The F-BAR Domain of Rga7 Relies on a Cooperative Mechanism of Membrane Binding with a Partner Protein during Fission Yeast Cytokinesis

Graphical Abstract

Highlights
- The Rga7 F-BAR domain binds Rng10 and the plasma membrane simultaneously
- The Rga7-Rng10 interaction significantly enhances Rga7 membrane avidity
- Rga7 F-BAR function can be bypassed by tethering the remainder of Rga7 to Rng10
- Some F-BAR domains are necessary but not sufficient for plasma membrane targeting

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In Brief
Liu et al. show that the Rga7 F-BAR domain binds an adaptor protein Rng10, which contains a second membrane-binding module, to enhance Rga7 membrane avidity and stabilize its membrane association. The authors reveal a mechanism by which F-BAR domains can achieve high-avidity binding with the plasma membrane.
The F-BAR Domain of Rga7 Relies on a Cooperative Mechanism of Membrane Binding with a Partner Protein during Fission Yeast Cytokinesis

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SUMMARY

F-BAR proteins bind the plasma membrane (PM) to scaffold and organize the actin cytoskeleton. To understand how F-BAR proteins achieve their PM association, we studied the localization of a Schizosaccharomyces pombe F-BAR protein Rga7, which requires the coiled-coil protein Rng10 for targeting to the division site during cytokinesis. We find that the Rga7 F-BAR domain directly binds a motif in Rng10 simultaneously with the PM, and that an adjacent Rng10 motif independently binds the PM. Together, these multivalent interactions significantly enhance Rga7 F-BAR avidity for membranes at physiological protein concentrations, ensuring the division site localization of Rga7. Moreover, the requirement for the F-BAR domain in Rga7 localization and function in cytokinesis is bypassed by tethering an Rga7 construct lacking its F-BAR to Rng10, indicating that at least some F-BAR domains are necessary but not sufficient for PM targeting and are stably localized to specific cortical positions through adaptor proteins.

INTRODUCTION

The F-BAR (Fer/CIP4 homology-Bin-Amphiphysin-Rvs)-domain superfamily broadly functions to link the plasma membrane (PM) to the actin cytoskeleton (Liu et al., 2015; Roberts-Galbraith and Gould, 2010; Salzer et al., 2017). As such, F-BAR proteins play major roles in membrane trafficking, cell morphology, cell motility, and cell division.

Membrane binding is an intrinsic property of all F-BAR domains (Begonja et al., 2015; Frost et al., 2009; Itoh and Takenawa, 2009; Liu et al., 2015; Takeda et al., 2013). These domains homodimerize to form crescent-shaped or flat modules that interact with anionic membranes with modest affinity (Almeida-Souza et al., 2018; Kelley et al., 2015a; Lefèvre et al., 2012; Moravecivc et al., 2015; Soulard et al., 2002). F-BAR domains can achieve stronger avidity for membranes by homo-oligomerizing through tip-to-tip or tip-to-core interactions (Frost et al., 2009; McDonald et al., 2015; Shimada et al., 2007). The majority of F-BAR proteins contain only F-BAR domains for membrane binding, with the exception of Fes and Fer, which have an adjacent FX domain as a second membrane-binding module (Itoh et al., 2009). Although interaction networks established through other domains such as SH3, μHD, RhoGAP (guanosine triphosphatase [GTPase]-activating protein), tyrosine kinase, and C1 can influence discrete localizations (Aspenström, 2009; Salzer et al., 2017; Roberts-Galbraith and Gould, 2010), in general, F-BAR proteins are thought to depend on the lipid binding of F-BAR domains for membrane association and proper intracellular targeting (Frost et al., 2009; McDonald and Gould, 2016b; Mim and Unger, 2012; Qualmann et al., 2011; Salzer et al., 2017). How F-BAR domains could mediate subcellular targeting is not clear, although several hypotheses have been suggested. One possibility is that F-BARs bind certain lipid head groups preferentially (e.g., phosphoinositides [PIPs]). However, few F-BAR domains contain specific PIP-binding pockets, and most are able to bind membranes with a wide range of compositions in vitro (Frost et al., 2009; Itoh and Takenawa, 2009; McDonald and Gould, 2016b). Another proposed localization mechanism is sensing subcellular membrane curvature (Mim and Unger, 2012). This hypothesis has seemed most relevant for F-BAR proteins involved in endocytosis, in which highly curved membrane intermediates are formed, and different F-BARs assemble on the budding vesicle in a defined order (Taylor et al., 2011). However, the localization timings do not correlate with the curvature of the F-BAR crescent (Qualmann et al., 2011), and in vitro, these F-BAR domains bind to liposomes with varied membrane curvatures (Itoh et al., 2005; McDonald and Gould, 2016b). Thus, it remains unclear mechanistically how most F-BAR proteins target to the correct subcellular localization.

The Schizosaccharomyces pombe F-BAR protein Rga7 localizes to the division site and functions during late cytokinesis; rga7 mutants lyse at cell separation due to defective septa (Ara-sada and Pollard, 2015; Liu et al., 2016; Martin-Garcia et al.,...
RESULTS AND DISCUSSION

The Rng10 C Terminus Is Required for Division-Site Localization of the F-BAR Protein Rga7

Rga7 localization and function during seption requires the coiled-coil protein Rng10 (Figure 1A; Liu et al., 2016). We defined the regions of Rng10 required for Rga7 localization. Rga7 localized to the division site and cell tips in all Rng10 N-terminal truncations tested (Figures 1B and S1A), but failed to localize in Rng10 C-terminal truncations (Figures 1B and S1B). Since Rga7 required minimally Rng10(751–1,038) for co-localization to the pre-constriction ring, constricting ring, and a disk lining the septum at the division site (Figures 1B and S1C), we concluded that the Rng10 C terminus is sufficient to localize Rga7.

