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Pivots of pluripotency: the roles of non-coding RNA in regulating embryonic and induced pluripotent stem cells

Jeffrey S. Huo and Elias T. Zambidis*

Institute for Cell Engineering, The Johns Hopkins University School of Medicine, and Divisions of Pediatric Oncology, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21205, USA

Abstract

BACKGROUND—Induced pluripotent stem cells (iPSC) derived from reprogrammed patient somatic cells possess enormous therapeutic potential. However, unlocking the full capabilities of iPSC will require an improved understanding of the molecular mechanisms which govern the induction and maintenance of pluripotency, as well as directed differentiation to clinically relevant lineages. Induced pluripotency of a differentiated cell is mediated by sequential cascades of genetic and epigenetic reprogramming of somatic histone and DNA CpG methylation marks. These genome-wide changes are mediated by a coordinated activity of transcription factors and epigenetic modifying enzymes. Non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are now recognized as an important third class of regulators of the pluripotent state.

SCOPE OF REVIEW—This review surveys the currently known roles and mechanisms of ncRNAs in regulating the embryonic and induced pluripotent states.

MAJOR CONCLUSIONS—Through a variety of mechanisms, ncRNAs regulate constellations of key pluripotency genes and epigenetic regulators, and thus critically determine induction and maintenance of the pluripotent state.

GENERAL SIGNIFICANCE—A further understanding of the roles of ncRNAs in regulating pluripotency may help assess the quality of human iPSC reprogramming. Additionally, ncRNA biology may help decipher potential transcriptional and epigenetic commonalities between the self renewal processes that govern both ESC and tumor initiating cancer stem cells (CSC).

Pluripotent stem cells are defined by their unlimited self-renewal and their potential to differentiate into derivatives of all three embryonic germ layer lineages. They were first recognized in invertebrates over 120 years ago, with the discovery by Driesch that blastomeres isolated from sea urchin embryos could by themselves form complete descendent sea urchins [1]. Pluripotent stem cell lines were subsequently generated from mouse blastocysts [2, 3] and human blastocysts [4]. These pluripotent embryonic stem cell (ESC) lines possessed the potential to develop into any type of tissue in the adult organism. Capitalizing on this potential through directed differentiation would allow the unlimited

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*Correspondence: Elias T. Zambidis MD, Ph.D, ezambid1@jhmi.edu, Institute for Cell Engineering, and Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, 733 N. Broadway, BRB 755, Baltimore, MD 21205, office: 410-614-0123; F: (443) 287-5611.

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repair or replacement of abnormal, damaged, or absent types of patient cells. However, although such capabilities would have enormous therapeutic potential, the isolation of pluripotent stem cells from human embryos, and their use in genetically unrelated patient recipients, entangle a host of medical, ethical, and political challenges.

The possibility of circumventing many of these challenges arose from the discovery of methods to reprogram fully differentiated somatic cells backwards into a pluripotent state. This was originally demonstrated using the nuclear reprogramming approach called somatic cell nuclear transfer (SCNT) [5, 6]. The subsequent landmark experiments of Takahashi and Yamanaka demonstrated that differentiated somatic cells could be epigenetically reprogrammed back into induced pluripotent stem cells (iPSC) using ectopic expression of defined reprogramming factors [7]. The development of efficient and accurate methods of generating a ready supply of genetically-matched, patient-specific iPSC from differentiated somatic donor cells would bypass many of the technical and ethical obstacles associated with human ESC derived from embryos. Thus, a more thorough understanding of the mechanisms that regulate induction, maintenance, and directed differentiation of pluripotent stem cells is central to unlocking the full therapeutic and research potential of patient-derived iPSC [8].

The ectopic expression of defined reprogramming factors in differentiated somatic cells triggers genome-wide expression cascades [9, 10], as well as an epigenetic remodeling of the differentiated genome that is mediated by a multitude of chromatin modifying and DNA methylation enzymes and factors [11, 12]. It has been increasingly recognized that a third class of actors, noncoding RNAs (ncRNAs), also play critical roles in regulating the normal and induced pluripotent state. These ncRNAs are highly abundant and may represent an even greater fraction of transcription across the human genome than protein coding RNAs [13]. They can be broadly classified into small (<200 bp) or large (>200 bp; lncRNAs). Initially, ncRNAs were thought to play limited roles in human biology, or perhaps even to represent transcriptional noise [14]. However, it has become increasingly recognized that ncRNAs are key players in the pathogenesis of human disease (reviewed in [15]). In parallel with this growing understanding of the importance of ncRNAs in human biology, a rapidly growing number of examples are being identified of the specific importance of short and long ncRNAs in regulating the induction and maintenance of the pluripotent state. Importantly, ncRNAs can modulate the activity of entire transcriptional networks, or coordinate concerted activities of constellations of master genetic and epigenetic regulators. Thus, ncRNAs serve as pivots around which the pluripotent state can be entered or exited. This brief review surveys the current yet swiftly expanding state of understanding of the roles of ncRNA in regulating embryonic and induced pluripotent states.

Small non-coding RNAs in the induction and maintenance of pluripotency

Small ncRNAs are ~20–30 nucleotide (nt) RNAs that are associated with the Argonaute (Ago)-family of proteins, and mediate post-transcriptional repression of target messenger RNA (mRNA). Most fall into one of three categories: 1) microRNAs (miRNAs), 2) endogenous small-interfering RNAs (endo-siRNAs), and 3) Piwi-interacting RNAs (piRNAs). MiRNAs are generated via sequential post-transcriptional processing by the Drosha-DGCR8 and the RNase III Dicer complexes, followed by assembly with Ago proteins into an RNA-induced silencing complex (RISC). The miRNA then guides the RISC complex to complementary “seed” sequences on target mRNAs, and mediates translational repression and exonucleolytic mRNA decay. A given miRNA can target mRNAs from dozens or hundreds of different genes. In contrast to miRNAs, the silencing effects of endo-siRNAs are dependent only on Dicer, and do not require Drosha-DGCR8 action. Finally, piRNAs require neither Drosha-DGCR8 nor Dicer, bind to PIWI-subfamily (instead of Ago-

subfamily) Argonaute proteins, and silence their targets by mediating mRNA degradation, or possibly DNA methylation. The biogenesis and function of all three of these ncRNA classes is dependent on a host of associated processing and regulatory proteins, and is reviewed in depth elsewhere [16].

The critical role of small ncRNAs in ESC has been most vividly demonstrated via genetic disruption of key small ncRNA processing enzymes. Gene targeting in murine ESC of *DGCR8*, necessary for normal miRNA maturation, resulted in severely delayed and abnormal patterns of expression of markers of differentiation, near-total disruption of normal teratoma formation *in vivo*, and failure to completely suppress markers of pluripotency following LIF withdrawal *in vitro* [17]. Furthermore, disruption of the gene encoding the enzyme Dicer, which is necessary not just for miRNA but also for endo-siRNA biogenesis, results in an even more severe phenotype. Gene targeting of *Dicer* in murine ESC resulted in total absence of teratoma formation *in vivo*, complete absence of expression of markers of differentiation after LIF withdrawal *in vitro*, and embryoid bodies that stopped growing altogether after 8–10 days in differentiation cultures [18]. These loss of function studies collectively underscored that, far from a niche role, the genetic regulatory action of small ncRNAs is both critical and indispensable for normal ESC function.

Mechanisms and targets of miRNAs that regulate ESC function (Figures 1, 2)

The murine *miR-290-295* and *miR-302-367* families were the first identified ESC-specific miRNAs [19]. Members of the *miR-290/295* cluster are among the most abundantly expressed miRNAs in mouse ESCs, and both *miR-290-295* and *miR-302-367* miRNA genes are occupied by key Core pluripotency transcription factors at their promoters (e.g., Oct4 and Sox2) [20, 21]. The importance of these miRNA families was first demonstrated via functional miRNAs screens, where reintroduction of these miRNAs to *DGCR8*-null mouse ESC was shown to partially rescue normal ESC self-renewal [22]. Additionally, *miR-290-295* miRNAs rescued proliferation in *Dicer*-null ESC [23], and were shown to be involved in protecting ESC from apoptosis following exposure to environmental stress (e.g., gamma irradiation or doxorubicin) [24]. Finally, *miR-290-295* and *miR-302* family members promoted an ESC-like rapid progression through the cell cycle, that was mediated in part through *miR-302* post-transcriptional inhibition of cyclin D1 [21, 25].

An important principle is that miRNAs can promote or inhibit maintenance of the pluripotent state by suppression of either key pro-differentiation or pro-ESC genes, respectively. For example, the pro-ESC *miR-290-295* family of miRNAs can suppress the expression of early differentiation markers [25]. *MiR-290-295* miRNAs were also found to maintain the pluripotent state in part by increasing expression of *Lin28* [26], which interferes with the normal function of the pro-differentiation *let-7* family of miRNAs [27]. In the absence of *miR-290-295* miRNAs, *let-7* miRNAs mediate changes in the expression of hundreds of target genes in mouse ESC, and promote rapid loss of pluripotency markers [26]. In addition to the *let-7* family, several other miRNAs involved in the differentiation of mouse ESCs suppress the activity of key pro-ESC genes (Figure 1). For example, expressions of *miR-134*, *miR-296* and *miR-470* are all increased during differentiation of mouse ESC with retinoic acid, and were identified as targeting mRNAs for the key pluripotency transcription genes *Nanog*, *Oct4* and *Sox2* [28]. Likewise, *miR-145* and *miR-34a*, which function downstream of p53, promoted differentiation of hESCs by decreasing *OCT4*, *SOX2*, and *KLF4* activity [29, 30]. Thus, the collective opposing actions of pro-ESC and pro-differentiation miRNAs on the function of key ESC genes contributes critically to the balance between maintenance and departure from the pluripotent state.

MiRNAs can also regulate the pluripotent state by mediating coordinated changes in the activity of key epigenetic regulators, such as the Polycomb Group (PcG) protein complexes.

PcG proteins are chromatin modifiers that are critical for both the induction and maintenance of the pluripotent state [31, 32]. Induction and maintenance of lineage-specific commitment depends on silencing a developmentally-inappropriate constellations of genes, and PcG chromatin complexes mediate such gene silencing through sequential histone modifications of their targeted genes in a cellular context-dependent manner [33]. For example, PRC2 complexes mediate gene silencing by trimethylating lysine 27 on histone H3 (H3K27me3), which subsequently recruits PRC1 complexes to add a lysine 119 monoubiquitylation mark on Histone 2A [34]. PRC1 complexes repress differentiation-associated genes in ESC, while silencing pluripotency associated genes in more differentiated cells.

