PNAS LETTER

Is Drp1 sufficient to catalyze membrane fission?

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Mahajan et al. report that the dynamin-related protein1 (Drp1) can constrict but not sever membrane nanotubes (1), which contradicts our results showing that Drp1 is sufficient for membrane fission (2). Drp1 functions to divide mitochondria and peroxisomes. Understanding its capacity for membrane remodeling is therefore important in constraining models on organelle division (3).

Mahajan et al. (1) attribute differences to the fact that we employed nanotubes supported on PEGylated glass coverslips (4), as opposed to their use of free-standing nanotubes. Time-lapse imaging of supported nanotubes exposed to Drp1 with guanosine triphosphate (GTP) showed fission (Fig. 1*A*), with a frequency of three to nine cuts per tube (n = 12). Tension caused the severed tubes to retract to foci where the nanotube was initially pinned to the surface. Importantly, free-standing nanotubes also get severed, with tubes wiggling in and out of focus and getting cut again (Fig. 1*B*, *Top*) or snapping out of the field of view entirely after the first cut (Fig. 1*B*, *Bottom*). This underestimated the fission frequency to one to four cuts per tube (n = 14), but both supported and free-standing nanotubes showed fission. We also compared the Drp1 construct used by Mahajan et al. in pRSET-C to ours in pET15b and find them both to catalyze fission of supported nanotubes (Fig. 1 *C* and *D*).

Fission assays employ time-lapse imaging of fluorescent probe-labeled nanotubes exposed to Drp1 and GTP (Fig. 1 *A* and *B* and refs. 1, 2, 5, and 6). Our assays showing fission were carried out in a buffer containing an enzymatic oxygen-scavenging (OS) mixture (2). Curiously, studies reporting lack of fission do not mention adding OS in their microscopy buffers (1, 5, 6). To test the importance of OS, we acquired time-lapse images of nanotubes exposed to Drp1 with GTP in a buffer lacking OS. Following this, we moved the microscope stage to simultaneously image the field that was exposed to light and the field that was in the dark. Remarkably, nanotubes in the light-exposed field showed prominent constrictions, reminiscent of results reported in Mahajan et al. (1), while those in the dark showed extensive cuts (Fig. 2*A*). OS depletes the buffer of dissolved O_2, thereby reducing phototoxicity from reactive oxygen species (ROS) generated upon excitation of fluorescent probes (7). ROS-mediated phototoxicity manifests from protein oxidation, most frequently of cysteine residues (8, 9). GTP hydrolysis is necessary for constrictions to progress toward fission (2) and it is possible

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The authors declare no competing interest.

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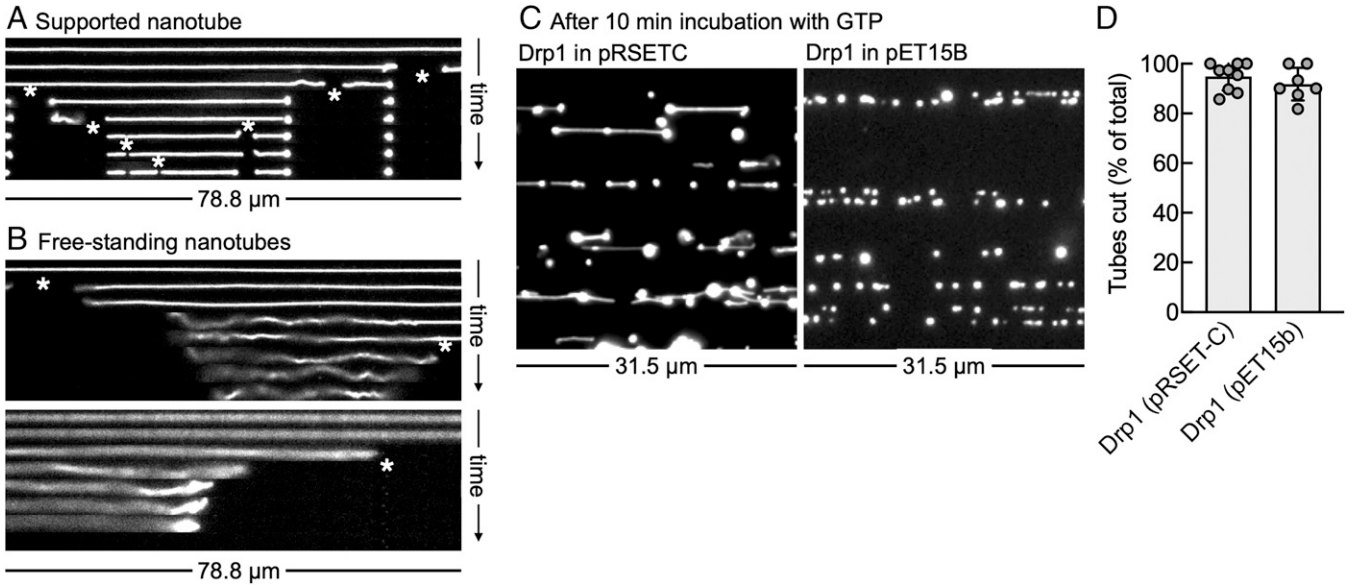