To confirm that the Rng10 C terminus is sufficient for Rga7 subcellular localization, we tested whether artificially mitochondria-localized Rng10(751–1,038) could recruit Rga7. The mitochondria outer membrane protein Tom20 was tagged with GFP-binding protein (GBP) (Rothbauer et al., 2008; Yamamoto et al., 2011) in combination with monomeric EGFP (mEGFP)/mECitrine-tagged Rng10 constructs. The Rng10 constructs were robustly recruited to the mitochondria by Tom20-GFP, while tdTomato, mScarlet-I, or mCherry tagged Rng10 were not (Figure S1D). As predicted, Rng10 and Rng10(751–1,038), but not Rng10(1–750), recruited Rga7 to mitochondria (Figure S1E). These data indicate that the Rng10 C terminus (amino acids [aa] 751–1,038) is sufficient to direct Rga7 to the division site.

The Rng10 C Terminus Directly Interacts with the Rga7 F-BAR Domain to Regulate Rga7 Localization

Because the Rga7 F-BAR domain (aa 1–320) is sufficient for Rga7 localization (Arasada and Pollard, 2015), we hypothesized that the F-BAR domain interacts with the Rng10 C terminus.
Mechanism of Rga7 F-BAR Membrane Binding

That Rng10 contains a membrane-binding motif linked to an Rga7 interaction motif raised the question of whether membrane binding by the Rga7 F-BAR is at all important for Rga7 localization and function. To determine whether and how the Rga7 F-BAR binds membranes, we generated a structural model of the Rga7 F-BAR dimer (Figure 3A) based on the closely related S. cerevisiae Rgd1 F-BAR that has been crystallized (Moravcevic et al., 2015). The model predicted the conservation of a PIP$_2$ binding pocket near the kinks in the F-BAR arms (blue residues,
Figure 3A). Additional positively charged residues were identified on the concave face of the Rga7 F-BAR that could contribute to membrane binding (orange and red residues, Figure 3A). We mutated patches of these basic residues and tested the mutants’ ability to bind membranes in vitro. While WT Rga7 F-BAR bound strongly to liposomes, mutation of the PIP2 pocket decreased its affinity (Figures 3B and 3C). Combining mutations in the PIP2 pocket and in positively charged patches along the core of the
domain and at its tips abolished membrane binding (Figures 3B and 3C). These mutations also prevented the Rga7 F-BAR from binding and tubulating giant unilamellar vesicles (GUVs) (Figure S3A), but they did not prevent binding to the Rng10 C terminus in vitro (Figures S2F and S2G), suggesting that the membrane- and Rng10-binding sites are on opposite faces of the Rga7 F-BAR domain. When expressed in COS-7 cells, the Rga7 F-BAR WT, but not membrane-binding mutants, induced tubules that also contained the Rng10 C terminus (Figures S3B and S3C).

We next tested whether Rga7 preferentially bound membranes of a specific lipid composition. As predicted by the presence of a PIP₂ binding pocket, the Rga7 F-BAR preferred membranes rich in PI(4)P and PI(4,5)P₂ (Figure 3D). The PIP₂ pocket mutant lost this specificity, binding membranes rich in phosphatidic acid (PA) equally well (Figure 3D).

To determine whether F-BAR membrane binding is required for Rga7 localization when Rng10 is present, we integrated the F-BAR lipid-binding mutants into rga7Δ cells under the endogenous rga7 promoter. The PIP₂ pocket mutant still localized to the division site, but with a lower level than WT (Figure 3E), consistent with its membrane-binding properties in vitro (Figure 3B). However, combining the PIP₂ binding mutations with core and tip patch mutations eliminated the localization of the Rga7 F-BAR to the division site (Figure 3E). Although the membrane-binding mutants were present at lower levels than WT Rga7 (Figure 3F), sufficient protein was produced for the detection of medial localization if it had occurred and overexpression of the mutants did not restore membrane localization (Figure S3D). We conclude that the Rga7 F-BAR interacts with membranes in a canonical fashion, and its membrane-binding ability is required but insufficient for Rga7 localization.

The Rng10-Rga7 Complex Enhances Rga7 Membrane Binding and Function at Physiological Concentrations

Since Rng10 and Rga7 directly interact and both bind membranes, we next asked whether the complex had different membrane-binding properties than the individual proteins. Rga7 F-BAR binding to liposomes was increased when Rng10(751–1,038) was present compared to the F-BAR alone (Figure 4A). The Rng10-Rga7 F-BAR binding to liposomes was increased when Rng10(751–1,038) was present compared to the F-BAR alone (Figure 4A). When Rng10 lacked either the Rga7- or membrane-binding motif, this increase was abolished. The increase was particularly pronounced at the lower protein concentrations (Figure 4A, inset) that match the global concentrations of Rng10 and Rga7 in cells at 0.38 and 1.00 μM, respectively (Figure 4B; see Method Details). The Rga7 level at the division site was only ~1.3× higher than Rng10, suggesting a 1:1 stoichiometry for Rng10 and Rga7 on the PM (Figure 4C). Thus, our results suggest that Rga7 F-BAR and Rng10(751–1,038) bind cooperatively to the PM for division-site localization.

