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Preview

MIMAS is a new giant multifunctional player in the mitochondrial megacomplex playground

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Mitochondria are rich in multi-protein assemblies that are usually dedicated to one function. In this issue of *Cell Reports,* Horten et al.[1] describe a 3-megadalton megacomplex in the mitochondrial inner membrane, which serves multiple functions integrating mitochondria biogenesis and metabolism.

The vast majority of the approximately one thousand mitochondrial proteins are imported from the cytosol.[2] Sorting and assembly of many imported proteins into multiprotein complexes within the organelle ensures the broad range of mitochondrial functions and cell fitness.[3] Early identification of mitochondrial proteins and protein import complexes relied on a combination of genetics and *in vitro* reconstitution assays. The development of high-resolution mass spectrometry allowed detailed complexome analyses for several mitochondrial complexes[4–6] that were initially showcased for respiratory supercomplexes.[7–9] An ever-increasing number of high-resolution structures provided an additional layer of systematic and comprehensive analysis of complexes ranging from OXPHOS, to protein translocases, and to machineries involved in protein folding or scaffolding and shaping of the inner membrane architecture. Underpinning the biochemical characterization of these complexes is our capacity to isolate them using mild detergents that preserve their integrity. The largest complexes known so far were the electron transfer chain supercomplexes (about 1 megadalton [MDa]) in the inner mitochondrial membrane (IM). However, despite the definition of many complexes, there are still some proteins (for example, assembly factors for the electron transfer complexes) that are not assigned to any. Additionally, these big complexes seem to be an assembly of proteins all dedicated to a specific function. Here, Horten et al. report a much larger complex, mitochondrial inner membrane (MIMAS) (3 MDa) that integrates several functions previously thought to be distinct and mediated by separate proteins or separate complexes.[1] Additionally, lipids crucially affect the integrity of MIMAS, rendering it a protein-lipid platform capable of integrating processes like protein biogenesis and mitochondrial metabolism. The presence of MIMAS in the mitochondrial IM is not entirely surprising given it is the most protein-rich membrane within cells. Nevertheless, it supports the emerging concept of a higher level of physical organization as a key principle to integrate apparently distinct functions.

The starting point for the discovery of MIMAS[1] was a biochemical analysis of the interaction of the ADP/ATP carrier (AAC2) and the COXIV assembly factor protein Rcf1. The two proteins formed a complex and comigrated within an assembly of 3 MDa in size. This megacomplex contained no respiratory chain subunits, and it was also different from the dimeric ATPase complex of 1.2 MDa. Such large complexes are not studied in native complex analysis, which could explain why it remained undetected so far. Further support for the interaction of the AAC and Rcf1 came from the MitCOM dataset.[9] Intriguingly, other metabolite carriers were also found comigrating in the same MIMAS complex, suggesting for the first time that at least some of these metabolite transporters (including some of the most abundant mitochondrial proteins) are part of the same megacomplex, despite their differential cargo selectivity. Does MIMAS contain only respiratory chain assembly factors and metabolite carriers? It turned out that this complex has a much more diverse composition. Several dehydrogenases and phospholipid biosynthesis enzymes are linked to the metabolic functions of the metabolite carriers, and, surprisingly, some of these proteins were also part of MIMAS. This evidence further supports MIMAS's function as an organized scaffold of proteins with diverse functions. Horten et al. elegantly demonstrate the proximity of these different proteins within MIMAS by performing chemical crosslinking and affinity pull-down experiments with tagged proteins. MIMAS can be isolated under a variety of conditions, suggesting it is a stable complex. The authors went a step further to investigate the presence of phospholipid biosynthesis enzymes within MIMAS. Cardiolipin is present in high amounts in the mitochondrial IM and stabilizes many protein-containing complexes. Surprisingly, the authors found that the enzymes catalyzing the early stages of cardiolipin (CL) synthesis (Pgs1 and Tam41) were not part of MIMAS, whereas the key enzymes for catalyzing phosphatidylethanolamine (PE) synthesis (Psd1) and cardiolipin synthase (Crd1) and cardiolipin transacylase (Taz1) were included. The presence of these enzymes begged the question as to whether any of these phospholipids affected the stability of MIMAS. This is a pertinent question because these nonbilayer lipids affect the stability of many IM complexes. By using relevant gene knockouts and catalytically inactive protein variants, the authors showed that PE, but not CL, is critical for the stability of MIMAS, which operates as a single-platform megacomplex involving several respiratory chain assembly factors, metabolite carriers, dehydrogenases, and phospholipid biosynthesis

enzymes. Its mere size, the diversity of functions, and the dependence of its integrity on PE distinguish MIMAS from other megacomplexes of the mitochondrial IM. The MitCOM complexome database[9] provided a useful tool to assess the interactions among these proteins within MIMAS and suggested other MIMAS components that can be analyzed further. The specificity of the complex, which is not just a randomly assembled entity of abundant mitochondrial proteins, presumably underpins specific and diverse functions that are integrated by MIMAS for optimal fitness of mitochondria. Horten et al. further isolate two versions of the MIMAS complex at about 3.3 MDa and 2.5 MDa, which will certainly be the subject of further analysis in the future.

The emerging number of megacomplexes in the mitochondria resembles the years-long process that led to the definition of different protein import translocases, challenging the existing assumption that there was only one translocase in the outer membrane (OM). It's now clear that there are at least three translocases in the OM (the TOM, Sam and Mim1 translocase), three in the IM (the TIM23, TIM22 and OXA1 translocase) and at least one in the IMS (The MIA translocase). The discovery of MIMAS suggests that there could be other such megacomplexes (after all, Mimas had a number of other giant siblings in Greek mythology). It also raises a number of key questions: what does the assembly of abundant metabolite carriers mean for their function, as they are known to work as monomers? Is the specific influence of PE linked to a functional role, or does it just affect complex integrity? What determines segregation of certain proteins (and possibly lipids) into MIMAS as opposed to others? The work by Horton et al. reinforces the idea that to gain a holistic understanding of the different functions of mitochondria, we need to think of large assemblies that are multifunctional and can integrate apparently distinct pathways of biogenesis and metabolism, and MIMAS exemplifies this concept. An additional role of such megacomplexes could be that of a protective assembly under stress to ensure optimal operation and mitochondrial fitness in health and disease.

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DECLARATION OF INTERESTS

The author declares no competing interests.

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