Golgi-derived PI(4)PI domains modulate mitochondrial dynamics.

Preview for "Nagashima S, *et al.* Golgi-derived PI(4)P-containing vesicles drive late steps of mitochondrial division. 2020. Science. 367, 6484."

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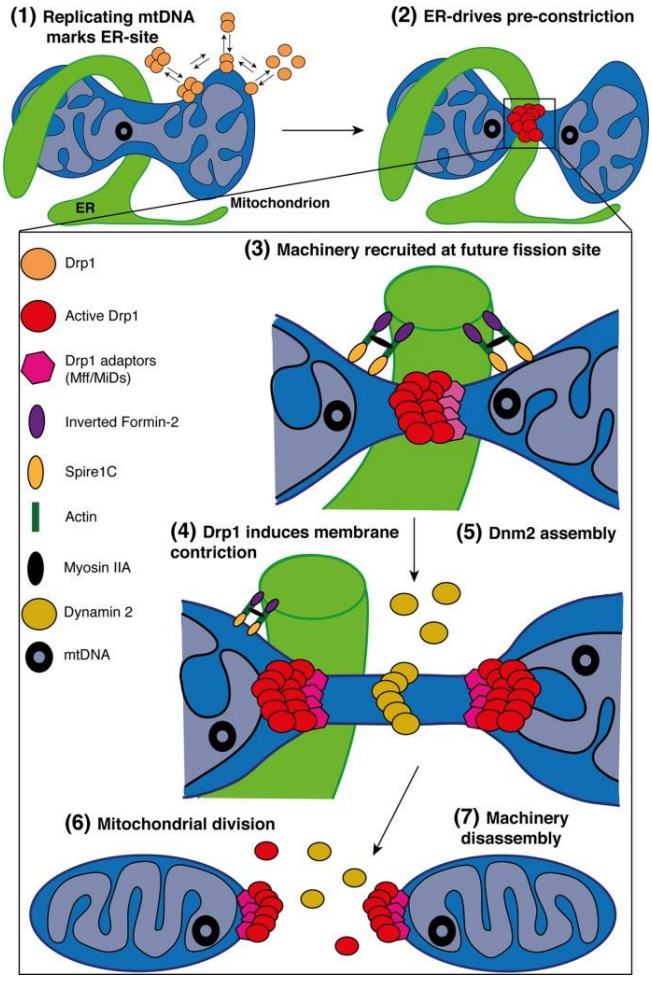
Introduction.

Mitochondria have always played crucial roles within a cell by coordinating biochemical processes including respiration and energy production. In order to maintain their shape, distribution and size, mitochondria undergo coordinated cycles of fission and fusion, which refers as "mitochondrial dynamics." These morphological adaptions together with a fine fusion/fission balance are critical for distinct cellular processes involved in disease, aging, and development. Elucidating the biological function and the mechanism by which mitochondrial dynamics are modulated is a fascinating area to explore. Mitochondrial fission and fusion processes are both mediated by large guanosine triphosphatases (GTPases). During the fusion process, different mitochondria fuse together forming an interconnected network, whereas during the fission process, mitochondria divide into smaller fragments. In this paper, the authors mainly focus on dissecting the molecular mechanism of late events in mitochondrial fission. Previously, researchers showed that mitochondrial fission begins with the recruitment of endoplasmic reticulum (ER) and mitochondria at specific pre-constriction sites, where subsequent additional factors will be participating and the fission will occur. Guanosine triphosphatase (GTPase) Dynamin-related protein-1 (Drp1) will be recruited to the site (1) and enhances mitochondrial constriction by GTP hydrolysis (2). The GTPase Dynamin 2 (Dnm2), downstream of Drp1, will then terminate the membrane scission (3). Although scientists have made extensively discoveries, the molecular details in the last events of mitochondrial fission remain still unclear. Recent evidences suggest that additional factors contribute on regulating mitochondrial division, such as phospholipids, calcium, and lysosomes (4). Phospholipids are the major components of membranes, and phosphoinositides play important roles in Golgi trafficking and structural integrity. PI(4)P and PI(4,5)P_2 are the most important phosphoinositide regulators at the Golgi complex and the phosphatidylinositol 4-kinase- III-b [PI(4)KIIIb], has been shown to mediate the phosphorylation of phosphatidylinositol to generate phosphatidylinositol 4-phosphate [PI(4)P]. The enrichment of PI(4)P in lipid microdomains is essential for membrane- remodeling events. Additionally, among GTPase families, ARF proteins facilitate membrane recruitment and transportation that modulate modifying enzymes and lipid composition in the Golgi. Moreover, a recent study in *C. elegans* showed that the knocking-down Arf1 led to mitochondrial hyperfusion (5). Because both Arf1 and PI(4)P play key roles in membrane dynamics, the author investigated the contribution of these enzymes in the regulation of mitochondrial morphology. Surprisingly, the four-way contact among mitochondria, ER, TGN, and lysosomal vesicles all contribute to the late events of mitochondrial fission.