Recent work has suggested that this context-dependent switch in PRC1 complex binding in ESCs is in part mediated by swapping of different Cbx paralogs into the PRC1 complex [35]. For example, *Cbx7* is highly expressed in ESC, and confers PRC1 complex targeting to lineage-associated genes. When ESC undergo differentiation, *Cbx7* levels drop, and PRC1 complexes form using alternative Cbx paralogs, conferring more differentiation-appropriate targeting of PRC1 complexes. This differentiation-associated loss of *Cbx7* in ESC was found to be secondary to differentiation-associated increases in *miR-125* and *miR-181* miRNAs. These data were consistent with previous observations that *miR-125* expression promoted differentiation of ESC into neural stem cells, and promoted maturation of pro-differentiation *let-7* miRNAs [36, 37]. Similarly, *miR-181* was shown to promote multi-lineage differentiation from hematopoietic stem-progenitors [38], and also to target *Lin28* expression [39]. Further work is likely to uncover more examples of the ability of miRNAs to serve as a pivot around which developmental stage-specific switching of key genetic regulators occurs.

MiRNAs that regulate the generation of iPSC

Consistent with their key role in maintaining the pluripotent state in ESCs, miRNAs have been further implicated in the induction of *de novo* induced pluripotency from reprogrammed somatic cells. For example, elimination of global miRNA function by shRNA knockdown of the key miRNA processing enzyme *Ago2* in mouse embryonic fibroblasts (MEF) led to a specific decrease in iPSC generation efficiency using ectopic expression of the Yamanaka factors (*Sox2*, *Oct4*, *Klf4*, and *c-Myc* (SOKM)), without affecting cell viability [40]. Such observations underscored the critical requirement for normal miRNA function in providing the necessary conditions for generating iPSC via ectopic Yamanaka factor expression. Additionally, one of the original factors used to drive induction of pluripotency from differentiated human tissues was LIN28 [41], the RNA binding protein already described above, that specifically blocks the normal formation of pro-differentiation *let-7* family miRNAs [42].

Several miRNA families have been shown to enhance the generation of iPSC in a manner consistent with their hypothesized critical roles in regulating pluripotency. For example, ectopic expression of *miR-290-295* family members in mouse embryonic fibroblasts (MEF) increased the efficiency of iPSC generation in conjunction with *Oct4*, *Sox2* and *Klf4* (OSK) transfection [43]. In contrast, direct inhibition of pro-differentiation *let-7* miRNA enhanced the generation of iPSC in a similar MEF/OSK system [26]. Finally, loss of p53-stimulated *miR-34* expression has been advanced as one mechanism by which down-regulation of p53 enhances iPSC generation [44].

More recently, iPSC reprogramming was reported via ectopic expression of miRNAs alone. Thus, instead of initiating the reprogramming process with ectopic transcription factors that initiate new gene expression, investigators instead used the inverse approach of initiating the reprogramming process by ectopic miRNA silencing of targeted genes. When primed with

the histone deacetylase inhibitor valproic acid [45], MEFs transduced with lentiviruses bearing mouse *miR-302-367* constructs generated mouse iPSCs that contributed to adult tissues and germline cells in chimeric mice [46]. In other studies, 7 candidate miRNAs were identified that had >2 fold greater expression in mouse ESC and iPSC vs. mouse adipose stromal cells, and these mature miRNAs were directly transfected into mouse adipose stromal cells [47]. Importantly, iPSC could not be generated from mouse adipose stromal cells with transfection of any single candidate miRNA, or any of the 21 possible combinations of two candidate miRNAs. However, using four serial transfections of mature *miR-302s*, *miR-200c*, and *miR-369s* over the first eight days of the reprogramming protocol, rare iPSC were generated from murine adipose stromal cells. Further understanding of the mechanisms by which miRNA-mediated gene silencing can enhance or even completely mediate reprogramming to a pluripotent state on their own may enable the development of optimized reprogramming methods which take advantage of both transcription factor activation and miRNA suppression of gene expression to maximize the efficiency and quality of iPSC generation. Together, this growing body of work demonstrates both the central importance and future potential of miRNA-mediated “tuning” of the pluripotent state.

Endo-siRNAs and piRNAs

Other classes of small ncRNAs, including endo-siRNAs and piRNAs, may also play important roles in the induction and maintenance of pluripotency. For example, endo-siRNAs have been isolated from mouse ESC [48], and *Dicer*-knockout ESC that lack both endo-siRNAs and miRNAs displayed more severe functional defects than *DCGR8*-knockouts which selectively lack only miRNAs [18]. Additionally, endo-siRNAs and piRNAs play important roles in the normal embryonic formation of fully pluripotent germline stem cells via de-differentiation of epiblast stem cells [49, 50]. This role of ncRNAs in reprogramming of epiblast cells into more primitive developmental states is of particular relevance to the study of ESC, in light of emerging questions regarding the comparative developmental states of mouse ESC, mouse post-implantation epiblast stem cells (EpiSC), and human ESC (which possess EpiSC-like qualities) [8, 51]. Finally, piwi-subfamily proteins, which mediate piRNA function, were also identified in human CD34⁺ hematopoietic stem progenitor cells [52]. Thus, this subclass of molecules may play roles not only in regulating germ cells, but also adult stem cells.

Regulation of naïve and primed pluripotent states by short ncRNAs

Pluripotent murine ESCs are defined by their unlimited self-renewal, and the capacity to differentiate to ectodermal, endodermal, and mesodermal cell lineages. Murine ESC can differentiate into multi-lineage embryoid bodies upon withdrawal of leukemia inhibitory factor (LIF) *in vitro*, or form tri-lineage teratomas upon injection *in vivo*. However, their *bona fide* pluripotency is most rigorously demonstrated by their ability to contribute to the formation of a chimeric mouse following injection into a murine host blastocyst [51]. In contrast to mouse ESC, mouse EpiSC are derived from the post-implantation stage of embryonic development [53, 54]. Although EpiSC demonstrate pluripotency during the less stringent *in vivo* teratoma assay, they appear to have a lesser potency when tested for ability to contribute to the cell lineages and germline of chimeric animals following blastocyst injection, or with the more rigorous tetraploid complementation chimerism assay [55]. These two distinct types of pluripotent stem cells – “naïve” ESC and “primed” EpiSC – also have distinct miRNA expression profiles [56]. This is of particular relevance because an increasing body of evidence suggests that existing human iPSC (hiPSC) and hESC are more akin to pluripotency-limited mouse EpiSC than they are to ‘ground state’ pluripotent “naïve” mouse ESC [53–55, 57–59]. It is possible that generating fully pluripotent “naïve” human iPSC may require reactivation of the same comprehensive epigenetic reprogramming

seen during generation of fully pluripotent germ cells from embryonic epiblast cells; a process in which endo-siRNAs and piRNAs play a critical role [49]. Further exploration of the differences in ncRNA function between these variant states of pluripotency, and their role during movement between pluripotent states may clarify the true developmental status of existing hESCs. It may also provide new clues to realizing a more robust differentiation potential for hiPSC that is ultimately necessary for fulfilling their promise in regenerative medicine.

Regulation of pluripotency by long non-coding RNAs (lncRNAs)

lncRNAs (Figure 2) are endogenous cellular RNAs that are >200 nucleotides in length. lncRNAs are similar to mRNAs, in that they are RNA polymerase II-promoted and polyadenylated. However, unlike mRNAs, lncRNAs lack experimental or evolutionary evidence for an open reading frame that can encode a functional protein [13, 60, 61]. Several types of lncRNA have been identified via a variety of search strategies. These types include intronic sense and antisense lncRNAs (transcribed from within introns of protein-coding genes) [62], antisense lncRNAs that overlap with protein-coding genes [63, 64], and lncRNAs that are found between protein-coding genes [61, 65, 66]. These diverse species of lncRNA have been shown to regulate cellular function through a variety of mechanisms that regulate the transcriptional machinery (reviewed in depth in [67], [68] and [69]). These mechanisms include 1) regulation of target mRNA degradation [70], 2) the recruitment, nucleation, assembly, and targeting of genetic and epigenetic regulatory protein complexes [71–73], and 3) by serving as competing endogenous RNA (ceRNAs) that bind and sequester miRNAs away from coding RNA targets, thus protecting target RNAs from repression [74]. It has been proposed that most ncRNAs may act as “integrators” of a variety of epigenetic and transcriptional processes by nucleating factors together into ncRNA-protein complexes [69].

Early microarray studies revealed the existence of lncRNAs that were differentially expressed during mouse ESC differentiation [75]. The transcription of lncRNAs in mouse ESC was subsequently found to be epigenetically regulated by promoter DNA CpG methylation in the same manner as protein coding mRNAs [76]. ESC have a repertoire of protein coding mRNA promoters bearing both H3K4me3 and H3K27me3 (“bivalent”) histone modification marks, resulting in genes which are silenced but “poised” for rapid activation in response to appropriate differentiation signals [77]. Interestingly, in one study, Wu et al. found that mouse ESCs also have suites of quiescent lncRNA promoters with similar bivalent marks. Furthermore, upon differentiation many lncRNAs lose the repressive H3K27me3 and are transcribed in a manner that is similar to protein-coding genes [76]. Knockdown of the gene for the H3K27me3 methyltransferase Ezh2 in mouse ESC led to activation of normally silenced H3K27me3 marked lncRNAs. These findings are consistent with the notion that ESC-specific lncRNAs are likely regulated in the same epigenetic mechanisms as protein-coding genes.

