Kinesin-5/Cut7 C-terminal tail phosphorylation is essential for microtubule sliding force and bipolar mitotic spindle assembly

Michele H. Jones[1,2,3], Zachary R. Gergely[1,2,3], Daniel Steckhahn[1], Bojun Zhou[1], and Meredith D. Betterton[1,2,*]

[1]Department of Physics, Colorado Avenue, University of Colorado Boulder, Boulder CO 80309, USA
[2]Department of Molecular, Cellular, and Developmental Biology, Colorado Avenue, University of Colorado Boulder, Boulder CO 80309, USA
[3]Equal contribution
[*]Corresponding author and lead contact, mdb@colorado.edu

SUMMARY

Kinesin-5 motors play an essential role during mitotic spindle assembly in many organisms:[1–11] they crosslink antiparallel spindle microtubules, step toward plus ends, and slide the microtubules apart[12–17]. This activity separates the spindle poles and chromosomes. Kinesin-5s are not only plus-end-directed, but can walk or be carried toward MT minus ends[18–34] where they show enhanced localization[3,5,7,27,29,32]. The kinesin-5 C-terminal tail interacts with and regulates the motor, affecting structure, motility, and sliding force of purified kinesin-5[35–37] along with motility and spindle assembly in cells[27,38,39]. The tail contains phosphorylation sites, particularly in the conserved BimC box[6,7,40–44]. Nine mitotic phosphorylation sites were identified in the kinesin-5 motor of the fission yeast *Schizosaccharomyces pombe*[45–48], suggesting that multi-site phosphorylation may regulate kinesin-5s. Here, we show that mutating all nine sites to either alanine or glutamate causes temperature-sensitive lethality due to a failure of bipolar spindle assembly. We characterize kinesin-5 localization and sliding force in the spindle, based on Cut7-dependent microtubule minus-end protrusions in cells lacking kinesin-14 motors[39,49–52]. Imaging and computational modeling show that Cut7p simultaneously moves toward minus ends of protrusion MTs and plus ends of spindle midzone MTs. Phosphorylation mutants show dramatic decreases in protrusions and sliding force. Comparison to a model of force to create protrusions suggests that tail truncation and phosphorylation mutants decrease Cut7p sliding force similarly to tail-truncated human Eg5[36]. Our results show that C-terminal tail phosphorylation is required for kinesin-5/Cut7 sliding force and bipolar spindle assembly in fission yeast.

RESULTS AND DISCUSSION

Kinesin-5/Cut7 C-terminal tail point mutants show defects in spindle assembly and mitotic progression

Kinesin-5 motors share a common domain structure (Figure 1A), with an N-terminal motor[53], central alpha-helical coiled-coil[16], and C-terminal tail[54]. Kinesin-5s form tetramers[12–17] in which the $\sim$100-amino-acid C-terminal tails of one dimer are near the motor domains opposite[15,36] (Figure 1B). Kinesin-5s crosslink anti-parallel microtubules (MTs) in the spindle midzone and move toward MT plus ends to separate spindle poles[1–11] (Figure 1C). Kinesin-5 depletion or loss of function inhibits sliding and can block spindle assembly[5,6,8,36,38,55–59]. In addition to its essential plus-end-directed motility, kinesin-5 can also move toward MT minus ends, both *in vitro*[18–25,28] and on the spindle[18,27,32,39,60]. Yeast kinesin-5s are intrinsically bidirectional[18–20,23–27], while vertebrate kinesin-5/Eg5 is transported toward spindle poles by dynein and its cofactors[29–34]. As a result, kinesin-5s can localize near mitotic spindle poles[3,5,7,27,29,32].

Recent work points to tail-motor interactions as important to kinesin-5 regulation. Purified tail fragment and motor domain directly interact[36]. Tail truncation can confer resistance to Eg5 inhibitors in cancer cells[61], and significantly impairs MT crosslinking, sliding, and force *in vitro* for metazoan kinesin-5s[35,36]. Similarly, the tail of fission-yeast kinesin-5/Cut7[62] is required for viability, spindle assembly, and sliding force[27,39]. Tail phosphorylation has been proposed as a possible regulator of tail-motor interactions and kinesin-5 activity[63].

The tail contains multiple phosphorylation sites[11,64] (Figure 1D). The BimC box[6] contains a conserved Cdk1 site, and alanine mutants of the consensus site threonine cause a loss of metazoan kinesin-5 spindle localization[7,40,42,43]. Antibodies to the phosphorylated *Drosophila* kinesin-5 Cdk1 site are enriched in the spindle midzone[55]. The human kinesin-5/Eg5 tail contains a site phosphorylated by the NIMA-family kinase Nek6[43]. Phosphorylation by Nek6 is specific to pole-localized Eg5, and alanine mutants at this site inhibit spindle assembly but not MT binding[43]. Instead, phosphorylation of the Eg5 tail and TPX2 promotes their binding and Eg5 centrosome localization, which is important for proper centrosome separation[44]. However, a broader picture of how tail phosphorylation affects kinesin-5s is not known. Therefore, we sought to further study the effects of fission-yeast kinesin-5/Cut7 tail phosphorylation.

Two tail point mutations in the BimC box of fission-yeast *cut7* have been previously studied (Figure 1E). The *cut7-22* allele has a temperature-sensitive lethal point mutation, P1021S, adjacent to two phosphorylation sites[52,65,66]. The *cut7-T1011A* allele contains the Cdk1-site T1011A mutation. This change has no apparent defect on its own[41], but causes temperature-sensitive growth defects when a C-terminal GFP fusion is added[67]. To our knowledge, the localization, dynamics, and motility of these *cut7* alleles have not been previously characterized. Therefore, we added C-terminal GFP tags to both point mutants in strains with low-level *mCherry-atb2* to image spindle MTs[68,69] (Key Resources Table). Both *cut7-P1021S* and *cut7-T1011A* cells showed temperature-sensitive defects (Figure 1F-J), consistent with previous work[41,65–67].

Cut7-P1021S-GFP fails to assemble a bipolar spindle at restrictive temperature, despite spindle localization. Persistent monopolar spindles occurred in 87% of *cut7-P1021S* cells observed at 37°C (Figure 1F). At 25°C, kymographs showed pole-localized Cut7-P1021S-GFP with midzone movement along with qualitatively normal spindle length and elongation (Figure 1G), as previously observed for Cut7-GFP[27]. However, at 37°C monopolar spindles showed pole-localized Cut7-P1021S-GFP and no observable plus-end-directed movement (Figure 1H), in marked contrast with Cut7-GFP motility on monopolar spindles[27]. This suggests a motility defect in Cut7-P1021S-GFP. The temperature-sensitive defect was reversible: after lowering the temperature from 37°C to 25°C, the monopolar spindles became bipolar and proceeded through mitosis.

