Apr 16th, 11:00 AM - 2:00 PM

Differential Regulation of Dynamin-related Protein 1 Splice Variants by Membrane Adaptors

Anthony J. Baglio
Cedarville University, abaglio@cedarville.edu

Differential regulation of Dynamin-related protein 1 splice variants by membrane adaptors

Patrick J. Macdonald, Anthony Baglio, Rajesh Ramachandran

Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH

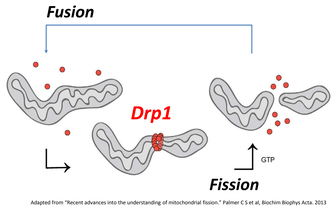
Abstract

We begin the first biochemical examination of the multiple splice variants of human Dynamin-related protein 1 (Drp1), a GTPase involved in mitochondrial fission. While eight such variants, generated through alternative mRNA splicing, have been identified, here we focus on two: the shortest variant (Short) which is ubiquitous, and the longest (Long), which piqued our interest since Drp1 Long is expressed exclusively in neurons. We now establish the various functional differences between these two Drp1 splice variants. Our data reveal that whereas Drp1 Short exhibits constitutively high GTPase activity, Drp1 Long does not. Interestingly, mitochondrial outer membrane proteins, mitochondrial fission protein 1 (Fis1) and mitochondrial fission factor (Mff) that putatively function as receptors for Drp1 differentially regulate the enzymatic activity of the two splice variants. It is possible that the roles of Mff and Fis1, which have conflicting reports in the literature, may vary across Drp1 splice variants and thus be tissue specific. To gain a better understanding of the role of such Drp1 effectors, we focus primarily on Mff, as it has the strongest observed effect on Drp1 enzymatic activity, and probe its mechanism of action using a variety of biochemical and biophysical tools.

Introduction

The dynamic nature of mitochondria

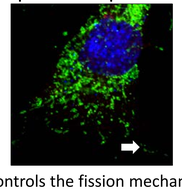
Mitochondria are more than bean-shaped organelles.



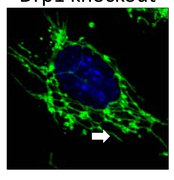
Adapted from "Recent advances into the understanding of mitochondrial fission." Palmer C.S et al, Biochim Biophys Acta. 2013

Mitochondria constantly divide and recombine, forming networks.

Drp1 overexpression



Drp1 knockout

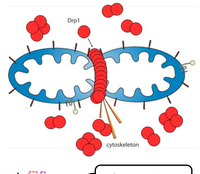


Drp1 controls the fission mechanism.

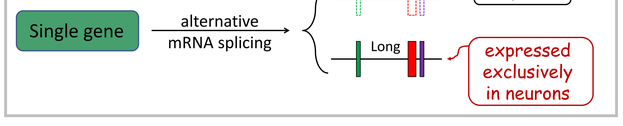
Drp1

Dynamin-related protein 1

- Self-assembling GTPase of 80-85 kDa
- Forms higher-order polymers on membranes
- Robust GTPase stimulation upon assembly on membranes

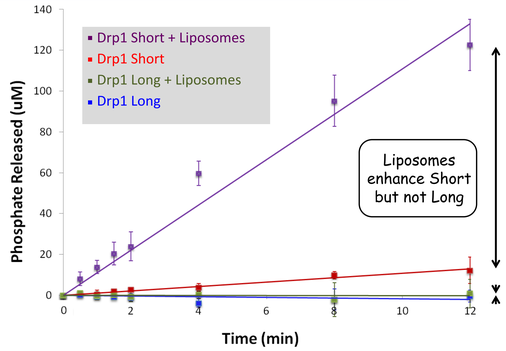


Drp1 splice variants

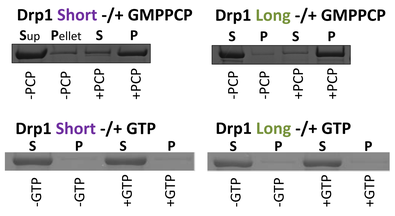


Comparison of splice variants

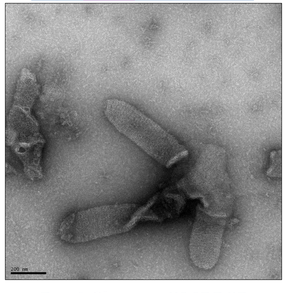
Drp1 splice variants: average GTPase activity



