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VIEWPOINT

Super-resolution microscopy: Insights into mitochondria–lysosome crosstalk in health and disease

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Live super-resolution microscopy has allowed for new insights into recently identified mitochondria–lysosome contact sites, which mediate crosstalk between mitochondria and lysosomes, including co-regulation of Rab7 GTP hydrolysis and Drp1 GTP hydrolysis. Here, we highlight recent findings and future perspectives on this dynamic pathway and its roles in health and disease.

Introduction

Advances in live super-resolution microscopy have allowed for the direct observation of cellular processes with unprecedented levels of spatiotemporal detail (Bond et al., 2022), including the crosstalk between mitochondria and lysosomes. Mitochondria, traditionally viewed as the powerhouse of the cell, play key roles in cell signaling. Lysosomes are vesicular lytic organelles that facilitate protein, metabolite, and lipid turnover, as well as nutrient sensing. Prior studies predominantly investigated mitochondria and lysosomes via fixed high-resolution ultrastructural analysis by electron microscopy, biochemical analysis at the bulk organ/cell level, or low-resolution imaging at the single-cell level. Thus, live-cell super-resolution microscopy is uniquely poised to address questions at the interface of these techniques, offering resolution capable of revealing new pathways at the individual organelle level (Fig. 1, A and B), including the dynamics and crosstalk occurring at mitochondria–lysosome contact sites.

Mitochondria–lysosome contact sites: Super-resolution imaging and cellular functions

Mitochondrial–lysosome contact sites were recently identified as a key site for the bidirectional regulation of mitochondrial and lysosomal dynamics, distinct from mitophagy or lysosomal engulfment of mitochondria (Wong et al., 2018; Cisneros et al., 2022). Multiple imaging approaches have demonstrated the formation of mitochondria–lysosome contact sites (reviewed in Cisneros et al., 2022), including super-resolution Airyscan and structured illumination microscopy (SIM; Fig. 1 D), which have provided further spatial and temporal evidence of dynamic tethering and subsequent untethering events between mitochondrial and lysosomal membranes at contact sites (Wong et al., 2018, 2023; Han et al., 2017; Kim et al., 2021; Peng et al., 2023). Live SIM also revealed inter-mitochondrial contacts involving dynamic membrane tethering between the outer mitochondrial membrane of two mitochondria independent of mitochondria fission or fusion (Wong et al., 2019). Similarly, inter-lysosomal contact sites, also recently documented with live SIM, are mediated by membrane tethering between two lysosomes and distinct from lysosomal fusion events (Wong et al., 2022).

Mitochondrial–lysosome contact sites allow for the coupled regulation of Rab7 GTP hydrolysis and Drp1 GTP hydrolysis pathways, which modulate mitochondrial and lysosomal dynamics (Fig. 1, A and C). Rab7 is a small, cytosolic GTPase recruited to the late endosomal/lysosomal membrane where, in its active GTP bound form, it binds effectors and promotes mitochondrial–lysosome contact formation. Subsequently, Rab7 GTP hydrolysis at contact sites is mediated by TBC1D15, a Rab7 GTPase activating protein (GAP) that is recruited to the mitochondria by Fis1 oligomerization. TBC1D15-mediated Rab7 GTP hydrolysis then drives both mitochondrial–lysosome contact untethering (Wong et al., 2018), and inter-lysosomal contact untethering upon contact with mitochondria (Wong et al., 2022). Similarly, Drp1 is a cytosolic GTPase recruited to the outer mitochondrial membrane by adaptors such as Mid51 oligomers, which drives Drp1 oligomerization. Drp1 oligomers can then bind Mff, which promotes Drp1 GTP hydrolysis to drive mitochondrial fission and inter-mitochondrial contact untethering upon contact with lysosomes (Wong et al., 2018, 2019). Importantly, a Mid51/Fis1 oligomerization complex on the outer mitochondrial membrane couples together Rab7 and Drp1 GTP hydrolysis pathways at mitochondria–lysosome contact sites, allowing for the co-regulation of both lysosomal and mitochondrial network dynamics (Wong et al., 2022; Fig. 1 C).

