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Investigating Interactions in the Drp1-Mff Copolymer that Contributes to Mitochondrial Fission

Kyle Whiddon, Ryan Clinton and Jason Mears

Case Western Reserve University, Cleveland, Ohio, United States

Mitochondrial dynamics, including fusion and fission, impact cellular respiration, regulation of apoptosis, and segregation of damaged mitochondrial components [1]. The process of mitochondrial fission is primarily governed by the function of a single protein, dynamin related protein 1 (Drp1), with homologs being found in all eukaryotes [2,3,4]. Highlighting its importance, mutations in Drp1 have been shown to cause neurological disorders, and dysregulation of Drp1 is associated with several diseases including cancer, neurodegenerative and ischemic disorders [1].

Prior to recruitment to the mitochondrial outer membrane (MOM), Drp1 is localized in the cytosol in proposed inactive storage forms [5,6,7]. During mitochondrial fission, functional Drp1 dimers are recruited to the MOM and form an oligomeric complex that can initiate membrane constriction [8]. This recruitment is accomplished via interactions with lipid species such as cardiolipin [9,10], as well partner proteins including mitochondrial fission factor (Mff), mitochondrial dynamics proteins (MiD49/51), and mitochondrial fission 1 protein (Fis1) [11].

Despite their importance in Drp1 recruitment, the specific sites of interaction between Drp1 and its partner proteins remain poorly understood, with only Drp1-MiD49 having a structural basis [12]. In light of this, we are investigating the structural and functional interactions between Drp1 and Mff. We have shown previously that the intrinsically disordered variable domain (VD) in Drp1 is responsible for interaction with cardiolipin [9], while evidence suggests that it acts as a negative regulator for interaction with Mff [13,14]. Supporting this, we have seen that deletion of the VD allows Mff to interact with Drp1 resulting in the formation of helical copolymers (Fig. 1). Using cryo-electron microscopy (cryo-EM), we aim to elucidate the specific interaction between Drp1 and Mff and the structural impact of this interaction on Drp1 polymerization compared other cofactors.

To perform helical reconstructions, we have assessed the helical symmetry (rise/twist per asymmetric unit and point group symmetry) from the layer line profiles of Fourier transforms of 2D class averages (Fig. 2). With this information, we are performing real space refinement using Iterative Helical Real Space Reconstruction (IHRSR [15]) and Relion [16] methods. Alternatively, cryo-electron tomography (cryo-ET) techniques can also be used to refine individual filaments, which would examine the uniformity of helical species for the polymers generated through Drp1-Mff interactions. Going forward, we plan to employ similar techniques to study interactions between Drp1-Mff copolymers on lipid templates to better evaluate this interaction in a membrane proximal environment [17].

