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SPOTLIGHT

Newborn daughters get a fresh start through PI(3,5)P_2-mediated vacuolar acidification

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Huda et al. (https://doi.org/10.1083/jcb.202406170) reveal a transient, cell cycle–dependent increase in PI(3,5)P_2 levels at the lysosome-like vacuole of yeast daughter cells. The resulting lipid asymmetry alters vacuolar pH in both daughter and mother cells and will impact multiple downstream functions.

During asymmetric cell division in *Saccharomyces cerevisiae*, mother cells provide organelles and nutrients to daughter cells, and daughters are "rejuvenated" relative to the mother. In this issue, Huda et al. (1) visualize cell cycle–dependent accumulation of the signaling lipid PI(3,5)P_2 in daughter cells and link this accumulation to increased acidification of the lysosome-like vacuole. Vacuole inheritance from mother to daughter occurs at a specific point in the yeast cell cycle (2). Using time-lapse microscopy, Huda et al. demonstrate that within a few minutes of vacuole segregation, a pronounced asymmetry in PI(3,5)P_2 levels emerges, with high levels at the daughter cell vacuole and reduced levels in the mother cell (1). This asymmetry is transient; PI(3,5)P_2 levels in both mother and daughter vacuoles recover to initial levels as cell division concludes and the cell cycle progresses (Fig. 1). Previous studies have linked both the growth of daughter vacuoles and PI(3,5)P_2 to cell cycle progression (2, 3). This work provides an additional connection by suggesting that a surge in PI(3,5)P_2 in daughters drives a drop in vacuolar pH at a specific point in the cell cycle. This may help newborn daughters to sequester nutrients released by the mother and support further growth.

Huda et al. find that while vacuolar pH in the daughter cell decreases as PI(3,5)P_2 levels increase, PI(3,5)P_2 decreases and vacuolar pH increases at the mother vacuole. The coordination between PI(3,5)P_2 and vacuole acidification may be explained by the activation of V-ATPase proton pumps in the vacuole by this lipid (4). Intriguingly, Okreglak et al. (5) recently described cell cycle–linked vacuolar pH dynamics that parallel those seen here. They proposed that oscillations of vacuolar pH in mother cells support the sequestration and mobilization of amino acids and envisioned the release of amino acids late in the cell cycle as a mechanism for supplying daughters with building blocks needed for rapid growth. These authors also propose that pH dynamics are driven by PI(3,5)P_2 levels but focus on cycling of the lipid and vacuolar pH in mother cells. Many of the results of these two studies are complementary. Lowering the pH of daughter cell vacuoles could facilitate the capture of amino acids released from the mother cell. Both studies also implicate the WD-repeat protein interacting with phosphoinositides family member Atg18 in the cell cycle–dependent changes in PI(3,5)P_2 and vacuolar pH. Huda et al. (1) find that Atg18 exhibits an asymmetric distribution between mother and daughter cells. In contrast, both Fab1, the lipid kinase that synthesizes PI(3,5)P_2, and its substrate PI(3)P are maintained uniformly at mother and daughter cell vacuoles. Atg18 is particularly important for the asymmetry between mother and daughter vacuoles; in an *atg18*Δ mutant, PI(3,5)P_2 asymmetry is lost and lipid levels are elevated in both mother and daughter vacuoles. This is consistent with evidence that Atg18 negatively regulates PI(3,5)P_2 levels (6) and with the inverse relationship between vacuolar Atg18 localization and pH in mother cells (1, 5). However, it is surprising that daughter vacuoles have a higher vacuolar pH than mother vacuoles in the *atg18*Δ mutant (1). This suggests that vacuole acidification and PI(3,5)P_2 levels are not always coupled and that recruitment of Atg18 to the daughter vacuole may be required to promote acidification. The previously described functions of Atg18 in autophagy and membrane bending/tubulation (7) do not readily explain these results. However, consistent with a critical role for Atg18 and acidification of daughter cell vacuoles, control of daughter cell size is lost in an *atg18*Δ mutant (5). The mechanisms connecting size control, cell cycle, PI(3,5)P_2, and vacuolar pH are almost certainly complex. Other players, including mTOR (2, 5, 8) are likely to be involved and will require further investigation.

