Aronov et al., http://www.jcb.org/cgi/content/full/jcb.201408088/DC1

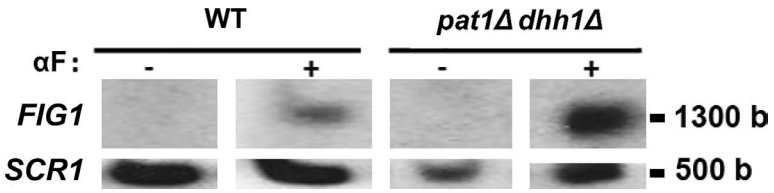


Figure S1. **The studied mutant strains respond to αF.** (A) WT and mutant cells respond to αF by transcriptional induction of *FIG1*. WT (yMC375) and *pat1Δ dhh1Δ* (yMC376) strains were treated with αF as described in Fig. 1. 1 h thereafter, the level of *FIG1* mRNA was determined by polyacrylamide-Northern analysis as in Materials and methods. The Pol III transcript *SCR1* RNA served as a control (Lotan et al., 2005).

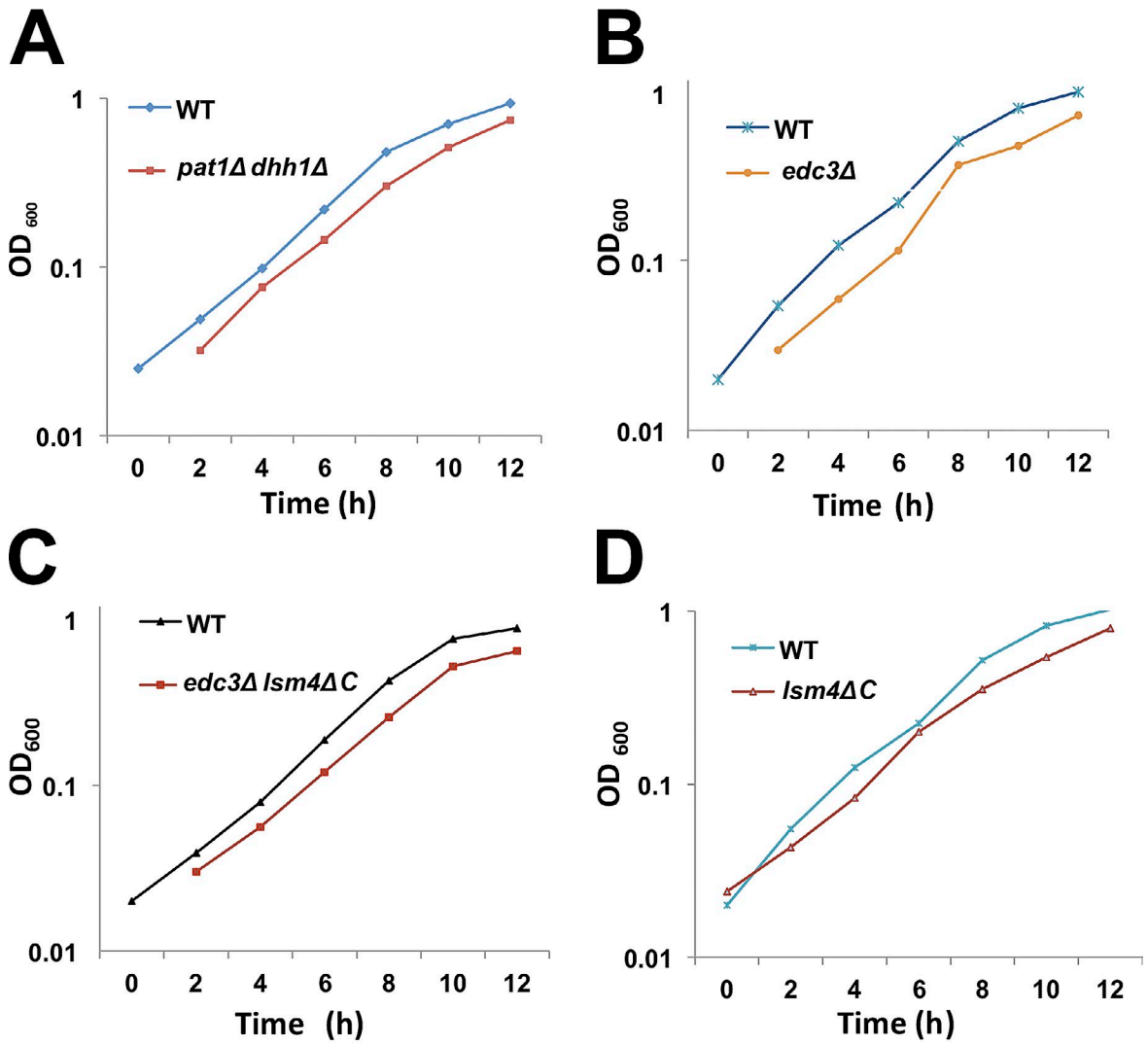


Figure S2. **Proliferation rate of the studied strains.** WT (yMC375), *pat1Δ dhh1Δ* (yMC376), *edc3Δ lsm4ΔC* (yMC524), *edc3Δ* cells (ySA196), and *lsm4ΔC* (ySA196) strains were examined. Cells were cultured in rich medium (YPD) at 30°C, with shaking at 180–200 rpm. The cultures' optical density (OD) at 600 nm was measured every 2 h for 12 h.

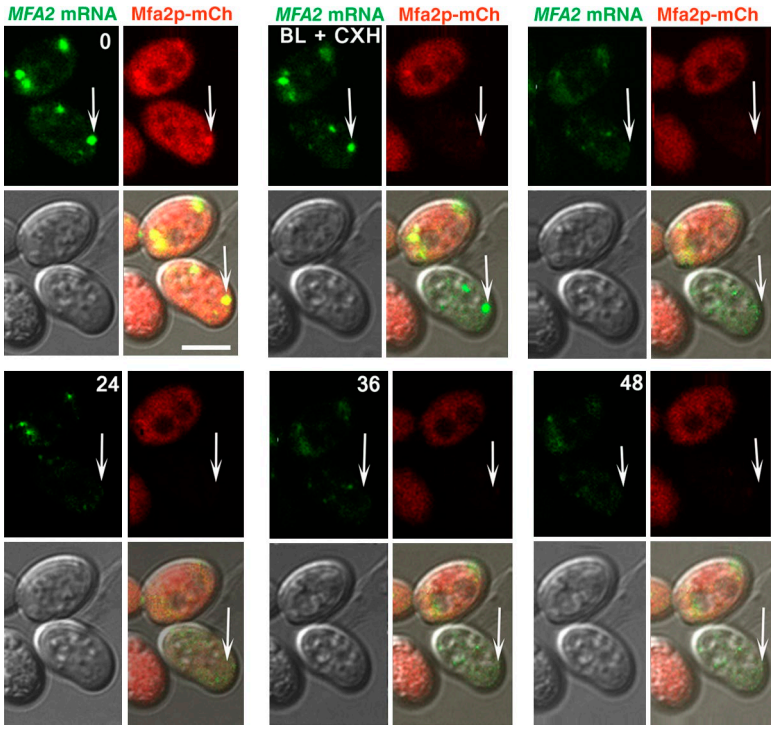
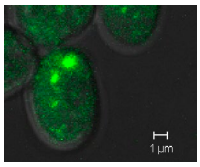
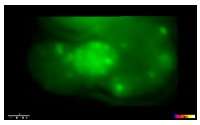


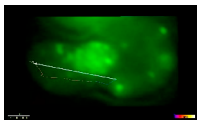
Figure S3. **CHX treatment prevents local accumulation of *Mfa2p-mCh* in the vicinity of the high-intensity granule in the shmoo.** (A–C) Mfa2p-mCh protein colocalizes with its mRNA in the shmoo tip after αF treatment. WT yeast cells cotransformed with *MFA2*p-mCh-UA1 and UA1p-GFP (ySA147) were grown to early logarithmic phase and treated with αF for 2 h. Bar, 5 µm. The lower cell was photobleached (BL). 100 µg/ml cycloheximide (CHX) was then added at the edge of the coverslip, allowing its diffusion inside (the shown field was near the edge of the coverslip). Fluorescence of the green channel and fluorescence recovery of the red channel were monitored by time-lapse microscopy, as in Fig. 8. Representative images from the time-lapse video: before photobleaching (top left); immediately after CHX addition and photobleaching (BL+CHX); 12, 24, 36, and 48 min after adding BL+ CHX. Each time point shows the green (top left), red (top right), bright field (bottom left), and the merged images (bottom right). Arrows point at the mating body. Times are given in minutes.



