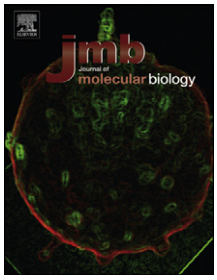
Featured Article

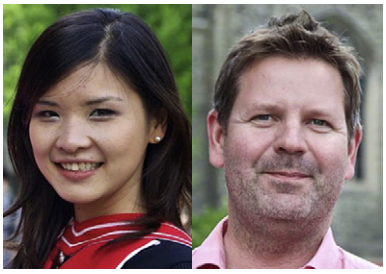


Mitochondrial Genome Maintenance 1 (Mgm1) Protein Alters Membrane Topology and Promotes Local Membrane Bending

Jarungjit Rujiviphat[1], Michael K. Wong[2], Amy Won[2], Yu-ling Shih[3], Christopher M. Yip[1,2,4] and G. Angus McQuibban[1]

*1 - **Department of Biochemistry,** University of Toronto, Toronto, Ontario, Canada M5S 1A8*
*2 - **Institute of Biomaterials and Biomedical Engineering,** University of Toronto, Toronto, Ontario, Canada M5S 3G9*
*3 - **Institute of Biological Chemistry,** Academia Sinica, Taipei 115, Taiwan*
*4 - **Department of Chemical Engineering and Applied Chemistry,** University of Toronto, Toronto, Ontario, Canada M5S 3E5*

***Correspondence to G. Angus McQuibban:** 1 King's College Circle, Medical Sciences Building Room 5342, Toronto, Ontario, Canada M5S 1A8. angus.mcquibban@utoronto.ca*
http://dx.doi.org/10.1016/j.jmb.2015.03.006
Edited by B. Poolman



Jarungjit Rujiviphat and G. Angus McQuibban

Abstract

Large GTPases of the dynamin superfamily promote membrane fusion and division, processes that are crucial for intracellular trafficking and organellar dynamics. To promote membrane scission, dynamin proteins polymerize, wrap around, and constrict the membrane; however, the mechanism underlying their role in membrane fusion remains unclear. We previously reported that the mitochondrial dynamin-related protein mitochondrial genome maintenance 1 (Mgm1) mediates fusion by first tethering opposing membranes and then undergoing a nucleotide-dependent structural transition. However, it is still unclear how Mgm1 directly affects the membrane to drive fusion of tethered membranes. Here, we show that Mgm1 association with the membrane alters the topography of the membrane, promoting local membrane bending. We also demonstrate that Mgm1 creates membrane ruffles resulting in the formation of tubular structures on both supported lipid bilayers and liposomes. These data suggest that Mgm1 membrane interactions impose a mechanical force on the membrane to overcome the hydrophilic repulsion of the phospholipid head groups and initiate the fusion reaction. The work reported here provides new insights into a possible mechanism of Mgm1-driven mitochondrial membrane fusion and sheds light into how members of the dynamin superfamily function as fusion molecules.

Legend: The cover is representing a giant liposome being remodeled by Mgm1.

Introduction

In the cell, membranes are dynamic architectures that undergo complex rearrangements during processes such as cell division, vesicle budding, and organelle restructuring. Mitochondria are double-membrane-bound organelles that continually fuse and divide. They serve as the power plant of the cell, generating most of the cellular supply of chemical energy in the form of adenosine triphosphate and are also major sites for cell signaling and regulation. For instance, cytochrome *c* is released from the mitochondria to activate apoptosis, the process of programmed cell death [1–3]. These important mitochondrial functions rely on a proper balance in the rates of mitochondrial fusion and division. Imbalances in mitochondrial dynamics cause mitochondrial misshape and dysfunction, resulting in a reduction in energy production and uncontrolled cell death [2]. Alterations in mitochondrial morphology and function have been linked to several neurodegenerative diseases including Parkinson's disease [4]. Mitochondrial inheritance, genome maintenance, and a quality control pathway termed mitophagy also require precise regulation of mitochondrial fusion and fission [5–7]. Proper regulation of mitochondrial dynamics is therefore crucial for both organellar and cellular integrity. To maintain mitochondrial dynamics, the opposing forces of mitochondrial fusion and fission require unique protein machineries.