Mitochondria–lysosome contact sites also have key roles in metabolite transfer. Calcium can be ferried from lysosomes to

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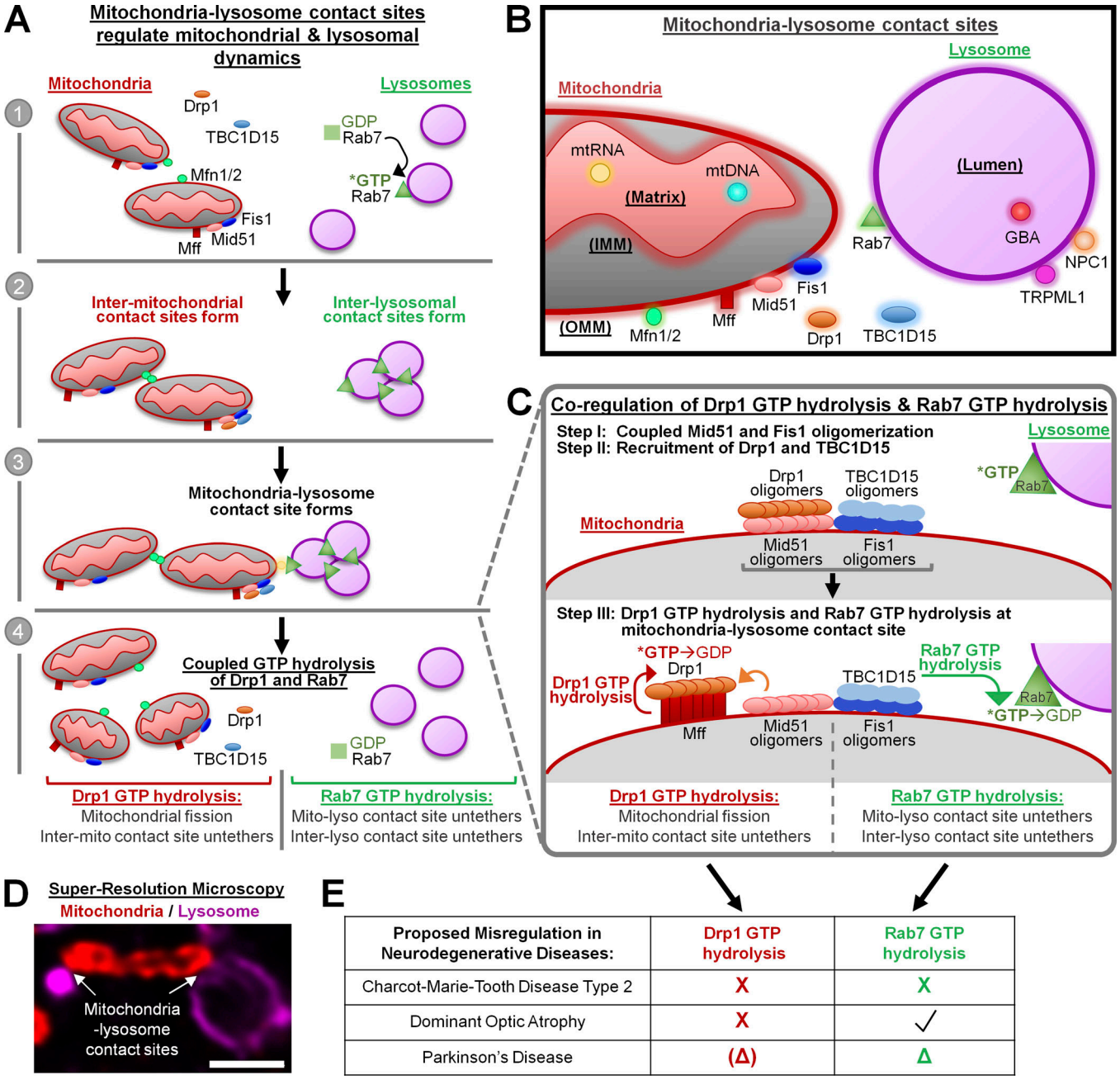

Proposed Misregulation in Neurodegenerative Diseases:	Drp1 GTP hydrolysis	Rab7 GTP hydrolysis
Charcot-Marie-Tooth Disease Type 2	X	X
Dominant Optic Atrophy	X	✓
Parkinson's Disease	(Δ)	Δ

