

Transcription Factor-mediated Epigenetic Reprogramming*

Published, JBC Papers in Press, September 5, 2012, DOI 10.1074/jbc.R111.319046

Camille Sindhu, Payman Samavarchi-Tehrani, and Alexander Meissner¹

From the Department of Stem Cell and Regenerative Biology, Harvard University and Harvard Stem Cell Institute, Cambridge, Massachusetts 02138 and the Broad Institute of Harvard and MIT, Cambridge, Massachusetts 02142

Input from various signaling pathways in conjunction with specific transcription factors (TFs), noncoding RNAs, and epigenetic modifiers governs the maintenance of cellular identity. Endogenous or exogenous TFs operate within certain boundaries, which are set, in part, by the cell type-specific epigenetic landscape. Ectopic expression of selected TFs can override the cellular identity and induce reprogramming to alternative fates. In this minireview, we summarize many of the classic examples and a large number of recent studies that have taken advantage of TF-mediated reprogramming to produce cell types of biomedical relevance.

For many years, it was unclear whether differentiation involves irreversible changes to the genome that would restrict a cell's developmental potential. Early work by Briggs and King (1) and later Gurdon (2) addressed this subject using somatic cell nuclear transfer (SCNT)² from various donor nuclei into enucleated frog oocytes. Successful generation of viable organisms by SCNT demonstrated that the genome of a differentiated cell does retain all genetic information necessary for normal development. Later experiments were able to expand these seminal findings to mammals (3). SCNT using donor nuclei from genetically marked lymphoid cells (4) and olfactory receptor neurons (5) demonstrated further that even terminally differentiated and post-mitotic genomes could be reprogrammed. More recently, human somatic cell nuclei were also shown to be amenable to reprogramming by SCNT. However, in contrast to other species, this could be achieved only without prior removal of the oocyte nucleus (6).

Other cell types besides oocytes have been shown to possess factors capable of activating silenced loci in a somatic genome. For instance, introduction of a chicken erythrocyte nucleus into the cytoplasm of a HeLa cell results in chromatin decondensation and initiation of RNA synthesis from the previously inac-

tive erythrocyte genome (7). Later studies in mice and humans demonstrated that fusion of somatic cells with embryonic stem cells (ESCs) or embryonic germ cells reactivates pluripotency-related genes from the somatic genome and creates pluripotent, albeit tetraploid (4N), cells (8–10). Although these have limited clinical value, their genesis has provided a useful experimental platform for studying cellular plasticity and reprogramming (11). Moreover, the fusion experiments offered evidence that ESCs, like oocytes, zygotes, and early blastomeres, contain factors that are sufficient to reprogram a somatic cell.

Initial evidence for the capacity of selected transcription factors (TFs) to direct cellular reprogramming came from the classic *myoD* experiments and subsequent lineage conversions in the hematopoietic system (reviewed in Ref. 12). However, a major advancement in the field occurred in 2006 with the landmark study by Takahashi and Yamanaka (13), who reported the generation of induced pluripotent stem cells (iPSCs) through ectopic expression of only four TFs.

Converting Cell States

In contrast to the limited plasticity that exists *in vivo*, there are many examples of ectopic TF-mediated reprogramming *in vitro* (Fig. 1). Early unrelated studies led to the discovery of the basic helix-loop-helix (bHLH) TF *myoD*, whose expression in mouse embryonic fibroblasts could induce their conversion to myoblasts (14). MyoD appears to be a master regulator of muscle-specific transcriptional programs, and its ectopic expression can induce activation of muscle-specific genes in a variety of non-muscle cells (15, 16). Interestingly, although MyoD could induce complete phenotypic conversion in mesodermal cell types, ectodermal and endodermal cells responded less effectively (16).

Valuable insights into TF-mediated reprogramming were gained through fate conversions within the hematopoietic lineage, where developmental hierarchies are well understood. Common lymphoid progenitors (CLPs) can be induced to give rise to myeloid lineage cells such as granulocytes and monocytes by ectopic expression of the IL-2 receptor, which in turn leads to expression of GM-CSF receptors and the associated responsiveness to myeloid commitment-inducing signals (17). Although both CLPs and pro-T-cells could undergo this form of lineage redirection, the further downstream and lineage-committed pre-T- and pro-B-cells could not, suggesting a possible link between differentiation state and amenability to cell fate conversion. In another example, ectopic expression of the zinc finger TF GATA-1, a key regulator of megakaryocyte-erythrocyte precursor lineage commitment, was found to elicit conversion of CLP and GM progenitors into megakaryocytes and erythrocytes (18, 19). GATA-1 activates megakaryocyte-erythrocyte precursor-specific genes while concomitantly down-regulating markers of the other lineages. Alternatively, when GATA-1 is expressed at low levels in GM progenitors, a fate decision is forced whereby eosinophils or mast cells are generated (20), suggesting a critical role for factor stoichiometry in fate determination.

* This work was supported, in whole or in part, by National Institutes of Health Grants P01GM099117 and U01ES017155 (to A. M.). This work was also supported by the Pew Charitable Trusts (to A. M.). This is the fifth article in the Thematic Minireview Series on Results from the ENCODE Project: Integrative Global Analyses of Regulatory Regions in the Human Genome.

¹ To whom correspondence should be addressed. E-mail: alexander_meissner@harvard.edu.

² The abbreviations used are: SCNT, somatic cell nuclear transfer; ESC, embryonic stem cell; TF, transcription factor; iPSC, induced pluripotent stem cell; bHLH, basic helix-loop-helix; CLP, common lymphoid progenitor; C/EBP, CCAAT enhancer-binding protein; iN, induced neuronal; OSKM, Oct4, Sox2, Klf4, and c-Myc; MET, mesenchymal-to-epithelial; 5-AZA, 5-azacytidine.

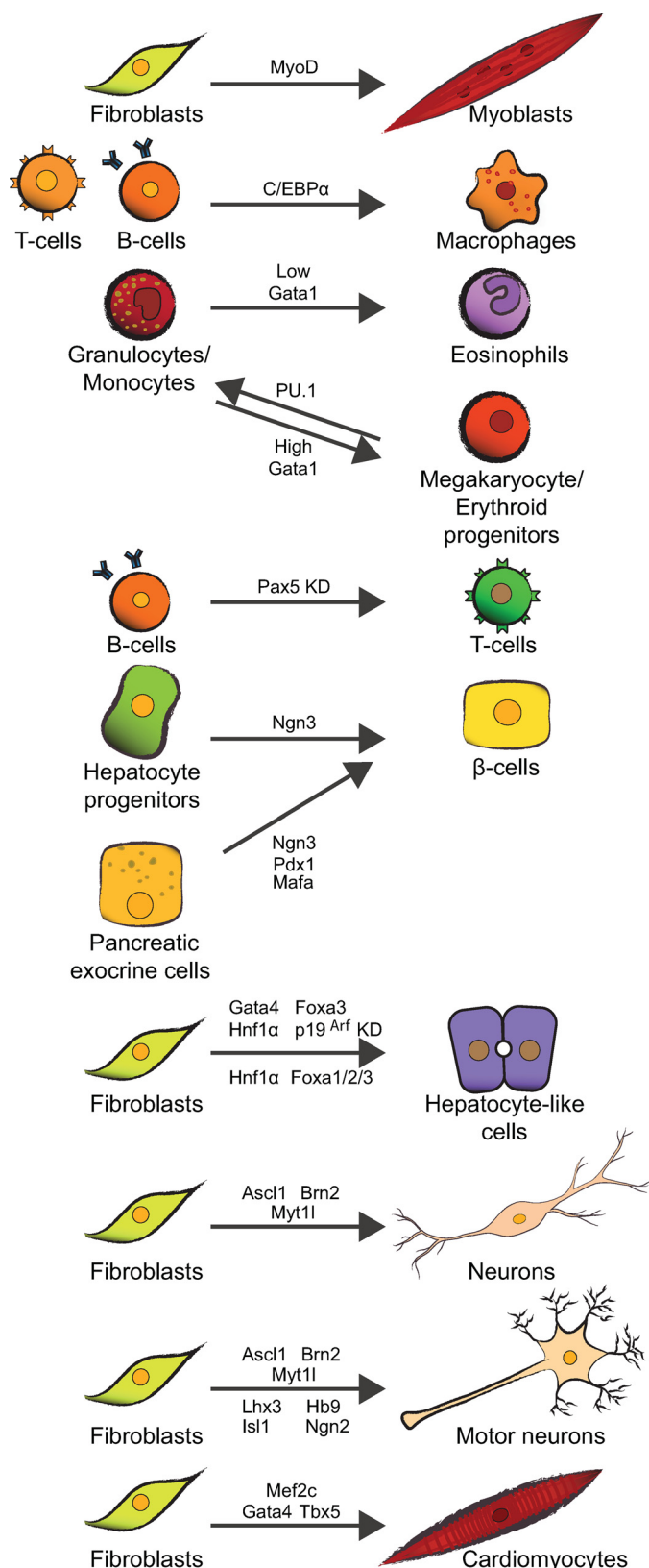


FIGURE 1. TF-mediated cell fate conversions in a variety of starting cell types. Shown are examples of published murine cell state conversions using modulation of TF expression. The majority utilize ectopic expression of one or more lineage-specific TFs, whereas others apply targeted depletion of TFs. Factors are shown next to the *arrows*. *KD*, knockdown.