Fig. 1. Drp1-catalyzed fission tested on various assay systems and using different constructs. Frames from a time-lapse movie showing fission of (*A*) supported and (*B*) free-standing nanotubes. White asterisks mark sites of fission. (*C*) Representative fields of supported nanotubes incubated with the Drp1 cloned in pRSET-C (1) or in pET15B (2). The pRSET-C construct carries an N-terminal 6xHis, XpressTM epitope and an enterokinase cleavage site, while the pET15b construct carries an N-terminal 6xHis tag with a TEV protease cleavage site and a C-terminal StrepII tag (Addgene plasmid #174421). (*D*) Quantitation of fission with the different Drp1 constructs. Data represent the mean ± SD of tubes cut in multiple fields across two independent experiments. All experiments were conducted with 1 μM Drp1, 1 mM GTP, and 1 mM Mg^{2+} in 20 mM Hepes, pH 7.4, 150 mM KCl, and 1 mM DTT with OS. See ref. 4 for OS composition. Membranes contained 1,2 dioleoyl *sn* glycero-3 phosphocholine (DOPC), heartcardiolipin (CL), and Texas Red DHPE in 74:25:1 mol % ratio.

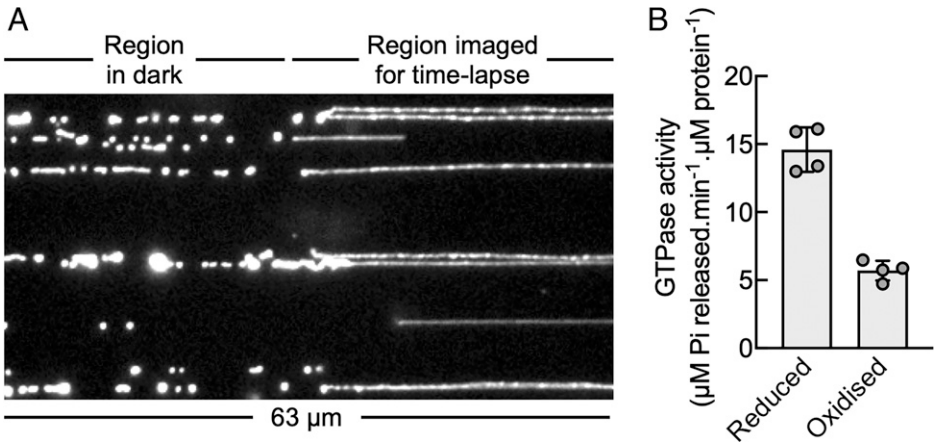

Fig. 2. Drp1-catalyzed fission is inhibited by phototoxicity and oxidation. (*A*) Representative image of supported nanotubes in the presence of Drp1 and GTP. Prior to taking this image, half of the field was exposed to light during time-lapse imaging while the other half was in the dark. Experiments were carried out in the same buffer as described in Fig. 1 but lacked OS. (*B*) Assembly-stimulated GTPase activity of Drp1 that was incubated with 1 mM DTT or H_2O_2 to reduce and oxidize cysteines, respectively (9). Data represent the mean ± SD of four independent experiments. GTPase assays were carried out as described in ref. 2.

that localized light-induced protein oxidation could have inhibited Drp1's GTPase activity, thereby stalling the fission reaction. Indeed, we find that forced oxidation of Drp1 with H_2O_2 shows 2.6-fold lower GTPase activity than Drp1 reduced with dithiothreitol (Fig. 2*C*).

Fission ensues from temporally coordinated reactions involving membrane binding, self-assembly, and stimulated GTPase activity. To consider it as a gain of function in Drp1 therefore seems implausible. Instead, a more logical explanation is that the lack of fission represents a loss of function caused by Drp1 oxidation. The redox state of the cell affects mitochondrial structure, and for such effects to manifest in part by redox control of Drp1's GTPase activity, as we report here, is an exciting possibility (10).

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