Cell Stem Cell
Review

Lin28: Primal Regulator of Growth and Metabolism in Stem Cells

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In recent years, the highly conserved Lin28 RNA-binding proteins have emerged as factors that define stemness in several tissue lineages. Lin28 proteins repress *let-7* microRNAs and influence mRNA translation, thereby regulating the self-renewal of mammalian embryonic stem cells. Subsequent discoveries revealed that Lin28a and Lin28b are also important in organismal growth and metabolism, tissue development, somatic reprogramming, and cancer. In this review, we discuss the Lin28 pathway and its regulation, outline its roles in stem cells, tissue development, and pathogenesis, and examine the ramifications for re-engineering mammalian physiology.

A central question in stem cell biology is whether common factors exist to define "stemness" in multiple tissue lineages. Arguably, one such candidate is the RNA-binding protein Lin28, which was first identified in the nematode *C. elegans* through screens for lineage-modifying genes that alter developmental timing or heterochrony (*lin-28*) (Ambros and Horvitz, 1984). Two other prominent heterochronic genes, *lin-4* and *let-7*, were the first microRNAs (miRNAs) to be discovered, and both miRNAs directly repress *lin-28* to suppress heterochronic reiterations of cell lineages. Heterochronic "reiteration" of nematode stem cells, as *C. elegans* geneticists first demonstrated, was strongly reminiscent of mammalian stem cell self-renewal (Chalfie et al., 1981; Ambros and Horvitz, 1984). This connection was reinforced by the discovery that mouse embryonic stem cells (ESCs) express high levels of mammalian Lin28, which decrease upon differentiation (Moss and Tang, 2003). Successful reprogramming of human fibroblasts into induced pluripotent stem cells (iPSCs) with the use of Lin28, along with Oct4, Sox2, and Nanog, further corroborated its role in pluripotent stem cells (Yu et al., 2007a), but the mechanism of action for Lin28 remained unclear. A subsequent flurry of studies showing that Lin28 directly inhibits *let-7* maturation in ESCs rapidly validated Lin28's function in ESC self-renewal (Viswanathan et al., 2008; Rybak et al., 2008; Heo et al., 2008; Newman et al., 2008). With the discovery that Lin28 is also important in cancer, the germ lineage, and cellular metabolism (Viswanathan et al., 2009; West et al., 2009; Zhu et al., 2011), understanding the role of Lin28 in stem cells during development and disease pathogenesis has emerged as a new field of research. In this review, we will discuss the Lin28 pathway and its complex molecular mechanisms, outline its known roles in stem cells, tissue development, and pathogenesis, and examine its ramifications for re-engineering mammalian physiology.

Lin28/*let-7*: A Conserved Bistable Switch

Current insights into Lin28 rest heavily on precedents in *C. elegans* genetics. *Lin-28* was first discovered through mutagenesis screens for heterochronic genes (Horvitz and Sulston, 1980; Sulston and Horvitz, 1981; Ambros and Horvitz, 1984). Loss of function in *lin-28* accelerates differentiation of the hypodermal and vulval stem cells (called seam cells and VPCs, respectively, in nematodes). In contrast, gain of function in *lin-28* promotes self-renewal and delays differentiation of the hypodermal and vulval stem cells, leading to the proliferation of hypodermal stem cells and a cell-cycle delay in vulval stem cells (Moss et al., 1997). *Lin-28* is highly expressed during embryogenesis and early larval development in the hypodermal, neural, and muscle cells but gradually diminishes and disappears by adulthood.

Two heterochronic miRNAs (*lin-4* and *let-7*) repress *lin-28* posttranscriptionally via direct binding sites in its 3′ untranslated region (3′ UTR) (Reinhart et al., 2000; Pasquinelli et al., 2000; Roush and Slack, 2008). Although canonical *let-7* is only expressed late in larval development to drive the transition to adulthood, three *let-7* homologs (*mir-48*, *mir-84*, and *mir-241*) display overlapping expression with *lin-28*. Indeed, loss of function in these *let-7* homologs phenocopied *lin-28* gain of function in the hypodermal stem cells and *lin-28* was epistatic to the three *let-7* homologs (Abbott et al., 2005). Mutation of the *let-7* binding site in the *lin-28* 3′ UTR also led to an increase in *lin-28* 3′ UTR-lacZ reporter expression (Morita and Han, 2006), suggesting that *let-7* binding contributes to *lin-28* repression and underlies their opposing roles in regulating differentiation.

The role of *lin-28* in mammalian stem cells was less clear until quite recently. The first glimpse of a connection came from the discovery that the mammalian *lin-28* ortholog is highly expressed in mouse ESCs and human embryonal carcinoma cells (Moss and Tang, 2003). The connection was further validated when human Lin28 was used along with Oct4, Sox2, and Nanog to reprogram human somatic fibroblasts into pluripotent stem cells (Yu et al., 2007a). Around the same time, a posttranscriptional mechanism was proposed to be responsible for the dramatic disparity between high levels of pri-*let-7* transcript and the deficiency of mature *let-7* miRNA in early mouse embryos

