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LIN28: roles and regulation in development and beyond

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ABSTRACT
LIN28 is an RNA-binding protein that is best known for its roles in promoting pluripotency via regulation of the microRNA let-7. However, recent studies have uncovered new roles for LIN28 and have revealed how it functions, suggesting that it is more than just a regulator of microRNA biogenesis. Together, these findings imply a new paradigm for LIN28—a gatekeeper molecule that regulates the transition between pluripotency and committed cell lineages, in both let-7-dependent and let-7-independent manners. Here, we provide an overview of LIN28 function in development and disease.

KEY WORDS: LIN-28, RNA-binding protein, Pluripotency

Introduction
LIN28 is one of the founding members of the C. elegans heterochronic pathway (Ambros and Horvitz, 1984), which comprises a network of genes that regulate the timing of developmental events. When these genes are mutated, whole larval stages are skipped or reiterated. In this pathway, LIN-28 promotes cell fates specific to early larval development while also regulating cell fates specific to later development, including the terminal differentiation of some cell types (Ambros, 1989; Ambros and Horvitz, 1984; Moss et al., 1997; Vadila et al., 2012).

In the 30 years since it was initially discovered in C. elegans, LIN28 has been shown to play expanding roles in development and it has generally been defined as a promoter of pluripotency. In mouse, human, C. elegans, Xenopus, zebrafish and Drosophila, LIN28 is expressed early during development and in undifferentiated tissues, and it is downregulated as development and cellular differentiation proceed (Faas et al., 2013; Moss et al., 1997; Moss and Tang, 2003; Ouchi et al., 2014; Yang and Moss, 2003). It is also highly expressed in embryonic stem cells (ESCs) and is downregulated in response to differentiation (Moss and Tang, 2003; Richards et al., 2004). Accordingly, LIN28 has been identified as one of several factors that can participate in the reprogramming of mammalian somatic cells to pluripotent cells (Hanna et al., 2009; Yu et al., 2007). Taken together, it appears that LIN28 has an evolutionarily conserved role promoting early, undifferentiated cell fates. In line with this, LIN28 expression in cancers is associated with less differentiated, more aggressive tumors (Hamano et al., 2012).

Despite its strong association with pluripotent cells, LIN28 has also been shown, in at least two cases, to be required for proper differentiation (Faas et al., 2013; Polesskaya et al., 2007). It was in light of these findings that LIN28 was first suggested to act as a molecular ‘gatekeeper’, regulating the transition from pluripotency to a committed cell lineage (Faas et al., 2013). Here, we review the roles of LIN28 in a variety of organisms and systems, in both normal development and diseased states.

LIN28 gene and protein structure
A single lin-28 gene is found in C. elegans and Drosophila melanogaster (Moss and Tang, 2003), whereas there are two Lin28 paralogs in all vertebrates: Lin28a and Lin28b (Fig. 1). All LIN28 proteins can be identified by their unique pairing of a cold shock domain (CSD) and a cysteine cysteine histidine cysteine (CCHC) zinc knuckle domain (Fig. 1), both of which can bind RNA; LIN28 is the only animal protein to contain this combination of motifs. The CSD is a beta barrel and is similar to that of Y-box proteins of frog and human (Moss and Tang, 2003). The CCHC zinc knuckle domain is a motif found in retroviruses (Moss and Tang, 2003). LIN28A and LIN28B have high sequence identity in these regions, but differ in a few respects. For example, LIN28B contains an extended tail region at the C-terminus of the protein (Lee et al., 2014) and it also contains a nuclear localization signal (Fig. 1A) (Piskounova et al., 2008). LIN28A is predominantly located in the cytoplasm of cells, although it can shuttle to and from the nucleus (Balzer and Moss, 2008). LIN28B, by contrast, is found in the nucleus, specifically in the nucleolus (Piskounova et al., 2011). Another difference between LIN28A and LIN28B is in how they inhibit the microRNA (miRNA) let-7, as discussed below. This review will focus on LIN28A, which is canonically known as LIN28 and is referred to as such hereafter.