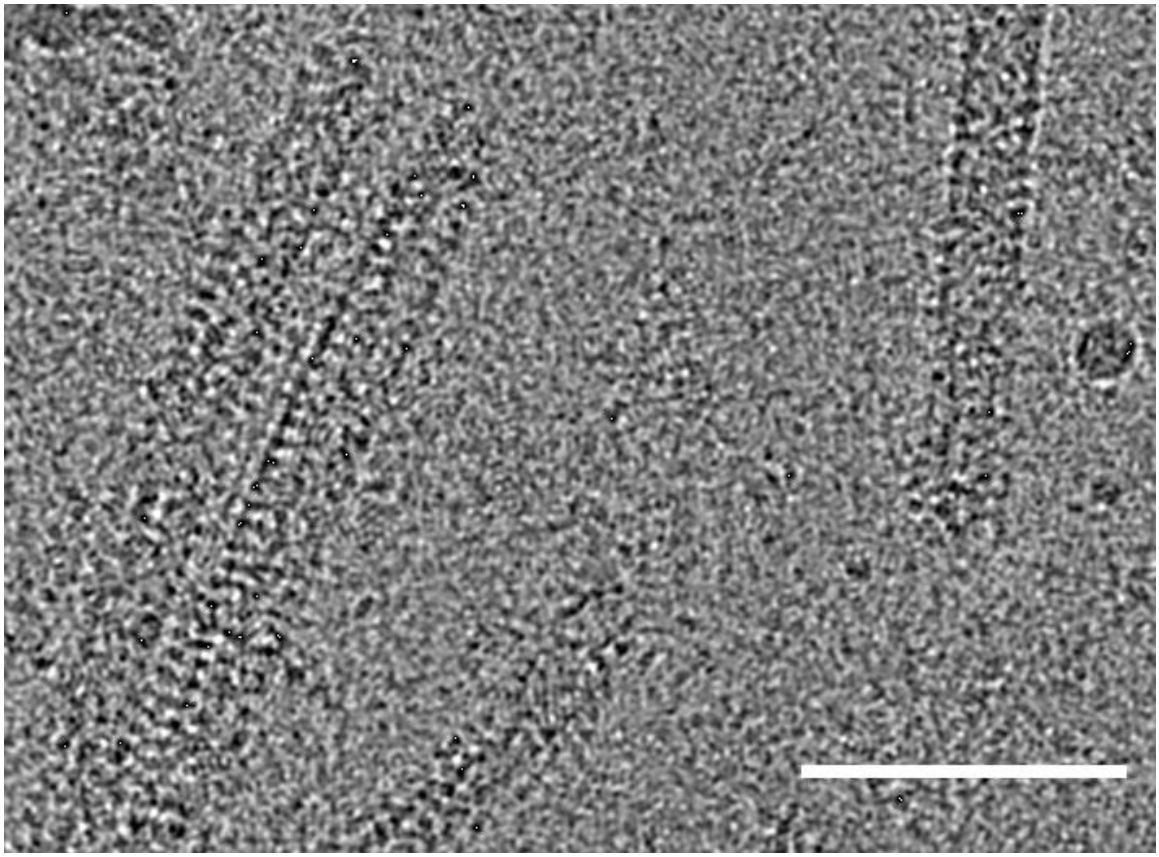

Figure 1. Cryo-EM micrographs of helical Drp1ΔVD-Mff assemblies. Ordered helices assemble spontaneously, suggesting that interactions are physiologically relevant. Scale bar represents 100nm.

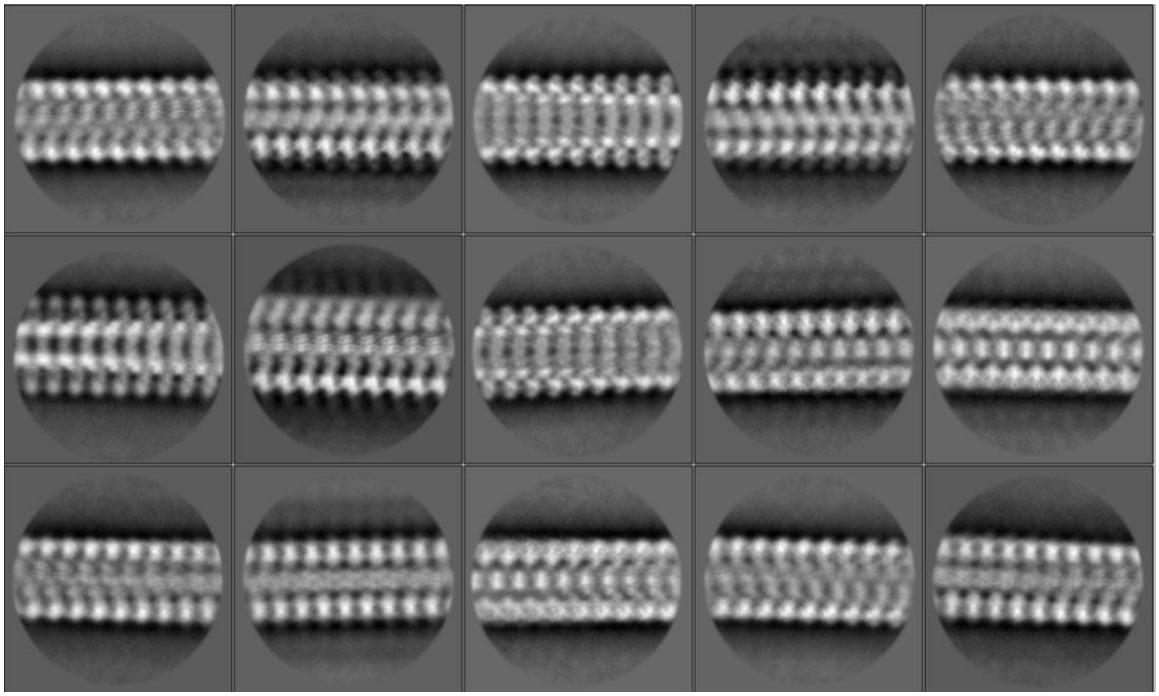

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Figure 2. 2D class averages of Drp1ΔVD-Mff copolymers. Layer lines seen in Fourier transforms of these averages can be used to investigate the helical symmetry of the filaments, facilitating 3D reconstructions.

References

[1] Chen, H., and Chan, D. C. Hum Mol Genet **18** (2009) p. R169-76
[2] Smirnova, E. *et al*. J Cell Biol **143** (1998) p. 351-358
[3] Smirnova, E. *et al*. Mol Biol Cell **12** (2001) p. 2245-2256
[4] Lee, J. E. *et al*. Nat **540** (2016) p. 139-143
[5] Macdonald, P. J. *et al*. Mol Biol Cell **25** (2014) p. 1905-1915
[6] Mehta, K. *et al*. J Biol Chem **294** (2019) p. 3385-3396
[7] Hatch, A. L. *et al*. Mol Biol Cell **27** (2016) p. 3109-3121
[8] Kraus, F., and Ryan, M. T. Cell Sci **130** (2017) p. 2953-2960
[9] Francy, C. A. *et al*. Sci Rep **7** (2017) p. 10744
[10] Stepanyats, N. *et al*. Mol Biol Cell **26** (2015) p. 3104-3116
[11] Ramachandran, R. Semin Cell Dev Biol **76** (2018) p. 201-212
[12] Kalia, R. *et al*. Nat **558** (2018) p. 401-405
[13] Clinton, R. W. *et al*. J Biol Chem **291** (2016) p. 478-92
[14] Lu, B. *et al*. Sci Rep **8** (2018) p. 10879
[15] Egelman, E. H. J Struct Biol **157** (2007) p. 83-94
[16] He, S., and Scheres, S. H. W. J Struc Biol **198** (2017) p. 163-176
[17] Francy, C. A. *et al*. J Biol Chem **290** (2015) p. 11692-11703

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