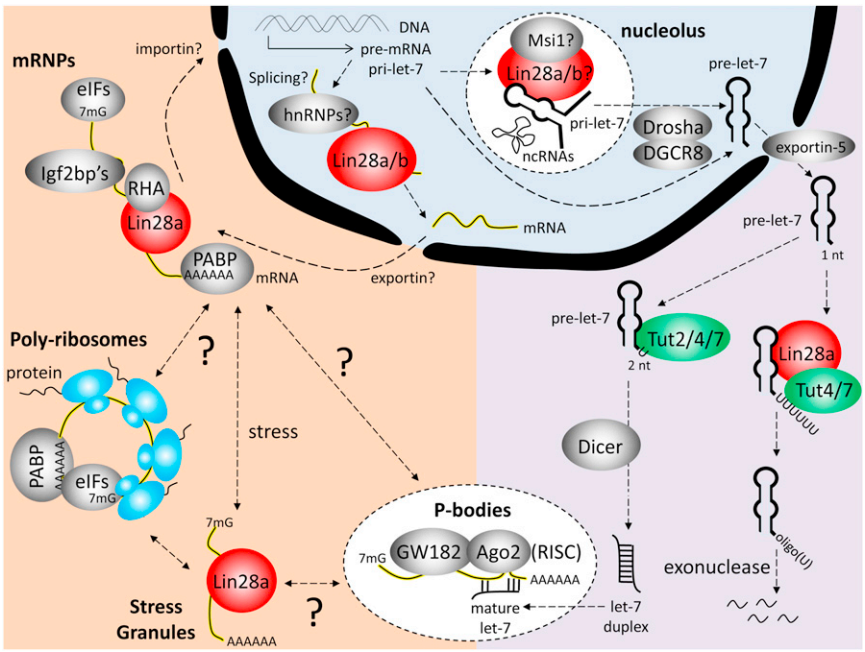

Figure 1. Overview of Molecular Mechanisms Underlying Lin28 Function

Both Lin28a and Lin28b have been observed to shuttle between the nucleus and cytoplasm, binding pri- and pre-*let-7*. In the nucleus, Lin28a and Lin28b could potentially work in tandem with the heterogeneous nuclear ribonucleoproteins (hnRNPs) to regulate splicing or with Musashi-1 (Msi1) to block pri-*let-7* processing. In the cytoplasm, Lin28a recruits Tut4 and Tut7 to oligo-uridylate pre-*let-7* and block Dicer processing in to mature *let-7* miRNA (right, violet). Lin28a also recruits RNA helicase A (RHA) to regulate mRNA processing in messenger ribonucleoprotein (mRNP) complexes, in tandem with the Igf2bps, poly(A)-binding protein (PABP), and the eukaryotic translation initiation factors (eIFs). In response to unknown signals and stimuli, the mRNAs are either shuttled into polyribosomes for translation, stress granules for temporary sequestering, or P-bodies for degradation, in part via miRNAs and the Ago2 endonuclease (left, orange).

and ESCs (Thomson et al., 2006; Wulczyn et al., 2007). These two lines of inquiry rapidly converged through a flurry of studies that showed that Lin28 (now routinely termed Lin28a) and its paralog Lin28b directly inhibit the posttranscriptional maturation of *let-7* in ESCs (Viswanathan et al., 2008; Rybak et al., 2008; Heo et al., 2008; Newman et al., 2008). A generally similar mechanism was later verified to be conserved in *C. elegans* (Lehrbach et al., 2009; Van Wynsberghe et al., 2011). Given that Lin28a and Lin28b inhibit the biogenesis of *let-7* miRNAs, which, in turn, repress Lin28a and Lin28b expression, it became clear that this bistable switch represents a central mechanism that governs stem cell self-renewal from worms to mammals.

Molecular Mechanisms of Lin28 Function

Following the discovery that Lin28a and Lin28b repress *let-7* biogenesis, several groups set about to determine the detailed biochemical mechanisms underlying *let-7* repression as a model for understanding miRNA biogenesis. Similar to the biogenesis of other miRNAs, *let-7* is first transcribed as part of long pri-*let-7* transcripts in the nucleus (Roush and Slack, 2008). Within the pri-*let-7* transcripts, there is a hairpin structure that is the precursor miRNA (pre-*let-7*). Drosha, in complex with its RNA-binding cofactor DGCR8, cleaves and releases the ~70 nt hairpin structure to produce pre-*let-7*. Like other pre-miRNAs, pre-*let-7* is then thought to be exported from the nucleus into the cytoplasm by exportin-5, although the majority of pre-*let-7* species lack the 3′ 2 nt overhang that exportin-5 presumably needs to export pre-miRNAs (Heo et al., 2012; Yi et al., 2003), suggesting that another mechanism might serve this function. In the cytoplasm, pre-*let-7* is further processed by Dicer to produce a 22 nt double-stranded RNA duplex. Mature single-stranded *let-7* is then incorporated from the duplex into the RNA-induced silencing complex to target mRNAs for translation inhibition and/or degradation in processing bodies (P-bodies) (Figure 1).

In a multipronged fashion, Lin28a and Lin28b bind to both pri-*let-7* and pre-*let-7*, effectively sabotaging the post-transcriptional processing of *let-7* (Viswanathan et al., 2008; Rybak et al., 2008; Heo et al., 2008; Newman et al., 2008). X-ray crystallography studies further revealed that Lin28a binds pre-*let-7* at the terminal loop and at the bulge GGAG motif where Dicer cleaves (Nam et al., 2011). Lin28a also recruits Tut4 (Zcchc11), a cytoplasmic terminal uridylyl transferase, to oligo-uridylate pre-*let-7* and prevents its processing by Dicer (Heo et al., 2009; Hagan et al., 2009). Recent studies have further elaborated on this mechanism, suggesting that Tut7 (Zcchc6) is a redundant homolog of Tut4 that can also oligo-uridylate pre-*let-7* in the presence of Lin28a (Thornton et al., 2012; Heo et al., 2012). In contrast, when Lin28a is absent, Tut4, Tut7, or Tut2 (Papd4/Gld2) mono-uridylates pre-*let-7*s at their 3′ 1 nt overhang to generate a 2 nt overhang, thereby enabling their processing by Dicer (Heo et al., 2012). Thus, one would expect oligo-uridylated pre-*let-7* to accumulate when pri-*let-7*, Lin28a, and Tut4 and Tut7 are present. But this was not observed, suggesting that an unknown nuclease must exist to degrade oligo-uridylated pre-*let-7*s (Heo et al., 2008, 2009) and/or that Lin28 can sequester pri-*let-7*s to prevent further processing (Viswanathan et al., 2008; Newman et al., 2008). Indeed, a study suggests that Lin28b is predominantly localized in the nucleolus where it can sequester pri-*let-7* away from Drosha and DGCR8 processing, whereas Lin28a is predominantly localized in the cytoplasm, where it can recruit Tut4 to oligo-uridylate pre-*let-7* and prevent Dicer processing (Piskounova et al., 2011). However, mammalian Lin28a and Lin28b and *C. elegans* lin-28 can all enter the nucleus as well as the cytoplasm (Moss et al., 1997; Guo et al., 2006; Balzer and Moss, 2007; Heo et al., 2008; Piskounova et al., 2011; Van Wynsberghe et al., 2011; Vogt et al., 2012; Hafner et al., 2013). Moreover, all three proteins possess a putative nucleolar localization signal, and all three proteins can bind to both pri- and pre-*let-7* (Viswanathan et al., 2008; Rybak et al., 2008; Heo et al., 2008; Newman et al., 2008; Lehrbach et al., 2009; Van Wynsberghe et al., 2011). Thus, the mode of

regulation of this division of labor between Lin28a and Lin28b remains unclear (Figure 1).