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Results.

In this paper, the authors discovered that recruitment of trans-Golgi network(TGN)-PI(4)P-containing vesicles to the ER-mitochondria contact sites, drives mitochondrial fission event downstream of Drp1. They first examined the mitochondrial morphology of HeLa cells and found that knocking down either Arf1 or PI(4)KIIIβ led to mitochondrial hyperfusion. They further confirmed this finding by utilizing mitochondrial matrix-targeted photoactivatable GFP probe diffuse in the mitochondrial network in cells lacking Arf1 or PI(4)KIIIβ upon light stimulation. Moreover, a deeper observation from transmission electron microscopy images showed that besides hyperfusion phenotype, mitochondria in Arf1 or PI(4)KIIIβ knockdown cells resulted in more constriction sites in proximal contact with ER. Moreover, these constriction sites highly co-localize with Drp1 in PI(4)KIIIβ knockdown cells. These together with the observation that mitochondrial fission events induced by CCCP treatment and mitochondrial-anchored protein ligase (MAPL) overexpression are reduced in Arf1 or PI(4)KIIIβ knockdown cells indicate mitochondrial fission is blocked despite Drp1 recruitment. The authors later investigated the subcellular localization of PI(4)KIIIβ and discover that in addition to Golgi apparatus, it also resides

in ER-mitochondria contact site, where mitochondrial fission occurs. Time-lapse imaging showed that over 70% of mitochondrial fission events are marked by GFP-Arf1, while Drp1 is recruited to the event site before GFP-Arf1 in 77% of the cases. These results indicate that Arf1 works downstream of Drp1 in mitochondrial fission events. Similarly, the authors utilized the GFP-PH^{FAPP1} probe to demonstrate that PI(4)P formation occurs at the ER-mitochondrial fission site in more than 70% of the events in an Arf1 and PI(4)KIIIβ-dependent manner, and a majority of them occurs after Drp1 accumulation. Interestingly, the authors revealed that GFP-Arf1 and GFP-PH^{FAPP1} are both recruited to the fission site via TGN vesicles, revealing a 3-way interaction between ER, mitochondria, and TGN during mitochondrial fission event.

Significance and Future Directions.

Mitochondrial dynamics play crucial roles in cell fate decisions. Understanding the molecular mechanism is crucial for deciphering how mitochondrial morphology contribute to cellular functions and homeostasis.

A groundbreaking discovery dates back to the identification of the ER required for the initial step of mitochondrial division. Recent evidences support the role of phospholipids, calcium transfer and lysosomes during this process, suggesting those ER-contact sites required not only for the mitochondrial membrane scission but also as a signaling platform for metabolite exchange.

Among phospholipids, phosphoinositides play important roles in Golgi traffic and structural integrity. PI(4)P and PI(4,5)P_2 are the most important phosphoinositide regulators at the Golgi complex and the kinase PI4KIIIβ transfers ceramide from the ER to the Golgi in a PI(4)P-dependent-manner. This event generates lipid microdomains enriched in PI(4)P required for membrane remodeling.

The detailed mechanism on how phospholipids or the Golgi Apparatus might contribute to the late events of mitochondrial fission is still unclear. Nagashima et. *al* introduced a novel key information by identifying Arf1 and PI4KIIIβ as crucial players on Golgi vesicles driving the late steps of mitochondrial divisions. These findings emphasize a three-organelle-contact among mitochondria, ER, and TGN at the fission sites.

This paper raises additional questions for uncovering all the key players involved in this process. The authors observed PI(4)P localization predominantly in the Golgi as well as ER- mitochondrial constriction sites in an Arf1 and PI4KIIIβ-dependent manner. Since the recruitment of PI(4)P pool at the restriction mitochondrial sites occurs in about 73% of events, it is unclear whether a distinct set of lipids or kinases might contribute to the remaining 30% of fission events. Therefore, it is crucial that functional PI(4)P pools accumulated in specific foci to trigger a local concentration of specific lipids or cargo proteins. How kinases or phosphatases may regulate PI(4)P metabolism upstream by distributing these local phosphoinositide gradients remains a challenging question to be addressed.

Furthermore, two different classes of PI(4)P effectors can be recruited to the Golgi. Nagashima et. *al* only tested the functional role of the ceramide transfer protein (CERT) not required for mitochondrial hyperfusion. In addition to CERT, lipid transfer proteins including OSBP, FAPP2 or clahtrin adaptors might be involved in this dynamic assembly. Because this mechanism might be independent on canonical PI(4)P effectors, it would be intriguing to identify adaptor proteins involved in driving local actin dynamics or novel components leading to mitochondrial fission.

The reason of an organelle—transition-complex requirement for mitochondrial division is unknown. Furthermore, the whole downstream pathway remains still elusive.

Lastly but not least, this paper opens up avenues of promising research that would lead to identify potential PI(4)P drug inhibitors/inducers that will potentially tune mitochondrial dynamics in numerous physiological and pathological conditions.

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