Stem Cell Niches: Famished Paneth Cells, Gluttonous Stem Cells

Adult tissue stem cells adjust to environmental changes. A new study in the mouse intestine reveals that caloric restriction causes Paneth cells to repress mTORC1 signaling; this in turn stimulates proliferation of neighboring stem cells.

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> "… And I, already going blind, groped over my brood
> Calling to them, though I had watched them die
> For two long days. And then the hunger had more
> Power than even sorrow over me."
> Dante, *Inferno*, Canto 33

Caloric restriction promotes longevity, in part by reducing the risks of cancer, diabetes, and atherosclerosis [1], and many cultures and religious groups fast regularly. But starvation is a powerful and primal trigger and, to promote survival, it elicits robust metabolic responses, including glycogenolysis and reduced insulin secretion. Another response might be to protect vulnerable stem cells, salvaging them for rapid tissue replenishment when energy is less scarce. A recent report from Yilmaz *et al.* [2] elucidates a novel signaling mechanism by which Paneth cells in the intestinal stem cell (ISC) niche help amplify ISC numbers during, and many months after, a period of prolonged caloric restriction. This occurs non-autonomously, through repression of mammalian target of rapamycin complex 1 (mTORC1) signaling in Paneth cells.

Self-renewing stem cells in the adult bone marrow, skin, and gut repopulate these tissues continually, producing first an intermediate population of transit-amplifying progenitors; these progenitors eventually differentiate into functional, post-mitotic cells. Stem cell activity must at some level respond to environmental changes, particularly toxic and deprivation states; indeed, starved fruitflies reduce proliferation of both germline stem cells and ISCs [3,4]. Alternatively, proliferation of some stem cells can increase at the expense of progenitors or differentiated cells; for example, caloric restriction enhances hematopoietic stem cell activity in BALB/c mice [5]. The signals recruited in the stem cell niche to achieve these diverse ends are mostly unknown, but the mTORC1 complex is one vital node in pathways that coordinate cell growth with nutrient and energy availability [6]. By revealing non-autonomous, mTORC1-mediated increases in ISC proliferation under fasting conditions, Yilmaz *et al.* [2] elaborate on this mechanism in the mouse intestine.

Stem cells located in intestinal crypts expand and differentiate to replace the entire surface lining of absorptive and secretory cells every few days. In this epithelium, enterocytes of a single type absorb nutrients, but there are three varieties of secretory cells; one of these, the Paneth cells, reside at the bottom of each crypt in the small intestine, where they produce microbicidal peptides to help maintain a sterile environment [7]. Judging by long-term lineage tracing studies, intestinal surface renewal seems to rely on two distinct ISC populations. The more abundant population is nestled among Paneth cells in the crypt base, expresses the surface protein LGR5, and replicates approximately once a day [8]. As LGR5$^+$ cells in mice can replenish the adjoining epithelium for months, they represent *bona fide* self-renewing stem cells. A second, less abundant population of quiescent, LGR5$^-$ stem cells replicates infrequently, expresses some combination of the crypt-restricted molecular markers BMI1, mTERT, HOPX and LRIG1 [9–12] and occupies a distinct niche higher in the crypt. This population of stem cells interconverts with LGR5$^+$ cells [11,13] and replenishes lost LGR5$^+$ cells [14].

Quiescent BMI1$^+$ LGR5$^-$ stem cells thus respond physiologically to damage or depletion of the LGR5$^+$ compartment, but what about the workhorse population of LGR5$^+$ stem cells? Does their ability to divide and differentiate respond to environmental cues? If so, LGR5$^+$ stem cells would likely receive the necessary signals from their niche, a unique mix of mesenchymal cells, myofibroblasts, Paneth cells, and other epithelial cells in or near the crypt base. In particular, Paneth cells produce both Wnt and Notch, drivers of ISC activity [7], and they abet optimal expansion of single cultured Lgr5$^+$ cells; because Lgr5$^+$ cell numbers fall in proportion to Paneth cell deficiency in some mouse models, recent attention has centered on these niche cells [15].

