degradation, suggesting that integrins also regulate localized degradation of the BM (Hagedorn et al., 2009). Future studies utilizing mammalian systems with greater integrin signaling complexity are now needed to build on and refine the current model, and, hopefully, to bridge the gap between mechanisms regulating controlled invasion during development and the complex signaling networks promoting human diseases such as cancer.

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LIN28 *lets* BLIMP1 Take the Right Course

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The transcription factor BLIMP1 is a master regulator of primordial germ cell (PGC) specification and is suppressed by the microRNA *let-7*. In a recent issue of *Nature*, West and colleagues use a unique in vitro ES cell differentiation strategy to show that LIN28 is an essential regulator of PGC formation through inhibition of *let-7* maturation and consequential induction of BLIMP1.

PGCs are the first cells in the mouse embryo to become committed to a specific fate. Cells in the early mouse embryo are pluripotent, but a transition occurs around embryonic day (E) 5.5–6.0 when signaling by bone morphogenetic proteins (BMP2, BMP4, and BMP8B) induces proximal epiblast cells to adopt a PGC fate. At about E6.25–6.5 founder PGCs emerge (Figure 1A), and by E7.5 about 40 PGCs can be identified by the expression of PGC-specific markers.

Identifying the factors required for PGC specification in the embryo has been challenging and so far only a few factors have been found. Early studies were rather laborious, relying on differential expression analyses, comparing gene expression in PGC and non-PGC cells, followed by gene knockout studies. These studies nevertheless were able to identify some important PGC regulators such as PRDM14 ([PRDI-BF1-RIZ]/SET domain containing 14) and BLIMP1 (B-Lymphocyte-induced maturation protein1, also known as PRDM1), which are expressed in PGCs starting at the founder stage and are essential for PGC specification (Ohinata, et al., 2005; Vincent, et al., 2005; Yamaji, et al., 2008). Two other proteins, TNAP (tissue nonspecific alkaline phosphatase) and STELLA (also known as DPPA3, developmental pluripotency-associated 3), are also expressed specifically in PGCs, starting at E7.5, but are not essential for PGC formation. West et al. (2009) build on this previous work to create, manipulate and study PGCs outside of the embryo, leading to the identification of LIN28 as a third major player in PGC specification.

To identify PGCs in vitro, West et al. (2009) and Wei et al. (2008) took advantage of 100% concordance of PGCs and STELLA expression. They created mouse ES cells that contain *Stella*-GFP reporter constructs. They then manipulated the cells (by formation of embryoid bodies) and found a small population that expressed GFP, indicating that founder PGCs were forming in vitro, and that the *Stella*-GFP constructs were working as expected (Figure 1B). West et al. (2009) were then able to identify PGC specification genes by testing the effects of lentivirus-delivered shRNA knockdown of 30 candidate genes, selected based on previous differential expression in PGCs versus somatic cells. Because TNAP more precisely marks true PGCs in vitro, the authors used formation of TNAP-positive colonies as their assays for the effects of each knockdown. As expected, knockdown of *Blimp1* dramatically reduced the number of TNAP-positive colonies. Equally effective was knockdown of *Lin28*, an evolutionarily conserved gene that was recently shown to play key roles in ES differentiation. Along with the three transcription factors, OCT4, SOX2, and NANOG, the RNA-binding protein LIN28 can reprogram human fibroblasts into induced pluripotent stem (iPS) cells that resemble human ES cells (Yu, et al., 2007).

Several recent studies are now starting to shed light on the mechanism by which LIN28 functions in ES and other cells. During microRNA biosynthesis, a primary miRNA (pri-miRNA) is processed to a

precursor miRNA (pre-miRNA) and eventually to a mature microRNA (miRNA) that functions to modulate mRNA stability and protein translation. Surprisingly, mouse and human ES cells have very high levels of pri-miRNAs, yet the levels of processed miRNAs are not always as high. In particular, there is a dramatic block in the processing of the abundant and evolutionarily-conserved *let-7* family pri-miRNAs to mature miRNAs (Thomson, et al., 2006). Last year, four groups discovered independently that LIN28 and its paralog LIN28B are key players in *let-7* processing, acting by binding to the *let-7* pre-miRNA and pri-miRNA and altering their processing or stability (reviewed in Bussing et al., 2008; Figure 1C). In a separate study, and in a different context, Nie, et al., (2008) discovered an association between BLIMP1 and *let-7a* in Hodgkin's lymphoma, a type of cancer involving abnormal B cells (i.e., Reed-Sternberg cells). BLIMP1 is a master regulator of B cell differentiation as well. In Reed-Sternberg cells, *let-7a* is highly expressed whereas BLIMP1 is suppressed. Nie et al. (2008) then showed that the *let-7a* miRNA bound to a target sites in the BLIMP1 3′ UTR to suppress BLIMP1 expression. Together these studies suggest that LIN28 and LIN28B suppress *let-7* synthesis in ES cells and likely also during formation of iPS cells, whereas *let-7* suppresses BLIMP1 in lymphoma cells.

