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# A Glycolytic Solution for Pluripotent Stem Cells

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## Abstract

Glycolysis is an essential component of cellular metabolism associated with pluripotent stem cells (PSCs). Two new papers, Gu et al. in Cell Stem Cell and Zhang et al. in Cell Reports, demonstrate that glycolytic flux is dynamically increased in human primed PSCs upon feeder-free cultivation or conversion into naïve state.

## **Main text**

Pluripotent stem cells (PSCs) preferentially rely on glycolytic metabolism, despite the relatively limited efficiency in energy output compared to oxidative metabolism (reviewed in (Ryall et al., 2015)). Several explanations for this counterintuitive regulation have been put forth, including the need to increase the biosynthetic pathways through the pentose phosphate pathway (PPP) to accommodate biomass requirements in rapidly cycling cells and the necessity to prevent genomic damage caused by free radicals generated as by-products of mitochondrial oxidative phosphorylation (OXPHOS). Moreover, glycolysis-derived metabolites may represent important modulators of stem cell epigenetics (Ryall et al., 2015). Consequently, a dramatic glycolytic shift occurs during cell fate conversion (from somatic cells to PSCs or from PSCs to differentiated progenies (Prigione et al., 2010). Two new papers in *Cell Stem Cell* and *Cell Reports* (Gu et al., 2016; Zhang et al., 2016) employ metabolic flux analysis to demonstrate that PSCs, even while maintaining pluripotency, dynamically modulate their glycolytic metabolism in response to changes in the culture conditions or conversion between different PSC states.

Gu et al. (2016) investigated the metabolic changes occurring during the transition between human naïve and primed pluripotent states. These two PSC states respectively represent pre-implantation cells in the inner cell mass and post-implantation cells in the epiblast (Takashima et al., 2014; Theunissen et al., 2014). Previous studies demonstrated that human and mouse naïve PSCs contain mitochondria with more immature morphology than primed PSCs (Zhou et al., 2012). At the same time, human and mouse naïve PSCs exhibit increased OXPHOS (Takashima et al., 2014; Zhou et al., 2012). Now Gu et al. (2016) demonstrate that human naïve PSCs, both newly derived and those cultured in previously reported reversion conditions (Takashima et al., 2014; Theunissen et al., 2014), in addition to higher mitochondrial oxygen consumption also display elevated glycolytic rates. Human naïve PSCs consumed more glucose, produced more lactate, and incorporated more glucose

carbons into lactate and into purine and pyrimidine nucleotides, implying increased flux towards glycolysis and the PPP. This is in contrast with the reduction of glycolytic metabolism previously found in mouse naïve PSCs (Zhou et al., 2012). Gu et al. (2016) suggest that this discrepancy may be due to species-related differences. In particular, they observed that nuclear C-MYC, which promotes glucose metabolism, is higher in primed PSCs in mice, while it is higher in naïve PSCs in humans. Accordingly, using RNA-Seq analysis, Gu et al. (2016) identified significant enrichment of MYC-regulated gene sets in human naïve versus primed PSCs.

The concomitant raise of OXPHOS and glycolysis observed by Gu et al. (2016) might be a consequence of the bivalent metabolic state of naïve PSCs, which can switch to one or the other metabolic route according to the demand (Zhou et al., 2012). In fact, it has been suggested that glycolysis may represent the basal energy source for naïve PSCs and mitochondrial respiration their spare capacity (Carbognin et al., 2016). Accordingly, inhibition of glycolysis is not as detrimental for naïve PSCs as it is for primed PSCs (Takashima et al., 2014). It would be interesting to compare the effects of OXPHOS inhibition. Perhaps, naïve and primed PSCs would equally survive as they both rely on glycolysis. Overall, naïve PSCs appear equipped with a metabolic program that enables efficient adaption to different environmental conditions, including those not favorable for glycolysis, which might also partially explain their enhanced clonogenic properties.

Signaling molecules and culture conditions play a contributing role in the primed to naïve conversion. LIF is employed in the majority of naïve media (Gu et al., 2016; Takashima et al., 2014) and activates mitochondrial respiration (Carbognin et al., 2016). Activin is included in some naïve media (Gu et al., 2016; Theunissen et al., 2014) and may stimulate glycolysis through the stabilization of Hypoxia-Inducible Factor-1 alpha (HIF1a) (Zhou et al., 2012). HIF1a is a master metabolic regulator contributing to the glycolytic shift associated

with the induction of pluripotency (Prigione et al., 2014). Its role in the metabolism of human naïve PSCs warrants further investigation.