LIN28 in the C. elegans heterochronic pathway
LIN28 plays two roles in C. elegans larval development
The C. elegans heterochronic pathway controls the specification of cell fates in diverse cell types at each larval stage. The four larval stages (L1–L4) of C. elegans are characterized by stage-specific patterns of cell division and differentiation, punctuated with the synthesis of a new cuticle and the subsequent molting of the existing cuticle. When heterochronic genes are mutated, developmental events for a specific stage are skipped or reiterated (Ambros and Horvitz, 1984). Null mutations in lin-28 cause precocious development, whereby L2 stage-specific events are skipped (Fig. 2) and later events occur one stage earlier relative to wild-type animals (Ambros and Horvitz, 1984). This is clearly seen in the lateral hypodermal seam cells, in which the proliferative L2 division is skipped and all later events in this lineage, including the terminal differentiation and synthesis of adult cuticle, occur precociously (Ambros and Horvitz, 1984). Furthermore, lin-28 mutants cease molting after the L3, going through only three stages instead of the normal four. Additional tissues are affected, such as the hypodermal vulval precursor cells, which divide precociously and form a deformed, nonfunctional egg-laying system (Euling and Ambros, 1996). LIN28 is also sufficient to cause reiteration of L2 cell fates (Moss et al., 1997); a constitutively expressed lin-28 transgene causes reiteration of the L2 cell division, resulting in extensive hypodermal proliferation (Fig. 2). Additionally, vulval divisions are delayed or completely blocked in this mutant. Thus, LIN-28 is sufficient to drive reiteration of L2 cell fates, thereby blocking the execution of later fates.

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The link between LIN-28 and let-7 was surprising because LIN-28 is part of a complex genetic pathway
Although many questions remain, much is now known about the relationship between LIN-28 and other members of the heterochronic pathway. In 1989, the first hierarchy in the heterochronic pathway was established, when it was found that lin-28 acted downstream of lin-4 (Fig. 3) (Ambros, 1989). It was not until 1997, after the discovery that lin-4 encoded the first known miRNA (Lee et al., 1993), that it was revealed that lin-28 is a molecular target of lin-4 (Moss et al., 1997). In particular, it was shown that lin-4 targets lin-28 via the lin-4-complementary element in its 3′ UTR, making lin-28 the second known miRNA target (Moss et al., 1997).

Another lin-4 target in the pathway is lin-14, which encodes a transcription factor that promotes both L1 and L2 cell fates during larval development (Fig. 2) (Ambros and Horvitz, 1984, 1987; Hristova et al., 2005). It was known that LIN-28 and LIN-14 acted at the same step of development (Ambros and Horvitz, 1984), but two main experiments elucidated their genetic relationship. First, it was shown that, in a lin-4 null background, both LIN-14 and LIN-28 are expressed abnormally late into development, causing retarded development (Arasu et al., 1991; Moss et al., 1997). However, the late expression of LIN-14, whether in a lin-4 null or a lin-14(gf) background, requires functional LIN-28 (Arasu et al., 1991). Second, it was shown that the late expression of LIN-28 in a lin-4 null requires functional LIN-14 (Moss et al., 1997). Together, these findings indicated that lin-14 and lin-28 are in a positive-feedback loop, each promoting the expression of the other (Fig. 3). These findings also suggested that there is lin-4-independent repression of each gene in the other’s absence (Arasu et al., 1991; Seggerson et al., 2002). To investigate this, Seggerson and colleagues used a strain containing a unique allele of lin-14 [lin-14(n355sd)] in which the lin-4-responsive elements of the lin-14 3′ UTR are missing and,
**Lin28 in vertebrates**

The expression of LIN28 has been studied extensively in the whole mouse as well as in different pluripotent cell lines. This has resulted in a better understanding of the temporal and spatial expression patterns of LIN28 over the course of normal development and differentiation and of its function in vertebrates.