Under different conditions of metabolic stress in embryonal carcinoma cells and myoblasts, Lin28a localizes specifically to cytoplasmic stress granules where mRNAs are sequestered and mRNA translation is temporarily stalled (Balzer and Moss, 2007; Polesskaya et al., 2007). Under normal growth conditions, cytoplasmic Lin28a can directly or indirectly associate with translation initiation factors eIF3B and eIF4E, elongation factors EF1α and EF1α2, ribosomal proteins, poly(A)-binding protein, Igf2bps, Musashi1 (Msi1), and RNA helicase A (RHA) in messenger ribonucleoprotein complexes to regulate mRNA translation (Balzer and Moss, 2007; Polesskaya et al., 2007; Jin et al., 2011). When point mutations are introduced into the RNA-binding motifs, Lin28a localizes to the nucleus (Balzer and Moss, 2007). These findings suggest a model in which Lin28a regulates the posttranscriptional processing of its mRNA targets, perhaps by first binding them in the nucleus and subsequently shuttling them between ribosomes, P-bodies, or stress granules for translational regulation, depending on the environmental conditions. It would be interesting to know which factors sense the environmental conditions for the regulation of this shuttling of Lin28a and whether these conditions alter the RNAs bound by Lin28a (Figure 1). One study, for example, suggests that retinoic acid-induced differentiation of ESCs triggers Msi1 expression, which recruits Lin28a to the nucleus to sequester and inhibit pri-*miR-98* but not pri-*let-7b* (Kawahara et al., 2011). But what else could Lin28a and Lin28b be doing in the nucleus? Nuclear Lin28 could also regulate the alternative splicing of pre-mRNAs, or processing of small nucleolar RNAs (snoRNAs) and long noncoding RNAs to generate greater RNA diversity. The likelihood for this additional role is supported by the RNA-dependent association between Lin28a protein and nuclear splicing factors, such as heterogeneous ribonucleoproteins F and H1 (Polesskaya et al., 2007), and its direct regulation of splicing factors and snoRNAs (Wilbert et al., 2012; Hafner et al., 2013). How these various RNA-processing mechanisms relate to stem cell self-renewal and plasticity in response to environmental changes remains an important avenue for future research.

Regulatory Signals Upstream of Lin28

Throughout their lifespans, stem cells must decide whether to self-renew, proliferate, differentiate or die. The regulation of stem cell homeostasis is a complex process that involves integrating intrinsic and extrinsic signals so that stem cells can correctly adapt to the environment. The central role of the Lin28/*let-7* bistable switch in governing stem cell self-renewal raises a provocative question: what signaling pathways converge upstream to regulate the switch?

In *C. elegans*, an important intrinsic signal upstream of *lin-28* that regulates hypodermal stem cell self-renewal is the miRNA *lin-4*. In vertebrates, *lin-4* is conserved as *miR-125a* and *miR-125b* (Lagos-Quintana et al., 2002). Vertebrate *miR-125/lin-4* has been shown to be critical for regulating processes as disparate as neurogenesis, somitogenesis, hematopoiesis, myogenesis, and epidermal stem cell self-renewal (Rybak et al., 2008; Le et al., 2009a; Le et al., 2009b; Klusmann et al., 2010; Bousquet et al., 2010; O'Connell et al., 2010; Ooi et al., 2010; Ge et al., 2011; Zhang et al., 2011a; Guo et al., 2010; Chaudhuri et al., 2012). During these developmental processes, *miR-125/lin-4* appears to regulate stem cell self-renewal and progenitor differentiation by repressing a variety of different targets, including Lin28a and Lin28b , as well as the p53 network (Le et al., 2011). In mammalian ESCs, the pluripotency factors Oct4, Sox2, Nanog, and Tcf3 have also been shown to regulate the transcription of *Lin28a* (Marson et al., 2008). Among these pluripotency factors, Sox2 appears to be the most critical for regulating *Lin28a* expression, on the basis of Bayesian probabilistic network modeling of single-cell gene expression data during iPSC reprogramming (Buganim et al., 2012). Interestingly, Sox2 directly binds to Lin28a in a nuclear protein-protein complex, suggesting a close relationship between Sox2 and Lin28a in pluripotency (Cox et al., 2010). Repression of the Dot1L H3K79 histone methyltransferase upregulates *Lin28a* during reprogramming, although the mechanism is indirect and occurs via mesenchymal regulators downstream of TGFβ signaling (Onder et al., 2012). Finally, c-Myc and NF-κB transactivate *Lin28b* in transformed cancer cells (Chang et al., 2009; Iliopoulos et al., 2009), suggesting that *Lin28a* and *Lin28b* possess distinct *cis*-regulatory elements to drive their transcription (Figure 2). Beyond

