OUTLOOK

A serine metabolic enzyme is flexing its muscle to help repair skeletal muscle

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Metabolic reprogramming of stem cells is a targetable pathway to control regeneration. Activation of stem cells results in down-regulation of oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) and turns on glycolysis to provide fuel for proliferation and specific signaling events. How cell type-specific events are regulated is unknown. In this issue of *Genes & Development* Ciuffoli and colleagues (pp. 151–167) use metabolomic, gene inactivation, and functional approaches to show that phosphoserine aminotransferase (Psat1), an enzyme in serine biosynthesis, is activated in muscle stem cells and contributes to cell expansion and skeletal muscle regeneration via the production of α-ketoglutarate and glutamine.

Metabolic pathways are complex and interrelated, as depicted in vast and complicated charts that are studied in biochemistry courses. Those charts usually contain all the possible pathways a cell can use. However, the importance and contribution of any given pathway to specific cell types and particular cellular mechanisms are harder to establish. Gene expression profiling and metabolic screens provide a plethora of information on regulated gene and metabolite levels and on rate-limiting steps. However, mechanistic and causal relationships are harder to establish due to the interrelatedness of metabolic pathways. In recent years the concept of metabolic reprogramming emerged, positing that shifts in sources of energy or activation of particular metabolic pathways not only represents the adaptation to extrinsic cues but also serve as regulatory pathways bringing about changes in cellular phenotypes. Such reprogramming events have been documented for immune cells and also for stem cells (Zhou et al. 2012; Takashima et al. 2014; Sperber et al. 2015) and represent a balancing act between the constraints provided by extrinsic conditions (inducing signal) and the intrinsic (metabolic) requirements of a given cellular state. In stem cells transitioning from the naïve state to the primed, the pluripotent state of mouse stem cells reduces OXPHOS and increases rates of glycolysis (Shyh-Chang and Ng 2017). This is initiated by high expression of glucose transporters (Leese 1995; Zhou et al. 2012). Similar observations have been made for adult stem cells as well, such as skeletal muscle stem cells, which in their quiescent state have an SIRT1-activated PGC1α to promote FAO in the mitochondria. PGC1α activates OXPHOS genes and suppresses glycolysis, resulting in high levels of NAD^+ and an active SIRT1 (Wu et al. 1999; Gerhart-Hines et al. 2007). Once activated, muscle stem cells deactivate FAO and turn on glycolysis (Ryall et al. 2015). This metabolic switch decreases NAD^+, deactivates SIRT1 and its histone H4K16 deacetylation activity, and activates myogenic transcription programs and muscle differentiation. However, the switch between these two metabolic states becomes less efficient over time, which leads to a reduced number of muscle stem cells and a reduced capacity to self-renew. Therefore, any mechanistic insight into the causative relationships between the metabolic switch, stem cell activation, and muscle fiber formation can have significant therapeutic implications.

Ciuffoli et al. (2024) succeeded in identifying such a pathway in muscle stem cells in mice. Their primary observation was that enzymes of the serine biosynthetic pathway (Phgdh, Psat1, and Psph) (Fig. 1) are barely detectable in quiescent muscle stem cells and rapidly induced upon activation or injury. Out of the three, only Psat1 proved to be essential for in vitro muscle cell line proliferation; thus, they focused on this one in their subsequent studies. Psat1 catalyzes 3-phosphohydroxypyruvate and

