

Status Anxiety among Pluripotent Stem Cells?

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Abundant cell death marks early embryonic development. New work reported in *Developmental Cell* from Diaz-Diaz and colleagues (2017) proposes that this death results from cell competition arising from differences in cellular differentiation status, thus providing a physiological mechanism for controlling the make-up of the pluripotent stem cell population.

Little is known, although much has been written, about the remarkable adaptability of the pre- and peri-implantation mammalian embryo. For instance, an embryo can split, or multiple pre-implantation embryos can merge, and nevertheless attain normal size shortly after implantation (Lewis and Rossant, 1982). Even single embryos can show unevenness in local growth rates, but this unevenness vanishes with the onset of gastrulation so that proper cell numbers are maintained (Gardner, 2014). Such growth stabilization would seem to entail a rapid, self-correcting mechanism, but developmental biologists do not yet have a model to account for this feat. Work from the Torres group in this issue of *Developmental Cell* (Diaz-Diaz and colleagues, 2017) now suggests that the adaptability of the developing embryo involves “cell competition,” a process thought to eliminate “less-fit” cells during tissue growth and homeostasis in a cell context-dependent manner.

Cell competition was first observed in chimeric analyses of *Drosophila* imaginal discs where so-called minute cells bearing ribosomal gene mutations surrounded by wild-type cells were lost at a rate not explainable solely based on autonomous effects (e.g., slower growth) (Morata and Ripoll, 1975). The cell competition model holds that cells somehow register each other's relative fitness, and “winners” eliminate the “less-fit” or “loser” cells even when the latter are otherwise perfectly viable and capable of executing developmental programs on their own. In other words, the model posits a form of Darwinian evolution at the level of ontogenesis—and subverts the developmental dictum that embryogenesis is mostly a matter of following a preprogrammed set of rules. This phe-

nomenon has been extremely difficult to study; progress has been made only slowly over the past three decades, mostly in *Drosophila*. Part of the problem is the challenge of studying the effects under physiological conditions rather than in an artificially perturbed system.

Diaz-Diaz et al. (2017) approached this challenge by observing development of whole murine embryos and embryonic stem cells (ESCs). Their study follows on previous work in which the authors found natural heterogeneity among murine epiblast cells in Myc expression levels and metabolic activity. The cells with higher Myc levels outcompeted those with lower levels (Clavería et al., 2013). In the current work, the authors devised a sensitive Myc-responsive reporter system and observed that, under steady-state conditions, ESCs did not switch much between Myc-high and Myc-low status, and Myc-high cells proliferated faster. Intriguingly, after cell sorting, the Myc-low cells died but the Myc-high cells replicated the original distribution of Myc-high and -low cells. There was a strong correlation between low Myc expression and the likelihood of the cell dying in culture: specifically, the Myc-low cells died via apoptosis when they were exposed to Myc-high cells for 10–12 hours. Furthermore, low-Myc cells survived as long as the neighboring cells' Myc levels were not too much higher, indicating that the relative expression matters more than the absolute levels. Finally, direct contact between Myc-low and -high cells was necessary for this effect to occur. According to the cell competition metaphor, Myc-high ESCs win over Myc-low cells if they are in direct contact for too long.

Why would there be two ESC populations in the first place? To address this

question, the authors profiled the transcriptomes of Myc-high and -low cells. The most significant changes in the gene signature pertained to genes associated with “pluripotency” control: the transcriptome of Myc-high cells matched the profile of so-called naive pluripotency, while the Myc-low cells had a profile typical of primed cells. (Naive and primed pluripotency are two differentiation states of early embryonic cells: naive cells are the more primitive ones, residing in blastocyst embryos prior to implantation, whereas primed cells can be found slightly later, just prior to the onset of gastrulation [Dejosez and Zwaka, 2012].) Myc levels also reflected other features of naive and primed pluripotency, such as the signal intensity of the STAT and ERK pathways, respectively. This observation provides a pivotal insight into the nature of cell competition: it occurs when cells of different differentiation status are mixed.

The authors then observed the developing mouse embryo and found consistent correlations between high Myc levels, naive signaling, and survival: Myc-low cells appeared at the onset of gastrulation in the vicinity of the primitive streak and died. The authors next sought to determine whether high Myc levels are sufficient to drive cell competition in the embryo. Mosaic overexpression of Myc is enough to trigger competition but not to influence the pluripotency status of the cells. This suggests that differential Myc levels arise as a consequence of the transition from the naive to the primed state and that the resulting high-low discrepancy causes the loss of Myc-low cells from the stem cell compartment.

As with all good science, these results raise more questions than they answer. One is whether cell competition actually

functions to ensure the purity of the stem cell population. Running counter to the idea that competition-induced apoptosis is important during development is the observation that blocking cell death has no appreciable effect on developmental trajectories in the mouse embryo or the *Drosophila* wing disc, where there is very little cell death in the first place (Milán et al., 1997). The interpretation of the results from Diaz-Diaz et al. (2017) is also complicated by the fact that, in culture, cells do not experience any developmental gradients, they are not properly positioned along any developmental axis, their numbers are not controlled, and they are missing most developmental cues. One alternative explanation for the current findings could be that the pluripotent cells produce metabolites that are toxic to primed cells, causing their death.

It is also possible that what we call competition between Myc-high and Myc-low cells might be better thought of in terms of cooperation between cells of the same developmental type at the right point in development (Dejosez et al., 2013). Long viewed as a conundrum for Darwinian evolution, cooperation is, in fact, essential to the transition to multicellularity; it is the driving force for greater levels of complexity, as new units of selection emerge from functional groupings of previously autonomous units. Perhaps, then, naive cells simply cooperate better among themselves than with primed cells,

and this lack of coordination and cooperation then leads to the loss of primed cells as part of the developmental plan. The fact that essentially the same effect occurs in seemingly unrelated developmental contexts (imaginal disc versus murine epiblast) certainly suggests an evolutionarily conserved root. Regardless, whichever metaphor best captures the phenomenon, competition or cooperation, the notion that developing cells are passive units receiving and responding to developmental triggers seems destined to yield some ground to the possibility that they actually participate in the developmental decision-making process.

Biologists have long recognized that to understand evolution we must understand the genetics of development and vice versa (Carroll, 2008; Moreno and Rhiner, 2014). Until recently, competition and exploitation have received far more attention than cooperation, as indicated by the gleeful relish with which the discoveries of cheaters, defectors, and supercompetitors in microorganisms and *Drosophila* have been discussed. (The gentle reader may ponder what this reveals about the present culture of science.) The dominant discourse is beginning to shift, however, and cooperation is now recognized as a primary driver of complexity and fitness in both biology and human culture. Accordingly, cell cooperation during development may help to ensure that the embryo is able to

continually adapt to changes in its environment such that the organism (rather than individual cells) achieves the best fit under the given circumstances. Cell cooperation mechanisms may better explain the remarkable flexibility of early mammalian embryogenesis—but in either case, competition or cooperation, we must be aware of the interpretive bias of our own metaphors.

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