Supplemental Materials

Molecular Biology of the Cell

Wang et al.
The Rho-GEF Gef3 interacts with the septin complex and activates the GTPase Rho4 during fission yeast cytokinesis

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Supplemental materials
## Supplemental Table S1: S. pombe strains used in this study.

| Strain   | Genotype                                                                 | Reference/Related figures/table |
|----------|--------------------------------------------------------------------------|---------------------------------|
| JW5520   | gef3-mECitrine-kanMX6 rlc1-mCFP-kanMX6 ade6-M210 leu1-32 ura4-D18         | Fig. 1A                         |
| JW5522   | gef3-mECitrine-kanMX6 sad1-mCFP-kanMX6 ade6-M210 leu1-32 ura4-D18         | Figs. 1B and S1A                |
| JW5568   | spn1-CFP-kanMX6 gef3-mECitrine-kanMX6 ade6-M210 leu1-32 ura4-D18          | Figs. 1C and S1B                |
| JW5493   | h' gef3-mECitrine-kanMX6 ade6-M210 leu1-32 ura4-D18                      | Figs. 1D, 2A, 7A, 7E, S2C, and S4E; Fig. 2A |
| JW5575   | mid2-Δ1::kanMX6 gef3-mECitrine-kanMX6 ade6-M210 leu1-32 ura4-D18          | Fig. 2A                         |
| JW5521   | spn1-Δ2::kanMX6 gef3-mECitrine-kanMX6 leu1-32 ura4-D18                   | Fig. 2A                         |
| JW5941   | spn3-Δ2::kanMX6 gef3-mECitrine-kanMX6 ade6-M210 leu1-32 ura4-D18          | Fig. 2A                         |
| JW1100   | h' spn1-mEGFP ade6-M210 leu1-32 ura4-D18                                 | Fig. 2A                         |
| JW5516   | gef3Δ:hphMX6 spn1-mEGFP-kanMX6 ade6-M210 leu1-32 ura4-D18                | Fig. 2A                         |
| JW1213   | h' mid2-mEGFP-kanMX6 ade6-M210 leu1-32 ura4-D18                          | Fig. 2A                         |
| JW6020   | gef3Δ:hphMX6 mid2-mEGFP-kanMX6 ade6-M210 leu1-32 ura4-D18                | Fig. 2B                         |
| JW5947   | h' gef3-13Myc-kanMX6 ade6-M210 leu1-32 ura4-D18                          | Fig. 2C                         |
| JW1171   | h' spn4-mYFP-kanMX6 ade6-M210 leu1-32 ura4-D18                           | Fig. 2C; Wu and Pollard, 2005   |
| JW6015   | spn1-mEGFP-kanMX6 gef3-13Myc-kanMX6 ade6-M210 leu1-32 ura4-D18           | Fig. 2C                         |
| JW6016   | spn4-mYFP-kanMX6 gef3-13Myc-kanMX6 ade6-M210 leu1-32 ura4-D18            | Fig. 2C                         |
| JW6017   | mid2-mEGFP-kanMX6 gef3-13Myc-kanMX6 ade6-M210 leu1-32 ura4-D18           | Fig. 2C                         |
| JW81     | h' ade6-M210 leu1-32 ura4-D18                                            | Figs. 3A, 3B, 5B², 6B, 6D, 6F, 8C², S2E, and S5B; Wu et al., 2003 |
| JW5492   | h' gef3Δ:hphMX6 ade6-M210 leu1-32 ura4-D18                               | Figs. 3A, 3B, 6B, 6D, 6F, 9D, 9E, S2A, and S2E; Table 1 |
