genome plasticity and the establishment of new heritable gene expression programs. As discussed below, the hyperdynamic nature of pluripotent chromatin may be essential to achieve rapid changes in transcriptional programs during lineage commitment and differentiation.

THE CORE PLURIPOTENCY CIRCUITRY

Recent studies have begun to uncover a transcriptional regulatory network in ES cells that provides insights into the molecular basis of how pluripotency is established and maintained. The genes essential or contributing to the pluripotent state are listed in **Table 1** and in an extended form in **Supplemental Table 1** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). To help the reader judge the quality of the data, null mutations that provide definitive evidence are given in bold, whereas RNAi studies are shown in plain type. Foremost among this list are the three key transcription factors, Oct4, Sox2, and Nanog, which form an intrinsic core-regulatory circuitry with positive feedback that maintains the pluripotent state of stem cells (Boiani & Scholer 2005; Boyer et al. 2005, 2006; Chew et al. 2005; Ivanova et al. 2006; Loh et al. 2006; Rao & Orkin 2006; Remenyi et al. 2003; Yeom et al. 1996). The POU family transcription factor Oct3/4 (encoded by *Pou5f1*) is a critical regulator of pluripotency. During mouse embryonic development, zygotic Oct4 expression begins at the four-cell stage of, and is subsequently restricted to, pluripotent stem cells (i.e., ICM, germ cells, and ES cells). Oct4 deficiency induces the differentiation of the ICM and ES cells into trophectoderm and later cell death, whereas its overexpression in ES cells promotes differentiation into the primitive endoderm and mesoderm lineages (highlighting the importance of negative feedback mechanisms) (Nichols et al. 1998; Niwa 2001, 2007; Niwa et al. 2000; Yeom et al. 1996). Nanog, a NK2-class homeobox transcription factor, is another component of the core pluripotency network that is required for the maintenance of pluripotency in both the ICM and ES cells (Mitsui et al. 2003). Nanog expression is restricted to pluripotent cells, and ES cells deficient for this gene spontaneously differentiate into the primitive endoderm lineage; yet, it is not essential for formation of ES cells (Chambers et al. 2003, Mitsui et al. 2003). Overexpression of Nanog in mouse ES cells can bypass the requirement for leukemia inhibitory factor in maintaining pluripotency in culture (Matsuda et al. 1999). Similarly, the SRY-related HMG-box transcription factor Sox2 is required for the maintenance of pluripotency

Table 1 Function of selected epigenetic regulators in mouse pluripotent ES cells

Gene	Gene product	Mouse mutant	Function in ES cells	Phenotype of mouse germline mutation	Reference(s)
Brg1	SWI/SNF subunit, ATPase	null and KD	Required for ES cell SR and pluripotency. Required for survival of the ICM and trophectoderm. KO ES cells cannot be derived from blastocysts*	KO embryos die during the pre-implantation stage	Bultman et al. (2000, 2006), Ho et al. (2009a,b), Kidder et al. (2009)
BAF250a/ Arid1a	SWI/SNF subunit	null	Required for ES cell pluripotency, SR and differentiation. KO ES cells are impaired in their ability to differentiate into functional mesoderm-derived cardiomyocytes and adipocytes but are capable of differentiating into ectoderm-derived neurons. KO ES cells are prone to differentiate into primitive endoderm-like cells under normal feeder-free culture conditions	KO embryos arrest development at E6.5; they form the ICM but do not gastrulate or form mesoderm	Gao et al. (2008)
BAF250b/ Arid1b	SWI/SNF subunit	null	Required for ES cell SR and proliferation. KO ES cells show a mild reduction in proliferation and more rapid differentiation	N/A; biallelic inactivation in ES cells	Yan et al. (2008)
BAF155/ Srg3	SWI/SNF subunit	null	Required for ICM outgrowth. KO ES cells cannot be derived from blastocysts*	KO embryos develop in the early implantation stage but undergo rapid degeneration thereafter	Kim et al. (2001)
BAF47/ Snf5/ini1	SWI/SNF subunit	null	Required for ICM outgrowth and formation of trophectoderm. KO ES cells cannot be derived from blastocysts*	KO embryos die between E3.5 and E5.5 at the peri-implantation stage	Klochendler-Yeivin et al. (2000), Guidi et al. (2001)
Snf2h	ISWI subunit, ATPase	null	Required for survival and growth of trophectoderm and ICM	KO embryos die during the periimplantation stage	Stopka & Skoultchi (2003)
Bptf	ISWI subunit	null	Required for ES cell differentiation. KO ES cells are deficient in their ability to form the mesodermal, endodermal, and ectodermal lineages	KO embryos manifest growth defects at the post-implantation stage and are reabsorbed by E8.5	Landry et al. (2008)
Mbd3	NuRD subunit	null	Required for ES cell pluripotency. KO ES cells can be maintained in the absence of leukemia inhibitory factor (LIF) and initiate differentiation in embryoid bodies or chimeric embryos, but fail to commit to specific lineages. ICM of KO blastocysts fails to develop into mature epiblast after implantation	KO embryos die at around the time of implantation	Kaji et al. (2006, 2007)

Ring1b/ Rnf2	Polycomb group, PRC1, H2A E3 monoubiquitin ligase	null	Required to stably maintain undifferentiated state of mouse ES cells	KO embryos show gastrulation arrest	Voncken et al. (2003), van der Stoop et al. (2008), Roman-Trufero et al. (2009)
Ezh2/Enx1	Polycomb group, PRC2, H3K27 HMTase	null	KO ES cells can be derived from blastocysts as well as self-renew	KO embryos stop developing after implantation or fail to complete gastrulation and die at around E8.5	Shen et al. (2008)
Eed	Polycomb group, PRC2	null	Eed null ES cells are pluripotent, even though they have a tendency to differentiate spontaneously in culture and display midly defective differentiation. Eed null chimeras have a paucity of mesoderm	KO embryos die at around E8.5 with all germ layers formed but defects in mesoderm formation	Faust et al. (1995), Montgomery et al. (2005)
Suz12	Polycomb group, PRC2	null	Required for ES cell differentiation in culture. KO ES cells cannot form neurons after in vitro differentiation and KO EBs fail to form a proper endodermal layer	KO embryos die during early postimplantation stages	Pasini et al. (2004, 2007)
Yy1	PRC2/3 interaction	null	KO ES cells cannot be derived from blastocysts*	KO embryos die at around the time of implantation	Donohoe et al. (1999)
Jarid2/ jumonji	Histone demethylase of jumonji family, PRC2 subunit	null	Required for ES cell differentiation. Modulates the balance between SR and differentiation. Lineage commitments are delayed in KO ES cells	KO embryos die before E15.5, required for neural tube formation	Takeuchi et al. (1995, 1999), Shen et al. (2009), Pasini et al. (2010)
Mll2/Wbp7	H3K4 HMTase	null	Required for ES cell proliferation, proper differentiation and survival but dispensable for SR and pluripotency	KO embryos fail to develop beyond around E9.5	Glaser et al. (2006), Lubitz et al. (2007)
G9a/Ehmt2	H3K9 HMTase	null	KO ES cells exhibit growth defects upon induction of differentiation with all-trans retinoic acid (RA)	KO embryos die at around E8.5–E9.5	Tachibana et al. (2002, 2005)
Glp/Ehmt1	H3K9 HMTase	null	N/A	KO embryos die at around E9.5	Tachibana et al. (2005)
Eset/Setdb1	H3K9 HMTase	null	Required for ICM outgrowth. KO ES cells cannot be derived from blastocysts*	KO embryos die at around E3.5–E5.5	Dodge et al. (2004), Bilodeau et al. (2009)
Dnmt1	Dnmt (maintenance)	null	Required for ES cell differentiation. KO ES cells proliferate normally but die upon induction of differentiation and cannot form teratomas	Development of KO embryos is arrested prior to the eight-somite stage	Lei et al. (1996), Tucker et al. (1996), Gaudet et al. (1998)
Dnmt3a/3b	Dnmt (de novo)	null	Required for ES cell differentiation. Late-passage KO ES cells cannot form teratomas	Dnmt3a KO mice become runted and die at around 4 weeks of age; Dnmt3b KO mice die after E9.5; dKO mice die before E11.5	Okano et al. (1999), Chen et al. (2003)

(*Continued*)

Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in ES cells	Phenotype of mouse germline mutation	Reference(s)
Dnmt1/3a/3b	Dnmt	null	Modest effect on ES cell proliferation. Triple KO ES cells grow robustly (although slightly slower than WT) and maintain their undifferentiated characteristics	N/A; triple-KO ES cells were studied	Tsumura et al. (2006)
p300	HAT and coactivator	null	Required for ES cell differentiation but dispensable for SR	KO embryos die at or before E11.5	Yao et al. (1998), Zhong & Jin (2009)
Thap11/Ronin	Thap and ZF-domain epigenetic regulator	null and OE	Promotes ES cell SR/proliferation, essential for pluripotency. Required for ICM outgrowth. KO ES cells cannot be derived from blastocysts.* OE inhibits ES cell differentiation	KO embryos die at periimplantation	Dejosez et al. (2008)

Abbreviations: dKO, double knockout; Dnmt, DNA methyltransferase; EB, embryoid body; ES, embryonic stem; HAT, histone acetyltransferase; ICM, inner cell mass; KD, knockdown; KO, knockout; N/A, not available; OE, overexpression; SR, self-renewal; WT, wild type; ZF, zinc finger.
*Deletion of these genes causes a failure of the ICM to give rise to ES cells in vitro, suggesting a direct role in the establishment or maintenance of pluripotency.

(Avilion et al. 2003, Masui et al. 2007). Sox2 expression is not restricted to pluripotent cells in the embryo (in contrast to Oct4 and Nanog) and is maintained in early neural cells (Avilion et al. 2003). Sox2-null embryos die immediately after implantation (Avilion et al. 2003), and shRNA-mediated knockdown of Sox2 in ES cells promotes their differentiation into multiple lineages (Ivanova et al. 2006). Oct4, Sox2, and Nanog biochemically interact with each other and coregulate the expression of many target genes (Boyer et al. 2005, Kuroda et al. 2005, Loh et al. 2006, Masui et al. 2007, Rodda et al. 2005) including histone-modification enzymes (Loh et al. 2006, 2007; Matoba et al. 2006). Oct4, Sox2, and Nanog are also direct transcriptional targets of SWI/SNF-like BAF chromatin-remodeling complexes (Ho et al. 2009a,b) and are found associated with these complexes in pluripotent ES cells (Boyer et al. 2005; Ho et al. 2009a,b; Liang et al. 2008; Zhou et al. 2007) (**Figure 1**). As discussed below, biochemical and functional interactions between the core pluripotency network and chromatin-remodeling enzymes may promote a permissive chromatin structure that is essential to preserve genomic plasticity and pluripotency (Loh et al. 2007).

EPIGENETIC MECHANISMS TO MAINTAIN PLURIPOTENCY

Chromatin-Remodeling Complexes and Pluripotency

Differentiation of ES cells or the cells of the ICM from pluripotent to developmentally more restricted states is accompanied by global epigenetic changes at the level of the chromatin structure and concomitant changes in gene expression. Stem-cell-specific genes are gradually silenced as differentiation occurs, whereas subsets of lineage-specific genes are turned on. This developmental transition occurs, at least in part, through chromatin regulatory mechanisms, which include covalent histone modification, DNA methylation of CpG

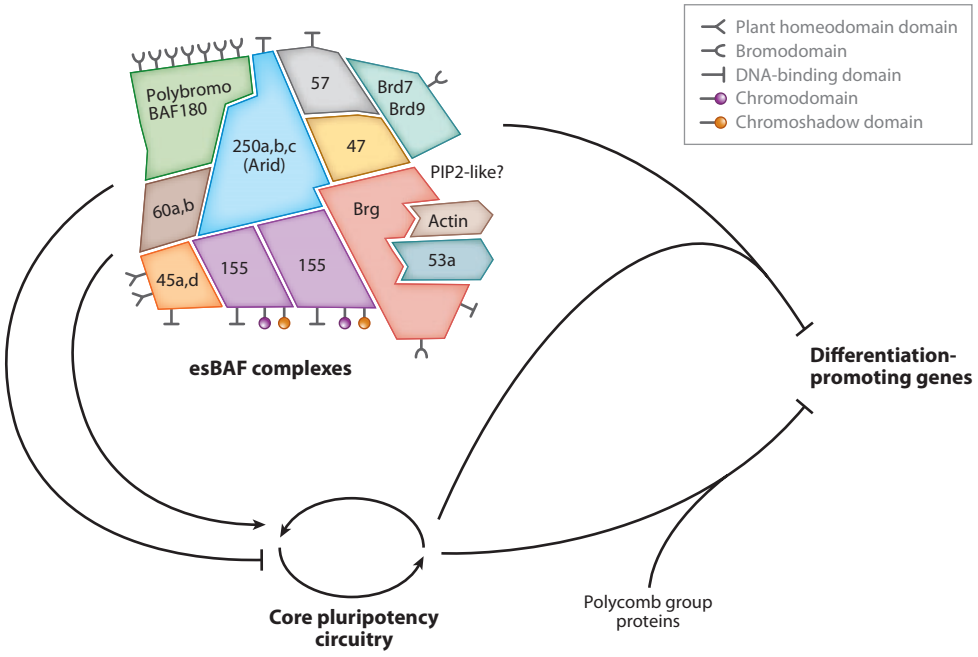

Figure 1

A functionally and structurally specialized SWI/SNF-like complex, esBAF, cobinds across the genome with the factors of the pluripotency transcriptional circuit as well as those that initiate and maintain pluripotency. esBAF complexes are distinguished by containing a homodimer of BAF155 but not 170; Brg but not Brm; BAF45a and d, but not b and c; and BAF53a but not BAF53b. Proteomic studies of endogenous complexes have demonstrated biochemical interactions with Sox2, Oct4, and many of the proteins involved in induced pluripotent stem (IPS) cell formation or embryonic stem (ES) cell maintenance. Of particular note was the absence of binding to general transcription factors or proteins such as Sp-1 or Fos that are present at high levels in ES cells, indicating that the interactions of esBAF are functionally dedicated to pluripotency. In addition, esBAF complexes occupy the promoters of nearly all genes of the core pluripotency network, such as Oct4, Sox2, c-myc, KLF4, Sall4, TCF3, and Nanog. esBAF complexes also co-occupy target genes of Oct4, Sox2, and Nanog, suggesting a functional interaction between esBAF complexes and the core pluripotency circuitry. Recently, components of esBAF were shown also to facilitate pluripotency (Singhal et al. 2010). The subunits are shown as interlocking pieces to indicate that they must be partially denatured (2 M urea) to dissociate from the complex. The positions are not necessarily accurate.

dinucleotides, and ATP-dependent chromatin remodeling.

ATP-dependent chromatin-remodeling complexes and pluripotency. Perhaps the genetically most well-documented chromatin regulators of the pluripotent state are the ATP-dependent chromatin-remodeling enzymes. In mammalian cells, approximately 30 genes encode ATP-dependent chromatin regulators that can be roughly grouped into families based on the structural features of the ATPase domain. These include Brg, Brahma/Brm, SNF2H, SNF2L, CHD1, and Mi2-beta, all of which play genetically non-redundant roles. These characterized ATPases are assembled into complexes such as BAF (also called mSWI/SNF), NuRD, ISWI, CDH1, and Tip60 and interact with several other subunits, indicating that perhaps several hundred genes are involved in ATP-dependent chromatin regulation.

In mammalian cells, the Brm (Brahma) and Brg ATPases are assembled with 12 other subunits into BAF or mSWI/SNF complexes that share certain homologs with yeast SWI/SNF

complexes, but have lost, gained, and shuffled subunits with other classes of ATPases. Highlighting fundamental mechanistic differences in the control of gene expression, mammalian BAF complexes often repress transcription from a distance, whereas the yeast SWI/SNF complex regulates all known targets by activation from promoters. Unlike the homologous complexes in yeast, flies, and worms, most subunits of mammalian BAF complexes are encoded by gene families and the complexes are combinatorially assembled (Ho et al. 2009b; Lemon et al. 2001; Lessard et al. 2007; Takeuchi & Bruneau 2009; Wang et al. 1996a,b; Wu et al. 2007, 2009). In certain cases (see below), complex composition confers functional specificity to these complexes.