Cells containing *cut7-T1011A* showed less severe temperature-sensitive spindle defects. At 37°C, most cells were able to assemble a bipolar spindle, but this typically occurred after a transient, sometimes prolonged, monopolar state (Figure 1F). While Cut7-T1011A-GFP localization and movement were qualitatively similar to those of Cut7-GFP at 25°C (Figure 1I), at 37°C, spindle pole separation was often delayed (Figure 1J). We occasionally observed spindle length fluctuations with transient spindle shortening, or delays in anaphase B onset.

The tail truncation of *cut7* causes subtle spindle defects at permissive temperature, including shorter spindles and delays in reaching steady anaphase elongation[39]. Therefore, we measured spindle length dynamics in the tail point mutants (Figure 1K-N). Both mutants showed shorter pre-anaphase spindle length (Figure 1K-L). The time after anaphase onset to reach steady elongation was not significantly different (Figure 1M). However, the anaphase spindle elongation speed showed significant differences: it was faster in *cut7-P1021S* and slower in *cut7-T1011A* compared to *cut7* (Figure 1N). This suggests that *cut7-P1021S* may have a time-dependent changes in sliding force. Cut7p containing either BimC box point mutant can localize to the spindle, but shows spindle defects that suggest problems with Cut7p motility and sliding force.

Cut7 multi-site tail phosphorylation mutants have growth and spindle defects

While most previous work on kinesin-5 tail phosphorylation has focused on single sites[7,29,40,41,43,67], the tail hotspot of kinesin-5/Cut7 contains multiple phosphorylation sites (Figure 1D). These nine sites were identified by mass spectrometry screens in *S. pombe*[45–48]. T1020, T1022, and T1024 phosphorylation was identified in mitotically arrested cells.[45] Phosphorylation at sites T1007, T1008, and T1011 decreased by a factor of 3-4 after Cdk1 inhibitor treatment[48]. Sites S1027 and S1028 showed decreased phosphorylation when the Aurora or DYRK/Pom1 kinases were inhibited[46,47]. Phosphorylation over time has been measured for the Cdk1 site T1011, which increased at the start of mitosis to over 90% phosphorylated, followed by a slow decrease[48]. Many of these sites are conserved in other organisms as phosphorylatable sites (S/T) or negatively charged residues (D/E), or have adjacent phosphorylatable sites (Figure 1D), supporting their potential importance in regulation of kinesin-5 activity.

We constructed mutants of all nine sites to alanine (phospho-deficient, *cut7-9A*) or aspartate (phospho-mimetic, *cut7-9D*, Figure 2A) with a C-terminal GFP fusion and low-level *mCherry-atb2*. We also made the same mutants with tail truncation beyond amino acid 1030[39] (Figure 2A). These phosphorylation mutant strains grew at 25°C, but showed temperature-sensitive lethality or growth defects at 37°C (Figure 2B). While *cut7-1030-9A* was temperature-sensitive lethal, *cut7-1030-9D* showed some growth at restrictive temperature. These growth defects correlated with spindle assembly failure: at 37°C, phosphorylation mutants showed predominantly monopolar spindles (Figure 2C-E). The ts-lethal alleles *cut7-9A*, *cut7-9D*, and *cut7-1030-9A* had $>$90% monopolar spindles, while *cut7-1030-9D* that showed some growth at 37°C had only $\sim$30% monopolar spindles (Figure 2F), showing a correlation with greater cell growth in *cut7-1030-9D* (Figure 2B). Therefore, tail truncation at a.a. 1030 partially rescues the phospho-mimetic mutant for unknown reasons. These results show that proper tail phosphorylation is required for bipolar spindle assembly and growth at restrictive temperature.

At permissive temperature, cells with phosphorylation mutants grew and formed bipolar spindles. Microscopy observations suggested that mutants had shorter spindles (Figure 2G-I), and quantification showed that pre-anaphase length of bipolar spindles decreased significantly in the phosphorylation mutants (Figure 2J,K). The time from anaphase onset to reach steady-state spindle elongation (Figure 2L) and anaphase spindle elongation speed (Figure 2M) showed fewer significant alterations. Therefore, Cut7 tail phosphorylation is required for normal spindle length at permissive temperature. These spindle defects occurred despite Cut7-GFP spindle localization and movement. Motor localization to the spindle and movement were qualitatively similar in all strains (Figure S1A-L). The phospho-deficient cells had similar or slightly higher total Cut7 intensity on the spindle relative to Cut7-GFP, while the phospho-mimetic mutants showed slightly lower total intensity (Figure S1M). This could occur if reduced negative charge of the phospho-ablated mutants increases their binding to negatively charged MTs, while increased negative charge of phospho-mimetic mutants lowers binding. Our observations of spindle localization in phosphorylation mutants were similar to previous work in fission yeast showing that single- or double-site alanine mutants localized to the spindle[41], but in contrast to metazoan tail Cdk-site alanine mutants, which lose spindle localization[7,40,42,43].

Because the amount of Cut7-GFP at the midzone relative to the pole can affect sliding force[27] and decreases in tail truncation mutants[39], we quantified the midzone-to-pole intensity ratio. This ratio was higher for phosphorylation mutants on 2-4 μm-long spindles (Figure S1N). This might occur because phosphorylation mutants had shorter spindles at anaphase onset (Figure 2J,K). We therefore compared midzone-to-pole ratio as a function of time relative to anaphase onset, and found similar profiles between all strains (Figure S1O). The similar midzone-to-pole ratio in phosphorylation mutants is consistent with our previous work demonstrating that the amino acids N-terminal to the phosphorylation sites (a.a. 988-1006) have the most significant effect on midzone localization[39]. In previous work, we found that Cut7p activity jumps at anaphase onset, with increases both in observable movement and direction switching[27]. While we speculated that this activity change could occur due to phosphorylation, the similar midzone-to-pole ratio in the phosphorylation mutants found here argues against tail site phosphorylation as a driver of the localization changes at anaphase onset (Figure S1O). Together, our results suggest the temperature-sensitive lethality of the phosphorylation mutants is not due to a defect in spindle localization.

Cut7-GFP accumulates at protrusion tips via minus end-directed motility and confinement by the nuclear envelope

Because Cut7 is essential for spindle assembly, its loss of function leads to monopolar spindles (Figure 1C). However, if kinesin-14/Pkl1 (or its binding partners Msd1 and Wdr8) is absent, Cut7 is no longer essential[52,70], allowing study of otherwise lethal *cut7* alleles[27,39,52]. In this background, MT minus-ends can detach from the spindle pole bodies (SPBs), and be driven by Cut7p sliding to protrude into the nuclear envelope[49–52] (Figure 3A). We hypothesized that protrusions can be used to assess Cut7-GFP directional movement, based on the intriguing observation that Cut7-GFP is frequently visible as a spot at the tip of the protruding MT(s)[39,50,51] (Figure 3B-H, S2). Since MTs in Cut7-dependent protrusions have their minus-ends distal to the spindle[49,50], this accumulation seemed likely to occur as the result of minus-end-directed motility.