Zhang et al. (2016) further explored the impact of culture conditions on pluripotency through a comprehensive metabolic assessment of human primed PSCs grown as feeder-free (FF) or as feeder-supported (FS) cultures. Elevated glucose flux towards glycolysis and the PPP was detected in FF versus FS PSCs. Similarly, Gu et al. (2016) observed increased glucose metabolism and glucose incorporation into biosynthetic pathways in FF PSCs. In addition, Gu et al. (2016) found that FF PSCs were more sensitive to the inhibition of MCT1, a target of MYC-driven glycolysis. This proliferation defect was rescued by the addition of feeder-conditioned media. The findings of Zhang et al. (2016) may provide a plausible explanation for this effect, since a lack of lipid supplementation was identified within the media commonly employed for FF cultivation. Zhang et al. (2016) demonstrate that FF PSCs increase glutamine consumption and glutamine-mediated reductive carboxylation. This latter pathway can be active upon HIF1a stabilization and is important for lipid biosynthesis. *De novo* lipogenesis was indeed increased in FF cultures. Remarkably, lipid supplementation was sufficient to convert key metabolic features of FF PSCs back to a state similar to that of FS PSCs. Therefore, nutrients contained within PSC media may dramatically influence PSC metabolism. In light of these findings, we speculate that lipid supplementation of naïve PSCs might potentially enable their FF cultivation.

Importantly, Zhang et al. (2016) showed that mitochondrial flux is also active in primed FS PSCs and significantly increased in FF PSCs upon lipid supplementation. Hence, the maintenance of human primed pluripotency might also depend on mitochondrial metabolism. In accordance, elevated alpha-ketoglutarate ( $\alpha$ KG), which promotes histone/DNA demethylation and blocks the mitochondrial ATP synthase, can support mouse naïve PSCs but significantly accelerates the differentiation of human primed PSCs (TeSlaa et al., 2016).

Altogether, glycolysis remains a defining feature of pluripotency. Nevertheless, PSCs can dynamically modulate their metabolism in response to media composition, oxygen tension, and signaling molecules (**Figure 1**). Since each cell fate is characterized by a defined transcriptional signature, we may expect the same for metabolic traits. The dynamic nature of metabolism indicates that assigning a unique *bona fide* metabolic signature to cell fates might turn out to be very challenging.