Genetic studies in mice have demonstrated that BAF complexes are essential for early embryonic development and pluripotency. In mice, inactivation of most BAF subunits including the ATPase Brg as well as the BAF47, BAF57, BAF60, BAF155, BAF180, and BAF250a subunits results in early embryonic lethality, and in the case of Brg, BAF47, and BAF155, a failure of formation of pluripotent cells (Bultman et al. 2006, Doan et al. 2004, Gao et al. 2008, Guidi et al. 2001, Kim et al. 2001, Klochendler-Yeivin et al. 2000, Lickert et al. 2004, Roberts et al. 2000). Conversely, mice with deletion of the alternative ATPase Brm are viable and approximately 15% larger than controls (Reyes et al. 1998). Maternally derived Brg is required for zygotic genome activation, a nuclear reprogramming event that establishes totipotency in the cleavage-stage embryo and is required for embryonic development (Bultman et al. 2000). Consistent with this, nuclear reprogramming of permeabilized somatic human cells using extracts from *Xenopus laevis* eggs and early embryos requires Brg, demonstrating the importance of these complexes in the establishment of pluripotency (Hansis et al. 2004). Brg, BAF155, and other components of the complex were also identified in a large-scale RNAi screen targeted against chromatin regulatory factors as being required for the maintenance of ES cell colony morphology (Fazzio et al. 2008) and in a screen for genes required for Nanog expression (Schaniel et al. 2009). Interestingly, in these screens, components not characteristic of esBAF were not detected. Recently, components of esBAF were found to facilitate pluripotency (Singhal et al. 2010).

BAF or mSWI/SNF complexes have been considered to be general regulators of transcription, suggesting that the essential roles of this complex could simply reflect a general role in transcription. However, several observations argue strongly against a general role, but rather for a specific and programmatic role. First, recent proteomics studies by Ho et al. (2009b) revealed that pluripotent ES cells express distinctive complexes (termed esBAF) defined by the presence of Brg, BAF155, and BAF60a and the absence of Brm, BAF170, and BAF60c subunits (**Figure 1**). These studies indicated that the ATPase Brg is essential for the self-renewal ability of pluripotent ES cells. shRNA-mediated depletion of Brg in ES cells generated small colonies with flattened morphology indicative of spontaneous differentiation. These studies also showed that ES cells require a specific esBAF composition with respect to BAF155 and BAF170 subunits. BAF155 depletion in ES cells diminished ES cell proliferation and increased cell death, whereas enforced expression of BAF170 decreased ES cell competitive self-renewal ability and teratoma formation in immunocompromised mice (Ho et al. 2009b). Similarly, combinatorial assembly of subunits of the BAF250 family regulates esBAF function. BAF250a and BAF250b subunits are both required to maintain ES cell pluripotency and self-renewal, but they differentially regulate the potential of ES cells to develop into specific lineages (Gao et al. 2008, Yan et al. 2008). BAF250a and b are alternative subunits and esBAF complexes contain either one or the other, which imply that these subtypes of complexes are dedicated to different, non-redundant pluripotency programs. Mouse embryos lacking BAF250a (ARID1a) form the ICM but do not gastrulate or form mesoderm. ES cells deficient

for BAF250a are capable of differentiating into primitive endoderm- and ectoderm-like cells but cannot generate mesoderm-derived cardiomyocytes (Gao et al. 2008). Conversely, disruption of BAF250b in ES cells results in downregulation of pluripotency genes, reduced proliferation, and increased expression of lineage-specific genes, including markers of mesodermal differentiation. Interestingly, deletion of components of the related PBAF complex, defined by the signature subunit BAF180 or polybromo, leads not to a reduction in pluripotency, but instead to specific late developmental effects (see below). Confirming the importance of the specific subunit composition of esBAF complexes, only esBAF subunits have been detected in RNAi screens for pluripotency of ES cells (Fazzio et al. 2008, Schaniel et al. 2009).

An important question regarding the role of esBAF complexes is whether their function is simply to act in a general way, promoting the transcription of whatever genes are active in a given cell type, or whether they function in a programmatic way as an essential component of the core pluripotency circuit. Genome-wide studies of direct targets also strongly support a programmatic and unexpected function. High-resolution genome-wide analysis of Brg-containing esBAF occupancy in ES cells revealed that these complexes bind approximately 3% of the murine genome with an average footprint of approximately 2.1 kb. Transcriptional start sites show a clear peak; however, most peaks are not at the transcriptional start site and many enhancers and silencers are also sites of Brg binding (Ho et al. 2009a). Although repression at a distance had been previously demonstrated for the CD4 gene in T cells (**Figure 2**), this finding was a surprise because the yeast SWI/SNF complex activates all its genomic targets by binding to promoters. This reinforces the apparent mechanistic difference between SWI/SNF and BAF complexes and suggests caution when generalizing between the two complexes. Biochemical and genetic studies indicated that Brg-containing esBAF complexes directly interact with Oct4

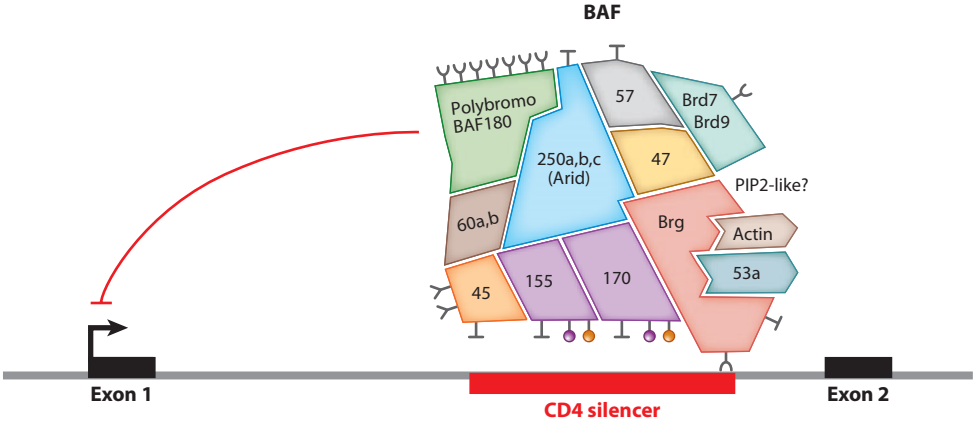

Figure 2

BAF complexes commonly repress their targets at a distance (indicated here for the CD4 gene). In developing T lymphocytes, BAF complexes bind to the CD4 silencer and repress transcription of the CD4 gene at a distance. Deletion of Brg or the silencer itself by homologous recombination results in similar phenotypes with derepression of the CD4 gene in common lymphoid progenitors. This mode of function is probably the norm for BAF complexes as shown from genome-wide studies of embryonic stem cells. Highlighting fundamental mechanistic differences in the control of gene expression, mammalian BAF complexes primarily repress transcription from a distance, whereas the yeast SWI/SNF complex regulates all known targets by activation from promoters.

and Sox2 and are required for ES self-renewal and pluripotency (Ho et al. 2009a,b). esBAF complexes occupy the enhancers and promoters of nearly all genes of the core pluripotency network, such as Oct4, Sox2, c-myc, KLF4, Sall4, TCF3, and Nanog. In addition, esBAF complexes co-occupy target genes of Oct4, Sox2, and Nanog, suggesting a functional interaction between esBAF complexes and the core pluripotency circuitry (**Figure 1**). Microarray analysis of the genes acutely affected by conditional deletion of *Brg* in ES cells revealed that Brg-containing esBAF complexes function mainly as transcriptional repressors in pluripotent ES cells. Consistent with a role for these complexes in maintaining the expression of stem-cell-specific genes within the correct range for ES cell function, Brg represses a significant number of differentiation-specific genes as well as many targets of the core pluripotency network in these cells (Ho et al. 2009a,b). Altogether, these studies suggest that esBAF functionally interacts with Sox2 and Oct4 to refine the expression of pluripotency genes, while repressing the transcription of differentiation-specific genes. This suggests a revision of the conventional view that Trithorax genes maintain the expression of developmental genes, whereas Polycomb group (PcG) genes repress them, and it implies that in the case of stem cells these regulatory circuitries may be more complex.

Combinatorial assembly of ATP-dependent BAF chromatin-remodeling complexes also orchestrates the development of the nervous system. A switch in subunit composition of neural, SWI/SNF-like BAF chromatin-remodeling complexes underlies the transition from proliferating neural stem/progenitors to postmitotic differentiated neurons (Lessard et al. 2007). Most compellingly, the self-renewal and proliferative activities of neural stem/progenitor cells require a specialized npBAF complex containing the double–plant-homeodomain (PHD) domain BAF45a/d subunit and the actin-related protein BAF53a assembled on the Brg/Brm ATPases. The dynamic exchange of these progenitor-specific subunits for the homologous BAF45b, BAF45c, and BAF53b subunits in postmitotic neurons orchestrates cell-cycle withdrawal and the acquisition of neuronal properties. The subunits of the npBAF complex are essential for neural-progenitor proliferation, and mice with reduced dosage for the genes encoding its subunits have defects in neural-tube closure similar to those in human spina bifida. BAF45a expression appears sufficient for inducing proliferation of neural progenitors, implying an instructive role of npBAF complexes. In contrast, the BAF45b/BAF53b-containing neuron-specific BAF (nBAF) complex is essential for postmitotic neuronal function, including activity-dependent dendritic outgrowth, via its association with the Ca^{2+}-responsive dendritic regulator CREST (Wu et al. 2007). Remarkably, these studies indicated that the highly homologous BAF53a protein, which is a component of neural-progenitor and non-neural BAF complexes, cannot replace BAF53b's role in dendritic development and that this functional specificity of BAF53b is conferred by its actin fold subdomain 2. More recent studies have found that microRNA-mediated regulation of specific subunits of BAF chromatin-remodeling complexes is essential for mitotic exit and the onset of dendritic morphogenesis in the vertebrate nervous system (Yoo et al. 2009) (**Figure 3**). In postmitotic neurons, BAF53a repression is mediated by sequences in the 3′ untranslated region corresponding to the recognition sites for microRNAs miR-9* and miR-124, which are selectively expressed in these cells. Mutation of these sites leads to persistent expression of BAF53a and defective activity-dependent dendritic outgrowth in neurons, whereas overexpression of miR-9* and miR-124 in neural stem/progenitor cells impaired cellular proliferation. Altogether, these studies indicate that functional specificity to ATP-dependent chromatin-remodeling complexes is achieved, at least in part, by miRNA-mediated switching of specific subunits, allowing differential interaction with specific factors that promote cell-lineage commitment and terminal differentiation.

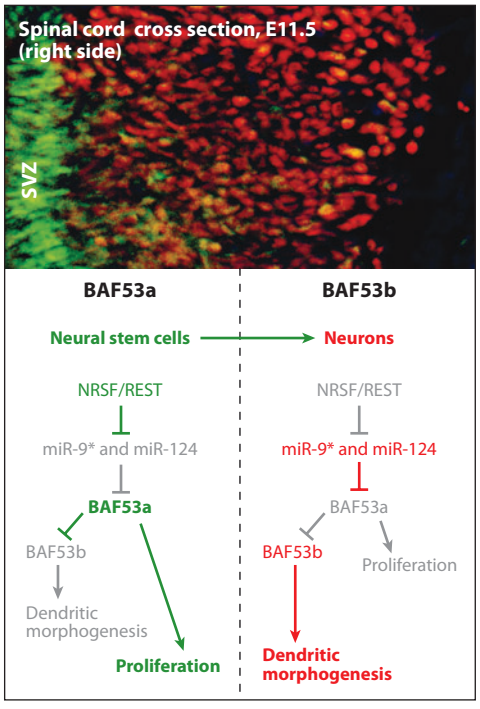

Figure 3

Genetic/epigenetic circuitry controlling mitotic exit of neural stem cells. (*left*) In neural stem cells in the subventricular zone (SVZ), NRSF/REST represses the microRNAs miR-9* and miR-124, allowing constitutive expression of BAF53a (*green*) and proliferation. npBAF complexes containing BAF53a repress BAF53b, preventing dendritic morphogenesis. Inactive paths are gray. (*right*) In postmitotic neurons, REST is repressed, leading to expression of miR-9* and miR-124, repression of BAF53a, and derepression of BAF53b (*red*). BAF53b is necessary for dendritic development in both mice and *Drosophila*. Photograph by Brett Staahl.

Finally, deletion of Brg, BAF180, and BAF60c subunits in the mouse has been associated with distinct cardiac developmental outcomes. Mice lacking *BAF180* or polybromo have specific defects in formation of the ventricular chambers of the heart that are consistent with a role for this subunit in response to retinoic acid. Interestingly, earlier retinoic acid–dependent processes do not seem to be affected (Wang et al. 2004). Conditional mutation of *Brg* in the heart indicated that Brg maintains cardiomyocytes in an embryonic state (promotes their proliferation and preserves differentiation) by interacting with histone deacetylases (HDACs) and poly (ADP ribose) polymerase (PARP) and controlling developmental gene expression. In adult cardiomyocytes, Brg is turned off but can be reactivated by cardiac stress to induce a pathological program of gene expression by interacting with HDAC and PARP (Hang et al. 2010). Similarly, RNAi interference of *BAF60c* in the early mouse embryo revealed a specific requirement in skeletal and cardiac development (Lickert et al. 2004). More recent studies have shown that BAF60c is critical to establish the regions of the embryo that give rise to the heart, a function quite different from that of BAF180 in cardiac development. Remarkably, BAF60c appears to have an instructive role in heart development, because its injection into non-cardiogenic regions of the embryo can result in the generation of beating cardiomyocytes (Takeuchi & Bruneau 2009). These studies suggest the existence of a specialized cBAF complex. However, purification of this putative cardiogenic complex has not yet been reported.

NuRD complexes. Mammalian nucleosome remodeling deacetylase (NuRD) complexes contain at least six subunits that are encoded by gene families (Bowen et al. 2004). These complexes possess both ATP-dependent chromatin-remodeling and HDAC activities (Denslow & Wade 2007). The activity of Hdac1 and Hdac2 within the complexes requires the presence of the chromodomain-containing Mi2a and Mi2b, which are SNF2/SWI2-like ATPase subunits. Other subunits of these complexes include the methyl-CpG-binding proteins Mbd 1/2/3, the metastasis-associated Mta1/2/3 proteins, the WD40-containing RbAP46 and RbAP48 proteins, and two zinc fingers proteins, p66a and p66b. Mi2b-containing NuRD complexes, which possess both transcriptional repressive and activating functions, are required for hematopoietic stem cell self-renewal and multilineage differentiation (Wade et al. 1999, Williams et al. 2004, Yoshida et al. 2008). Several subunits

of these complexes are also important for ES cell pluripotency and differentiation. ES cells lacking Mbd3 are viable but fail to form a stable NuRD complex and display a profound defect in differentiation that results in persistent self-renewal. Mbd3-deficient ES cells can be maintained in the absence of leukemia inhibitory factor and can initiate differentiation in embryoid bodies or chimeric embryos, but they fail to commit to developmental lineages, except when induced with retinoic acid (Kaji et al. 2006). Recent studies indicated that Mbd3 is required for the ICM of blastocysts to develop into mature epiblast after implantation. Expression of the pluripotency factors Oct4, Nanog, or Sox2 and their targets did not seem to be affected in the absence of MBD3 (methyl-binding domain 3), but transcription of genes that are normally expressed at the preimplantation stage and then silenced failed to be repressed. Unlike Mbd3-null ES cells, Mbd3-deficient ICMs grown ex vivo fail to expand Oct4-positive pluripotent cells despite producing robust endoderm outgrowth (Kaji et al. 2007). Together, these findings define a role for MBD3 in cell-fate commitment of pluripotent ES cells and epiblast formation after implantation.