The transcription factor PU.1 (Spi-1) belongs to the Ets family and is a key regulator of myeloid and B-lymphoid lineage specification, functioning through interaction with other TFs such as GATA-1/2 and CCAAT enhancer-binding protein (C/EBP) α/β (21–23). Interestingly, lineage choice between these two fates is determined in part by graded expression of PU.1 (24). Through its interaction with GATA proteins, PU.1 transactivation of myeloid target genes is suppressed (25). Conversely, PU.1 inhibits the erythroid program by binding GATA-1 and inducing the formation of repressive chromatin structure at GATA-1 target loci (26, 27). Consistently, ectopic expression of PU.1 in multipotent progenitor cell lines leads to suppression of GATA-1 and concomitant conversion into myeloid cells (28). The antagonism between GATA-1 and PU.1 dictates one of two fates and highlights the key role for TF interactions in the process.

Another classic example involves the basic leucine zipper transcription factor C/EBP α , which is required for the common myeloid progenitor-to-GM progenitor transition *in vivo* (29). Ectopic expression of C/EBP α in primary bone marrow cells, lymphocytes (30, 31), or fibroblasts (32) can elicit myelomonocytic cell-type characteristics, a classification that includes both macrophages and granulocyte precursors. The function of C/EBP α in this context is dependent on synergism with PU.1, which is required for deposition of H3K4me1 at enhancer elements of target genes, suggesting that these factors cooperate to define cell type-specific binding patterns at regulatory elements (33, 34).

In addition to ectopic expression, loss of fate determinants can also induce cell fate changes. Differentiation of CLPs into the B-cell lineage depends on the TFs PU.1, E2A, and EBF1, which induce Pax5 to activate B-cell-specific genes while repressing genes associated with alternative lineages. In agreement with this model, Pax5^{-/-} pro-B-cells fail to complete B-lymphopoiesis but are capable of differentiating into other hematopoietic cell types such as macrophages, dendritic cells, and granulocytes in response to specific signaling cascades (35–37). Additionally, Pax5 ablation in mature B-cells results in dedifferentiation into an uncommitted progenitor cell population, which can then undergo T-lymphopoiesis (38). Notably, the order in which factors are expressed can also impact the outcome. For example, altering the sequential expression of C/EBP α and GATA-2 in GM progenitors can instruct commitment to different hematopoietic cell types (39).

In summary, lessons from the hematopoietic system have provided strong evidence for the ability of TFs to redirect cell fate across related lineages derived from one germ layer or between specialized cell types of a particular lineage. Below, we will briefly review many of the more recent reprogramming studies.

Using a candidate gene approach, Melton and co-workers (40) identified three bHLH TFs, Ngn3 (or NeuroD1), Pdx1, and MafA, whose forced expression can convert exocrine pancreas tissue into insulin-secreting endocrine β -cells *in vivo*. Expressing only the endocrine progenitor-defining TF Ngn3 in hepatic progenitor cells generated physiologically responsive pancreatic endocrine cells. In contrast, expression of Ngn3 in mature hepatocytes induced only insulin expression (but not transdif-

ferentiation) in islet cells (41). An additional example of reprogramming *in vivo* comes from the inner ear, where the TFs Atoh1 and Prox1, among others, were found to regulate the development of sensory hair cells and supporting cells from a common progenitor (42, 43). Ectopic expression of the bHLH TF Atoh1 (also known as Math1) results in conversion of non-sensory cochlear cells into functional sensory hair cells (44, 45). Conversely, expression of Prox1 in sensory hair cells leads to the repression of Gfi1 and Atoh1, factors required for sensory hair cell specification, resulting in cellular degeneration (43).

Recently, other groups have demonstrated direct reprogramming *in vitro* into additional endodermal cells types. The ectopic expression of GATA-4, Hnf1a, and Foxa3 in fibroblasts, along with inactivation of p19^{Arf}, could give rise to induced hepatocyte-like cells (46). A separate study found that ectopic expression of Hnf4 α and one of the three *foxA* genes in mouse embryonic or adult fibroblasts could also induce expression of multiple hepatocyte-specific features, converting the fibroblasts to hepatocyte-like cells (47).

Employing a similar candidate gene approach as Yamanaka and Melton, Wernig and co-workers (48) demonstrated that expression of three factors, Ascl1, Brn2 (also known as Pou3f2), and Myt1l, in mouse embryonic and postnatal fibroblasts induced conversion to neural cells, termed induced neuronal (iN) cells. Although the exact identity of these cells remains unclear, iN cells can form functional synapses and are physiologically responsive (48). These three factors could also induce neuronal differentiation of human ESCs, although reprogramming of human fetal fibroblasts to functional iN cells required additional coexpression of NeuroD1 (49). To definitively demonstrate the neural conversion of a differentiated non-ectodermal cell type, Marro *et al.* (50) recently reprogrammed mouse hepatocytes into iN cells. The hepatic transcriptome was largely repressed in the iN cells, and they retained only a limited epigenetic signature of their starting state. These initial findings demonstrate the ability of ectopic TFs to reprogram somatic cells across germ layers. More recently, others have shown that human and mouse fibroblasts can be reprogrammed to specialized functional neural subtypes such as dopaminergic neurons (51, 52) and spinal motor neurons (53). Interestingly, fibroblasts can be induced to express neural lineage markers and exhibit neuronal morphology solely by forced expression of microRNAs miR-9/9* and miR-124, which repress the Baf53a subunit of the BAF chromatin-remodeling complex (54). However, generating functional iN cells required expression of additional TFs (54, 55). Two of these recent iN studies also noted that reprogramming occurred in the absence of continued cell division, as the majority of reprogramming cells became post-mitotic within 24 h of factor induction (48, 50).

Along with the ability to generate a variety of neuronal cell types, effective production of cardiac muscle cells from ESCs or non-cardiac somatic cells is a compelling target of translational medicine. The first evidence of reprogramming non-cardiogenic tissues to a cardiac fate was provided by *in vivo* transfection of mouse embryos with core transcriptional regulators of cardiac development (56). More recently, Srivastava and co-workers (57) reported the TFs GATA-4, Mef2C, and Tbx5

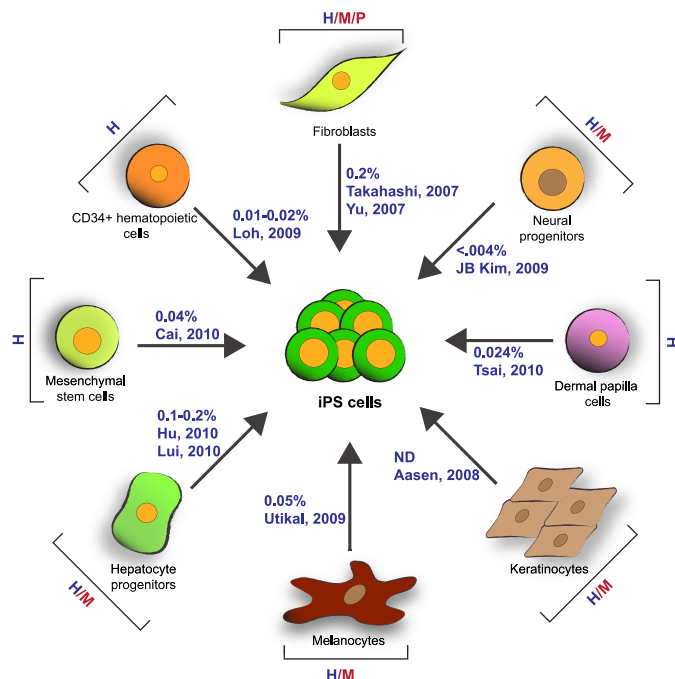


FIGURE 2. Diverse somatic origin and efficiencies of iPSC generation. A wide variety of starting cell types originating from different species and representing all three embryonic germ layers can be reprogrammed to pluripotency with variable but consistently low efficiency. *H*, human; *M*, mouse; *P*, pig. Only human reprogramming efficiencies are indicated next to the arrows (highlighted in blue).

could reprogram embryonic and adult fibroblasts directly into induced cardiomyocytes.

