Figures and figure supplements

Mechanical force induces mitochondrial fission

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Figure 1. Mitochondria undergo DRP1-mediated fission upon encountering actin-propelled Shigella. (A) U2OS KERMIT cells (stably expressing mtBFP) were transfected with mCherry-Lifeact plasmid and infected with mCherry-labelled S. flexneri. Left, overview of time-lapse microscopy results presented at 1 min interval. Right, magnifications of selected areas from Left at the indicated times. Note that the times in the right inset do not necessarily match the times in the overview on the left. Arrowheads indicate mitochondria positions before and after impact with bacterium. (B) COS7 cells transduced with lentiviruses expressing GFP-Lifeact and mtBFP were infected with GFP-labelled S. flexneri. Imaging was performed as in A. Shown are four individual fission events upon encounter with S. flexneri. Blue and orange arrowheads indicate mitochondria before and after fission, respectively. (C) DRP1-CRISPR U2OS KERMIT cells were subjected to the same treatment and analysis as in A. Numbered boxes as in A. Blue arrowheads, thinning mitochondrial tubules due to impact by S. flexneri, followed by recovery of mitochondrial tubules without fission. (D) mCherry-DRP1-expressing U2OS cells were treated as in B. Blue arrowheads, recruitment of DRP1 (white) at sites of encounter with S. flexneri. Orange arrowheads, subsequent fission events. Scale bars, 2 μm.

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Figure 1—figure supplement 1. All detectable forms of DRP1 are depleted by siRNA, shRNA or CRISPR-induced mutations. (A) KERMIT cells were transfected three times consecutively within 72 hr, each with 40 nM of scrambled (SCR) or DRP1 siRNA #1 or #2. 40 μg of total lysate was loaded per lane onto a 10% polyacrylamide gel and subjected to SDS-PAGE and immunoblotting. Tubulin immunoblot is shown as loading control. (B) Total lysates were prepared from KERMIT cells (WT clone 1), from a second clone derived from U2OS cells (WT clone 2) and from DRP1 CRISPR KERMIT cells. 20 μg of lysate per sample was analyzed on 10% polyacrylamide gel and subjected to SDS-PAGE and immunoblotting. Proteins were detected using specific antibodies. Tubulin immunoblot is shown as loading control. (C) U2OS mtBFP cells were incubated with either virus encoding shRNA targeted against DRP1 or a virus transduction control overnight at 37°C. Subsequently the medium was exchanged and puromycin (1 μg/ml) was added. Cells were grown under selection for four days and subsequently prepared for SDS-PAGE and immunoblotting. Equal amounts of protein were loaded and proteins were detected using specific antibodies. Tubulin immunoblot is shown as loading control. (D) KERMIT cells were transfected with 10 nM of scrambled (SCR) or MFF-targeting siRNA. Cells were fixed 2 days post transfection using 4% paraformaldehyde and stained with DAPI and MFF-specific antibody. All images were acquired using the same excitation and acquisition settings, and presented at the same intensity settings. Scale Bar, 10 μm.

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Figure 2. Mitochondria undergo DRP1-dependent fission upon AFM-mediated force application. (A) Scheme of the experimental setup. (B) Quantification of fission events elicited by the application of AFM-induced mechanical forces observed in cells treated either with scrambled or DRP1 siRNA. Successful force application to an individual mitochondrion was defined as visible constriction of the mitochondrial matrix following tip approach (examples shown with blue arrowhead in C and D). (C) U2OS cells stained with Mitotracker Deep Red and transduced with viruses encoding mCherry-FIS1TM and a control shRNA were imaged by time-lapse microscopy. Two examples are shown. At t = 0 s, the cantilever of the AFM approached the cell in Contact mode, with a force set at 15 nN, at the position of the red ring. Green rings mark the time and area of tip retraction. Blue arrowheads indicate mitochondria that are visibly thinned by the pressure but have not yet undergone fission. Fission events are Figure 2 continued on next page
Figure 2 continued

indicated by an orange arrowhead. OMM panels, mCherry-FIS1TM (red). Matrix panel, mtBFP (green). Scale bar, 5 μm. (D) As in (C) except that the cells were treated with a virus encoding DRP1 shRNA. Scale bar, 5 μm.

