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SPOTLIGHT

Cnm1: A bridge between mitochondria and nuclear ER

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Few membrane contact sites have been defined at the molecular level. By using a high-throughput, microscopy-based screen, Eisenberg-Bord, Zung et al. (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202104100) identify Cnm1 as a novel tethering protein that mediates contact between mitochondria and the nuclear ER in response to phospholipid levels.

Organelles communicate through the exchange of biological materials by vesicular trafficking or at sites of close membrane apposition known as membrane contact sites (MCSs). While the molecular machinery mediating vesicular trafficking has been well characterized, our knowledge of the molecules involved in forming and regulating MCSs is limited. MCSs physically tether two or more organelles via protein–protein or protein–lipid interactions, contain defined proteomes, and perform specific biological functions (1). While MCSs have been appreciated microscopically since the 1950s, only recently have advances in technology permitted the discovery of the molecular composition of some MCSs (2). A major breakthrough occurred when a synthetic biology screen identified the ER–mitochondria encounter structure (ERMES), which forms an MCS between the ER and mitochondria (3). ERMES has since been shown to be involved in phospholipid transport between mitochondria and the ER (4). While ERMES is one of the best characterized MCSs, there are still many questions as to the precise molecules being transported at ER–mitochondria contacts and how directionality of transport is achieved. Subsequent studies using split fluorescent proteins revealed that nearly all organelles appear to form MCSs of some kind (5). Thus, despite progress in defining the components and functions of a few MCSs, there are still many MCSs whose molecular identities are completely unknown.

Recently, a study in mammalian cells identified an MCS between the nucleus and mitochondria that plays a role in adapting cells to stress via the mitochondrial retrograde signaling response (6). The proteins that form this MCS are not conserved in yeast, however, suggesting that alternative mechanisms for nucleus–mitochondria contacts exist in other organisms. In this issue, Eisenberg-Bord, Zung et al., set out to identify proteins involved in forming an MCS between mitochondria and the nuclear ER that is distinct from ERMES-mediated ER–mitochondria contacts (7). First, high-resolution cryo-electron tomographs revealed that mitochondria form contacts with the nucleus that have an average separation of ∼20 nm, which is within the expected range for a bona fide MCS (1). To identify the molecular composition of this contact site, the authors generated a synthetic reporter that is specific to nucleus-mitochondria contacts by fusing one part of a split fluorescent protein to an outer mitochondrial membrane protein and the other to a peripheral nuclear protein. A high-throughput, microscopy-based genetic screen was then used to compare the localization of the synthetic reporter to fluorescently tagged versions of all yeast proteins. Candidates were refined by determining which proteins caused an expansion of the nucleus–mitochondria contact site upon overexpression, a phenotype that has been observed with other MCS proteins (8). Based on these results, the best candidate for a molecular tether between mitochondria and the nucleus was Ybr063c.

Ybr063c is a 46-kD nonessential protein of uncharacterized function that contains predicted transmembrane domains. The authors first demonstrated that Ybr063c is an integral membrane protein residing on the nuclear membrane. In support of Ybr063c forming a nucleus–mitochondria contact site that is distinct from ERMES, Ybr063c did not colocalize with ERMES subunits nor did overexpression of Ybr063c alter the size of ERMES patches. Remarkably, overexpression of Ybr063c resulted in the mitochondrial network becoming tightly associated with the nuclear membrane. Based on these results, the authors concluded that Ybr063c functions as a molecular tether between mitochondria and the nucleus and the protein was renamed Cnm1 for contact nucleus mitochondria 1.

Through further genetic screens, Eisenberg-Bord, Zung et al., identified several genes that are required to cluster mitochondria around the nucleus when Cnm1 is overexpressed. Interestingly, several of these genes are known to function in phosphatidylcholine (PC) metabolism. Deletion of these components resulted in a decrease in Cnm1 expression, which alters the extent of nucleus-mitochondria contacts. Overexpression of Cnm1 in genetic conditions that reduce PC

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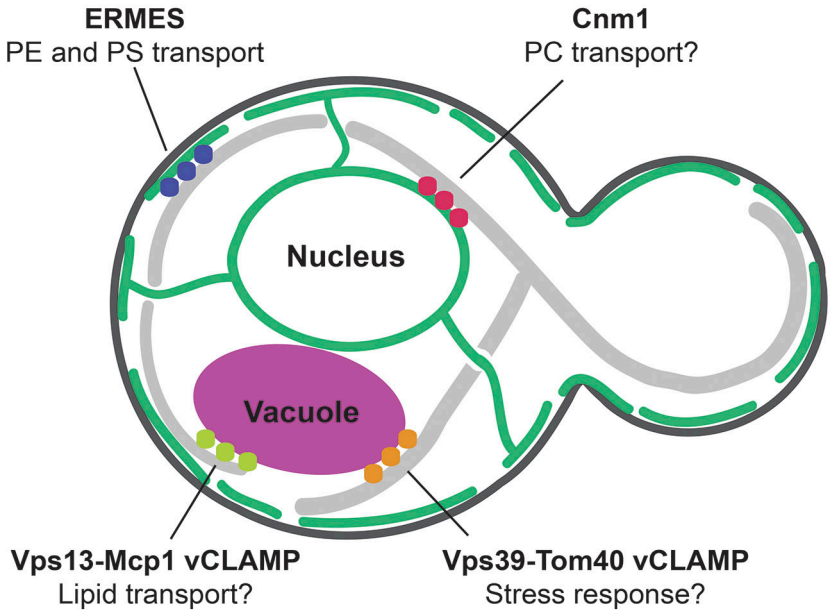

