Kar9 Condensates Prevent Caravan Sway While Towing Nuclear Envelope Cargo

The version of this poster is adapted for a general audience

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REFERENCES

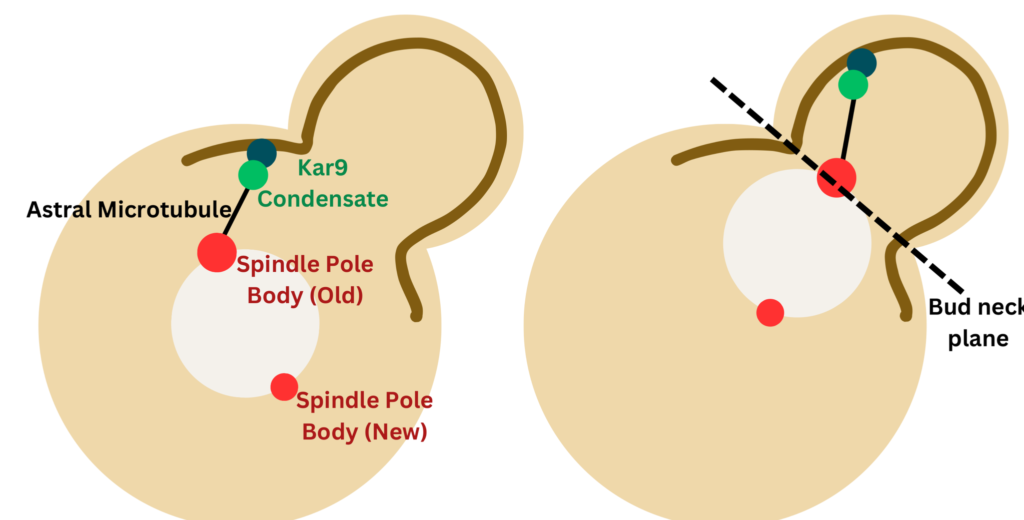
Meier, S. M., Farcas, A. M., Kumar, A., Ijavi, M., Bill, R. T., Stelling, J., Dufresne, E. R., Steinmetz, M. O., & Barral, Y. (2023). Multivalency ensures persistence of a +TIP body at specialized microtubule ends. Nature cell biology, 25(1), 56–67. https://doi.org/10.1038/s41556-022-01035-2

Miller, R. K., & Rose, M. D. (01 1998). Kar9p Is a Novel Cortical Protein Required for Cytoplasmic Microtubule Orientation in Yeast. *Journal of Cell Biology*, *140*(2), 377–390. doi:10.1083/jcb.140.2.377

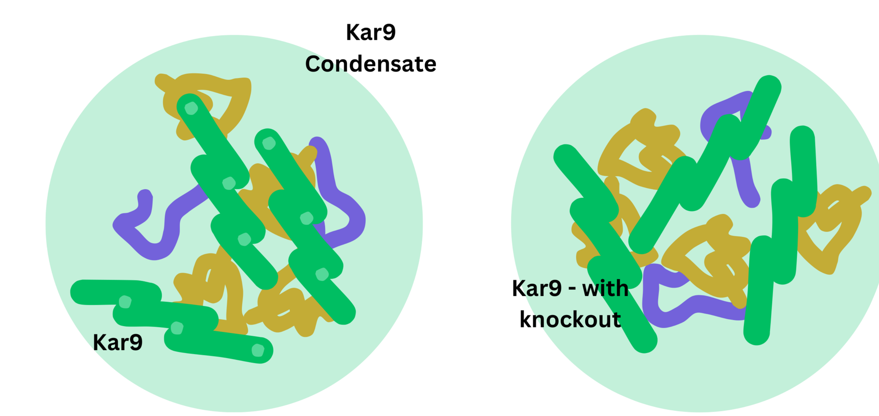
Meziane, M., Genthial, R., & Vogel, J. (2021). Kar9 symmetry breaking alone is insufficient to ensure spindle alignment. Scientific Reports, 11(1), 4227. doi:10.1038/s41598-021-83136-w

What is Kar9?