The work of West et al. (2009) now connects all of these players in PGCs. Since knockdown of LIN28 or LIN28B in the *Stella*-GFP ES cells by West and colleagues resulted in fewer TNAP-positive colonies, the authors postulated that LIN28 and LIN28B could be acting through *let-7* to suppress BLIMP1 expression. Indeed, expression of a *Blimp1* construct lacking the *let-7* binding site in the *Blimp1* 3′ UTR overrode the block in formation of PGCs caused by LIN28 or LIN28B knockdown. Furthermore, overexpression of *Lin28* induced more TNAP-positive PGCs expressing the correct repertoire of PGC markers (e.g., *Prdm14*). These findings not only confirm the requirement for LIN28 and BLIMP1 in PGC formation, but also demonstrate for the first time that suppression of miRNA biosynthesis is critical for PGC specification (Figure 1C).

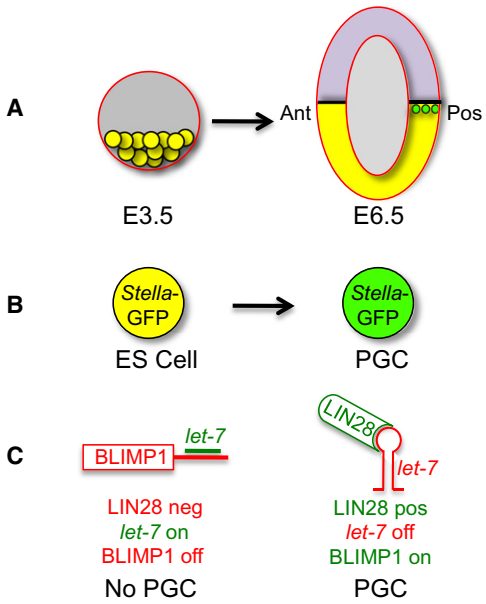

Figure 1. PGC Specification and Interactions of LIN28, *let-7*, and BLIMP1

(A) Mouse ES cells (yellow), derived from E3.5 blastocysts, are pluripotent. At ~E6.5, the founder population of PGCs (green), the first cells in the embryo to commit to a particular fate, forms from epiblast cells (yellow) in the posterior (pos) but not the anterior (ant) part of the embryo.

(B) The *Stella*-GFP transgene is dormant in ES cells, but is transcriptional active when the ES cells are allowed to differentiate into PGCs in vitro.

(C) In the absence of LIN28, *let-7* will bind to the 3′UTR of the BLIMP1 mRNA to block its translation and prevent PGCs from developing (left). During PGC formation, LIN28 binds to the *let-7* family pri-miRNA/pre-miRNA loop to prevent processing of these precursor forms into the mature *let-7* miRNA, allowing BLIMP1 translation, and permitting PGC specification (right).

Several questions arise from the above studies. Is LIN28 expressed continuously in ES cells through formation of PGCs? Since BMPs play such critical permissive roles in the formation of PGC precursors, do they directly regulate LIN28 expression? Would transient suppression of LIN28 in PGCs be useful for dedifferentiating the cells into pluripotent ES cells? These studies may also have implications in the field of reproduction. Because overexpression of LIN28 in mouse ES cells is an effective way to direct the cells toward a PGC fate, it may be possible to discover additional genes required during PGC specification as well as downstream genes that can cause the PGCs to fully differentiate into fertilizable male or female germ cells. For example, sequential expression of stage-specific reporters may make it easier to identify growth conditions and gene products required for completion of oogenesis and spermatogenesis. Ultimately, the results of such studies could be applied to human ES cells to generate a ready supply of human oocytes and spermatozoa.

Besides their roles in the germline, Daley's laboratory also implicates LIN28 and LIN28B as major players in cancer. While the West et al. (2009) study demonstrated that multiple human testicular germ cell tumors express these proteins, Viswanathan, et al., (2009) showed that 15% of cancers overexpress LIN28 and/or LIN28B and repress *let-7*. Furthermore, during key developmental junctures, *LIN28* is also a target of *let-7*, resulting in an important feedback to downregulate LIN28. Although it is unknown whether overexpression of LIN28 is a driver or a passenger in BLIMP1-mediated oncogenesis and other cancers, the recent studies implicate LIN28 and LIN28B as important control proteins, low levels of which may dictate a "good year" for the patient.

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