# Mitochondria Are Clamped to Vacuoles for Lipid Transport

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http://dx.doi.org/10.1016/j.devcel.2014.06.024

**In this issue of *Developmental Cell*, Elbaz-Alon et al. (2014) and Hönscher et al. (2014) describe a contact site between mitochondria and the lysosome-like yeast vacuole named vCLAMP (*v*acuo*l*e *a*nd *m*itochondria *p*atch). They show that vCLAMP plays a role in lipid exchange, thereby linking mitochondria to the endomembrane system.**

Mitochondria are endosymbiotic cell organelles that arose from once free-living bacteria taken up and enslaved by an ancient Archaea-like cell. During ~1.5 billion years of coevolution with their host, these bacteria became essential organelles and hallmarks of eukaryotic cells. Contemporary mitochondria have lost most of their autonomy, and their biogenesis and behavior strictly depend on multiple interactions with other organelles. Most of the endosymbiont's genes were transferred to the host genome, and thus the vast majority of mitochondrial proteins have to be synthesized by cytosolic ribosomes and imported post-translationally. Furthermore, most of the mitochondrial membrane lipids are synthesized in the endoplasmic reticulum (ER) and must be imported into the organelle (Tatsuta et al., 2014). However, mitochondria are not connected to the vesicular transport system that distributes membrane lipids among the endomembranes of eukaryotic cells. Instead, intimate physical contacts with the ER, termed mitochondria-associated membranes (MAMs), are thought to mediate nonvesicular lipid exchange between both organelles. Our understanding of the molecular nature of mitochondria-ER contacts is most advanced in baker's yeast *Saccharomyces cerevisiae*. Contact sites in yeast were recently shown to be established by the ER-mitochondria encounter structure (ERMES) complex, which consists of the ER membrane protein Mmm1, a soluble factor, Mdm12, and two mitochondrial outer membrane proteins, Mdm10 and Mdm34 (Kornmann et al., 2009). Mitochondria participate in numerous biochemical pathways and therefore are essential for viability even in organisms such as yeast that can live without oxidative phosphorylation. At the same time, their biogenesis strictly depends on the import of ER-synthesized lipids. Thus, it was surprising to see that ERMES mutants are viable (Kornmann et al., 2009) and display an unexpectedly mild lipid phenotype (Nguyen et al., 2012). These findings demand an alternative route for phospholipid import into mitochondria, but the molecular components and cellular pathways involved remained enigmatic. In this issue of *Developmental Cell*, two papers—Elbaz-Alon et al. (2014) and Hönscher et al. (2014)—solve this mystery by reporting the identification of a molecular hug between the yeast vacuole and mitochondria called the vacuole and mitochondria patch (vCLAMP). These studies provide compelling evidence that vCLAMP can compensate for the loss of mitochondria-ER contacts by providing membrane lipids for mitochondrial biogenesis (Figure 1).

The two groups headed by Maya Schuldiner and Christian Ungermann identified vCLAMP by different approaches. Elbaz-Alon et al. (2014) reasoned that loss of an ERMES-independent lipid transport pathway would result in upregulation of ERMES-dependent contact sites. To identify this pathway, the authors screened the ~4,800 viable yeast gene deletions for mutants that display an increased number of ERMES foci per cell. Hönscher et al. (2014), on the other hand, first observed mitochondria-vacuole contact sites by fluorescence microscopy and electron tomography before using a candidate gene overexpression approach to look for strains that showed an expansion of mitochondria-vacuole contacts. Strikingly, both approaches led to the identification of the same gene: *VPS39*. The Vps39 protein is a well-known subunit of the homotypic fusion and vacuole protein sorting (HOPS) tethering machinery that is required for the fusion of vacuoles. While HOPS is a multi-subunit complex, Vps39 and the small Rab GTPase Ypt7 appear to be the only components that are shared between the vacuolar fusion machinery and vCLAMP. Consistent with the anticipated tethering function of Vps39, mitochondrial and vacuolar membranes are found in close proximity in yeast cells, and Vps39 is localized exactly at these sites, as revealed by immunoelectron microscopy (Elbaz-Alon et al., 2014; Hönscher et al., 2014).

