Supplementary information

Supplementary Methods

Isolation of conditional lethal mutants with polarity defects

Mutants that showed an altered morphology were selected visually by fluorescence microscopy after staining with Calcofluor white, as described previously (Hirata *et al.*, 1998; Radcliffe *et al.*, 1998). The isolated round mutants were further examined for sensitivity to the protein kinase inhibitor staurosporine (1.5 μg/ml). The temperature-sensitive (ts) *pmo25* mutant was created by error-prone PCR mutagenesis as follows: The DNA fragments containing the *pmo25*$^{+}$*:GFP:kan*^{r} gene amplified by PCR from the *pmo25*$^{+}$*:GFP:kan*^{r} strain were integrated into the *pmo25*$^{+}$ locus in wild-type cells; and from the resultant kan^{r} cells, the ts *pmo25*-35 mutant was isolated at 36°C.

Immunochemical and kinase assays

Preparation of cell extracts, immunoprecipitation, immunodetection, and kinase assays were performed as previously described (Matsusaka *et al.*, 1995; Bähler and Nurse, 2001; Huang *et al.*, 2003; Wiley *et al.*, 2003). Immunoprecipitation was done by using anti-HA antibody (HA.11, BabCO), anti-GFP antibody (8362-1, Clontech), anti-Myc antibody (9E10, Calbiochem), and magnetizable beads conjugated to protein A or G (Dynabeads, DYNAL).

Microscopy techniques

For the observation of Pmo25-GFP, the cells expressing Pmo25-GFP were fixed with methanol (-20°C) for 10 min and washed three times with PEM buffer (Alfa *et al.* 1993). Fixed-cell images were collected with an Axiophot 2 MOT (ZEISS), the ApoTome sectioning system, and AxioCam MRm CCD camera; and the images were further processed with AxioVision software. For time-lapse microscopy, a 35 mm glass-bottomed culture dish (MatTek Corporation, P35G-1.5-10-C) was coated with 100 μg/ml concanavalin A. The culture of logarithmically growing cells (50 μl) was deposited in the well for a couple of minutes and then removed. The dish was filled with 3 ml of EMM medium and the cells that were attached to the bottom of the well were subjected to microscopic analysis. Live-cell images were collected with an IX70 (OLYMPUS) and DeltaVision sectioning system. Cytological techniques were performed according to Matsusaka *et al.* (1995).

In vitro binding assay

The expression plasmids for HA-Pmo25 or Myc-Nak1 proteins were generated as follow: An HA-tagged *pmo25* cDNA or Myc-tagged *nak1* cDNA was amplified from pACT2-HA-Pmo25 or pGBKT7-Myc-Nak1, respectively, by PCR. The PCR products were inserted into the *Not*I and *Xhol*I sites of the plasmid pTWIN1. The HA-Pmo25 and Myc-Nak1 proteins were synthesized using IMPACT-TWIN System (New England Biolabs). The HA-Pmo25 protein (10 μg/ml) was incubated with Myc-Nak1 protein (10

μg/ml) in Buffer A (Boudeau *et al*., 2004), which contained 50 mM Tris-HCl (pH7.5), 0.27 M Sucrose, 0.1 mM EDTA (pH8.0), 0.1%(v/v) mercaptoethanol and protease inhibitors, for 1 h at 4˚C. The mixture was incubated in 1:1000 dilution of a mouse anti-HA antibody (HA.11, Babco) and magnetizable beads conjugated to protein A (Dynabeads, DYNAL) for 1.5 h at 4˚C. The beads were washed three times with Buffer A, and the bound proteins were subjected to an immunoblot analysis.

Supplementary References

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Bähler, J. and Nurse, P. (2001) Fission yeast Pom1p kinase activity is cell cycle regulated and essential for cellular symmetry during growth and division. *EMBO J*. **20**, 1064-1073.

Matsusaka, T., Hirata, D., Yanagida, M. and Toda, T. (1995) A novel protein kinase *ssp1*$^{+}$ is required for alteration of growth polarity and actin localization in fission yeast. *EMBO J*., **14**, 3325-3338.