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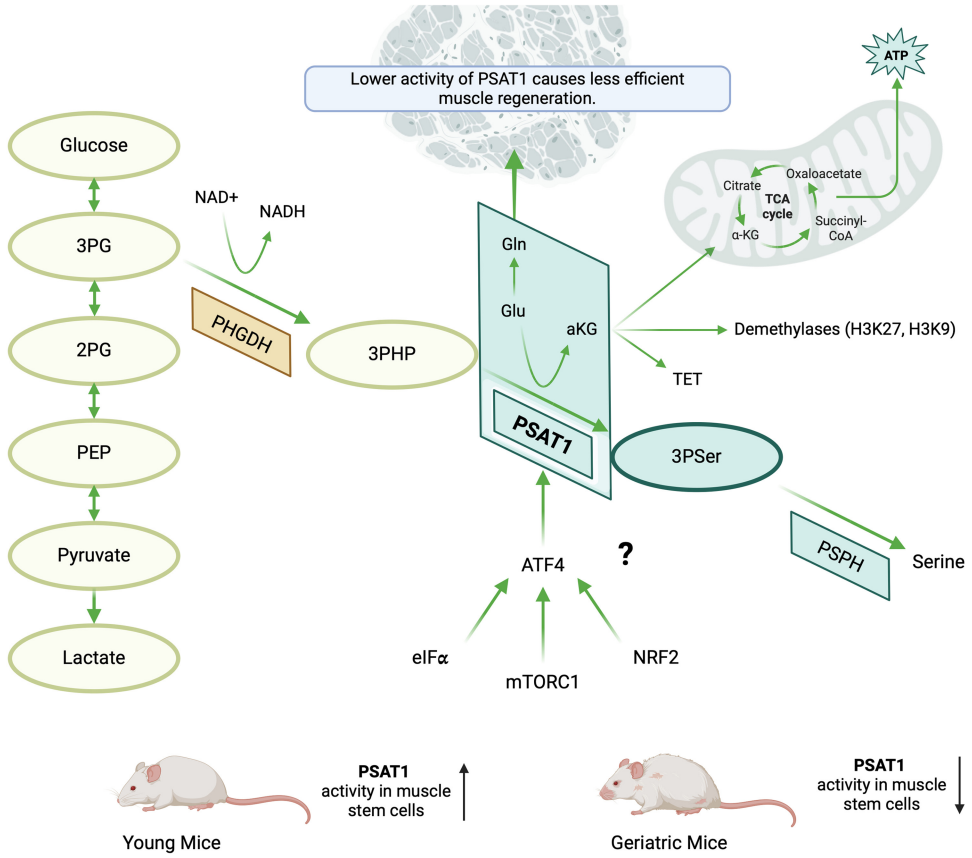

Figure 1. The integration of Psat1 into glycolysis and the serine biosynthetic pathway and its contribution to additional signaling mechanisms. Glycolysis converts glucose to lactate via 3-phosphoglycerate (3PG), 2-phosphoglycerate (2PG), phospho-enol-pyruvate (PEP), and pyruvate. De novo serine biosynthesis from 3PG to 3-phosphohydroxypyruvate (3PHP) to 3-phosphoserine (3P-Ser) to serine via the enzymes phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase (Psat1), and phosphoserine phosphatase (PSPH). α-Ketoglutarate (αKG) is converted to glutamate, and glutamine synthase 2 converts it to glutamine. αKG is required for demethylases and ten eleven translocation (TET), a methylcytosine dioxygenase. αKG also contributes to the tricarboxylic acid (TCA) cycle in the mitochondrion. PSAT1 is known to be regulated by the transcription factor ATF4 via input from mTORC1, eIFα, and NRF2 (He et al. 2023). It is not known whether this pathway is active in activated muscle stem cells. The green-shaded rectangle shows the pathway identified by Ciuffoli et al. (2024) as contributing to muscle stem cell proliferation and tissue regeneration. (Figure created with BioRender.com.)

glutamate into 3-phosphoserine and α-ketoglutarate. It integrates metabolic pathways critical for cell proliferation, survival, migration, and epigenetics, such as glycolysis, de novo serine synthesis, citric acid cycle, and one-carbon metabolism. Therefore, all these activities had to be considered in rigorous mechanistic studies. The role of Psat1 was further focused by their metabolomic findings showing that aminomalonate, glycine, glucose-1-phosphate, and α-ketoglutarate (αKG) were significantly reduced upon siRNA-mediated knockdown of *Psat1* while relevant amino acid (serine, methionine, glutamic acid, cysteine, and alanine) levels were not changed. By generating an inducible *Pax7*-driven *Cre* knockout mouse line, they could demonstrate further that Psat1 is indeed required for muscle regeneration in an acute muscle injury paradigm. It is well established that αKG is linked to dioxygenases, including histone H3K27 and H3K9 demethylases and the TET family of DNA demethylases (Klose and Zhang 2007; Carey et al. 2015; Islam et al. 2018). These observations naturally raised the intriguing and very plausible hypothesis that one of these pathways might be involved in the phenotype switch. The investigators painstakingly examined these pathways using epigenomic methods and came up empty-handed. They could not detect meaningful changes; thus, the culprit must be somewhere else. Another plausible scenario for the observed changes is that αKG is also a key tricarboxylic acid (TCA) cycle intermediate, so its reduced level might affect mitochondrial TCA (Fig. 1). Relevant mitochondrial parameters (mitochondrial content and basal, maximal, and spare mitochondrial respiratory capacities) were unchanged in the Psat1-depleted myoblasts. This left one more pathway to examine: the conversion of αKG to glutamate. They hit the jackpot there. They found that glutamate levels were reduced and the addition of αKG normalized the glutamate levels. In addition, glutamine could improve *Psat1* knockout cell proliferation. Finally, to support the in vivo relevance of their findings and open up potential therapeutic utility, they demonstrated that both αKG and glutamine can improve muscle