| DM1274   | h' scw1Δ::ura4² ade6-M210 leu1-32 ura4-D18                               | Figs. 3A, 6A, 6B, S5C², and S5D²; Table 1; Jin and McCollum, 2003 |
| JW5790   | gef3Δ:kanMX6 scw1Δ::ura4² ade6-M210 leu1-32 ura4-D18                     | Figs. 3A, 6A, 6B, 8F², and 8G²; Table 1 |
| PPG2601  | h' gef1Δ::ura4² leu1-32 ura4-D18                                         | Figs. 3B, 6C, 6D, 9D, and 9E; Table 1; Coll et al., 2003 |
| JW5783   | gef3Δ:kanMX6 gef1Δ::ura4² ade6-M210 leu1-32 ura4-D18                     | Figs. 3B, 6C, 6D, 9D, and 9E; Table 1 |
| MBY887   | h' sec8-1 ura4-D18 leu1-32                                               | Figs. 3C, 6E, 6F; Table 1       |
| JW5958   | sec8-1 gef3Δ::hphMX6 ade6-M210? leu1-32 ura4-D18                         | Figs. 3C, 6E, 6F; Table 1       |
| JW2716   | h' exo70Δ::kanR ade6 leu1-32 ura4-D18                                    | Figs. 3D, S2D, and S2E; Table 1 |
| JW5954   | exo70Δ::kanR gef3Δ::hphMX6 ade6 leu1-32 ura4-D18                         | Figs. 3D and S2E; Table 1       |
| JW6030   | spn1-Δ2::kanMX6 kanMX6-3nmt1-3FLAG-gef3 ade6-M210 leu1-32 ura4-D18       | Figs. 4, 5A, 5D, and 5E         |
| JW6002   | h' rho3Δ::natMX6 ade6-210 leu1-32 ura4-D18                               | Fig. 6, A-D; Table 1            |
JW6039  
 rho3Δ::natMX6  sec11Δ::ura4Δ ade6-M210  leu1-32  ura4-D18
JW6047  
 rho4Δ::kanMX6  sec11Δ::ura4Δ ade6  leu1-32  ura4-D18
JW6037  
 rho4Δ::kanMX6  gef1Δ::ura4Δ ade6  leu1-32  ura4-D18
JW6038  
 rho3Δ::natMX6  gef1Δ::ura4Δ ade6  leu1-32  ura4-D18
JW6035  
 rho4Δ::kanMX6  sec8-1  leu1-32  ura4-D18
PPG1580
 h⁻  rho4Δ::kanMX6  leu1⁺::GFP-rho4  leu1-32  ura4-D18
PPG3723
 h⁺  eng1-GFP-kan8  leu1-32  ura4-D18
JW6532
 gef3Δ::hphMX6  rho4Δ::kanMX6  leu1⁺::GFP-rho4  leu1-32  ura4-D18
JW6540
 rho4Δ::kanMX6  eng1-GFP-kan8  ade6-M216  leu1-32  ura4-D18
JW6584
 Pgef3-mECitrine-gef3  ade6-210  ura4-D18  leu1-32
JW6024
 h⁻  kanMX6  gef3-3-mECitrine-kanMX6  ade6-M210  leu1-32  ura4-D18
JW6053
 h⁻  gef3(ΔDH)-mECitrine-kanMX6  ade6-M210  leu1-32  ura4-D18
JW5901
 gef1Δ::ura4Δ  gef3(1-270)-mECitrine-kanMX6  ade6  leu1-32  ura4-D18
JW5904
 gef1Δ::ura4Δ  gef3(1-69)-mECitrine-kanMX6  ade6-M210  leu1-32  ura4-D18
JW6576
 gef1Δ::ura4Δ  gef3(ΔDH)-mECitrine-kanMX6  ade6  leu1-32  ura4-D18
YSM836
 h⁺  cdc42-1625(A158V)-kanMX  leu1-32  ura4-D18
JW5960
 cdc42-1625(A158V)-kanMX  gef3Δ::hphMX6  ade6-M210?  leu1-32  ura4-D18
JW1272
 h⁻  myo52Δ::ura4Δ  ade6-M210  leu1-32  ura4-D18
JW6624
 myo52Δ::ura4Δ  rho4Δ::kanMX6  ade6-M21X  leu1-32  ura4-D18
JW6625
 myo52Δ::ura4Δ  gef3Δ::hphMX6  ade6-M210  leu1-32  ura4-D18
JW5955
 exo70Δ::kan8  gef3-3-mECitrine-kanMX6  ade6  leu1-32  ura4-D18
JW5956
 sec8-1  gef3-3-mECitrine-kanMX6  ade6-M210?  leu1-32  ura4-D18
JW6036
 rho4Δ::kanMX6  exo70Δ::kan8  ade6?  leu1-32  ura4-D18
JW5990
 h⁻  kanMX6-Prho3-mECitrine-rho3  ade6-M216  leu1-32  ura4-D18
JW6009
 spn1Δ2::kanMX6  kanMX6-Prho3-mECitrine-rho3  ade6-M210  leu1-32  his3-27?  ura4-D18
Table 1