Other important and early studies defined specific executive roles of lncRNAs by identifying two lncRNAs in mouse ESC that were direct targets of Oct4 and Nanog [78]. The knockdown or over-expression of these lncRNAs in turn influenced the expression of *Oct4* and/or *Nanog*, as well as other markers of pluripotency. Furthermore, it was demonstrated that NRSF/REST, a key repressor of activation of neuronal genes, regulated the expression of two neural-specific lncRNAs in neural stem cells. These studies were important in collectively demonstrating that lncRNAs in stem cells not only regulated pluripotency networks, but could also regulate lineage-associated genetic regulators, and thus may play a direct role in lineage specification [79]. Ng et al. examined differential lncRNA expression in human ESC [80] using microarrays for human lncRNAs as defined

by Jia et al. [61]. In these studies, Ng et al. identified three lncRNAs that were exclusively expressed in hESC or iPSC, and had decreased expression following RNAi suppression of *NANOG* and/or *OCT4*. Knock down of these lncRNAs in hESC, resulted in loss of OCT4 protein, down-regulation of pluripotency markers, and upregulation of lineage markers. Two of these lncRNAs were found to bind the epigenetic regulator SUZ12 and SOX2. Together, these findings demonstrated that lncRNAs in both mouse and human ESC could play important roles in the maintenance of the pluripotent state.

In other studies, Guttman et al. focused on the action in stem cells of a subset of lncRNAs unassociated with protein-coding loci [65]. Such lncRNAs were predicted by identifying regions of the mouse genome between protein coding genes that had chromatin states actively transcribed by RNA Polymerase II. This subset of lncRNAs, termed long intergenic noncoding RNAs (lincRNAs), demonstrated strong evolutionary conservation in both promoter and exonic sequences. Like other lncRNAs, lincRNAs possessed the ability to serve as molecular scaffolds for coordinated assembly of chromatin regulatory complexes (e.g. Polycomb group complexes) and regulation of their localization in a genome-wide manner [66]. Thus, they likely play critical roles in transcriptional repression and activation. Individual expression knock-down of members of the lincRNA subclass of lncRNAs in mouse ESC identified dozens of lincRNAs that induced either differentiation or a specific bias in lineage commitment [81]. Strikingly, the knockdown of some lincRNAs resulted in global ESC gene expression patterns that were comparable in degree to the silencing of key pluripotency factors. LincRNAs were not only able to regulate genes that were physically contiguous to their genomic location, but also across the entire genome in *trans*. The promoters of these lincRNAs bound pluripotency-associated transcription factors, and shRNA knockdown of pluripotency transcription factors caused expression of many lincRNAs to decrease to the same degree as protein-coding RNAs. Furthermore, crosslinking of RNA to chromatin modifying proteins identified many lincRNAs that were directly associated with them. Finally, shRNA knockdown of either an individual lincRNA or its correspondingly bound chromatin modifying protein resulted in overlapping patterns of gene expression. Collectively, these data suggest that in mouse ESC, lincRNAs can regulate the actions of multiple chromatin remodeling proteins that in turn coordinately regulate patterns of gene expression necessary for either the maintenance of pluripotency, or alternatively the repression of lineage specificity.

Regulation of factor-driven induced pluripotency by lncRNAs

To probe the role of lncRNAs during the reprogramming of somatic cells into iPSC, Loewer et al. systematically examined the changes in lincRNA expression that were elicited during induction of pluripotency of human fibroblasts via retroviral SOKM transduction [82]. The authors began by identifying 234 lincRNAs that were differentially expressed in either fibroblast iPSCs or hESCs vs. starting fibroblasts. They next sought to distinguish between those lincRNAs important for the pluripotent state in general, and those uniquely important for the process of reprogramming differentiated fibroblasts back to a pluripotent state. To identify iPSC-specific lincRNAs important for reprogramming, a subset of 28 pluripotency-associated lincRNAs that were even more highly expressed in iPSC than hESC were isolated, screened via responsiveness to shRNA knockdown of Oct4, and then tested for their ability to modulate reprogramming efficiency. Knockdown of one particular lincRNA termed lincRNA-ST8SIA3 (later renamed lincRNA-RoR) was found to decrease the efficiency of reprogramming. Alternatively, over-expression of lincRNA-RoR in fibroblasts prior to induction of pluripotency increased the efficiency of iPSC generation. This work provided evidence that long ncRNA can specifically directly regulate the process of reprogramming of differentiated cells into a pluripotent state.

LncRNAs that regulate somatic stem-progenitor cells

As described above, the lineage-specific expression patterns of many lncRNAs suggest that they are also under precise tissue-specific control in somatic stem-progenitors. Several studies have further implicated lncRNA in the maintenance and function of lineage-committed somatic stem-progenitor cells. For example, in human epidermal progenitors, expression of the lncRNA gene *ANCR* decreased during terminal differentiation to keratinocytes [83]. RNAi knockdown of *ANCR* in epidermal progenitors by itself triggered robust expression of skin-specific differentiation genes, suggesting that *ANCR* played a key role in maintaining the undifferentiated epidermal progenitor state. Furthermore, in mouse erythroid progenitors, a screen of lncRNAs expressed during the terminal stage of erythropoiesis identified a lncRNA, *LincRNA-EPS*, whose inhibition resulted in blockade of erythroid differentiation and increased apoptosis [84]. Microarray analyses suggested that *LincRNA-EPS* acted through suppression of apoptosis-associated genes (*e.g.*, *Pycard*) via obscure mechanisms. In human neural stem cells, neuronal-specific lncRNAs were identified [80]. Knockdown of these lncRNAs led to inhibition of neuronal differentiation, via a mechanism that possibly acted via association with SUZ12 or REST binding, or perhaps indirectly via associated decreases in *mir-125* and *let7b*. Understanding the sequential roles of lncRNAs along the entire length of the differentiation process will be critical to the ultimate goal of optimizing the differentiation of pluripotent stem cells to useful clinical products.

LncRNAs can also work in concert with small ncRNAs in progenitor cells to modulate gene expression programs. For example, lncRNAs were shown to act as endogenous RNAs that could modify the regulation of myogenic progenitor cell function by competing with miRNAs [85]. In myoblasts, linc-MD1 can bind and sequester *miR-133* and *miR-135* away from their coding RNA targets, preventing these anti-differentiation miRNAs from inhibiting the coding RNAs for transcription factors that are associated with myocyte differentiation. In neural stem cells, lncRNA_N2 siRNA knockdown inhibits normal neuronal differentiation and results in decreases in the levels of pro-differentiation *miR-125* and *LET7a* miRNAs found within the introns of lncRNA_N2. However, the relationship between these two findings remains to be fully delineated. [80]. It will be interesting to explore whether there are also gene regulatory systems in progenitor cells where both short and long ncRNAs collaborate to regulate in trans gene expression by mechanisms beyond direct interaction. One example is the epigenetic regulator Cbx7, which plays a key role in maintenance of ESC pluripotency [86], and whose function has been demonstrated to be regulated by both small ncRNA [35] and long ncRNA [73] dependent mechanisms.

X-chromosome inactivation (XCI)

Finally, a special note is made of ncRNA regulation of X-chromosome inactivation in stem cells via the concerted actions of the *XIST* lncRNA transcript (and many other lncRNAs). Importantly, X-chromosome inactivation (XCI) was one of the earliest identified and best characterized biological roles for lncRNAs (reviewed in [49, 87]). XCI is of particular relevance to ESC biology, since one of the hallmarks of “naïve” pluripotent stem cells is the absence of XCI [51]. In contrast, XCI is typically found in “primed” murine EpiSC [51, 55]. Since naïve murine ESC lack XCI [58], it has been expected that ground state pluripotent “naïve” human pluripotent stem cells should similarly lack XCI. However, there remain important, yet incompletely characterized differences in the developmental timing and mechanisms of XCI in humans and mice that are directly relevant to ESC biology and that are incompletely elucidated (reviewed in [88]). Accordingly, there has been found to be substantial variation in the initial XCI status among various human ESC and iPSC lines, as well as development of XCI instability and loss of *XIST* function following progressive passage in culture [89, 90].

Recent papers have emphasized the importance of acquiring a better understanding of the lncRNA-dependent regulation of XCI in stem cells. One recent study examined XCI and *XIST* in 136 hESC and 69 hiPSC lines [91], reconfirming previous observations of *XIST* function loss and XCI instability in some pluripotent stem cell lines. Furthermore, it was found that resultant epigenetic and transcriptional aberrations in genes normally subject to XCI persisted in differentiated cells derived from XCI-aberrant pluripotent stem cells. Another recent study showed that this progressive loss with passage of *XIST* and XCI function in pluripotent stem cells led to ectopic reactivation of previously silenced X-linked loci in a hiPSC model of Lesch-Nyhan syndrome [92]. Loss of normal *XIST* function in human iPSC derived from females was associated with upregulated oncogene expression, accelerated proliferation, and decreased differentiation potential [93]. Finally, using *LIF*-expressing SNL feeders during reprogramming of fibroblasts into hiPSC, it was found that there was an increased frequency of generation of hiPSC that both had a more stable, ESC-like pluripotent state like without XCI [94]. Furthermore, these hiPSC lines demonstrated proper reestablishment of *XIST*-mediated X-inactivation following differentiation. This work suggested that micro-environmental derivation conditions might be key to the establishment of hiPSC with proper, developmentally appropriate XCI and *XIST* function. While a full discussion of the many complex unanswered questions regarding lncRNA-regulated XCI status in hESC and hiPSC is beyond the scope of this review (recent reviews include [95], [96] and [97]), it is clear that a greater understanding of the mechanisms underlying the proper regulation of XCI status and *XIST* function in hESC and iPSC is a prerequisite for their ultimate therapeutic application.

Collectively, it has become clear from this rapidly expanding body of knowledge that, like short ncRNAs, long ncRNAs similarly play a central role in the coordination of key ESC regulators to either promote or repress the pluripotent state. With increased understanding of the diversity of lncRNA biology, and the greater use of genomics survey techniques that better capture lncRNA expression data, the number of assigned roles and mechanisms for lncRNAs in regulation of pluripotency should continue to grow.

Predicting the quality of the pluripotent state via expression profiling of non-coding RNAs

One of the ultimate goals of studying human pluripotent stem cells is to use them for generating clinically useful cell lineages. However, it has become increasingly understood that hESC and hiPSC vary considerably in the quality and quantity of their differentiation potential [98, 99]. Efforts to characterize the molecular mechanisms responsible for such variability in quality between individual hESC and iPSC lines have initially focused on variation between levels of expression of protein-coding genes [100]. However, there is evidence that differences in ncRNA expression also impact the differentiation potential of particular ESC and iPSC lines.

