On the move, lysosomal CAX drives Ca\textsuperscript{2+} transport and motility

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Acidic Ca\textsuperscript{2+} stores are important sources of Ca\textsuperscript{2+} during cell signaling but little is known about how Ca\textsuperscript{2+} enters these stores. In this issue, Melchionda et al. (2016. J. Cell Biol. http://dx.doi.org/10.1083/jcb.201510019) identify a Ca\textsuperscript{2+}/H\textsuperscript{+} exchanger (CAX) that is required for Ca\textsuperscript{2+} uptake and cell migration in vertebrates.

Intracellular Ca\textsuperscript{2+} signaling is of fundamental importance in processes such as cell migration but we do not fully understand the contribution made by different intracellular Ca\textsuperscript{2+} stores to this particular function. Elevation of cytosolic Ca\textsuperscript{2+} by 10- to 100-fold the normal resting levels can occur by entry of external Ca\textsuperscript{2+} across the plasma membrane and release of Ca\textsuperscript{2+} from intracellular organelles such as the ER. Ca\textsuperscript{2+} ions are transported across membranes by ligand-gated ion channels, energy-dependent pumps, and transporters (Berridge et al., 2003; Lloyd-Evans et al., 2010). Intracellular Ca\textsuperscript{2+} levels are regulated in this manner from simple organisms, such as yeast, through to complex multicellular organisms, suggesting a degree of conservation across the taxonomic kingdoms (Patel and Cai, 2015). Recent evidence has indicated that “acidic Ca\textsuperscript{2+} stores” such as lysosomes in mammalian cells are a key intracellular Ca\textsuperscript{2+} signaling store, like the ER (Lloyd-Evans and Platt, 2011; Patel and Mualem, 2011). The Ca\textsuperscript{2+} concentration of the lysosome (500 μM) is similar to the ER (Christensen et al., 2002; Lloyd-Evans et al., 2008) but lysosomes are smaller in volume and their impact on cellular Ca\textsuperscript{2+} signaling seems localized to events that regulate endocytosis, vesicular fusion, and recycling (Ruas et al., 2010; López-Sanjurjo et al., 2013). However, there is a significant amount of evidence emerging that lysosomes are capable of triggering much larger changes in cytosolic Ca\textsuperscript{2+} during signaling via the induction of Ca\textsuperscript{2+} release from the ER. This effect appears to be mediated by the most potent intracellular Ca\textsuperscript{2+}-releasing second messenger nicotinic acid adenine dinucleotide phosphate (NAADP), which triggers Ca\textsuperscript{2+} release from lysosomes via two-pore channels (Brailoiu et al., 2009; Calcra et al., 2009). In addition to two-pore channels, acidic stores also express other Ca\textsuperscript{2+}-permeable channels (summarized in Fig. 1).

Despite recent advances in our knowledge of lysosomal Ca\textsuperscript{2+} release channels, we have so far failed to identify the transport proteins that fill the lysosome with Ca\textsuperscript{2+}. Ca\textsuperscript{2+} entering the cell by endocytosis is removed by the early endosome after the initiation of endosomal acidification by the vATPase; therefore, it is likely that lysosomes have their own transporters or pumps to take up Ca\textsuperscript{2+} (Gerasimenko et al., 1998; Christensen et al., 2002). Although there have been studies suggesting the presence of ATPases and putative ion exchangers on mammalian cells (Styrt et al., 1988), the identity of the proteins that mediate lysosomal Ca\textsuperscript{2+} uptake remains elusive. In this issue, Melchionda et al. describe the first lysosomal CAX in nonplacental mammals and link lysosomal Ca\textsuperscript{2+} import via CAX to the maintenance of normal cellular migration during development.

To identify novel regulators of Ca\textsuperscript{2+} transport in vertebrates, Melchionda et al. (2016) searched gene databases for homologues of the CAX proteins, which are known to use the proton gradient across the vacuole to drive Ca\textsuperscript{2+} uptake in plant and yeast cells (Dunn et al., 1994). They identified putative CAX genes in many species, from sea urchins and frogs to reptiles and birds. The CAX homologues discovered in the genomes of the platypus and Tasmanian devil are the first lysosomal Ca\textsuperscript{2+} exchangers to be identified in any mammalian species. This new work is a significant finding as it suggests that these mechanisms do clearly exist in some mammalian cells and are required for lysosomal Ca\textsuperscript{2+} store filling. To examine the regulation of lysosomal Ca\textsuperscript{2+} uptake by vertebrate CAX transporters, the authors cloned full-length CAX from the frog and found that expression of frog CAX could rescue Ca\textsuperscript{2+} transport in yeast lacking their own CAX. Furthermore, the authors show that the frog CAX channels correctly localize to lysosomes when expressed in human cell lines and that these CAX are capable of manipulating lysosomal and cytosolic Ca\textsuperscript{2+} levels (in a manner perhaps comparable to plasma membrane Ca\textsuperscript{2+} ATPases). The findings reported in Melchionda et al. (2016) also have significance for researchers who are using simpler model organisms to characterize mechanisms regulating acidic store Ca\textsuperscript{2+}. A study by Churchill et al. (2002) that used acidic stores purified from sea urchin egg homogenate to monitor acidic store Ca\textsuperscript{2+} entry concluded that vanadate-sensitive Ca\textsuperscript{2+} pumps were absent and suggested instead the presence of a CAX. This now appears to be the case through the reported cloning of sea urchin CAX. The findings of Melchionda et al. (2016) are a step forward in unraveling the molecular mechanisms of Ca\textsuperscript{2+} handling in model animals.