The diversity of cell types generated by TF-mediated reprogramming demonstrates the potential utility of this approach for therapeutic purposes. However, several key aspects of these converted cell types need further investigation. In particular, the stability and relative completion of functional, transcriptional, and epigenetic remodeling, as well as the *in vivo* equivalence of the generated cell types, remain unclear. A better understanding of potential risks posed by incomplete reprogramming or cellular memory is an important benchmark for the translational application of induced cell types.

Reprogramming Somatic Cells to Pluripotency

Perhaps the most striking example of factor-induced changes in cell state remains the reprogramming of somatic cells into pluripotent embryonic stem-like cells, termed iPSCs. The seminal work by Takahashi and Yamanaka (13) demonstrated that ectopic expression of Oct4, Sox2, Klf4, and c-Myc (OSKM) in embryonic and adult fibroblasts could convert these cells to iPSCs. Subsequently, numerous laboratories have improved the original protocol and generated iPSCs from a variety of species and cell types (58), highlighting the robustness and general conservation of the approach (Fig. 2).

Forced expression of OSKM initiates dramatic phenotypic (59) and molecular (60–63) changes in the targeted somatic cell. Transcriptional and epigenetic changes eventually lead to the reactivation of endogenous pluripotency genes and acquisition of molecular and, in some cases, functional pluripotency. Commonly used markers of reprogramming include alkaline phosphatase and SSEA1 (stage-specific embryonic antigen-1)

(62, 64). Although these markers appear earlier in the process, they are not indicative of complete reprogramming and are of relatively low stringency (65). Retroactive tracing of individual reprogramming fibroblasts has demonstrated that within the set of cells that undergo complete reprogramming, the initial response to reprogramming factor induction is characterized by a marked decrease in cell size and increased proliferation rate (59). The early response to the four TFs is marked by the transcriptional reversion from the somatic state, as evidenced by the down-regulation of the fibroblast-specific genes *Snai1/2*, *Zeb1/2*, and *Cdh2* and the surface marker *Thy1* (60, 63, 64). The loss of a mesenchymal phenotype coincides with the emerging expression of epithelium-associated markers such as E-cadherin and EpCAM, known as the mesenchymal-to-epithelial (MET) transition (63, 66). The importance of MET during reprogramming is highlighted by acceleration of reprogramming using bone morphogenetic protein ligand stimulation or expression of microRNAs from the miR-200 family (63), which have been implicated in MET (67). Notably, *Klf4*, the main driver of this transition, synergizes with *Oct4* and *Sox2* to direct reprogramming, and its absence can be replaced by stimulation with bone morphogenetic protein (68).

Establishment of the iPSC state in mice requires continuous expression of the OSKM transgenes for 8–10 days (64), leading to the eventual activation of endogenous pluripotency genes such as *Oct4*, *Sox2*, and *Nanog* after ~2 weeks. The final progression of reprogramming occurs only in a small percentage of SSEA1-positive cells (62). The mechanism whereby the endogenous stem cell network is transcriptionally reactivated in the final transition to pluripotency remains poorly understood.

Although *c-Myc* is routinely included in the reprogramming factor mixture, and expression of this gene enhances the efficiency of somatic cell reprogramming, it is not a core member of the ESC pluripotency network. The network controlled by *c-Myc* is largely separate from the core ESC pluripotency network and primarily includes targets involved in metabolic, cell cycle, and proliferative processes (69–71). In ESCs, *c-Myc* target gene promoters are highly enriched for the active histone modification H3K4me3 and depleted of the repressive modification H3K27me3; this is a striking pattern that is not exhibited to the same extent by targets of the core pluripotency factors (69). The core factors reside at targets enriched for both activating and repressive histone modifications, reflecting their role in maintaining ESC identity by activating ESC-specific genes while repressing expression of lineage-specific transcriptional regulators (72). Conversely, *c-Myc* targets are more expansive in function, encompassing genes not unique to the pluripotent state (61, 69). The recent discovery that *c-Myc* plays a role in regulating pause release of polymerase II transcriptional elongation is in agreement with many of the early phenotypic and molecular responses to reprogramming factor induction (73). Notably, the latter suggests that *c-Myc* may not be directly involved in reactivating the pluripotency network but rather act more broadly to drive robust transcription of paused genes during the early stages of reprogramming. Genome-wide localization studies of pluripotency factors, along with affinity-based identification of their interacting partners, have revealed an interconnection between TFs (74,

TABLE 1
Genome-wide localization studies of TFs and chromatin remodelers

Factor	Function	mES/hES	Reference
p300	Histone acetyltransferase	mES	96
Suz12	PRC2 core component	mES, hES	96, 97
Eed	PRC2 core component	mES, hES	97, 98
Rnf2	PRC1 component	mES	97
Phe1	PRC1 core component	mES	97
Brg1	esBAF component (SWI/SNF chromatin remodeling complex)	mES	99, 102
Tet1	Catalyzes conversion of 5mC to 5hmC	mES	111
Ezh2	PRC2 subunit	mES, hES	100
Ring1B/Bmi1	PRC1 subunit	mES, hES	100
Jarid2	PRC2 component	mES	101
Mt12	PRC2 component	mES	101
Baf155	esBAF component (SWI/SNF chromatin remodeling complex)	mES	102
Wdr5	Set/MLL histone methyltransferase	mES	79
Rbbp5	Set/MLL histone methyltransferase	mES	79
Kdm5B	H3K4me3 demethylase	mES	103
Oct4	Core ES cell circuitry; reprogramming factor	hES	104
Sox2	Core ES cell circuitry; reprogramming factor	hES	104
Nanog	Core ES cell circuitry	hES	104
Klf4	LIF signaling; Nanog regulation	mES	74
c-Myc	Cell proliferation; metabolism; cell cycle regulation	mES	74
Dax1	Oct4 inhibition	mES	74
Rex1	JAK/STAT3 pathway inhibition; primitive endoderm differentiation	mES	74
Zfp281	GATA6 repression; cell proliferation; differentiation	mES	74
Nac1	GATA6 repression	mES	74
STAT3	BMP signaling to core pluripotency circuitry	mES	70
Smad1	LIF signaling to core pluripotency circuitry	mES	70
Esrrb	Steroid hormone receptor; self-renewal	mES	70
Zfx	Self-renewal	mES	70
n-Myc	Cell proliferation; metabolism; cell cycle regulation	mES	70
Tefcp211	[uncharacterized]; upregulated in ES cells	mES	70
E2f1	Cell-cycle progression	mES	70
CTCF	Transcriptional insulation	mES	70
Sall4	Regulation pluripotency; early embryonic development	mES	105
Cnot3	Zfx/c-Myc cofactor; cell cycle regulation	mES	106
Trim28	Zfx/c-Myc cofactor; cell cycle regulation	mES	106
Ronin	Metabolic/biosynthetic regulation	mES	107
Te3	Connects Wnt signaling to core pluripotency factors	mES	108
Prdm14	Germ cell lineage development	hES	109
Smad2/3/4	TGF- β /Activin/Nodal signaling	hES	110
FoxH1	SMAD cofactor; activating signaling transducer	hES	110

75), chromatin-remodeling, and modifying complexes (Table 1). Although it is well established that ESC chromatin is predominantly euchromatic (76, 77) and possess characteristic bivalent enrichment of both active and repressive histone modifications at developmental genes (78), functional links between specific epigenetic modifiers and members of the ESC core transcriptional network have only recently come to light. *Wdr5*, a member of the mammalian Trithorax complex and an effector of activating H3K4 methylation, is an interacting partner of *Oct4* (79). This provides a framework for conceptualizing how transcriptional regulators of pluripotency may cooperate with chromatin-associated factors to regulate the balance between self-renewal and differentiation.