To evaluate how the Rng10-Rga7 interaction affects cell division, we quantified cell lysis, indicative of septation failure (Liu et al., 2016), in the Rng10 and Rga7 mutants described above. The rga7Δ and rga7Δ/C14Δ mutants had mild lysis at 25°C and severe lysis at 36°C, as did Rng10 mutants lacking Rga7 and/or membrane-binding motifs. In contrast, Rng10(751–1,038) had low levels of lysis (Figure 4D). Thus, Rng10 interactions with both Rga7 and the PM are critical for Rga7 function.

Although the Rga7 F-BAR alone localizes to the division site in rga7Δ cells (Figure 3E; Arasada and Pollard, 2015), >40% rga7Δ(1–320) cells lysed at 36°C, comparable to the ~50% lysis of rga7Δ cells (Figure 4D), indicating that the Rga7 central region and/or GAP domain contribute to Rga7 function (Figure 1A). This result was unexpected because Rga7 GAP catalytic activity was reported to be dispensable for Rga7 function (Martín-García et al., 2014). We therefore tested whether localizing the Rga7 central and GAP domains to the PM is the critical function of its F-BAR domain by artificially recruiting them to the division site with Rng10-GBP. GFP-Rga7(277–695) alone, lacking the majority of the F-BAR domain, could not localize to the PM, resulting in massive cell lysis (58%) similar to rga7Δ (Figures 4E and S3E; Martín-García et al., 2014). However, Rng10-GBP restored GFP-Rga7(277–695) localization to the division site and significantly reduced cell lysis (Figures 4E and S3E). Thus, targeting Rga7 central and GAP domains to the PM, but not other possible activities such as membrane tubulation (Figures S3A and S3B) or curvature sensing, is the critical function of Rga7 F-BAR.

Our data indicate that the interaction between the Rga7 F-BAR and Rng10 ensures the division site localization of Rga7 by elevating its membrane-binding avidity, and this supports efficient septation during cytokinesis. Analogous examples of such cooperativity between intramolecular dual membrane-binding domains exist in a few F-BAR and N-BAR proteins (Carman and Domínguez, 2016). The Fes F-BAR domain and adjacent FX domain cooperate to increase membrane avidity (Itcho et al., 2009). APPL1 contains an N-BAR domain and an immediately adjacent pleckstrin homology domain, which also binds phospholipids (Lemmon, 2004). The sorting nexin Snx9 contains a phox homology domain that binds phosphatidylinositol precursors preceding the N-BAR domain (Pylpypenko et al., 2007). The Rga7 F-BAR appears to use a similar strategy of a second membrane-binding domain, but in this case, within its partner, Rng10. In general, individual F-BARs are relatively weak membrane-binding domains; it is only by interacting with another membrane-binding domain or by extensive oligomerization as seen in many other F-BARs (McDonald and...
Gould, 2016b) that a stable interaction with the membrane can be established. Furthermore, we have mechanistically illustrated a case in which the ability of an F-BAR domain to bind membranes is necessary but not sufficient for cellular localization. Instead, the domain relies on linkage to an adaptor protein that has additional membrane-binding potential to cooperatively achieve stable and specific membrane associations (Figure 4F).
strategy of membrane localization could be particularly useful for exerting spatial and temporal control of dynamic F-BAR localization since interactions between different adapters can be regulated. Because the majority of the BAR superfamily proteins contains only the F-BAR/BAR domains as a low-affinity membrane-binding module (Liu et al., 2015; McDonald et al., 2016b; Salzer et al., 2017), we expect that additional examples of protein adapters that modulate F-BAR/BAR protein localization and function will be identified in the future.

STAR METHODS

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Supplemental Information can be found with this article online at https://doi.org/10.1016/j.celrep.2019.01.112.

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AUTHOR CONTRIBUTIONS

Y.L., N.A.M., K.L.G., and J.-Q.W. designed the experiments. Y.L., N.A.M., and S.M.N. performed the experiments and analyzed the data. Y.L., N.A.M., K.L.G., and J.-Q.W. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCE TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Anti-GFP            | Roche  | 11814460001; RRID: AB_390913 |
| TAT1                | Keith Gull (Woods et al., 1989) | N/A |
| Anti-mouse immunoglobulin G | Sigma-Aldrich | A4416; RRID: AB_258167 |
| Monoclonal anti-His | Clontech | 631212; RRID: AB_2729105 |
| Anti-GST            | Novus Biologicals | NB600-446; RRID: AB_10003107 |
| Anti-mouse fluorescent antibody | LI-COR | 92568070; RRID: AB_2651128 |
| Bacterial and Virus Strains |        |            |
| BL21(DE3)pLysS      | Novagen | 694513 |
| Chemicals, Peptides, and Recombinant Proteins |        |            |
| Gelatin             | Sigma-Aldrich | G2500 |
| Protease inhibitor  | Roche  | 11873580001 |
| Lipid chloroform stocks | Avanti Polar Lipids | N/A |
| CellMask Deep Red   | Thermo Fisher Scientific | C10046 |
| SuperSignal West Maximum Sensitivity Substrate | Thermo Fisher Scientific | 34096 |
| Glutathione-Sepharose beads | GE Healthcare | 17-5132-01 |
| Talon metal affinity resin | Clontech | 635501 |
| Experimental Models: Organisms/Strains |        |            |
| See Table S1        | This study | N/A |
| Software and Algorithms |        |            |
| ImageJ              | NIH    | https://imagej.nih.gov/ij/ |
| Other               |        |            |
| Liposome extruder   | Avanti Polar Lipids | 610023 |
| BioMax MR film      | Kodak  | Z350370 |
| Epifluorescence microscope | Nikon | Eclipse Ti |
| UltraVIEW spinning disk confocal system | PerkinElmer | Vox CSUX1 |
| Personal Deltavision system | GE Healthcare | N/A |

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagent should be directed to and will be fulfilled by the Lead Contact, Jian-Qiu Wu (wu.620@osu.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Fission Yeast
Table S1 lists the S. pombe strains used in this study. Cells were grown exponentially in YE5S or EMM5S liquid media for 1~2 days. Detailed growth conditions see individual experiments in Method Details.