Figure 1. **Model of crosstalk between mitochondrial and lysosomal network dynamics at mitochondria–lysosome contact sites. (A)** Mitochondria and lysosomes are bidirectionally regulated to ensure proper organelle distribution. **(1)** Mitochondria and lysosomes are initially properly distributed. Cytosolic inactive Rab7 GDP is recruited to lysosomes as active Rab7 GTP. **(2)** Rab7 GTP promotes inter-lysosomal contact tethering (potentially via the HOPS complex), while Mfn1/Mfn2 promotes inter-mitochondrial contact tethering. **(3)** Mitochondria–lysosome contacts form promoted by Rab7 GTP. **(4)** Coupled GTP hydrolysis of Drp1 and Rab7 (see panel C for details) drives mitochondrial fission and inter-mitochondrial contact untethering events via Drp1 GTP hydrolysis, and mitochondria–lysosome contact and inter-lysosomal contact untethering events via Rab7 GTP hydrolysis. **(B)** Regulation of mitochondria–lysosome contact sites by multiple mitochondrial and lysosomal proteins, including those mutated in various neurodegenerative diseases. (IMM: inner mitochondrial membrane; OMM: outer mitochondrial membrane). **(C)** Coupled co-regulation of Drp1 GTP hydrolysis and Rab7 GTP hydrolysis at mitochondria–lysosome contact sites: Mid51 and Fis1 undergo coupled oligomerization together on the outer mitochondrial membrane (Step I), which allows for the respective recruitment of Drp1 oligomers and TBC1D15 oligomers to mitochondria (Step II), and the subsequent transfer of Drp1 oligomers to Mff. This leads to Mff-driven Drp1 GTP hydrolysis and TBC1D15-driven Rab7 GTP hydrolysis at mitochondria–lysosome contact sites (Step III), and the downstream regulation of both mitochondrial and lysosomal network dynamics. **(D)** Super-resolution live SIM of mitochondria (red) and lysosomes (purple) tethered at mitochondria–lysosome contact sites (white arrows). Mitochondria: tdTomato-TOMM20; lysosome: LAMP1-Halo. Scale bar: 1 µm. **(E)** Proposed misregulation in different neurodegenerative diseases of the co-regulation of Drp1 GTP hydrolysis and Rab7 GTP hydrolysis at mitochondria–lysosome contact sites: In Charcot-Marie-Tooth Disease Type 2, both Drp1 and Rab7 GTP hydrolysis are inhibited, leading to defects in both mitochondrial and lysosomal dynamics. In Dominant Optic Atrophy, Drp1 GTP hydrolysis is selectively inhibited, leading to defects in mitochondrial dynamics. In Parkinson's disease, Rab7 GTP hydrolysis events are misregulated, leading to disrupted lysosomal dynamics and potential loss of proper co-regulation of Drp1 GTP hydrolysis and mitochondrial dynamics at mitochondria–lysosome contact sites. X = Inhibited; ✓ = Properly regulated; Δ = Misregulated.

mitochondria via the lysosomal channel TRPML1 and mitochondrial channels VDAC1 and mitochondrial calcium uniporter at mitochondria–lysosome contacts (Peng et al., 2020). Additionally, subcellular metabolomics revealed changes in amino acid profiles of lysosomes and mitochondria upon modulation of mitochondria–lysosome contact sites (Peng et al., 2023). Moreover, misregulated cholesterol trafficking may further alter mitochondria–lysosome contact formation (Höglinger et al., 2019). Finally, contacts between mitochondria and late endosomes can mediate RNA translation in axons (Cioni et al., 2019). Thus, mitochondria–lysosome contact sites are important hubs for regulating cellular homeostasis.

Roles for mitochondria–lysosome contact site dysfunction in human diseases

Dysregulated mitochondria–lysosome contact sites due to the disruption of co-regulated Rab7 and Drp1 GTP hydrolysis may have a contributing role in various neurodegenerative diseases (Fig. 1 E). Charcot Marie Tooth Disease Type 2 (CMT2) is characterized by axonal degeneration in peripheral sensory and motor neurons. Autosomal dominant mutations in the late endosomal/lysosomal-localized Rab7 and mitochondrial MFN2 and GDAP1 are genetic causes for CMT2, and disrupt mitochondria–lysosome contact sites and functions including both mitochondrial and lysosomal dynamics (Wong et al., 2019, 2023; Cantarero et al., 2021; Khalil et al., 2017; Cioni et al., 2019). Live super-resolution microscopy of mitochondria–lysosome contact sites in peripheral sensory neurons showed defective cycling of CMT2B mutant Rab7 onto lysosomes in contact with mitochondria, consistent with its impaired Rab7 GTP hydrolysis. Importantly, downstream axonal mitochondrial motility was disrupted but could be rescued by promoting Rab7 GTP hydrolysis at mitochondria–lysosome contacts via TBC1D15 (Rab7-GAP) expression (Wong et al., 2023). Thus, CMT2 mutations may simultaneously inhibit both co-regulated Drp1 GTP hydrolysis and Rab7 GTP hydrolysis, leading to the defects observed in both mitochondrial and lysosomal network dynamics (Fig. 1 E) and contributing to peripheral neuropathy.