Early Dynamics in TF-induced Reprogramming

Genome-wide chromatin mapping studies of selected epigenetic marks in populations of induced fibroblasts have provided many useful insights into early reprogramming dynamics (80). One of the earliest cellular responses to ectopic factor expression is *de novo* deposition or enhanced enrichment of euchromatin-associated H3K4me2 at a large number of promoters. Many of the target genes include developmental regulators and

pluripotency genes (80). Importantly, these changes appear to precede transcriptional activation, which, in the case of the pluripotency-associated genes, will not occur until the late stages of reprogramming. Many of these dynamics can be observed in the absence of cell division. The fact that these regions are enriched for both targets of Oct4 and Sox2 suggests that one of the early steps in somatic cell reprogramming involves global coordinated epigenetic “priming.” The notable enrichment for H3K4 methylation at Oct4 target sites accords well with the aforementioned study by Ang *et al.* (79) connecting Oct4, Wdr5, and histone methylation of Oct4 targets. Importantly, the reestablishment and erasure of repressive marks such as H3K27me₃ occur at a notably slower rate (80).

Interestingly, the majority of expression changes that are detectable during the first few cell divisions occur almost solely within regions of H3K4me₃-enriched accessible chromatin environments (60, 80). This suggests that the action of the reprogramming factors is restricted, at least initially, to transcriptionally permissive genomic loci. Moreover, at these early stages, up-regulated (but not down-regulated) genes are strongly enriched for targets of c-Myc. Unlike Oct4 and Sox2, c-Myc is already endogenously expressed in somatic fibroblasts (13) and primarily regulates targets involved in cellular metabolism and proliferation. The observed transcriptional response to increased ectopic c-Myc is consistent with its proposed role in stabilizing transcriptional elongation, thereby enabling productive transcription at many shared (somatic and pluripotent) target genes (73). Consistent with these data and in accordance with a distinct binding pattern for c-Myc in ESCs (69), Plath and co-workers (61) showed that the most dramatic early changes in fibroblast gene levels were largely attributable to c-Myc expression.

Subsequent to the first few cell divisions, molecular events leading to up-regulation of endogenous pluripotency genes and transition to the iPSC state remain hard to define due to population heterogeneity. Treatment of reprogramming cells with chromatin-modifying agents can positively affect efficiency. For instance, the Dnmt inhibitor 5-azacytidine (5-AZA), which decreases overall DNA methylation levels and promotes chromatin decondensation, can improve overall efficiency and facilitate the transition of partially reprogrammed cells to pluripotency (60). Similarly, inhibitors of histone deacetylases and methyltransferases can elicit a similar improvement (81), as can members of ATP-dependent chromatin-remodeling complexes. In a recent screen for ESC factors able to facilitate the reprogramming process, Schöler and co-workers (82) identified components of the BAF chromatin-remodeling complex that act in part by facilitating Oct4 binding to target loci during reprogramming, perhaps by improving TF access to target DNA.

Full transition to pluripotency can also be mediated by kinase inhibition in partially reprogrammed cells that lack the expression of true pluripotency markers. Treatment of these cells with inhibitors of GSK3 β and MEK (dual inhibition) induces the up-regulation of endogenous Nanog and Oct4 and demethylates the Nanog promoter to ESC-comparable levels, eventually leading to stable pluripotency (83). It is worth noting that although both 5-AZA treatment and dual inhibition culture can

enhance reprogramming, their effects may be attributable in part to the selection against somatic or non-pluripotent intermediates. Both the toxicity of 5-AZA and the lethality of kinase inhibition for any mitogen-dependent cells could cause an apparent increase in efficiency. The acquisition of an ESC-like proliferative state, which is uniquely resistant to the growth inhibitory effects of these small molecules, may provide a selective advantage to successfully reprogramming cells compared with those that retain somatic cell cycle characteristics.

Some iPSCs Are Equivalent to ESCs

The functional and molecular equivalence of ESCs and iPSCs has been a key question since the initial report of iPSC derivation (13). Developmental potential can be determined with low stringency by *in vitro* differentiation and teratoma formation and with higher stringency in non-human species by chimera formation, germ-line contribution (84), or the production of entirely iPSC/ESC-derived animals by tetraploid complementation (85, 86). Multiple molecular screening strategies have been employed to compare ESC and iPSC lines quantitatively, including profiling transcriptional and epigenetic signatures on a genome scale. At the morphological, molecular, and functional levels, ESCs and iPSCs show a high degree of similarity (87), although the complete equivalence of these cell types remains controversial. Numerous studies have described molecular differences between iPSCs and ESCs (88–90). We have recently used three genomic assays to profile 20 human ESC and 12 iPSC lines and generated a reference map of DNA methylation, gene expression, and *in vitro* differentiation potential for the tested lines (91). Surprisingly, much of the variation observed within iPSC lines could be explained by the general variation between the different pluripotent lines tested, including the ESC lines. These findings accord with functional studies in the mouse, which have established that some (but not all) iPSC lines are both functionally and molecularly equivalent to ESCs (86, 89, 92).

Experimentally determined variations in gene expression levels and epigenetic profiles have yet to provide a molecular signature specific to iPSCs that can consistently discriminate between the various pluripotent populations (91, 93). Establishing the criteria for such a signature has proven challenging, given that variability exists among individual ESC and iPSC lines. To avoid confounding effects of differences in genetic background, a recent study derived and compared the molecular and functional characteristics of genetically matched mouse ESCs and iPSCs. The findings suggest that the expression patterns of ESCs and iPSCs are remarkably similar, with the exception of a few genes of the imprinted *Dlk1-Dio3* locus, which appeared to be aberrantly silenced in the majority of iPSC clones examined (89). A more recent study has shown this disparity to be greatly affected by the stoichiometry of the TFs (OSKM), suggesting the inactivation of this locus may be linked to the specific strategy or conditions in which the cells are generated and is not necessarily inherent to reprogramming (94). An additional caveat to the generality of the observations summarized here is the relatively small number of ESC and iPSC lines that are used in many studies (Table 2). In humans, another concern is the limited number of ESC lines, such as H1

TABLE 2

Summary of recent studies comparing molecular characteristics of various human ESC and iPSC lines

	noncoding RNA Profiling	Gene Expression Profiling	Chromatin State Profiling	DNA Methylation Profiling	Comparison of Genetic Stability	Number ES/iPSC Lines Profiled	
Takahashi, K et al. (2007)	■	■	■			1 / 1	1,276 genes exhibit >5-fold differences in gene expression; pluripotency gene promoters demethylated to an ESC-comparable level
Doi, A. et al. (2009)	■		■			3 / 9	ESC/iPSC methylomes are very similar but distinguished by 71 differentially methylated regions
Chin, M.H. et al. (2009)	■	■	■			4 / 5	Detected conserved, iPSC-specific gene expression signature distinguishing iPSCs from ESCs
Marchetto, M.C. et al. (2009)	■					2 / 2	iPSCs insufficiently repress somatic cell-of-origin genes, incompletely upregulate early embryonic development genes, aberrantly upregulate a set of iPSC-specific genes silent in ESCs and somatic cells of origin
Wilson, K.D. et al. (2009)	■					1 / 1	Expression miR-371/372/373 cluster higher in ESCs; miR-886-5p higher in iPSCs
Ghosh, Z. et al. (2010)	■					6 / 4	iPSC lines of diverse somatic origin retain residual somatic gene expression which distinguishes them from ESCs; some reprogrammed somatic cells recapitulate ESC-like expression more closely than others
Guenther, M.G. et al. (2010)	■	■				6 / 6	Detected no consistent expression signature distinguishing iPSCs/ESCs
Hawkins, R.D. et al. (2010)		■				1 / 1	Subtle ESC/iPSC differences in H3K27me3-marked repressive chromatin restricted to intergenic regions
Loewer, S. et al. (2010)	■					2 / 7	10 lincRNA genes exhibit elevated expression in iPSCs relative to ESCs
Mayshar, Y. et al. (2010)				■		17 / 46	Provided evidence the reprogramming process can rapidly select for iPSC-specific chromosomal abnormalities not detected in parental fibroblasts or in ESCs
Neveu, P. et al. (2010)	■					6 / 16	Detected no consistent miRNA expression signature distinguishing iPSCs/ESCs
Newman, A.M. & Cooper, J.B. et al. (2010)	■					23 / 68	ESC/iPSC lines exhibit laboratory of origin-specific expression signatures
Bock, C. et al. (2011)	■		■			20 / 12	Most ESC/iPSC lines exhibit subtle differences with comparison to an ESC reference while some lines are highly similar; most iPSC variability falls within the spectrum of ESC variability
Hussein, S.M. et al. (2011)				■		17 / 22	Early-passage iPSCs exhibit increased levels of copy number variations (CNVs) and genetic mosaicism compared to ESCs; iPSC CNV levels decrease over passaging
Laurent, L.C. et al. (2011)				■		69 / 37	All iPSC samples tested exhibit moderate numbers of genetic deletions and duplications, whereas a large number of duplications were found in only a few ESC samples
Lister, R. et al. (2011)	■	■	■	■		2 / 5	Distribution of genomic aberrations across iPSC samples relatively even; skewed in ESCs due to small ESC subset exhibiting large numbers of gene duplications
Bar-Nur, O. et al. (2011)	■	■	■	■		3 / 11	Gene expression of β cell-derived iPSCs globally similar to ESCs and other iPSCs; β -iPSCs retain epigenetic signatures of somatic origin at β -cell genes distinguishing them from other iPSCs