Escherichia coli
E. coli strain BL21(DE3)pLysS (694513; Novagen, EMD Chemicals, Darmstadt, Germany) were grown in LB media at 37° C and induced with 1 mM isopropyl-β-D-thiogalactoside (IPTG) for 2-4 h at 30 or 37° C.

Cell Line
COS-7 cells were cultured in DMEM media containing 10% fetal bovine serum (FBS), and transfected with a Lipofectamine 2000 reagent according to the manufacturer’s protocols.
METHOD DETAILS

Molecular Biology Methods

Full length and truncated genes were tagged C-terminally at their native chromosomal loci and expressed under their endogenous promoters by PCR-based gene targeting (Sähler et al., 1998). N-terminal truncations and tagging of mg10 were constructed with pFA6a-kanMX6-Pmg10-mECitrine (JQW908) as the PCR template, resulting in strains under the control of the native promoter of mg10 (−510 to +6 bp). N-terminal tagging of Rga7 F-BAR mutants was constructed using pFA6a-kanMX6-P3mt1-mECitrine (JQW331). All strains from gene targeting were confirmed by PCR and visual screening if possible. To minimize cell lysis, some spores from crosses were germinated on medium with 1.2 M sorbitol.

The mutants are: PIP2 mutant, R144D/K148E; PIP2 + Core mutant, K60E/K67E/K78E/K82E/R144D/K148E; PIP2 + Core + Tips mutant, K60E/K67E/K78E/K82E/R144D/K148E/K167E/R181D/K194E. To integrate these mutations into the rga7 locus, the whole rga7 ORF was deleted using pFA6a-ura4+ (JQW328) to obtain the rga7Δ::ura4+ strain. Then rga7Δ::ura4+ sequences with the desired mutations were amplified from plasmids JQW923-JQW925 using primers containing sequences of the 5’ and 3’ UTRs of rga7. The PCR products were transformed into the rga7Δ::ura4+ cells. Positive transformants were selected by resistance to 5-fluoorotic acid (5-FOA) and confirmed by sequencing.

To construct protein expression plasmids, mg10 and rga7 fragments were amplified from genomic DNA. Rng10 fragments were cloned into pQE-80L (His tag) at SacI and PstI sites, or pGEX-4T-1 (GST tag) at EcoRI and NotI sites. Rga7(1-320) was cloned into pQE-80L at BamHI and NdeI sites. Synthesized Rga7 F-BAR lipid-binding mutant DNAs were amplified and re-cloned into pEGFP-C1 at EcoRI and BamHI sites and pET15b-GFP at NdeI and BamHI sites.

Microscopy

Confocal microscopy was performed as previously described (Liu et al., 2016). For analyses of cell lysis, cells were first grown exponentially in YE5S liquid medium at 25 °C for ~40 h and then imaged or shifted to 36 °C for 4 h before imaging. Cells were collected by centrifugation at 3000 rpm for 30 s and imaged on the glass slide with a pad of YE5S with 20% gelatin and 50 nM n-propyl-gallate (n-PG) at room temperature. For fluorescence microscopy, cells were grown exponentially in YE5S liquid medium at 25 °C for 2 days, or in EMM5S liquid medium at 25 °C for 24 h to induce overexpression of Rga7 F-BAR mutants. Cells were centrifuged at 3000 rpm for 30 s and then washed twice with EMM5S to reduce autofluorescence. n-PG from a 10 × stock made in EMM5S were added to EMM5S in the second wash to a final concentration of 50 nM to reduce phototoxicity and photobleaching (Giloh and Sedat, 1982; Laporte et al., 2011). Live cells were imaged on a glass slide with a pad of EMM5S with 20% gelatin (Sigma-Aldrich, St. Louis, MO) and 50 nM n-PG at 23-24 °C.

Fission yeast cells were imaged on two microscopy systems with 100x/1.4 numerical aperture (NA) Plan-Apo objective lenses (Nikon, Melville, NY). Differential interference contrast (DIC) images for quantification of cell lysis were taken using a Nikon Eclipse Ti inverted microscope equipped with a Nikon DS-Q1 cooled digital camera. Fluorescence images were taken using a spinning-disk confocal system (UltraVIEW Vox CSUX1 system; PerkinElmer, Waltham, MA) with 440-, 488-, 515-, and 561-nm solid-state lasers and back thinned electron-multiplying charge-coupled device (EMCCD) cameras (C9100-13 or C9100-23B; Hamamatsu Photonics, Bridgewater, NJ) on a Nikon Ti-E microscope. COS-7 cells and giant unilamellar vesicles were imaged on a Personal Deltavision system (GE) equipped with 63X 1.4NA objective, CoolSnap2 CCD camera, and 488-, 561-, and 647-nm lasers.

Image Analysis

Images were processed and analyzed using Velocity (PerkinElmer) and ImageJ (National Institutes of Health, Bethesda, MD). Fluorescence images in figures are maximum-intensity projections with z sections spaced at 0.5 μm except where noted. Some images contain lysed cells, which have bright autofluorescence.

