Supplemental Materials

Molecular Biology of the Cell

Fong et al.

Direct measurement of microtubule attachment strength to yeast centrosomes

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Running Head: MT attachment strength to centrosomes

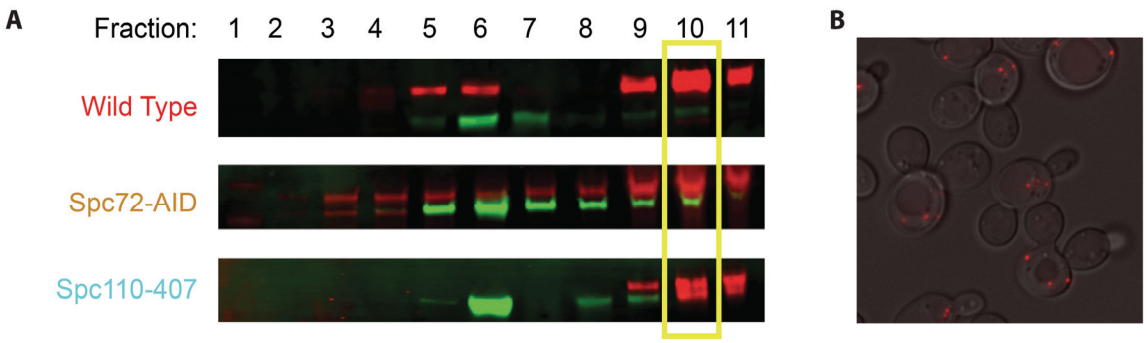
Abbreviations: SPB (spindle pole body), MT (microtubule), VE-DIC (video enhanced differential interference contrast), TAP (tandem affinity purification), AID (auxin-inducible degron)

SUPPLEMENTAL MATERIALS

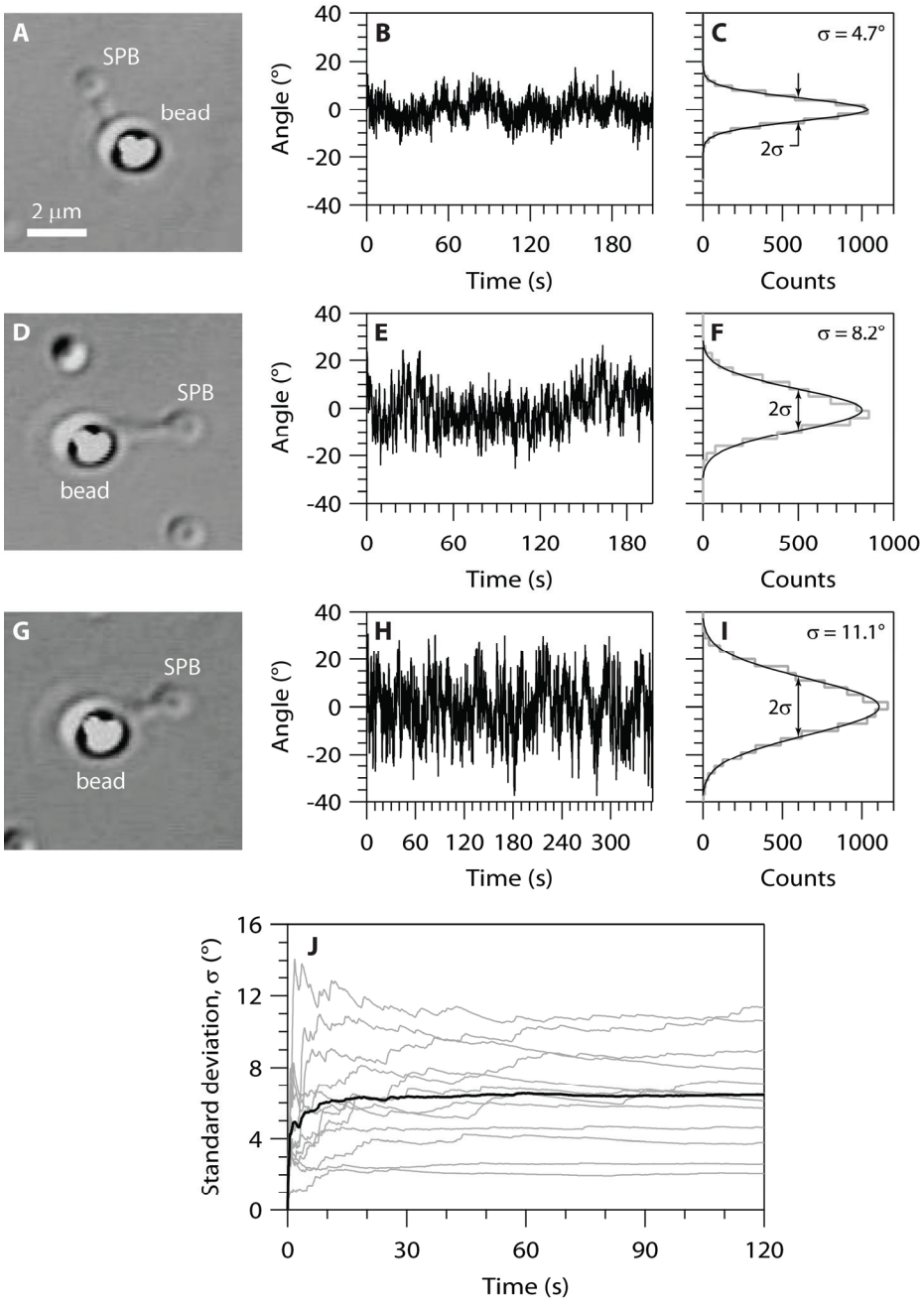
Supplemental Data File 1 | **Excel table of all individual rupture force values.** All individual force values for ruptures at the SPB-microtubule interface, failures at the bead-microtubule interface, and for events that reached the load-limit of the trap are reported in this Excel spreadsheet file.