■ Notable ESC / iPSC differences detected

■ Global similarity; subtle ESC / iPSC differences detected

■ No iPSC-specific signatures detected

and H9, which have been used as references or controls in many studies. For instance, a recent study reported aberrant hypomethylation of promoter regions in iPSC lines compared with the H1 and H9 ESC lines (95). However, comparison with a panel of 32 pluripotent cell lines (91) demonstrated that hypomethylation of the reported regions is a general characteristic of both ESCs and iPSCs, with the exception of a few cell lines, including the H1 and H9 ESC lines used as controls in the original report. This suggests that rather than aberrant hypomethylation in the iPSCs, these loci exhibit possibly aberrant hypermethylation in the control ESC lines.

Conclusion

Coordinated activity of TFs and epigenetic modifiers, acting downstream or in concert with signaling molecules, establishes and maintains stable cellular identities by driving cell type-spe-

cific gene expression programs. Epigenetic modifications affect the ability of the transcriptional machinery to access the chromatin template, thereby influencing gene expression patterns that ultimately determine cellular identity. Although cellular differentiation appears to be largely unidirectional during development and fixed in adult organisms, elegant experiments over the recent decades have demonstrated the remarkable plasticity by which differentiated cells can be redirected to alternative fates. Cell state can be considered the phenotypic output of regulatory networks, which are in turn influenced by and reflected in cell-specific epigenetic landscapes. By manipulating transcriptional programs, it should be theoretically possible to reprogram a cell's identity to any desired alternative state.

Many important questions remain open in the field of cell fate conversion experiments. It is still unknown how ectopic

factors access their binding sites in a foreign chromatin context and how their function is influenced by the starting cell's molecular ensemble. A mechanistic description for the relevance of epigenetic or transcriptional similarities between starting and intended cell types in cell fate reprogramming is also lacking. For example, the higher efficiency of conversion between closely related cell types may be partly attributable to higher transcriptional similarity and thus availability of essential cofactors between the starting and intended cell types. In addition to the transcriptional repertoire, however, a more closely related epigenetic landscape may also enable more efficient conversions. Ultimately, the knowledge gained from experimental manipulation of cell fates will have far-reaching implications not only for the basic understanding of the molecular mechanisms behind cellular identity but also for the eventual application to personalized regenerative medicine.

Acknowledgments—We thank members of the Meissner laboratory (in particular, Zachary D. Smith and Casey A. Gifford) for critical comments.

REFERENCES

- Briggs, R., and King, T. J. (1952) Transplantation of living nuclei from blastula cells into enucleated frog eggs. *Proc. Natl. Acad. Sci. U.S.A.* **38**, 455–463
- Gurdon, J. B. (1962) Adult frogs derived from the nuclei of single somatic cells. *Dev. Biol.* **4**, 256–273
- Wilmut, L., Schnieke, A. E., McWhir, J., Kind, A. J., and Campbell, K. H. (1997) Viable offspring derived from fetal and adult mammalian cells. *Nature* **385**, 810–813
- Hochedlinger, K., and Jaenisch, R. (2002) Monoclonal mice generated by nuclear transfer from mature B and T donor cells. *Nature* **415**, 1035–1038
- Eggan, K., Baldwin, K., Tackett, M., Osborne, J., Gogos, J., Chess, A., Axel, R., and Jaenisch, R. (2004) Mice cloned from olfactory sensory neurons. *Nature* **428**, 44–49
- Noggle, S., Fung, H. L., Gore, A., Martinez, H., Satriani, K. C., Prosser, R., Oum, K., Paull, D., Druckenmiller, S., Freeby, M., Greenberg, E., Zhang, K., Golland, R., Sauer, M. V., Leibel, R. L., and Egli, D. (2011) Human oocytes reprogram somatic cells to a pluripotent state. *Nature* **478**, 70–75
- Bolund, L., Ringertz, N. R., and Harris, H. (1969) Changes in the cytochemical properties of erythrocyte nuclei reactivated by cell fusion. *J. Cell Sci.* **4**, 71–87
- Tada, M., Tada, T., Lefebvre, L., Barton, S. C., and Surani, M. A. (1997) Embryonic germ cells induce epigenetic reprogramming of somatic nucleus in hybrid cells. *EMBO J.* **16**, 6510–6520
- Cowan, C. A., Atienza, J., Melton, D. A., and Eggan, K. (2005) Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science* **309**, 1369–1373
- Tada, M., Takahama, Y., Abe, K., Nakatsuji, N., and Tada, T. (2001) Nuclear reprogramming of somatic cells by *in vitro* hybridization with ES cells. *Curr. Biol.* **11**, 1553–1558
- Pereira, C. F., Terranova, R., Ryan, N. K., Santos, J., Morris, K. J., Cui, W., Merkenschlager, M., and Fisher, A. G. (2008) Heterokaryon-based reprogramming of human B-lymphocytes for pluripotency requires Oct4 but not Sox2. *PLoS Genet.* **4**, e1000170
- Graf, T. (2011) Historical origins of transdifferentiation and reprogramming. *Cell Stem Cell* **9**, 504–516
- Takahashi, K., and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126**, 663–676
- Davis, R. L., Weintraub, H., and Lassar, A. B. (1987) Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* **51**, 987–1000
- Choi, J., Costa, M. L., Mermelstein, C. S., Chagas, C., Holtzer, S., and Holtzer, H. (1990) MyoD converts primary dermal fibroblasts, chondroblasts, smooth muscle, and retinal pigmented epithelial cells into striated mononucleated myoblasts and multinucleated myotubes. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7988–7992
- Weintraub, H., Tapscott, S. J., Davis, R. L., Thayer, M. J., Adam, M. A., Lassar, A. B., and Miller, A. D. (1989) Activation of muscle-specific genes in pigment, nerve, fat, liver, and fibroblast cell lines by forced expression of MyoD. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5434–5438
- Kondo, M., Scherer, D. C., Miyamoto, T., King, A. G., Akashi, K., Sugamura, K., and Weissman, I. L. (2000) Cell fate conversion of lymphoid-committed progenitors by instructive actions of cytokines. *Nature* **407**, 383–386
- Heyworth, C., Pearson, S., May, G., and Enver, T. (2002) Transcription factor-mediated lineage switching reveals plasticity in primary committed progenitor cells. *EMBO J.* **21**, 3770–3781
- Iwasaki, H., Mizuno, S., Wells, R. A., Cantor, A. B., Watanabe, S., and Akashi, K. (2003) GATA-1 converts lymphoid and myelomonocytic progenitors into the megakaryocyte/erythrocyte lineages. *Immunity* **19**, 451–462
- Kulesa, H., Frampton, J., and Graf, T. (1995) GATA-1 reprograms avian myelomonocytic cell lines into eosinophils, thromboplasts, and erythroblasts. *Genes Dev.* **9**, 1250–1262
- Klemsz, M. J., McKercher, S. R., Celada, A., Van Beveren, C., and Maki, R. A. (1990) The macrophage- and B-cell-specific transcription factor PU.1 is related to the *ets* oncogene. *Cell* **61**, 113–124
- Gupta, P., Gurudutta, G. U., Saluja, D., and Tripathi, R. P. (2009) PU.1 and partners: regulation of hematopoietic stem cell fate in normal and malignant hematopoiesis. *J. Cell. Mol. Med.* **13**, 4349–4363
- Scott, E. W., Fisher, R. C., Olson, M. C., Kehrl, E. W., Simon, M. C., and Singh, H. (1997) PU.1 functions in a cell-autonomous manner to control the differentiation of multipotential lymphoid-myeloid progenitors. *Immunity* **6**, 437–447
- DeKoter, R. P., and Singh, H. (2000) Regulation of B-lymphocyte and macrophage development by graded expression of PU.1. *Science* **288**, 1439–1441
- Zhang, P., Behre, G., Pan, J., Iwama, A., Wara-Aswapati, N., Radomska, H. S., Auron, P. E., Tenen, D. G., and Sun, Z. (1999) Negative cross-talk between hematopoietic regulators: GATA proteins repress PU.1. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 8705–8710
- Stopka, T., Amanatullah, D. F., Papetti, M., and Skoultchi, A. I. (2005) PU.1 inhibits the erythroid program by binding to GATA-1 on DNA and creating a repressive chromatin structure. *EMBO J.* **24**, 3712–3723
- Rekhtman, N., Radparvar, F., Evans, T., and Skoultchi, A. I. (1999) Direct interaction of hematopoietic transcription factors PU.1 and GATA-1: functional antagonism in erythroid cells. *Genes Dev.* **13**, 1398–1411
- Nerlov, C., and Graf, T. (1998) PU.1 induces myeloid lineage commitment in multipotent hematopoietic progenitors. *Genes Dev.* **12**, 2403–2412
- Zhang, P., Iwasaki-Arai, J., Iwasaki, H., Fenyus, M. L., Dayaram, T., Owens, B. M., Shigematsu, H., Levantini, E., Huettner, C. S., Leksstrom-Himes, J. A., Akashi, K., and Tenen, D. G. (2004) Enhancement of hematopoietic stem cell repopulating capacity and self-renewal in the absence of the transcription factor C/EBP α . *Immunity* **21**, 853–863
- Xie, H., Ye, M., Feng, R., and Graf, T. (2004) Stepwise reprogramming of B-cells into macrophages. *Cell* **117**, 663–676
- Laiosa, C. V., Stadtfeld, M., Xie, H., de Andres-Aguayo, L., and Graf, T. (2006) Reprogramming of committed T-cell progenitors to macrophages and dendritic cells by C/EBP α and PU.1 transcription factors. *Immunity* **25**, 731–744
- Feng, R., Desbordes, S. C., Xie, H., Tillo, E. S., Pixley, F., Stanley, E. R., and Graf, T. (2008) PU.1 and C/EBP α/β convert fibroblasts into macrophage-like cells. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 6057–6062
- Ghisletti, S., Barozzi, L., Mietton, F., Polletti, S., De Santa, F., Venturini, E., Gregory, L., Lonie, L., Chew, A., Wei, C. L., Ragoussis, J., and Natoli, G. (2010) Identification and characterization of enhancers controlling the inflammatory gene expression program in macrophages. *Immunity* **32**,