In miRNA microarray analysis of a panel of human pluripotent stem cell lines, the initial expression levels of members of the *miR-371-3* family in individual ESC or iPSC lines was found to be a predictor of neural differentiation capacity [101]. Pluripotent stem cell lines with less initial expression levels of *miR-371-3* differentiated more effectively into neural lineages. Given the growing number of examples of the central role of ncRNAs in regulating pluripotency, future comparisons of ncRNAs from different ESC and iPSC lines will likely uncover additional examples of ncRNA activity that can predict relative differentiation capacity.

Surveying ncRNA expression as a tool for predicting the malignant potential of iPSC

There is also increasing recent recognition that the process of generating iPSC with viral integrating factors introduces malignant genetic and epigenetic alterations [102–104]. It is ultimately essential to minimize any risk of tumorigenesis associated with the use iPSC lines before they can be safely used for human therapies [105]. Neveu et al. profiled 330 miRNAs in 22 hESC and iPSC lines, 21 tumor cell lines and samples, and 6 differentiated cell types. Strikingly, a p53 related miRNA signature divided the studied ESC and iPSC lines into two distinct groups [106]. One group of ESC and iPSC clustered in similarity together with malignant tumor samples, while the remaining ESC and iPSC lines instead clustered closer to normal non-malignant, differentiated cells. Interestingly, these investigators demonstrated that an iPSC line could be converted from a normal to a tumor-like state by virtue of over-expression of *miR-92* and *miR-141*, which produced decreases in p53 transcript levels. Both findings by Neveu et al. hint at the potentially significant influence of miRNAs in defining the relative tumorigenic potential of ESC and iPSC of different originating methods, and the importance of including miRNA analysis alongside more traditional protein-coding RNA surveys in future studies. Systematic survey of lncRNAs is likewise likely to reveal important roles in defining ESC and iPSC tumorigenic potential.

The potential role of ncRNA in regulating epigenetic memory

The phenomenon of retention of epigenetic and transcriptional somatic memory is becoming increasingly relevant to the generation of iPSC from differentiated somatic cells. The differentiation of pluripotent stem cells into more specialized lineages requires establishment of an epigenetic state specific to that specialized lineage. If these lineage-specific epigenetic changes are not fully reversed during the pluripotency reprogramming of differentiated cells, the residual, lineage-specific epigenetic changes may form an epigenetic “memory” of the donor cell in the resultant iPSC [107–110]. Current evidence suggests that this failure to completely erase the epigenetic memory of the somatic donor during induction of pluripotency may limit the differentiation capacity of the resultant human and murine iPSC [109–112]. In addition to the key role of lncRNAs in XCI and imprinting discussed earlier, ncRNAs may also play a role in establishment and retention of such epigenetic memory (reviewed in [113]). It is possible that the failure to completely reprogram protein-coding genes and chromatin states to an ESC-like state may be secondary to a failure to completely reprogram ESC-like expression states of ncRNA master regulators. As future studies of retention of epigenetic memory in iPSC begin to include both ncRNAs along with protein-coding RNAs in their surveys of expression, this hypothesis should be able to be explored more fully.

Pluripotency-associated microRNAs and the cancer stem cell

It is hypothesized that some tumors arise from a sub-population of cancer stem cells (CSC) that possess stem-cell like self-renewal capabilities [114]. Such CSCs are postulated to possess different physiological characteristics than the more developed tumor cells that they give rise to. For example, unlike their differentiated progeny, CSC may not be as sensitive to conventional chemotherapy, and malignant relapse may in part arise from the failure of chemotherapy to target CSC. One hypothesis suggests that molecular mechanisms of unlimited self-renewal that promote or suppress pluripotency in ESC may be correspondingly important in promotion or suppression of CSC and tumorigenic phenotypes [115, 116]. Consistent with this hypothesis, there are examples of ncRNAs with important roles in initiation or suppression of self-renewal and pluripotency in ESCs, that have also been found to have corresponding roles in initiation or suppression of self-renewal and tumorigenesis in CSCs.

Among the better developed roles for pluripotency-associated ncRNAs in cancer are the downregulation in CSCs of such miRNAs that are also known to suppress ESC self-renewal. For example, downregulation of members of the *let-7* family of miRNAs, with their pro-differentiation, anti-pluripotency roles in ESCs, is seen in a wide variety of tumors [117]. A specific role of *let-7* family miRNAs in CSC was identified in breast cancer stem cells that were enriched from human primary breast tumor samples by passaging through multiple rounds of chemotherapy *in vivo* in NOD/SCID mice to select for the relatively chemotherapy-resistant, highly metastatic, and rapidly self-renewing breast CSC fraction [118]. This highly proliferative, poorly differentiated CSC fraction was found to have suppressed levels of members of the *let-7* family miRNAs. In contrast, over-expression of pro-differentiation *let-7* family members in this breast CSC fraction reduced proliferation, increased differentiation, diminished *in vivo* tumor formation, and decreased the metastatic potential by breast CSC in NOD/SCID mice.

Members of the p53-regulated *miR-34* family, which similarly suppress the pluripotent phenotype in ESC, also inhibits CSC self renewal and *in vivo* tumor formation of a pancreatic tumor [119] and glioma cell lines [120]. Consistent with this, loss of activity of anti-pluripotency *miR-34* family members is also seen in a wide variety of other tumors [121]. A recent paper also demonstrated a similar role for p53-regulated *miR-145* in hepatocarcinoma CSC [122]. Finally, *miR-200* family members that suppress *Sox2*, *Klf4*, and *Bmi1* (a PcG-group protein key for maintenance of the stem cell state) were found to suppress tumorigenicity of both breast CSC and pancreatic CSC [123, 124].

Furthermore, miRNA signatures were identified that could distinguish CSC from non-CSC by comparing six different prostate CSC fractions with their corresponding non-CSC counterparts [125]. Downregulation of members of three of the anti-pluripotency miRNA families discussed previously (*let-7*, *miR-200*, and *miR-34*) was seen in at least five of the six CSC fractions, consistent with previously discussed work. However, many of the other miRNAs identified as having distinct differences in expression between multiple lines of prostate CSC vs. non-prostate CSC do not yet have clearly identified roles in regulation of pluripotency in ESC, and make intriguing candidates for further examination.

One aspect of these studies that complicates the relationship between ncRNA function in ESC and CSC is the possibility that cancer stem cells may actually be more closely related to lineage-committed, tissue-specific stem cells than pluripotent stem cells. This may contribute to the explanation of why ncRNAs that promote pluripotency in pluripotent stem cells have apparently contradictory effects in potentially more differentiated CSC that may represent more lineage-committed progenitors. For example, members of the *miR-302/367* family of miRNAs, which play a key role in promoting self-renewal and pluripotency in ESC, were recently found to suppress self-renewal and tumorigenicity of glioma CSC [126]. Likewise, *let-7* and *miR-181* miRNA family members that inhibit the ESC pluripotent state were found have high levels of expression in hepatocellular CSC [127]. An improved understanding of the biology underlying “discrepancies” in ncRNA roles between various CSC and ESC may contribute to a better characterization of where along the developmental continuum the CSC of various tumors exists.

Continuing advances in understanding of the roles of miRNAs in CSCs and tumorigenesis will continue to provide fertile ground for new hypotheses regarding their corresponding function in ESCs, and vice versa. Likewise, the wide range of roles identified for lncRNAs in cancer [128] should provide many further opportunities for cross-fertilization between ESC and CSC biology. Additionally, the possibility of a role for piRNAs in cancer as well as germ cell function has also been recently suggested [50, 129]. The further employment of emerging high throughput genomic technologies that can more comprehensively

characterize ncRNAs will accelerate the elucidation of a more complete transcriptomic signature that sets CSC apart from the rest of the tumor. Such knowledge is expected to advance efforts for developing CSC-specific therapies to overcome chemotherapy resistance, metastasis, and relapse.

Conclusions

A growing literature is identifying more and more short and long ncRNAs as central “regulators of the regulators” of pluripotency. Through an increasing number of identified targets and mechanisms, ncRNAs coordinate the activity of groups of genetic regulators and modulate the expression of a constellation of key ESC genes. Yet the individual examples of ncRNAs as key pivots of pluripotency discovered so far are certainly only the tip of the iceberg, and full dissection of the constellations of targets they regulate lags even further behind.

Recognition of the importance of ncRNAs in regulating pluripotency lagged in part because ncRNAs were less well represented, or absent altogether, from earlier generations of genomic-wide survey technologies that were designed with a focus on protein coding RNAs. Next-generation genomics techniques, with less of an initial selection bias regarding what RNA transcripts are biologically relevant, are rapidly becoming more practical and affordable [130, 131], capturing information about transcripts which might have once been overlooked. Including analysis of these once often-overlooked ncRNA transcripts in future studies of pluripotency, and delineation of the full scope of protein-coding RNAs and genetic regulators they coordinate, will be key to addressing key problems such as how to assess ‘quality’ between various classes of pluripotent cells and determining commonalities between induced pluripotent stem cells and the origin of CSC from normal tissues. As the use of earlier protein-coding RNA-focused microarrays and other less comprehensive genomics methods in the study of pluripotency gives way to routine use of more global transcriptomic survey methods (*e.g.*, comprehensive RNA sequencing (RNA-Seq) methods), the full role of known and yet-to-be-discovered ncRNAs as pivots of pluripotency will no longer be “lost in translation”.