We considered two possibilities for protrusion-tip localization: direct Cut7-GFP binding to MT minus ends, or minus-end-directed movement along the protruding MT to its end. In imaging of protrusion dynamics, Cut7-GFP at protrusion tips often became brighter over time (Figure 3B,C, S2) and was sometimes visible between the SPB and the protrusion end on the spindle (Figure 3C, S2D). The tubulin signal along the protrusion was consistently dimmer than in the spindle, showing that relatively few MTs are in the protrusion, consistent with previous work[39,49–52] (Figure 3D, S2). EM analysis identified protrusions with 3 MTs[49], while a fission-yeast half-spindle has $\sim$14 MTs attached to a single SPB[71,72]. Cut7-GFP signal at protrusion tips was often brighter than at the neighboring SPB (Figure 3C,D, S3), which would be surprising if Cut7-GFP accumulation occurred solely by direct binding to MT minus ends.

These results suggested that Cut7-GFP moves to protrusion tips by minus-end-directed motility (Figure 3E). In this picture, Cut7-GFP localized near the SPBs could have one motor head bind to an MT in a protrusion. If the other head unbinds, the motor can step toward the MT minus end and move to the protrusion tip. Over time, this Cut7-GFP accumulates at the protrusion tip while the signal at the SPB decreases. To test whether this mechanism can explain our observations, we photobleached Cut7-GFP in protrusions and monitored fluorescence recovery after photobleaching (FRAP, Figure 3F-H). We then observed Cut7-GFP spots moving toward the protrusion tip, visible in kymographs as dim streaks of Cut7-GFP fluorescence moving at $\sim$20 nm/s (Figure 3G,H), similar to previous measurements[27].

We further tested this mechanism in a computational model of Cut7p movement on protrusions (Figure 3I). We fixed our simulated bipolar spindle length and nuclear diameter at 2 μm, then created a protrusion with a prescribed number of MTs that extends over time. After initialization of Cut7 binding, the protrusion MT(s) moved outward beyond the SPB at speed 10 nm/s, determined from the average protrusion growth speed we observed (Figure S3A,B). As the protruding MT(s) extended, a cylindrical nuclear-envelope tube of specified radius increased in length to accommodate the protrusion (Methods, Table S1). A model with purely minus-end-directed motors led to Cut7 accumulation at SPBs and protrusion tips (Figure 3J,L). When Cut7 was modeled with purely plus-end-directed motility, it accumulated in the center of the spindle and not at protrusion tips (Figure 3K,L). This suggests that Cut7p minus-end-directed movement leads to its accumulation at protrusion tips, consistent with our conceptual model.

We used the computational model to determine what mechanisms control the extent of Cut7-GFP accumulation at protrusion tips. Because we observed a range in Cut7-GFP brightness at the protrusion tip (Figure 3B-D, S2), we simulated protrusions with 1, 2, or 3 MTs[49]. The model with 1 protrusion MT showed dim signal at the protrusion tip (Figure 3M), while 3 protrusion MTs had a bright protrusion tip and dim SPB (Figure 3N). We measured the fraction of Cut7 localized within 0.5 μm of the protrusion tip (Figure 3J) and found a linear increase with number of protrusion MTs (Figure 3O). Therefore, varying protrusion MT number could tune Cut7-GFP brightness at protrusion tips. In addition, we observed in simulations that Cut7 that unbinds from a protrusion MT diffuses freely, but then typically re-binds due to confinement by the nuclear envelope. To test the effects of confinement, we varied the protrusion radius in the model. Fission yeast nuclear MT bundles had radius 50 nm[73] and a 3-MT protruding bundle had a 70 nm radius[49]. Therefore, we considered 50-70 nm as a typical protrusion radius. The fraction of Cut7 localized at the protrusion tip decreased as the protrusion radius increased, dropping to less than 1 percent for a 1 μm protrusion that is the same width as the nucleus (Figure 3P). Thus, confinement by the nuclear envelope increases the effective Cut7 processivity to promote accumulation on protrusions.

Our data and modeling are consistent with minus-end-directed Cut7-GFP motility driving accumulation at protrusion tips. Interestingly, at the same time, protrusions form due to sliding force from *plus-end*-directed Cut7p crosslinking antiparallel MTs in the spindle midzone[39,50,51]. Therefore, our results extend previous observations of Cut7p bidirectionality and minus-end directed movement *in vitro*[23–25] and on the fission-yeast spindle[27,74]. It remains unclear what regulates Cut7p on the spindle to move toward MT minus ends in protrusions and toward MT plus ends in the spindle midzone. One possibility is minus-end bias on single MTs or when crosslinking parallel MTs, and plus-end bias when crosslinking antiparallel MTs, as has been observed for budding yeast Cin8[18].

Phosphorylation of the C-terminal tail is required for Cut7 sliding force to generate protrusions

Protrusion formation depends on Cut7p sliding force[50–52]: protrusions do not form when Cut7 is deleted[50,51], and are independent of the other fission-yeast spindle-elongation motor, kinesin-6/Klp9[50]. Our recent results suggest that protrusions can vary as a read-out of Cut7 sliding force: the fraction of cells with protrusions decreased sequentially with increasing *cut7* tail truncation, dropping to zero when the BimC motif was deleted[39]. Tail truncation past the BimC motif also led to lethality in *pkl1+* cells due to spindle assembly failure. Therefore, Cut7 motors with insufficient force to generate protrusions (in *pkl1*Δ cells) also have insufficient force to assemble the spindle (in *pkl1+* cells).

Because protrusions in tail truncation mutants require the BimC box, we hypothesized that phosphorylation site mutants might also affect protrusions. We constructed *cut7-1030*, *cut7-1030-9A*, and *cut7-1030-9D* alleles in the *pkl1*Δ *klp2*Δ background with low-level *mCherry-atb2*. All strains grew similarly and were viable at 37°C, although the 1030-9A strain showed slight growth inhibition (Figure 4A). This shows that deletion of kinesin-14s can rescue phosphorylation mutant ts-lethality, as seen previously with other *cut7* mutations[39,52,65]. In live-cell imaging at 37°C, monopolar spindles were frequent in these strains despite their improved growth: all *cut7-1030-9A* cells and ~65% of *cut7-1030-9D* cells contained persistent or transient monopolar spindles (Figure 4B). These results are consistent with our observations of more severe defects in the alanine mutants (Figure 2). Consistent with the growth and spindle defects, the phosphorylation mutants showed few or no protrusions at 25°C (Figure 4C-E). Strikingly, only ~5% of *cut7-1030-9A* and no *cut7-1030-9D* cells showed protrusions, compared to >50% of *cut7-1030* and *cut7-FL* phospho-wild type cells (Figure 4F). This shows that proper tail phosphorylation is required for Cut7p sliding force.

