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Embryos burn fat in standby

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Embryonic and adult stem cells enable development and regeneration. Embryonic cells, like adult stem cells, can enter dormancy as part of their lifecycle. Recent evidence suggests that this cellular transition to dormancy requires active rewiring of metabolism. The dormancy-induced metabolic switches in embryonic and adult stem cells are explored here.

Development on hold

Over 130 mammalian species control the timing of the development of their offspring by delaying implantation. This phenomenon, known as embryonic diapause, preserves fertilized embryos in a reversibly dormant state, thereby temporarily halting further development (Box 1) [1]. In most mammals, diapause occurs at the blastocyst stage, after the specification of embryonic and extraembryonic cells and just before implantation and further tissue specification [1]. Laboratory models of diapause mimicking the maternal hormonal regulation (through ovariectomy) or signals suppressing cell growth in the embryo [through mammalian target of rapamycin (mTOR) inhibition] are used to understand cellular mechanisms that enable a successful transition in and out of this dormant state [2]. Embryos in diapause share features with adult stem cells, including metabolic and epigenetic adaptations to sustain cellular dormancy.

A metabolic switch is necessary to endure dormancy

While the blastocyst-stage embryo as a whole becomes dormant during diapause, the embryonic and extraembryonic cells within the embryo show different growth dynamics during this transition [2,3]. While the inner cell mass (ICM), which contains the pluripotent cells that are progenitors of embryonic tissues, maintains or reduces the number of cells, the extraembryonic trophectoderm (TE) continues to proliferate at residual levels even during diapause [4]. This is likely due to the higher mTOR pathway activity in the TE compared with the ICM [5]. *In vitro* derivatives of embryonic and extraembryonic cells [i.e., embryonic stem cells (ESCs) and trophoblast stem cells (TSCs)] reflect the distinct dynamics of ICM and TE and also respond differently to induction of dormancy in culture. When cultured in the presence of a catalytic mTOR inhibitor, ESCs efficiently establish and maintain a dormant pluripotent state, which is characterized by reduced proliferation, maintenance of cellular identity and developmental competence, and the ability to reactivate and resume proliferation upon release from mTORi [3]. TSCs, however, gradually lose stemness and start to differentiate under mTORi, despite successfully reducing proliferation and overall metabolic activity. These findings suggest that the transition to dormancy is a multi-step process that entails more than a first-line response to reduce energy expenditure and is regulated in a tissue-specific manner even within the pre-implantation embryo.

The laboratory models of diapause were recently used to systematically identify these adaptive events in the cellular transition to dormancy [3]. Time-resolved proteomics, together with metabolic profiling, revealed a metabolic switch in pluripotent cells as they enter dormancy. Following the first-line response to mTORi, such as reducing protein synthesis, ESCs switch from the energy-dense glycolytic metabolism to using lipids stored in lipid droplets instead. Lipid droplets are lipid storage organelles that also serve as 'emergency energy reserves'. Fatty acids are released from lipid droplets as required and are typically transported from the cytosol into mitochondria via the enzyme carnitine palmitoyltransferase 1a (CPT1a), which conjugates them with carnitine. In stark contrast to pluripotent embryonic cells that consume fatty acids during dormancy, extraembryonic TE cells instead accumulate lipids in large lipid droplets. Prolonged dormancy under mTORi compromises TE quality and embryo survival, which may be due to the inefficient use of lipids in this tissue [3,6]. In support of this argument, supplementation of dormant embryos with carnitine-conjugated fatty acids, carnitine itself, or carnitine-precursor branched chain amino acids significantly improves embryo survival in culture, whereas CPT1a inhibition or delipidation of embryos is detrimental [3,7]. Thus, fatty acid usage appears to be critical in maintaining mouse embryos in a dormant state for later reactivation (Figure 1).

Fatty acid oxidation is also upregulated in dormant adult stem cells such as skeletal muscle stem cells (MuSCs), hippocampal neural/progenitor cells (NSPCs), hematopoietic stem cells (HSCs), endothelial cells, and cardiomyocytes [8–12]. The promyelocytic leukemia–peroxisome proliferator-activated receptor δ (PPARδ)–fatty-acid oxidation axis is essential for the maintenance of dormancy in HSCs and inhibition of CPT1a induces HSC overactivation and exhaustion [8]. Similarly, elevating cellular malonyl-CoA, a natural CPT1a inhibitor, leads to activation of NSPCs [10]. The transition from dormancy to proliferation of MuSCs is also accompanied by a switch from fatty acid oxidation back to glycolysis [9]. Loss of the muscle-specific CPT1 isoform CPT1b stimulates cardiomyocyte proliferation [11]. The critical role of fatty acid oxidation is best exemplified in cardiomyocytes, which normally cease proliferation during the first weeks after birth, however, they regain their proliferative potential upon inhibition of fatty acid oxidation. Thereby, this metabolic switch may contribute to heart regeneration. More generally, fine-tuned fatty acid oxidation