Do ERMES and vCLAMP execute partially redundant functions in lipid transport? Several lines of evidence suggest that the answer to this question is yes. Contacts between mitochondria and ER or vacuoles appear to be coregulated. Elbaz-Alon et al. (2014) show that disruption of ERMES causes strong vCLAMP proliferation and vice versa. Consistently, Hönscher et al. (2014) report that vCLAMP and ERMES formation are antagonistically regulated in response to the carbon source of the growth medium. Unlike mitochondria, vacuoles are connected to the ER by the vesicular transport system. Thus, it was tempting to speculate that contacts between mitochondria and vacuoles could bypass ERMES function and supply mitochondria with ER-derived lipids. Consistent with this idea, double mutants lacking crucial vCLAMP and ERMES components are inviable. Using conditional ERMES/vCLAMP double mutants, Elbaz-Alon et al. (2014) show that vCLAMP indeed

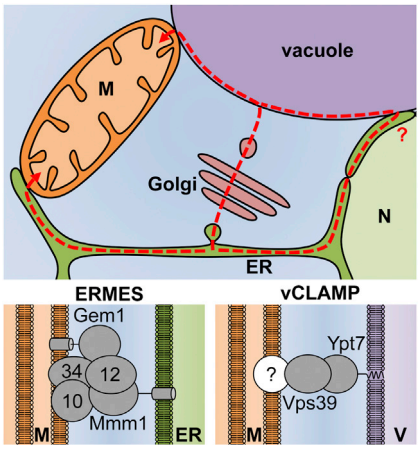


**Figure 1. Model for ERMES and vCLAMP Cooperation in Supplying Mitochondria with Lipids**

The ER is the source of phospholipids that are either directly imported into mitochondria (M) or make a detour through the vacuole (V). Mitochondria are connected to the ER by ERMES and to the vacuole by vCLAMP. Lipids are imported into mitochondria through both contact sites (red arrows). Vacuoles obtain ER-synthesized lipids via Golgi-dependent vesicular traffic and possibly through direct contacts with the ER at the nucleus (N) vacuole junction. ERMES consists of the ER residing subunit Mmm1, two mitochondrial outer membrane proteins, Mdm10 (10) and Mdm34 (34), a soluble subunit, Mdm12 (12), and the mitochondrial outer membrane GTPase, Gem1. vCLAMP is formed by the soluble HOPS complex subunit Vps39, the small prenylated Rab GTPase Ypt7, and an unknown mitochondrial receptor (?).

serves as a route for phospholipid transport to mitochondria. Thus, vCLAMP constitutes an alternative pathway for import of lipids into mitochondria that maintains the mitochondrial lipid supply in ERMES mutants.

The dynamic interplay between vCLAMP and ERMES demands some crosstalk between the organelles to regulate contact site formation. While the signaling cascades are yet unknown, there are some promising candidates. The regulatory GTPase Gem1 is associated with ERMES, and cells lacking Gem1 display fewer and larger ERMES foci, pointing to a role of Gem1 as a regulator of ERMES assembly (Kornmann et al., 2011). Furthermore, Hönscher et al. (2014) provide evidence that vCLAMP formation is negatively regulated by phosphorylation of Vps39 by an unknown kinase. Recently, the genes *MCP1* and *MCP2* were identified as multicopy suppressors of the ERMES component Mdm10 deletion phenotype (Tan et al., 2013). Mcp2 is a conserved protein homologous to an uncharacterized human putative kinase, ADCK-1, and thus could play a role in phosphorylation of contact site proteins. It will be exciting to reveal how cells sense existing contact sites, integrate intra- and extracellular stimuli, and finally respond by the regulated formation of new interorganellar contacts.

An intimate functional relationship between the vacuole and mitochondria has been anticipated for many years. For example, several mutants with impaired vacuolar functions, such as V-ATPase mutants, suffer from rapid loss of mitochondrial respiratory activity, and the Δ*vps39* mutant was found previously to be respiratory deficient (Merz and Westermann, 2009). Furthermore, age-induced decline of vacuolar acidity is tightly connected to mitochondrial dysfunction (Hughes and Gottschling, 2012). The identification of vCLAMP opens the door to an understanding of this interorganellar dependence in yeast. It will be exciting to see whether a similar organellar crosstalk exists in higher eukaryotes. Intriguingly, mouse mitochondria establish physical contacts to melanosomes, lysosome-related organelles of pigment cells, and these contacts are involved in melanosome biogenesis and maturation (Daniele et al., 2014). Thus, spatial and functional connections between mitochondria and late endomembrane organelles may be common to both yeast and higher eukaryotes. The discovery of vCLAMP will certainly stimulate the exploration of a so far poorly understood area of cell biology.

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