Formation or elongation of protrusions was sometimes followed by spindle shortening (Figure 3D, S2). This could occur for two reasons: movement of Cut7p onto protrusions could deplete crosslinking Cut7p in the midzone that elongates the spindle, and/or the inward force on the spindle from nuclear envelope deformation during protrusion extension might be large enough to affect spindle length. The protrusion growth speed of 10 nm/s (Figure S3A,B) is typically slower than Cut7-GFP movement speed of 20 nm/s[39], suggesting that protrusion growth occurs against a hindering force. To characterize the dynamics and force balance underlying protrusion growth and shrinkage, we measured protrusion length dynamics for prototypical examples (Figure 4G) and developed a force-balance model of protrusion length dynamics. We assumed that a protruding MT has its minus end outward and plus end overlapping spindle MTs from the opposite SPB. In the antiparallel overlap region, plus-end-directed Cut7p drives outward sliding, while nuclear envelope distortion creates an inward force and friction resists any movement (Figure 4H). This leads to a protrusion velocity $v_{\text{slide}} = (f_{\text{slide}} L_{\text{overlap}} - F_{\text{NE}})/\gamma$, where f_{slide} is the force per unit length of Cut7 sliding, L_{overlap} is the overlap length of the protrusion with antiparallel MTs in the spindle, F_{NE} is the inward nuclear envelope force, and γ is the drag coefficient (Methods). Dynamic instability changes protrusion length: MT polymerization at the plus end increases L_{overlap}, causing an increase in outward sliding force, and the protrusion grows; conversely, depolymerization shortens the MT and the protrusion.

Our comparison of model and experimental results was best explained by sliding of dynamic protrusion MTs. A model with steady MT growth up to a maximum length resembled some experimental observations (Figure 4I). We then considered how to model protrusions that shrink rapidly, then grow again (Figure 4G). These protrusions sometimes tilted relative to the spindle axis prior to shortening (Figure S3C), suggesting that the protrusion MT may have extended until it has little or no crosslinking to spindle MTs. We modeled this loss of connection to the spindle zone of antiparallel overlap by setting the midzone sliding force to zero for a period of time. This leads to modeled protrusion length with rapid shortening (Figure 4I). Finally, to test the effects that phosphorylation mutants may have on sliding force, we considered a model in which the Cut7 sliding force was lowered by a factor of 5, comparable to the factor of 8 reduction in force measured for the human Eg5 tail truncation mutant[36]. With low sliding force, the protrusion never significantly extended due to the opposing force from the nuclear envelope (Figure 4I). Therefore, both our experimental and model results suggest that phosphorylation of the C-terminal tail has a profound effect on Cut7p sliding force, because mutating these nine sites has a similar effect on protrusions as deleting the entire tail to beyond the BimC motif (79 amino acids)[39].

Previous work and our results suggest two possible models for the effects of tail phosphorylation on kinesin-5 function. The tail directly interacts with the motor domain, and tail truncation leads to defects in MT crosslinking, sliding, force, spindle assembly and protrusions[27,35,36,39,63]. Given the similar phenotypes of our phosphorylation mutants to the tail truncation, we support the previous proposal that phosphorylation modulates tail-motor interactions[63]. Effects of changing tail-motor interactions could occur within a single kinesin-5 tetramer or between motors (Figure 4J,K). Phosphorylation mutants might disrupt tail-motor interactions intra-tetramer, leading to low force of single motors similar to the tail truncation. In the intra-tetramer picture, proper phosphorylation leads to tail-motor binding and higher sliding force of individual motors (Figure 4J). In the inter-tetramer model previously proposed[63], the important tail-motor interaction for sliding force occurs between tetramers to promote clustering. This may be disrupted in phosphorylation mutants (Figure 4K).

In summary, we studied phosphorylation mutants of 9 kinesin-5/Cut7 C-terminal tail phosphorylation sites. These mutations cause more severe phenotypes than single-site mutants previously studied, suggesting that multi-site phosphorylation is important to regulate Cut7. The growth defects in phosphorylation mutants occur due to a failure of bipolar spindle assembly, despite Cut7p spindle localization and similar midzone-to-pole ratio as wildtype versions of Cut7p. At permissive temperature, phosphorylation mutants show spindle length and elongation defects consistent with decreased sliding force. The 9A mutants show more severe phenotypes than the 9D in the context of the partial tail truncation. This suggests that substitution of alanine with aspartate can partially but not completely rescue the defects. This may occur because aspartate does not provide the full negative charge necessary to mimic phosphorylation, or because dynamic phosphorylation during the cell cycle is important to Cut7p function. In the kinesin-14 deletion background, Cut7p moves to protrusion tips via minus-end-directed motility. Phosphorylation mutants show a striking decrease in protrusions in the kinesin-14 deletion background, and comparison to a computational model suggests that they have a significant decrease in sliding force. These results show that multi-site tail phosphorylation is required for proper spindle assembly and sliding force by kinesin-5/Cut7.

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Author contributions

Conceptualization, M.H.J., Z.R.G., and M.D.B.; Methodology, M.H.J., Z.R.G., D.S., B.Z., and M.D.B.; Software, D.S., B.Z., and M.D.B.; Validation, M.H.J., Z.R.G., D.S., and B.Z.; Formal Analysis, Z.R.G., D.S., B.Z., and M.D.B.; Investigation, M.H.J. and Z.R.G.; Resources, M.D.B.; Data Curation, Z.R.G., D.S., and B.Z.; Writing - Original Draft, M.H.J., Z.R.G., D.S., B.Z., and M.D.B.; Writing - Review & Editing, M.H.J., Z.R.G., D.S., B.Z., and M.D.B.; Visualization, M.H.J., Z.R.G., D.S., B.Z., and M.D.B.; Supervision, M.H.J., Z.R.G., and M.D.B.; Project Administration, M.H.J., Z.R.G., and M.D.B.; Funding Acquisition, M.D.B.

Declaration of interests

The authors declare no competing interests.