Supplemental Movie 1 | **Polarity of a reconstituted SPB-microtubule attachment tested using kinesin motility.** A kinesin-bead bound to an SPB-attached microtubule in the presence of ATP moves away from the SPB, demonstrating that the microtubule is attached to the SPB via its minus end, as in the physiological situation.

Supplemental Movie 2 | **Rupture of an SPB-microtubule attachment.** An SPB-attached microtubule is initially placed under compression, because the laser trap (not visible) is pushing the microtubule-bound bead toward the SPB. (Due to the compressive force, the microtubule is initially buckled.) Then the direction of force is reversed and the magnitude of tension is gradually increased, until the SPB-microtubule interface ruptures.



Supplemental Figure 1 | **Validation of spindle pole body purifications.** (**A**) Western blot analysis verified the presence of spindle pole body components (Spc110 in red and Spc97 in green) after velocity sedimentation. Fractions were removed from the top of the sucrose gradient, with low sucrose (~10%) in fraction 1 and high sucrose (~55%) in fraction 11. Fraction 10, outlined in yellow, was used for spindle pole body rupture force experiments. (**B**) Fluorescence microscopy of Spc42-mCherry in Spc72-AID cells after auxin-induced degradation of Spc72 for 3 hours. The cells were multi-budded and contained multiple spindle pole bodies, consistent with the multinuclear phenotype of Spc72 mutants (Chen *et al.*, 1998; Souès and Adams, 1998).



Supplemental Figure 2 | **Microtubules pivot about their attachments to spindle pole bodies.** (**A**) Example image from a VE-DIC movie of an SPB-attached microtubule with a kinesin-coated bead attached near the plus end. The orientation of the microtubule over time was determined from positions of the bead and SPB, which were tracked at 30 Hz using custom LabView software. (**B**) Orientation versus time for the SPB-attached microtubule shown in A. (**C**) Distribution of angles for the SPB-attached microtubule shown in A (*gray histogram*), fit with a normal distribution (*black curve*; standard deviation, σ = 4.7°). (**D**, **E**, **F**) and (**G**, **H**, **I**) show two additional examples of SPB-microtubule attachments. (**J**) Evolution of the standard deviation of the angle for twelve SPB-microtubule attachments (gray curves). The standard deviation typically reached a steady value after 2 min, indicating that the microtubules pivoted about a stable mean orientation. Black line shows average of all the curves, which plateaus at 7 ± 3° (mean ± s.dev., *N* = 12 SPB-microtubule attachments). We speculate that the pivoting of SPB-attached microtubules might occur via bending or kinking of the tethering molecule, Spc110, which contains a long, rod-like central region with predicted coiled-coil structure (Kilmartin *et al.*, 1993).

	Protein coverage (%)
Bbp1	66.8
Cdc31	71.4
Cmd1	63.3
Cnm67	70.7
Kar1	62.6
Mps2	48.8
Mps3	49.0
Nbp1	52.7
Ndc1	34.7
Nud1	49.4
Sfi1	40.8
Spc29	75.9
Spc42	76.6
Spc72	57.7
Spc97	51.5
Spc98	42.2
Spc110	69.3
Tub4	46.3

Supplemental Table 1 | Mass spectrometry analysis of purified wild type spindle pole bodies verifies high coverage of all spindle pole body components. Purified spindle pole bodies were digested with trypsin and mass spectrometry was performed on a Q-Exactive (Thermo Fisher Scientific). All detectable proteins were identified by searching high-resolution MS/MS spectra against whole proteome databases using Comet (Eng *et al.*, 2013). Peptide identifications were processed with Percolator (Käll *et al.*, 2007) and MSDaPl was used to visually inspect the results (Sharma *et al.*, 2012).

SUPPLEMENTAL REFERENCES

Eng, J. K., Jahan, T. A., and Hoopmann, M. R. (2013). Comet: An open-source MS/MS sequence database search tool. Proteomics 13, 22–24.

Käll, L., Canterbury, J. D., Weston, J., Noble, W. S., and MacCoss, M. J. (2007). Semi-supervised learning for peptide identification from shotgun proteomics datasets. Nat. Methods 4, 923–925.

Sharma, V., Eng, J. K., Maccoss, M. J., and Riffle, M. (2012). A mass spectrometry proteomics data management platform. Mol. Cell. Proteomics 11, 824–831.