In contrast, selective impairment of Drp1 GTP hydrolysis may underlie other neurological diseases (Fig. 1 E). Dominant optic atrophy is a neurodegenerative condition that primarily affects the optic nerves, resulting in vision loss, and can be caused by a dominant Mid51 (Y240N) mutation in its Drp1-binding domain (Charif et al., 2021). Mid51 (Y240N) induces defective Drp1 GTP hydrolysis as observed by misregulated mitochondrial dynamics (Charif et al., 2021), but has normal Mid51 and Fis1 oligomerization such that Rab7 GTP hydrolysis-mediated untethering of mitochondria–lysosome contacts and inter-lysosomal contacts are not disrupted (Wong et al., 2022). Thus, dominant optic atrophy mutations may uncouple Drp1 GTP hydrolysis from Rab7 GTP hydrolysis, resulting in normal Rab7 GTP hydrolysis, but selectively inhibit Drp1 GTP hydrolysis, leading to preferentially misregulated mitochondrial dynamics (Fig. 1 E).

Conversely, other diseases may primarily disrupt Rab7 GTP hydrolysis and prevent it from properly co-regulating Drp1 GTP hydrolysis, leading to primary defects in lysosomal dynamics with potential downstream misregulation of mitochondrial dynamics. Parkinson's disease is a movement disorder characterized by progressive neurodegeneration of dopaminergic neurons in the basal ganglia. A recessive Mid51 (R169W) mutation in its oligomerization domain, potentially linked to Parkinson's disease, could still drive Drp1 GTP hydrolysis-mediated mitochondrial dynamics (Lubbe et al., 2020, *Preprint*). However, it disrupted the normal co-oligomerization of Mid51 and Fis1, ultimately leading to misregulated Rab7 GTP hydrolysis-mediated untethering of mitochondria–lysosome contacts and inter-lysosomal contacts (Wong et al., 2022), suggesting that misregulated Rab7 GTP hydrolysis and its uncoupling from Drp1 GTP hydrolysis may contribute to disease. Indeed, Parkinson's patient neurons with mutant lysosomal enzyme glucocerebrosidase showed disrupted mitochondria–lysosome contact dynamics due to inhibited Rab7 GTP hydrolysis, resulting in aberrant downstream mitochondrial distribution and function, which could be rescued by TBC1D15 expression (Kim et al., 2021). Moreover, super-resolution microscopy of Parkinson's disease–associated mutant Parkin neurons also showed misregulated mitochondria–lysosome contact dynamics due to upregulated Rab7 GTP hydrolysis, and TBC1D15 knockdown restored mitochondria–lysosome tethering and ameliorated cellular and subcellular amino acid profiles in Parkin mutant neurons (Peng et al., 2023). Thus, Parkinson's disease mutations may differentially disrupt the proper regulation of Rab7 GTP hydrolysis events, ultimately leading to misregulated mitochondria–lysosome contact and inter-lysosomal contact tethering dynamics and function. This may further result in the loss of proper co-regulation of Drp1 GTP hydrolysis at mitochondria–lysosome contact sites, potentially contributing to aberrant mitochondrial dynamics and function in Parkinson's disease (Fig. 1 E).

Finally, lysosomal storage disorders such as mucolipidosis type IV and Niemann-Pick Type C result in multisystem disease and progressive neurodegeneration. Loss-of-function mutations in the lysosomal calcium efflux channel TRPML1 lead to mucolipidosis type IV, and result in defective calcium transfer from lysosomes to mitochondria at mitochondria–lysosome contact sites (Peng et al., 2020). Similarly, loss-of-function mutations in NPC1, which regulates lysosomal cholesterol transport, leads to Niemann-Pick Type C and may result in increased percentage of lysosomes contacting mitochondria (Höglinger et al., 2019). Thus, multiple disease mutations affecting mitochondria–lysosome contact sites highlight its significant role in the pathophysiology of human disorders.

