


SPOTLIGHT

Stressed-out yeast do not pass GO

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Using microfluidics and imaging, Argüello-Miranda et al. (2021. *J. Cell Biol.* <https://doi.org/10.1083/jcb.202103171>) monitor the response of individual yeast cells to nutrient withdrawal. They discover that cells arrest not only in the early G1 phase as expected, but also later in the cell cycle, and that Xbp1 is critical for arrest at other cell cycle phases.

Cells can enter a quiescent, nondividing state in response to extracellular signals (1). This cellular state allows unicellular organisms to survive under harsh conditions, while in multicellular organisms, it facilitates the formation of tissues with complex architectures that are maintained even as individual cells die and repaired upon injury. Early studies from the '70s revealed that cells exit the cell cycle before replicating their DNA. Budding yeast devoid of nutrients arrest right after mitosis and before the formation of a new bud—that is, before the “start” point in the cell cycle (2). Mammalian cells undergo cell cycle arrest before reaching the restriction point early in G1, but once beyond this point, they are committed to progressing through the cell cycle so neither their genome integrity nor their energy status are reassessed until the next G1 (3). This model seems intuitive; why should a cell commit to the energy-intensive process of DNA replication if it is not going to generate a new cell? The same rationale applies at later stages in the cell cycle. If, after commitment into the S phase, entering or completing mitosis imposes a risk for the viability of the cell (e.g., because the newly synthesized DNA is damaged or because chromosome misalignment could result in deleterious genomic aberrations), different checkpoints, such as the G2 or the spindle assembly checkpoint, get activated and arrest the cell (4). Besides these established

checkpoints, there are additional override systems in numerous organisms. In yeast, glucose or nitrogen depletion, as well as exhaustion of carbon sources, can lead cells into quiescence at any stage of the cell cycle (5, 6). *C. elegans* embryos deprived of oxygen stop cycling in interphase, prophase and metaphase, indicating that there are multiple checkpoints in the cell cycle for these cells to arrest as well (7). Similarly, frog and mouse oocytes can arrest in metaphase before fertilization (8, 9), and *Drosophila* neural stem cells can enter quiescence in both G0 and G2 cell cycle phases (10).

Cells that follow the archetype of entering quiescence in early G1 are characterized by low levels of activity for the cyclin-dependent kinases (CDKs), which upon interaction with their cognate cyclins, phosphorylate target proteins to drive the cell cycle forward (11). Yet, the findings above suggest that some cells stop cycling despite high levels of CDK activity present at other phases than G1 (5, 7, 8, 9, 10). These cells are particularly challenging to characterize as they usually represent rare cells in complex populations or tissues, and we have limited ability to physically isolate them for further investigation. Thus, it has been difficult to distinguish cells that are cycling slowly from those that are quiescent. In addition, for microorganisms, entry into a quiescent state is achieved through nutrient limitation, hence the effects of food scarcity on cell cycle and stress are challenging to disentangle.

Further, in mixed populations, dissecting how cells influence each other—for instance, whether stressed or quiescent cells in turn induce stress or quiescence in neighboring cells—can be difficult. As a result, many questions remain about the pathways through which cells exit the cell cycle when conditions are unfavorable. Which cells and how many arrest at noncanonical checkpoints? Do these arrested cells activate the same gene expression programs, and are they functionally equivalent to those that enter quiescence in early G1? What molecules or pathways, if any, determine whether a cell will arrest before start or at a different point within the cell cycle? How do these factors supersede the cycle-promoting signals the cell is receiving from active CDKs?

In this issue, Argüello-Miranda et al. elegantly interrogate the response of budding yeast *Saccharomyces cerevisiae* cells to an acute loss of nutrients (12). Using a combination of real-time imaging with different fluorescent markers and microfluidics, they tracked individual cells that were cultured in a flow cell with rich medium and their fate after they were transferred to a medium devoid of nutrients. The advantage of this experimental design is that individual cells were monitored in isolation, which removes potential cell–cell communication effects and allows each cell to respond to nutrient cues individually. Cells depleted of nutrients stopped cycling and retained high viability