Interestingly, a subfamily of NuRD complexes (termed NODE for Nanog and Oct4 associated deacetylase) containing Hdac1/2- and Mta1/2- and near absence (or substoichiometric levels) of Mbd3 and Rbbp7 interacts with the pluripotency factors Nanog and Oct4 (Liang et al. 2008). NODE HDAC activity seems to be comparable to NuRD, and NODE is recruited to Nanog/Oct4 target genes independently of Mbd3 in ES cells. In contrast to Mbd3 loss-of-function, knockdown of NODE subunits in ES cells increased expression of developmentally regulated genes and promoted differentiation. shRNA-mediated depletion of Mta1 also has different effects than MBD3 depletion on target genes. In contrast to Mbd3, which is required to repress preimplantation genes, Mta1 is required to repress lineage-specific factors, such as Gata6 and Foxa2. Thus, a subfamily of NuRD complexes containing Hdac1/2- and Mta1/2 is essential to maintain pluripotency by interacting with components of the core pluripotency circuitry. The question remains whether different NuRD-related complexes possess distinct enzymatic activities and play generic or specialized roles in the regulation of stem cell self-renewal, proliferation, and differentiation.

ISWI complexes. The ISWI family of chromatin remodelers contains two to four subunits based on the alternative ATPases SNF2L and SNF2H, the mammalian homologs of the *Drosophila* ISWI ATPase (Eberharter & Becker 2004). ISWI subunits differ in their expression pattern and assemble into at least seven distinct complexes. SNF2L is a component of the NURF complex, together with BPTF and RbpAp46/48. The PHD-domain-containing BPTF subunit appears to mediate the selective recruitment of ISWI complexes to target genes with transcriptionally active histone marks such as H3K4me3 (Wysocka et al. 2006), but genetic studies on mice lacking the BPTF PHD domain will be essential to confirm this result. BPTF null embryos have growth defects leading to their death by E8.5 (Goller et al. 2008), and BPTF deletion in ES cells impairs their ability to form the mesodermal, endodermal, and ectodermal lineages (Landry et al. 2008).

The chromatin-remodeling activity of at least six subfamilies of ISWI complexes, namely hACF, hCHRAC, hWICH, RSF, NoRC, and SNF2H/cohesin, is regulated by the presence of the alternative ATPase subunit SNF2H (Eberharter & Becker 2004). Snf2h$^{-/-}$ embryos die during the periimplantation stage, and Snf2h is required for the survival and proliferation of both the trophectoderm and ICM (Stopka & Skoultchi 2003). As genetic analyses indicate that ISWI complexes play important roles in diverse biological processes (such as transcriptional regulation, heterochromatin replication, chromatin assembly, and the formation of higher-order chromatin structure), it will be interesting to investigate whether combinatorial assembly of ISWI subunits assembled on SNF2H and SNF2L generates a family of heterogeneous complexes with distinct and

specialized functions in embryonic and adult stem cells (Bozhenok et al. 2002, Eberharter et al. 2001, Hamiche et al. 1999, Ito et al. 1999, Langst et al. 1999, Poot et al. 2004, Strohner et al. 2001).

Tip60-p400 complexes. The Tip60-p400 family of complexes [whose subunits, on the basis of tagging overexpressed proteins, appear to be composed of Ruvbl1, Ruvbl2, Dmap1, Ep400 (p400), Htatip (tip60), Trrap, Tip49 (TAP54α), Tip48 (TAP54β), BAF53a, β-actin, E(Pc), and MRGBP] possesses both histone acetyltransferase and chromatin-remodeling activities and can act either as positive or negative regulators of transcription (Ikura et al. 2000, Cai et al. 2003). Tip60-p400 transcriptional activity seems to be mediated, at least in part, by the incorporation of the histone variant H2AZ into nucleosomes and by the catalysis of histone acetylation at target genes (Sapountzi et al. 2006, Squatrito et al. 2006). Embryos lacking Tip60 and Trrap, two components of the Tip60-p400 complexes, also die before implantation (Gorrini et al. 2007, Herceg et al. 2001), suggesting a role in early development. Interestingly, Tip60-p400 was recently identified in a large-scale RNAi screen for chromatin-remodeling proteins involved in ES cell function (Fazzio et al. 2008). Depletion of several subunits of Tip60-p400 complexes inhibited the self-renewal ability of ES cells, impaired their ability to differentiate, and/or generated ES cell colonies with altered morphology without affecting the expression of the pluripotency transcription factors. Chromatin immunoprecipitation experiments indicated that Tip60-p400 colocalizes with the pluripotency factor Nanog and the transcriptionally active histone mark H3K4me3 in ES cells. Interestingly, the authors observed a significant overlap between Tip60-p400 target genes and that of Nanog and further demonstrated that both Nanog and H3K4me3 are required for Tip600-p400 binding at target promoters in ES cells, whereas binding of Tip60-p400 is required to mediate histone H4 acetylation at both activated and repressed target genes in ES cells.

CHD1 complexes. Although there is a strong correlation between open chromatin and the undifferentiated state of stem cells, it has long been debated whether open chromatin is necessary for stem cell potential. In support of this idea, RNAi knockdown of the chromatin remodeler Chd1 reduced chromatin decondensation and pluripotency of ES cells (Gaspar-Maia et al. 2009). Chd1 contains an ATPase SNF2-like helicase domain and belongs to the chromodomain family of proteins (Woodage et al. 1997). The two chromodomains in Chd1 are essential for recognition of H3K4me2/3 (Sims et al. 2005) and Chd1 is involved in transcriptional activation in several organisms (Simic et al. 2003, Sims et al. 2007, Stokes et al. 1996). Chromatin immunoprecipitation studies in mouse ES cells indicated that the *Chd1* promoter is bound by several pluripotency-associated factors such as Oct4, Nanog, Sox2, and Zfx (Chen et al. 2008), highlighting a potential mechanism by which CHD1 complexes function downstream of the pluripotency factors to maintain open chromatin of mouse ES cells and regulate their pluripotency.

Polycomb group genes regulate pluripotency by suppressing developmental as well as metabolic pathways. PcG proteins are an evolutionarily conserved family of chromatin regulators known best for their role in establishing and maintaining the silent state of homeotic gene expression during embryonic development (Ringrose & Paro 2004). Mammalian PcG proteins assemble into at least three biochemically distinct complexes: PRC1, PRC2, and PhoRC. The four core subunits (PHC, CBX, Bmi1, and RING1) of mammalian PRC1 complexes are homologs of *Drosophila* Ph, Pc, Psc, and dRing, respectively. Mammalian PRC2 complexes contain EED, SUZ12, and either EZH1 or EZH2. The SET-domain-containing proteins EZH2 and potentially EZH1 of PRC2 are required for the initiation of silencing through the di- and tri-methylation of the K27 residue of histone H3. This modification forms the recruiting mark for the PRC1

complex, which is implicated in the maintenance of gene repression through the formation of higher-order chromatin structures (Valk-Lingbeek et al. 2004). This process appears to involve Ring1b-mediated monoubiquitination of H2AK119, an activity that is stimulated by the Bmi1 and Mel18 PRC1 subunits (Elderkin et al. 2007). Although this simple relationship between the two biochemical activities of PRC2 and PRC1 is appealing, genetic evidence in mammals indicates that this sequential action is not used broadly (see below).

A role for PcG proteins in maintaining ES cell identity and pluripotency was first suggested on the basis that most PcG components are required for early embryonic development (mainly PRC2 subunits, see below) (Pasini et al. 2004, Shumacher et al. 1996, Voncken et al. 2003), the self-renewal/maintenance of different types of adult stem cells (Molofsky et al. 2003, Park et al. 2003), and the formation of the bivalent chromatin state of stem cells (Bernstein et al. 2006). EED is required for PRC2 activity and early embryonic development in mice (Faust et al. 1995, Shumacher et al. 1996). *Eed*^{null} embryos, which lack all detectable H3K27 methylation, display disrupted A/P patterning of the primitive streak during gastrulation and contain excess extraembryonic mesoderm but reduced embryonic mesoderm. Despite the absence of the repressive H3K27me3 mark, *Eed*^{null} ES cells can be derived from blastocysts, and chimeric embryo analyses indicated that they are pluripotent, even though they have a tendency to express differentiation-promoting genes (and differentiate spontaneously) in culture (Boyer et al. 2006, Chamberlain et al. 2008). Primordial germ cells are specified in *Eed*^{null} embryos, suggesting that they can contribute to the germline (Faust et al. 1995). However, high-contribution *Eed*^{null} chimeras have a paucity of mesoderm, suggesting that Eed is required for the specification of embryonic mesoderm (Faust et al. 1995) and/or for the differentiation or maintenance of multipotent progenitors (Chamberlain et al. 2008). Similarly, Suz12 is essential for PRC2 activity and its inactivation results in early lethality of mouse embryos (Pasini et al. 2004). ES cells and the ICM form in the absence of Suz12, and embryos lacking Suz12 produce all three germ layers. *Suz12*$^{-/-}$ ES cells are also characterized by global loss of H3K27 tri-methylation (H3K27me3) and higher expression levels of differentiation-specific genes. However, in contrast to Eed, Suz12 is apparently required for differentiation of ES cells in culture, as Suz12$^{-/-}$ ES cells cannot form neurons after in vitro differentiation, and Suz12$^{-/-}$ Embryoid bodies fail to form a proper endodermal layer (Pasini et al. 2007). A molecular explanation for this apparent paradox is not clear, but it may be related to a role of Suz12 in other complexes. Despite the crucial role of EZH2 in the di- and tri-methylation of H3K27 in ES cells, a recent study by Orkin and colleagues showed that EZH2-deficient ES cells can be derived from blastocysts as well as self-renew (Shen et al. 2008). Surprisingly, known PcG targets (derepressed in EED-deficient ES cells) remained unaffected in EZH2-deficient ES cells and still contained the H3K27me3 repressive mark. This work also revealed that EZH1 exhibits histone methyltransferase activity in vitro and colocalizes with EED at PcG targets. Depletion of EZH1 in EZH2$^{-/-}$ ES cells was sufficient to remove the repressive H3K27me3 mark from these important developmental targets, demonstrating functional complementation between these two PRC2 subunits. The PRC2-associated PCL2 (Polycomb-like 2) protein was identified in a genome-wide screen for regulators of ES cell self-renewal and pluripotency. Knockdown of *Pcl2* in mouse ES cells resulted in enhanced self-renewal, differentiation defects, and altered patterns of histone methylation (Walker et al. 2010). Although these studies suggest that PcG proteins may be dispensable for the establishment of pluripotency in ES cells, they suggest that at least some components of PRC2 complexes are required for the maintenance of pluripotency in its strictest meaning (i.e., potential of ES cells to generate all differentiated cell types in a cell-autonomous fashion as well as chimeras with germline

potential). At present, it is still not clear why PRC2 mutant embryos die, but it may relate to a failure to assemble mesodermally derived tissues such as blood vessels or withdrawal of essential cytokines and growth factors.

How might PcG genes be involved in regulating aspects of ES cell identity? Genome-wide studies indicated that PcG targets are preferentially activated upon ES cell differentiation, suggesting that they regulate pluripotency by repressing the premature expression of lineage-specific genes (Bernstein et al. 2006, Boyer et al. 2006, Buszczak & Spradling 2006, Lee et al. 2006) (**Figure 1**). Consistently, PRC1 and PRC2 targets in ES cells were enriched in genes involved in developmental patterning, signaling, morphogenesis, and organogenesis (Boyer et al. 2006, Lee et al. 2006). A significant subset of PcG target genes was co-occupied by Oct4, Sox2, and Nanog (Bernstein et al. 2006, Boyer et al. 2006, Lee et al. 2006), suggesting functional interaction between PcG proteins and the core pluripotency network (**Figure 1**). However, a much larger fraction of combined Oct4/Sox2/Nanog targets are co-occupied by Brg (Ho et al. 2009a). Finally, recent studies revealed that one of the founding members of the Jumonji C (JmjC) domain protein family, JARID2, forms a stable complex with PRC2 in pluripotent ES cells and promotes its recruitment to target genes while inhibiting its histone methyltransferase activity (Pasini et al. 2010, Peng et al. 2009, Shen et al. 2009). Jarid2-deficient mice form all germ layers and die with defects in the organization of the cardiovascular system at approximately E10.5. In other genetic backgrounds, the mice survive until birth and are fully formed, indicating that pluripotency in the early embryo is not significantly compromised. Surprisingly, Jarid2 is required for the differentiation of mouse ES cells, and activation of genes marked by H3K27me3 and lineage commitments are delayed in JARID2$^{-/-}$ ES cells. However, one group of investigators found the opposite result, i.e., that Jmjd1a or Jmjd2c depletion leads to enhanced ES cell differentiation (Loh et al. 2007). One interpretation is that the dynamic regulation of PRC2 activity by JARID2 fine-tunes the relative balance between self-renewal and differentiation decisions in pluripotent ES cells. Why these defects in pluripotency are not seen or are dramatically blunted in the embryo is not clear, but this may become apparent upon a focused analysis of the Jarid2 embryonic phenotype.

One curious feature of the phenotype of PRC2-deficient mice is that the embryos die significantly after gastrulation and slightly before or at the time that an organized vasculature becomes essential for viability (the vascular/oxygenation checkpoint). For example, VEGF-, VEGF receptor–, and calcineurin-deficient mice die at about the same time with a similar appearance (Carmeliet et al. 1996, Graef et al. 2001, Fong et al. 1995, Shalaby et al. 1995). Because cells that simply fail to differentiate properly do not necessarily die, this suggests a fundamental defect in either the metabolism of PRC2-deficient cells or the initiation of a checkpoint-induced cell death. For these reasons, reanalysis of PRC2-deficient embryos may be quite informative and provide a framework for possible mechanisms underlying PRC2 action.

Whereas deletion of any of the PRC2 subunits in mice is embryonic lethal (embryos die with defects in gastrulation 7 to 9 days postfertilization), mice with deletion of PRC1 subunits, with the exception of Ring1b, are viable, suggesting that the PRC1 complex may be redundant with another mechanism in early development (Faust et al. 1995, Pasini et al. 2007). In any case, these genetic observations indicate that it is unlikely that PRC2 functions only to set up later repression by PRC1 (**Figure 4**), because this sequential mechanism would lead to similar phenotypes for PRC1 and PRC2 complex family members. However, several PRC1 components are required for the self-renewal/maintenance of different types of multipotent adult stem cells. For example, Bmi1 is required for the maintenance of hematopoietic stem cells (Lessard & Sauvageau 2003, Park et al. 2003); leukemic hematopoietic stem cells (Lessard & Sauvageau 2003); and neural, mammary, lung, and intestinal stem cells

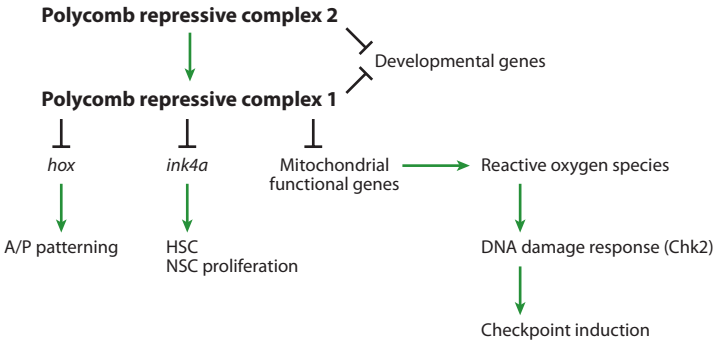

Figure 4

Potential roles of the Polycomb repressive complex 1 (PRC1) and PRC2 complexes in the maintenance of multipotent and pluripotent cells. A/P, anterior/posterior; HSC, hemopoietic stem cell; NSC, neural stem cell.