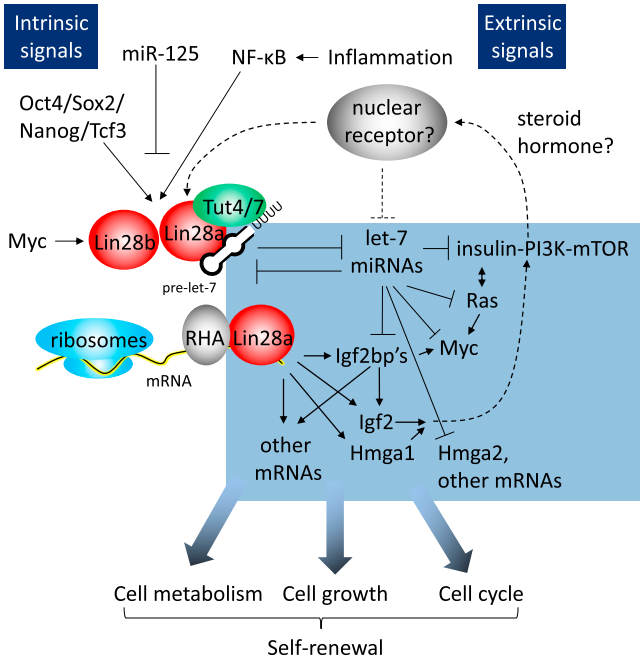

Figure 2. Signals Upstream and Targets Downstream of Lin28 in the Lin28 Pathway

The *lin-4* homolog *miR-125a/miR-125b* represses both Lin28a and Lin28b during stem cell differentiation. The core pluripotency transcription factors Oct4, Sox2, Nanog, and Tcf3 can activate *Lin28a* transcription in ESCs and iPSCs, whereas the growth regulator Myc and the inflammation- and stress-responsive NF-κB can transactivate *Lin28b*. A putative steroid hormone-activated nuclear receptor, conserved from *C. elegans daf-12*, might also regulate Lin28a and Lin28b and *let-7* expression. Downstream of Lin28a and Lin28b, the *let-7* family represses a network of proto-oncogenes, including the insulin-PI3K-mTOR pathway, Ras, Myc, Hmga2, and the Igf2bps. At the same time, Lin28a can also directly bind to and regulate translation of mRNAs, including Igf2bps, Igf2, Hmga1, and mRNAs encoding metabolic enzymes, ribosomal peptides, and cell-cycle regulators. Altogether, this broad network of targets allows Lin28 to program both metabolism and growth to regulate self-renewal.

these studies, relatively little is known about the transcription factors that regulate *Lin28a* and *Lin28b* expression during mammalian development, and additional investigation is warranted.

The *C. elegans* nuclear receptor *daf-12* feeds extrinsic signals from steroid hormones to *lin-28* and *let-7* to regulate diapause or dauer arrest (Antebi et al., 1998, 2000; Gerisch et al., 2007; Bethke et al., 2009; Hammell et al., 2009). Although this mechanism is well-characterized in *C. elegans* development and a similar ecdysone-*let-7* mechanism operates in *Drosophila* metamorphosis (Sokol et al., 2008; Chawla and Sokol, 2012), it is unclear if a similar mechanism exists to hormonally regulate the Lin28/*let-7* switch in mammals (Figure 2). Studies have shown that the homologous retinoic acid receptors and estrogen receptor α regulate *let-7* expression in vitro (Thomson et al., 2006; Wulczyn et al., 2007; Gehrke et al., 2010; Schulz et al., 2011; Bhat-Nakshatri et al., 2009), but both the directness and the physiological relevance of these mechanisms remain to be shown. It would also be interesting to test if any of the nuclear hormone receptors implicated in pluripotency and especially the naive state (Feng et al., 2009; Heng et al., 2010; Guo and Smith, 2010; Wang et al., 2011; Martello et al., 2012), a state associated with rodent diapause (Nichols et al., 2001; Nichols and Smith, 2009), also regulate the Lin28/*let-7* switch (Figure 2).

Coordinate Regulation of Metabolism and Cell Cycle by Downstream Targets of Lin28

Given our wealth of understanding of the upstream regulators of *lin-28* in worms, it is surprising that relatively little was discerned about the downstream RNA targets in the *C. elegans* literature. It was only after the discovery that *lin-28* directly binds to and represses pre-*let-7* that the more well-known *let-7* targets could be placed downstream of *lin-28.* Only some of these *let-7* targets are known to be conserved in mammals, including *lin-41* (Trim71), *let-60* (Ras), and Lin28 itself. The regulation of *NRAS* and *KRAS* by *let-7* in human cancer cells has led to the proposal that *let-7* functions as a tumor suppressor in humans (Johnson et al., 2005). Indeed, a network of *let-7* targets involving numerous other proto-oncogenes has been uncovered in mammalian cells, including Myc, Hmga2, Igf2bps, cyclins (Sampson et al., 2007; Mayr et al., 2007; Lee and Dutta, 2007; Johnson et al., 2007; Yu et al., 2007b; Boyerinas et al., 2008; Iliopoulos et al., 2009; Legesse-Miller et al., 2009; Chang et al., 2012), and components of the insulin-PI3K-mTOR pathway, such as Igf1r, Insr, Irs2, Akt2, and Rictor (Zhu et al., 2011; Frost and Olson, 2011). These studies fit with the suggestion that Lin28a and Lin28b function as oncogenes in multiple cancers by repressing the *let-7* network (Viswanathan et al., 2009). However, a majority of these studies were conducted in vitro, and many of these claims still await validation in vivo through *Lin28a* and *Lin28b* or *let-7* mouse models.

Adding a second layer of complexity downstream of *Lin28*, some studies have shown that Lin28a directly binds to many mRNAs, including *Igf2* in myoblasts and neural progenitors, and *cyclin A* and *cyclin B* in ESCs, to directly enhance their translation independently of *let-7* (Polesskaya et al., 2007; Xu et al., 2009; Balzer et al., 2010). Genome-wide RNA immunoprecipitation studies revealed thousands of mRNA targets bound directly by Lin28. In human ESCs and cancer cells, LIN28A directly binds and promotes the mRNA translation of numerous metabolic enzymes, ribosomal peptides, and cyclins as well as splicing factors (Peng et al., 2011; Li et al., 2012; Wilbert et al., 2012; Hafner et al., 2013). Curiously, in mouse ESCs, Lin28a was also recently found to bind and subtly repress the ribosomal occupancy of numerous membrane protein mRNAs (Cho et al., 2012). Given the plethora of mRNA targets that are emerging for Lin28a, including nearly 50% of the human transcriptome in one study (Cho et al., 2012), an important task lying ahead is to determine whether all these targets or only a subset contribute to the Lin28 phenotypes observed in vivo. Such an undertaking might require a return to the powerful genetics of *C. elegans* to search for conserved mRNA targets of *lin-28*. This idea is supported by the finding that *let-7*-independent mechanisms must account for the *lin-28* phenotype in *C. elegans* as well (Vadla et al., 2012).