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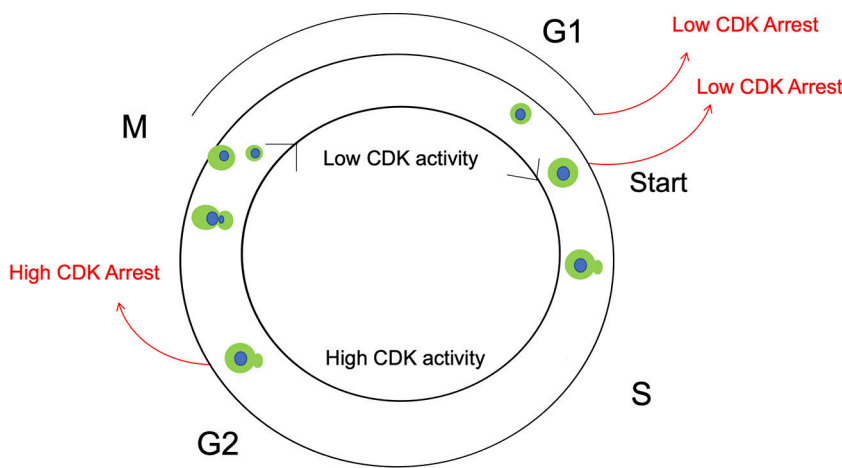


Figure 1. Schematic of arrest positions. The *S. cerevisiae* budding yeast cell cycle is shown. Portions of the cell cycle with low and high CDK activity are depicted. Three different pathways to cell cycle arrest in response to nutrient withdrawal are described by Argüello-Miranda et al. (12). Cells that were in the G1 phase of the cell cycle with low CDK activity arrested in that phase. Many cells that were in a different phase of the cell cycle completed a cell division and then arrested. In addition, Argüello-Miranda and colleagues discovered that another population of yeast cells, the high CDK-arrested cells, exited the cell cycle and arrested with high CDK activity without completing a cell cycle.

when nutrients were re-added. The cells exhibited changes previously associated with quiescence in yeast such as activation of trehalose and glycogen metabolism. Further, they became resistant to stress and survived high salt conditions. Most of the cells that experienced low nutrient conditions for 20 h were unbudded, consistent with being arrested in early G1, with low CDK activity. Some of these cells were already in a low CDK state in G1 when the starvation began, and they remained in that state, while others were in a high CDK state and completed a cell cycle, returned to the next low CDK G1 phase, and arrested. However, in addition to those two categories of cells, there was also a minority of cells, ~7%, that arrested with a small bud, indicating that they were in a later point in the cell cycle, after start (Fig. 1). These cells did not progress through the cell cycle, as the small bud did not grow or disappear. The authors confirmed that CDK activity remained high in these cells despite the fact that they were not progressing through the cell cycle. High CDK-arrested cells had duplicated their DNA, suggesting they were stopped in G2, and were able to resume proliferation with a similar efficiency as cells arrested in a low CDK activity state. Entry into a high CDK quiescent state was not simply a function of cell cycle status, as even if the cells were synchronized using nocodazole and then deprived of nutrients,

high and low CDK-arrested cells were both observed. Thus, the researchers concluded that the cells arrested with a small bud were in a high CDK quiescence state.

Using a set of imaging-based readouts, Argüello-Miranda et al. discovered that many of the stress response signals that had been previously associated with quiescence were similar in both low and high CDK-arrested cells (12). By monitoring reporters for calcium signaling, overall stress, cell wall stress, carbon stress, osmoregulation, and histone deacetylase activity, they found that cells that had stopped dividing with low or high CDK activity exhibited similar patterns. In addition, both cell populations displayed induction of autophagosomes and clumping of an aggregation-prone protein after nutrient depletion. The two cohorts of cells also exhibited comparable increases in mitochondrial biomass with starvation and similar levels of DNA damage. Thus, in many aspects, low and high CDK-arrested cells were alike, and shared many properties with yeast induced into quiescence in batch culture (13). In contrast, the accumulation of nuclear Sfp1, a regulator of ribosomal protein gene expression, Gln3, a nitrogen stress transcription factor, and Xbp1, a stress-induced histone deacetylase regulator/transcriptional repressor, did differ between the two cell groups. Because these are stress response transcription factors, the authors hypothesized that the presence of high CDK-arrested cells could

