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Fission yeast mod5p regulates polarised growth via anchoring of tea1p at cell tips

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1. Supplementary Methods

Fluorescence intensity measurements

To measure amounts of tea1p at cell tips in live and fixed cells, average projections were constructed from confocal image stacks (for anti-tea1p staining) or from wide-field image stacks (for tea1-GFP), in all cases containing the entire cell volume. A region was drawn around the cell tip, using NIH Image 1.62, and the total tea1p signal was calculated as the mean intensity within the region multiplied by its area, after subtraction of the mean background intensity of a nearby area of comparable size. In all cases, exposures conditions within an experiment were identical; arbitrary units are shown. Numbers of examples scored are shown in Supplementary Figure legends.

To measure tea1-GFP particle intensity, the total fluorescent signal from each particle was measured and the cellular background signal subtracted.

To measure the degree of spreading of GFP-mod5 away from cell tips, single Z-sections through the middle of live cells were used. The intensity of GFP-mod5p fluorescence at cell tips vs. cell sides was determined by measuring line scans 10 pixels (0.65 μm) wide, along the long and short axes of live cells, using MetaMorph software. After background subtraction, the peak tip intensity divided by the peak side intensity was calculated (tip:side intensity ratio). A high ratio indicates highly polarised GFP-mod5, restricted to cell tips; a ratio of 1 indicates an isotropic distribution of GFP-mod5p.

60 cell tips were measured for each time point during MBC treatment, and in each mutant strain.

Characterisation of anti-mod5p antibody

Protein G-purified anti-mod5p IgG was used for immunoblotting at a dilution of 1/1000, probing Western blots of total cell lysates (50 µg/lane). mod5p migrated as a broad, diffuse band, which was absent from *mod5Δ* cells, and GFP-mod5p also migrated as a broad, diffuse band. A single unrelated cross-reacting band that comigrated with mod5p was observed in both wild-type and *mod5Δ* cells. However, *mod5Δ* mutants stained with the anti-mod5p antibody yielded only faint background staining, in contrast to the cell tip staining observed in wild-type cells (see Supplementary Figures).

2. Legends for Supplementary Tables

Supplementary Table 1: Strains used in this study

Supplementary Table 2: Oligonucleotide primers used in this study

Supplementary Table 3: Percent cells with altered morphology during polarity re-establishment on solid media. Morphological defects in *mod5Δ* mutants are significantly less than in *tea1Δ* mutants.

Supplementary Table 4: Percent cells with a curling microtubule at cell ends, at different temperatures. In all strains, including wild-type cells, branching increases with temperature. *mod5Δ* cells show more branching than wild-type cells, but significantly less than *tea1Δ* cells, consistent with the interpretation that *tea1p* function is compromised but not obliterated in *mod5Δ* cells.

3. Legends for Supplementary Figures

Supplementary Figure 1: Integrated *tea1p* immunofluorescence intensity at cell tips in wild-type and *mod5Δ* mutants during exponential growth. Average levels of *tea1p* at cell tips are reduced by approximately 50% in *mod5Δ* mutants. In a small number of *mod5Δ* mutant cells with high levels of *tea1p* away from cell tips, values are less than zero because of background subtraction.

Supplementary Figure 2: Integrated tea1p immunofluorescence intensity at cell tips during polarity re-establishment, in the presence and absence of the microtubule inhibitor MBC. In the presence of MBC, *mod5Δ* mutants show essentially no tea1p staining at cell tips, while significant levels are found in wild-type cells.

Supplementary Figure 3: Integrated tea1p-GFP intensity at cell tips in wild-type and *mod5Δ* mutants during exponential growth. The reduction in tea1p-GFP localisation at cell tips seen here in *mod5Δ* mutants is similar to the reduction of tea1p observed by immunofluorescence in Suppl. Fig. 1.

Supplementary Figure 4: Intensity of tea1p-GFP particles in wild-type and *mod5Δ* mutants during exponential growth. tea1p-GFP particles show a wide range of intensities, with a mode intensity 2-3 times higher than in wild-type cells.

Supplementary Figure 5: Characterisation of anti-mod5p antibody by immunofluorescence and immunoblotting. A. Immunofluorescent staining of *mod5Δ* cells gives no signal. B. mod5p runs as a broad diffuse band on Western blots, comigrating with an unrelated band (asterisk).

Supplementary Figure 6: Ratio of tip:side cortical GFP-mod5p in exponentially growing wild-type cells after microtubule disruption. GFP-mod5p is significantly enriched at cell tips vs. cell sides before drug treatment. After 30 min MBC treatment this is reduced approximately 3-fold, although some degree of polarisation remains. Control DMSO treatment alone has a small effect on enrichment.

Supplementary Figure 7: Integrated immunofluorescence intensity at cell tips in exponentially growing wild-type cells after 30 min MBC treatment. tea1p staining is reduced on average by approximately 50-60% after MBC treatment. Thus upon microtubule disruption with MBC, neither mod5p nor tea1p is completely delocalised.

Supplementary Figure 8: Ratio of tip:side cortical GFP-mod5p intensity in wild-type and mutant strains during exponential growth. In contrast to wild-type cells, *tea1Δ* and *tip1Δ* mutants show an almost isotropic membrane localisation of GFP-mod5p; *tea3Δ* mutants show a clear decrease in the enrichment of GFP-mod5p at cell tips, and *bud6Δ*, *pom1Δ*, and *tea2-1* mutants are only very slightly affected.

Supplementary Figure 9: Integrated tea1p immunofluorescence intensity at cell tips in wild-type and mutant strains during exponential growth. Average levels of tea1p at cell tips in *mod5Δ*, *tip1Δ*, and *tea2-1* mutants are roughly similar. The absence of signal in *tea1Δ* mutants indicates that the antibody is highly specific for tea1p

Supplementary Figure 10: Anti-tea1p immunofluorescence in wild-type and mutant strains. In *tip1Δ* and *tea2-1* mutants, the low levels of tea1p at cell tips show a relatively smooth distribution, in contrast to the more uneven distribution of tea1p at cell tips in *mod5Δ* mutants.

Supplementary Figure 11: Formal structure of a positive feedback loop in which mod5p and tea1p regulate each other's localisation. Details are presented with the model.

4. Legends for Movies

Movie 1: Movie of the cell shown in Fig. 3a.

Movie 2: Movie of the cell shown in Fig. 3b.

Movie 3: Movie of the cell shown in Fig. 3c.

Movie 4: Movie of the cell shown in Fig 3d.