Several key insights into Lin28 function are emerging from the small set of well-validated targets, most notably the insight that Lin28 coordinates both proliferative growth and metabolism. Lin28a and Lin28b can upregulate a large number of cell-cycle regulators through *let-7* repression, including *Myc*, *Ras*, *cyclin D1*, *cyclin D2*, *Cdk6*, *Cdc25a*, *Cdc34*, *Trim71* (which represses $p21^{Cip1}$), *Hmga2* (which represses $p16^{Ink4a}$ and $p19^{Arf}$), and PI3K-Akt signaling (Johnson et al., 2005; Sampson et al., 2007; Johnson et al., 2007; Chang et al., 2012; Mayr et al., 2007; Nishino et al., 2008; Zhu et al., 2011). A recent study even suggested that *let-7* can directly bind to and silence Rb1 and E2F target genes via heterochromatin during senescence (Benhamed et al., 2012). Lin28a also directly binds and promotes the translation of mRNAs encoding *cyclin A*, *cyclin B*, *cyclin D*, *Cdk1*, *Cdk2*, *Cdk4*, *Cdc2*, and *Cdc20*, thereby coordinating the cell cycle at multiple checkpoints (Xu et al., 2009; Li et al., 2012; Hafner et al., 2013). Besides the cell cycle, Lin28a and Lin28b might also control cellular growth by regulating ribosomal synthesis of proteins. Lin28a directly binds to the mRNAs of numerous ribosomal peptides in human ESCs (Peng et al., 2011). In addition, Lin28a and Lin28b increase mTOR signaling via *let-7* (Zhu et al., 2011; Frost and Olson, 2011), which can activate ribosomal biogenesis and translation in many contexts. In parallel with its extensive control of cell-cycle and cell-growth regulators, Lin28a and Lin28b appear to also coordinate cellular metabolism, both via *let-7* and by directly stimulating mRNA translation. Through *let-7*, Lin28a and Lin28b upregulate the insulin-PI3K, Ras, and Myc pathways – all of which are oncogenic regulators of metabolism (Vander Heiden et al., 2009; Dang, 2011). By directly binding mRNAs and influencing the translation of Igf2bps, Igf2, glycolysis enzymes, and mitochondrial enzymes, Lin28a can directly potentiate cellular metabolism (Zhu et al., 2011; Peng et al., 2011; Polesskaya et al., 2007; Janiszewska et al., 2012; Hafner et al., 2013). *Hmga1*, another mRNA target of Lin28a, can also upregulate insulin-PI3K signaling (Liau et al., 2006; Chiefari et al., 2011; Peng et al., 2011). Given how growth signaling pathways are intertwined with cellular metabolism, it is perhaps not surprising that Lin28 would have to program both arms of genes to regulate self-renewal. Thus, a model, albeit an inchoate one, is emerging whereby Lin28 programs both metabolism and proliferative growth to regulate stem and progenitor cell self-renewal (Figure 2).

Lin28 in Embryonic Stem Cell Metabolism

The functional role of Lin28 in cellular metabolism is evidenced by recent studies in ESCs. Recent work has shown that aerobic glycolysis, akin to the Warburg effect in cancer, is critical to ESCs and iPSCs (Folmes et al., 2011). This is perhaps not surprising, given the high proliferative capacity of ESCs and the importance of glycolysis in providing carbon intermediates for anabolic growth (Vander Heiden et al., 2009). What is surprising is that some studies have shown that mitochondrial oxidative metabolism is also critical to ESCs (Wang et al., 2009; Alexander et al., 2011; Zhang et al., 2011b) despite the immature morphology of ESC mitochondria. Interestingly, Lin28a binds to a large number of mRNAs encoding mitochondrial enzymes in human ESCs (Peng et al., 2011). One possibility is that ESC mitochondrial oxidation could be operating to recycle mitochondrial NAD^+ and keep the Krebs cycle running in order to generate fatty acids and various amino acids for ESCs (Shyh-Chang et al., 2011). Curiously, mouse ESCs rely uniquely upon mitochondrial oxidation of threonine (Thr) into glycine (Gly) via threonine dehydrogenase to generate one carbon and folate intermediates to fuel rapid nucleotide synthesis (Wang et al., 2009). This seminal early work led to findings that the 5-methyl-THF generated by mitochondrial Thr oxidation also fuels the synthesis of S-adenosyl methionine (SAM) to regulate histone H3K4 methylation and the pluripotency of ESCs (Shyh-Chang et al., 2013a). Surprisingly, *Lin28a* overexpression in ESCs leads to a dramatic accumulation of many metabolites in the Thr-Gly-SAM pathway, whereas overexpression of *let-7* reduces the abundance of these metabolites, suggesting that the Thr-Gly-SAM pathway is at least indirectly regulated by the Lin28/*let-7* switch to maintain ESC self-renewal. These findings might also have relevance to cancer, given that lung cancer stem cells have been found to express and depend upon high levels of both Lin28b and glycine decarboxylase in the Thr-Gly pathway to initiate tumorigenesis (Zhang et al., 2012). In fact, several enzymes in Gly metabolism have recently been implicated in human tumorigenesis (Locasale et al., 2011; Possemato et al., 2011; Jain et al., 2012). Thus, Lin28 could potentially regulate glucose and amino acid metabolism in a variety of stem and progenitor cells, including those that are both normal and malignant (Shyh-Chang et al., 2013b).

Lin28 in Early Embryogenesis, Pluripotent Stem Cells, and Reprogramming

The earliest phases of embryogenesis feature high levels of Lin28a because of protein inheritance through the maternal oocyte. From the mouse zygote to the preimplantation blastocyst, Lin28a is exclusively localized in the nucleolus where it is thought to regulate nucleolar maturation (Vogt et al., 2012). Morpholino knockdown of Lin28a in the zygote produces defects in nucleolar morphology and developmental arrest at the 2-cell and 4-cell stages, suggesting that Lin28a is required for proper nucleolar genesis and function and early embryogenesis. Curiously, Lin28a is localized in the nucleolus of mouse ESCs as well but is not localized in primate ESCs (Vogt et al., 2012). Given that Lin28a is an RNA-binding protein, these observations suggest that maternal Lin28a might also be involved in ribosomal RNA processing in the nucleolus for the regulation of zygotic genome activation during the maternal to zygotic transition, although this remains speculative.