Super-resolution microscopy approaches to study mitochondria–lysosome crosstalk

Looking forward, additional live super-resolution microscopy studies can offer new insights into the dynamic crosstalk occurring at mitochondria–lysosome contact sites. These include live super-resolution approaches such as SIM and spinning disk super resolution by optical pixel reassignment (SoRa; Bond et al., 2022). SIM relies on widefield fluorescence microscopy combined with patterned illumination to offer 2× resolution enhancement beyond the diffraction limit and acquisition speeds between 1 and 15 fps, with Lattice SIM capable of 4× resolution enhancement and imaging speeds

up to 255 fps via interleaved reconstruction. SIM can also be combined with other imaging modalities such as total internal reflection or lattice light sheet (LLS) microscopy. In contrast, SoRa systems are based on confocal microscopy and can achieve ∼1.4× resolution enhancement (up to 2× with deconvolution) and imaging speeds up to 200 fps, with deeper imaging up to 100 µm compared to ∼20 µm for SIM. Thus, these super-resolution approaches can help better elucidate the dynamic nature of mitochondria–lysosome contacts.

Live super-resolution microscopy can also be performed by utilizing arrayed detector confocal microscopy, such as Zeiss Airyscan or Nikon Spatial Array Confocal (NSPARC), which rely on photon reassignment conducted digitally after acquisition rather than optically as in SoRa. Airyscan and NSPARC can achieve ∼1.7× resolution enhancement (up to 2.6× with deconvolution) with slightly slower acquisition speeds than SoRa. Moreover, stimulated emission depletion (STED)–based microscopy can be used for live-cell imaging and offers localization precision between 3 and 20 nm, but requires exceptionally high laser powers, bright photostable fluorophores, and has limited temporal resolution. However, new STED-like modalities such as MINFLUX and MINSTED allow for tracking of molecules on exceptionally small temporospatial scales (Bond et al., 2022). Finally, techniques such as LLS-SIM, single objective light sheet SIM, and grazing incidence SIM (GI-SIM) may offer ways to retain the speed and resolution benefits of SIM while limiting phototoxicity by restricting the illumination plane to smaller areas. Additionally, on-the-fly spectral unmixing in confocal microscopy can allow for multiplexing beyond four colors. Together, a combination of different super-resolution live approaches (Bond et al., 2022) can shed additional light on the proteins and cellular pathways regulating mitochondria–lysosome contact tethering and function.

In addition, advances in image processing and analysis will expand our understanding of mitochondria–lysosome crosstalk. Analysis of entire organelle networks at the individual organelle level is challenging and may require multiple processing steps to enhance organellar structure and segment individual mitochondria or lysosomes. Moreover, current toolsets for organelle segmentation include classical segmentation algorithms and cutting-edge, deep-learning-based segmentation (e.g., Cellpose, StarDist), which were designed for whole-cell segmentation, rather than subcellular structures. However, recent developments in deep-learning-based tools for segmenting specific organelles (e.g., mitochondria, ER) or specific structures defined by user training may help investigate mitochondrial and lysosomal network regulation. Finally, analysis tools to quantify the relationship between segmented organelles will also be key to understanding the highly dynamic crosstalk occurring at mitochondria–lysosome contact sites at both the individual organelle and sub-organellar level.

Conclusion: Future perspectives on super-resolution imaging of mitochondrial and lysosomal dynamics

Future studies of mitochondria–lysosome crosstalk are crucial for understanding the dynamic pathways underlying cellular homeostasis. Super-resolution live microscopy offers improved spatial and temporal resolutions to uncover functions at mitochondria–lysosome contact sites which might be missed by basic confocal microscopy approaches. Functional probes targeted to different compartments within mitochondria or lysosomes may reveal additional metabolic changes at contacts. Additionally, single-molecule super-resolution imaging of mitochondrial and lysosomal proteins may provide further details into contact site formation and regulation. Furthermore, recent super-resolution studies have highlighted new dynamics in lysosomal biology (Kuchitsu et al., 2023), and in mitochondria that contain dynamic mitochondrial DNA nucleoids and mitochondrial RNA granules (Long et al., 2021; Rey et al., 2020; Fig. 1 B). Finally, live GI-SIM has been used to study the crosstalk between multiple organelles including mitochondria and lysosomes (Guo et al., 2018). Thus, super-resolution live microscopy offers exciting angles to further probe how components of mitochondria and lysosomes are modulated and regulated at contact sites. Finally, only a few studies have used live super-resolution microscopy to elucidate how mitochondria–lysosome contacts are misregulated in disease, which may reveal additional pathways driving the etiology of human disorders. Ultimately, further insights into mitochondria–lysosome contacts will continue to reveal new roles in health and disease.

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