- 317–328
34. Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y. C., Laslo, P., Cheng, J. X., Murre, C., Singh, H., and Glass, C. K. (2010) Simple combinations of lineage-determining transcription factors prime *cis*-regulatory elements required for macrophage and B-cell identities. *Mol. Cell* **38**, 576–589
 35. Nutt, S. L., Heavey, B., Rolink, A. G., and Busslinger, M. (1999) Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. *Nature* **401**, 556–562
 36. Rolink, A. G., Nutt, S. L., Melchers, F., and Busslinger, M. (1999) Long-term *in vivo* reconstitution of T-cell development by Pax5-deficient B-cell progenitors. *Nature* **401**, 603–606
 37. Mikkola, I., Heavey, B., Horcher, M., and Busslinger, M. (2002) Reversion of B-cell commitment upon loss of Pax5 expression. *Science* **297**, 110–113
 38. Cobaleda, C., Jochum, W., and Busslinger, M. (2007) Conversion of mature B-cells into T-cells by dedifferentiation to uncommitted progenitors. *Nature* **449**, 473–477
 39. Iwasaki, H., Mizuno, S., Arinobu, Y., Ozawa, H., Mori, Y., Shigematsu, H., Takatsu, K., Tenen, D. G., and Akashi, K. (2006) The order of expression of transcription factors directs hierarchical specification of hematopoietic lineages. *Genes Dev.* **20**, 3010–3021
 40. Zhou, Q., Brown, J., Kanarek, A., Rajagopal, J., and Melton, D. A. (2008) *In vivo* reprogramming of adult pancreatic exocrine cells to β -cells. *Nature* **455**, 627–632
 41. Yeohor, V., Liu, V., Espiritu, C., Paul, A., Oka, K., Kojima, H., and Chan, L. (2009) Neurogenin-3 is sufficient for transdetermination of hepatic progenitor cells into neo-islets *in vivo* but not transdifferentiation of hepatocytes. *Dev. Cell* **16**, 358–373
 42. Edge, A. S., and Chen, Z. Y. (2008) Hair cell regeneration. *Curr. Opin. Neurobiol.* **18**, 377–382
 43. Kirjavainen, A., Sulg, M., Heyd, F., Alitalo, K., Ylä-Herttua, S., Möröy, T., Petrova, T. V., and Pirvola, U. (2008) Prox1 interacts with Atoh1 and Gfi1 and regulates cellular differentiation in the inner ear sensory epithelia. *Dev. Biol.* **322**, 33–45
 44. Zheng, J. L., and Gao, W. Q. (2000) Overexpression of Math1 induces robust production of extra hair cells in postnatal rat inner ears. *Nat. Neurosci.* **3**, 580–586
 45. Woods, C., Montcouquiol, M., and Kelley, M. W. (2004) Math1 regulates development of the sensory epithelium in the mammalian cochlea. *Nat. Neurosci.* **7**, 1310–1318
 46. Huang, P., He, Z., Ji, S., Sun, H., Xiang, D., Liu, C., Hu, Y., Wang, X., and Hui, L. (2011) Induction of functional hepatocyte-like cells from mouse fibroblasts by defined factors. *Nature* **475**, 386–389
 47. Sekiya, S., and Suzuki, A. (2011) Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. *Nature* **475**, 390–393
 48. Vierbuchen, T., Ostermeier, A., Pang, Z. P., Kokubu, Y., Südhof, T. C., and Wernig, M. (2010) Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **463**, 1035–1041
 49. Pang, Z. P., Yang, N., Vierbuchen, T., Ostermeier, A., Fuentes, D. R., Yang, T. Q., Citri, A., Sebastiano, V., Marro, S., Südhof, T. C., and Wernig, M. (2011) Induction of human neuronal cells by defined transcription factors. *Nature* **476**, 220–223
 50. Marro, S., Pang, Z. P., Yang, N., Tsai, M. C., Qu, K., Chang, H. Y., Südhof, T. C., and Wernig, M. (2011) Direct lineage conversion of terminally differentiated hepatocytes to functional neurons. *Cell Stem Cell* **9**, 374–382
 51. Caiazzo, M., Dell'Anno, M. T., Dvoretzka, E., Lazarevic, D., Taverna, S., Leo, D., Sotnikova, T. D., Menegon, A., Roncaglia, P., Colciago, G., Russo, G., Carninci, P., Pezzoli, G., Gainetdinov, R. R., Gustincich, S., Dityatev, A., and Broccoli, V. (2011) Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature* **476**, 224–227
 52. Pfisterer, U., Kirkeby, A., Torper, O., Wood, J., Nelander, J., Dufour, A., Björklund, A., Lindvall, O., Jakobsson, J., and Parmar, M. (2011) Direct conversion of human fibroblasts to dopaminergic neurons. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 10343–10348
 53. Son, E. Y., Ichida, J. K., Wainger, B. J., Toma, J. S., Rafuse, V. F., Woolf, C. J., and Egan, K. (2011) Conversion of mouse and human fibroblasts into functional spinal motor neurons. *Cell Stem Cell* **9**, 205–218
 54. Yoo, A. S., Sun, A. X., Li, L., Shcheglovitov, A., Portmann, T., Li, Y., Lee-Messer, C., Dolmetsch, R. E., Tsien, R. W., and Crabtree, G. R. (2011) MicroRNA-mediated conversion of human fibroblasts to neurons. *Nature* **476**, 228–231
 55. Ambasadhan, R., Talantova, M., Coleman, R., Yuan, X., Zhu, S., Lipton, S. A., and Ding, S. (2011) Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions. *Cell Stem Cell* **9**, 113–118
 56. Takeuchi, J. K., and Bruneau, B. G. (2009) Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors. *Nature* **459**, 708–711
 57. Ieda, M., Fu, J. D., Delgado-Olguin, P., Vedantham, V., Hayashi, Y., Bruneau, B. G., and Srivastava, D. (2010) Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* **142**, 375–386
 58. Masip, M., Veiga, A., Izpisua Belmonte, J. C., and Simón, C. (2010) Reprogramming with defined factors: from induced pluripotency to induced transdifferentiation. *Mol. Human Reprod.* **16**, 856–868
 59. Smith, Z. D., Nachman, I., Regev, A., and Meissner, A. (2010) Dynamic single-cell imaging of direct reprogramming reveals an early specifying event. *Nat. Biotechnol.* **28**, 521–526
 60. Mikkelsen, T. S., Hanna, J., Zhang, X., Ku, M., Wernig, M., Schorderet, P., Bernstein, B. E., Jaenisch, R., Lander, E. S., and Meissner, A. (2008) Dissecting direct reprogramming through integrative genomic analysis. *Nature* **454**, 49–55
 61. Sridharan, R., Tchieu, J., Mason, M. J., Yachechko, R., Kuoy, E., Horvath, S., Zhou, Q., and Plath, K. (2009) Role of the murine reprogramming factors in the induction of pluripotency. *Cell* **136**, 364–377
 62. Brambrink, T., Foreman, R., Welstead, G. G., Lengner, C. J., Wernig, M., Suh, H., and Jaenisch, R. (2008) Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. *Cell Stem Cell* **2**, 151–159
 63. Samavarchi-Tehrani, P., Golipour, A., David, L., Sung, H. K., Beyer, T. A., Datti, A., Woltjen, K., Nagy, A., and Wrana, J. L. (2010) Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. *Cell Stem Cell* **7**, 64–77
 64. Stadtfeld, M., Maherali, N., Breault, D. T., and Hochedlinger, K. (2008) Defining molecular cornerstones during fibroblast to iPS cell reprogramming in mouse. *Cell Stem Cell* **2**, 230–240
 65. Meissner, A. (2010) Epigenetic modifications in pluripotent and differentiated cells. *Nat. Biotechnol.* **28**, 1079–1088
 66. Li, R., Liang, J., Ni, S., Zhou, T., Qing, X., Li, H., He, W., Chen, J., Li, F., Zhuang, Q., Qin, B., Xu, J., Li, W., Yang, J., Gan, Y., Qin, D., Feng, S., Song, H., Yang, D., Zhang, B., Zeng, L., Lai, L., Esteban, M. A., and Pei, D. (2010) A mesenchymal-to-epithelial transition initiates and is required for the nuclear reprogramming of mouse fibroblasts. *Cell Stem Cell* **7**, 51–63
 67. Gregory, P. A., Bert, A. G., Paterson, E. L., Barry, S. C., Tsykin, A., Farshid, G., Vadas, M. A., Khew-Goodall, Y., and Goodall, G. J. (2008) The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. *Nat. Cell Biol.* **10**, 593–601
 68. Chen, J., Liu, J., Yang, J., Chen, Y., Chen, J., Ni, S., Song, H., Zeng, L., Ding, K., and Pei, D. (2011) BMPs functionally replace Klf4 and support efficient reprogramming of mouse fibroblasts by Oct4 alone. *Cell Res.* **21**, 205–212
 69. Kim, J., Chu, J., Shen, X., Wang, J., and Orkin, S. H. (2008) An extended transcriptional network for pluripotency of embryonic stem cells. *Cell* **132**, 1049–1061
 70. Liu, X., Huang, J., Chen, T., Wang, Y., Xin, S., Li, J., Pei, G., and Kang, J. (2008) Yamanaka factors critically regulate the developmental signaling network in mouse embryonic stem cells. *Cell Res.* **18**, 1177–1189
 71. Papp, B., and Plath, K. (2011) Reprogramming to pluripotency: stepwise resetting of the epigenetic landscape. *Cell Res.* **21**, 486–501
 72. Jaenisch, R., and Young, R. (2008) Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell* **132**, 567–582
 73. Rahl, P. B., Lin, C. Y., Seila, A. C., Flynn, R. A., McCuine, S., Burge, C. B., Sharp, P. A., and Young, R. A. (2010) c-Myc regulates transcriptional pause release. *Cell* **141**, 432–445