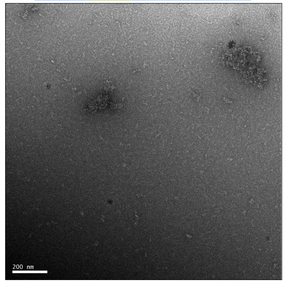
Spin sedimentation assay



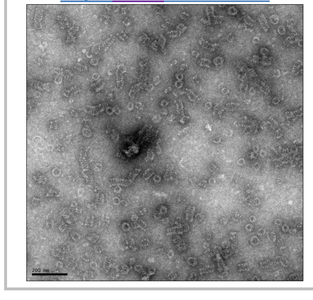
Drp1 Short + liposomes



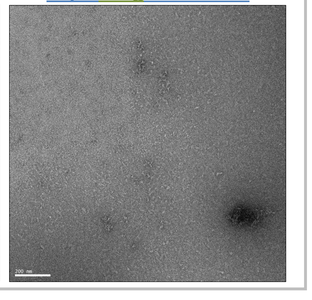
Drp1 Long + liposomes



Drp1 Short + GMPPCP

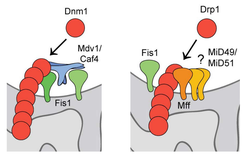


Drp1 Long + GMPPCP



Drp1 adaptors

Yeast versus humans



Mitochondrial fission protein 1 (Fis1)

- tail-anchored membrane protein
- critical Dnm1 receptor in yeast
- not critical in mammals

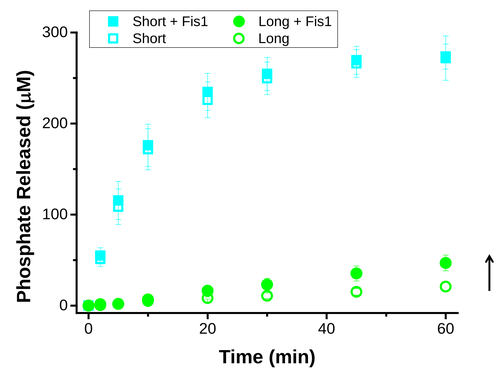
Mitochondria fission factor (Mff)

- tail-anchored membrane protein
- essential Drp1 recruiter in mammals
- no yeast homolog

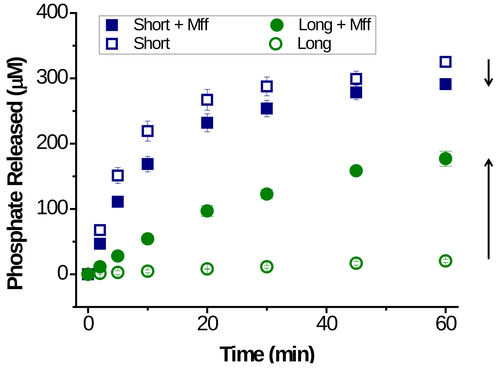
What are the roles of these adaptors?

Splice variants & Adaptors

GTPase assay with liposomes ± Fis1

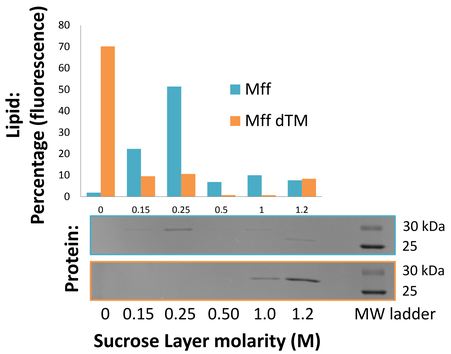


GTPase assay with liposomes ± Mff

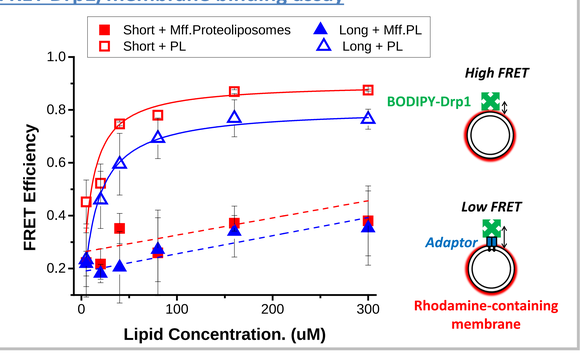


Proteoliposomes, Membrane binding

Floatation assay for Mff membrane incorporation



FRET Drp1/membrane binding assay



Conclusions

- Drp1 Short and Long are differentially stimulated by membranes and adaptor proteins.
- Drp1 Short GTPase activity is stimulated by assembly on membranes, unlike Drp1 Long, despite similar membrane binding and oligomerization properties.
- Alternatively, Drp1 Long activity is enhanced by adaptor proteins, marginally by Fis1, and robustly by Mff. Drp1 Short does not show any change.
- Although both variants bind Mff similarly, as detected by FRET.

Future work

- Further biophysical examination of Drp1/adaptor interactions to determine the mechanism of GTPase activation and membrane fission.

Acknowledgments

- We thank Jason Mears and Xin Qi (CWRU) for help with EM and cell biology experiments.