Box 1. Features of embryonic diapause

Embryonic diapause is a reversible dormant state during pre-implantation embryo development [1]. When in diapause, embryo implantation and further development is delayed. Diapause can last from several days up to 11 months, depending on the species. In most mammals, the possibility of diapause is restricted to the blastocyst stage. The blastocyst comprises of the embryonic epiblast cells and extraembryonic cells, which give rise to the embryo proper and the extraembryonic tissues such as the placenta and extraembryonic endoderm, respectively. *In vivo*, diapause is initiated by maternal signals to the embryo, such as micro-RNAs, polyamines, and lack of amino acids [1]. The uterus becomes nonreceptive, while providing an embryonic microenvironment that supports survival, but temporarily restricts proliferation. A diapause-like state can be induced *in vitro* in both embryos and ESCs through chemical inhibition of the cellular nutrient sensor mammalian target of rapamycin (mTOR) [2]. A successful switch in and out of dormancy is made possible by first reducing anabolic activity (reduced translation, translation, metabolic output), followed by epigenetic and metabolic rewiring, including increase in heterochromatin and reliance on fatty acid degradation [2,3,15]. Diapaused embryos reactivate in response to replenishing of nutrients and growth factors and lifting of mTOR inhibition.

appears essential for the maintenance of dormant stem cell pools in adult tissues and may be harnessed for novel regenerative therapeutic strategies.

Epigenetic adaptations accompanying the metabolic state of dormant stem cells

Cellular metabolic pathways produce metabolites, such as acetyl-CoA, NAD+, or α-ketoglutarate, that serve as substrates or cofactors to chromatin-modifying enzymes [13]. Epigenetic modifications alter transcriptional programs, thereby affecting gene expression and cellular states. Nuclear acetyl-CoA is used by histone acetyl-transferases to acetylate histones. Nuclear acetyl-CoA is predominantly synthesized from citrate by ATP-citrate lyase (ACLY) and acetate by acetyl-CoA synthetase 2 (ACSS2). Typically, increasing cellular acetate or glucose levels increases global histone acetylation. In mammalian cells, histone acetylation at gene promoters and enhancers is mostly associated with active transcription. Elevating global histone acetylation could create conflict with repression of genetic activity and, as such, would be undesirable in dormancy. In line with this notion, histones are normally lowly acetylated in the diapause state [2]. The aforementioned metabolic switch from glycolysis to fatty acid oxidation would be expected to reduce nuclear acetyl-CoA levels due to less glycolytic activity. Indeed, carnitine supplementation of dormant embryos in culture significantly reduced histone acetylation levels in both the embryonic and extraembryonic tissues (Figure 1, the specific residue analyzed was histone H4 lysine 16, H4K16ac) [3]. Thus, the metabolic switch that is required during dormancy transition may in turn facilitate repression of transcriptional programs. Notably, carnitine supplementation appeared to place the embryos in a deeper dormant state, which allowed the embryos to be preserved longer; however, they were harder to reactivate. Likewise, deeper dormancy in adult stem cells creates a similar dilemma of setting a higher barrier for reactivation, which at times compromises regeneration (e.g., [14]), meanwhile conferring better protection against stress, providing a more stable stem cell pool.

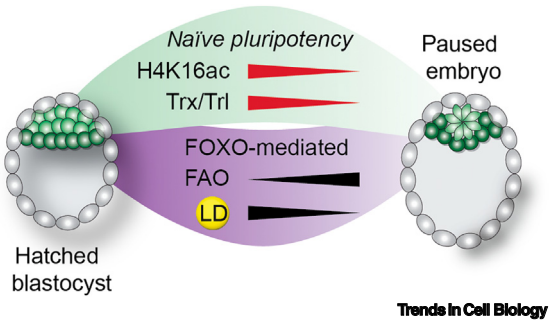

Figure 1. Fatty acid oxidation functions as a metabolic switch in embryonic diapause. Mammalian pre-implantation embryos can enter a reversible dormant state called diapause. Diapause is characterized by a reduction in H4K16ac levels and hypo transcription and translation (Trx/Trl). Metabolically, embryos in diapause switch their metabolic profile and use fatty acids stored in lipid droplets (LDs) instead of sugars. The transcription factor FOXO1 is required for this switch. Enhanced fatty acid oxidation (FAO) facilitates the establishment of a more dormant state, characterized by globally low histone acetylation.