regeneration upon injury and in geriatric mice. The success of this tour de force approach to examine all facets of a complex metabolic hub could yield not only exciting novel insights into muscle stem cell activation and proliferation but also therapeutically relevant findings. Further work is needed to determine the upstream signal(s) and pathways initiating the induction of Psat1. Some promising candidates include ATF4, either via the mTORC1, eIF2α, or NRF2 pathways (Fig. 1; He et al. 2023). The identity of downstream mechanisms and to what extent the pathway identified by Ciuffoli et al. (2024) is involved in other stem cell niches remain to be explored. Finally, the translatability of such mechanistic, metabolic studies affords new opportunities to target human diseases such as age-related muscle atrophy, sarcopenia, and cachexia and would be of great value to researchers and physicians alike.

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References

Carey BW, Finley LW, Cross JR, Allis CD, Thompson CB. 2015. Intracellular α-ketoglutarate maintains the pluripotency of embryonic stem cells. *Nature* **518:** 413–416. doi:10.1038/nature13981

Ciuffoli V, Feng X, Jiang K, Acevedo-Luna N, Ko KD, Wang AHJ, Riparini G, Khateb M, Glancy B, Dell'Orso S, et al. 2024. Psat1-generated α-ketoglutarate and glutamine promote muscle stem cell activation and regeneration. *Genes Dev* (this issue). doi:10.1101/gad.351428.123

Gerhart-Hines Z, Rodgers JT, Bare O, Lerin C, Kim SH, Mostoslavsky R, Alt FW, Wu Z, Puigserver P. 2007. Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1α. *EMBO J* **26:** 1913–1923. doi:10.1038/sj.emboj.7601633

He L, Ding Y, Zhou X, Li T, Yin Y. 2023. Serine signaling governs metabolic homeostasis and health. *Trends Endocrinol Metab* **34:** 361–372. doi:10.1016/j.tem.2023.03.001

Islam MS, Leissing TM, Chowdhury R, Hopkinson RJ, Schofield CJ. 2018. 2-Oxoglutarate-dependent oxygenases. *Annu Rev Biochem* **87:** 585–620. doi:10.1146/annurev-biochem-061516-044724

Klose RJ, Zhang Y. 2007. Regulation of histone methylation by demethylimination and demethylation. *Nat Rev Mol Cell Biol* **8:** 307–318. doi:10.1038/nrm2143

Leese HJ. 1995. Metabolic control during preimplantation mammalian development. *Hum Reprod Update* **1:** 63–72. doi:10.1093/humupd/1.1.63

Ryall JG, Cliff T, Dalton S, Sartorelli V. 2015. Metabolic reprogramming of stem cell epigenetics. *Cell Stem Cell* **17:** 651–662. doi:10.1016/j.stem.2015.11.012

Shyh-Chang N, Ng HH. 2017. The metabolic programming of stem cells. *Genes Dev* **31:** 336–346. doi:10.1101/gad.293167.116

Sperber H, Mathieu J, Wang Y, Ferreccio A, Hesson J, Xu Z, Fischer KA, Devi A, Detraux D, Gu H, et al. 2015. The metabolome regulates the epigenetic landscape during naive-to-primed human embryonic stem cell transition. *Nat Cell Biol* **17:** 1523–1535. doi:10.1038/ncb3264

Takashima Y, Guo G, Loos R, Nichols J, Ficz G, Krueger F, Oxley D, Santos F, Clarke J, Mansfield W, et al. 2014. Resetting transcription factor control circuitry toward ground-state pluripotency in human. *Cell* **158:** 1254–1269. doi:10.1016/j.cell.2014.08.029

Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, et al. 1999. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* **98:** 115–124. doi:10.1016/S0092-8674(00)80611-X

Zhou W, Choi M, Margineantu D, Margaretha L, Hesson J, Cavanaugh C, Blau CA, Horwitz MS, Hockenbery D, Ware C, et al. 2012. HIF1α induced switch from bivalent to exclusively glycolytic metabolism during ESC-to-EpiSC/hESC transition. *EMBO J* **31:** 2103–2116. doi:10.1038/emboj.2012.71

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