**Developmental expression patterns**

LIN28 is expressed early in development, with the protein showing accumulation in mouse nucleolar precursor bodies during the two-cell stage of embryogenesis (Vogt et al., 2012). Expression is seen throughout the developing embryo by E6.5, and extends to the extra-embryonic tissue as well (Yang and Moss, 2003). As development progresses, the previously broad expression of LIN28 becomes restricted to just some tissues: a subset of epithelial cells, including those of the branchial arches, the lungs and the kidney, cardiac muscle cells of the myocardium, and neuroepithelium; LIN28 is not expressed in all epithelial cells nor in the other two tissues of the developing heart (the endocardium and pericardium) (Yang and Moss, 2003). Expression in the lung epithelium decreases as differentiation occurs and is absent entirely by E17.5. Once development is complete, LIN28 remains expressed in the adult in the epithelial cells of the loop of Henle and collecting duct of the kidney, in cardiac and skeletal muscle (Yang and Moss, 2003) and in erythrocytes (de Vasconcellos et al., 2014).

In most instances, LIN28 is expressed in undifferentiated, pluripotent cell types. One notable exception is the cells of the small intestine. Here, LIN28 expression is restricted to a specific group of cells: the transit-amplifying population. These are cells that are committed to villous cell fates but that have not yet fully differentiated, establishing a virtual boundary zone in the tissue between more and less differentiated cell types (Yang and Moss, 2003). Therefore, in the small intestine, and perhaps elsewhere, LIN28 is expressed in a transit-amplifying population of cells within a tissue, rather than in its stem cells.

**LIN28 in pluripotent stem cells**

LIN28 is also highly expressed in ESCs, as well as in the NT-2 human teratocarcinoma cell line and in the mouse P19 embryonal carcinoma cell line, both of which exhibit pluripotent properties. When these cells differentiate, LIN28 expression is downregulated (Balzer et al., 2010; Yang and Moss, 2003). In ESCs, LIN28 regulates overall cell number and proliferation (Xu et al., 2009). Further investigation by Peng and colleagues identified a number of mRNAs regulated by LIN28 that are important for both growth and translation, supporting its role in the regulation of proliferation (Peng et al., 2011). When LIN28 is expressed, it regulates self-renewal in mouse ESCs as a function of AIRE, the autoimmune regulator (Bin et al., 2012). As in ESCs, proliferation in neural precursor cells is promoted by SOX2 through LIN28 (Cimadamore et al., 2013). LIN28 also regulates neural precursor cell proliferation promoted by MASH1 (ASCL1), via its inhibition of let-7.
(Cimadamore et al., 2013). Accordingly, the loss of LIN28 in neural progenitor cells results in fewer cells due to a reduction in proliferation (Yang et al., 2015).

Developmental and physiological roles for LIN28
At the organismal level, LIN28 has consequences for diverse biological processes. LIN28 overexpression causes an increase in total body size along with a proportional increase in organ size, which is likely to be due to an increase in cell number and proliferation. It also delays the onset of puberty, including postponement of the vaginal opening and first estrus (Zhu et al., 2010). LIN28 loss, by contrast, results in embryonic lethality, reduced growth and fat accumulation, and reduced brain size (Yang et al., 2015; Zhu et al., 2010). Additionally, LIN28 regulates glucose metabolism; its overexpression increases the ability of muscle cells to take up glucose (Zhu et al., 2010), while its loss results in insulin resistance (Shinoda et al., 2013; Zhu et al., 2011). Furthermore, sensitivity to insulin can be restored in obese adipose stem cells with the introduction of LIN28 (Perez et al., 2013). Finally, LIN28 overexpression increases regeneration during digit repair, epidermal hair regrowth and pinnal tissue regrowth in the mouse (Shyh-Chang et al., 2013). In many of these cases, the cellular basis and the mechanism of action of LIN28 are not fully understood.

