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Metabolic RemodeLIN of Pluripotency

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Cellular metabolism is a key regulator of cell fate, including fate in pluripotent stem cells. Now in *Cell Stem Cell*, Zhang et al. (2016) show that Lin28 controls the metabolic transition from naive to primed pluripotency by directly repressing oxidative metabolism genes and metabolic intermediates involved in epigenetic regulation independently of let-7.

Metabolism, the processes required to generate cellular energy, has long been known to be essential for cellular work and enzymatic reactions that keep the cells alive and replicating. Cellular metabolism consists of oxidative processes to harness the energy of macromolecules. Different cell types, due to different environmental, functional, and cellular demands, have evolved to utilize different substrates or different levels of mitochondrial activity to fulfill their cellular energy demands. Perhaps surprisingly, changes in cellular metabolism have turned out to be a leading, and perhaps causal, event during cellular fate changes. Metabolism of pluripotent stem cells (PSCs) has recently been the focus of intense study. Two main stable pluripotent stages, albeit with some heterogeneity among the populations, have been derived from mouse and human PSCs. These naive and primed PSC stages, representing preimplantation and postimplantation cells, respectively, utilize very different strategies for energy production (Greer et al., 2012; Sperber et al., 2015; Zhou et al., 2012). Although both populations utilize glycolysis, naive PSCs further use fatty acid beta-oxidation to generate ATP through mitochondrial respiration. Although some factors, such as HIF, are known to be important for this metabolic shift, the determinants of how cells enter and exit this very unique metabolic state are poorly understood. In this issue of *Cell Stem Cell*, Zhang et al. (2016) reveal a new let-7-independent function for Lin28 in the regulation of PSC metabolism. They show that LIN28 directly binds mRNAs of oxidative phosphorylation genes and has a repressive effect of their corresponding protein abundance, correlating with the induction of a metabolic shift from a fibroblast to reprogrammed iPSC and from a naive to primed metabolic state.

Lin28 is a conserved RNA-binding protein that was first identified in *C. elegans* in a screen for genes causing defects in developmental timing (Tsialikas and Romer-Seibert, 2015). Further studies revealed that Lin28 plays an important role in promoting cellular proliferation. Transgenic mice overexpressing Lin28 exhibit larger body size while Lin28A KO mice weigh 20% less than wild-type at birth. Lin28a/b double knockout embryos show developmental delay and die at E10.5–12.5 (Shinoda et al., 2013). *Lin28a* and *Lin28B* paralogs are both highly expressed in mammalian pluripotent stem cells compared to differentiated cells. Furthermore, Lin28a was revealed to be a critical reprogramming factor (Tsialikas and Romer-Seibert, 2015), partly due to its effects on increasing cell division rate. These studies have shown that Lin28 is critical for PSC self-renewal and have suggested discrete roles for these paralogs. Lin28 regulates the stability of a microRNA called let-7. Although the Lin28/let-7 axis is important for cellular metabolism and regulation of pluripotency, Lin28 can directly bind and regulate the translation of many mRNA encoding proteins involved in cell cycle, splicing factors, and metabolism (Tsialikas and Romer-Seibert, 2015) independently of let-7.

