mRNA Regulators Put the Brakes on Cell Division

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GE Zentner

While the progression of cells through the cell cycle has been well studied, comparatively little is known about how cells exit from the cell cycle and achieve a prolonged, reversible quiescent state. Metazoans depend on resting stem cell populations for growth and tissue renewal, and failure to exit or improper reentry into the cell cycle is characteristic of most if not all cancers (Hanahan and Weinberg 2011). Work recently published in Molecular Biology of the Cell, by postdoctoral fellow Dr. Lihong Li and colleagues in the laboratory of Dr. Linda Breeden (Basic Sciences Division), has now identified critical regulatory factors involved in the transition from rapid growth to quiescence in the budding yeast Saccharomyces cerevisiae. "Knowing when to stop dividing and how to survive in a quiescent state is critical for all cells, and these very basic processes are usually conserved from yeast to humans," says Dr. Breeden. "Now that we can monitor this transition and purify quiescent yeast, we can begin to define this universal state."

During rapid growth, yeast exhaust the glucose in their medium and switch from glycolysis to respiration, a process called diauxic shift. This leads to carbohydrate accumulation in cells and a shift in cellular density, which facilitates the purification of quiescent cells in the lab. The authors first assessed the properties of cells undergoing the transition from rapid growth to quiescence following the diauxic shift. Quiescent cells were found to store carbohydrates and resist heat stress at much higher levels than their non-quiescent counterparts. Strikingly, pre-quiescent cells also display increased inheritance of mitochondrial DNA, and respire at approximately five times the rate of non-quiescent cells.

The authors found that the transition to quiescence in yeast was accompanied by alterations in the cell wall that could be detected by flow cytometry. The authors first observed that quiescent cells treated with a fluorescent DNA dye displayed lower fluorescence than their growing counterparts. As the DNA content of cells entering quiescence is not expected to change upon the attainment of quiescence, this observation suggests a reorganization of the cell wall that renders it less permeable to dyes. The authors next analyzed the light scattering profiles of growing and quiescent cells. While a culture of growing cells displayed a highly uniform light scattering profile, a culture of cells grown for 24 hours (and therefore post-diauxic shift) showed three distinct profiles. These profiles were assigned to cell populations designated R1, R2, and R3, with each

group displaying a different light scattering profile (see figure). Strikingly, the R3 population showed a DNA fluorescence profile that was highly similar to that of purified quiescent cells. The authors went on to confirm that cell wall changes were responsible for the DNA fluorescence and light scattering profiles observed, both by repeating the experiments in the presence of a reducing agent to weaken cell wall integrity and by using a yeast strain carrying a mutation in a gene important for cell wall strengthening in quiescent cells.

The authors' previous work had demonstrated that cells carrying a truncated allele of the gene encoding the Ssd1 RNA-binding protein results in drastically reduced generation of quiescent cells (Li et al., 2009). Notably, Ssd1 binds to mRNAs encoding cell wall components. The authors therefore assessed DNA fluorescence and light scattering in wild-type and mutant Ssd1 cells. Truncation of Ssd1 did not affect light scattering, but did cause a drastic slowdown in the shift from growing to quiescent DNA fluorescence profiles, indicating that these two cell wall phenotypes are mechanistically distinct. The authors also found that deletion of the ECM33 gene, encoding an Ssd1-bound mRNA, caused similar defects as the Ssd1 mutation, arguing for the importance of the RNA regulatory function of Ssd1 in shaping the quiescent cell wall.

The authors also analyzed the efficiency of conversion of growing to quiescent cells by assessing a strain of yeast lacking the Mpt5 RNA-binding protein, which has overlapping functions with Ssd1. Loss of Mpt5 reduced the number of quiescent cells generated by about half. Interestingly, loss of Mpt5 did not affect DNA fluorescence or light scattering, but did reduce quiescent cell lifespan. However, when combined with Ssd1 truncation, loss of Mpt5 resulted in aberrant DNA fluorescence and light scattering, confirming functional redundancy of Mpt5 and Ssd1 during the transition to quiescence.

This work identifies critical regulators of the transition from growth to quiescence, and may have important implications for this cellular transition in cancer. The Warburg effect is the observation that cancer cells predominantly produce energy via glycolysis rather than respiration. In this way, the non-quiescent cells studied here are similar to cancer cells, as they accumulate lower levels of carbohydrates than quiescent cells. Further molecular characterization of the pathways leading to stable cellular quiescence, including those involving RNA-binding proteins, will undoubtedly shed further light on the dysregulation of cell proliferation in cancer.

Li L, Miles S, Melville Z, Prasad A, Bradley G, Breeden LL. 2013. Key events during the transition from rapid growth to quiescence in budding yeast require posttranscriptional regulators. *Mol Biol Cell* 2013 Oct 2.

See also: Hanahan D, Weinberg RA. 2011. Hallmarks of Cancer: The Next Generation. *Cell* 144(5):646-674.

See also: Li L, Lu Y, Qin LX, Bar-Joseph Z, Werner-Washburne M, Breeden LL. 2009. Budding yeast SSD1-V regulates transcript levels of many longevity genes and extends chronological life span in purified quiescent cells. *Mol Biol Cell* 20(17):3851-3864.

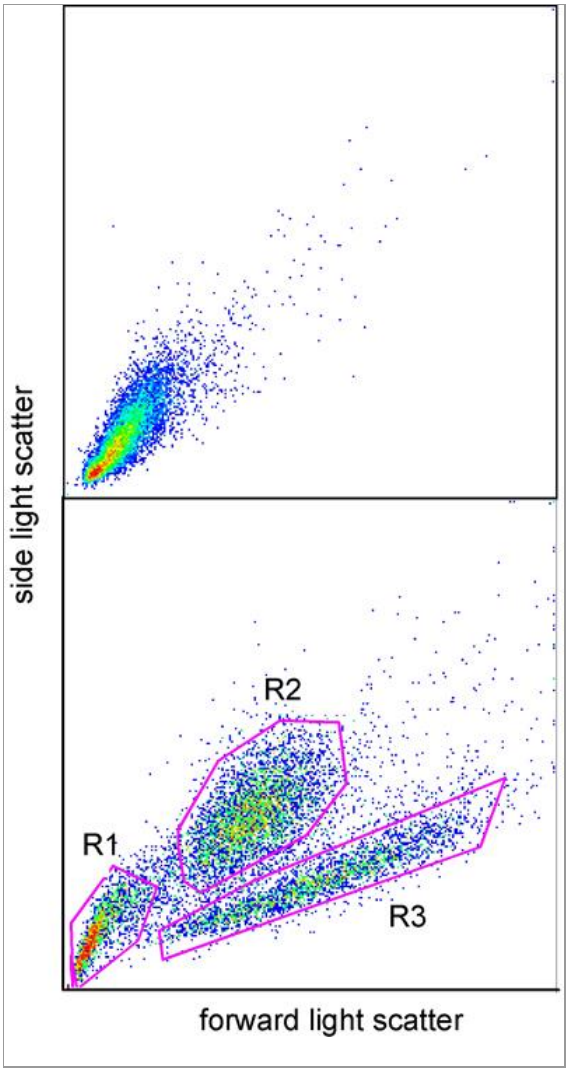

Image provided by Dr. Linda Breeden

Light scattering profiles of rapidly growing (upper panel) and quiescent (lower panel) yeast. Rapidly growing yeast generate a uniform light scatter profile, but when signaled to stop division they differentiate into three distinct cell types based on light scattering.