MAIN FIGURE TITLES AND LEGENDS

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Figure 1: **Overview of kinesin-5/Cut7 domains, function, tail sequence, and BimC box point mutants.**
(A) Cut7 domains. (B) Schematic of Cut7p tetramer. (C) Schematic of Cut7p function in spindle assembly. Upper, Cut7p-driven spindle pole separation. Lower, failure of spindle pole separation in the absence of Cut7. (D) ClustalW sequence alignment of kinesin-5 C-terminal tails near the BimC box, adapted from previous work[43] with data from multiple studies[6,7,29,40,41,45–48,66,67]. The organisms, genes, and tail amino acids are *Schizosaccaharomyces pombe* Cut7 964-1085, *Homo sapiens* Eg5 880-1056, *Mus musculus* Eg5 879-1052, *Xenopus laevis* Eg5 891-1067, *Drosophila melanogaster* KLP61F 887-1066, *Aspergillus nidulas* BimC 959-1184, and *Saccharomyces cerevisiae* KIP1 932-111 and CIN8 915-1038. BimC motif, bracket; conserved residues, purple; negatively charged residues, blue; phosphorylated residues, bright pink; phosphorylatable residues, pink; Cdk consensus motif, small box; *S. pombe* temperature-sensitive mutations, cyan. (E) BimC box sequence showing sites of two previously studied point mutations[41,66,67] (cyan). (F) Fraction of cells grown at 37°C with bipolar spindles (green), delayed bipolar spindles (yellow), or persistent monopolar spindles (red) for 78 *cut7-P1021S* and 32 *cut7-T1011A* cells. (G-J) Kymographs of Cut7-GFP on the spindle. Bright regions are SPB-localized motor and dim streaks show moving clusters. Scale bars, 1 μm horizontal, 60 sec vertical. (G-H) Cut7-P1021S-GFP on bipolar spindle at 25°C (G) and monopolar spindle at 37°C (H). (I-J) Cut7-T1011A-GFP on bipolar spindle at 25°C (I) and on monopolar spindle that becomes bipolar (magenta arrow) at 37°C (J). (K-N) Spindle length dynamics. Spindle length as a function of time was averaged for 9 *cut7-FL*, 7 *cut7-P1021S*, and 8 *cut7-T1011A* cells. We defined $t = 0$ as the time of anaphase onset to align the curves (dashed vertical line, Methods). Time at which steady anaphase elongation speed was reached marked on each curve by the vertical bar. (L) Pre-anaphase spindle length, (M) the time between anaphase onset and start of steady elongation, and (N) anaphase spindle elongation speed. Error bars in L-N are standard error of the mean. Statistical significance was assessed by 1-way ANOVA (L-N) and multiple-comparison testing using Fisher's least-significant-difference procedure. Ns: not significant, *: $p < 0.05$, **: $p < 0.01$, *****: $p < 10^{-5}$, *******: $p < 10^{-20}$.

Figure 2: **Mutation of multiple Cut7 BimC box phosphorylation sites compromises cell viability and spindle assembly and function.**
(A) Schematic of phosphorylation mutants aligned with the BimC box region amino acid sequence (upper), showing phosphorylation sites (pink). Each allele is labeled with retained or altered amino acids (T, threonine; S, serine; A alanine; D, aspartate), amino acids retained (gray bar and numbers), C-terminal GFP (green bar) (B) Serial dilution assays of cells grown at 25°C (upper) and 37°C (lower). (C-E) Fluorescence images showing mCherry-atb2 spindle MTs (red, left), Cut7-GFP (green, center), and merge (right) for cells grown and imaged at 37°C, displayed as maximum-intensity projections of confocal sections. (C) Cut7-GFP on bipolar spindle. (D,E) Cut7-9A-GFP and Cut7-9D-GFP on monopolar spindles. Scale bar, 1 μm. (F) Fraction of cells grown at 37°C that formed bipolar spindles (green), monopolar spindles that became bipolar (yellow), or persistent monopolar spindles (red) for 23 *cut7-FL*, 23 *cut7-9A*, 23 *cut7-9D*, 23 *cut7-1030*, 23 *cut7-1030-9A*, and 16 *cut7-1030-9D* cells. (G-I) Fluorescence images showing mCherry-atb2 spindle MTs (red, left), Cut7-GFP (green, center), and merge (right) for cells grown and imaged at 25°C, displayed as maximum-intensity projections of confocal sections showing bipolar spindles with (G) Cut7-GFP, (H) Cut7-9A-GFP, and (I) Cut7-9D-GFP. Scale bar, 1 μm. (J-M) Spindle length dynamics. Spindle length as a function of time was averaged for 9 *cut7-FL*, 9 *cut7-9A*, 7 *cut7-9D*, 11 *cut7-1030*, 9 *cut7-1030-9A*, and 11 *cut7-1030-9D* cells. We defined $t = 0$ as the time of anaphase onset to align the curves (dashed vertical line, Methods). Time at which steady anaphase elongation speed was reached marked on each curve by the vertical bar. (K) Pre-anaphase spindle length, (L) the time between anaphase onset and start of steady elongation, and (M) anaphase spindle elongation speed. Error bars in J-M are standard error of the mean. Statistical significance was assessed by 1-way ANOVA (H-J) and multiple-comparison testing using Fisher's least-significant-difference procedure. Significance denoted ns: not significant, *: $p < 0.05$, **: $p < 0.01$, *****: $p < 10^{-5}$, *******: $p < 10^{-20}$. In H all comparisons were statistically significantly different with $p < 10^{-20}$, with the exception of the nonsignificant pairs labeled. See also Figure S1.

Figure 3: **Comparison of experimental and computational modeling results suggests that Cut7-GFP accumulates at protrusion tips via minus-end-directed motility.**
(A) Schematic of spindle assembly in the kinesin-14/Pkl1 deletion background and Cut7-driven protrusions of spindle MTs beyond the SPBs (right). MTs, red; Cut7p, green; SPBs, grey. (B-C) Fluorescence image time series showing mCherry-atb2 spindle MTs (red, upper), Cut7-GFP (green, center), and merge (lower), displayed as maximum-intensity projections of confocal sections. Cut7-1-1032-GFP shown in B, Cut7-GFP in C. Time between images 17.3 sec in B and 8.9 sec in C. Scale bar, 1 μm. (D) Kymograph of spindle shortening and Cut7-GFP relocalization during protrusion formation showing mCherry-atb2 spindle MTs (red), Cut7-GFP (green), SPB position (white dots), protrusion formation (right pointing arrow), spindle shortening (left pointing arrow). Scale bars, 1 μm horizontal, 60 sec vertical. (E) Schematic of proposed mechanism of Cut7-GFP accumulation at protrusion tips. Motors near SPB step toward protrusion MT minus ends and accumulate. (F-H) Images and kymographs with Sid4-mCherry (SPB marker, red) and Cut7-GFP (green). Cut7-GFP at the protrusion tip was bleached at the time indicated by the lightning bolt symbol. (F) Image sequence. Scale bar, 1 μm. Images 9.7 seconds apart. (G,H) Kymographs. Scale bars, 1 μm horizontal, 60s vertical. (I) Schematic of computational model of Cut7 movement on dynamic protrusions, with parameters Cut7 speed v_{s}, unbound diffusion coefficient D_{u}, protrusion radius R_{pro}, and protrusion growth speed v_{pg}. (J,K) Simulated fluorescence images (left) and schematic of motor distribution from simulations (right) for minus-end-directed (J) and plus-end-directed (K) Cut7. Scale bars, 1 μm. (L) Cut7 distribution along the spindle axis for simulations shown in G and H. (M, N) Simulated fluorescence image time series showing spindle MTs (red) and Cut7 (green) for protrusions with one (M) and 3 (N) MTs. Time between images is 16.7 sec, scale bars, 1 μm. (O) Fraction of Cut7 at protrusion tip from simulations with varying number of MTs in protrusion. (P) Fraction of Cut7 at protrusion tip from simulations with varying protrusion radius. Error bars in O, P are standard error of the mean. See also Figure S2, Table S1.