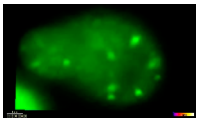
Video 1. **In response to pheromone treatment, low-intensity *MFA2* mRNA-containing granules are distributed all over the cell, whereas a high-intensity granule localizes to the shmoo before and during its growth.** Cells coexpressing *MFA2*-U1A and U1A-GFP (yAS20) were treated with 3 nM αF for 1 h. The distribution of *MFA2* mRNA-containing granules (green) was analyzed by time-lapse confocal microscopy, 30 min after addition of αF using a laser-scanning confocal microscope (LSM 510; Carl Zeiss). Images were captured every 3 min during 1 h, for a total of 20 frames. The video shows a fast-forward version, in which the 60 min are shown in 4 s (speed rate 5 frames/s). Note that during αF treatment, the high-intensity and immotile granules were localized in the shmoo as early as 30 min after αF addition and remained associated with the shmoo tip during its growth. These granules remained immotile during the entire course of the experiment. In parallel experiments, we noticed that their size first increased during the first 2 h after αF addition, and later it decreased. In contrast, the low-intensity granules were distributed all over the cell and were motile throughout imaging. Fig. 2 C shows representative snapshots that were chosen from the Video 1.



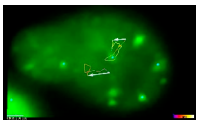
Video 2. **Distribution and motility features of *MFA2* mRNA-containing granules.** Related to Fig. 3 C. Cells coexpressing *MFA2*-U1A and U1A-GFP (yAS20) were treated with 3 nM αF for 2 h. Images were analyzed by time-lapse fluorescent microscopy that was acquired using the microscope (Fast Acquisition AxioVision; Carl Zeiss). The video was taken at 113 ms/frame, without intervals between shots, for a total of 27 frames. Bar, 1 µm. Note that one the low-intensity *MFA2* mRNA-containing granule move directly and rapidly over long distances from the cell body to the shmoo tip.



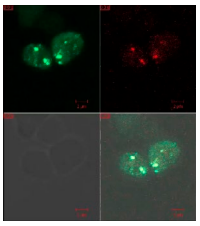
Video 3. **Tracking analysis of low-intensity *MFA2* mRNA-containing granules of Video 2.** The arrow shows the directed movement of the granule from the cell body to the shmoo region. Related to Fig. 3 C and Video 2. The tracking was obtained by Imaris program version 7.1 (Bitplane AG) on 2D videos. Bar, 1 µm.



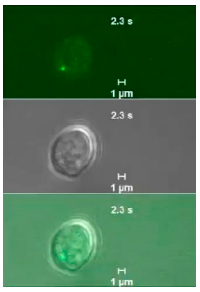
Video 4. **Movement of *PGK1* mRNA-containing granules in αF-treated cells.** Related to Fig. 3 B. Cells coexpressing *PGK1*-U1A and U1A-GFP (ySA32) were treated with 3 nM αF for 3 h. *PGK1* mRNA-containing granules (green) were analyzed by time-lapse fluorescent microscopy that was acquired using a microscope (Fast Acquisition AxioVision; Carl Zeiss). The video was taken at 73 ms/frame, without intervals between shots. Only oscillating movement can be observed; the long distance movement, which characterizes the *MFA2* mRNA granules, could not be observed.



Video 5. **Tracking analysis of *PGK1* mRNA-containing granules of Video 4**. Related to Fig. 3 B. The tracking of *PGK1* mRNA granules was analyzed by Imaris program version 7.1 (Bitplane AG) on 2D videos.



Video 6. **Colocalization of *MFA2* mRNA and Dcp2p-RFP during movement in αF-treated cells**. Related to Figs. 4 and 5. (A) Cells coexpressing *MFA2*-U1A, U1A-GFP, and the PB marker, Dcp2p-RFP (ySA22), were treated with 3 nM αF for 2 h. The movement *MFA2* mRNA (green) and Dcp2p-RFP (red) was analyzed by time-lapse fluorescent microscopy that was acquired using a confocal microscope (Fast Acquisition AxioVision; Carl Zeiss). To demonstrate colocalization, a slow motion is shown.



Video 7. **Motility of *MFA2* mRNA-containing granules in αF-treated *pat1Δ dhh1Δ* mutant cells.** Related to Fig. 6. Mutant cells (*pat1Δ dhh1Δ*) coexpressing *MFA2*-U1A and U1A-GFP (yAS25) were treated with 3 nM αF for 4 h. Motility of *MFA2* mRNA-containing granules (green) was analyzed by time-lapse fluorescent microscopy that was acquired using a microscope (Fast Acquisition AxioVision; Carl Zeiss). A video was taken at 2.3 s/frame, without intervals, over 73 s. The video shows a fast-forward version, in which the 73 s are shown in 6 s. Note the slow movement of the granules, relative to the movement in WT cells.

Reference

Lotan, R., V.G. Bar-On, L. Harel-Sharvit, L. Duek, D. Melamed, and M. Choder. 2005. The RNA polymerase II subunit Rpb4p mediates decay of a specific class of mRNAs. *Genes Dev.* 19:3004–3016. http://dx.doi.org/10.1101/gad.353205