Examining the response to caloric restriction in mouse intestines, Yilmaz *et al.* [2] observed mild mucosal atrophy and reduced proliferation of transit amplifying progenitors, similar to prior studies [16]. Surprisingly, LGR5$^+$ and Paneth cells increased in number, and through elegant study of mutant mice and organoid cultures, Yilmaz *et al.* [2] traced the trophic effect on LGR5$^+$ cells largely to fasting-induced reduction of mTORC1 activity in their Paneth cell neighbors. For example, Paneth cells isolated from starved mice promoted expansion of LGR5$^+$ ISCs in culture more efficiently than cells from well-fed mice. Forced mTORC1 activity reduced Paneth cell numbers and, indirectly, their salutary effect on LGR5$^+$ ISCs, even under calorie restriction. Conversely, specific inhibition of mTORC1 by rapamycin in well-fed mice enhanced crypt clonogenicity non-autonomously. Expression profiling of calorie-restricted Paneth cells did not reveal obvious changes in Wnt or Notch ligands. Rather, it identified an excess of bone stromal antigen 1 (Bst1), a secreted enzyme that elevates local concentrations of cyclic ADP ribose (cADPR). Bst1 deficiency abrogated the capacity of calorie-restricted intestinal crypts to form organoids, and exogenous cADPR rescued this effect.

Yilmaz *et al.* [2] thus shed new light on how the ISC niche facilitates a response to starvation. Their work also raises important questions for further research, both on how Paneth cells sense starvation and how different crypt cell populations maintain intestinal homeostasis. Insulin and insulin-like growth factors (IGFs) are prominent inducers of mTORC1 activity [6]. IGF-1 levels decrease in fasting rodents and rise during feeding [17]. Indeed, Yilmaz *et al.* [2] report that insulin and refeeding activated

mTORC1 to similar degrees in Paneth cells in fasting mice. Perhaps Paneth cells are especially sensitive to insulin in distinguishing starved from fed states. Second, only Paneth and LGR5$^+$ cell numbers rise in calorie-deprived mouse intestines; organ mass, progenitors and enterocyte numbers all decline. As both enterocytes and Paneth cells derive from LGR5$^+$ ISCs, one question is whether caloric restriction promotes Paneth cell differentiation or prolongs the life of these cells.

Although LGR5$^+$ ISC numbers faithfully follow those of Paneth cells in some mouse models [15], they replicate and function normally in other mice with severe or total Paneth cell deficiency [18,19]. Thus, dependence on Paneth cells, which Yilmaz *et al.* [2] confirm in organoid cultures, may be masked in some contexts *in vivo*. Their work, however, leads to the testable prediction that LGR5$^+$ ISC proliferation will be attenuated in fasting Paneth-cell-deficient mice. Notably, the trophic effect of caloric restriction on LGR5$^+$ ISCs is selective; replication of transit-amplifying progenitors falters. Is this because of spatial proximity to Paneth cells, because LGR5$^+$ ISCs are uniquely responsive to cADPR, or because cADPR regulates only certain modes of cell division? LGR5$^+$ ISCs divide symmetrically [20] and, at least in feeding *Drosophila*, ISC divisions shift from asymmetric to symmetrical modes [4]. It will be interesting to know whether mammalian ISCs also change replication modes in fed or starved states. Lastly, LGR5$^+$ and quiescent LGR5$^-$ ISCs function in a complex, non-linear hierarchy [11,13,14]. It will be useful to know whether cADPR-mediated effects on cell growth extend to other ISC populations or stimulate interconversion with LGR5$^+$ cells. Insights into how different crypt cells respond to environmental stresses and affect one another will inform strategies for combating intestinal disorders of reduced nutrient absorption, inflammation, and cancer.

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