After zygotic genome activation, mammalian blastocysts show high levels of *Lin28a* and *Lin28b* transcription in the pluripotent cells of the inner cell mass (ICM) and epiblast and their in vitro correlates—the indefinitely self-renewing ESCs. Studies suggest that Lin28a and Lin28b act as repressors of *let-7* miRNAs to prevent premature differentiation in the pluripotent ICM and epiblast (Suh et al., 2010; Melton et al., 2010). When the pluripotent ICM is cultured in vitro for ESC derivation, Lin28a is further upregulated, in parallel with the acquisition of indefinite self-renewal capacity in vitro (Tang et al., 2010). Furthermore, overexpression of Lin28a with a cocktail of the core pluripotency-associated transcription factors Oct4, Sox2, and Nanog helps promote the reprogramming of human somatic fibroblasts into indefinitely self-renewing iPSCs (Yu et al., 2007a; Hanna et al., 2009). These data suggest that Lin28a is critical to pluripotent stem cell self-renewal.

But is Lin28 also required for pluripotency? Lin28 knockout mouse models suggest that the answer is no. *Lin28a* knockout mice progress through the blastocyst stage without obvious developmental defects in utero, although they weigh 20% less at birth (Zhu et al., 2010). *Lin28b* knockout mice are viable and fertile. Thus, Lin28a and Lin28b do not appear to be essential per se for pluripotency in vivo.

Do pluripotent stem cells then require Lin28a and Lin28b for indefinite self-renewal? The answer depends on the context, given that overexpression of mature *let-7* does not inhibit mouse ESC self-renewal unless DGCR8 is knocked out and miRNA biogenesis is prevented (Melton et al., 2010). Another class of miRNAs called the miR-290 family can respond to and compensate for the effects of *let-7* overexpression. Although the breadth of genes targeted by the miR-290 family and their connections with Lin28a and Lin28b (if any) remain unclear, it is thought that *let-7* promotes ESC differentiation only in the absence of DGCR8 by directly repressing *Sall4*, *Nmyc*, and *Lin28a*. Conversely, Lin28a or Lin28b knockout in the presence of DGCR8 should not lead to defects in self-renewal via *let-7* upregulation alone, even if we ignore the compensatory redundancy observed between Lin28a and Lin28b (Wilbert et al., 2012). However, RNA interference against Lin28 does lead to proliferative defects in both mouse and human ESCs (Xu et al., 2009; Peng et al., 2011), suggesting that Lin28a and Lin28b might synergistically promote ESC self-renewal through a combination of *let-7* repression and *let-7*-independent mechanisms, such as direct binding of mRNAs involved in metabolism and growth. Thus, it will also be interesting to see if Lin28a and Lin28b double knockout leads to defects in ESC self-renewal. On the other hand, *let-7* knockdown in fibroblasts promotes iPSC reprogramming (Melton et al., 2010), suggesting that Lin28a might promote self-renewal via repression of *let-7* during iPSC reprogramming without compensatory effects from ESC-specific *miR-290*. Indeed, studies have shown that Lin28a can accelerate the early stochastic phase of iPSC reprogramming by accelerating the cell cycle and that Lin28a is one of the earliest markers of the deterministic phase of iPSC reprogramming after endogenous Sox2 expression is induced (Hanna et al., 2009; Buganim et al., 2012; Golipour et al., 2012). However, it remains to be verified whether *let-7* is the relevant target of Lin28a during reprogramming and what downstream targets of *let-7* drive iPSC reprogramming. It is also unknown whether Lin28/*let-7* is implicated

in the much more rapid and deterministic process of reprogramming by somatic cell nuclear transfer.

Lin28 in Normal and Transformed Tissue Progenitors

Contrary to popular belief, the expression of Lin28a and Lin28b and their influence on development is far from unique to pluripotent cells in the blastocyst ICM, but, rather, extends to a variety of tissues. For instance, the trophoblast (Vogt et al. 2012) and the resultant placental tissues (Sangiao-Alvarellos et al. 2013) show high levels of Lin28a and Lin28b. Moreover, the LIN28B locus shows imprinting and paternal monoallelic expression in the human placenta (Barbaux et al., 2012). However, the placental function of Lin28 remains unclear in vivo.

Germline stem cells also retain high levels of Lin28 expression during mammalian development. Lin28a promotes primordial germ cell (PGC) specification via *let-7* regulation of the master regulator Blimp1 (West et al., 2009) and remains high specifically in the spermatogonial stem cells of adult male testes (Zheng et al., 2009). Both Lin28a knockout and *let-7* overexpression led to a reduction in PGCs during embryogenesis and a reduction in proliferating spermatogonia and germ cells before adulthood (Shinoda et al., 2013). Interestingly, aberrant overexpression of Lin28a and Lin28b is associated with the malignancy of human germ cell tumors, such as choriocarcinomas, embryonal carcinomas, seminomas, yolk sac tumors, and mixed germ cell tumors (West et al., 2009; Cao et al., 2011a; Cao et al., 2011b; Gillis et al., 2011; Xue et al., 2011). Overexpression of Lin28a produces higher-grade teratomas, whereas Lin28a knockdown leads to smaller teratomas, suggesting that Lin28a acts as an oncogene in germ cell tumors by enhancing the self-renewal of PGCs and spermatogonial stem cells (West et al., 2009).

Although Lin28a and Lin28b decline rapidly upon implantation (Tang et al., 2010), high levels of Lin28 persist in the neural tube and neural crest (Yang and Moss, 2003; Balzer et al., 2010). *miR-125* is thought to promote neural differentiation, in part by downregulating Lin28a in neural stem cells (Wulczyn et al., 2007; Rybak et al., 2008). Lin28a and Lin28b overexpression, in turn, regulates the balance of neurogenesis and gliogenesis in vitro (Balzer et al., 2010) and leads to an abundance of primitive neural tissue in teratomas formed by ESCs (West et al., 2009). By increasing Nmyc, conditional overexpression of Lin28b in neural crest progenitors in mice could inhibit neuronal differentiation and lead to neuroblastoma (Molenaar et al., 2012). Interestingly, a genome-wide association study also found a Lin28b variant with higher expression is associated with higher neuroblastoma risk in humans (Diskin et al., 2012). This suggests that dysregulation of Lin28b in neural crest progenitors, which normally show only limited self-renewal, can provoke transformation into neuroblastoma. Lin28a is also highly expressed in aggressive primitive neuroectodermal brain tumors and medulloblastoma, although its oncogenic role in these tumors remains less clear (Picard et al., 2012; Rodini et al., 2012).