Figure 4: **Spindle MT minus-end protrusions are largely absent in phosphorylation mutants, suggesting reduced sliding force.**
(A) Serial dilution assays of cells grown at 25°C (upper) and 37°C (lower). (B) Fraction of cells grown at 37°C that formed bipolar spindles (green), monopolar spindles that became bipolar (yellow), or persistent monopolar spindles (red) for 7 *cut7-FL*, 9 *cut7-1030*, 23 *cut7-1030-9A*, and 23 *cut7-1030-9D* cells. (C-E) Fluorescence image time series taken at 25°C showing mCherry-atb2 spindle MTs (red, upper), Cut7-GFP (green, center), and merge (lower), displayed as maximum-intensity projections of confocal sections. Cut7-1030-GFP shown in C, Cut7-1030-9A-GFP in D, Cut7-1030-9D-GFP in E. Time between successive images 45.3s in C, 17.9s in D, and 18.3s in E. Scale bar, 1 μm. (F) Fraction of cells with visible protrusions. Data from 75 *cut7-FL*, 39 *cut7-1030*, 39 *cut7-1030-9A*, and 28 *cut7-1030-9D* cells. Error bars are are estimated from Poisson counting statistics. (G) The length of experimental protrusions over time. (H) Schematic of model of protrusion length dynamics. (I) Protrusion length as a function of time in protrusion model. (J,K) Schematics of possible models of phosphorylation effects on kinesin-5 sliding activity. (J) Intra-tetramer interaction model. Left, alanine mutation lowers tail-motor interactions within a motor tetramer, causing alanine mutants to produce lower force similar to tail truncation mutants. Right, phosphorylation promotes tail-motor interactions that increase motor force. (K) Inter-tetramer interaction model. Left, alanine mutation lowers tail bridging between motor tetramers and cluster formation, leading to decreased sliding force. Right, phosphorylation promotes tail interaction with motor domains of adjacent tetramers, leading to increased clustering and sliding force. See also Figure S3, Table S2.

STAR METHODS

Key Resources Table

See Word Document

Resource Availability

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Meredith Betterton (mdb@colorado.edu).

Materials availability

Yeast strains and bacterial plasmids generated in this study are available upon request from the Betterton Lab.

Data and code availability

- All microscopy data and analysis reported in this paper are available at the Center for Research Data & Digital Scholarship Petalibrary: (DOI in process)
- All original code has been deposited in GitHub repositories and is publically available as of the date of publication. URLs are cited in the text and listed in the key resources table.
- Additional information required to reanalyze the data will be supplied upon request to the lead contact.

Experimental Model and Subject Details

Schizosaccharomyces pombe (fission yeast) was the experimental model for this study. Cell stocks were maintained using standard techniques on YE5S (Yeast Extract plus 5 Supplements) nutritionally rich media.[75] Cells were maintained at 25°C for standard growth. In preparation for microscopy cells were placed at either 25°C for general imaging or 37°C to test temperature sensitivity.

Method Details

Reagents and techniques

We purchased oligonucleotide primers from Integrated DNA Technologies (Coralville, IA) and restriction enzymes and Phusion HF DNA polymerase from New England Biolabs (Ipswich, MA). DNA was prepared using Qiaprep Spin Miniprep Kit and polymerase chain reaction (PCR) products were purified using Qiaquick PCR Purification Kit, both from Qiagen (Germantown, MD). Fission yeast genomic DNA was prepared using the YeaStar Genomic DNA Kit from Zymo Research (Irvine, CA). DNA sequencing was performed by Quintarabio (Hayward, CA). We determined DNA concentration using a Thermo Scientific Nanodrop 2000. Strains were verified by PCR and sequence analysis of genomic DNA compared to the *S. pombe* sequence[76]. At least two different transformant strains were analyzed in experiments, and cells were cultured using standard techniques[75]. Growth phenotypes were analyzed after 3-4 days on YE5S plates with 5-fold serial dilution starting with 5 μL of culture at 0.1 optical density.

Strain construction for strains containing Cut7-GFP, 1030 truncation phosphorylation mutants, Cut7-22-GFP, and Cut7-T1011A-GFP

In the full-length (FL) and 1030 truncated (1-1030) *cut7* genes, we added four silent restriction sites for ease of cloning. The 1030 phosphorylation mutations were constructed starting with a modified pFA6a-GFP(S65T)-kanmx plasmid containing *cut7* sequences (bp2810-bp3090) in the BamH1-Pac1 region. This insert contained either wild type sequence with silent restriction sites (pMB50-Cut7-1030), or the 9A (pMB51-9A) or 9D (pMB52-9D) mutations described above. PCR products were made from these plasmids and used to transform either yMB1084 (*pkl1+ klp2+*) yielding strains yMB1181 (1-1030), yMB1183 (1-1030-9A) and yMB1185 (1-1030-9D)or yMB1147 (*pkl1*Δ *klp2*Δ) yielding strains yMB1187 (1-1030), yMB1189 (1-1030-9A) and yMB1192 (1-1030-9D). Two gift strains from Yoshi Watanabe (University of Tokyo, Yayoi, Tokyo) were crossed to obtain cut7-T1011A-GFP, yMB1028. A gift *cut7-22* strain from Janet Paluh (Suny Polytechnic Institute, Utica, NY), was transformed with a PCR product using pFA6a-GFP-kanmx and then crossed to a strain containing *mCherry-atb2* to produce strain yMB1097. Crosses were completed using the random spore analysis method described previously[27] where genes were identified using Edinburgh minimal medium (MP Biomedicals, Solon, OH) plates without the relevant supplement for auxotrophic mutants or YE5S plates with the relevant antibiotic. The fluorescent label for MTs was obtained by expressing an mCherry–α-tubulin chimera at a low level ($\sim$10 % of wild-type α-tubulin). This low-level labeling reduces possible tag-related perturbations to MT dynamics. Cells with kinesin-5 mutation are susceptible to chromosome missegregation and other mitotic abnormalities. To avoid problems resulting from these abnormalities, cells for each experiment were stored at 4℃ and reisolated from frozen stocks every 2-4 weeks.