Protein Purification and in vitro Binding Assay

Purifications of His6-tagged proteins from E. coli were carried out as previously described (Zhu et al., 2013). His6-tagged proteins were purified with Talon metal affinity resin (635501; Clontech, Mountain View, CA) in extraction buffer (50 mM sodium phosphate, pH 8.0, 500 mM NaCl, 10 mM β-mercaptoethanol, 1 mM PMSF, and 10 mM imidazole) with EDTA-free protease inhibitor tablet (Roche) and eluted with elution buffer (50 mM sodium phosphate, pH 8.0, 500 mM NaCl, 10 mM β-mercaptoethanol, 1 mM PMSF, and 200 mM imidazole). GST-tagged proteins were purified with Glutathione-Sepharose beads (17-7132-01; GE Healthcare) in phosphate extraction buffer (50 mM sodium phosphate, pH 6.0, 200 mM NaCl, 1 mM PMSF, and 1 mM DTT) with EDTA-free protease inhibitor tablet and eluted with glutathione (50 mM sodium phosphate, pH 6.1, 200 mM NaCl, 1 mM PMSF, 1 mM DTT, 100 μM glutathione). The purified proteins were then dialyzed into the final reaction buffer.

For in vitro binding assay between GST-Rng10(751-1038) and His6-Rga7(1-320), purified proteins were dialyzed into the binding buffer (100 mM NaCl, 20 mM imidazole, 1 mM DTT, pH 7.0). GST-Rng10(751-1038) or GST control (Zhu et al., 2013) was incubated with glutathione-Sepharose beads for 2 h at 4 °C and washed 4 times with the binding buffer to remove unbound proteins. His6-Rga7(1-320) or His6-mEGFP control (Wu and Pollard, 2005) was then incubated with the beads for 2 h at 4 °C. After 5 washes...
with the binding buffer, the beads were boiled with sample buffer for 5 min. Then the samples were run on SDS-PAGE gel and detected with Coomassie Blue staining.

For in vitro binding assays between GST-Rng10(751-950/1038) and His$_6$-Rga7(1-320) mutants, GST-Rng10(751-950/1038) was first purified and dialyzed into the binding buffer (50 mM sodium phosphate, pH 7, 200 mM NaCl, 1 mM PMSF, and 1 mM DTT) and then incubated with glutathione-Sepharose beads for 1 h at 4°C. The beads were washed once, resuspended, and aliquoted in equal volume. His$_6$-Rga7(1-320) wild-type control and mutants were prepared in small scale by resuspending cells in the binding buffer and sonicating to break the cells. Cell lysates were centrifuged at 15,000 rpm for 15 min at 4°C. Rga7 proteins in the supernatant were normalized to similar protein levels and added to the aliquoted GST-Rng10 bound beads. After 1 h incubation at 4°C, beads were washed 3 times with the binding buffer, and boiled with sample buffer. Then the samples were run on SDS-PAGE gel and detected with western blotting.

For measuring the $K_d$ between Rng10(751-950/1038) and Rga7(1-320), we followed the described methods and guidelines (Lee et al., 1999; Pollard, 2010). The procedure was similar to the in vitro binding assays described in the previous paragraph except different concentrations of beads and purified GST-Rng10(751-950/1038) were incubated with the same concentration of His$_6$-Rga7(1-320) from large-scale purification. Total amount of bead bound GST-Rng10(751-950/1038) was estimated by subtracting the remaining proteins in the supernatant (including proteins released during washing) from the total input. We formed a GST-Rng10(751-950/1038) concentration gradient by adding different volumes of GST-Rng10(751-950/1038) bound beads (0, 20, 50, 80, 100, 150, 200, 250, 300, 350, and 400 μl) to each tube. The same volumes of GST bound beads were used as the control. Binding buffer and a final concentration of 1 μM His$_6$-Rga7(1-320) were added to a final volume of 500 μl for each tube. After incubation for 10 min at 4°C, the beads were spun down at 15,000 rpm for 5 min, supernatant samples removed from the reactions were boiled with sample buffer, run on an SDS-PAGE gel, and detected with Coomassie Blue staining.

**Liposome Co-pelleting Assays**

Liposomes were prepared as previously described (McDonald et al., 2015). Lipid chloroform stocks (Avanti Polar Lipids, Alabaster, AL) were mixed at the desired ratios, vacuum dried into a thin film in a glass tube, and resuspended at 1 mg/ml in 20 mM Tris, pH 8.0, 150 mM NaCl buffer with vortexing. The resulting liposome mix was freeze-thawed 10x and passed through an 800 nm liposome extruder (Avanti Polar Lipids, Alabaster, AL). 100 μl liposomes were mixed with 100 μl purified Rga7 or Rng10 protein at the indicated concentrations (or 20 μg when not indicated) and incubated for 15 min at room temperature. Bound liposomes were centrifuged at 150,000 g for 15 min and supernatant (unbound) and pellet (bound) fractions were separated and run on an SDS-PAGE for Coomassie blue detection.

Giant unilamellar vesicles were formed as previously described (McDonald and Gould, 2016a). Chloroform lipid stocks of the desired composition were evaporated on ITO-coated glass coverslides. Coverslides were assembled into a 1 mm thick chamber filled with a 20 mM HEPES, pH 7.4, 500 mM sucrose solution. A 10 Hz, 2.5 V sine current was passed across the chamber for 2 h to electroform the GUVs.