The key components driving mitochondrial fusion and fission belong to the dynamin protein superfamily. This superfamily includes classical dynamin and dynamin-related proteins (DRPs), large GTPases that undergo GTP cycling and act as mechanoenzymes to mediate intracellular membrane-remodeling events such as vesicle budding, as well as organelle fusion and fission [8,9]. Members of the dynamin superfamily are composed of three specific domains: a GTPase domain, a middle domain, and a GTP effector domain (GED) [9]. X-ray crystallography has revealed that the head region, which consists of the GTPase domain, and the stalk region, which consists of the middle domain and the GED, can self-interact to promote oligomerization and polymerization into highly ordered helical structures [8,10–13]. The foot region, which bridges the middle domain and GED, is typically composed of a pleckstrin homology domain that interacts with lipids [8]. To induce fission, dynamin binds to the membrane and polymerizes and constricts the membrane upon GTP-induced rearrangement of the head and stalk regions [10]. The stages of tubulation, constriction, and scission have been observed by electron microscopy [14–16]. While this model of dynamin-mediated membrane fission is widely accepted, it is still unclear how members of the DRP superfamily promote membrane fusion.

In humans, DRP1 mediates scission of both outer and inner mitochondrial membranes with mitofusins (MFN1 and MFN2) and optic atrophy 1 (OPA1) facilitating outer and inner mitochondrial membrane fusion, respectively. Mutations in these DRPs cause neurodegenerative diseases, underscoring their importance in mitochondrial function [17–19]. Other membrane fusion proteins are believed to act via similar steps of membrane tethering and nucleotide-dependent structural changes, as in the case of atlastin-mediated membrane fusion of the endoplasmic reticulum [20,21]. Mitofusins mediate fusion of the outer mitochondrial membrane by tethering membranes via their coiled-coil domains, which is then followed by GTP binding and hydrolysis [22]. In the case of OPA1, very little is known about how it mediates inner mitochondrial membrane fusion. The yeast homologue of OPA1 is mitochondrial genome maintenance 1 (Mgm1), which has two isoforms s-Mgm1 and l-Mgm1. Notably, both isoforms are crucial for inner mitochondrial membrane fusion. l-Mgm1 is anchored to the inner membrane (IM) while s-Mgm1 resides in the intermembrane space. Since l-Mgm1 does not require a functional GTPase domain to function, it is proposed that l-Mgm1 serves a structural role while s-Mgm1 acts as the mechanoenzyme in the fusion reaction [23]. To address these questions, we focused on the mechanistic actions of s-Mgm1 in this study.

We have previously shown that s-Mgm1 has GTPase activity and binds to specific phospholipids, behavior similar to that of other DRPs [24]. Unlike a soluble isoform of OPA1, which has been shown to cause liposome tubulation, s-Mgm1 has been reported to only assemble on the membrane as a crystalline array, failing to initiate liposome tubulation [25–27]. We demonstrated that, similar to atlastin and the mitofusins, s-Mgm1 tethers membranes and undergoes a striking GTP-dependent structural transition that might be the mechanism to promote membrane fusion [28]. Since the fundamental difference between pro-fusion and pro-fission dynamins is that the pro-fusion dynamins are membrane anchored, it is plausible that the transmembrane domains of the mitofusins, atlastin, and l-Mgm1 are essential for destabilizing the phospholipid bilayer in order to facilitate fusion. However, it is still unclear how s-Mgm1, which is only peripherally associated to the membrane unlike other pro-fusion dynamins, can act directly on the membrane to drive the fusion of tethered membranes. In this work, we applied real-time confocal and scanning probe microscopies to characterize how s-Mgm1 promotes membrane fusion *in vitro* using model membrane substrates. Here, we show that s-Mgm1 binds to the membrane, alters membrane topology, and promotes local membrane bending, which could serve as crucial steps to orchestrate membrane fusion. This work provides new insights into the possible mechanism