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Figure 2—figure supplement 1. Mitochondria undergo DRP1-dependent fission upon AFM-mediated force application. (A) U2OS KERMIT cells stably expressing mtBFP and Sec61β-GFP and treated with DRP1 siRNA were imaged by time-lapse microscopy. Two examples are shown. At t = 0 s, the cantilever of the AFM approached the cell in Contact mode, with a force set at 15 nN, at the position of the red ring. Green rings mark the time and area of tip retraction. Blue arrowheads indicate mitochondria that are visibly thinned by the pressure. Mito panels, mtBFP. Scale bar, 5 μm.
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Figure 3. Mitochondrial fission upon cell deformation. (A) Scheme of the experimental setup. (B) 3D projection of a U2OS cell expressing Sec61β-GFP showing thinning of the cytoplasm at the groove’s edge (arrowhead). (C) Mitochondria of indicated cells grown on vinyl records. Mitochondria are color-coded according to their Z-position (red in the groove, green on the ledge). The dashed lines indicate the approximate position of the edge. (D) Quantification of the number of cells showing a divided mitochondrial network, defined as having no mitochondria spanning the edge between the groove and the ledge. Number of cells analyzed are indicated on each bar. (E) Time-lapse microscopy of the fission events leading to divided mitochondrial network. Top panel, low magnification of the start- and end-points of the recording. Lower panels, individual fission events captured during the time course. Blue arrowheads, mitochondria before fission; Orange arrowheads, mitochondria after fission. Scale bars, 5 μm.

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Figure 4. Force-induced mitochondrial fission upon ER dynamics perturbations. (A) U2OS KERMIT cells stably expressing mtBFP and Sec61β-GFP and treated with scrambled siRNA were imaged by time-lapse microscopy. Two examples are shown. At $t = 0$ s, the cantilever of the AFM approached the
Figure 4 continued

cell in Contact mode, with a force set at 15 nN, at the position of the red ring. Green rings mark the time and area of tip retraction. Blue arrowheads indicate mitochondria that are visibly thinned by the pressure but have not yet undergone fission. Fission events are indicated by an orange arrowhead.

(B) Cells were seeded on vinyl records and transfected with a CLIMP-63-overexpressing plasmid, effectively converting large fractions of the ER to sheets. Mitochondria underwent fission when spanning over the edge of the groove whether or not the ER had been converted to sheets at the site of fission. The two facing arrowheads indicate the position of the groove’s edge. (C) As in (B) but cells were instead transfected with a construct expressing a dominant-negative form of ATL1-K80A, which inhibits ER interconnection and increases the size of gaps in the ER network. As a result, mitochondria can be observed undergoing fission at sites devoid of ER while spanning the edge of the groove. (D) U2OS KERMIT cells were transfected with a lentivirus expressing RFP-Lifeact (blue) and transfected with a cyto-ATL2 expression plasmid. Cells were then infected with mCherry-labelled \textit{S. flexneri} (blue). Mitochondria can be observed undergoing fission at sites stimulated by motile bacterium. The blue and yellow arrowheads represent mechanically constricted sites before and after fission, respectively. Mito panels, mtBFP. ER panel, Sec61β-GFP. Scale bars 5 μm. (E) U2OS cells transduced with GFP-Lifeact and matrix-targeted RFP were treated with 5 μg/ml of Cytochalasin D for 90 min. (F) U2OS cells transduced with GFP-Lifeact and matrix-targeted RFP were treated for 90 min with 1 μg/ml (upper panel) or 5 μg/ml Cytochalasin D (lower panel), respectively. At t = 0 s, the cantilever of the AFM approached the cell in Contact mode, with a force set at 15 nN, at the position of the red ring. Green rings mark the time and area of tip retraction. Blue arrowheads indicate mitochondria that are visibly thinned by the pressure but have not yet undergone fission. Fission events are indicated by an orange arrowhead.

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**Figure 4—figure supplement 1.** Control for INF2 knockdown efficiency. U2OS cells stably expressing mitochondrial matrix-targeted BFP (mtBFP) were reverse-transfected with scrambled (SCR) siRNA or siRNA specifically targeting INF2-CAAX isoform. A day later all cells were transfected with a plasmid expressing GFP-INF2-CAAX. 48 hr post siRNA transfection cells were fixed and imaged on a DeltaVision epifluorescence microscope. Scale bar, 5 μm.