**Circular Dichroism and Structural Modeling**

The CD spectrum was collected using Jasco J815 spectropolarimeter (Easton, MD, USA) under UV spectroscopy in 1-cm quartz cuvettes at 25°C. CD scans ranged from 180 to 300 nm at a scan rate of 200 nm/min with 1 s interval. Reactions were performed in 25 mM potassium phosphate, pH 7.0 with a protein concentration of at least 10 μM. Data analyses were carried out using K2D3 algorithm to predict secondary structures (Louis-Jeune et al., 2012). The Rga7 F-BAR structure was modeled using the Phyre2 server (Kelley et al., 2015b) based on the S. cerevisiae Rgd1 crystal structure (Moravcevic et al., 2015).
detected with (1) a monoclonal anti-GFP antibody (1:2000 dilution; 11814460001; Roche, Mannheim, Germany) or a TAT1 antibody for tubulin (1:20,000 dilution; (Woods et al., 1989); and (2) a secondary antibody anti-mouse immunoglobulin G (1:5000 dilution; A4416, Sigma-Aldrich). ECL (SuperSignal Maximum Sensitivity Substrate, Thermo Fisher Scientific) was used to develop the protein bands on film (BioMax MR; Kodak, Rochester, NY).

For in vitro binding assays between GST-Rng10(751-950/1038) and His6-Rga7(1-320) wild-type control and three mutants, inputs and bound proteins were detected with monoclonal anti-His (1:20,000 dilution; 631212, Clontech) or anti-GST (1:20,000 dilution; NB600-446, Novus Biologicals) antibodies. Secondary anti-mouse fluorescent antibody (92568070, LI-COR) was used at 1:10,000 dilution. Protein bands were detected using LI-COR (Odyssey CLx) at 700 nm channel.

COS-7 cell lysates were separated on an SDS-PAGE gel and detected with a monoclonal anti-GFP primary antibody (1:1000; Roche) and anti-mouse fluorescent secondary antibody (LI-COR) before detection using a LI-COR Odyssey.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Protein molecular numbers were calculated from quantifying fluorescence intensity (Deng et al., 2014; Liu et al., 2016). Only the central ~75% of cells in the imaging field were analyzed to minimize the effects of uneven illumination. We summed intensity from 13 z sections at 0.5 μm spacing. For global protein level quantification, the polygon region of interest (ROI) tool in ImageJ was applied to trace cell boundaries. Then WT cells with no fluorescent tag were used to deduct the background. Some of the intensities were converted to concentrations and molecule numbers using a protein standard curve generated from proteins with known molecule numbers (Liu et al., 2016; Wu and Pollard, 2005). The global concentration was calculated using mean molecules and a cytoplasm volume of 27 μm³ that occupies ~30% of a total cell volume (Wu and Pollard, 2005).

To quantify protein levels on the PM at the cell side, mean intensity of the PM between the division site and the cell tips was measured at the middle z section. Background was subtracted using cytoplasm mean intensity (avoiding nucleus region).

For measuring the Kd between two proteins, intensity for each Coomassie-stained protein band was measured and background subtracted. We defined the intensity of the sample without beads as 100% and normalized others accordingly, which corresponded to unbound proteins. The percentages of bound His6-Rga7 (1-320) were calculated as a function of the GST-Rng10(751-950/1038) concentrations. Analyzed data were plotted and fitted in KaleidaGraph (Synergy Software) using the equation $y = B_{\text{max}} \left(\frac{x}{x + K_d}\right)$, where $B_{\text{max}}$ is the maximal specific binding.

Data in figures are mean ± SEM except where noted. The $P$ values in statistical analysis were calculated using a one-way ANOVA and Tukey’s post hoc analysis.
Supplemental Information

The F-BAR Domain of Rga7 Relies on a Cooperative Mechanism of Membrane Binding with a Partner Protein during Fission Yeast Cytokinesis

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Figure S1. Characterization of Rng10 Motifs That Mediate Rga7 Localization. Related to Figure 1.

(A and B) Localization of Rga7 in Rng10 N-terminal (A) and C-terminal (B) truncations.

(C) End-on views of Rga7 division-site localization in cells expressing FL Rng10 and Rng10(751-1038).

(D) Tom20-GBP recruits proteins tagged with mEGFP and mECitrine, but not tdTomato, mScarlet-I, or mCherry to mitochondria and lack of signal bleedthrough between fluorescence channels.

(E) The Rng10 C-, but not N-terminus, mislocalizes Rga7 to mitochondria by Tom20-GBP. Scale bars, 5 μm.
Figure S2. Rga7 and Rng10 In Vitro Binding Assays and Global Rga7 Concentrations in Cells with FL and Truncated Rng10. Related to Figures 1-3.

(A) SDS-PAGE gel showing purified proteins used in binding assays. Asterisks mark the predicted FL fragments.

(B) Coomassie-stained gels of supernatant depletion assays for measuring binding isotherms between Rga7(1-320) and Rng10(751-1038) or Rng10(751-950). Numbers above each lane are the concentration (µM) of GST or GST-tagged proteins in the reaction.

(C) Analytical ultracentrifugation traces of purified Rga7 F-BAR (left) and Rga7 F-BAR + GST-Rng10(751-1038) (right). The arrows below indicate species present in each peak based on molecular weight.

(D) In vitro binding of GST-Rng10(951-1038) and His6-Rga7(1-320).

(E) Global Rga7 concentrations in cells with FL and truncated Rng10. Mean ± 1 SD. *, P < 0.01; **, P < 0.001; ***, P < 0.0001 compared to FL Rng10.