(Dovey et al. 2008, Liu et al. 2006, Molofsky et al. 2003, Pietersen et al. 2008, Sangiorgi & Capecchi 2008). In addition to Bmi1, several other subunits of PRC1 (Mel18, Phc1/Rae28, Ring1b) and PRC2 (EZH2) complexes are required for hemopoietic stem cell function (Kajiume et al. 2004, Kamminga et al. 2006, Kim et al. 2004, Ohta et al. 2002). Even though the targets of Polycomb complexes are commonly thought to be developmental genes, a recent study demonstrated that Bmi1 mutant mice show defects in mitochondrial function resulting in the release of reactive oxygen species with subsequent DNA damage. Remarkably, the Bmi defect in many stem cell populations could be repressed with a second mutation in the DNA damage checkpoint gene, CHK2 (Liu et al. 2009), indicating that a substantial role of Bmi1 in stem cell populations is to control the generation of reactive oxygen species in mitochondria (**Figure 4**). If indeed PRC2 functions upstream of PRC1, then there should also be defective mitochondrial function in Suz12, Eed, and Ezh2 mutant mice, possibly explaining early embryonic death. Altogether, these findings support a model in which Polycomb repression could act not only in pluripotent stem cells to ensure proper lineage choice, but also in progenitor cells to guide their further developmental potential by ensuring proper regulation of subtype-specific genes (**Figure 4**).

DNA Methylation and Pluripotency

DNA methylation is a covalent modification of cytosine at position C5 in CpG dinucleotides. In mammals, DNA methylation has been implicated in processes as diverse as tissue-specific gene expression, cell-fate determination, cellular differentiation, X chromosome inactivation, and imprinting (Farthing et al. 2008). In the genome of mammalian cells, nearly all DNA methylation occurs on CpG dinucleotides, more than 70%–80% of which are methylated predominantly in areas of repetitive sequences (Bird 2002). This epigenetic modification is catalyzed by several DNA methyltransferases (Dnmts). Dnmt3a and Dnmt3b are de novo methyltransferases responsible for remethylating the genome in postimplantation mouse embryos and primordial germ cells (Okano et al. 1999), whereas the maintenance of methylation relies on Dnmt1, which favors hemimethylated DNA and methylates the complementary strand (Bestor 2000). Dnmt3l lacks enzymatic activity but may act as a cofactor for the de novo Dnmts (Dnmt3a and Dnmt3b). Recent studies indicated that unmethylated H3K4 is specifically recognized by Dnmt3l (Ooi et al. 2007). Dnmt2 does not have methyltransferase activity and its function remains obscure (Okano et al. 1998). Recent studies suggest that, in addition to Dnmts, the epigenetic regulator Hells (Lsh, lymphoid-specific helicase) is directly involved in the control of de novo methylation of DNA (Zhu et al. 2006). Silencing of gene expression upon DNA methylation could occur through the recruitment of methyl-CpG binding proteins (such as MBD1, MBD2, MBD3, MBD4, MECP2, and Kaiso) or, alternatively, by blocking the binding of transcription factors to their cognate response elements. The maintenance DNA methylation enzyme (Dnmt1) can act as transcriptional repressor and associate with HDACs to silence gene expression (Robertson et al. 2000). Although DNA demethylase activity has been reported for MBD2 (Bhattacharya et al. 1999), whether DNA demethylation is a reversible process remains to be determined (see below).

Several studies suggest that DNA methylation may play a key role in cell-fate determination and pluripotency (Reik et al. 2001). Dnmt1 and Dnmt3b knockout mice die by E10.5, whereas Dnmt3a-deficient mice, which are born occasionally, suffer from serious malformations and die within weeks after birth (Li et al. 1992, Okano et al. 1999). Dnmt1-deficient ES cells are viable but undergo cell death upon induction of differentiation (Panning & Jaenisch 1996). Dnmt3a and Dnmt3b inactivation in ES cells results in progressive loss of DNA methylation patterns at both single-copy genes and repetitive sequences. In mouse ES cells, both of these enzymes directly interact (Li et al. 2007) and function synergistically to methylate the promoters of pluripotency genes such as Oct4 and Nanog. Hypomethylation of the Oct4 promoter region in ES cells allows cells to maintain high levels of Oct4 expression, thus keeping them in a pluripotent state, whereas hypermethylation of its promoter in differentiating cells correlates with its silencing. Together, these studies indicate that DNA methylation/demethylation may regulate the expression of master developmental regulators in ES cells. Interestingly, recent genome-wide studies revealed that DNA methylation at CpG-rich sequences is very low in stem cells and that methylation can occur at CpG island promoters and at CpG-rich sequences outside of promoter regions during lineage determination (Farthing et al. 2008, Fouse et al. 2008, Illingworth et al. 2008, Meissner et al. 2008, Mohn et al. 2008). Interestingly, many of the genes that are de novo methylated upon cellular differentiation are stem-cell- and germline-specific genes (Farthing et al. 2008, Mohn et al. 2008, Weber et al. 2007). These studies collectively suggest that DNA methylation is involved (either causally or as a result of) in shutting down the pluripotency program upon lineage specification and in preventing its aberrant reactivation under physiological conditions.

Recent studies have highlighted a critical role for DNA methylation in regulating adult stem cell function. For example, de novo Dnmts Dnmt3a and Dnmt3b are required to promote hemopoietic stem cell self-renewal (but not differentiation) (Tadokoro et al. 2007). Similarly, Dnmt1, MBD1, and MeCP2 are essential for fetal or adult neural stem cell function (Fan et al. 2005, Kishi & Macklis 2004, Zhao et al. 2003). However, how DNA methylation specifically contributes to pluripotency, commitment, and phenotypic maturation of specific differentiated cells is not well understood.

As mentioned above, DNA methylation has been generally considered to be irreversible, raising the following question: What removes the methylation during the induction of pluripotency? Recently, the work of Blau and colleagues has indicated that the cytosine deaminase AID (activation-induced cytidine deaminase) is required for active DNA methylation and nuclear reprogramming of somatic cell nuclei toward pluripotency (Bhutani et al. 2009). The mechanism proposed involves AID-mediated promoter demethylation and induction of OCT4 and NANOG gene expression. Base-excision repair mechanisms seem a risky way of removing methylation because mutations may result from the extensive removal of methyl marks at thousands of sites over the genome. If this is indeed the case, such mutations may reduce the therapeutic potential for induced pluripotency.

Chromatin Modifications: The Generation of Histone Marks

The diversity and complexity of histone modifications, which together act as "marks" that can signal transcriptional activation or repression, are being studied intensively. The core histones (H2A, H2B, H3, and H4) are subject to dozens of different modifications (including acetylation, methylation, phosphorylation, and ubiquitination) that can be epigenetically inherited. Lysine acetylation, the most studied modification, is generally associated with gene expression, whereas lysine methylation can lead to either gene activation or repression, depending on the residue involved. The level of

methylation of a particular lysine residue (i.e., mono-, di-, and tri-methylation) influences the levels of gene expression or repression by recruiting different effector proteins. Each histone modification can induce or inhibit subsequent modification, and this cross-talk can operate both in *cis*, on the same histone, or in *trans*, between histones. As discussed below, histone modifications can impinge on transcription by promoting the binding of transcriptional regulators and by directly altering chromatin structure. Understanding of histone modifications is undergoing revision owing to the finding that these modifications are reversible by specific demethylases. In addition, results of genome-wide studies have demonstrated remarkable lability of acetylation marks (Wang et al. 2009).

At active promoters (H3K4me3 and H3/H4Ac). Recent studies have highlighted the molecular mechanisms responsible for generating, removing, and recognizing the histone marks located at active promoters. H3/H4Ac, H3K4me3, or H4K4me2 marks are generally associated with accessible chromatin structures and gene activation (Santos-Rosa et al. 2002, Schubeler et al. 2004). These active marks are found in the promoters of nearly all transcribed genes, whereas H3K36me3 and H3K79me3 appear to be located along the actively transcribed regions (Edmunds et al. 2008). In mammals, the trimethylation of H3K4 is catalyzed by SET-domain-containing proteins of the Trithorax group, which are encoded by at least six genes in the mouse (MLL1–4, SET1a, and SET1b). The recent discovery of histone demethylases revealed that this modification is more dynamic than previously thought (Klose & Zhang 2007). Several histone demethylases belonging to the Jumonji domain-containing (Jmjd) protein family [such as lysine-specific demethylase 1 (LSD1), JHDM1A, JHDM2A, JHDM3/JMJD2] catalyze the demethylation of H3K4me2/3, H3K27me2/3, or H3k9me2/3 marks and play important roles in promoting ES cell self-renewal, pluripotency, and differentiation (Christensen et al. 2007; Cloos et al. 2008; Iwase et al. 2007; Klose & Zhang 2007; Loh et al. 2007; Pasini et al. 2008, 2010; Peng et al. 2009; Shen et al. 2009; Tsukada et al. 2006; Yamane et al. 2006, 2007). PRC2 and Rbp2 are both displaced from promoters that are activated during ES cell differentiation, resulting in removal of the H3K27me3 mark and deposition of the H3K4me3 mark (Pasini et al. 2008). The H3K4me3 mark seems to be specifically recognized by PHD-domain-containing proteins. For example, the BPTF subunit of NURF complexes is specifically recruited to H3K4me3 at the *HOXC8* promoter leading to its activation (Wysocka et al. 2006). In ES cells, removal of the H3K4me3 mark by the RBP2 demethylase leads to the silencing of *HOX* gene expression (Christensen et al. 2007). The PHD-domain-containing TAF3 subunit of the general transcription factor TFIID also recognizes the H3K4me3 mark and may contribute to the assembly of the polymerase II initiation complex at active or poised promoters (Vermeulen et al. 2007). Notably, a role for this mark in protecting inactive CG-rich promoters from de novo DNA methylation by Dnmt3L has been proposed (Ooi et al. 2007, Weber et al. 2007).

Recent genome-wide studies in ES cells have indicated that the abundance of the H3K36me3 mark better correlates with levels of gene expression than does the H3K4me3 mark. In these studies, H3K4 tri-methylation in ES cells was found at more than 80% of the annotated promoters (Guenther et al. 2007). Similarly, RNA polymerase II was detected at more than 50% of the annotated promoters in ES cells, including many silent genes. The discrepancy between RNA pol II binding, H3K4me3 levels, and gene activation may be explained by the recent observation that short abortive transcripts are synthesized at these promoters (Guenther et al. 2007). Although the underlying mechanisms are still obscure, H3K27 methylation by PcG proteins may be responsible for blocking elongation at these promoters (Bernstein et al. 2006, Boyer et al. 2006, Lee et al. 2006). As the presence of a "poised polymerase" at silent promoters was also observed in B and T lymphocytes and *Drosophila* (Barski et al. 2007, Guenther et al. 2007), inhibition of gene

elongation may represent a general mechanism to keep inactive genes "poised" for activation. In agreement with a role for histone methyltransferases (HMTases) in regulating adult stem cell populations, MLL1, MLL2, and MLL5 are required for some aspects of hemopoietic (MLL1 and MLL5) (Ernst et al. 2004, Heuser et al. 2009, Lim et al. 2009, McMahon et al. 2007), neural (MLL1) (Lim et al. 2009), and ES cell function (MLL2) (Lubitz et al. 2007).

The acetylation of histones H3 and H4, which is catalyzed by interplay between histone acetyltransferase (HAT) and HDAC enzymes (Lee & Workman 2007, Xu et al. 2007), is also associated with gene activation. Many active transcription factors either recruit HATs or utilize their own internal HAT domains (e.g., CREB binding protein) to catalyze H3 and H4 acetylation and lead to accessible chromatin structure and transcriptional activation. Bromodomain-containing proteins (such as Brg and the BAF180 subunit of BAF chromatin-remodeling complexes) are generally targeted to acetylated histone residues and may be involved in opening the chromatin structure at these sites. Interestingly, the HAT p300 is required for proper ES cell differentiation and Nanog expression (Zhong & Jin 2009), and a role for the Querkopf (Qkf) (Merson et al. 2006, Thomas et al. 2000), Moz, and CBP HATs in regulating neural and hemopoietic stem cell function has been reported (Katsumoto et al. 2006, Rebel et al. 2002, Thomas et al. 2006).

At silenced promoters (H3K27me3 and H3K9me3). Methylated H3K9, H3K27, or H4K20 residues are mainly associated with transposons, repetitive sequences, and pericentromeres and usually link to gene repression (Mikkelsen et al. 2007). The enzymes responsible for making these repressive chromatin marks are currently being elucidated (Swigut & Wysocka 2007). The best studied of these marks, H3K9me3, is catalyzed by SUV39h (mouse Suv39H1, Suv39H2), SetDB (mouse ESET), and G9a. These HMTases are likely recruited to methylated DNA by MBD proteins. H3K9 methylation allows the recruitment of heterochromatin protein-1 (HP1) and the formation of higher-order chromatin structures (Agarwal et al. 2007, Fujita et al. 2003). Heterochromatin-mediated gene silencing is propagated through cell division by an interaction between HP1, HDACs, and Dnmts (Lachner & Jenuwein 2002). Several H3K9me3 demethylases have been discovered including LSD1, Jmjd1a, and Jmjd2c (Klose et al. 2006, Loh et al. 2007, Whetstine et al. 2006). Removal of the H3K9me3 marks at the promoter of the pluripotency factor Nanog by Jmjd2c is required to prevent HP1 and KAP1 repressor binding (Loh et al. 2007).

H3K27me3 is another repressive histone mark, which is catalyzed by the SET-domain-containing EZH2 subunit of the PRC2 (Barski et al. 2007, Mikkelsen et al. 2007). Subsequent recognition of this mark by the PRC1 at the silenced promoters ensures the formation of higher-order chromatin structures and its propagation through mitosis (Cao & Zhang 2004). In ES cells, several PRC2 subunits are essential for lineage specification, suggesting an important role for H3K27 tri-methylation (Lee et al. 2006). Jmjd proteins, notably UTX1, UTY1, and JMJD3, have been identified as H3K27 demethylases (Agger et al. 2007, De et al. 2007, Lan et al. 2007, Lee et al. 2007). Interplay between histone demethylases and methyltransferases in gene activation is suggested by the recent observation that UTX1 and MLL2 (an H3K4 HMT) biochemically interact (Agger et al. 2007, Issaeva et al. 2007, Lee et al. 2007). Interestingly, a role for the HMTases Carm1, Mll2/Wbp7, G9a/Ehmt2, Glp/Ehmt1, and Setdb1 (mouse Eset) has recently been demonstrated in pluripotent ES cells, and several of those HMTases are required for ICM outgrowth (Dodge et al. 2004; Lubitz et al. 2007; Tachibana et al. 2002, 2005; Wu et al. 2009) (see **Table 1** and **Supplemental Table 1**).

Supplemental Material

Bivalent domains and pluripotency. The ES cell genome has a specific epigenetic profile characterized by a general abundance of transcriptionally active chromatin marks, such as

H3K4me3, H3K9ac3, and H4Ac, and a more localized distribution of histone marks associated with gene silencing, such as H3K27me3 (Azuara et al. 2006, Mikkelsen et al. 2007, Bernstein et al. 2006). These short active and long silent clusters of histone marks are associated with highly conserved noncoding elements termed bivalent domains. As bivalent domains frequently overlap the binding sites of the core pluripotency factors Oct3/4, Sox2, and Nanog, it has been proposed that they promote pluripotency in undifferentiated cells by maintaining the expression of lineage-specific factors in a silent state, but poised for transcription. Consistently, the "primed" gene loci replicate earlier in S phase than in their differentiated progeny (Azuara et al. 2006, Perry et al. 2004) and can be enriched for key developmental regulators that are silenced in pluripotent ES cells but activated upon differentiation (Bernstein et al. 2006). Upon ES cell differentiation, repressive marks (H3K27me3) are removed from the promoters of activated genes, whereas activating marks (H3K4me3) are erased from genes that remain silent (Bernstein et al. 2006). Several subunits of the PcG PRC2 complexes, such as Eed and Suz12, are detected at these bivalent domains, and repression of developmentally regulated genes at bivalent domains is dependent on Eed (Boyer et al. 2006, Loh et al. 2006).