Within the developing mesodermal tissues, fetal hematopoietic stem and progenitor cells (HSPCs) express high levels of Lin28b, whereas adult HSPCs do not (Yuan et al., 2012). Overexpression of Lin28a alone in adult Lin^- bone marrow cells can reprogram some of them into fetal-like lymphoid progenitors (Yuan et al., 2012), which may be relevant to the oncogenic role of Lin28 in T cell lymphoma and leukemia (Beachy et al., 2012; Rao et al., 2012). However, in acute myeloid leukemia (AML), Lin28a appears to act as an oncogene in MLL-driven AML on one end of the spectrum and as a tumor suppressor in *miR-125*-driven AML on the other (Jiang et al., 2012; Chaudhuri et al., 2012). It is especially interesting that *miR-125* overexpression alone can promote the self-renewal of long-term adult HSCs to cause a variety of myeloid and lymphoid malignancies in both mice and humans (Bousquet et al., 2008, 2010; Guo et al., 2010; O'Connell et al., 2010; Ooi et al., 2010; Chaudhuri et al., 2012), even though the *miR-125* homolog *lin-4* promotes differentiation in nematode stem cells. The unresolved questions surrounding *miR-125* and Lin28's roles in hematopoiesis indicate that our understanding of how the *miR-125-Lin28-let-7* pathway regulates hematopoiesis remains incomplete.

In another mesoderm-derived tissue, muscle stem cells or satellite cells have not been observed to express Lin28a and Lin28b , but proliferative myoblasts do upregulate Lin28a during muscle regeneration (Polesskaya et al., 2007). Loss of Lin28a by small interfering RNA knockdown inhibits myogenesis, whereas Lin28a overexpression promotes myogenesis, at least in vitro. This process depends on the direct stimulation of *Igf2* translation (Polesskaya et al., 2007) and probably other *Lin28a* targets in cellular growth and metabolism (Zhu et al., 2011). Although muscle development does not seem overtly affected in *Lin28a* transgenic or knockout mice (Zhu et al., 2010; Zhu et al., 2011), it remains to be tested whether *Lin28a* and *Lin28b* are functionally important in muscle regeneration upon injury or in rhabdomyosarcoma growth in vivo.

Despite tremendous progress in our knowledge of Lin28 function in tissues of germline, ectodermal, and mesodermal origin, little is known about Lin28 function in endodermal tissues. Lin28 expression has been detected in the fetal liver, kidney, intestines, and lung by immunohistochemistry (Yang and Moss, 2003). A variety of cancers involving these tissues express Lin28b, including hepatocellular carcinoma, Wilm's tumor, colorectal cancer and lung cancer—suggesting that Lin28a and Lin28b might play a role in both the normal development and malignancy of endodermal tissues (Guo et al., 2006; Viswanathan et al., 2009; King et al., 2011; Zhang et al., 2012). Given the limitations of immunohistochemistry in small cellular compartments and the expectation that Lin28 may only be active in stem or progenitor cells, careful analyses using tissue specific Cre- or Cre-ER-driven mouse models are needed to rigorously address the role of Lin28a and Lin28b in tissue development by lineage tracing.

Lin28 in Re-engineering of Mammalian Physiology

It has been proposed that Lin28 is an oncofetal gene with little physiological relevance in normal adult tissues (Boyerinas et al., 2008; Peter 2009). Given that Lin28a and Lin28b are primarily expressed during embryogenesis and largely silent in most adult tissues, one could argue that these proteins bear little relevance to normal adult human physiology except when reactivated in the setting of malignancy. Evolutionarily, this could be because of Lin28's potency in promoting stem cell self-renewal, and, hence, tumorigenesis, if dysregulated. Another gene endowed with similar properties is the catalytic component of telomerase, Tert, which is likewise predominantly expressed

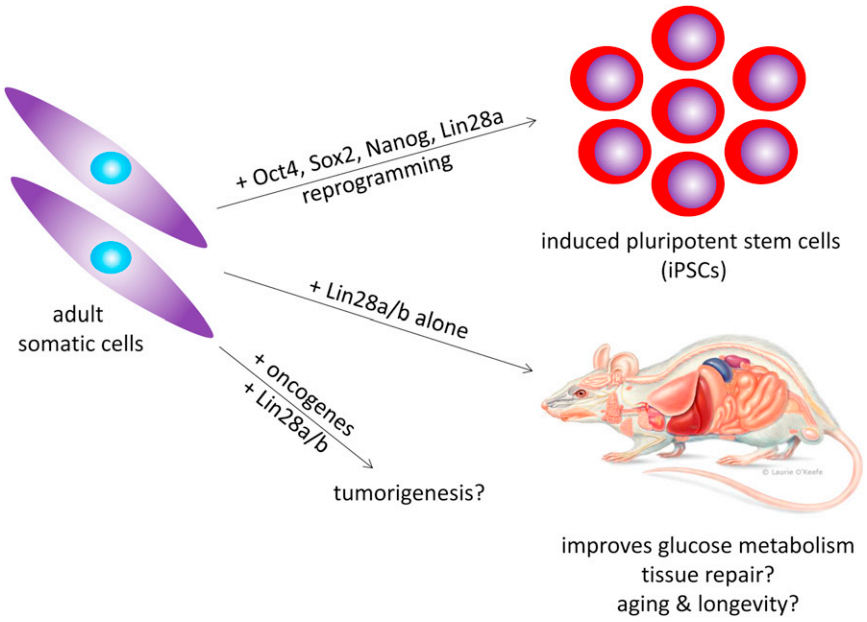

Figure 3. Potential of Lin28 in Re-engineering Adult Mammalian Physiology

Lin28a, in conjunction with the pluripotency factors Oct4, Sox2, and Nanog, can reprogram somatic cells into iPSCs. Alone, Lin28a and Lin28b can reprogram adult HSPCs into a fetal-like state and enhance insulin sensitivity in the skeletal muscles to improve glucose homeostasis, resist obesity, and prevent diabetes. Emergent clues suggest that optimal doses of Lin28a and Lin28b might have the potential to re-engineer adult mammalian tissue repair capacities and extend longevity, although Lin28a and Lin28b could also cooperate with oncogenes to initiate tumorigenesis. Future work might elucidate these mysteries.

during early embryogenesis, in small compartments of adult stem cells, and in cancers (Kolquist et al., 1998; Schaetzlein et al., 2004). Despite its initial apparent irrelevance to adult physiology—telomerase knockout mice are healthy and viable for the first few generations (Blasco et al., 1997; Yuan et al., 1999)—Tert has gained preeminence as an agent for re-engineering mammalian adult cells by immortalizing cells and extending lifespan in vivo while being a potential target in cancer therapy (Sahin and Depinho, 2010). Could Lin28 show similar potential in re-engineering adult human cells (Figure 3)?