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References

1. Driesch H. Entwicklungsmechanische Studien. I. Der Werth der beiden ersten Furchungs-zellen in der Echinodermentwicklung. II. fiber die Beziehung des Lichtes zur ersten Etage der theirischen Formenbilciung. *Z f Wiss Zool.* 1891; 53:160–183.
2. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature.* 1981; 292:154–156. [PubMed: 7242681]
3. Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci U S A.* 1981; 78:7634–7638. [PubMed: 6950406]
4. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science.* 1998; 282:1145–1147. [PubMed: 9804556]

5. Gurdon JB, Elsdale TR, Fischberg M. Sexually Mature Individuals of *Xenopus laevis* from the Transplantation of Single Somatic Nuclei. *Nature*. 1958; 182:64–65. [PubMed: 13566187]
6. Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KHS. Viable offspring derived from fetal and adult mammalian cells. *Nature*. 1997; 385:810–813. [PubMed: 9039911]
7. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
8. Robinton DA, Daley GQ. The promise of induced pluripotent stem cells in research and therapy. *Nature*. 2012; 481:295–305. [PubMed: 22258608]
9. Kim J, Chu J, Shen X, Wang J, Orkin SH. An extended transcriptional network for pluripotency of embryonic stem cells. *Cell*. 2008; 132:1049–1061. [PubMed: 18358816]
10. Kim J, Woo AJ, Chu J, Snow JW, Fujiwara Y, Kim CG, Cantor AB, Orkin SH. A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs. *Cell*. 2010; 143:313–324. [PubMed: 20946988]
11. Fisher CL, Fisher AG. Chromatin states in pluripotent, differentiated, and reprogrammed cells. *Current Opinion in Genetics & Development*. 2011; 21:140–146. [PubMed: 21316216]
12. Onder TT, Kara N, Cherry A, Sinha AU, Zhu N, Bernt KM, Cahan P, Mancarci OB, Unternaehrer J, Gupta PB, Lander ES, Armstrong SA, Daley GQ. Chromatin-modifying enzymes as modulators of reprogramming. *Nature*. 2012; 483:598–602. [PubMed: 22388813]
13. Carninci P, Kasukawa T, Katayama S, Gough J, Frith MC, Maeda N, Oyama R, Ravasi T, Lenhard B, Wells C, Kodzius R, Shimokawa K, Bajic VB, Brenner SE, Batalov S, Forrest ARR, Zavolan M, Davis MJ, Wilming LG, Aidinis V, Allen JE, Ambesi-Impimbato A, Apweiler R, Aturaliya RN, Bailey TL, Bansal M, Baxter L, Beisel KW, Bersano T, Bono H, Chalk AM, Chiu KP, Choudhary V, Christoffels A, Clutterbuck DR, Crowe ML, Dalla E, Dalrymple BP, de Bono B, Gatta GD, di Bernardo D, Down T, Engstrom P, Fagiolini M, Faulkner G, Fletcher CF, Fukushima T, Furuno M, Futaki S, Gariboldi M, Georgii-Hemming P, Gingeras TR, Gojobori T, Green RE, Gustincich S, Harbers M, Hayashi Y, Hensch TK, Hirokawa N, Hill D, Huminiecki L, Iacono M, Ikeo K, Iwama A, Ishikawa T, Jakt M, Kanapin A, Katoh M, Kawasaki Y, Kelso J, Kitamura H, Kitano H, Kollias G, Krishnan SPT, Kruger A, Kummerfeld SK, Kurochkin IV, Lareau LF, Lazarevic D, Lipovich L, Liu J, Liuni S, McWilliam S, Babu MM, Madera M, Marchionni L, Matsuda H, Matsuzawa S, Miki H, Mignone F, Miyake S, Morris K, Mottagui-Tabar S, Mulder N, Nakano N, Nakauchi H, Ng P, Nilsson R, Nishiguchi S, Nishikawa S, Nori F, Ohara O, Okazaki Y, Orlando V, Pang KC, Pavan WJ, Pavesi G, Pesole G, Petrovsky N, Piazza S, Reed J, Reid JF, Ring BZ, Ringwald M, Rost B, Ruan Y, Salzberg SL, Sandelin A, Schneider C, Schönbach C, Sekiguchi K, Sempere CAM, Seno S, Sessa L, Sheng Y, Shibata Y, Shimada H, Shimada K, Silva D, Sinclair B, Sperling S, Stupka E, Sugiura K, Sultana R, Takenaka Y, Taki K, Tammoja K, Tan SL, Tang S, Taylor MS, Tegner J, Teichmann SA, Ueda HR, van Nimwegen E, Verardo R, Wei CL, Yagi K, Yamanishi H, Zabarovsky E, Zhu S, Zimmer A, Hide W, Bult C, Grimmond SM, Teasdale RD, Liu ET, Brusic V, Quackenbush J, Wahlestedt C, Mattick JS, Hume DA, Group RGER, Genome Science G, Kai C, Sasaki D, Tomaru Y, Fukuda S, Kanamori-Katayama M, Suzuki M, Aoki J, Arakawa T, Iida J, Imamura K, Itoh M, Kato T, Kawaji H, Kawagashira N, Kawashima T, Kojima M, Kondo S, Konno H, Nakano K, Ninomiya N, Nishio T, Okada M, Plessy C, Shibata K, Shiraki T, Suzuki S, Tagami M, Waki K, Watahiki A, Okamura-Oho Y, Suzuki H, Kawai J, Hayashizaki Y. The Transcriptional Landscape of the Mammalian Genome. *Science*. 2005; 309:1559–1563. [PubMed: 16141072]
14. Costa, FcF. Non-coding RNAs: Lost in translation? *Gene*. 2007; 386:1–10. [PubMed: 17113247]
15. Esteller M. Non-coding RNAs in human disease. *Nat Rev Genet*. 2011; 12:861–874. [PubMed: 22094949]
16. Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol*. 2009; 10:126–139. [PubMed: 19165215]
17. Wang Y, Medvid R, Melton C, Jaenisch R, Blueloch R. DGCR8 is essential for microRNA biogenesis and silencing of embryonic stem cell self-renewal. *Nat Genet*. 2007; 39:380–385. [PubMed: 17259983]
18. Kanellopoulou C, Muljo SA, Kung AL, Ganesan S, Drapkin R, Jenuwein T, Livingston DM, Rajewsky K. Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes & Development*. 2005; 19:489–501. [PubMed: 15713842]

19. Houbaviiy HB, Murray MF, Sharp PA. Embryonic stem cell-specific MicroRNAs. *Dev Cell*. 2003; 5:351–358. [PubMed: 12919684]
20. Marson A, Levine SS, Cole MF, Frampton GM, Brambrink T, Johnstone S, Guenther MG, Johnston WK, Wernig M, Newman J, Calabrese JM, Dennis LM, Volkert TL, Gupta S, Love J, Hannett N, Sharp PA, Bartel DP, Jaenisch R, Young RA. Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells. *Cell*. 2008; 134:521–533. [PubMed: 18692474]
21. Card DA, Hebbar PB, Li L, Trotter KW, Komatsu Y, Mishina Y, Archer TK. Oct4/Sox2-regulated miR-302 targets cyclin D1 in human embryonic stem cells. *Mol Cell Biol*. 2008; 28:6426–6438. [PubMed: 18710938]
22. Wang Y, Baskerville S, Shenoy A, Babiarz JE, Baehner L, Bluelloch R. Embryonic stem cell-specific microRNAs regulate the G1-S transition and promote rapid proliferation. *Nat Genet*. 2008; 40:1478–1483. [PubMed: 18978791]
23. Sinkkonen L, Hugenschmidt T, Berninger P, Gaidatzis D, Mohn F, Artus-Revel CG, Zavolan M, Svoboda P, Filipowicz W. MicroRNAs control de novo DNA methylation through regulation of transcriptional repressors in mouse embryonic stem cells. *Nat Struct Mol Biol*. 2008; 15:259–267. [PubMed: 18311153]
24. Zheng GXY, Ravi A, Calabrese JM, Medeiros LA, Kirak O, Dennis LM, Jaenisch R, Burge CB, Sharp PA. A Latent Pro-Survival Function for the Mir-290–295 Cluster in Mouse Embryonic Stem Cells. *PLoS Genet*. 2011; 7:11.
25. Lichner Z, Páll Ek, Kerekes A, Pállinger Eva, Maraghechi P, Bösze Z, Göcza E. The miR-290–295 cluster promotes pluripotency maintenance by regulating cell cycle phase distribution in mouse embryonic stem cells. *Differentiation*. 2011; 81:11–24. [PubMed: 20864249]
26. Melton C, Judson RL, Bluelloch R. Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. *Nature*. 2010; 463:621–626. [PubMed: 20054295]
27. Piskounova E, Polytarchou C, Thornton JE, LaPierre RJ, Pothoulakis C, Hagan JP, Iliopoulos D, Gregory RI. Lin28A and Lin28B inhibit let-7 microRNA biogenesis by distinct mechanisms. *Cell*. 2011; 147:1066–1079. [PubMed: 22118463]
28. Tay Y, Zhang J, Thomson AM, Lim B, Rigoutsos I. MicroRNAs to Nanog, Oct4 and Sox2 coding regions modulate embryonic stem cell differentiation. *Nature*. 2008; 455:1124–1128. [PubMed: 18806776]
29. Xu N, Papagiannakopoulos T, Pan G, Thomson JA, Kosik KS. MicroRNA-145 Regulates OCT4, SOX2, and KLF4 and Represses Pluripotency in Human Embryonic Stem Cells. *Cell*. 2009; 137:647–658. [PubMed: 19409607]
30. Jain AK, Allton K, Iacovino M, Mahen E, Milczarek RJ, Zwaka TP, Kyba M, Barton MC. p53 Regulates Cell Cycle and MicroRNAs to Promote Differentiation of Human Embryonic Stem Cells. *PLoS Biol*. 2012; 10:e1001268. [PubMed: 22389628]
31. Richly H, Aloia L, Di Croce L. Roles of the Polycomb group proteins in stem cells and cancer. *Cell Death Dis*. 2011; 2:e204. [PubMed: 21881606]
32. Sauvageau M, Sauvageau G. Polycomb group proteins: multi-faceted regulators of somatic stem cells and cancer. *Cell Stem Cell*. 2010; 7:299–313. [PubMed: 20804967]
33. Sparmann A, Van Lohuizen M. Polycomb silencers control cell fate, development and cancer. *Nature Reviews Cancer*. 2006; 6:846–856.
34. Morey L, Helin K. Polycomb group protein-mediated repression of transcription. *Trends in Biochemical Sciences*. 2010; 35:323–332. [PubMed: 20346678]
35. O’Loughlen A, Munoz-Cabello AM, Gaspar-Maia A, Wu HA, Banito A, Kunowska N, Racek T, Pemberton HN, Beolchi P, Lavial F, Masui O, Vermeulen M, Carroll T, Graumann J, Heard E, Dillon N, Azuara V, Snijders AP, Peters G, Bernstein E, Gil J. MicroRNA regulation of Cbx7 mediates a switch of Polycomb orthologs during ESC differentiation. *Cell Stem Cell*. 2012; 10:33–46. [PubMed: 22226354]
36. Rybak A, Fuchs H, Smirnova L, Brandt C, Pohl EE, Nitsch R, Wulczyn FG. A feedback loop comprising lin-28 and let-7 controls pre-let-7 maturation during neural stem-cell commitment. *Nat Cell Biol*. 2008; 10:987–993. [PubMed: 18604195]