LIN28 and the control of cell differentiation
In most mammalian cell culture models, cell differentiation is associated with downregulation of LIN28; in fact, LIN28 has been cited as a marker of ‘stemness’ (Richards et al., 2004). However, there are cases in which LIN28 appears to be required for efficient differentiation, notably in specifying mesodermal cell fates, as seen in both Xenopus tropicalis and mouse models (Faas et al., 2013; Polesskaya et al., 2007). Interestingly, in both these species, LIN28 does not require let-7 for this role.

For example, using X. tropicalis embryos Faas et al. (2013) found that knocking down LIN28 causes abnormalities in the differentiation and patterning of mesodermal structures at early larval stages. Several genes expressed in the very early mesoderm, including Xbra, chordin and myoD, were reduced both in their expression domains and expression levels. Furthermore, LIN28 knockdown inhibits the ability of pluripotent cells from the Xenopus embryo to differentiate into mesoderm in response to the growth factors FGF and activin (Faas et al., 2013). All of these effects occur in early development. Although Xenopus LIN28 can bind let-7, it was shown that, at this stage, there were no significant changes in let-7 expression following LIN28 knockout (Faas et al., 2013). Therefore, the effects of LIN28 on mesoderm specification are unlikely to be mediated through a LIN28/let-7 pathway.

Another notable exception in which differentiation is associated with LIN28 induction is skeletal muscle (Polesskaya et al., 2007). Using in vitro gain- and loss-of-function assays, Polesskaya et al. uncovered the importance of LIN28 during myogenic differentiation. They found that overexpression of LIN28 in a myoblast cell line had a stimulating effect on terminal differentiation and, conversely, that repression of LIN28 dramatically decreased the efficiency of muscle differentiation (Polesskaya et al., 2007). Additionally, they found that LIN28 is upregulated during the differentiation and maturation of newly formed skeletal muscle fibers in vivo. Finally, they found that LIN28 is likely to act by promoting the translation of Igf2 in this system, an mRNA that does not contain any predicted let-7 binding sites (Balzer et al., 2010). Taken together, these data suggest that LIN28 has an essential role in differentiating myoblasts and one that is not dependent on the regulation of let-7.

Molecular mechanisms of LIN28 action: two separate mechanistic pathways
The let-7-dependent pathway
Currently, the regulation of let-7 miRNAs is by far the best characterized mechanism of LIN28. Accordingly, a number of genetic pathways have been discovered in which LIN28 influences development via its regulation of let-7. The molecular aspects of the LIN28/let-7 axis have been studied in exquisite detail (Loughlin et al., 2012; Mayr and Heinemann, 2013; Nam et al., 2011). In brief, studies have shown that LIN28 can bind to both pri- and pre-let-7 in vivo and block their processing (Fig. 4) (Newman et al., 2008; Rybak et al., 2008; Viswanathan et al., 2008). LIN28 binds an evolutionarily conserved motif within let-7: GGAG. In mammals, this is present within the let-7 stem loop (Heo et al., 2009; Loughlin et al., 2012; Nam et al., 2011) and in nematodes it is found in the primary transcript, downstream of pre-let-7 (Stefani et al., 2015).