Strain construction for strains containing full length cut7 alleles, silent restriction sites (FL), 9A or 9D mutations, or sid4-mCherry markers

Plasmid pMB23 was made in a Bluescript backbone (gift of Wayne Wahls, University of Arkansas for Medical Sciences, Little Rock, AR) and contains *cut7* coding sequence from Bgl2 (bp1835) to the C-terminus (bp3255) and downstream sequences through *his1*. Synthetic *cut7* alleles (Genscript) were inserted into pMB23, from Bcl1 (bp2356) to BsrG1 (bp3361) containing either wild type sequence with four silent restriction sites (SS, pMB31-FL), 9 alanine substitutions (pMB34, AA1007, 1008, 1011, 1013, 1020, 1022, 1024, 1027, 1028), or 9 Aspartate substitutions (pMB40, same sites). These were cloned into a related plasmid containing a C-terminal GFP (pMB44-FL, pMB53-9A, and pMB54-9D respectively) and a fragment from these plasmids was transformed into yeast strain MB1091 (in which the downstream *his1* had been replaced with *ura4*) and *his+* colonies were characterized. This yielded strains yMB1199 (FL), yMB1201 (9A), and yMB1203 (9D). Yeast with a *cut7* allele containing silent restriction sites grew the same as yeast with an allele containing no silent restriction sites. The strain containing a sid4-mCherry marker was a gift of Dr. Anne Paoletti (Institut Curie, Paris, France). It was crossed with existing lab strains to make strain yMP1302.

Live-cell imaging

All microscopy images and related datasets except those for Figure 3F were shown as smoothed maximum-intensity projections using the Smooth and Z-project functions in FIJI/ImageJ. Images were obtained using live-cell preparation and time-lapse spinning-disk confocal microscopy on a Nikon Eclipse Ti microscope (Nikon USA, Melville, NY). We exclusively used a Nikon 100x 1.45NA Plan Apo λ OIL WD 0.13 (mm) objective. Nikon NIS Elements software settings included an EM gain of 300 with exposure times of 100 ms for both the 488 nm and the 561 nm lasers. Lasers were set to 100% laser power, and 7 z planes were acquired with 500 nm distance between each plane. The triggered acquisition setting was used to prevent photobleaching when switching between z planes and between lasers. Rapid movement between z planes was accomplished with a Physik Instrumente (Auburn, MA) P-736 piezoelectric actuator. Imaging was performed as fast as possible with a time of approximately 9 seconds between each successive z stack.

To prepare for microscopy cells were grown on YE5S at 25 °C as previously described[27,39,77]. Cells were incubated on YE5S plates for 48 h and restreaked every 12 h. A small volume of exponentially growing cells was removed from the petri dish and placed in 10 μL of Edinburgh minimal medium (MPBio, Solon, OH) which was previously filtered with a 0.2 μm cellulose acetate filter (VWR International, Radnor, PA) to reduce background fluorescence. Cells in Edinburgh minimal medium were placed on 22×60-mm coverslips pretreated with 8 μL of lectins from *Bandeiraea simplicifolia* (Sigma-Aldrich, Burlington, MA). These coverslips were pre-equilibrated to the appropriate temperature of either 25 or 37°C. To check for temperature sensitivity and the presence of monopolar spindles, cells were placed at 37°C for 2–4 hours and then imaged at 37°C. Cells were transferred from a 37°C incubator to the pre-warmed microscope in less than 30 seconds to temperature shift that could allow monopolar spindles to become bipolar. Temperature was maintained with ±0.1 °C precision using a CherryTemp temperature controller (Cherry Biotech, Rennes, France). The controller was equipped with a 100X objective temperature sensor which continually maintained the temperature at the coverslip by sensing the objective temperature and making incremental adjustments at the coverslip to compensate for temperature changes at the objective.

For the cell shown in Figure 3F, live-cell preparation was performed as above on an Olympus Spin SR (Olympus, Waltham, MA) spinning-disk confocal microscope configured with a CSU-W1 confocal scanner unit. Olympus CellSense software settings included an exposure time of 200 ms for both the 488 nm and the 561 nm lasers. Lasers were set to 60% laser power, and 7 z planes were acquired with 500 nm distance between each plane. Images were obtained with a 60X Olympus apochromatic objective and processed with OlympusOlyVIA image viewer software.

Fluorescence photobleaching experiments and kymographs

For fluorescence photobleaching experiments, a 405 nm photobleaching laser was used on a Nikon Eclipse Ti spinning-disk confocal described above. Before photobleaching, four image stacks with illumination from the 488 nm laser were obtained to record the location of the spindle poles and Cut7–GFP. Photobleaching was then performed on the area of interest with the laser at 50–100% power and an exposure time of 100 ms. After photobleaching, signal was measured with time-lapse image stacks obtained continuously using the 488 nm illumination laser.

The two color kymographs shown in Figure 3G-H were constructed by first creating maximum intensity projections in FIJI/ImageJ from the Nikon nd2 datasets. A cell of interest was then cropped and the line tool was used to draw a line through a spindle of interest that included Cut7-GFP on protruding MTs. The spindle data was then aligned using the StackReg plugin with the rigid body transformation option selected in FIJI/ImageJ. A kymograph was produced from the aligned imaging using the reslice menu command.

Quantification and Statistical Analysis

Spindle length measurement and kymograph construction

For each phosphorylation mutant, we selected mitotic cells with a visible bipolar spindle based on the two bright spots of Cut7-GFP localized at spindle poles. We identified the Cut7-GFP intensity peaks and fit to a Gaussian intensity distribution to identify the maxima with subpixel accuracy. The length of the spindle was the distance between maximum. We fit the spindle length versus time curves with a 5th order polynomial and identified spurious spot detection by finding frames with unreasonable length fluctuation larger than 0.5 μm per frame ($\sim$9 s). These points were removed as outliers. We then smoothed the length as a function of time with a rolling average time window of 2 minutes.

To measure pre-anaphase spindle length and anaphase spindle elongation speed, for each cell we manually identified the time of pre-anaphase approximately steady spindle length, the time of anaphase B onset, and times at which steady spindle elongation begins and ends. Pre-anaphase spindle length includes all data points in the pre-anaphase steady spindle length window. The anaphase spindle elongation speed was determined from a linear fit to the region of steady elongation. We also determined the time to steady-state growth as the time from anaphase B onset to the time of steady spindle elongation. To determine average spindle length as a function of time, we aligned data from each cell by the time of anaphase onset. We then collected averaged data points in 15s time bins.

Kymographs and measurements of Cut7-GFP localization along the spindle were performed as in previous work and published code[39,78]. We selected the mitotic cells that formed a bipolar spindle. We manually segmented an elliptical region to include our cell of interest; any region outside this segmentation was not considered. The position of both Cut7-GFP spots was tracked to subpixel accuracy for each mitotic frame. We constructed kymographs by interpolating the Cut7-GFP intensity along the line connecting the spots in each frame and subtracting the background intensity. The brighter spot was used as a fixed reference point plotted on the left side of the kymograph, while the other spot varied in position. In some cases the line along the spindle axis extends beyond the boundary of the cell, into the region that has been cropped. In this case the kymograph includes pixels that have been cropped and therefore have zero intensity, which are visible in the kymographs as completely black areas.