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Figure 5. MFF recruitment to mechanically strained sites. (A) Immunofluorescence of Kermit cells transduced with shDRP1, using an anti-MFF antibody (green). Mitochondrial matrix (mtBFP) is shown in red. Insets on the right correspond to the framed areas on the left. Arrowheads point at naturally...
occuring constrictions on the mitochondria. Plots are linescans of the mitochondria (red) and MFF (green) signals around the constriction. X-axis is in μm. Y-axis is normalized fluorescence in arbitrary units. (B) as in A, but the cells were grown on gramophone records. Top left panel is a 3D projection of the same cell rotated by 90° around the x-axis. The white box on the left corresponds to the magnified area on the right. Arrowheads indicate mitochondrial constrictions at the groove’s edge. (C) U2OS cells were transduced with lentiviral vectors expressing mtBFP, RFP-Lifeact, GFP-MFF as well as shRNA targeting DRP1. Puromycin-resistant cells (shRNA-positive) were then infected with RFP-labeled S. flexneri, and imaged by time-lapse microscopy. MFF is recruited to sites of encounter with S. flexneri (white arrowheads). Right panels also show two examples of MFF enrichment at sites of mitochondria thinning (curly brackets), as indicated by reduction of matrix mtBFP signal, independent of Shigella encounter. (D) Line scan of mtBFP and MFF signal of the white dotted line in (C). Arrowhead and curly bracket correspond to same zones in (C). Normalized background-subtracted pixel values are plotted as arbitrary units. (E) Z-projected image of a transduced U2OS cell spanning the edge of a vinyl groove expressing mtBFP, GFP-MFF as well as shRNA targeting DRP1. The groove’s edge is indicated by two facing arrowheads on top right panel. Two stabilized individual mitochondrial tubules span over the edge (white line), and show loss of matrix BFP signal and increased MFF signal. Another example of GFP-MFF enrichment to a constricted mitochondrial tubule outside of the edge area is indicated by a curly bracket. (F) Dotted white lines 1 and 2 in (E) are selected for line plots as in (D). (G) U2OS cells were transduced with lentiviral vectors expressing mtBFP, mCherry-Fis1TM (OMM), as well as shRNA targeting DRP1. Puromycin-resistant cells (shRNA-positive) were then transfected with GFP-MFF, and imaged by time-lapse microscopy. MFF spontaneously stabilizes thin mitochondrial section (curly brackets) that are devoid of matrix staining but retain continuous OMM signal. (H) Line scan of mtBFP, GFP-MFF and OMM signal of the curly bracket in (G). Scale bars, A-B, 5 μm, C-H 2 μm.

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Figure 5—figure supplement 1. MFF immunostaining in WT and DRP1-deficient cells. (A) KERMIT cells were transduced with lentiviruses expressing either a non-targeting shRNA (shCTRL) or a DRP1-targeting shRNA. Cells were then cultured under puromycin selective pressure for 10 days before...
they were fixed, immunostained using a MFF-specific antibody, and imaged on a DeltaVision epifluorescence microscope. Scale bar, 5 μm. (B) More examples of cells grown of gramophone records and imaged as in Figure 5B. Arrowheads indicate mitochondrial constrictions at grooves’ edges. For each example, the rightmost panel is a 3D projection of the image rotated by 90° about the Y-axis.

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Figure 6. Monte Carlo simulation of protein-membrane interactions for different conditions. (A) Proteins were modelled as a linear chain built out of five spheres positioned on a circular arc. Each sphere has a radius of $s$. The center-to-center distance between the spheres within a protein was adjusted to $2/3$ of $s$. $R_{pr}$ is the radius of the arc, for which we found the optimal value to be $3.5s$ (see supplement), and $\theta_{pr}$ is the protein-membrane contact angle, which we set to $\pi/4$, in order for the proteins to attract the membrane only by the inner part of their structure. (B) 20 proteins as in A with an optimized $R_{pr}$ were allowed to reach equilibrium on a membrane tube with a radius of $10s$ and a length of $100s$. (C) 20 proteins as in A were simulated on a membrane tube with a pre-constriction (radius at the center of the constriction = $3s$) and allowed to equilibrate. (D) 50 proteins as in A were allowed to reach equilibrium on a membrane tube as in B (without pre-constriction).

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Figure 6—figure supplement 1. Optimization of the binding curvature of the protein during Monte Carlo simulation. Proteins were modelled as in Figure 6D. Proteins with different radii of curvatures R_{pr} were allowed to reach equilibrium on a membrane tube with a radius of 10σ and a length of 100σ. Proteins adopted different assembly behaviors at different radii. (A) At R_{pr} = 3σ, proteins attracted each other, but only in the longitudinal, and not angular, direction. (B) At R_{pr} = 4σ, proteins did not self-assemble at all.

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