37. Boissart C, Nissan X, Giraud-Triboulet K, Peschanski M, Benchoua A. miR-125 potentiates early neural specification of human embryonic stem cells. *Development*. 2012; 139:1247–1257. [PubMed: 22357933]
38. Chen C-Z, Li L, Lodish HF, Bartel DP. MicroRNAs Modulate Hematopoietic Lineage Differentiation. *Science*. 2004; 303:83–86. [PubMed: 14657504]
39. Li X, Zhang J, Gao L, McClellan S, Finan MA, Butler TW, Owen LB, Piazza GA, Xi Y. MiR-181 mediates cell differentiation by interrupting the Lin28 and let-7 feedback circuit. *Cell Death Differ*. 2012; 19:378–386. [PubMed: 21979467]
40. Li Z, Yang CS, Nakashima K, Rana TM. Small RNA-mediated regulation of iPS cell generation. *EMBO J*. 2011; 30:823–834. [PubMed: 21285944]
41. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007; 318:1917–1920. [PubMed: 18029452]
42. Viswanathan SR, Daley GQ, Gregory RI. Selective blockade of microRNA processing by Lin28. *Science*. 2008; 320:97–100. [PubMed: 18292307]
43. Judson RL, Babiarz JE, Venere M, Blueloch R. Embryonic stem cell-specific microRNAs promote induced pluripotency. *Nat Biotechnol*. 2009; 27:459–461. [PubMed: 19363475]
44. Choi YJ, Lin CP, Ho JJ, He X, Okada N, Bu P, Zhong Y, Kim SY, Bennett MJ, Chen C, Ozturk A, Hicks GG, Hannon GJ, He L. miR-34 miRNAs provide a barrier for somatic cell reprogramming. *Nat Cell Biol*. 2011; 13:1353–1360. [PubMed: 22020437]
45. Huangfu D, Maehr R, Guo W, Eijkelenboom A, Snitow M, Chen AE, Melton DA. Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat Biotechnol*. 2008; 26:795–797. [PubMed: 18568017]
46. Anokye-Danso F, Trivedi CM, Juhr D, Gupta M, Cui Z, Tian Y, Zhang Y, Yang W, Gruber PJ, Epstein JA, Morrisey EE. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. *Cell Stem Cell*. 2011; 8:376–388. [PubMed: 21474102]
47. Miyoshi N, Ishii H, Nagano H, Haraguchi N, Dewi DL, Kano Y, Nishikawa S, Tanemura M, Mimori K, Tanaka F, Saito T, Nishimura J, Takemasa I, Mizushima T, Ikeda M, Yamamoto H, Sekimoto M, Doki Y, Mori M. Reprogramming of mouse and human cells to pluripotency using mature microRNAs. *Cell Stem Cell*. 2011; 8:633–638. [PubMed: 21620789]
48. Babiarz JE, Ruby JG, Wang Y, Bartel DP, Blueloch R. Mouse ES cells express endogenous shRNAs, siRNAs, and other Microprocessor-independent, Dicer-dependent small RNAs. *Genes & Development*. 2008; 22:2773–2785. [PubMed: 18923076]
49. Hayashi K, Surani MA. Resetting the Epigenome beyond Pluripotency in the Germline. *Cell Stem Cell*. 2009; 4:493–498. [PubMed: 19497276]
50. Juliano C, Wang J, Lin H. Uniting germline and stem cells: the function of Piwi proteins and the piRNA pathway in diverse organisms. *Annu Rev Genet*. 2011; 45:447–469. [PubMed: 21942366]
51. Nichols J, Smith A. Naive and Primed Pluripotent States. *Cell Stem Cell*. 2009; 4:487–492. [PubMed: 19497275]
52. Sharma AK, Nelson MC, Brandt JE, Wessman M, Mahmud N, Weller KP, Hoffman R. Human CD34(+) stem cells express the hiwi gene, a human homologue of the Drosophila gene piwi. *Blood*. 2001; 97:426–434. [PubMed: 11154219]
53. Brons IG, Smithers LE, Trotter MW, Rugg-Gunn P, Sun B, Chuva de Sousa Lopes SM, Howlett SK, Clarkson A, Ahrlund-Richter L, Pedersen RA, Vallier L. Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature*. 2007; 448:191–195. [PubMed: 17597762]
54. Tesar PJ, Chenoweth JG, Brook FA, Davies TJ, Evans EP, Mack DL, Gardner RL, McKay RD. New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature*. 2007; 448:196–199. [PubMed: 17597760]
55. Buecker C, Geijsen N. Different flavors of pluripotency, molecular mechanisms, and practical implications. *Cell Stem Cell*. 2010; 7:559–564. [PubMed: 21040897]
56. Jouneau A, Ciaudo C, Sismeiro O, Brochard V, Jouneau L, Vandormael-Pournin S, Coppee JY, Zhou Q, Heard E, Antoniewski C, Cohen-Tannoudji M. Naive and primed murine pluripotent stem cells have distinct miRNA expression profiles. *RNA*. 2012; 18:253–264. [PubMed: 22201644]

57. Ying QL, Wray J, Nichols J, Battle-Morera L, Doble B, Woodgett J, Cohen P, Smith A. The ground state of embryonic stem cell self-renewal. *Nature*. 2008; 453:519–523. [PubMed: 18497825]
58. Silva J, Nichols J, Theunissen TW, Guo G, van Oosten AL, Barrandon O, Wray J, Yamanaka S, Chambers I, Smith A. Nanog Is the Gateway to the Pluripotent Ground State. *Cell*. 2009; 138:722–737. [PubMed: 19703398]
59. Hanna J, Cheng AW, Saha K, Kim J, Lengner CJ, Soldner F, Cassady JP, Muffat J, Carey BW, Jaenisch R. Human embryonic stem cells with biological and epigenetic characteristics similar to those of mouse ESCs. *Proc Natl Acad Sci U S A*. 2010; 107:9222–9227. [PubMed: 20442331]
60. Okazaki Y, Furuno M, Kasukawa T, Adachi J, Bono H, Kondo S, Nikaido I, Osato N, Saito R, Suzuki H, Yamanaka I, Kiyosawa H, Yagi K, Tomaru Y, Hasegawa Y, Nogami A, Schonbach C, Gojobori T, Baldarelli R, Hill DP, Bult C, Hume DA, Quackenbush J, Schriml LM, Kanapin A, Matsuda H, Batalov S, Beisel KW, Blake JA, Bradt D, Brusica V, Chothia C, Corbani LE, Cousins S, Dalla E, Dragani TA, Fletcher CF, Forrest A, Frazer KS, Gaasterland T, Gariboldi M, Gissi C, Godzik A, Gough J, Grimmond S, Gustincich S, Hirokawa N, Jackson JJ, Jarvis ED, Kanai A, Kawaji H, Kawasawa Y, Kedzierski RM, King BL, Konagaya A, Kurochkin IV, Lee Y, Lenhard B, Lyons PA, Maglott DR, Maltais L, Marchionni L, McKenzie L, Miki H, Nagashima T, Numata K, Okido T, Pavan WJ, Pertea G, Pesole G, Petrovsky N, Pillai R, Pontius JU, Qi D, Ramachandran S, Ravasi T, Reed JC, Reed DJ, Reid J, Ring BZ, Ringwald M, Sandelin A, Schneider C, Semple CA, Setou M, Shimada K, Sultana R, Takenaka Y, Taylor MS, Teasdale RD, Tomita M, Verardo R, Wagner L, Wahlestedt C, Wang Y, Watanabe Y, Wells C, Wilming LG, Wynshaw-Boris A, Yanagisawa M, Yang I, Yang L, Yuan Z, Zavolan M, Zhu Y, Zimmer A, Carninci P, Hayatsu N, Hirozane-Kishikawa T, Konno H, Nakamura M, Sakazume N, Sato K, Shiraki T, Waki K, Kawai J, Aizawa K, Arakawa T, Fukuda S, Hara A, Hashizume W, Imotani K, Ishii Y, Itoh M, Kagawa I, Miyazaki A, Sakai K, Sasaki D, Shibata K, Shinagawa A, Yasunishi A, Yoshino M, Waterston R, Lander ES, Rogers J, Birney E, Hayashizaki Y. Analysis of the mouse transcriptome based on functional annotation of 60,770 full-length cDNAs. *Nature*. 2002; 420:563–573. [PubMed: 12466851]
61. Jia H, Osak M, Bogu GK, Stanton LW, Johnson R, Lipovich L. Genome-wide computational identification and manual annotation of human long noncoding RNA genes. *RNA*. 2010; 16:1478–1487. [PubMed: 20587619]
62. Kampa D, Cheng J, Kapranov P, Yamanaka M, Brubaker S, Cawley S, Drenkow J, Piccolboni A, Bekiranov S, Helt G, Tammana H, Gingeras TR. Novel RNAs Identified From an In-Depth Analysis of the Transcriptome of Human Chromosomes 21 and 22. *Genome Research*. 2004; 14:331–342. [PubMed: 14993201]
63. Katayama S, Tomaru Y, Kasukawa T, Waki K, Nakanishi M, Nakamura M, Nishida H, Yap CC, Suzuki M, Kawai J, Suzuki H, Carninci P, Hayashizaki Y, Wells C, Frith M, Ravasi T, Pang KC, Hallinan J, Mattick J, Hume DA, Lipovich L, Batalov S, Engstrom PG, Mizuno Y, Faghihi MA, Sandelin A, Chalk AM, Mottagui-Tabar S, Liang Z, Lenhard B, Wahlestedt C. Antisense transcription in the mammalian transcriptome. *Science*. 2005; 309:1564–1566. [PubMed: 16141073]
64. He Y, Vogelstein B, Velculescu VE, Papadopoulos N, Kinzler KW. The Antisense Transcriptomes of Human Cells. *Science*. 2008; 322:1855–1857. [PubMed: 19056939]
65. Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassady JP, Cabili MN, Jaenisch R, Mikkelsen TS, Jacks T, Hacohen N, Bernstein BE, Kellis M, Regev A, Rinn JL, Lander ES. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. *Nature*. 2009; 458:223–227. [PubMed: 19182780]
66. Khalil AM, Guttman M, Huarte M, Garber M, Raj A, Rivea Morales D, Thomas K, Presser A, Bernstein BE, van Oudenaarden A, Regev A, Lander ES, Rinn JL. Many human large intergenic noncoding RNAs associate with chromatin-modifying complexes and affect gene expression. *Proc Natl Acad Sci U S A*. 2009; 106:11667–11672. [PubMed: 19571010]
67. Ponting CP, Oliver PL, Reik W. Evolution and Functions of Long Noncoding RNAs. *Cell*. 2009; 136:629–641. [PubMed: 19239885]