(F and G) Western blots (F) and quantifications (G) of in vitro binding between GST-Rng10(751-950/1038) and His6-Rga7 F-BAR mutants.
Figure S3. Membrane Tubulation by Rga7 F-BAR, Overexpression of Rga7 F-BAR Mutants, and Restoration of GFP-Rga7(277-695) Localization to the Division Site by Rng10-GBP, Related to Figures 3 and 4.

(A) Giant unilamellar vesicle (GUV) binding assays with GFP-Rga7 F-BAR wildtype and mutants. Percentage of GUVs with tubules is shown on the right.

(B) Rga7 membrane binding is required for tubulation even in the presence of Rng10 C terminus. Wildtype GFP-Rga7 F-BAR (the 1st row) or GFP-Rga7 F-BAR constructs (wildtype and mutants) and mCherry-Rng10(751-1038) were expressed in COS-7 cells. The PM was stained with Cellmask Deep Red.

(C) Expression levels of Rga7 constructs used in (B).

(D) Micrographs showing that overexpression of Rga7 F-BAR mutants cannot restore Rga7 division-site localization.

(E) GFP-Rga7(277-695) localization to the division site is restored by Rng10-GBP-RFP. Scale bars, 10 μm (A and B), 5 μm (D and E).
Table S1. *S. pombe* strains used in this study. Related to the STAR Methods.