In the nucleus, LIN28 co-transcriptionally binds pri-let-7 and blocks its processing by the miRNA-processing enzyme Drosha (Van Wijnen et al., 2011). In human ESCs and neuronal stem/progenitor cells, it has been shown that the RNA-binding protein musashi 1 (MSI1) enhances the localization of LIN28 to the nucleus in vivo. LIN28 inhibits let-7 processing. LIN28 binds to both pri- and pre-let-7 in vivo and blocks their processing (Newman et al., 2008; Rybak et al., 2008; Viswanathan et al., 2008). In the nucleus, LIN28 co-transcriptionally binds pri-let-7 and blocks its processing by Drosha in complex with DGCR8 (Van Wijnen et al., 2011). In human ESCs and neuronal stem/progenitor cells, the RNA-binding protein MSI1 enhances the localization of LIN28 to the nucleus and, synergistically with LIN28A, blocks the cropping of pri-let-7 (Kawahara et al., 2011). By contrast, LIN28B predominantly localizes to the nucleolus, where it sequesters pri-let-7 to block further processing (Piskounova et al., 2011). In the cytoplasm, LIN28 binds pre-let-7 to block its processing by Dicer and instead induce its oligo-uridylation (Heo et al., 2008). In mammals, TUT4 and, to a lesser extent, TUT7 catalyze the oligo-uridylation (Hagan et al., 2009; Heo et al., 2009; Thornton et al., 2012). Once oligo-uridylated, pre-let-7 is more rapidly degraded than unmodified pre-let-7 (Heo et al., 2008); in mouse ESCs, the 3′-5′ exonuclease DIS3L2 catalyzes this decay (Chang et al., 2013).
and, acting synergistically with LIN28, blocks the cropping of pri-
let-7, which occurs in the nucleus (Kawahara et al., 2011). By
contrast, LIN28B predominantly localizes to the nucleolus owing to
its nuclear and nucleolar localization signals, where it sequesters
pri-let-7 and further blocks its processing (Piskounova et al., 2011).

In the cytoplasm, LIN28 binds pre-let-7 to block its processing by
Dicer and induces the oligo-uridylation of the 3’ overhang of pre-
let-7 (Heo et al., 2008). In mammals, terminal uridy ltransferase 4
(TUT4; also known as ZCCHC11) and, to a lesser extent, TUT7
(ZCCHC6) catalyze this oligo-uridylation (Hagan et al., 2009; Heo
et al., 2009; Thornton et al., 2012), whereas in C. elegans this is
carried out by the poly(U) polymerase PUP-2 (Lehrbach et al.,
2009). Once oligo-uridy late, pre-let-7 is more rapidly degraded
than unmodified pre-let-7 (Heo et al., 2008), and it has been shown
that, in mouse ESCs, the 3’-5’ exonuclease DIS3L2 catalyzes the
decay of oligo-uridy lated pre-let-7 (Chang et al., 2013). For a recent,
in-depth review of LIN28-mediated control of let-7, the reader is
referred to Mayr and Heinemann (2013).

The let-7-independent pathway

Although the let-7-dependent mechanism is important for LIN28
function in development, it is only half the story of how LIN28
works. A number of papers have demonstrated that, just like in
C. elegans, mammalian LIN28 exhibits let-7-independent roles. For
example, Zhu and colleagues found that transgenic mice with a
skeletal muscle-specific knockout of Lin28 showed impaired glucose
tolerance and insulin resistance, despite no significant change in let-
7 levels (Zhu et al., 2011). In another study, transgenic mice that
overexpressed LIN28 showed increased organ size compared with
their wild-type littermates, even in tissues in which let-7 expression
was unaf fected (Zhu et al., 2010). LIN28 has also been found to alter
gene expression during neurogenesis, prior to let-7 upregulation
(Balzer et al., 2010). Finally, two studies found that LIN28 promotes
ESC proliferation in part by binding to, and increasing the translation
of, cell cycle-related mRNAs (Peng et al., 2011; Xu et al., 2009).
Taken together, it is clear that let-7-independent roles of LIN28 are
important in a number of developmental contexts.

In the search to understand these let-7-independent functions,
small-scale studies have identified a handful of potential LIN28
targets and have begun to elucidate how some of these might
function (Balzer et al., 2010; Polesskaya et al., 2007; Qiu et al.,
2010). However, although most studies found that
association with LIN28 enhances translation (Peng et al., 2011;
Polesskaya et al., 2007; Qiu et al., 2010; Xu and Huang, 2009; Xu
et al., 2009), one demonstrated both enhancing and repressive
effects (Wilbert et al., 2012), one primarily repressive effects (Cho
et al., 2012), while another showed no statistically significant
changes (Hafner et al., 2013). These discrepancies underscore our
limited understanding of the molecular details of let-7-independent
LIN28 functions.