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Table 1; Wu et al., 2010

Table 1; Wu et al., 2010

Table 1; Liu et al., 1999

Table 1; Wu et al., 2010

Table 1; Wu et al., 2010

Fig. S4, C and D

Fig. S4E

Fig. S5A; Alonso-Nunez et al., 2005

Fig. S5B; Table 1;

Table 1

Table 1

Table 1

Table 1
| Strain   | Genetic Background                                                                 | Table |
|----------|------------------------------------------------------------------------------------|-------|
| JW2642   | h<sup>+</sup> rho5Δ::kan<sup>8</sup> ade6-M210 leu1-32 ura4-D18                   | 1     |
| JW5940   | gef3Δ::hphMX6 rho5Δ::kan<sup>8</sup> ade6 leu1-32 ura4-D18                        | 1     |
| JW6602   | gef3(ΔDH)-mECitrine-kanMX6 scw1Δ::ura4Δ ade6-M210 leu1-32 ura4-D18               | 1     |
| JW6603   | gef3(ΔDH)-mECitrine-kanMX6 exo70Δ::kan<sup>8</sup> ade6 leu1-32 ura4-D18          | 1     |
| JW6604   | gef3(ΔDH)-mECitrine-kanMX6 sec8-1 ade6-M210 leu1-32 ura4-D18                      | 1     |
| JW6605   | for3Δ::kanMX6 rho4Δ::kanMX6 ade6 leu1-32 ura4D-18                                 | 1     |
| JW6645   | art1Δ::ura4<sup>+</sup> rho4Δ::kanMX6 ade6-M216 leu1-32 ura4D-18                  | 1     |
| JW6646   | plo1.ts18::ura4<sup>+</sup> rho4Δ::kanMX6 ade6leu1-32 ura4-D18                    | 1     |
| JW6648   | bgs1-191 rho4Δ::kanMX6 ade6 leu1-32 ura4-D18                                     | 1     |
| JW6649   | scd1Δ::kan<sup>8</sup> rho4Δ::kanMX6 ade6 leu1-32 ura4-D18                        | 1     |
| JW6677   | rho1-596-natMX6 rho4Δ::kanMX6 ade6-M216 leu1-32 ura4D-18                          | 1     |
| JW6677   | rho2Δ::kan<sup>8</sup> rho4Δ::kanMX6 ade6? leu1-32 ura4-D18                       | 1     |
| JW6647   | rho5Δ::kan<sup>8</sup> rho4Δ::kanMX6 ade6 leu1-32 ura4-D18                        | 1     |

*Because we used fresh mutant cells transformed with overexpression plasmids for this experiment, only the parent strain without a plasmid is listed.*
Supplemental References