Figure 1. **The ER and vacuole form multiple MCSs with mitochondria in budding yeast.** The ER is depicted in green, and the mitochondrial network is depicted in gray. ERMES mediates an MCS between tubular ER and mitochondria. In addition to functions that are distinct from lipid trafficking, ERMES-mediated MCSs likely function to transport PS or PE between the organelles. Cnm1 mediates an MCS specifically between the nuclear ER and mitochondria and potentially functions in PC transport. The Vps13-Mcp1 vCLAMP mediates an MCS between mitochondria and the vacuole that likely functions in lipid transport and may have redundant functions with ERMES. The Vps39-Tom40 vCLAMP is a separate MCS between mitochondria and the vacuole that responds to different stress conditions, though its function is unknown.

levels resulted in exaggerated growth defects. These results raise the possibility that Cnm1-mediated nuclear–mitochondria contacts may be involved in the transport of PC from the ER to mitochondria. Thus, while the functional importance is unknown, Cnm1-mediated nuclear–mitochondria contacts respond to PC levels.

The genetic screens also identified a single resident mitochondrial protein, Tom70, as affecting the ability of overexpressed Cnm1 to cluster mitochondria around the nucleus. Subsequent experiments demonstrated that localization of Cnm1 to the nuclear membrane and Tom70 to the mitochondrial membrane is required to tether mitochondria to the nucleus upon overexpression of Cnm1. Thus, Cnm1 and Tom70 mediate an MCS between mitochondria and the nucleus.

The identification of Cnm1-mediated nucleus–mitochondria contacts opens many questions about the function and composition of the contact site and how it operates in the broader context of mitochondrial–nuclear communication. While identifying the functions of MCSs has proven challenging, the genetic screens conducted in this study provide an excellent starting point by elucidating a link between Cnm1 and PC metabolism. The authors propose that Cnm1-mediated contacts could function in the direct transport of PC from the ER to mitochondria (Fig. 1). In this model, ERMES, which likely functions in earlier steps of PC synthesis by transporting phosphatidylethanolamine (PE) or phosphatidylserine (PS), would have a distinct but related function in organizing and maintaining a pipeline for the transport of lipids between the ER and mitochondria (Fig. 1). This model is speculative, however, and future experiments will be necessary to define the role of Cnm1 in PC metabolism.

There is a growing body of evidence that two organelles can form multiple MCSs that are spatially and functionally distinct. In addition to ERMES and Cnm1-mediated mitochondria–ER contacts, in yeast, two distinct MCSs have been described between mitochondria and the vacuole that are referred to as vacuolar and mitochondrial patches, or vCLAMPs. One, mediated by Vam6 and Tom40, has been implicated in responding to cellular stress while the other, mediated by Mcp1 and Vps13, may have overlapping functions with the ERMES complex (8, 9; Fig. 1). Interestingly, many of the proteins present at MCSs have been shown to be multifunctional (2). For example, the vCLAMP component Vam6 is also a subunit of the homotypic fusion and protein-sorting (HOPS) complex while its binding partner Tom40 is the central subunit of the translocase of outer membrane (TOM) complex (8). Thus, while these complexes have distinct biological functions in vacuolar protein sorting and mitochondrial protein import respectively, individual subunits have moonlighting functions in the formation, and perhaps function, of MCSs. Eisenberg-Bord, Zung et al., now reveal that Tom70, another component of the TOM complex, also plays a role in the formation of nucleus–mitochondria contacts. This raises the exciting possibility that cells use these multifunctional proteins to coordinate functions such as mitochondrial protein import with lipid trafficking. A crucial next step will be to determine how the multiple functions of these proteins are coordinated to maintain organelle homeostasis.

Nuclear–mitochondrial communication is a critical aspect of eukaryotic cellular life that allows cells to adapt to different environmental conditions and energy needs. A breakdown in communication between mitochondria and the nucleus has been implicated in several diseases, including cancers (10). The formation of a nucleus–mitochondria MCS likely facilitates the exchange of lipids or small molecules that stimulate signaling pathways to help cells respond to environmental changes or mitochondrial damage (6, 7). Identifying the molecules that regulate these contacts and clarifying the physiological contexts under which these contacts function is crucial to our understanding of human disease. Thus, the identification of a nucleus-mitochondria MCS represents a

significant breakthrough in our understanding of nucleus–mitochondria communication.

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References

1. Scorrano, L., et al. 2019. *Nat. Commun.* 10:1287. https://doi.org/10.1038/s41467-019-09253-3
2. Lackner, L.L. 2019. *Trends Cell Biol.* https://doi.org/10.1016/j.tcb.2019.02.009
3. Kornmann, B., et al. 2009. *Science.* https://doi.org/10.1126/science.1175088
4. Kawano, S., et al. 2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201704119
5. Shai, N., et al. 2018. *Nat. Commun.* https://doi.org/10.1038/s41467-018-03957-8
6. Desai, R., et al. 2020. *Sci. Adv.* https://doi.org/10.1126/sciadv.abc9955
7. Eisenberg-Bord, M., et al. 2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202104100
8. González Montoro, A., et al. 2018. *Dev. Cell.* https://doi.org/10.1016/j.devcel.2018.05.011
9. John Peter, A.T., et al. 2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201610055
10. Xia, M., et al. 2019. *Cell Biosci.* https://doi.org/10.1186/s13578-019-0289-8