| Strain name | Genotype | Figure/reference |
|-------------|----------|------------------|
| JW5943      | h' rng10-mEGFP-kanMX6 rga7-tdTomato-natMX6 ade6 leu1-32 ura4-D18 | Figure 1B |
| JW6587      | rng10(1-750)-mEGFP-kanMX6 rga7-tdTomato-natMX6 ade6-M210 leu1-32 ura4-D18 | Figure 1B |
| JW8602      | kanMX6-Prng10-mECitrine-rng10(751-1038) rga7-mCherry-natMX6 ade6-M210 leu1-32 ura4-D18 | Figures 1B, 2A, S1C and S2E |
| JW8268      | kanMX6-Prng10-mECitrine-rng10(751-1038) rga7(1-320)-mScarlet-I-hphMX6 ade6-M210 leu1-32 ura4-D18 | Figures 1C and 4D |
| JW7642      | tom20-GBP-hphMX6 Prng10-mECitrine-rng10(751-950)-kanMX6 rga7-mCherry-natMX6 ade6-M210 leu1-32 ura4-D18 | Figure 1H |
| JW7616      | Prng10-mECitrine-rng10(751-950)-kanMX6 rga7-mCherry-natMX6 ade6-M210 leu1-32 ura4-D18 | Figures 2A and S2E |
| JW8600      | rng10-mEGFP-kanMX6 rga7-mCherry-natMX6 ade6-M210 leu1-32 ura4-D18 | Figures 2A, S1C and S2E |
| JW8634      | rng10(1-750)-mEGFP-kanMX6 rga7-mCherry-natMX6 ade6-M210 leu1-32 ura4-D18 | Figures 2A and S2E |
| JW8636      | kanMX6-Prng10-mECitrine-rng10(951-1038) rga7-mCherry-natMX6 ade6-M210 leu1-32 ura4-D18 | Figures 2A and S2E |
| JW5669      | h' rng10-mECitrine-kanMX6 ade6-M210 leu1-32 ura4-D18 | Figure 2E |
| JW8163      | h' kanMX6-Prng10-mECitrine-rng10(951-1038) ade6-M210 leu1-32 ura4-D18 | Figures 2E and 4D |
| JW8860      | kanMX6-Prng10-mECitrine-rng10(951-1038) rga7Δ::natMX6 ade6 leu1-32 ura4-D18 | Figure 2E |
| JW8786      | rga7(1-320)-mECitrine-kanMX6 rng10-mCherry-natMX6 ade6-M21X leu1-32 ura4-D18 | Figure 3E |
| JW8816      | rga7 F-BAR(1-320 aa; K60E/K67E/K78E/K82E/R144D/K148E)-mECitrine-kanMX6 rng10-mCherry-hphMX6 ade6-M21X leu1-32 ura4-D18 | Figure 3E |
| JW8817      | rga7 F-BAR(1-320 aa; K60E/K67E/K78E/K82E/R144D/K148E)-mECitrine-kanMX6 rng10-mCherry-natMX6 ade6-M21X leu1-32 ura4-D18 | Figure 3E |
| JW8818      | rga7 F-BAR(1-320 aa; K60E/K67E/K78E/K82E/R144D/K148E/K167E/R181D/K194E)-mECitrine-kanMX6 rng10-mCherry-natMX6 ade6-M21X leu1-32 | Figure 3E |
| Strain  | Genetic Information                                                                 | Notes                                                                 |
|---------|-------------------------------------------------------------------------------------|----------------------------------------------------------------------|
| AR783   | h+ rga7FBD-mEGFP-kanMX6 rlc1-tdTomato-natMX6 sad1-mCherry-natMX6 ade6-M21X leu1-32 ura4-D18 | Figure 3F (Arasada and Pollard, 2015)                                |
| JW7690  | h+ rga7 F-BAR(1-320 aa; R144D/K148E)-mEGFP-kanMX6 ade6-210 leu1-32 ura4-D18          | Figure 3F                                                            |
| JW7691  | h+ rga7 F-BAR(1-320 aa; K60E/K67E/K78E/K82E/R144D/K148E)-mEGFP-kanMX6 ade6-210 leu1-32 ura4-D18 | Figure 3F                                                            |
| JW7692  | h+ rga7 F-BAR(1-320 aa; K60E/K67E/K78E/K82E/R144D/K148E/K167E/R181D/K194E)-mEGFP-kanMX6 ade6-210 leu1-32 ura4-D18 | Figure 3F                                                            |
| JW3660  | h+ rga7-mEGFP-kanMX6 ade6-M210 leu1-32 ura4-D18                                      | Figures 4B and 4C                                                     |
| JW5675  | h- rng10-mEGFP-kanMX6 ade6-M210 leu1-32 ura4-D18                                     | Figures 4B and 4C                                                     |
| JW81    | h- ade6-M210 ura4-D18 leu1-32                                                       | Figures 4D and 4E                                                     |
| JW4028  | h+ rga7Δ::kanMX6 ade6-M210 leu1-32 ura4-D18                                         | Figures 4D and 4E                                                     |
| JW5670  | h+ rng10Δ::kanMX6 ade6-M210 ura4-D18 leu1-32                                        | Figure 4D                                                            |
| JW6188  | h+ rng10(1-750)-mEGFP-kanMX6 ade6-M210 leu1-32 ura4-D18                            | Figure 4D                                                            |
| JW7195  | Prng10-mECitrine-rng10(751-1038) ade6-M210 leu1-32 ura4-D18                         | Figure 4D                                                            |
| JW8225  | h+ rga7(1-320) ade6-M21X leu1-32 ura4-D18                                           | Figure 4D                                                            |
| JW8161  | h+ rng10-GBP-RFP-hphMX6 ade6-M210 leu1-32 ura4-D18                                  | Figure 4E                                                            |
| PPG13207| h+ rga7::kanMX6 GFP-rga7ΔF-BAR:leu1+ leu1-32 ura4-D18                               | Figures 4E and S3E (Martin-Garcia et al., 2014)                       |
| JW8198  | rng10-GBP-RFP-hphMX6 rga7::kanMX6 GFP-rga7ΔF-BAR:leu1+ leu1-32 ura4-D18             | Figures 4E and S3E                                                    |
| JW8601  | kanMX6-Prng10-mECitrine-rng10(201-1038) rga7-mCherry-natMX6 ade6-M210 leu1-32 ura4-D18 | Figure S1A                                                           |
| JW8877  | kanMX6-Prng10-mECitrine-rng10(451-1038) rga7-mCherry-natMX6 ade6-M210 leu1-32 ura4-D18 | Figure S1A                                                           |
| JW6585  | rga7-idTomato-natMX6 rng10(1-200)-mEGFP-kanMX6 ade6-M210 leu1-32 ura4-D18           | Figure S1B                                                           |
| JW6586  | rga7-idTomato-natMX6 rng10(1-450)-mEGFP-kanMX6 ade6- | Figure S1B                                                           |
| Strain        | Description                                                                 | Figure   |
|--------------|-----------------------------------------------------------------------------|----------|
| JW6955       | `tom20-GBP-hphMX6 rng10-3EGFP-kanMX6 ade6 leu1-32 ura4-D18`                 | S1D      |
| JW6959       | `tom20-GBP-hphMX6 rga7-tdTomato-natMX6 ade6 leu1-32 ura4-D18`               | S1D      |
| JW7636       | `tom20-GBP-hphMX6 kanMX6-Prng10-mECitrine-rng10(751-1038) ade6-M210 leu1-32 ura4-D18` | S1D      |
| JW7638       | `tom20-GBP-hphMX6 rga7-mCherry-natMX6 ade6-M210 leu1-32 ura4-D18`           | S1D      |
| JW7639       | `tom20-GBP-hphMX6 Prng10-mECitrine-rng10(751-950)-kanMX6 ade6-M210 leu1-32 ura4-D18` | S1D      |
| JW7673       | `tom20-GBP-hphMX6 rng10(1-750)-mEGFP-kanMX6 ade6-210 leu1-32 ura4-D18`      | S1D      |
| JW8498       | `tom20-GBP-hphMX6 rga7-mScarlet-I-hphMX6 ade6-M210 leu1-32 ura4-D18`        | S1D      |
| JW6960       | `tom20-GBP-hphMX6 rng10-3EGFP-kanMX6 rga7-tdTomato-natMX6 ade6 leu1-32 ura4-D18` | S1E      |
| JW7672       | `tom20-GBP-hphMX6 rng10(1-750)-mEGFP-kanMX6 rga7-tdTomato-natMX6 ade6-210 leu1-32 ura4-D18` | S1E      |
| JW8386       | `tom20-GBP-hphMX6 kanMX6-Prng10-mECitrine-rng10(751-1038) rga7-mScarlet-I-hphMX6 ade6-M210 leu1-32 ura4-D18` | S1E      |
| JW7842       | `kanMX6-P3nmt1-mECitrine-rga7 F-BAR(1-320 aa; R144D/K148E) ade6-210 leu1-32 ura4-D18` | S3D      |
| JW7843       | `kanMX6-P3nmt1-mECitrine-rga7 F-BAR(1-320 aa; K60E/K67E/K78E/K82E/R144D/K148E) ade6-210 leu1-32 ura4-D18` | S3D      |
| JW7844       | `kanMX6-P3nmt1-mECitrine-rga7 F-BAR(1-320 aa; K60E/K67E/K78E/K82E/R144D/K148E/K167E/R181D/K194E) ade6-210 leu1-32 ura4-D18` | S3D      |
| PPG9553      | `h- rga7::kanMX6 GFP-rga7::leu1+ leu1-32 ura4-D18`                         | S3E (Martin-Garcia et al., 2014) |