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**Supplemental Figure S1**: Localization of Gef3 at cell-division site. (A) Timing of Gef3-mECitrine localization to the division site. Sad1-mCFP was used as an SPB marker. The arrow marks the faint Gef3 signal appeared at the division site during late anaphase B; the arrowhead and asterisk indicate the single ring or double ring of Gef3, respectively, at later stages of cytokinesis. (B) Time course (in minutes) showing that Gef3 colocalizes with Spn1 during septation in cells expressing both Gef3-mECitrine and Spn1-CFP. Time 0 is the start of the movie. Bars, 5 µm.
Supplemental Figure S2: Synthetic genetic interactions of gef3Δ or rho4Δ with mutations in cdc42, myo52, and exo70, and localization independence of Gef3 on the exocyst complex. (A, B) DIC images showing the synthetic interactions between gef3Δ/rho4Δ and cdc42-1625 (A) or myo52Δ (B) at 25°C. (C) Gef3 localization is normal in exocyst mutants exo70Δ and sec8-1. Cells were grown at 36°C for 4 h and imaged at 36°C. (D, E) DIC images (D) and quantifications of septation index (E) showing the synthetic interactions of exo70Δ with gef3Δ and rho4Δ at 36°C. Cells were grown at 36°C for 6 h before imaging. (E) n > 600 cells for each strain. Bars, 5 µm.
Supplemental Figure S3: Gef3 binds to Rho4 and has GEF activity toward Rho4 in vitro. (A) Coomassie blue staining of SDS-PAGE showing purified 6His-Gef3 from *E. coli*. The asterisk marks 6His-Gef3 band with the expected size. (B, C) Rho4 is most efficient at pulling down Gef3 in vitro. Purified GST-Rho GTPases and GST control were bound to the beads and then incubated with purified 6His-Gef3. The amount of pulled-down Gef3 was detected by Western blotting (B) and quantified (C). The intensities of 6His-Gef3 bands were normalized by the intensities of pulled-down Rho GTPases. The intensity of 6His-Gef3 band in GST control was set as 1. (D) Gef3 efficiently catalyzes the nucleotide exchange on GST-Rho4. Raw data from a representative experiment (n = 3) were plotted since the fluorescence intensity dropped too fast (upon 1 μM 6His-Gef3 addition) to do the curve fit. Broken line marks the first readings after Gef3 or the same amount of buffer is added.
Supplemental Figure S4: Localization of Rho4 but not Rho3 at the division site is affected in spn1Δ or gef3Δ. (A) Localization of Rho3 shown in maximum intensity projection, the middle slice of z-stacks, or vertical view of the color-boxed regions. Red box, a cell at the start of septum formation; blue box, a cell with complete septum. (B) Time courses of GFP-Rho4 localization in wt, gef3Δ, and spn1Δ cells during cytokinesis. Time 0 is the time point just before Rho4 appears at the division site. Arrows mark the cells with peak Rho4 intensity. Note that the Rho4 intensity between different cells is not comparable due to signal bleaching. (C, D) Localization (C) and intensity quantification (D) of Rho3 at the division site in wt, gef3Δ, and spn1Δ cells. (C) The maximum projection and middle slice of z-stacks were shown. Arrows, cells at the start of septum formation (ring stage); arrowheads, cells with complete septa (disk stage). (D) Fluorescence intensities (mean ± 1 SD) at the ring and disk stages are shown. n ≥ 30 cells for each strain at each stage. (E) Localization of Gef3 is not affected by rho3Δ. Arrowheads mark cells with complete septa. Bars, 5 µm.
**Supplemental Figure S5**: Agn1 localization in gef3Δ, synthetic genetic interactions of agn1Δ with gef3Δ and rho4Δ, and failure of rescuing scw1Δ by Rho4 overexpression. (A) Agn1 localization is defective in gef3Δ cells. DIC (left) and maximum intensity projection (right) of wt and gef3Δ cells expressing Agn1-GFP. Arrows mark cells with complete septa for comparison. (B) DIC images showing the synthetic interactions of agn1Δ with gef3Δ or rho4Δ after 6 h at 36°C. (C, D) DIC images (C) and quantification of septation index (D) showing that Rho4 overexpression does not rescue the septation defects in scw1Δ cells. Cells with plasmids were grown in EMM5S – leucine medium for 24 h 25°C. (D) Mean ± 1 SD from three independent experiments is plotted, and n > 500 for each strain in each experiment. Bars, 5 µm.