The enrichment for bivalent marks at conserved elements in pluripotent mouse ES cells (versus adult tissues) initially suggested a functional relationship between bivalent domains and pluripotency (Bernstein et al. 2006). However, it was recently shown that bivalent domains are not a unique feature of pluripotent cells but are also present in differentiated cell types and can even form de novo during cellular differentiation (Azuara et al. 2006, Barski et al. 2007, Mikkelsen et al. 2007, Pan et al. 2007, Roh et al. 2006, Zhao et al. 2007). In addition, the genetic studies of Magnuson and colleagues has shown that ES cells can be formed in the absence of H3K27me3 (Chamberlain et al. 2008), indicating that bivalent marks are not essential for pluripotency, but rather mark genes that will become activated during differentiation. Based on these observations and the fact that the number of promoters with a bivalent domain configuration gradually decreases during ES cell differentiation, Bernstein and colleagues recently proposed an alternative model whereby the relative abundance of bivalent domains in a given cell type corresponds to its degree of pluripotency (Mikkelsen et al. 2007). It is important to keep in mind that current studies have examined only a small fraction of the known histone modifications in the human genome (for which we know the relationship to gene expression) and only in a small number of cell types. More comprehensive genome-wide maps of histone modifications in ES cells and their differentiated progeny as well as their impact on gene expression may help decipher the molecular mechanisms underlying stem cell pluripotency and lineage specification.

CONCLUSIONS AND PERSPECTIVES

During the past five years, genome-wide analysis combined with proteomic studies and genetics in mice have provided important advances in our understanding of the molecular basis of the stable heritable state of pluripotency. A more dynamic picture of chromatin has emerged from the discovery of demethylases and deacetylases, prompting investigations into the mechanisms stabilizing competing activities that control histone modifications. In addition, specialized assemblies of ATP-dependent chromatin-remodeling complexes, such as esBAF, appear to give robustness and stability to the pluripotent state by interacting directly with pluripotency proteins, interacting with their regulatory regions, and binding across the genome with pluripotent factors such as Oct4, Nanog, and Sox2. These ATP-dependent chromatin-remodeling complexes undergo sequential changes in subunit composition in the development of the vertebrate nervous system to coordinate mitotic exit and the onset of postmitotic neural functions. Whether such changes occur in the

development of other tissues remains to be determined. Genome-wide studies have also challenged the traditional view of the opposing action of Polycomb and Trithorax genes, revealing that the Trithorax gene *Brg* and esBAF complexes repress most of their targets, including many developmentally regulated genes, a function that was thought to be largely due to Polycomb action. The view that ATP-dependent chromatin remodeling is a permissive mechanism is being challenged by the observation that specific subunits, such as BAF45a and BAF53a, play instructive roles in directing progenitor division in the vertebrate nervous system, whereas subunits such as BAF60c appear to play instructive roles in the initiation of cardiac development. Finally, subunits of esBAF complexes facilitate reprogramming of induced pluripotent stem cells. Although chromatin regulation has generally been considered to be global and to affect vast numbers of genes, the recent discovery that most phenotypes of PRC1 mutations can be repressed by mutation of a single gene indicates that a few critical targets may mediate most of the actions of these chromatin regulators. Similar observations for the neural nBAF complex indicate that this may be a general feature of chromatin regulators. A final area of future investigations must be directed at understanding the mechanisms used by ATP-dependent chromatin-remodeling complexes. Although genetic studies strongly implicate several ATP-dependent chromatin-remodeling complexes in pluripotency, the biochemical mechanisms involved remain a mystery. Could it really be that these complexes, which in the case of the esBAF complex are 12 times the mass of a nucleosome and contain two highly active ATPases, function in vivo to move nucleosomes, a task that can be produced by the binding of a transcription factor? The development of better assays to explore the mechanisms of chromatin regulatory complexes will be critical to understanding their role in stem cells and as potential therapeutic targets.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review, although they may be hugely biased by their egos.

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Contents

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Supplemental Table 1 Function of selected mouse genes in pluripotent ES cells, NSCs, and HSCs

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
ES cells						
Chd1*	SNF2-like ATPase	KD	Required for ES cell pluripotency and differentiation. KO ES cells are incapable of giving rise to primitive endoderm and have a high propensity for neural differentiation	N/A	Gaspar-Maia et al. 2009	
Snf2h*	ISWI family ATPase	null	Required for survival and growth of TE and ICM	KO embryos die during the periimplantation stage	Stopka & Skoultchi 2003	
Bptf*	Subunit of ISWI complexes	null	Required for ES cell differentiation. KO ES cells are deficient in their ability to form the mesodermal, endodermal, and ectodermal lineages	KO embryos manifest growth defects at the postimplantation stage and are reabsorbed by E8.5	Landry et al. 2008	
Mbd3*	Subunit of NuRD complexes	null	Required for ES cell pluripotency. KO ES can be maintained in the absence of leukaemia inhibitory factor (LIF) and initiate differentiation in embryoid bodies or chimeric embryos, but fail to commit to specific lineages. ICM of KO blastocysts fails to develop into mature epiblast after implantation	KO embryos die at around the time of implantation	Kaji et al. 2006, 2007	
Mll2/Wbp7*	H3K4 HMTase	null	Required for ES cell proliferation, proper differentiation and survival but dispensable for SR and pluripotency	KO embryos fail to develop beyond around E9.5	Glaser et al. 2006, Lubitz et al. 2007	
Carm1*	H3 HMTase	KD	Required to maintain ES cell pluripotency	N/A	Wu et al. 2009	

(*Continued*)

Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
G9a/Ehmt2*	H3K9 HMTase	null	KO ES cells exhibit growth defects upon induction of differentiation with all-trans retinoic acid (RA)	KO embryos die at around E8.5-9.5	Tachibana et al. 2002, 2005	HMTases G9a and GLP form heteromeric complexes
Glp/Ehmt1*	H3K9 HMTase	null	N/A	KO embryos die at around E9.5	Tachibana et al. 2005	
Eset/Setdb1*	H3K9 HMTase	null	Required for ICM outgrowth. KO ES cells cannot be derived from blastocysts**	KO embryos die at around E3.5–E5.5	Dodge et al. 2004, Bilodeau et al. 2009	
Ring1b/ Rnf2*	Polycomb Group, PRC1, H2A E3 monoubiquitin ligase	null	Required to stably maintain undifferentiated state of mouse ES cells	KO embryos show gastrulation arrest	Voncken et al. 2003, van der Stoop et al. 2008, Roman-Trufero et al. 2009	
Ezh2/Enx1*	Polycomb Group, PRC2, H3K27 HMTase	null	KO ES cells can be derived from blastocysts as well as self-renew	KO embryos stop developing after implantation or fail to complete gastrulation and die at around E8.5	Shen et al. 2008	
Eed*	Polycomb Group, PRC2	null	Eed null ES cells are pluripotent, even though they have a tendency to differentiate spontaneously in culture and display midly defective differentiation. Eed null chimeras have a paucity of mesoderm	KO embryos die at around E8.5 with all germ layers formed but defects in mesoderm formation	Faust et al. 1998, Montgomery et al. 2005	
Suz12*	Polycomb Group, PRC2	null	Required for ES cell differentiation in culture. KO ES cells cannot form neurons after in vitro differentiation and KO EBs fail to form a proper endodermal layer	KO embryos die during early postimplantation stages	Pasini et al. 2004, 2007	

(*Continued*)

Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Yy1*	PRC2/3 interaction	null	KO ES cells cannot be derived from blastocysts**	KO embryos die at around the time of implantation	Donohoe et al. 1999	
Dnmt1*	Dnmt (maintenance)	null	Required for ES cell differentiation. KO ES cells proliferate normally but die upon induction of differentiation and cannot form teratomas	Development of KO embryos is arrested prior to the eight-somite stage	Lei et al. 1996, Tucker et al. 1996, Gaudet et al. 1998	
Dnmt3a/3b*	Dnmt (de novo)	null	Required for ES cell differentiation. Late-passage KO ES cells cannot form teratomas	Dnmt3a KO mice become runted and die at around 4 weeks of age; Dnmt3b KO mice die after E9.5; dKO mice die before E11.5	Okano et al. 1999, Chen et al. 2003	
Dnmt1/3a/3b*	Dnmt	null	Modest effect on ES cell proliferation. Triple KO ES cells grow robustly (although slightly slower than WT) and maintain their undifferentiated characteristics	N/A; triple-KO ES cells were studied	Tsumura et al. 2006	
Brg1*	SWI/SNF epigenetic regulator; ATPase	null and KD	Required for ES cell SR and pluripotency. Required for survival of the ICM and TE. KO ES cells canot be derived from blastocysts**	KO embryos die during the preimplantation stage	Bultman et al. 2000, Bultman et al. 2006, Kidder et al. 2009, Ho et al. 2009	
BAF155/Srg3*	SWI/SNF epigenetic regulator	null	Required for ICM outgrowth. KO ES cells cannot be derived from blastocysts**	KO embryos develop in the early implantation stage but undergo rapid degeneration thereafter	Kim et al. 2001	

(*Continued*)

Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
BAF47/Snf5/ini1*	SWI/SNF epigenetic regulator	null	Required for ICM outgrowth and formation of TE. KO ES cells cannot be derived from blastocysts**	KO embryos die between E3.5 and E5.5 at the periimplantation stage	Klochendler-Yeivin et al. 2000, Guidi et al. 2001	
BAF250a/Arid1a*	SWI/SNF epigenetic regulator	null	Required for ES cell pluripotency, SR and differentiation. KO ES cells are impaired in their ability to differentiate into functional mesoderm-derived cardiomyocytes and adipocytes but are capable of differentiating into ectoderm-derived neurons. KO ES cells are prone to differentiate into primitive endoderm-like cells under normal feeder-free culture conditions	KO embryos arrest development at E6.5; they form the ICM but do not gastrulate or form mesoderm	Gao et al. 2008	
BAF250b/Arid1b*	SWI/SNF epigenetic regulator	null	Required for ES cell SR and proliferation. KO ES cells show a mild reduction in proliferation and more rapid differentiation	N/A; biallelic inactivation in ES cells	Yan et al. 2008	
p300*	HAT and coactivator	null	Required for ES cell differentiation but dispensable for SR	KO embryos die at or before E11.5	Yao et al. 1998, Zhong et al. 2009	
Jarid2/jumonji*	Histone demethylase of jumonji family, PRC2 subunit	null	Required for ES cell differentiation. Modulates the balance between SR and differentiation. Lineage commitments are delayed in KO ESCs.	KO embryos die before E15.5; required for neural tube formation	Takeuchi et al. 1995, 1999; Shen et al. 2009; Pasini et al. 2010	Founding member of the Jumonji family

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Jmjd1a*	Histone demethylase of jumonji family	KD	KD leads to ES cell differentiation	N/A	Loh et al. 2007	Positively regulated by Oct4
Jmjd2c*	Histone demethylase of jumonji family	KD	KD leads to ES cell differentiation	N/A	Loh et al. 2007	Positively regulated by Oct4; Jmjd2c is a positive regulator of Nanog
Utf1*	Chromatin-associated protein, Myb/SANT domain TF	KD	Required for ES cell differentiaiton. KD results in substantial delay or block in ES cell differentiation	N/A	van den Boom et al. 2007	
Thap11/ Ronin*	Thap and ZF domain epigenetic regulator	null and OE	Promotes ES cell SR/proliferation, essential for pluripotency. KO ES cells canot be derived from blastocysts**. Required for ICM outgrowth. OE inhibits ES cell differentiation	KO embryos die at periimplantation	Dejosez et al. 2008	
H2AZ	H2A histone variant	KD	Required for lineage commitment and differentiation	KO embryos die before E7.5	Faast et al. 2001, Creyghton et al. 2008	
Nanog	Homeodomain TF	null and OE	Dispensible for expression of somatic pluripotency but is specifically required for formation of germ cells. KO ES are prone to differentiate, although they can self-renew indefinitely in the permanent absence of Nanog. Nanog is capable of maintaining ES cell SR independently of LIF/Stat3	KO embryos display early embryonic lethality	Mitsui et al. 2003; Chambers et al. 2003, 2007	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Pouf1/Oct4	Pou domain TF	null, KD, and OE	Essential for ES cell SR and pluripotency. Depletion induces differentiation into TE lineage, whereas a less than twofold increase in expression causes differentiation into primitive endoderm and mesoderm	KO embryos develop to the blastocyst stage, but the ICM is not pluripotent and embryos die around the time of implantation	Nichols et al. 1998, Niwa et al. 2000	
Sox2	SRY-related HMG box protein	null and KD	Required for maintenance of ES cell pluripotency, epiblast, and extraembryonic ectoderm development. KD in ES cells promotes their differentiation into multiple lineages, including trophoectoderm	KO embryos die after implantation	Avilion et al. 2003, Ivanova et al. 2006	
Bcor	Bcl6 corepressor	null	Regulates ES cell differentiation	KO mice show a strong parent-of-origin effect, most likely indicating a requirement in extraembryonic development	Wamstad et al. 2008	Mutated in patients with X-linked Oculofacio-cardiodental (OFCD) syndrome. Regulates gene expression in association with PcG proteins, SCF ubiquitin ligase components, and Jmjc HMTases

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Caspase-3	Cysteine protease	null	Promotes differentiation of ES cells. KO ES cells show a marked delay in differentiation (upon RA treatment)	N/A	Fujita et al. 2008	Caspase-induced cleavage of Nanog in differentiating ES cells
Zfx	Zinc finger TF	null and OE	Promotes ES cell SR and survival. KO ES cells are impaired in their SR but not their differentiation capacity and show increased apoptosis. OE facilitates SR by opposing differentiation	KO embryos develop normally until E9.5 and subsequently die owing to extraembryonic tissue abnormalities	Galan-Caridad et al. 2007	
Cgbp	Transcriptional activator CpG binding protein (unmethylated)	null	Promotes differentiation of ES cells. KO ES cells show defective differentiation (unable to achieve in vitro differentiation following removal of LIF), and increased apoptosis. KO blastocysts are viable and capable of hatching and forming both an ICM and a TE	KO embryos die between E6.5 and E12.5	Carlone et al. 2001, 2005	
Caf-1	Histone chaperone	null	KO ES cells cannot be derived from blastocysts**	KO embryos arrest development at the 16-cell stage	Houlard et al. 2006	
Npm2	Histone chaperone	null	KO ES cells cannot be derived from blastocysts**	KO females have fertility defects owing to failed preimplantation embryo development	Burns et al. 2003	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Cdx2	Caudal-type homeodomain protein	null and OE	Required for SR of TS cells and blastocyst differentiation into TE. KO blastocyst display normal contribution to all cell lineages except TE and intestinal cells. Dispensable for ES cell derivation. OE is sufficient to generate proper TS cells	KO embryos die around the time of implantation	Chawengsaksophak et al. 2004, Strumpf et al. 2005, Niwa et al. 2005	
Eomes	The T-box TF	null	KO blastocysts display a block in early TE differentiation but can implant	KO embryos die around the time of implantation	Strumpf et al. 2005	
Gata6	GATA-binding protein	null and OE	Required (together with Gata4) to generate visceral endoderm and definitive endoderm of foregut. Forced expression in ES cells is sufficient to induce the proper differentiation program towards extraembryonic endoderm	KO embryos die at E5.5–E7.5 because of defects in VE formation and subsequent extraembryonic development	Morrisey et al. 1998, Fujikura et al. 2002, Capo-Chichi et al. 2005	
Gata4	GATA-binding protein	null and OE	Required (together with Gata6) to generate visceral endoderm and definitive endoderm of foregut. Forced expression in ES cells is sufficient to induce the proper differentiation program towards extraembryonic endoderm	KO embryos die between E8 and E9 because of defects in heart morphogenesis	Molkentin et al. 1997, Kuo et al. 1997, Fujikura et al. 2002, Capo-Chichi et al. 2005	
Cyclin a2	Cell cycle regulator	null	Essential for ES cell cycle progression	KO embryos die shortly after implantation	Murphy et al. 1997, Kalaszczynska et al. 2009	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Klf5	Zinc-finger TF of the Kruppel-like family	null, OE, and KD	Essential for ES cell SR/proliferation. KO ES cells cannot be derived from blastocysts**. KD in ES cells prevents their correct differentiation. OE in ES cells maintains pluripotency in the absence of LIF	KO embryos show early embryonic lethality due to implantation defects	Ema et al. 2008, Parisi et al. 2008	
Klf2, 4 and 5	Zinc-finger TF of the Kruppel-like family	KD	Promote ES cell SR. Simultaneous depletion leads to ES cell differentiation	N/A	Jiang et al. 2008	
Zfp281	Zinc finger TF	KD	Required to maintain ES cell pluripotency	N/A	Wang et al. 2008	Interacts with Nanog
Sall4	Zinc finger TF of the splat family	null	Essential for ES cell pluripotency and proliferation but dispensable for differentiation. Reduced growth of KO ICM	KO embryos show lethality during periim-plantation	Sakaki-Yumoto et al. 2006, Zhang et al. 2006, Lim et al. 2008, Yang et al. 2008	Interacts with Nanog
Nac1	BTB domain-containing TF	KD	Required for ES cell proliferation	N/A	Wang et al. 2006	Interacts with Nanog
Foxd3	Forhead TF	null	Promotes ES cell SR, represses differentiation and maintains survival. KO ES cells cannot be derived from blastocysts**. KO ES cells display normal proliferation rate, increased apoptosis, strong precocious differentiation along multiple lineages including TE, endoderm and mesoderm	KO embryos die shortly after implantation	Hanna et al. 2002, Liu et al. 2008	Interacts with Oct4