reflect the stress status of the cells. Indeed, when they prestressed yeast with glucose or nitrogen depletion before withdrawing nutrients, a higher fraction of cells entered the high CDK-arrested state and expressed higher levels of Xbp1 compared with their non-prestressed counterparts, suggesting that Xbp1 is a regulator of high CDK quiescence. A comprehensive, machine learning-based analysis of the real-time images revealed that high nuclear XBP1 levels predicted whether a cell would arrest with high CDK in response to nutrient withdrawal. Accordingly, overexpression of XBP1 resulted in a higher fraction of cells arresting in high versus low CDK conditions, an effect that required the ability of XBP1 to transcriptionally activate downstream genes. Importantly, deletion of Xbp1 resulted in a smaller fraction of high CDK-arrested cells and reduced viability of those cells that did arrest, further solidifying Xbp1 as a key mediator of high CDK quiescence. Not only did Xbp1 function as an important regulator of the high CDK-arrested state, but its levels also served as a mechanism for the cell to “remember” prior stress signals. As yeast were subjected to cycles of glucose depletion, nuclear Xbp1 levels increased rather than reverting to baseline, providing each cell with a molecular memory of its previous stress history. Consistent with this model, in cells with intact Xbp1, but not cells with Xbp1 loss, a second nutrient depletion trigger soon after a first nutrient withdrawal resulted in a greater accumulation of high CDK-arrested cells, thus demonstrating that the commitment to a high CDK-arrested state is affected by the cell history of preceding stressors in an Xbp1-dependent manner.

These findings provide additional insight into the choices that cells make in response to nutrient deprivation. The powerful combination of real-time fluorescence imaging and microfluidics used by the authors allowed them to determine the behavior of individual cells without the complexities added by batch culture. The characterization of a high CDK quiescent state in yeast that shares similarities to the low CDK quiescent state points toward cell cycle mechanisms that may be shared by other cell types. The authors’ finding that stress regulator Xbp1 determines how cells respond to starvation—by arresting with a low or high CDK state—supports the existence of a molecular system that can

override cell cycle control by CDKs. Additional studies will likely shed light on the transcriptional targets of Xbp1 that are mediating this high CDK arrest and on whether Xbp1-driven non-G1 arrest occurs in other cell types and in response to additional anti-proliferative triggers. Investigating whether other types of cells display a similar ability to integrate historical cues to set different, cell-specific thresholds for completing or arresting the cell cycle will also be of interest.

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References

1. Coller, H.A. 2011. *Science*. <https://doi.org/10.1126/science.1216242>
2. Hartwell, L.H., et al. 1974. *Science*. <https://doi.org/10.1126/science.183.4120.46>
3. Pardee, A.B. 1974. *Proc. Natl. Acad. Sci. USA*. <https://doi.org/10.1073/pnas.71.4.1286>
4. Hartwell, L.H., and T.A. Weinert. 1989. *Science*. <https://doi.org/10.1126/science.2683079>
5. Wei, W., et al. 1993. Yeast cells can enter a quiescent state through G1, S, G2, or M phase of the cell cycle. *Cancer Res*.
6. Laporte, D., et al. 2011. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201009028>
7. Hajeri, V.A., et al. 2005. *BMC Cell Biol.* <https://doi.org/10.1186/1471-2121-6-47>
8. Lorca, T., et al. 1993. *Nature*. <https://doi.org/10.1038/366270a0>
9. Nixon, V.L., et al. 2002. *Curr. Biol.* [https://doi.org/10.1016/S0960-9822\(02\)00811-4](https://doi.org/10.1016/S0960-9822(02)00811-4)
10. Otsuki, L., and A.H. Brand. 2018. *Science*. <https://doi.org/10.1126/science.aan8795>
11. Morgan, D.O. 2007. *The cell cycle: principles of control*. New Science Press, London.
12. Argüello-Miranda, O., et al. 2021. *J. Cell Biol.* <https://doi.org/10.1101/2021.03.13.434817>
13. Gray, J.V., et al. 2004. *Microbiol. Mol. Biol. Rev.* <https://doi.org/10.1128/MMBR.68.2.187-206.2004>