- *Saccharomyces cerevisiae* (Budding yeast) undergoes a unique mitosis process where the nuclear envelope does not dissolve.
- Instead, the nuclear envelope is towed across the bud neck.
  - Astral microtubules are anchored to spindle pole bodies on the ends of the envelope, and Kar9 condensates.
  - Kar9 functions as pivotal hitches connecting the astral microtubule tow lines allowing them to be pulled.
- It is known that Kar9 is an essential part of the spindle positioning process but a more complete understanding of how its physical properties contribute to the metaphase process is necessary.
- A triple interface mutant of Kar9 is tested, which knocks out Kar9's ability to interact with itself, changing the properties within the condensate like viscosity.



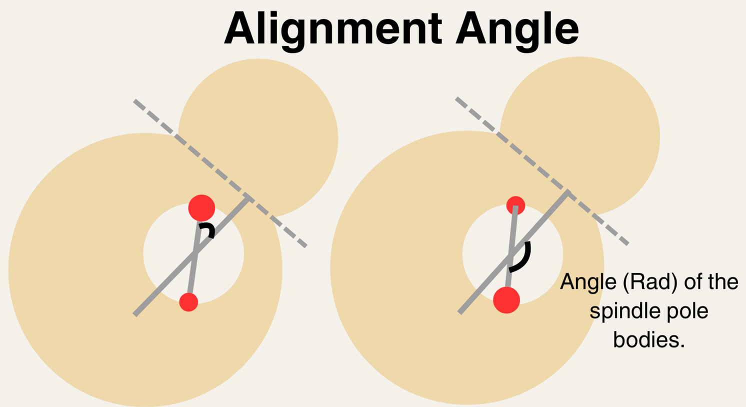
Nuclear envelope transport across the bud neck.



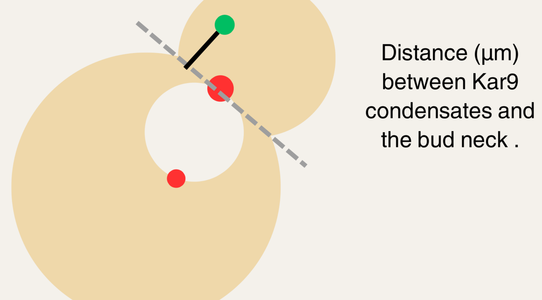
Kar9 condensates with and without triple interfaces.