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Tcl1	T-cell leukemia/ lymphoma TF. Cofactor of the Akt1 kinase	KD	Required to sustain the undifferentiated state of ES cells and for efficient SR. Downregulation induces differentiation of ES cells along specific lineages	N/A	Ivanova et al. 2006	
Tcf3	HMG-domain containing TF, DNA-binding effector of Wnt signaling	null and KD	Inhibits ES cell SR. KO ES cells can self-renew in absence of LIF and display delayed differentiation in embryoid bodies. Depletion delays ES cell differentiation (Cole)	KO embryos die at around E7.5–E9.5 from early gastrulation defects	Merrill et al. 2004, Yi et al. 2008, Cole et al. 2008	
Esrrb	Estrogen-related receptor	KD	KD promotes ES cell differentiation into a mixture of extraembryonic and embryonic lineages	N/A	Ivanova et al. 2006	Target of Oct4 and Nanog in ES cells
Tbx3	T-box TF	KD	KD triggers ES cell differentiation into lineages derived from the primitive ectoderm	N/A	Ivanova et al. 2006	Target of Oct4 and Nanog in ES cells
Rest	RE1-silencing TF	HET +/− and null	Dispensable for ES cell SR (Jorgensen et al. 2009). However, Singh et al. 2008 claimed that REST promotes ES cell SR and pluripotency	KO embryos die at around E9.5	Chen et al. 1998, Singh et al. 2008, Jorgensen et al. 2009	May act through suppression of miR-21, which specifically suppresses ES SR
Pim-1, Pim-3	Serine/threonine kinases	KD	KD increases the rate of spontaneous ES cell differentiation, impairs growth and increases apoptosis	N/A	Aksoy et al. 2007	
Dicer	RNAi machinery	null	KO ES cells cannot be derived from blastocysts**	KO embrryos die early in development, depleted of stem cells	Bernstein et al. 2003	Oct4 staining is much reduced in KO embryos