Ca\textsuperscript{2+} signaling plays an important role in development, particularly for cellular migration, where localized elevations in intracellular Ca\textsuperscript{2+} drive rearrangement of the cytoskeleton, cellular contraction, and adhesion (Wei et al., 2009; Sumoza-Toledo et al., 2011; Praitis et al., 2013). A concentration gradient
of Ca\textsuperscript{2+} exists across the migrating cell, with higher levels at the rear that contribute to cellular detachment and contraction (Praitis et al., 2013). Recent evidence has highlighted the presence of Ca\textsuperscript{2+} flickers at the leading edge of the migrating cell that have been shown to underlie changes in direction (Wei et al., 2009). Despite the clear importance of Ca\textsuperscript{2+} in mediating cellular migration events and the emergent role of lysosomes in maintaining intracellular Ca\textsuperscript{2+} signaling, very little is known about the roles of lysosomal Ca\textsuperscript{2+} stores in cellular migration. ER Ca\textsuperscript{2+} channels including the inositol 1,4,5-trisphosphate receptors and ryanodine receptors as well as the secretory pathway Ca\textsuperscript{2+} ATPase and lysosomal TRPM2 have all been implicated in regulating changes in intracellular Ca\textsuperscript{2+} to mediate cellular migration, but to date no lysosomal transporters have been implicated in this process (Wei et al., 2009; Somoza-Toledo et al., 2011; Praitis et al., 2013). Melchionda et al. (2016) investigated the migration of neural crest cells during frog development to find out whether or not Ca\textsuperscript{2+} transporters control motility. CAX proteins are expressed in the neural crest of developing frogs and morpholino-mediated knockdown of CAX expression reduced the ability of neural crest cells to form stable protrusions that contain focal adhesion complexes at the leading edge of the migrating neural cells. Loss of CAX protein expression reduced the ability of neural crest cells to form stable focal adhesions and undergo the initial cell spreading required for migration. The work presented by Melchionda et al. (2016) is a significant discovery providing evidence that lysosomal Ca\textsuperscript{2+} uptake is involved in cell migration and that lower organisms are useful model systems to investigate the role of acidic store Ca\textsuperscript{2+} in this critical cellular function during embryo development.

Melchionda et al. (2016) have made a significant step forward in our understanding of the mechanisms that regulate lysosomal/vacuolar Ca\textsuperscript{2+} entry. However, we remain in the dark about the identity of the transporters that pump Ca\textsuperscript{2+} into the lysosomes of placental mammals. What led to the loss of CAX genes in these organisms is as much a mystery as the identity of the transporters that have replaced CAX. Evidence from a study using purified mammalian lysosomes to observe Ca\textsuperscript{2+} uptake indicates that the process is ATP-dependent (Styrt et al., 1988). Placental mammals may have completely different ATP-dependent mechanisms governing lysosomal Ca\textsuperscript{2+} uptake compared with lower order organisms and nonplacental mammals. Interestingly, defects in lysosomal Ca\textsuperscript{2+} uptake are associated with two human diseases, Niemann-Pick type C and Chediak-Higashi syndrome (CHS; Styrt et al., 1988; Lloyd-Evans et al., 2008). The lysosomal accumulation of sphingosine, a Ca\textsuperscript{2+} ATPase inhibitor (Lloyd-Evans and Platt, 2011), leads to reduced lysosomal Ca\textsuperscript{2+} levels in Niemann-Pick type C disease cells and defects in NAADP-mediated lysosomal Ca\textsuperscript{2+} release (Lloyd-Evans et al., 2008). In CHS, there have been reports of enhanced lysosomal Ca\textsuperscript{2+} ATPase transporter activity in neutrophils (Styrt et al., 1988). Interestingly, CHS leukocytes show alterations in chemotaxis with a reduced response to chemotactic factors (Clark and Kimball, 1971), which is supportive of the findings of Melchionda et al. (2016). Much remains to be elucidated about the enigma of mammalian lysosomal Ca\textsuperscript{2+} uptake, but the work of Melchionda et al. (2016) begins to pick this mystery apart.

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