It is intriguing to speculate that asymmetry in PI(3,5)P_2 and vacuolar acidification contribute to the rejuvenation of daughter cells. Huda et al. suggest that loss of acidification in mother cells during the cell cycle could contribute to aging (1). This aligns with data indicating that pH homeostasis and lysosomal function are compromised in aging cells (9). However, Huda et al. mainly follow events through a single-cell cycle in this work. Although they show that the vacuolar pH is slightly higher in mother cells than in daughters, both loss of PI(3,5)P_2 and vacuole alkalinization in mother cells are transient (1). Okreglak et al. monitored

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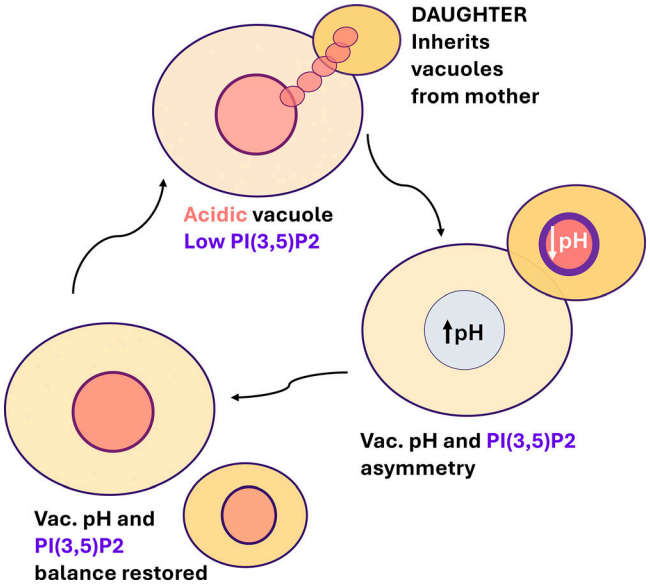

Figure 1. **The balance of vacuolar acidification and PI(3,5)P_2 levels between mother and newborn daughter cells changes during the yeast cell cycle.** Top: A daughter cell buds from the mother, which transfers vacuoles at a defined point in the cell cycle. At this point, the mother vacuole is acidified (light pink) and vacuolar levels of PI(3,5)P_2 (purple) are relatively low. Right: In early anaphase, pronounced asymmetry between the mother and daughter vacuoles emerges. The daughter vacuole exhibits high levels of PI(3,5)P_2 and becomes more acidic (dark pink) than the mother vacuole, which is transiently alkalinized (blue). Bottom left: After cell division, PI(3,5)P_2 asymmetry disappears and both mother and daughter vacuoles are acidified.

pH dynamics for longer times and found that mother cells re-establish vacuolar acidification across multiple cell cycles (5). It is possible that repeated cycles of alkalinization and reacidification ultimately lead to increased vacuolar pH or compromise vacuolar integrity during aging, but further experiments are needed to connect the cell cycle–related events described here with long-term loss of organelle pH homeostasis in aging cells.

In yeast, as well as other eukaryotic cells, PI(3,5)P_2 is present at very low levels, and monitoring its dynamics in living cells has been challenging. The authors adapt a recently developed PI(3,5)P_2 sensor, SnxA (10), for use in yeast and validate its use by demonstrating that mutants that alter PI(3,5)P_2 levels affect the recruitment of SnxA from the cytosol to the vacuole as expected. They show that salt stress, a well-established trigger of PI(3,5)P_2 synthesis (8), stimulates the recruitment of SnxA to the membrane. Interestingly, under these conditions, recruitment is uneven, suggesting localized zones of PI(3,5)P_2 synthesis near sites of vacuole fragmentation. Application of this sensor in yeast is a major advance that was critical for the experiments described here and promises future insights into the cellular roles of this enigmatic lipid.

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