LIN28 in disease and therapy

Understanding the role of LIN28 in disease is a very exciting area
of research. Initial investigations have revealed roles for LIN28 in
glucose uptake and tolerance, diabetes, sickle cell anemia and
cancer (de Vasconcellos et al., 2014; Perez et al., 2013; Shinoda
et al., 2013; Slyh-Chang et al., 2013; Zhu et al., 2011), suggesting
that the modulation of LIN28 activity might be an attractive
therapeutic approach. For example, the expression of LIN28 in
cultured, sickle-shaped erythrocytes resulted in a significant
decrease in their sickle morphology compared with control
erthrocytes (de Vasconcellos et al., 2014). LIN28 has also
proven to be useful in the fields of cellular reprogramming and
regeneration. In each of these scenarios, however, the research is still
in its infancy and many questions remain to be answered.

LIN28 and cancer

LIN28 has a powerful effect on the ability of the cell to maintain a
pluripotent state. Aberrant LIN28 expression can therefore impact
normal development, mainly the switch from the maintenance of a

Table 1. Classes of mRNAs targeted by LIN28

<table>
<thead>
<tr>
<th>Cell cycle regulation</th>
<th>RNA-binding proteins</th>
<th>Histone components</th>
<th>Glucose metabolism</th>
<th>Early embryonic genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclin A, cyclin B, CDK4, CDK12, CCND1/2, CCNB1</td>
<td>FUS/TLS, hnRNP F, TDP-43, TIA-1</td>
<td>Histone H2A, histone H4H, linker histone, H1FX</td>
<td>Insulin receptors, IGF receptors, IRS2/4, AKT1-3, IGF2BP1, IGF2BP3, HMGA2</td>
<td>Peg3, SalI4, Oct4, Lin28b</td>
</tr>
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</table>

Genome-wide as well as smaller scale studies (Balzer et al., 2010; Hafner et al., 2013; Peng et al., 2011; Polesskaya et al., 2007; Qiu et al., 2010; Wilbert et al., 2012; Xu and Huang, 2009; Xu et al., 2009; Yang et al., 2015) have identified thousands of potential LIN28 targets, a subset of which are shown here.
pluripotent state to a cancerous state. Both LIN28 and LIN28B have been identified in a number of tumor and cancer cell types (for reviews see Thornton and Gregory, 2012; Zhou et al., 2013). The inappropriate expression of each LIN28 paralog in these different tumor types results in various combinations of cancer characteristics including invasiveness, larger tumor size, metastasis, poorer prognosis, and increased cell number (Feng et al., 2012; Hamano et al., 2012; King et al., 2011a, b; Liu et al., 2013; Qin et al., 2014). LIN28-mediated control of proliferation and cell division seems to be a key feature in the aggressive nature of these cancers (Feng et al., 2012; Nguyen et al., 2014; Urbach et al., 2014; Wang et al., 2015). For example, in breast cancer, high LIN28 expression increases the size of the tumor and increases cell growth, and this is likely to be a result of increased cell division and proliferation (Feng et al., 2012). LIN28 overexpression in the primitive cap mesenchyme cells of the kidney results in prolonged cell proliferation into adulthood and the prevention of normal postnatal differentiation (Urbach et al., 2014). Not only does the continued expression of LIN28 result in Wilms tumor (pediatric kidney cancer), but its continued expression is required for maintaining the proliferative nature of the cap mesenchyme cells within the tumor (Urbach et al., 2014).