SAMPLE OF THE PROPERTIES TESTED

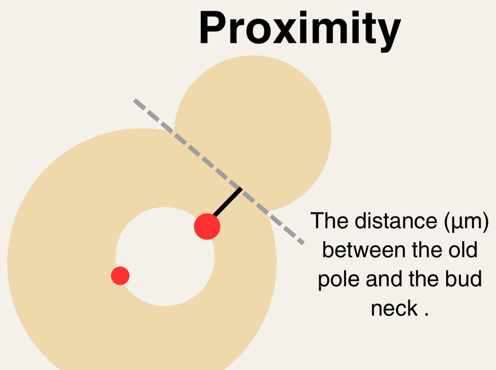
Alignment Angle



Kar9-Bud Neck Proximity



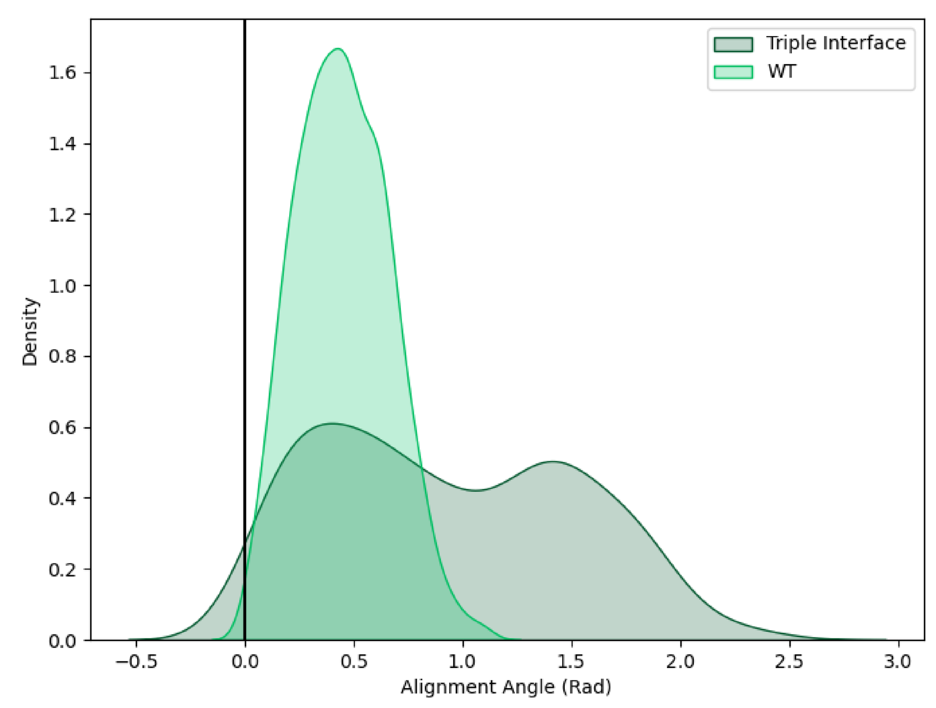
Old Spindle Pole - Bud Neck Proximity

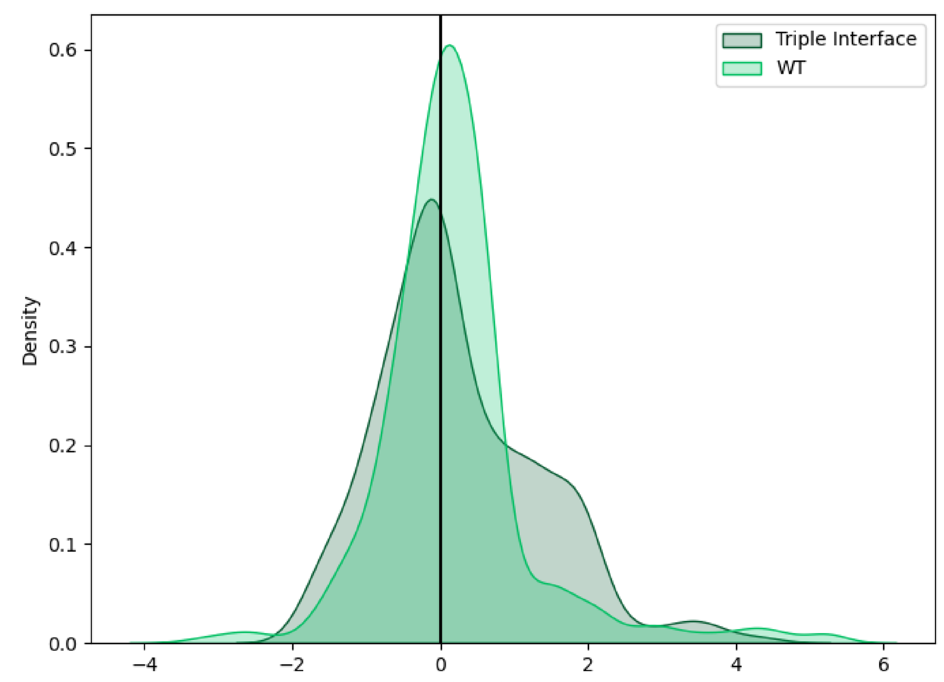


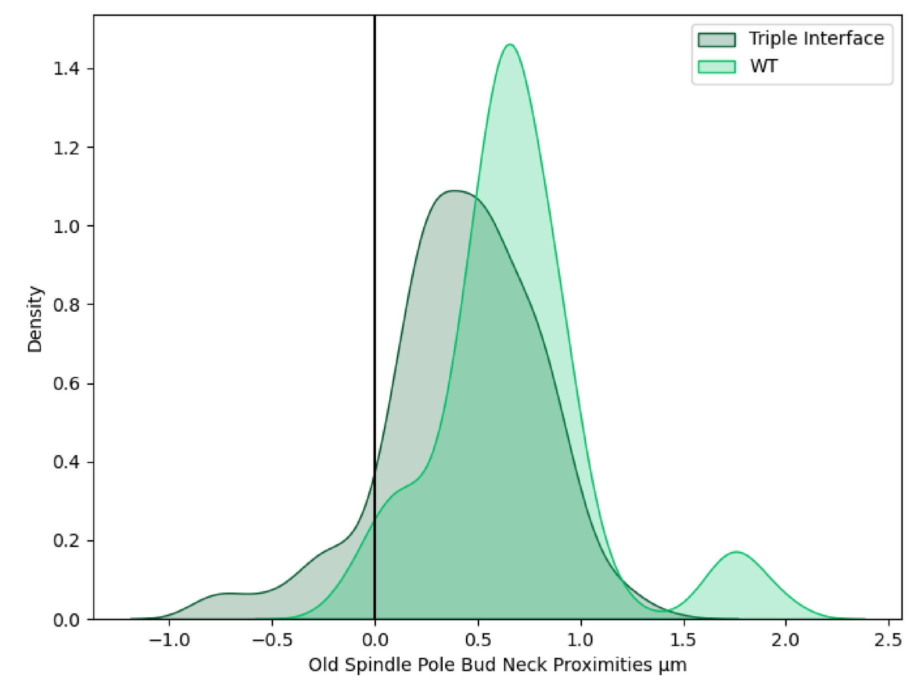
METHODS

- Sample: 6 wild-type cells, 3 mutant cells.
  - Small sample size so far.
- Image Acquisition: Elyra Lattice SIM.
  - Super high-resolution 3D fluorescence microscopy.
- Analysis: TrackMate, Python

Results



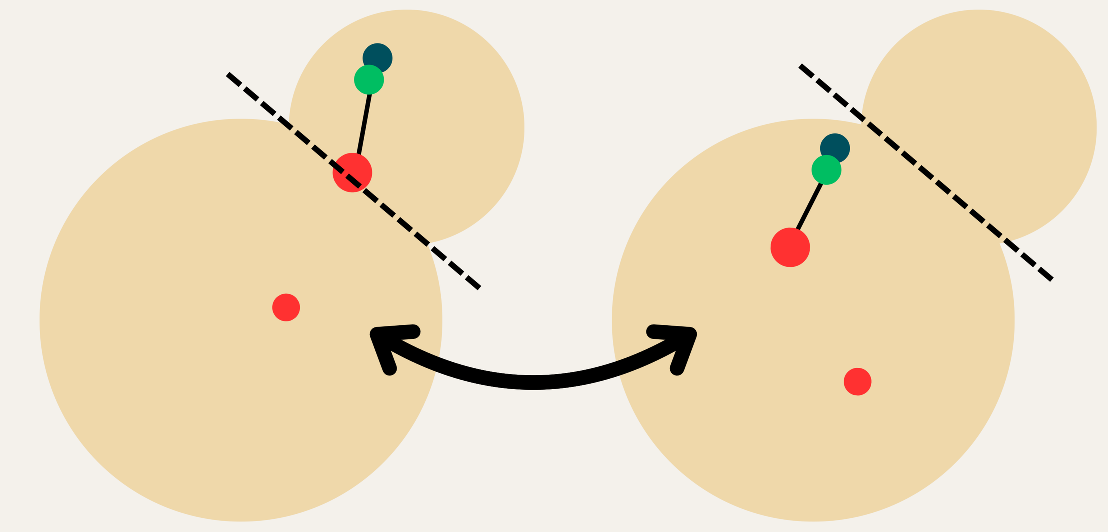




- Strikingly, the interface mutation causes alignment angles to vary greatly.
  - Kar9-Kar9 interactions are needed for aligned towing.
- Changes to the proximity of the spindle pole bodies / Kar9 to the bud neck are more subtle.
  - The mutation appears to cause a slightly extended presence of Kar9 inside the bud neck.
- The position of the spindle is similar between the strains but the rotation differs greatly which might be an indicator of an increase of Kar9 being associated with both.

Future Direction

In future studies, leveraging additional positional data tracks of the spindle pole bodies alongside Kar9 positional data tracks and their associated properties a custom machine learning classifier could be trained. This could be done with a normalized/augmented dataset from clips of track data and their associated properties. A classifier like this could be used to facilitate the arrangement of clips of various cells temporally, to get an average metaphase trajectory of mitotic budding yeast.



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