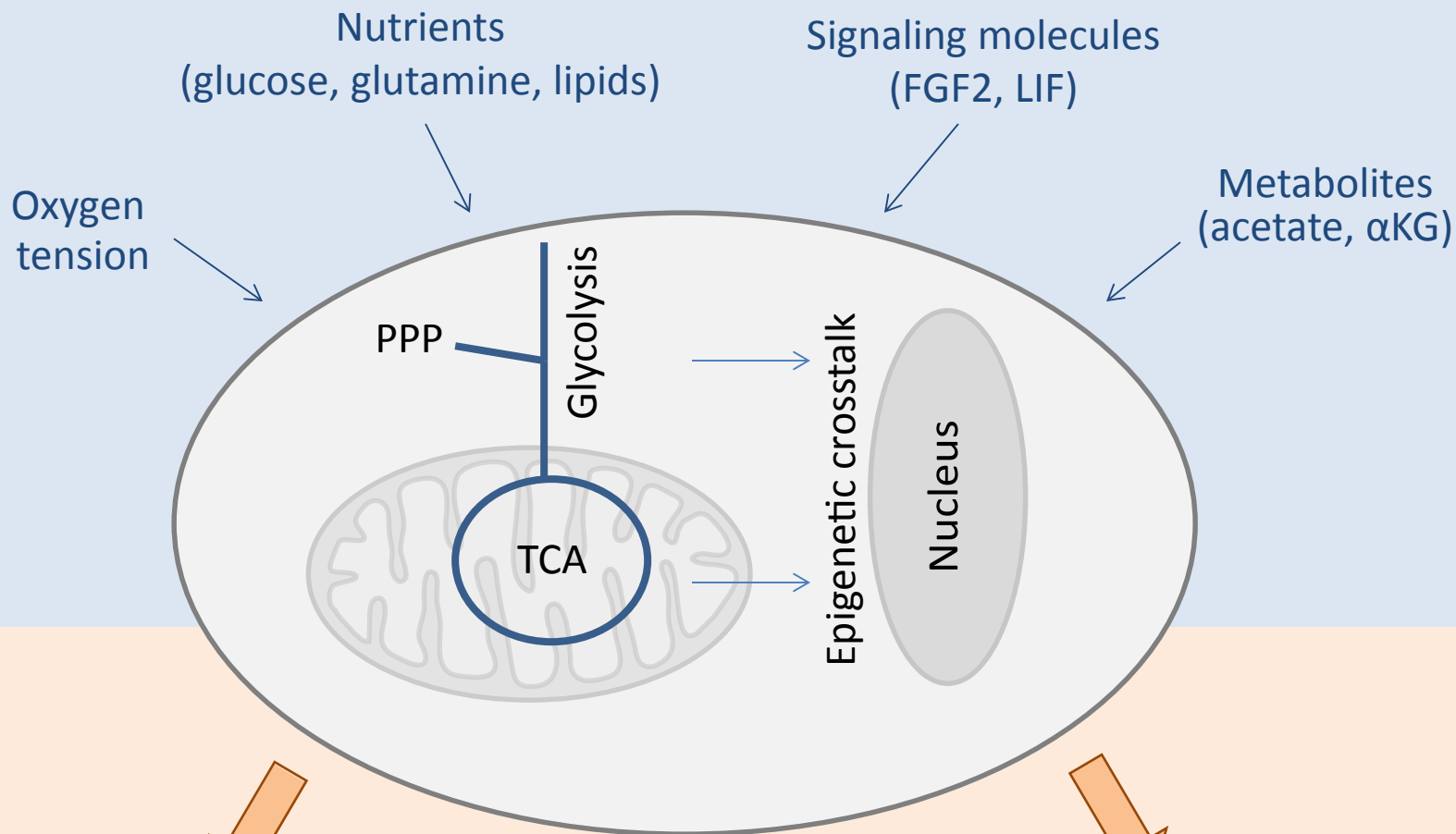
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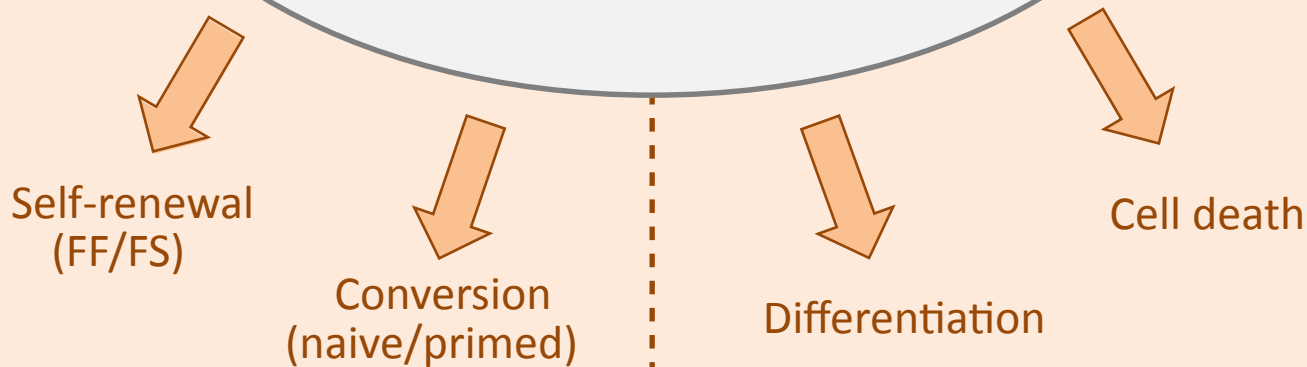
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**Figure legend. Metabolic control of pluripotent cell fates.** The energy metabolism of pluripotent stem cells (PSCs) is modulated by environmental cues, including nutrients within the media, oxygen tension, and signaling molecules. Metabolism can in turn influence cellular epigenetics through metabolites derived from glycolysis and the tricarboxylic acid (TCA) cycle or exogenously provided. This metabolic-epigenetic crosstalk contributes to define the cellular identity. As a result, the fate of PSCs can be the maintenance or the loss of pluripotency. PPP: pentose phosphate pathway; FF: feeder-free; FS: feeder-supported;  $\alpha$ KG: alpha-ketoglutarate.

ENVIRONMENTAL CUES



CELL FATE OUTCOMES



**Pluripotency**

**Loss of pluripotency**