LIN28 in reprogramming and regeneration

Currently, LIN28 is well known for its role in induced pluripotent stem cells (iPSCs). In combination with NANOG, OCT4 (POU5F1) and SOX2, it can reprogram somatic cells to pluripotent stem cells (Yu et al., 2007). OCT4 and SOX2 were shown to be essential components for this induction process, whereas LIN28 and NANOG are non-essential factors. LIN28 and NANOG each increase the reprogramming efficiency when inducing dedifferentiation – NANOG to a greater extent than LIN28 (Yu et al., 2007). In support of this proposed role, it has been shown that LIN28 promotes increased reprogramming by inducing a greater cell division rate and an increased number of cell divisions (Hanna et al., 2009). In the study of tissue regeneration, the overexpression of LIN28 results in increased digit repair, epidermal hair regrowth and pinnal tissue regrowth in the mouse (Shyh-Chang et al., 2013). The success of tissue regeneration is likely to be due to LIN28-mediated control of proliferation of the stem cell and transit-amplifying populations. LIN28 promotes expansion of these cell types leading to an increase in tissue size and regrowth of the damaged region.

Perspectives

In C. elegans, LIN-28 acts in a two-step mechanism: first, regulating early, proliferative cell fates; and second, promoting later differentiating cell fates via regulation of let-7 (Vadla et al., 2012). There is growing evidence that vertebrate LIN28 acts in a similar two-step fashion: first, promoting pluripotency by increasing proliferation; and second, directing cells down specific differentiation pathways.

Several studies suggest that LIN28 promotes pluripotency by promoting cellular proliferation. Human and mouse ESCs rapidly proliferate in a unique cell cycle that is characterized by a shortened G1 phase and a high proportion of cells in S phase. This unique cell cycle and rapid proliferation are thought to be biologically coupled to pluripotency (White and Dalton, 2005). Although it has not been shown that LIN28 promotes this specific cell cycle step, it does promote proliferation in several contexts. First, two separate studies have found that LIN28 expression promotes ESC proliferation (Peng et al., 2011; Xu et al., 2009). Second, LIN28 expression promotes reprogramming (to iPSCs) by accelerating the reprogramming process through more rapid cell divisions (Hanna et al., 2009; Yu et al., 2007). Third, adult mice overexpressing LIN28 are significantly larger (in height and weight) than their wild-type littermates, a phenotype that results from increased cell number (Zhu et al., 2010). Finally, LIN28 knockout mice weigh 20% less at birth than wild-type pups (Zhu et al., 2010), and they also have reduced brain size due to decreased proliferation and enhanced cell cycle exit of neural progenitors (Yang et al., 2015).

The role of LIN28 in development is not limited to promoting pluripotency; it also plays a key role in directing cells to specific committed lineages. During skeletal muscle differentiation, LIN28 induction is essential for terminal differentiation (Polesskaya et al., 2007). Additionally, in Xenopus embryos, cells require LIN28 in order to respond to mesoderm-inducing growth factors and to differentiate properly (Faas et al., 2013). Finally, there is evidence of at least one case in which LIN28 appears to act somewhere between pluripotency and differentiation; in murine adult intestinal crypts, the transition from stem cell to differentiated villus can be spatially visualized and, here, LIN28 is highly expressed in a population of cells transitioning from pluripotency to a differentiated state, possibly the transit-amplifying cells (Yang and Moss, 2003).

Based on this evidence, we believe that the two-step mechanism first elucidated in C. elegans is an evolutionarily conserved feature
of LIN28, placing it as a gatekeeper between pluripotency and differentiation (Faas et al., 2013; Vadla et al., 2012). In C. elegans, the distinct let-7-dependent and let-7-independent molecular mechanisms of LIN28 strongly correlate with its two separate roles in development, but in vertebrates this distinction is less clear. Elucidating how the let-7-independent and let-7-dependent functions of LIN28 work separately and together to execute its two roles in development will provide the key to understanding this unusual regulator of gene expression and cell behavior.

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Competing interests
The authors declare no competing or financial interests.

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