Histone deacetylation and (de)methylation are other exemplary reactions at the interface of metabolism and epigenetics, in which NAD+, *S*-adenosylmethionine, and α-ketoglutarate are used as necessary coenzymes or substrates for the activity of histone deacetylases, methyltransferases, and demethylases, respectively. In dormant MuSCs, high levels of fatty acid oxidation lead to elevated activity of the histone deacetylase Sirtuin 1 (SIRT1) [9]. CPT1b-deficient cardiomyocytes, mentioned earlier, have increased α-ketoglutarate activity along with reduced levels of the transcription-associated H3K4me3 modification at cardiac differentiation and maturation genes [11]. Though far from providing actual mechanistic links, these findings underline the fact that lipid metabolism is not only important for adjusting cellular energy levels, but likely also impacts the epigenome and stem cell function and must therefore be quantitatively fine-tuned (Figure 1).

Dormancy depth and dormancy regulators

It is now becoming increasingly clear that dormancy is not a steady state, but rather a continuum between shallow and deep states [14]. Different depths of dormancy reflect differing capacities and timelines to

resume proliferation [14]. Lipid consumption puts dormant mouse embryos in a deeper dormant state. Mechanistically, this metabolic switch and the depth of dormancy, depends on Forkhead box protein O1 (FOXO1) activity (Figure 1) [3]. Known as DAF16 in *Caenorhabditis elegans*, this protein family regulates longevity, metabolism, and stress responses. FOXO1 is also upregulated in dormant adult (stem) cells across different tissues, including MuSCs, neuronal stem cells, hair follicle stem cells, HSCs, B cells, and hepatic stellate cells [3]. FOXO1 thus presents itself as a molecular candidate driving the metabolic switch from dormancy to proliferation of both embryonic and adult stem cells in mammals. Together, investigating metabolism-directed stem cell states holds great potential to understand how the epigenetic landscape is formed and how it contributes to longevity and disease to eventually exploit this to develop novel tools to tackle disease and assure healthy development.

Declaration of interests

No interests are declared.

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References

1. Renfree, M.B. and Fenelon, J.C. (2017) The enigma of embryonic diapause. *Development* 144, 3199–3210
2. Bulut-Karslioglu, A. *et al.* (2016) Inhibition of mTOR induces a paused pluripotent state. *Nature* 540, 119–123
3. van der Weijden, V.A. *et al.* (2024) FOXO1-mediated lipid metabolism maintains mammalian embryos in dormancy. *Nat. Cell Biol.* 26, 181–193
4. Kamemizu, C. and Fujimori, T. (2019) Distinct dormancy progression depending on embryonic regions during mouse embryonic diapause†. *Biol. Reprod.* 100, 1204–1214
5. Iyer, D.P. *et al.* (2023) Delay of human early development via in vitro diapause. *bioRxiv*, Published online May 30, 2023. https://doi.org/10.1101/2023.05.29.541316
6. Ma, C. *et al.* (2023) mTOR hypoactivity leads to trophectoderm cell failure by enhancing lysosomal activation and disrupting the cytoskeleton in preimplantation embryo. *Cell Biosci.* 13, 219
7. Arena, R. *et al.* (2021) Lipid droplets in mammalian eggs are utilized during embryonic diapause. *Proc. Natl. Acad. Sci. U. S. A.* 118, e2018362118
8. Ito, K. *et al.* (2012) A PML–PPAR-δ pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance. *Nat. Med.* 18, 1350–1358
9. Ryall, J.G. *et al.* (2015) The NAD+-dependent SIRT1 deacetylase translates a metabolic switch into regulatory epigenetics in skeletal muscle stem cells. *Cell Stem Cell* 16, 171–183
10. Giger, S. *et al.* (2020) A single metabolite which modulates lipid metabolism alters hematopoietic stem/progenitor cell behavior and promotes lymphoid reconstitution. *Stem Cell Rep.* 15, 566–576
11. Li, X. *et al.* (2023) Inhibition of fatty acid oxidation enables heart regeneration in adult mice. *Nature* 622, 619–626
12. Kalucka, J. *et al.* (2018) Quiescent endothelial cells upregulate fatty acid β-oxidation for vasculoprotection via redox homeostasis. *Cell Metab.* 28, 881–894.e13
13. Dai, Z. *et al.* (2020) The evolving metabolic landscape of chromatin biology and epigenetics. *Nat. Rev. Genet.* 21, 737–753
14. Eames, A. and Chandrasekaran, S. (2024) Leveraging metabolic modeling and machine learning to uncover modulators of quiescence depth. *Proc. Natl. Acad. Sci. U. S. A. Nexus* 3, pgae013
15. Stötzel, M. *et al.* (2024) TET activity safeguards pluripotency throughout embryonic dormancy. *Nat. Struct. Mol. Biol.*, Published online May 23, 2024. https://doi.org/10.1038/s41594-024-01313-7