68. Lipovich L, Johnson R, Lin CY. MacroRNA underdogs in a microRNA world: evolutionary, regulatory, and biomedical significance of mammalian long non-protein-coding RNA. *Biochim Biophys Acta*. 2010; 1799:597–615. [PubMed: 20951849]
69. Wang X, Song X, Glass CK, Rosenfeld MG. The long arm of long noncoding RNAs: roles as sensors regulating gene transcriptional programs. *Cold Spring Harb Perspect Biol*. 2011; 3:a003756. [PubMed: 20573714]
70. Gong C, Maquat LE. lncRNAs transactivate STAU1-mediated mRNA decay by duplexing with 3 [prime] UTRs via Alu elements. *Nature*. 2011; 470:284–288. [PubMed: 21307942]
71. Bond AM, Vangompel MJ, Sametsky EA, Clark MF, Savage JC, Disterhoft JF, Kohtz JD. Balanced gene regulation by an embryonic brain ncRNA is critical for adult hippocampal GABA circuitry. *Nat Neurosci*. 2009; 12:1020–1027. [PubMed: 19620975]
72. Tsai M-C, Manor O, Wan Y, Mosammaparast N, Wang JK, Lan F, Shi Y, Segal E, Chang HY. Long Noncoding RNA as Modular Scaffold of Histone Modification Complexes. *Science*. 2010; 329:689–693. [PubMed: 20616235]
73. Yap KL, Li S, Munoz-Cabello AM, Raguz S, Zeng L, Mujtaba S, Gil J, Walsh MJ, Zhou MM. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. *Mol Cell*. 2010; 38:662–674. [PubMed: 20541999]
74. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell*. 2011; 146:353–358. [PubMed: 21802130]
75. Dinger ME, Amaral PP, Mercer TR, Pang KC, Bruce SJ, Gardiner BB, Askarian-Amiri ME, Ru K, Solda G, Simons C, Sunkin SM, Crowe ML, Grimmond SM, Perkins AC, Mattick JS. Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. *Genome Res*. 2008; 18:1433–1445. [PubMed: 18562676]
76. Wu SC, Kallin EM, Zhang Y. Role of H3K27 methylation in the regulation of lncRNA expression. *Cell Res*. 2010; 20:1109–1116. [PubMed: 20680032]
77. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell*. 2006; 125:315–326. [PubMed: 16630819]
78. Sheik Mohamed J, Gaughwin PM, Lim B, Robson P, Lipovich L. Conserved long noncoding RNAs transcriptionally regulated by Oct4 and Nanog modulate pluripotency in mouse embryonic stem cells. *RNA*. 2010; 16:324–337. [PubMed: 20026622]
79. Johnson R, Teh CH, Jia H, Vanisri RR, Pandey T, Lu ZH, Buckley NJ, Stanton LW, Lipovich L. Regulation of neural macroRNAs by the transcriptional repressor REST. *RNA*. 2009; 15:85–96. [PubMed: 19050060]
80. Ng S-Y, Johnson R, Stanton LW. Human long non-coding RNAs promote pluripotency and neuronal differentiation by association with chromatin modifiers and transcription factors. *EMBO J*. 2012; 31:522–533. [PubMed: 22193719]
81. Guttman M, Donaghey J, Carey BW, Garber M, Grenier JK, Munson G, Young G, Lucas AB, Ach R, Bruhn L, Yang X, Amit I, Meissner A, Regev A, Rinn JL, Root DE, Lander ES. lincRNAs act in the circuitry controlling pluripotency and differentiation. *Nature*. 2011; 477:295–300. [PubMed: 21874018]
82. Loewer S, Cabili MN, Guttman M, Loh YH, Thomas K, Park IH, Garber M, Curran M, Onder T, Agarwal S, Manos PD, Datta S, Lander ES, Schlaeger TM, Daley GQ, Rinn JL. Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells. *Nat Genet*. 2010; 42:1113–1117. [PubMed: 21057500]
83. Kretz M, Webster DE, Flockhart RJ, Lee CS, Zehnder A, Lopez-Pajares V, Qu K, Zheng GX, Chow J, Kim GE, Rinn JL, Chang HY, Siprashvili Z, Khavari PA. Suppression of progenitor differentiation requires the long noncoding RNA ANCR. *Genes Dev*. 2012; 26:338–343. [PubMed: 22302877]
84. Hu W, Yuan B, Flygare J, Lodish HF. Long noncoding RNA-mediated anti-apoptotic activity in murine erythroid terminal differentiation. *Genes Dev*. 2011; 25:2573–2578. [PubMed: 22155924]

85. Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, Chinappi M, Tramontano A, Bozzoni I. A Long Noncoding RNA Controls Muscle Differentiation by Functioning as a Competing Endogenous RNA. *Cell*. 2011; 147:358–369. [PubMed: 22000014]
86. Morey L, Pascual G, Cozzuto L, Roma G, Wutz A, Benitah SA, Di Croce L. Nonoverlapping functions of the Polycomb group Cbx family of proteins in embryonic stem cells. *Cell Stem Cell*. 2012; 10:47–62. [PubMed: 22226355]
87. Lee JT. Lessons from X-chromosome inactivation: long ncRNA as guides and tethers to the epigenome. *Genes Dev*. 2009; 23:1831–1842. [PubMed: 19684108]
88. Bermejo-Alvarez P, Ramos-Ibeas P, Gutierrez-Adan A. Solving the “X” in embryos and stem cells. *Stem Cells Dev*. 2012; 21:1215–1224. [PubMed: 22309156]
89. Silva SS, Rowntree RK, Mekhoubad S, Lee JT. X-chromosome inactivation and epigenetic fluidity in human embryonic stem cells. *Proc Natl Acad Sci U S A*. 2008; 105:4820–4825. [PubMed: 18339803]
90. Tchieu J, Kuoy E, Chin MH, Trinh H, Patterson M, Sherman SP, Aimiwu O, Lindgren A, Hakimian S, Zack JA, Clark AT, Pyle AD, Lowry WE, Plath K. Female human iPSCs retain an inactive X chromosome. *Cell Stem Cell*. 2010; 7:329–342. [PubMed: 20727844]
91. Nazor KL, Altun G, Lynch C, Tran H, Harness JV, Slavin I, Garitaonandia I, Muller FJ, Wang YC, Boscolo FS, Fakunle E, Dumevska B, Lee S, Park HS, Olee T, D’Lima DD, Semechkin R, Parast MM, Galat V, Laslett AL, Schmidt U, Keirstead HS, Loring JF, Laurent LC. Recurrent variations in DNA methylation in human pluripotent stem cells and their differentiated derivatives. *Cell Stem Cell*. 2012; 10:620–634. [PubMed: 22560082]
92. Mekhoubad S, Bock C, de Boer AS, Kiskinis E, Meissner A, Eggan K. Erosion of dosage compensation impacts human iPSC disease modeling. *Cell Stem Cell*. 2012; 10:595–609. [PubMed: 22560080]
93. Anguera MC, Sadreyev R, Zhang Z, Szanto A, Payer B, Sheridan SD, Kwok S, Haggarty SJ, Sur M, Alvarez J, Gimelbrant A, Mitalipova M, Kirby JE, Lee JT. Molecular signatures of human induced pluripotent stem cells highlight sex differences and cancer genes. *Cell Stem Cell*. 2012; 11:75–90. [PubMed: 22770242]
94. Tomoda K, Takahashi K, Leung K, Okada A, Narita M, Yamada NA, Eilertson KE, Tsang P, Baba S, White MP, Sami S, Srivastava D, Conklin BR, Panning B, Yamanaka S. Derivation conditions impact x-inactivation status in female human induced pluripotent stem cells. *Cell Stem Cell*. 2012; 11:91–99. [PubMed: 22770243]
95. Kim DH, Jeon Y, Anguera MC, Lee JT. X-chromosome epigenetic reprogramming in pluripotent stem cells via noncoding genes. *Semin Cell Dev Biol*. 2011; 22:336–342. [PubMed: 21376830]
96. Minkovsky A, Patel S, Plath K. Concise review: Pluripotency and the transcriptional inactivation of the female Mammalian X chromosome. *Stem Cells*. 2012; 30:48–54. [PubMed: 21997775]
97. Wutz A. Epigenetic Alterations in Human Pluripotent Stem Cells: A Tale of Two Cultures. *Cell Stem Cell*. 2012; 11:9–15. [PubMed: 22770238]
98. Osafune K, Caron L, Borowiak M, Martinez RJ, Fitz-Gerald CS, Sato Y, Cowan CA, Chien KR, Melton DA. Marked differences in differentiation propensity among human embryonic stem cell lines. *Nat Biotechnol*. 2008; 26:313–315. [PubMed: 18278034]
99. Chang KH, Nelson AM, Fields PA, Hesson JL, Ulyanova T, Cao H, Nakamoto B, Ware CB, Papayannopoulou T. Diverse hematopoietic potentials of five human embryonic stem cell lines. *Exp Cell Res*. 2008; 314:2930–2940. [PubMed: 18692044]
100. Bock C, Kiskinis E, Verstappen G, Gu H, Boulting G, Smith ZD, Ziller M, Croft GF, Amoroso MW, Oakley DH, Gnirke A, Eggan K, Meissner A. Reference Maps of human ES and iPSC cell variation enable high-throughput characterization of pluripotent cell lines. *Cell*. 2011; 144:439–452. [PubMed: 21295703]
101. Kim H, Lee G, Ganat Y, Papapetrou EP, Lipchina I, Socci ND, Sadelain M, Studer L. miR-371–3 expression predicts neural differentiation propensity in human pluripotent stem cells. *Cell Stem Cell*. 2011; 8:695–706. [PubMed: 21624813]
102. Ghosh Z, Huang M, Hu S, Wilson KD, Dey D, Wu JC. Dissecting the Oncogenic and Tumorigenic Potential of Differentiated Human Induced Pluripotent Stem Cells and Human Embryonic Stem Cells. *Cancer Research*. 2011; 71:5030–5039. [PubMed: 21646469]

