Supporting Online Material

Materials and Methods

Growing *Dictyostelium* cells on EM grids

*D. discoideum* cells, strain AX2-214, were cultivated to near confluence at 23°C in 8 cm diameter polystyrene cell culture dishes containing nutrient medium (S1). Cells were harvested from the plates by washing twice in 17 mM K/Na-phosphate buffer, pH 6.0, and resuspending them in the buffer.

Carbon-coated grids were prepared using conventional deposition methods. It turned out to be important for survival of the cells that both sides of the copper grid were coated with carbon film. Cells were incubated on the grids for 30 to 60 minutes. Cells on the grids were inspected by phase contrast using an Axiovert 135 inverse microscope (Zeiss).

Cryo-electron tomography

The grids were rapidly frozen in liquid ethane as described by (S2) and transferred to a ±70° tilt cryoholder (Gatan). The specimens were introduced into a Philips CM 300 FEG microscope equipped with a Gatan post column GIF 2002 energy filter. Tilt series were collected, typically covering an angular range from of -60° to 60°, sampled with 1.5° tilt increments and at 15 µm underfocus. As a result, the optimal resolution was 5.5 nm and the pixel size at the specimen level was 0.85 nm. The data acquisition was fully automated as described previously (9, S3). To account for the increased effective thickness the exposure time was increased by a factor of $1/(\cos \alpha)$. 
**Image processing**

The projection images were transferred to an SGI workstation (SGI), aligned and reconstructed by a weighted back-projection algorithm as implemented by EM image processing software package (S4). For improvement of the visualization, the 3-D reconstructed volumes were further processed by an anisotropic denoising algorithm (S5). Surface rendering was performed using the options provided in the Amira 2.2 software package (ZIB, Germany and Indeed-Visual Concepts Berlin, Germany).

**Movie Legend**

**Movie S1.** Cells of *Dictyostelium discoideum* spread on a carbon-coated grid for electron microscopy

The cells, assuming on the grid a typical amoeboid shape, move and divide. To visualize actin assemblies in vivo, cells expressing GFP-actin (S6) were used for confocal fluorescence scanning. The fluorescence images (green) are superimposed on phase-contrast images showing cell shape and carbon-coated grid structure (blue). Frame-to-frame intervals are 5 seconds. Bar, 10 µm. The first section of the movie shows migrating cells with actin accumulated primary at the front regions. Frequent turning is paralleled by the accumulation of actin at new sites of the cell cortex. In the second section, one cell is undergoing mitotic cell division (cytokinesis). Specimens like the ones shown were vitrified and subjected to cryo-electron tomography (except that cells not expressing GFP-actin were used).
References