Zhang et al. (2016) asked how LIN28 functions mechanistically to control reprogramming and whether both isoforms play equivalent roles during reprogramming of fibroblasts into induced pluripotent stem cells (iPSCs). They found that both isoforms of LIN28, a and b, are required for maximal reprogramming efficiency, but that iPSCs could also be generated from MEFs in which Lin28a and Lin28b (*Lin28ab*$^{-/-}$) have been deleted. These findings suggested that LIN28 is not required for acquisition of naive pluripotency in mouse cells. However, *Lin28ab*$^{-/-}$ iPSCs in serum/LIF media displayed lower expression of some stem cell markers (such as Nanog, Sox2, and Tbx3) than wild-type iPSCs. Compared to microarray gene expression profiles of wild-type counterparts, *Lin28ab*$^{-/-}$ iPSCs were enriched for metabolism-related GO terms. Consistently, *Lin28ab*$^{-/-}$ iPSCs displayed an increased reliance on oxidative metabolism. Since the metabolic switch from oxidative metabolism used in MEFs to glycolysis is an important step during reprogramming into primed PSCs (Mathieu et al., 2014; Prigione et al., 2010), the authors asked whether Lin28 is required for the conversion to a primed pluripotent state. They found that overexpression of Lin28 directly facilitates conversion of naive PSCs to a primed state and that knockout of Lin28 in naive PSCs inhibits their conversion into primed pluripotency. Zhang et al. then performed a powerful combination of genomics, proteomics, and metabolomic analyses to directly show that Lin28 inhibits mitochondrial oxidative function in primed ESCs, at least in part by directly binding the mRNAs of OXPHOS genes to control their translation.

The authors also assessed how LIN28 may influence the epigenetic status of PSCs. Deposition of key epigenetic marks is known to be dependent on the availability of several metabolites. S-adenosyl methionine (SAM) metabolism is a key component of histone methylation marks and is required for maintenance of the undifferentiated state in mouse and human PSCs (Shiraki et al., 2014; Shyh-Chang et al., 2013). Furthermore, SAM levels in naive human PSCs are reduced by Nicotinamide N methyl transferase (NNMT) to maintain low levels of H3K27me3 marks (Sperber et al., 2015). NNMT consumes

SAM in naive cells, making it unavailable for histone methylation. Zhang et al. found reduced generation of SAM in Lin28 KO iPSCs compared to controls, which correlated with hypomethylation of H3K9 and H3K27me3 epigenetic histone marks that are known to change in transitions between naive and primed pluripotent states. Since the catalytic component of PRC2, histone methyltransferase EZH2, is a known let-7 target, it will be interesting to specifically define the mechanisms of how LIN28 regulates H3K27me3 deposition.

Additional pathways are involved in the metabolic shift from naive to primed ESC transition and during the reprogramming process, including that of HIF, which can activate glycolysis (Zhou et al., 2012; Mathieu et al., 2014). However, it was previously not clear how HIF could reduce mitochondrial OXPHOS gene expression. The studies in the present paper have revealed a potential explanation for this outstanding question, suggesting that HIF may activate glycolysis while Lin28 reduces oxidative mitochondrial activity. It will therefore be very informative in the future to further dissect the mechanisms of Lin28-dependent regulation of mitochondrial activity as well as its interactions with other pathways known to control the metabolism of pluripotency.

While the new findings are extremely tantalizing, many questions remain. It is unclear how Lin28 specifically binds mRNAs of electron transport chain genes. Since these functional analyses were performed in different stages of mouse PSCs, analyzing Lin28 function in different human PSCs is an important next step. Furthermore, it is important to establish if let-7 plays a role in naive to primed transition in human PSCs, since let-7 is differentially expressed in naive and primed hESCs (Sperber et al., 2015). Since recent data have shown differences in naive and primed states between human and mouse cells, it is important to further reveal which conclusions can be extended to human pluripotency. Future studies should shed light onto these intriguing, outstanding questions.

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Modeling Zika Virus Infection in Mice

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Understanding the link between Zika virus (ZIKV) infection and microcephaly requires in vivo models of ZIKV infection in pregnant adults and fetuses. Three studies recently generated such mouse models of ZIKV infection, which corroborate previous in vitro evidence linking ZIKV infection and apoptosis induction in neurons and progenitors to microcephaly.

Zika virus (ZIKV) transmission in the Americas continues to cause international concerns and the upcoming 2016 Olympics in Brazil only highlight these ever-growing worries. The more researchers study the natural history of the virus, the more troubling the results appear. The development of an animal model to begin to understand the underlying pathology of ZIKV infection, especially within pregnant individuals and fetuses, is absolutely critical. Luckily, a herculean effort by several groups has resulted in the timely reporting of several murine models to study ZIKV, including adult models