The answer appears to be in the affirmative. Altogether, the demonstrations that Lin28a overexpression with Oct4, Sox2, and Nanog can help reprogram adult human fibroblasts into ESC-like iPSCs (Yu et al., 2007a), that Lin28a overexpression can reprogram adult HSPCs into a fetal-like HSPCs (Yuan et al., 2012), and that Lin28b overexpression can expand neural crest progenitors (Molenaar et al., 2012) suggest that Lin28 overexpression might be useful for promoting stem cell or progenitor self-renewal in vitro. This is conceptually distinct from Tert-based immortalization of any somatic cell, given that Lin28 appears to counteract cellular differentiation, whereas Tert counteracts replicative senescence. If Lin28 can enhance self-renewal, what effects would it exert on adult tissue repair in vivo? Although Lin28's effects on mammalian tissue repair remain unexplored, a study has shown that zebrafish *Lin28* can promote retinal regeneration by repressing *let-7* (Ramachandran et al., 2010). This finding hints at the possibility that Lin28 might also extend the limits of mammalian tissue repair upon injury—a hypothesis that awaits further testing (Figure 3).

In the same vein, the Lin28/*let-7* pathway could impact longevity, although it remains unclear whether Lin28 overexpression would promote or delay aging. Given that Lin28 upregulates and *let-7* downregulates insulin-PI3K signaling in mammals (Zhu et al., 2011) and that insulin-PI3K signaling also regulates mammalian aging in an evolutionarily conserved fashion, one might expect Lin28 to promote and *let-7* to delay aging in mammals. This would concur with several studies showing that several long-lived mouse strains like the Ames, Snell, GHRKO, and *Igfr1*$^{+/-}$ mice are all dwarfed (Bartke 2012), similar to mice overexpressing *let-7* (Zhu et al., 2011; Frost and Olson, 2011). However, improved insulin sensitivity, a prominent phenotype of mice overexpressing Lin28a or Lin28b, is also associated with longevity (Barbieri et al., 2003) and is indicative of how longevity can be regulated by a multifaceted network of factors. Adding to this complexity, deficiency in tissue repair can accelerate aging as shown in telomerase-deficient and p53-overexpressing mice (Rudolph et al., 1999; Tyner et al., 2002), but hyperactive tissue regeneration can also lead to stem cell exhaustion and shorter lifespans, as shown in models of *Pten*$^{-/-}$ myelodysplasias and muscular dystrophy (Yilmaz et al., 2006; Sacco et al., 2010). It could well be that only the precise dosage of Lin28/*let-7* that strikes an optimal equilibrium between insulin signaling and tissue repair would enhance mammalian longevity.

As is the case with telomerase, a wide variety of cancers reactivate Lin28 to re-engineer their cellular states (Viswanathan et al., 2009). Although the oncogenic role of Lin28a and Lin28b has only been demonstrated in vivo for a small subset of cancers, including neuroblastoma, T cell acute lymphoblastic leukemia, and peripheral T cell lymphoma (Molenaar et al., 2012; Beachy et al., 2012; Rao et al., 2012), the cancer stem cell model seems to apply in all these cases, Lin28 being the stem cell factor promoting self-renewal. The cancer stem cell model posits that cancers are maintained by a small population of tumor-sustaining cancer stem cells with self-renewal capacity (Rosen and Jordan, 2009). Although the cancer stem cell model has engendered debate and does not apply universally to all cancers, as shown most convincingly in advanced melanoma (Quintana et al., 2008, 2010; Boiko et al., 2010), leukemias, germ cell tumors, and many other solid cancers appear to follow the cancer stem cell model (Ishizawa et al., 2010). Boiko et al., 2010, have proposed that, in the earliest stages of melanoma, rare cancer stem cells differentiate into nonmalignant progeny to form the bulk of the tumor, whereas, in the advanced stages, cancer stem cell clones dominate and constitute the bulk of the tumor (Boiko et al., 2010). These findings suggest that targeting cancer cell heterogeneity may be a relevant approach for eradicating

tumors during a cancer's early stages. If the role of Lin28 in promoting stem cell and progenitor self-renewal is also its essential mechanism for promoting tumorigenesis, then Lin28 would represent a promising universal factor for therapeutic targeting in a wide variety of cancer stem cells. This exciting prospect warrants attempts to drug the Lin28 pathway.

Conclusion

Seminal observations made nearly three decades ago by Ambros and Horvitz on the role of Lin28 in *C. elegans* heterochronic reiterations have spawned great leaps in our understanding of how posttranscriptional RNA processing can regulate stem cells. Recent advances are painting a detailed picture of how Lin28 regulates *let-7* miRNA biogenesis and mRNA translation to coordinate both cellular metabolism and proliferative growth pathways for the purpose of stem cell self-renewal. Although much effort has been expended to elucidate the *let-7* miRNA regulatory mechanism, it is imperative that we also understand how Lin28a and Lin28b regulate mRNA processing and trafficking between the nucleus, ribosomes, P-bodies, and stress granules—a mystery that remains unresolved. What conditions or pathways regulate the mechanism of Lin28a or Lin28b within the nucleolus, the cytoplasm, and P-bodies? How do these mechanisms affect Lin28a and Lin28b function in tissue stem cells and progenitors, especially those derived from the endoderm, given its preponderance in colon, kidney, liver, and lung cancers? Can we harness Lin28 to re-engineer and improve mammalian tissue repair and longevity or target it for cancer therapy? We hope to answer these questions, and more, in the coming decade.

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