103. Doi A, Park IH, Wen B, Murakami P, Aryee MJ, Irizarry R, Herb B, Ladd-Acosta C, Rho J, Loewer S, Miller J, Schlaeger T, Daley GQ, Feinberg AP. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat Genet.* 2009; 41:1350–1353. [PubMed: 19881528]
104. Ohm JE, Mali P, Van Neste L, Berman DM, Liang L, Pandiyan K, Briggs KJ, Zhang W, Argani P, Simons B, Yu W, Matsui W, Van Criekinge W, Rassool FV, Zambidis E, Schuebel KE, Cope L, Yen J, Mohammad HP, Cheng L, Baylin SB. Cancer-related epigenome changes associated with reprogramming to induced pluripotent stem cells. *Cancer Res.* 2010; 70:7662–7673. [PubMed: 20841480]
105. Ben-David U, Benvenisty N. The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat Rev Cancer.* 2011; 11:268–277. [PubMed: 21390058]
106. Neveu P, Kye MJ, Qi S, Buchholz DE, Clegg DO, Sahin M, Park IH, Kim KS, Daley GQ, Kornblum HI, Shraiman BI, Kosik KS. MicroRNA profiling reveals two distinct p53-related human pluripotent stem cell states. *Cell Stem Cell.* 2010; 7:671–681. [PubMed: 21112562]
107. Ng RK, Gurdon JB. Epigenetic memory of active gene transcription is inherited through somatic cell nuclear transfer. *Proc Natl Acad Sci U S A.* 2005; 102:1957–1962. [PubMed: 15684086]
108. Ng RK, Gurdon JB. Epigenetic memory of an active gene state depends on histone H3.3 incorporation into chromatin in the absence of transcription. *Nat Cell Biol.* 2008; 10:102–109. [PubMed: 18066050]
109. Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P, Kim J, Aryee MJ, Ji H, Ehrlich LI, Yabuuchi A, Takeuchi A, Cunniff KC, Hongguang H, McKinney-Freeman S, Naveiras O, Yoon TJ, Irizarry RA, Jung N, Seita J, Hanna J, Murakami P, Jaenisch R, Weissleder R, Orkin SH, Weissman IL, Feinberg AP, Daley GQ. Epigenetic memory in induced pluripotent stem cells. *Nature.* 2010; 467:285–290. [PubMed: 20644535]
110. Kim K, Zhao R, Doi A, Ng K, Unternaehrer J, Cahan P, Hongguang H, Loh Y-H, Aryee MJ, Lensch MW, Li H, Collins JJ, Feinberg AP, Daley GQ. Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. *Nat Biotech.* 2011; 29:1117–1119.
111. Ohi Y, Qin H, Hong C, Blouin L, Polo JM, Guo T, Qi Z, Downey SL, Manos PD, Rossi DJ, Yu J, Hebrok M, Hochedlinger K, Costello JF, Song JS, Ramalho-Santos M. Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. *Nat Cell Biol.* 2011; 13:541–549. [PubMed: 21499256]
112. Polo JM, Liu S, Figueroa ME, Kulalert W, Eminli S, Tan KY, Apostolou E, Stadtfeld M, Li Y, Shioda T, Natesan S, Wagers AJ, Melnick A, Evans T, Hochedlinger K. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat Biotechnol.* 2010; 28:848–855. [PubMed: 20644536]
113. Malecova B, Morris KV. Transcriptional gene silencing through epigenetic changes mediated by non-coding RNAs. *Curr Opin Mol Ther.* 2010; 12:214–222. [PubMed: 20373265]
114. Clevers H. The cancer stem cell: premises, promises and challenges. *Nat Med.* 2011; 17:313–319. [PubMed: 21386835]
115. Ben-Porath I, Thomson MW, Carey VJ, Ge R, Bell GW, Regev A, Weinberg RA. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. *Nat Genet.* 2008; 40:499–507. [PubMed: 18443585]
116. Kim J, Orkin S. Embryonic stem cell-specific signatures in cancer: insights into genomic regulatory networks and implications for medicine. *Genome Medicine.* 2011; 3:75. [PubMed: 22126538]
117. Boyerinas B, Park SM, Hau A, Murmann AE, Peter ME. The role of let-7 in cell differentiation and cancer. *Endocr Relat Cancer.* 2010; 17:F19–36. [PubMed: 19779035]
118. Yu F, Yao H, Zhu P, Zhang X, Pan Q, Gong C, Huang Y, Hu X, Su F, Lieberman J, Song E. let-7 regulates self renewal and tumorigenicity of breast cancer cells. *Cell.* 2007; 131:1109–1123. [PubMed: 18083101]
119. Ji Q, Hao X, Zhang M, Tang W, Yang M, Li L, Xiang D, Desano JT, Bommer GT, Fan D, Fearon ER, Lawrence TS, Xu L. MicroRNA miR-34 inhibits human pancreatic cancer tumor-initiating cells. *PLoS One.* 2009; 4:e6816. [PubMed: 19714243]

120. Li Y, Guessous F, Zhang Y, Dipierro C, Kefas B, Johnson E, Marcinkiewicz L, Jiang J, Yang Y, Schmittgen TD, Lopes B, Schiff D, Purow B, Abounader R. MicroRNA-34a inhibits glioblastoma growth by targeting multiple oncogenes. *Cancer Res.* 2009; 69:7569–7576. [PubMed: 19773441]
121. Vogt M, Munding J, Gruner M, Liffers ST, Verdoodt B, Hauk J, Steinstraesser L, Tannapfel A, Hermeking H. Frequent concomitant inactivation of miR-34a and miR-34b/c by CpG methylation in colorectal, pancreatic, mammary, ovarian, urothelial, and renal cell carcinomas and soft tissue sarcomas. *Virchows Arch.* 2011; 458:313–322. [PubMed: 21225432]
122. Jia Y, Liu H, Zhuang Q, Xu S, Yang Z, Li J, Lou J, Zhang W. Tumorigenicity of cancer stem-like cells derived from hepatocarcinoma is regulated by microRNA-145. *Oncol Rep.* 2012; 27:1865–1872. [PubMed: 22378186]
123. Shimono Y, Zabala M, Cho RW, Lobo N, Dalerba P, Qian D, Diehn M, Liu H, Panula SP, Chiao E, Dirbas FM, Somlo G, Pera RAR, Lao K, Clarke MF. Downregulation of miRNA-200c Links Breast Cancer Stem Cells with Normal Stem Cells. *Cell.* 2009; 138:592–603. [PubMed: 19665978]
124. Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, Waldvogel B, Vannier C, Darling D, Hausen Az, Brunton VG, Morton J, Sansom O, Schuler J, Stemmler MP, Herzberger C, Hopt U, Keck T, Brabletz S, Brabletz T. The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. *Nat Cell Biol.* 2009; 11:1487–1495. [PubMed: 19935649]
125. Liu C, Kelnar K, Vlassov AV, Brown D, Wang J, Tang DG. Distinct microRNA Expression Profiles in Prostate Cancer Stem/Progenitor Cells and Tumor-Suppressive Functions of let-7. *Cancer Res.* 2012; 72:3393–3404. [PubMed: 22719071]
126. Fareh M, Turchi L, Virolle V, Debruyne D, Almairac F, de-la-Forest Divonne S, Paquis P, Preynat-Seauve O, Krause KH, Chneiweiss H, Virolle T. The miR 302–367 cluster drastically affects self-renewal and infiltration properties of glioma-initiating cells through CXCR4 repression and consequent disruption of the SHH-GLI-NANOG network. *Cell Death Differ.* 2012; 19:232–244. [PubMed: 21720384]
127. Meng F, Glaser SS, Francis H, DeMorrow S, Han Y, Passarini JD, Stokes A, Cleary JP, Liu X, Venter J, Kumar P, Priester S, Hubble L, Staloch D, Sharma J, Liu CG, Alpini G. Functional analysis of microRNAs in human hepatocellular cancer stem cells. *J Cell Mol Med.* 2012; 16:160–173. [PubMed: 21352471]
128. Gutschner T, Diederichs S. The Hallmarks of Cancer: A long non-coding RNA point of view. *RNA Biology.* 2012; 9:703–719. [PubMed: 22664915]
129. Siddiqi S, Matushansky I. Piwis and piwi-interacting RNAs in the epigenetics of cancer. *J Cell Biochem.* 2012; 113:373–380. [PubMed: 21928326]
130. Ozsolak F, Milos PM. RNA sequencing: advances, challenges and opportunities. *Nat Rev Genet.* 2011; 12:87–98. [PubMed: 21191423]
131. Hawkins RD, Hon GC, Ren B. Next-generation genomics: an integrative approach. *Nat Rev Genet.* 2010; 11:476–486. [PubMed: 20531367]

Highlights

- Regulatory non-coding RNAs play important roles in embryonic stem cell processes
- Opposing pro- and anti-differentiation miRNAs regulate pluripotency
- miRNAs can catalyze or enhance the generation of induced pluripotent stem cells
- Long noncoding RNAs are increasingly recognized as regulators of pluripotency
- ncRNAs may define stem cell quality and may also regulate cancer stem cells

Figure 1.

Summary of known microRNAs that regulate Core pluripotency factors and the pluripotent state. Solid lines denote known direct regulation, and dashed lines indicate indirect or incompletely characterized mechanisms of regulation.

Figure 2.

Summary of mechanisms of ncRNA regulation of gene expression identified in ESCs. (a) MiRNAs (green) can post-transcriptionally inhibit constellations of target mRNAs directly, (b) target mRNA for transcription factors (like *Sox2*, *Oct4*, or *Klf4*), or (c) target mRNA for epigenetic regulators (like *Cbx7*). (d) MiRNAs can also inhibit other miRNAs through Lin28 lncRNAs, (e) catalyze the assembly of specific genetic regulators into complexes for establishment or erasure of epigenetic states, or (f) inhibit the activity of other miRNAs by mechanisms such as sequestration of target miRNA (*linc-MD1*), targeting of mRNA degradation, or enhancement of transcription (not shown). Additional mechanisms of genetic regulation by ncRNAs observed in other cell lines may ultimately also be identified in stem cells.