

Reply to Roy and Pucadyil: A gain of function by a GTPase-impaired Drp1

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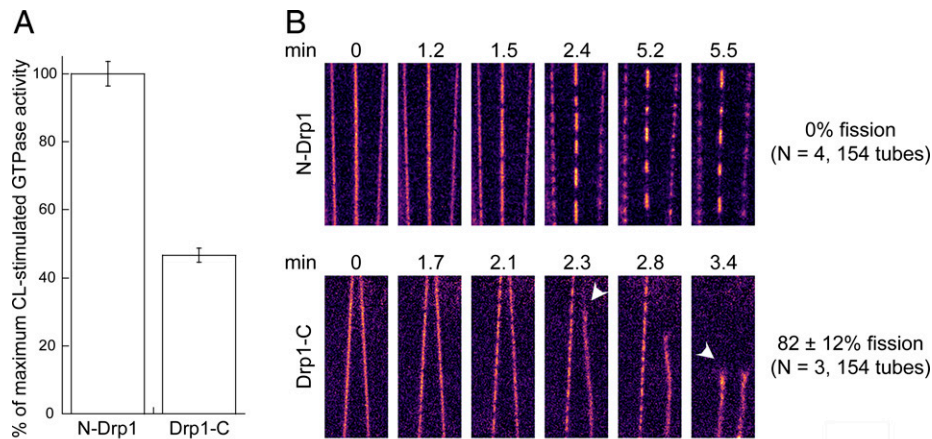


Fig. 1. Gain of function by Drp1-C. (A) Relative cardiophilin (CL)-stimulated GTPase activities of 0.5 μM N-Drp1 and Drp1-C on 25 mol % CL-containing liposomes as performed in our published study (1). (B) Time sequence of GTP-dependent remodeling of freely suspended lipid nanotubes (NT) upon addition of 0.5 μM unlabeled N-Drp1 or Drp1-C as performed in our published study (1). Assay buffer contained 1 mM DTT and 0.5 mM nPG final. Membrane composition was 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE), bovine heart CL, and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (RhPE) at 39.5:35:25:0.5 mol % (similar results were obtained with a DOPC:CL:RhPE 74.5:25:0.5 mol % mixture). Tube radii ranged from 5 to 35 nm. White arrows point to sites of NT scission. RhPE fluorescence is observed. Pseudocolor is used for clarity.

Whether Drp1 is self-sufficient for mitochondrial fission remains disputed (1, 2). Roy and Pucadyil (3) assert that a lethal combination of Drp1 oxidation and phototoxicity led to failed membrane fission in our assay system (1). While we acknowledge that unresolved differences in our *in vitro* assays could explain the discrepancy, we rule out Drp1 oxidation and phototoxicity as relevant issues. Here, we argue that alterations in Drp1 behavior and the reaction micro-environment may be the cause.

- 1) Dithiothreitol (DTT) was present in our Drp1 buffers as stated (1, 4, 5). Cys oxidation, therefore, is unlikely.
- 2) Under the same conditions, even in the presence of an oxygen scavenger (OS), the fission efficiency was zero for our N-terminally tagged Drp1 (N-Drp1), while being $82 \pm 12\%$ for a C-terminally tagged Drp1 construct (Drp1-C) comparable to Kamerkar et al.'s (2) (Fig. 1). Susceptibility to photooxidation should remain the same for both constructs. Phototoxicity hence cannot explain the observed functional differences.
- 3) We demonstrate robust membrane constriction with N-Drp1 both with (Fig. 1) and without OS (1), indicating that neither Drp1 helical self-assembly nor GTPase activity is affected. By contrast, Roy and Pucadyil (3) do not detect comparable membrane constriction using our Drp1 construct under our purported conditions. Furthermore, in Kamerkar et al. (2), a GTPase-inactive K38A Drp1 mutant incapable of fission *in vivo* mediates residual fission *in vitro*. Thus, we believe that the assay system used by Pucadyil et al. (2, 3) may be biased toward fission.

- 4) We summarize other methodological differences that warrant further investigation to resolve this controversy and better understand Drp1 physiology:

- i) Effect of OS agents: We use *n*-propyl gallate (nPG), specific for peroxy radical scavenging on lipid surfaces (6, 7) (Fig. 1), whereas Roy and Pucadyil (3) use the enzymatic glucose oxidase/catalase system. The level of photoprotection afforded by these two disparate OS systems at membranes remains to be assessed. Besides, the potential effect of these OS mixture enzymes and reagents on Drp1 and membrane properties should be considered (8–10). Crucially, under our conditions (1) (Fig. 1), we observe robust GTP-dependent membrane constriction irrespective of the presence of nPG, indicating no effect on Drp1 self-assembly.

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The authors declare no competing interest.

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ii) Effect of protein tag: Kamerkar et al. (2) found that a widely used N-terminally green fluorescent protein-tagged Drp1 construct fully capable of supporting mitochondrial fission in vivo was incapable of mediating membrane fission in vitro. In contrast, a C-terminally tagged Drp1 construct with drastically reduced GTPase activity was found to disrupt membranes (2). We observe a similar effect of tag position on Drp1 activity (Fig. 1). Whether this fission reaction reflects Drp1 function in vivo (potentially mimicking C terminus targeted effectors) or is simply an in vitro artifact remains to be determined.

iii) Other factors: Other differences include Pucadyil and coworkers' use of polyethylene glycol, a known protein crowder (11), to support membrane nanotubes and assay our N-Drp1. While Roy and Pucadyil claim to utilize "free-standing" tubes to assay their Drp1 construct, the environment of these templates was not described, precluding any meaningful comparison to ours.

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