74. Wang, J., Rao, S., Chu, J., Shen, X., Levasseur, D. N., Theunissen, T. W., and Orkin, S. H. (2006) A protein interaction network for pluripotency of embryonic stem cells. *Nature* **444**, 364–368
75. van den Berg, D. L., Snoek, T., Mullin, N. P., Yates, A., Bezstarosti, K., Demmers, J., Chambers, I., and Poot, R. A. (2010) An Oct4-centered protein interaction network in embryonic stem cells. *Cell Stem Cell* **6**, 369–381
76. Efroni, S., Duttagupta, R., Cheng, J., Dehghani, H., Hoepfner, D. J., Dash, C., Bazett-Jones, D. P., Le Grice, S., McKay, R. D., Buetow, K. H., Gingeras, T. R., Misteli, T., and Meshorer, E. (2008) Global transcription in pluripotent embryonic stem cells. *Cell Stem Cell* **2**, 437–447
77. Fisher, C. L., and Fisher, A. G. (2011) Chromatin states in pluripotent, differentiated, and reprogrammed cells. *Curr. Opin. Genet. Dev.* **21**, 140–146
78. Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K., Jaenisch, R., Wagschal, A., Feil, R., Schreiber, S. L., and Lander, E. S. (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315–326
79. Ang, Y. S., Tsai, S. Y., Lee, D. F., Monk, J., Su, J., Ratnakumar, K., Ding, J., Ge, Y., Darr, H., Chang, B., Wang, J., Rendl, M., Bernstein, E., Schaniel, C., and Lemischka, I. R. (2011) Wdr5 mediates self-renewal and reprogramming via the embryonic stem cell core transcriptional network. *Cell* **145**, 183–197
80. Koche, R. P., Smith, Z. D., Adli, M., Gu, H., Ku, M., Gnirke, A., Bernstein, B. E., and Meissner, A. (2011) Reprogramming factor expression initiates widespread targeted chromatin remodeling. *Cell Stem Cell* **8**, 96–105
81. Huangfu, D., Maehr, R., Guo, W., Eijkelenboom, A., Snitow, M., Chen, A. E., and Melton, D. A. (2008) Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat. Biotechnol.* **26**, 795–797
82. Singhal, N., Graumann, J., Wu, G., Araúzo-Bravo, M. J., Han, D. W., Greber, B., Gentile, L., Mann, M., and Schöler, H. R. (2010) Chromatin-remodeling components of the BAF complex facilitate reprogramming. *Cell* **141**, 943–955
83. Theunissen, T. W., van Oosten, A. L., Castelo-Branco, G., Hall, J., Smith, A., and Silva, J. C. (2011) Nanog overcomes reprogramming barriers and induces pluripotency in minimal conditions. *Curr. Biol.* **21**, 65–71
84. Okita, K., Ichisaka, T., and Yamanaka, S. (2007) Generation of germ line-competent induced pluripotent stem cells. *Nature* **448**, 313–317
85. Boland, M. J., Hazen, J. L., Nazor, K. L., Rodriguez, A. R., Gifford, W., Martin, G., Kupriyanov, S., and Baldwin, K. K. (2009) Adult mice generated from induced pluripotent stem cells. *Nature* **461**, 91–94
86. Zhao, X. Y., Li, W., Lv, Z., Liu, L., Tong, M., Hai, T., Hao, J., Guo, C. L., Ma, Q. W., Wang, L., Zeng, F., and Zhou, Q. (2009) iPS cells produce viable mice through tetraploid complementation. *Nature* **461**, 86–90
87. Plath, K., and Lowry, W. E. (2011) Progress in understanding reprogramming to the induced pluripotent state. *Nat. Rev. Genet.* **12**, 253–265
88. Chin, M. H., Mason, M. J., Xie, W., Volinia, S., Singer, M., Peterson, C., Ambartsumyan, G., Aimiuwu, O., Richter, L., Zhang, J., Khvorostov, I., Ott, V., Grunstein, M., Lavon, N., Benvenisty, N., Croce, C. M., Clark, A. T., Baxter, T., Pyle, A. D., Teitell, M. A., Pelegrini, M., Plath, K., and Lowry, W. E. (2009) Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell* **5**, 111–123
89. Stadtfeld, M., Apostolou, E., Akutsu, H., Fukuda, A., Follett, P., Natesan, S., Kono, T., Shioda, T., and Hochedlinger, K. (2010) Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature* **465**, 175–181
90. Kim, K., Doi, A., Wen, B., Ng, K., Zhao, R., Cahan, P., Kim, J., Aryee, M. J., Ji, H., Ehrlich, L. I., Yabuuchi, A., Takeuchi, A., Cunniff, K. C., Hongguang, H., McKinney-Freeman, S., Naveiras, O., Yoon, T. J., Irizarry, R. A., Jung, N., Seita, J., Hanna, J., Murakami, P., Jaenisch, R., Weissleder, R., Orkin, S. H., Weissman, I. L., Feinberg, A. P., and Daley, G. Q. (2010) Epigenetic memory in induced pluripotent stem cells. *Nature* **467**, 285–290
91. Bock, C., Kiskinis, E., Verstappen, G., Gu, H., Boulting, G., Smith, Z. D., Ziller, M., Croft, G. F., Amoroso, M. W., Oakley, D. H., Gnirke, A., Eggan, K., and Meissner, A. (2011) Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell* **144**, 439–452
92. Kang, L., Wang, J., Zhang, Y., Kou, Z., and Gao, S. (2009) iPS cells can support full-term development of tetraploid blastocyst-complemented embryos. *Cell Stem Cell* **5**, 135–138
93. Guenther, M. G., Frampton, G. M., Soldner, F., Hockemeyer, D., Mitalipova, M., Jaenisch, R., and Young, R. A. (2010) Chromatin structure and gene expression programs of human embryonic and induced pluripotent stem cells. *Cell Stem Cell* **7**, 249–257
94. Carey, B. W., Markoulaki, S., Hanna, J. H., Faddah, D. A., Buganim, Y., Kim, J., Ganz, K., Steine, E. J., Cassidy, J. P., Creighton, M. P., Welstead, G. G., Gao, Q., and Jaenisch, R. (2011) Reprogramming factor stoichiometry influences the epigenetic state and biological properties of induced pluripotent stem cells. *Cell Stem Cell* **9**, 588–598
95. Ohi, Y., Qin, H., Hong, C., Blouin, L., Polo, J. M., Guo, T., Qi, Z., Downey, S. L., Manos, P. D., Rossi, D. J., Yu, J., Hebrok, M., Hochedlinger, K., Costello, J. F., Song, J. S., and Ramalho-Santos, M. (2011) Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. *Nat. Cell Biol.* **13**, 541–549
96. Chen, X., Xu, H., Yuan, P., Fang, F., Huss, M., Vega, V. B., Wong, E., Orlov, Y. L., Zhang, W., Jiang, J., Loh, Y. H., Yeo, H. C., Yeo, Z. X., Narang, V., Govindarajan, K. R., Leong, B., Shahab, A., Ruan, Y., Bourque, G., Sung, W. K., Clarke, N. D., Wei, C. L., and Ng, H. H. (2008) Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. *Cell* **133**, 1106–1117
97. Boyer, L. A., Plath, K., Zeitlinger, J., Brambrink, T., Medeiros, L. A., Lee, T. I., Levine, S. S., Wernig, M., Tajonar, A., Ray, M. K., Bell, G. W., Otte, A. P., Vidal, M., Gifford, D. K., Young, R. A., and Jaenisch, R. (2006) Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* **441**, 349–353
98. Lee, T. I., Jenner, R. G., Boyer, L. A., Guenther, M. G., Levine, S. S., Kumar, R. M., Chevalier, B., Johnstone, S. E., Cole, M. F., Isono, K., Koseki, H., Fuchikami, T., Abe, K., Murray, H. L., Zucker, J. P., Yuan, B., Bell, G. W., Herbolsheimer, E., Hannett, N. M., Sun, K., Odom, D. T., Otte, A. P., Volkert, T. L., Bartel, D. P., Melton, D. A., Gifford, D. K., Jaenisch, R., and Young, R. A. (2006) Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* **125**, 301–313
99. Kidder, B. L., Palmer, S., and Knott, J. G. (2009) SWI/SNF-Brg1 regulates self-renewal and occupies core pluripotency-related genes in embryonic stem cells. *Stem cells* **27**, 317–328
100. Ku, M., Koche, R. P., Rheinbay, E., Mendenhall, E. M., Endoh, M., Mikkelsen, T. S., Presser, A., Nusbaum, C., Xie, X., Chi, A. S., Adli, M., Kasif, S., Ptaszek, L. M., Cowan, C. A., Lander, E. S., Koseki, H., and Bernstein, B. E. (2008) Genome-wide analysis of PRC1 and PRC2 occupancy identifies two classes of bivalent domains. *PLoS Genet.* **4**, e1000242
101. Li, G., Margueron, R., Ku, M., Chambon, P., Bernstein, B. E., and Reinberg, D. (2010) Jarid2 and PRC2, partners in regulating gene expression. *Genes Dev.* **24**, 368–380
102. Ho, L., Jothi, R., Ronan, J. L., Cui, K., Zhao, K., and Crabtree, G. R. (2009) An embryonic stem cell chromatin-remodeling complex, esBAF, is an essential component of the core pluripotency transcriptional network. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 5187–5191
103. Xie, L., Pelz, C., Wang, W., Bashar, A., Varlamova, O., Shadle, S., and Impey, S. (2011) KDM5B regulates embryonic stem cell self-renewal and represses cryptic intragenic transcription. *EMBO J.* **30**, 1473–1484
104. Boyer, L. A., Lee, T. I., Cole, M. F., Johnstone, S. E., Levine, S. S., Zucker, J. P., Guenther, M. G., Kumar, R. M., Murray, H. L., Jenner, R. G., Gifford, D. K., Melton, D. A., Jaenisch, R., and Young, R. A. (2005) Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* **122**, 947–956
105. Yang, J., Chai, L., Fowles, T. C., Alipio, Z., Xu, D., Fink, L. M., Ward, D. C., and Ma, Y. (2008) Genome-wide analysis reveals Sall4 to be a major regulator of pluripotency in murine embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 19756–19761
106. Hu, G., Kim, J., Xu, Q., Leng, Y., Orkin, S. H., and Elledge, S. J. (2009) A genome-wide RNAi screen identifies a new transcriptional module required for self-renewal. *Genes Dev.* **23**, 837–848
107. Dejosez, M., Levine, S. S., Frampton, G. M., Whyte, W. A., Stratton, S. A.,