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Fetal NSC						
N-CoR*	Nuclear receptor co-repressor	null	Promotes SR/proliferation. KO cortical progenitors show reduced SR/proliferation and premature differentiation into astrocytes	KO embryos generally die before E16	Jepsen et al. 2000, Hermanson et al. 2002	
Dnmt1*	DNA methyl-transferase (maintenance)	null	Controls the timing and magnitude of astroglial differentiation. KO cells display precocious astroglial differentiation	KO embryos die at gastrulation	Li et al. 1992, Fan et al. 2005	
Ring1b/ Rnf2*	Polycomb Group PRC1, H2A E3 monoubiquitin ligase	null	Promotes maintenance/SR of embryonic olfactory bulb NSCs; KO NSCs display impaired SR/proliferation and multipotential abilities	KO embryos show gastrulation arrest	Voncken et al. 2003, Roman-Trufero et al. 2009	
Bmi1*	Polycomb Group, PRC1	null and OE	Essential for the SR and maintenance of NSCs from the CNS and PNS	KO mice die at around 4 months of age	van der Lugt et al. 1994, Molofsky et al. 2003, He et al. 2009	
Hmga2*	Chromatin regulator	null	Promotes SR (in young but not old mice). KO embryos show reduced NSC numbers and SR throughout the central and peripheral nervous systems of fetal and young-adult mice but not old-adult mice	KO mice exhibit a dwarf phenotype	Zhou et al. 1995, Nishino et al. 2008	
Querkopf (Qkf/Myst4/ Morf)*	Querkopf mutation is due to an insertion into a MYST family HAT gene	gene trap allele produc-ing 5% normal mRNA levels	Homozygous mice for Qkf mutation show reduced numbers of embryonic neural precursors	Qkf homozygous mice have craniofacial abnormalities and fail to thrive in the postnatal period	Thomas et al. 2000	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Presenilin/ Ps1	Notch pathway	null	Required for the maintenance of NSCs; decreased neurosphere frequency at E14.5	KO mice die shortly after natural birth or Caesarean section	Shen et al. 1997, Hitoshi et al. 2002	
Presenilin1, 2	Notch pathway	null	Required for the maintenance of NSCs; no neurospheres formed at E14.5	Homozygous mice for a targeted null mutation in PS2 exhibit no obvious defects	Donoviel et al. 1999, Hitoshi et al. 2002	
Dll1	Notch pathway	null	Regulates NSC differentiation. Increased neurons and decreased glial cells in differentiated KO neurosphere cultures at E10.5	KO embryos die at E11.5, before gliogenesis begins	Hrabé de Angelis et al. 1997, Grandbarbe et al. 2003	
Notch1	Notch pathway, transmembrane receptor	null and OE	Required for NSC maintenance. KO embryos show precocious neuronal differentiation, earlier neural progenitor pool depletion and decreased neurosphere frequency at E10.5 and E12.5. OE of constitutively active form of Notch1 in early neural progenitor cells induces apoptosis	KO embryos die between E10.5 and E11.5	Conlon et al. 1995, Hitoshi et al. 2002, Yoon et al. 2004, Yang et al. 2004	
RBP-Jκ	Notch pathway	null	Required for the maintenance (but not generation) of NSCs	KO embryos die soon after E8.5	Oka et al. 1995, Hitoshi et al. 2002	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Hes1, 3, 5	Notch pathway, bHLH TF repressor	null	Hes1 and Hes5 are required for NSC maintenance and control the timing of NSC differentiation. Decreased neurosphere frequency at E11.5. Neuroepithelial cells are initially independent of, but become dependent on, Hes gene activities for their maintenance before changing to radial glia. Hes1 and Hes 5 KO embryos show premature neuronal differentiation. doule KOs show increase severity of premature neuronal differentiation	Hes1-Hes5 dKO embryos survive until E10.5; the majority of Hes1–Hes3 dKO embryos survive until E10.5 but most of them die by E15.5	Ishibashi et al. 1995, Tomita et al. 1996, Ohtsuka et al. 1999, Cau et al. 2000, Hirata et al. 2001, Hatakeyama et al. 2004	
Numb and numblike	Adaptor protein, inhibitor of Notch signaling	null	Required to maintain progenitor cells during the initial progenitor versus neuronal fate decision and for the polarity of neural progenitors. KO embryos show premature progenitor cell depletion and malformation of neocortex and hippocampus after initial waves of neurogenesis	Numb KO embryos die around E11.5; numblike KO mice are viable, fertile, and exhibit no obvious phenotypes; dKO embryos die at around E9.5	Zhong et al. 2000; Petersen et al. 2002, 2004; Rasin et al. 2007	
B-catenin	Wnt signaling	null and OE of activated B-catenin	Essential for the maintenance and proliferation of neuronal progenitors. OE of activated B-catenin increases the size of the neuronal precursor population	KO embryos lack mesoderm and head structures and die before E7.5	Huelsken et al. 2000, Zechner et al. 2003	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Wnt3a	Wnt pathway	null	Essential for caudomedial cortical progenitor proliferation. KO mice show under-development of the hippocampus because of lack of proliferation. Caudomedial cortical progenitor cells appear to be specified normally, but then underproliferate	KO embryos die between E10.5 and E12.5 of gastrulation defects	Takada et al. 1994, Lee et al. 2000	
Lrp6	Co-receptor for Wnt signaling	null	Regulates the number of precursors setting up the dentate anlage and the radial glial network. Formation of the dorsal thalamus is disrupted due to failure to produce certain types of thalamic neurons	KO embryos die at birth	Pinson et al. 2000, Zhou et al. 2004	
Shh	Shh pathway	null	Promotes proliferation and inhibits differentiation of CNS precursor cells and granule cell precursors (GCPs) in the cerebellum. KO telencephalon is dysmorphic and reduced in size	KO embryos die at or just prior to birth	Chiang et al. 1996, Rowitch et al. 1999, Wechsler-Reya et al. 1999, Rallu et al. 2002	
Pax6	Paired box and homeobox TF	null	Essential for cortical NSC SR/ proliferation, multipotency and neurogenesis. KO reduces SR by decreasing expression of key cell cycle regulators resulting in excess early neurogenesis	KO mice have a phenotype similar to *Small eye* mutants and die a few minutes after birth	St-Onge et al. 1997, Sansom et al. 2009	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Sox1	TF, high-mobility-group DNA binding protein	null and OE in telencephalon and neural tube of chick embryos	Maintains neural progenitor cells in an undifferentiated state. Telencephalic neural progenitor cells isolated from KO embryos formed neurospheres normally, but were deficient in neuronal differentiation. Overxpression in the telencephalon expanded the progenitor pool and biased neural progenitor cells towards neuronal lineage commitment.	KO mice are viable, have small eyes with opaque lenses, and suffer from spontaneous seizures	Nishiguchi et al. 1998, Bylund et al. 2003, Kan et al. 2007	
Sox2	TF, high-mobility-group DNA binding protein	null and OE in chick neural tube	Required for NSC maintenance and hippocampal development. In KO mice, NSCs and neurogenesis are completely lost in the hippocampus, leading to dentate gyrus hypoplasia. OE in chick neural tube inhibits neuronal differentiation and results in the maintenance of progenitor characteristics	KO embryos show periim-plantation lethality	Graham et al. 2003, Bylund et al. 2003, Avilion et al. 2003, Favaro et al. 2009	
Sox 3	TF, high-mobility-group DNA binding protein	OE in neural tubes of chick embryos	Chick in ovo electroporation experiments suggest that Sox3 maintains neural progenitor cells in an undifferentiated state	KO embryos show early lethality due to gastrulation defects	Bylund et al. 2003, Rizzoti et al. 2004	
Mash1	bHLH TF	null	Positively regulates early steps of differentiation in NSCs	KO mice die at birth with apparent breathing and feeding defects	Guillemot et al. 1993, Torii et al. 1999	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Trim32	TRIM-NHL protein, E3 ubiquitin ligase	KD and OE	Reduces SR of NSCs. Depletion causes both daughter cells to retain progenitor cell fate. OE induces neuronal differentiation of cultured NSCs	KO mice are viable but replicate human muscular dystrophy phenotypes with age	Schwamborn et al. 2009, Kudryashova et al. 2009	
PPARγ	Peroxisome proliferator-activated receptor γ	HET, KD, and domi-nant negative	Promotes NSC proliferation. HET NSC have significantly reduced proliferation. Activation by agonists inhibits the differentiation of NSCs into neurons	KO embryos die at E10–E11 because of placental dysfunction and disordered development	Kubota et al. 1999, Wada et al. 2006	
Egfr	Growth factor receptor	null	Regulates the proliferation and/or differentiation of astrocytes and survival of post-mitotic neurons. KO causes forebrain cortical dysgenesis at late embryonic and postnatal ages	KO leads to peri-implantation death due to degeneration of the ICM on the CF-1 background, death at midgestation due to placental defects on 129/Sv background, and mice live for up to 3 weeks with abnormalities in several organs on CD-1 background	Threadgill et al. 1995; Sibilia et al. 1995, 1998	
Fgf2	Growth factor	null	Necessary for neural progenitor cell proliferation and neurogenesis. KO embryos significant reduction in cortical progenitor cell proliferation before neurogenesis begins	KO mice are viable and appear grossly normal	Zhou et al. 1998, Raballo et al. 2000	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Pten	Tumor suppressor, phosphatase	null	Negatively regulates SR/proliferation of neural stem/ progenitor cells. KO NSCs show increased SR/proliferation due in part to shortened cell cycle, decreased cell death and enlarged cell size. Increased neurosphere frequency at E14.5	KO embryos die at around E9.5	Suzuki et al 1998; Groszer et al. 2001, 2006	
p53	Tumor suppressor	null	Negatively regulates SR/proliferation of olfactory bulb NSCs. KO embryos show increased number of neurosphere-forming cells at E13.5, increased stem/ progenitor cell proliferation and differentiation biased toward neuronal precursors	KO mice are developmentally normal but susceptible to spontaneous tumors	Donehower et al. 1992, Armesilla-Diaz et al. 2009	
Adult NSC						
Bmi1*	Polycomb Group, PRC1	null	Required for postnatal NSC SR	KO mice die at around 4 months of age	van der Lugt et al. 1994; Molofsky et al. 2003, 2005; Bruggeman et al. 2005; Fasano et al. 2009	
Mll1/ All-1/Hrx*	TrxG Group, H3K4 HMTase	null	Required for neurogenesis from post-natal NSCs. KO SVZ NSCs have impaired neuronal differentiation but exhibit normal survival, proliferation and differentiation into glial lineages	KO embryos die at around E10.5	Yu et al. 1995, Lim et al. 2009	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Mecp2*	Methylated CpG binding protein, transcriptional repressor	null	Involved in the maturation and maintenance of neurons (such as dendritic arborization), not in their differentiation	KO mice have neurological symptoms that mimic Rett syndrome and die at around 6 weeks of age	Guy et al. 2001, Kishi et al. 2004, Matarazzo et al. 2004, Martin et al. 2009	
Mbd1*	Methyl-CpG binding protein, subunit of NuRD complexes	null	Regulates NSC differentiation. KO NSCs exhibit reduced neurogenesis and increased genomic instability	KO mice are viable and fertile	Zhao et al. 2003	
Querkopf (Qkf/Myst4/ Morf)*	HAT of the MYST family	gene trap allele producing 5% normal mRNA	Promotes SR and regulates differentiation. LOF phenotype includes reduction in adult NSC numbers, SR/proliferation and neuronal differentiation defect	Qkf homozygous mice have craniofacial abnormalities and fail to thrive in the postnatal period	Thomas et al. 2000, Merson et al. 2006	
Hmga2*	Chromatin regulator, high-mobility-group protein	null	Promotes SR (in young but not old mice). Reduced NSC numbers and SR throughout the central and peripheral nervous systems of fetal and young-adult KO mice but not old-adult mice	KO mice exhibit a dwarf phenotype	Zhou et al. 1995, Nishino et al. 2008	let-7b microRNA is known to target HMGA2
Presenilin1/ Ps1	Notch pathway	HET	Essential for the maintenance of NSCs. NSCs are reduced in the brains of HET mice	KO mice die shortly after natural birth or Caesarean section	Shen et al. 1997, Hitoshi et al. 2002	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Numb and numblike	Supresses Notch signaling	null	Regulate SVZ neural progenitor survival, polarity and cell adhesion	Numb KO embryos die around E11.5; numblike KO mice are viable, fertile, and exhibit no obvious phenotypes; dKO embryos die at around E9.5	Zhong et al. 2000, Kuo et al. 2006	
Smoothened (smo)	Shh pathway	null	Essential for the maintenance of postnatal telencephalic NSCs. KO progenitors from the P15 SVZ form significantly fewer neurospheres, proliferate less and show increased cell death. Generation of oligodendrocytes is compromised. P15 cortex, hippocampus and olfactory bulb are abnormal	KO mice do not survive beyond E9.5 and exhibit ventral cyclopia and holoprosencephaly	Zhang et al. 2001, Machold et al. 2003	
EGF-R	Growth factor receptor	null	Involved in the proliferation and/or differentiation of astrocytes and in the survival of postmitotic neurons. KO causes forebrain cortical dysgenesis at late embryonic and postnatal ages	KO leads to periimplantation death due to degeneration of the ICM on the CF-1 background, death at midgestation due to placental defects on 129/Sv background, and mice live for up to 3 weeks with abnormalities in several organs on CD-1 background	Threadgill et al. 1995; Sibilia et al. 1995, 1998	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
TGFα	Growth factor	null	Promotes NSC proliferation. KO mice show decreased proliferation within the SVZ (severity increases with age)	KO mice are viable and fertile	Luetteke et al. 1993, Tropepe et al. 1997	
Tlx	Orphan nuclear receptor	null	Essential for the maintenance and the proliferation of adult NSCs	Mature KO mice suffer from retinopathies, severe limbic defects, aggressiveness, reduced copulation, and progressively violent behavior	Shi et al. 2004	
ERβ	Estrogen receptor β	null	Essential for neuronal maintenance. KO mice show significant neuronal loss	KO mice are viable and fertile	Krege et al. 1998, Wang et al. 2001	
TRα	Thyroid hormone receptor α	null	Essential for NSC progression through cell cycle, suggesting a role in neurogenesis	KO mice die within 5 weeks after birth	Fraichard et al. 1997, Lemkine et al. 2005	
Sox2	TF, high-mobility-group DNA binding protein	null	Promotes the maintenance/proliferation of adult NSCs and maintenance of neurons in specific regions. KO causes hippocampal neurogenesis loss	KO embryos show periimplantation lethality	Graham et al. 2003, Bylund et al. 2003, Avilion et al. 2003, Ferri et al. 2004, Episkopou et al. 2005, Favaro et al. 2009	
p53	Tumor suppressor	null	Negatively regulates SR/proliferation and survival of adult NSCs	KO mice are developmentally normal but susceptible to spontaneous tumors	Donehower et al. 1992, Meletis et al. 2006	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Pten	Tumor suppressor, lipid phosphatase	null	Suppresses SR of adult NSCs. KO leads to persistently enhanced SR of NSCs in the subependymal (SEZ) zone (without signs of exhaustion) and constitutive neurogenesis in the olfactory bulb	KO embryos die at around E9.5	Suzuki et al 1998, Gregorian et al. 2009	
p21/cip1/ waf1	Cyclin dependent kinase inhibitor	null	Essential for the life-long maintenance of adult NSC SR. KO leads to loss of adult forebrain NSCs under proliferative stress (exhaustion). KO NSCs display limited in vitro SR (exhaust after few passages)	KO mice survive into late adulthood with a low incidence of tumorigenesis	Deng et al. 1995, Kippin et al. 2005	
Cdk2	Cyclin-dependent kinase	null	Required for SR/proliferation of adult SVZ NSCs. KO SVZ cells in culture display decreased SR/proliferation and enhanced differentiation	KO mice are viable but sterile	Berthet et al. 2003, Jablonska et al. 2007	
Ink4a/p16	Cyclin-dependent kinase inhibitor, tumor suppressor	null	Required for long-term SR capacity of SVZ NSCs. Aging KO mice show a significantly smaller decline in the frequency and SR/proliferation potential of SVZ multipotent progenitors and olfactory bulb neurogenesis than controls	KO mice are viable and fertile but have increased incidence of spontaneous and carcinogen-induced cancers	Sharpless et al. 2001; Molofsky et al. 2005, 2006	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Hmga2*	Chromatin regulator, high-mobility-group protein	null	Promotes SR (in young but not old mice). Reduced NSC numbers and SR throughout the central and peripheral nervous systems of fetal and young-adult KO mice but not old-adult mice	KO mice exhibit a dwarf phenotype	Zhou et al. 1995, Nishino et al. 2008	let-7b microRNA is known to target HMGA2
E-Cadherin	Cell adhesion protein	null, OE, and adhesion-blocking antibodies	Promotes SR/proliferation of adult NSCs	KO embryos die around the time of implantation	Larue et al. 1994, Karpowicz et al. 2009	
Fetal HSC						
Scl/ tal-1	bHLH TF	null	Essential for primitive hemopoiesis in the yolk sac, essential for HSC identity, promotes HSC SR	KO embryos die between E8.5 and E10.5	Shivdasani et al. 1995, Robb et al. 1995, Porcher et al. 1996	
Aml1/Runx1/ Cbfα*	TF, core binding factor (CBF) alpha subunit of a heterodimeric TF complex	null	Essential for definitive hematopoiesis, promotes HSC SR. KO embryos show normal morphogenesis and yolk sac-derived erythropoiesis, but lack FL hematopoiesis	KO embryos die at around E12.5	Okuda et al. 1996	
Cbfβ	non-DNA binding protein, core binding factor (CBF) beta subunit of a heterodimeric TF complex	null	Required for HSC emergence and normal differentiation of lymphoid and myeloid lineage cells	KO embryos die between E12.5 and E13.5 with extensive hemorrhages	Wang et al. 1996, Sasaki et al. 1996, Miller et al. 2002	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Mll/ All-1/Hrx*	Trithorax Group, H3K4 HMTase	null	Essential for definitive hemopoiesis, promotes SR, essential for the generation of HSCs in the embryo; KO HSC are reduced in number and unable to compete with WT cells in tx assays	KO embryos die at around E10.5	Yu et al. 1995, Ernst et al. 2004, McMahon et al. 2007	
Moz*	Transcriptional coactivator, MYST family of HAT	null	Necessary for HSC maintenance. KO FL HSCs fail to reconstitute a lethally irradiated host, reduced number of progenitor cells, partial block in late stage of erythroblast maturation	KO mice die at birth	Thomas et al. 2006, Katsumoto et al. 2006	
Cbp*	Co-activator HAT, CREB-binding protein	null	Necessary for HSC SR (embryonic) not for HSC generation per se	KO embryos die around E10.5–E12.5, apparently as a result of massive hemorrhage	Tanaka et al. 2000, Rebel et al. 2002	
Cdx4	Caudal related homeobox TF	OE	Brief pulses of ectopic Cdx4 expression are sufficient to enhance hematopoiesis during ESC differentiation	KO embryos are born healthy and appear morphologically normal	van Nes et al. 2006, Lengerke et al. 2007	
Rae28/mph1*	Polycomb Group, PRC1	null	Necessary for HSC SR	KO mice exhibit perinatal lethality	Takihara et al. 1997, Ohta et al. 2002, Kim et al. 2004	
Gata1	Zinc finger TF	null	Essential for embryonic erythropoiesis	N/A; ES cell chimeras, no germline transmission	Pevny et al. 1991, 1995; Fujiwara et al. 1996	
Gata2	Zinc finger TF	null	Essential for embryonic hemopoiesis. KO mice have a profound deficit in definitive HSC/ progenitors due to poor expansion in response to hemopoietic GF	KO embryos die at around E10–E11 with severe anemia	Tsai et al. 1994	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Lmo2/rbtn2	Cysteine-rich LIM domain protein	null	Essential for embryonic (yolk sac) erythropoiesis	KO embryos die at around E10.5	Warren et al. 1994	
Sox17	HMG-box TF	null	Required for the maintenance of fetal and neonatal, but not adult HSCs. Necessary for HSC SR. KO: loss of fetal and neonatal but not adult HSCs	KO mice die before E10.5	Kanai-Azuma et al. 2002, Kim et al. 2007	
Wnt3a	Growth factor, Wnt pathway	null	Promotes HSC SR. LOF embryos show defective HSC SR and defects in progenitor cell differentiation	KO embryos die between E10.5 and E12.5 of gastrulation defects	Takada et al. 1994, Luis et al. 2009	Canonical and noncanonical
C-mpl	Thrombopoietin (Tpo) receptor	null	Promotes HSC SR. Defect in amplification/SR of KO lin-AA4.1+Sca+ HSCs	KO mice are viable, healthy, and display no overt abnormalities	Gurney et al. 1994, Petit-Cocault et al. 2007	
Meis1	Homeodomain HOX co-factor	null	Essential for definitive (FL) hemopoiesis. KO HSC population in FL is reduced. KO HSC fail to radioprotect lethally irradiated animals and compete poorly in repopulation assays even though they can repopulate all hematopoietic lineages	KO embryos die between E11.5 and E14.5	Hisa et al. 2004, Kirito et al. 2004, Azcoitia et al. 2005	
Pu.1	ETS family TF	null	Necessary for SR. KO FL HSC can home to the BM, but have a defect in long-term reconstitution of adult BM as well as commitment and maturation of myeloid and lymphoid lineages	KO embryos die at a late gestational stage	Scott et al. 1994, Iwasaki et al. 2005	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Cited2	Transcriptional modulator	null	Promotes SR. Reduced numbers of KO KLS and progenitor cells of different lineages. KO HSCs are less competitive in tx assays and show compromised reconstitution of B, T and myeloid lineages	KO embryos die with cardiac malformations, adrenal agenesis, abnormal cranial ganglia, and exencephaly	Bamforth et al. 2001, Chen et al. 2007	Cited2: cAMP-responsive element binding protein CBP/p300-interacting transactivators with E- and D-rich tail
c-myb	TF	null	Essential for definitive hematopoiesis. KO FL does contain some cells with a hematopoietic progenitor phenotype, albeit at a reduced number. Multilineage defects are observed	KO embros die by E15 and are severely anemic	Mucenski et al. 1991, Sumner et al. 2000, Sandberg et al. 2005	
Prep1/ pKnox1	Homeodomain protein, Hox co-factor	hypomorphic allele that produces 3–10% of the normal Prep1 protein level	Required for the establishment of definitive hematopoiesis. KO FL cells compete inefficiently with WT BM in competitive repopulation experiments, suggesting that the major defect lies in LTR-HSCs	KO embryos die at gastrulation. Hypomorphic (Prep1i/i) mutation is embryonic lethal	Azcoitia et al. 2005, Ferretti et al. 2006, Di Rosa et al. 2007	
Evi-1	TF (SET/PR domain family)	null	Promotes HSC SR/proliferation. KO HSCs are severely reduced in number and have defective proliferative and repopulating capacity	KO embryos die at around E10.5	Hoyt et al. 1997, Goyama et al. 2008	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Car	Calcium-sensing receptor (CaR)	null	Essential for proper HSC localization in the niche. KO HSCs are highly defective in localizing anatomically to the endosteal niche	KO mice become hypercalcaemic and die by the age of 7–10 days	Adams et al. 2006	
Lyl-1	bHLH TF	null	Essential for the maintenace of HSCs. Decreased frequency of KO LSK HSCs. KO HSCs are impaired in their competitive reconstituting abilities, especially with respect to B and T lineage reconstitution	KO mice are viable and fertile	Capron et al. 2006	
Adult HSC						
Bmi-1*	Polycomb Group, PRC1	null	Required for the maintenance of adult but not fetal HSCs. Promotes HSC SR	KO mice die at around 4 months of age	van der Lugt et al. 1994, Park et al. 2003, Lessard et al. 2003	
Mel-18*	Polycomb Group, PRC1	null	Represses HSC SR		Kajiume et al. 2004	
Ezh2/enx1*	Polycomb Group, PRC2, H3K27 HMTase	null	Necessary for HSC SR, prevents adult BM HSC exhaustion	KO embryos stop developing after implantation or fail to complete gastrulation and die at around E8.5	O'Carroll et al. 2001, Kamminga et al. 2006	
Ring1b/Rnf2*	Polycomb Group, PRC1, H2A ubiquitinase	null	Restricts the proliferation of early progenitors and promotes the proliferation of their maturing progeny	KO embryos show gastrulation arrest	Voncken et al. 2003, Calés et al. 2008	
Mll-1/ All-1/hrx*	Trithorax Group, H3K4 HMTase	null	Promotes HSC SR. KO HSCs are highly compromised in their ability to competitively reconstitute irradiated recipients	KO embryos die at around E10.5	Yu et al. 1995, McMahon et al. 2007	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Mll5*	Trithorax Group	null	Promotes HSC SR, involved in terminal myeloid differentiation. KO HSCs have impaired competitive repopulating capacity	KO mice are viable but males are infertile	Heuser et al. 2009, Madan et al. 2009	May act through a mechanism involving DNA methylation
Hmga2*	Chromatin regulator, high-mobility-group protein	null	Promotes HSC SR	KO mice exhibit a dwarf phenotype	Zhou et al. 1995	
Mi-2β*	SNF2-like ATPase of the NuRD complex	null	Essential for HSC SR and multilineage differentiation. Initial expansion of KO HSCs and erythroid progenitors that are later depleted as more differentiated proerythroblast accumulate (signs of erythroid leukemia)	N/A; inducible deletion strategy in the adult BM was used	Yoshida et al. 2008	
Dnmt3a/b*	Dnmt (de novo)	null	Essential for HSC SR. dKO HSCs, but not single KO, are incapable of long-term reconstitution in tx assays	Dnmt3a KO mice become runted and die at around 4 weeks of age; Dnmt3b KO mice die after E9.5; dKO mice die before E11.5	Okano et al. 1999, Tadokoro et al. 2007	CRE-mediated deletion in CD34-KLS-purified cells
Ink4a/p16	Cyclin-dependent kinase inhibitor	null	Regulates HSC SR. In young mice, reduced HSC SR capacity relative to WT but no significant change in proliferaton. In old mice, increased number and SR function relative to WT and increased cell cycle entry	KO mice are viable and fertile but have increased incidence of spontaneous and carcinogen-induced cancers	Sharpless et al. 2001, Janzen et al. 2006	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Ink4c/p18	Cell cycle dependent kinase inhibitor	null	Decreases HSC SR. Increased KO HSC number and function, increased cell cycle entry, strikingly improved long-term engraftment largely by increasing SR divisions of the primitive cells	KO mice are viable but develop gigantism and widespread organomegaly	Franklin et al. 1998, Yuan et al. 2004, Yu et al. 2006	
Cyclin a2	Cell cycle regulator	null	Essential for HSC proliferation	KO embryos die shortly after implantation	Murphy et al. 1997, Kalaszczynska et al. 2009	
p21/Cip1/ Waf1	Cell-cycle-dependent kinase inhibitor	null	Decreases HSC SR (on mixed but not pure genetic background). Loss of KO HSCs with proliferative stress (exhaustion), increased sensitivity of primitive cells to chemotherapeutics, increased cell cycle entry	KO mice survive into late adulthood with a low incidence of tumorigenesis	Deng et al. 1995, Cheng et al. 2000, van Os et al. 2007	
Pten	Cell cycle regulator, tumor suppressor gene	null	Decreases HSC SR. Short-term increase in immunophenotypic KO HSCs, long-term loss of HSCs, increased cell cycle entry	KO embryos die at around E9.5	Suzuki et al. 1998, Yilmaz et al. 2006, Zhang et al. 2006	
p27/Kip1 + MAD1	Cell cycle dependent kinase inhibitor/ myc antagonist	null	Regulates HSC SR. Increase in the frequency of dKO HSCs, expanded pool of quiescent dKO HSCs	p27 KO mice are viable but show multiorgan hyperplasia and female sterility; Mad1 KO mice are viable and ferile	Fero et al. 1996, Foley et al. 1998, Walkley et al. 2005	
p53	Cell cycle regulator, tumor suppressor gene	null	Decreases HSC SR. Deletion expands BM LSK CD34-cells and the overall activity of HSCs	KO mice are developmentally normal but susceptible to spontaneous tumors	Donehower et al. 1992, TeKippe et al. 2003	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Tie2/tek	Receptor tyrosine kinase	null and Ang-1 OE	Essential for adult HSC maintenance, Tie2/Ang-1 signaling regulates HSC quiescence in the BM niche	KO embryos die at around E9.5 as a consequence of underdeveloped vasculature	Puri et al. 2003, Arai et al. 2004, Dumont et al. 1994	Angiopoietin-1 receptor
c-mpl	Thrombopoietin (Tpo) receptor	null, neutralizing antibody, and recombinant Tpo treatment	Tpo/MPL signaling regulates HSC quiescence and interaction with the osteoblastic niche. KO HSCs have reduced competitive repopulating capacity. Tpo is required to maintain adult quiescent HSCs	KO mice are viable and display no overt abnormalities	Gurney et al. 1994, Qian et al. 2007, Yoshihara et al. 2007	
Rac1	Rho GTPase	null	Required for HSC engraftment. Reduced in vitro HSC proliferation associated with impaired GF-stimulated cyclin D1 induction	KO mice die at around E8.5	Sugihara et al. 1998, Gu et al. 2003, Cancelas et al. 2005	
Cdc42	Rho GTPase	null	Required for HSC engraftment. Impaired adhesion, homing, lodging and retention of KO HSCs. Cell cycle activation of KO HSCs resulting in increased number of BM stem/progenitor cells	KO mice have significantly reduced body and organ sizes	Wang et al. 2005, Yang et al. 2007	
Mcl-1	Bcl-2 family member	null	Regulates HSC survival	KO embryos do not implant in utero but could be recovered at E3.5–E4.0	Rinkenberger et al. 2000, Opferman et al. 2005	
CD47	Immunoglobulin-like protein	null	Essential for HSC engraftment	CD47-deficient IAP−/− mice are viable and fertile	Jaiswal et al. 2009	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
RBP-JK and NICD	Notch pathway	null and OE	Not necessary for HSC SR but sufficient	RBPJ KO embryos die soon after E8.5	Oka et al. 1995, Varnum-Finney et al. 1998, Mancini et al. 2005, Stier et al. 2002, Maillard et al. 2008	
Wnt4	Wnt pathway	null	Expands multipotent hemopoietic progenitors. Low frequency of KO BM LSKs. Effects on hematopoietic cells are mainly non-cell-autonomous	KO mice die of renal failure shortly after birth	Stark et al. 1994, Louis et al. 2008	
B-catenin	Wnt pathway	null and OE	Might be required and sufficient for HSC SR. In one study, KO HSCs were deficient in long-term growth and maintenance (Zhao et al. 2007)	KO embryos die before E7.5 with defects in AP patterning	Huelsken et al. 2000, Zhao et al. 2007, Koch et al. 2008	
Apc	Wnt pathway, tumor suppressor gene	null	Essential for HSC maintenance, survival and cell cycle entry. Increased apoptosis and cell cycle entry of KO HSCs/progenitors, leading to their rapid disappearance and BM failure	Several germline mutations in the mouse have been studied	Fodde et al. 2001, Qian et al. 2008	
Klotho	Wnt pathway antagonist	null	Controls SR of old HSCs. Reduced number of KO KLS HSCs	KO mice die at 8–9 weeks of a syndrome resembling aging	Kuro-o et al. 1997, Liu et al. 2007	
Mk2	MAPK-activated protein kinase	null	Essential for HSC maintenance. Reduced number of KO HSCs, impaired ability for competitive repopulation in vivo	KO mice are viable and fertile	Kotlyarov et al. 1999, Schwermann et al. 2009	May act through chromatin remodeling by the PcG complex