We identified the Cut7-GFP peaks near the SPBs and measured the distance between the peaks. We extract all lines from the kymographs within ± 0.1 μm around the relevant peak separation, and plotted their average intensity as the group profiles of Cut7-GFP intensity along the spindle. We computed total Cut7-GFP intensity by summing the intensity from each spindle intensity profile extracted from the kymographs. We determined the Cut7-GFP midzone-to-pole ratio by first defining the pole and midzone. We summed pole intensity from a square box 0.75 μm on a side centered on the pole intensity peak. The midzone intensity is summed in a rectangular box of width 0.75 μm with long axis centered on the spindle axis that runs between the two poles, including all of the spindle except the pole boxes. The total Cut7-GFP intensity is the sum of the pole and midzone signals. The midzone-to-pole ratio is the total midzone Cut7-GFP intensity, divided by total pole intensity, computed for each frame.

Statistical analysis

Statistical analysis was performed in MATLAB. Details are presented in each figure caption. Statistical significance was assessed by 1-way ANOVA and multiple-comparison testing using Fisher's least-significant-difference procedure.

Model of Cut7 accumulation in protrusions

We built the model in the Coarse-Grained Living Active Systems Simulator (C-GLASS)[79,80] with extensions to model motors and crosslinkers[77,81–87]. MTs were modeled as rigid rods, with specified position. Spindle MTs were placed in a bipolar array approximating the fission-yeast spindle structure[71,72]. Protrusion MT(s) were initially fixed for a time t_i, then followed a prescribed sliding path with their minus-ends leading at constant velocity, until the protrusion tip reached 2 μm past the SPB. The protrusion MT(s) position was then fixed. To measure the Cut7 density and fraction of Cut7 at protrusion tips, the protrusion MTs were held at a length of 2 μm during data collection. We modeled the nuclear envelope as a sphere of radius R_{NE} connected to a cylinder to contain the protrusion of radius R_{pro} and variable length as the protrusion extends. The nuclear envelope cylinder length was chosen to surround the protrusion MT tip(s). The cylindrical and spherical sections of the envelope were connected at the point along the spindle axis where the edge of the cylinder reached the surface of the sphere.

Cut7 in the model can be unbound, bound to one MT, or crosslinking two MTs, in previous work[70,77,83–86]. We modeled unbound Cut7 and bound Cut7 movement with Brownian dynamics and state change (binding and unbinding) with kinetic Monte Carlo sampling. The initial state was constructed by randomly inserting unbound Cut7 molecules into the nucleoplasm. When an MT is within the binding radius R_{bind}, an unbound Cut7 can bind at rate $\ell c k_{\mathrm{u}\to\mathrm{s}}$ where ℓ is the length of the microtubule within the sphere of radius R_{bind} centered on the Cut7 molecule, c is the linear binding site density, and $k_{\mathrm{u}\to\mathrm{s}}$ is the binding rate constant. While bound to a single MT, Cut7 moves with velocity v_{s} in one direction (typically toward MT minus ends unless otherwise specified). Other aspects of the model are as in previous work[77,83], with parameters shown in Table S1.

Protrusion dynamics model

In the one-dimensional protrusion model[88], three forces act on the protruding MT bundle: outward force from Cut7-driven sliding, inward force from nuclear envelope distortion, and drag. The force-balance equation is

$$F_{\mathsf{s}} - F_{\mathsf{NE}} - \gamma v_o = 0, \tag{1}$$

where F_{s} is the Cut7-driven sliding force, F_{NE} is the nuclear envelope force, γ is the drag coefficient, and v_o is the sliding velocity. Outward is the positive direction. This force balance gives a sliding velocity

$$v_o = \frac{(F_{\mathsf{s}} - F_{\mathsf{NE}})}{\gamma}. \tag{2}$$

We assume that the sliding force is proportional the length of the overlap, as observed experimentally for kinesin-5 motors[36,59]. Therefore $F_{\mathsf{slide}} = f_{\mathsf{slide}} L_{\mathsf{overlap}}$, where f_{slide} is a constant Cut7 sliding force per unit length and L_{overlap} is the length of protrusion bundle antiparallel overlap with spindle MTs. The drag coefficient of MTs is[89]

$$\gamma_{\mathsf{mt}} = \frac{2\pi\eta}{L}\left(\ln\left(\frac{L}{2R}\right) - 0.114\right), \tag{3}$$

As in previous work, the nuclear envelope force is[39,77,84,90]

$$F_{\mathsf{NE}} = 2\pi R\left(\frac{\kappa}{2R^2} + \sigma\right)\left(1 + 2a_1a_2\exp\left(-\frac{L}{\sqrt{2}R}\right)\cos\left(\frac{L}{\sqrt{2}R} + \alpha_1 + \alpha_2\right)\right), \tag{4}$$

where R is the radius of the nuclear envelope tube, κ is the nuclear envelope bending modulus, σ is the nuclear envelope surface tension, and $a_1 = 0.746$, $a_2 = 0.726$, $\alpha_1 = 0.347$, and $\alpha_2 = 3.691$ are constants.

The total MT length $L = L_{\mathrm{overlap}} + L_{\mathrm{protr}}$, the sum of the overlap length and protrusion length. We assume that the MT length between the SPB and overlap edge is negligible and set it to zero. Protrusion MTs can slide and also may change length due to polymerization or depolymerization at speed v_p, where $v_p > 0$ corresponds to MT growth and $v_p < 0$ corresponds to MT shrinking.

MT polymerization or depolymerization occurs at the plus end and changes the overlap length. Therefore

$$\begin{aligned} \frac{dL}{dt} &= v_p, && (5) \\ \frac{dL_{\text{overlap}}}{dt} &= v_p - v_o, && (6) \\ \frac{dL_{\text{protr}}}{dt} &= v_o. && (7) \end{aligned}$$

We stochastically model MT dynamic instability: in the growing state the MT plus end grows at speed v_{g} and switches to the shrinking state with frequency f_{c}. In the shrinking state the MT plus end shortens at a speed v_{s} and switches to the growing state with frequency f_{r}. Our observations of protrusions showed that most protrusions slowed in growth and stabilized at a constant length. Therefore we added a maximum MT length in the model L_{m}. If in the model an MT grows beyond L_{m}, the MT switches to the shrinking state.

The protrusion MTs had initial length $L = L_{\mathrm{i}}$ and no protrusion. We then numerically integrated the model using Euler's method with a kinetic Monte Carlo step at each time step to compute state switches between growing and shrinking. Model parameters are shown in Table S2.

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