- Barton, M. C., Gunaratne, P. H., Young, R. A., and Zwaka, T. P. (2010) Ronin/Hcf-1 binds to a hyperconserved enhancer element and regulates genes involved in the growth of embryonic stem cells. *Genes Dev.* **24**, 1479–1484
108. Cole, M. F., Johnstone, S. E., Newman, J. J., Kagey, M. H., and Young, R. A. (2008) Tcf3 is an integral component of the core regulatory circuitry of embryonic stem cells. *Genes Dev.* **22**, 746–755
109. Chia, N. Y., Chan, Y. S., Feng, B., Lu, X., Orlov, Y. L., Moreau, D., Kumar, P., Yang, L., Jiang, J., Lau, M. S., Huss, M., Soh, B. S., Kraus, P., Li, P., Lufkin, T., Lim, B., Clarke, N. D., Bard, F., and Ng, H. H. (2010) A genome-wide RNAi screen reveals determinants of human embryonic stem cell identity. *Nature* **468**, 316–320
110. Kim, S. W., Yoon, S. J., Chuong, E., Oyolu, C., Wills, A. E., Gupta, R., and Baker, J. (2011) Chromatin and transcriptional signatures for Nodal signaling during endoderm formation in hESCs. *Dev. Biol.* **357**, 492–504
111. Wu, H., D'Alessio, A. C., Ito, S., Xia, K., Wang, Z., Cui, K., Zhao, K., Sun, Y. E., and Zhang, Y. (2011) Dual functions of Tet1 in transcriptional regulation in mouse embryonic stem cells. *Nature* **473**, 389–393