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Smad4/dpc4	TGF-β superfamily of GF	null	Necessary for HSC SR and reconstituting capacity, dispensable for homing potential, viability and differentiation	KO embryos die at E7.5 because of gastrulation defects	Sirard et al. 1998, Karlsson et al. 2007	
Stat5	Signal transducer	null	Maintains HSC quiescence during steady state hematopoiesis. Increased cell cycling of KO HSCs, gradual reduction in survival and depletion of long-term HSCs	KO mice die perinatally with severe anemia	Cui et al. 2004, Wang et al. 2009	
Cul4a	Core subunit of an ubiquitin ligase	null	Essential for HSC SR and engraftment. HET HSCs exhibit defects in engraftment and SR capacity	KO embryos die between E4.5 and E7.5	Li et al. 2002, Li et al. 2007	
c-cbl	E3 ubiquitin ligase	null	Decreases HSC SR. Increased KO HSC pool size, hyperproliferation, greater competence and enhanced long-term repopulating capacity	KO mice are viable and fertile	Naramura et al. 1998, Rathinam et al. 2008	
Fbw7/ Sel-10/ Cdc4	E3 ubiquitin ligase, F-box protein	null	Controls HSC quiescence. KO HSCs show defective maintenance of quiescence, leading to impaired SR and a severe loss of competitive repopulation capacity	KO mice die at around E10.5 due to defects in vascular development	Tetzlaff et al. 2004, Thompson et al. 2008	
Sca-1 (Ly-6A/E)	Glycosylphosphatidylinositol-anchored membrane protein	null	Regulates hematopoietic progenitor/stem cell lineage fate. KO HSCs appeared normal but lineage skewing observed in B, NK and G/M	KO mice are viable and fertile	Stanford et al. 1997, Bradfute et al. 2005	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Lyl-1	bHLH TF	null	Essential for HSC maintenance. Decreased frequency of KO LSKs, impaired competitive reconstituting abilities, especially with respect to B and T lineage reconstitution	KO mice are viable and fertile	Capron et al. 2006	
Zfx	Zinc finger TF	null	Necessary for adult HSC SR. Essential for the maintenance of adult HSCs but not erythromyeloid progenitors and fetal HSCs. Increased apoptosis of KO HSCs.	KO embryos develop normally until E9.5 and subsequently die due to extraembryonic tissue abnormalities	Galan-Caridad et al. 2007	
Pbx1	Non-Hox homeodomain TF, hox cofactor	null	Necessary for HSC SR. Progressive loss of KO LT-HSCs associated with reduction of their quiescence	KO embryos die at E15–E16 with hypoplasia/ aplasia of multiple organs	Selleri et al. 2001, Ficara et al. 2008	
Foxo3a	Forkhead TF	null	Essential for HSC SR. KO HSCs are impaired in their ability to support long-term reconstitution in a competitive tx assay	KO mice are viable and females show an age-dependent infertility	Castrillon et al. 2003, Miyamoto et al. 2007	
Foxo1, 3 and 4	Forkhead TF	null	Essential for HSC SR. Defective long-term repopulating activity of KO HSCs that correlates with increased cell cycling and apoptosis	Foxo1 KO mice die at E10.5 owing to defective angiogenesis; Foxo3a KO mice are viable and show an age-dependent female infertility; Foxo4 KO mice are viable and do not have an overt phenotype	Castrillon et al. 2003, Furuyama et al. 2004, Hosaka et al. 2004, Tothova et al. 2007	Elevated TOS, reduction of ros with antioxidant reverses HSC phenotype

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
MafB	bZip type TF	null	Controls the rate of specific HSC commitment divisions without compromising other lineages or SR. Myeloid repopulation bias of KO HSCs and increased proliferation upon M-CSF treatment	KO mice die at birth from central apnea	Blanchi et al. 2003, Sarrazin et al. 2009	Specific upregulation of the early myeloid selector gene PU.1
Mef1/elf4	ETS family TF	null	Regulates HSC quiescence. Increased KO HSC number and function, decreased cell cycle entry, enhanced recovery from chemotherapeutic ablation of cycling cells	KO mice are born healthy and develop normally throughout adulthood	Lacorazza et al. 2002, 2006	
Hoxb4	Homeodomain TF	null and OE	Promotes HSC SR. Mild proliferation defect of KO HSCs. OE studies revealed an extraordinary ex vivo expansion of HSCs, highly competitive repopulation ability and increased cell cycle entry. Full reconstitution after transplant while respecting the total niche size (does not expand HSC pool beyond normal size)	KO mice are viable and fertile	Thorsteinsdottir et al. 1999, Antonchuk et al. 2001, 2002, Kyba et al. 2002, Bjornsson et al. 2003, Brun et al. 2004, Schmittwolf et al. 2005, Bowles et al. 2006	Not required for the generation or maintenance of HSC
Pu.1	ETS family TF	null	Necessary for HSC maintenance. KO HSCs exhibit an arrest at the transition from the HSC to CLP and CMP stages and are outcompeted by normal HSCs in tx assays	KO embryos die at a late gestational stage	Scott et al. 1994, Iwasaki et al. 2005	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
c-myb	TF	Point mutation in transac-tivation domain (M303V)	Suppresses HSC proliferation. LOF (point mutant) results in a high increase in HSCs frequency and cycling activity	KO embros die by E15	Mucenski et al. 1991, Sandberg et al. 2005	
Evi-1	SET/PR domain family TF	null	Essential for HSC maintenance. KO BM HSCs cannot maintain hematopoiesis and lose their repopulating ability	KO embryos die at around E10.5	Hoyt et al. 1997, Goyama et al. 2008	
Gfi1	Zinc finger TF, repressor	null	Maintains adult but not fetal HSCs. Essential to restrict HSC proliferation and to preserve HSC functional integrity. HO HSCs display elevated proliferation rates, are functionally compromised in competitive repopulation and serial tx assays, are unable to engraft in the competitive repopulation assay and can initiate but do not sustain hematopoiesis in chimeric mice	KO mice are small and have a median survival of 11 weeks	Hock et al. 2003, 2004, Zeng et al. 2004	
Tel/Etv6	ETS family TF	null	Maintains adult but not fetal HSCs. Promotes HSC SR, necessary for adult HSC survival	KO mice die by E11.5 owing to vascular abnormalities	Wang et al. 1997, Hock et al. 2004	
Gata-2	Zinc finger TF	HET	Promotes HSC proliferation and survival. Compromised proliferation and survival of HET HSCs without a change in their differentiation or SR capacity	KO embryos die at around E10–E11 with severe anemia	Tsai et al. 1994, Rodrigues et al. 2005	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Jun b	AP-1 family TF	null	Limits HSC proliferation and differentiation without affecting SR. Increased numbers of KO LT-HSC and GMP due to increased proliferation and blockade of apoptosis while the numbers of committed progenitors remain normal	KO embryos die between E8.5 and E10 from severe vascular defects in the placenta	Schorpp-Kistner et al. 1999, Passegue et al. 2004, Santaguida et al. 2009	
Scl/tal-1	b HLH TF	null	Required for normal function of short-term repopulating HSCs (Curtis et al. 2004). Not essential for adult hemopoiesis and HSC function (Mikkola et al. 2003). Increased number of phenotypic KO HSCs and severe multilineage defect in repopulation capacity	KO embryos die between E8.5 and E10.5	Shivdasani et al. 1995, Robb et al. 1995, Mikkola et al. 2003, Curtis et al. 2004	
C/ebpα	bZIP TF	null	Suppresses HSC SR. Enhancement of KO HSC repopulating capacity and SR	KO mice die from hypoglycemia within 8 h after birth	Wang et al. 1995, Zhang et al. 2004	
c-myc	Cell cycle regulator, bHLH TF	null	Decreases HSC SR (Wilson et al. 2004). Decreased number and proliferation of KO progenitors. Increased number of functionally defective KO HSCs due to niche-dependent differentiation defects, no apparent change in proliferation	KO embryos die between E9.5 and E10.5 and are smaller	Davis et al. 1993, Satoh et al. 2004, Wilson et al. 2004	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
n-myc	Cell cycle regulator, bHLH TF	null	Might be dispensable for adult hemopoiesis	KO embryos die between E10.5 and E12.5	Charron et al. 1992, Laurenti et al. 2008	
Caspase-3	Cysteine protease	null	Controls HSC SR, contributes to HSC quiescence by dampening cell responsiveness to microenvironmental stimuli. Increased numbers of immunophenotypic KO LT-HSC in association with multiple functional changes, most prominently cell cycling	N/A	Janzen et al. 2008	
Birc5/ survivin	Member of inhibitor of apoptosis protein (IAP) family	null	Promotes HSC maintenance. Deletion leads to BM ablation with widespread loss of hematopoietic progenitors and rapid mortality	KO embryos die before E4.5	Conway et al. 2002, Leung et al. 2007	
Alpha 4 integrin/ CD49d/ CD29	VLA4, the heterodimer of α4 and β1 integrin (CD49d/CD29)	null	Essential for HSC homing and cell adhesion. KO long-term repopulating HSCs display a competitive disadvantage, impaired homing and short-term engraftment after tx	KO embryos die at around E14.5	Yang et al. 1995, Priestley et al. 2006	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Other types of multipotent stem cells						
Jmjd3*	Histone demethylase of jumonji family	KD	Regulates differentiation of epidermal progenitor cells. KD in mammalian epidermal tissue blocks differentiation	N/A	Sen et al. 2008	
Bmi1*	Polycomb Group, PRC1	null	Regulates SR/proliferation of lung stem/progenitor cells. KO in BASCs (adult epithelial tissue of the lung) impairs SR/proliferation in culture and after lung injury in vivo, but lung development occurs normally	KO mice die at around 4 months of age	van der Lugt et al. 1994, Dovey et al. 2008	
Bmi1*	Polycomb Group, PRC1	null	Regulates intestinal stem/progenitor cell function. KO leads to crypt loss in small intestinal tissue	KO mice die at around 4 months of age	van der Lugt et al. 1994, Sangiorgi et al. 2008	
Blimp1/ Prdm1	PR/SET domain protein	null	Regulates germ cell precursor function. KO leads to loss of germ cell precursors	KO causes a block early in the process of primordial germ-cell formation	Ohinata et al. 2005, Vincent et al. 2005	
p38α	MAP kinase	null	Regulates proliferation and differentiation of lung stem/progenitor cells. Increased proliferation and defective differentiation of lung KO stem cells and progenitor cells	KO embryos die at midgestation due to defects in placental development	Adams et al. 2000, Ventura et al. 2007	
Sox10	TF, high-mobility-group DNA binding protein	null	Regulates neural crest stem cell function	KO embryos die during gestation with failure of migration and/or differentiation of multiple neural crest derivatives	Southard-Smith et al. 1998, Kim et al. 2003	

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Supplemental Table 1 (*Continued*)

Gene	Gene product	Mouse mutant	Function in stem cells	Phenotype of mouse germline mutation	Reference(s)	Other features
Cdc42	Small rho GTPase	null	Promotes SR of neural crest stem cells. Reduces SR and proliferation of later stage NCSCs, but not early migratory NCSCs. Increases cell cycle exit	KO mice have significantly reduced body and organ sizes	Wang et al. 2005, Fuchs et al. 2009	
Rac1	Small rho GTPase	null	Promotes SR of neural crest stem cells. Reduces SR and proliferation of later stage NCSCs, but not early migratory NCSCs. Increases cell cycle exit	KO mice die at around E8.5	Sugihara et al. 1998, Fuchs et al. 2009	
Nf1 (Neurofibro-matosis1)	GTPase activating protein, tumor suppressor	null	Decreases SR of neural crest stem cells. Transient increase in KO neural crest stem cells frequency and SR	KO embryos die from a cardiac defect by E14.5	Brannan et al. 1994, Joseph et al. 2008	Negatively regulates Ras signaling
Ets2	Ets family of TF	null	Required for SR/ proliferation of TS cells. Slower growth of KO TS cells	KO embryos die before E8.5 and fail to form extraembryonic ectoderm (EXE) markers	Yamamoto et al. 1998, Georgiades et al. 2006, Wen et al. 2007	

ABBREVIATIONS: BM, bone marrow; ES, embryonic stem; FL, fetal liver; HSC, hemopoietic stem cell; KD, knockdown; KO, knockout; ICM, inner cell mass; LSK, Lin(-)Sca-1(+)c-kit(+); NSC, neural stem cell; OE, overexpression; SR, self-renewal; TE, trophectoderm; TF, transcription factor; TS, trophoblast stem; tx, transplantation; WT, wild type.

*Genes with a demonstrated role in epigenetic regulatory mechanisms. **In bold**: genes for which a null allele was studied; in regular font: genes for which either a KD, heterozygote, point mutation, hypomorphic allele, gene trap allele, dominant negative, OE, or neutralizing antibody was used to study its function.

**Deletion of these genes causes a failure of the ICM to give rise to ES cells in vitro, suggesting a direct role in the establishment or maintenance of pluripotency.

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