Vacular H⁺-ATPase (V-ATPase) is a glucose-responsive, ATP-driven proton pump that controls the acidity of cellular organelles. Increases in glucose stimulate V-ATPase assembly and activity, and glucose deprivation triggers rapid V-ATPase disassembly and inactivation in yeast. McGuire and Forgac describe the opposite phenomenon in mammalian cells, specifically that V-ATPase assembly and activation increases when glucose is lost, raising new questions about mammalian mechanisms of energy conservation.

Glucose is the preferred energy source for most organisms, and cells possess sophisticated mechanisms for sensing and responding to it. One of these mechanisms is the glucose-mediated regulation of vacuolar H⁺-ATPase (V-ATPase), an ATP-driven proton pump that generates pH gradients in the endomembrane system of eukaryotes. At normal glucose concentrations, V-ATPase maintains an acidic pH in organelles that allows for proper function of the Golgi, endosomes, lysosomes, and vacuoles (1). As glucose availability changes, however, V-ATPase responds quickly via regulated disassembly and reassembly (2). The response of V-ATPase to glucose depletion has been most thoroughly studied in yeast, and the mechanisms driving this response are still under investigation. In this issue, McGuire and Forgac (3) show that mammalian V-ATPase responds to glucose deprivation in a novel manner, highlighting fascinating species-specific differences in the ways that organisms respond to energy deficits.

In yeast, the peripheral domain of V-ATPase (V₁) rapidly dissociates from the membrane-bound domain (V₀) when glucose is not present (2). This disassembly completely inactivates V-ATPase, because V₁ cannot hydrolyze ATP without V₀, and V₀ cannot transport protons from the cytosol into the yeast vacuole without V₁ (1). Glucose readdition reverses disassembly, and reassembled V₁V₀ resumes ATP-driven proton transport to restore vacuolar and cytosolic pH homeostasis (2). It is believed that this regulated assembly cycle is essential for maintaining energy and pH homeostasis across the cell: V-ATPase disassembly conserves ATP under nutrient-limiting conditions (2), whereas glucose-induced V-ATPase reassembly allows cells to remove acid equivalents from the cytosol during enhanced glycolysis (4). Perhaps because eukaryotic V-ATPase complexes are highly conserved, studies in yeast have remained the primary focus for this process, and disassembly mechanisms in response to glucose deprivation have been understudied in mammalian systems. Furthermore, the molecular mechanisms driving V-ATPase disassembly in any organism are poorly understood.

McGuire and Forgac describe a new, mammalian-specific phenomenon (3): activation and increased assembly of mammalian V-ATPase in response to acute glucose depletion. Compared with baseline levels of assembled V-ATPase (5 mM glucose), the authors detected a 2-fold increase in assembled V₁V₀ complexes in membrane fractions from HEK293T cells deprived of glucose for 10 min. Notably, the magnitude of this effect was comparable to the increase observed following treatment with elevated glucose (25 mM). The increased V-ATPase assembly upon glucose starvation resulted in a corresponding increase in lysosomal V-ATPase activity and decrease in pH both in vitro (as measured by increased FITC-dextran quenching in isolated membranes) and in vivo (as measured by increased Lyso-Tracker fluorescence in intact cells). The authors also confirmed that V-ATPase is responsible for this phenomenon by adding 2-deoxyglucose, a V-ATPase inhibitor, which prevented lysosomal acidification in glucose-deprived cells. Notably, these observations were reproduced in a second cell line, LLC-PK1, suggesting that starvation-induced increases in V-ATPase assembly and activity may be a generalized response to glucose depletion in mammalian cells (Fig. 1).

McGuire and Forgac (3) next identified the signaling pathways that enable V-ATPase up-regulation under glucose scarcity. First, they showed that acute starvation activates ERK and AMPK signaling. Additional time-course studies clarified that activation of AMPK precedes lysosomal V-ATPase activation, which led the authors to conclude that activation of AMPK is required for V-ATPase assembly following glucose depletion. This hypothesis was further corroborated with inhibitor studies demonstrating that treatment with dorsomorphin (an AMPK inhibitor) prevented V-ATPase assembly and downstream lysosomal acidification during acute glucose starvation. Similar results with LY294002 (a PI3K inhibitor) and MK2206 (an Akt inhibitor) suggest involvement of the PI3K/Akt signaling pathway in this process as well (Fig. 1).

McGuire and Forgac (3) are the first to show V-ATPase activation under low glucose conditions. However, this discovery is
at odds with our current understanding of the molecular nature of regulated assembly. Specifically, V-ATPase appears primed to disassemble, and reassembly may require some form of energy (e.g. ATP) or assembly factor(s) to reform functional V1V0 interactions (5, 6). In yeast, glucose-driven V1V0 reassembly is aided by a V-ATPase-exclusive chaperone, regulator of ATPase of vacuoles and endosomes (RAVE). It will be interesting to explore whether the functions of RAVE are conserved in mammalian cells in the form of rabconnectins, RAVE sequence homologues that are involved in acidification of endosomes (7).

In a more general sense, why would cells use energy, under conditions of low energy input, to reassemble V-ATPase? McGuire and Forgac offer the intriguing hypothesis that V-ATPase-induced lysosomal acidification may promote autophagy by activating the corresponding lysosomal enzymes, providing new building blocks for cellular energy needs (8). Another important question that remains largely unaddressed is the relationship between V-ATPase and AMPK during glucose deprivation. It was earlier reported that, during glucose starvation, V-ATPase is required for noncanonical glucose sensing by AMPK on lysosomal membranes (9). In their study, McGuire and Forgac (3) demonstrated the opposite relationship, with AMPK signaling required for V-ATPase assembly and activation. Whether V-ATPase assembly upon glucose

depletion involves canonical AMP-mediated AMPK activation, and whether both canonical and noncanonical AMPK activation mechanisms operate independently on lysosomes, remains to be elucidated.

The findings by McGuire and Forgac unveil a critical difference in how fungal and mammalian systems regulate V-ATPase in response to glucose depletion. Despite these differences, both systems clearly adjust their V-ATPase assembly status to align with the glucose level and energy needs of the cell, and this process may underlie numerous disease pathologies. For example, enhanced glucose uptake is a characteristic of cancer, and cancer cells must adapt to low glucose to survive in nutrient-deprived environments or following anti-angiogenic therapy (10).

Therefore, understanding the molecular mechanisms by which cells determine how much V1V0 to disassemble or reassemble, so as to maintain sufficient V-ATPase activity during low and high energy periods, should lead to critical advances in metabolic therapy.

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