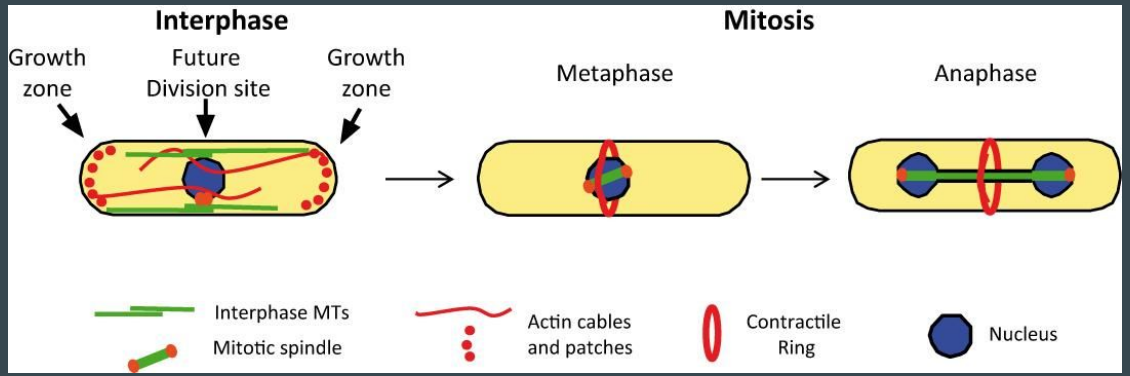
Quantifying Nuclear Movement in Wild Type and mmb1-delta and klp4-delta Fission Yeast Cells.

IISc Bangalore

Background

Fission yeast typically divide symmetrically after a period of growth.

Microtubules contribute to this process through positioning the nucleus at the center of the cell, which becomes the site of cell division.

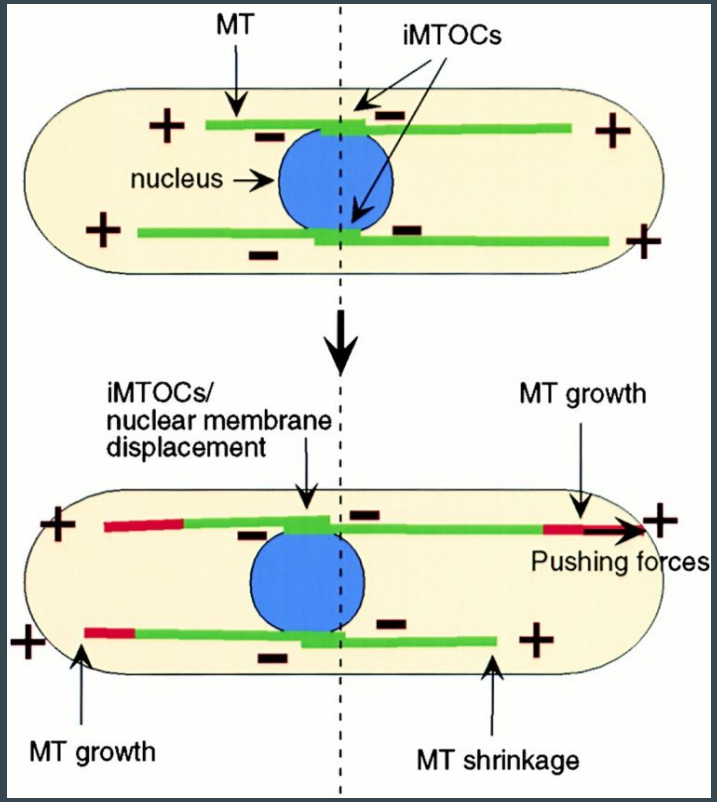


http://www.labex-celtisphybio.fr/cytoskeleton-architecture-and-cellular-morphogenesis/

Background

Microtubule pushing:

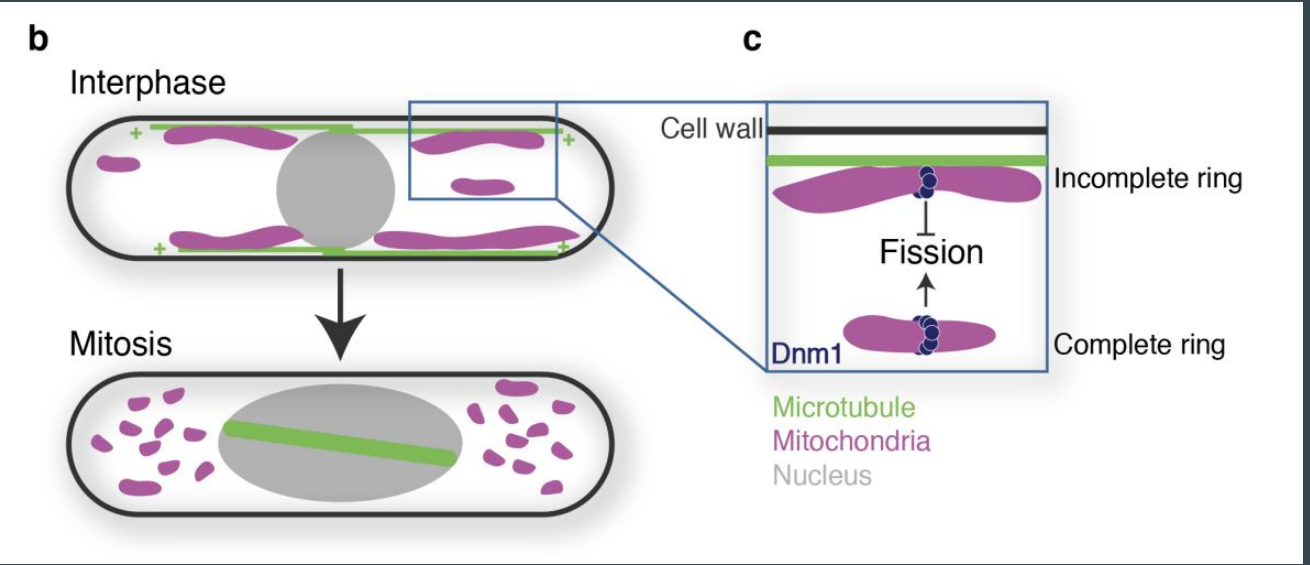
Growth and catastrophe of microtubules helps to center the nucleus of the cell.



Tran PT, Marsh L, Doye V, Inoué S, Chang F. A mechanism for nuclear
positioning in fission yeast based on microtubule pushing. J Cell Biol. 2001;153(2):397-411. doi:10.1083/jcb.153.2.397.

Background

Microtubules and mitochondria are associated through the protein mmb1 during interphase. During mitosis mitochondria undergo fission by dnm1.



Mehta K, Chug MK, Jhunjhunwala S, Ananthanarayanan V. Microtubule dynamics regulates mitochondrial fission. bioRxiv. 2017. doi:10.1101/178913.

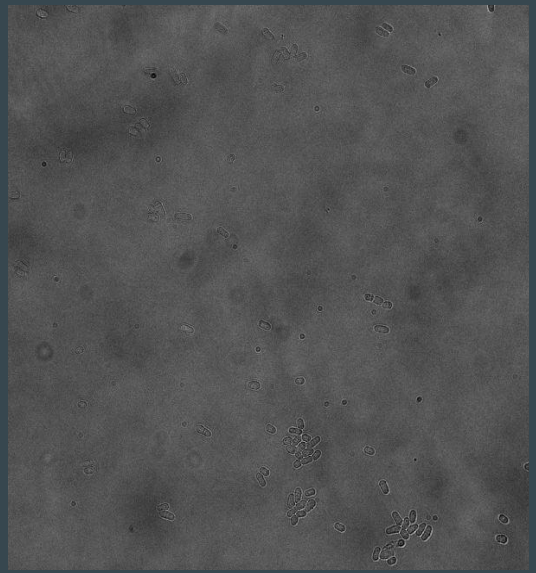
Klp4

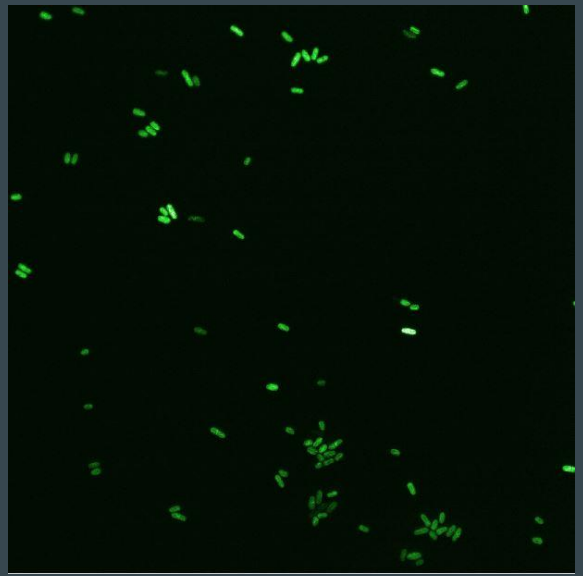
Kinesin protein that stabilizes microtubules

Deletion leads to short microtubules

Methods

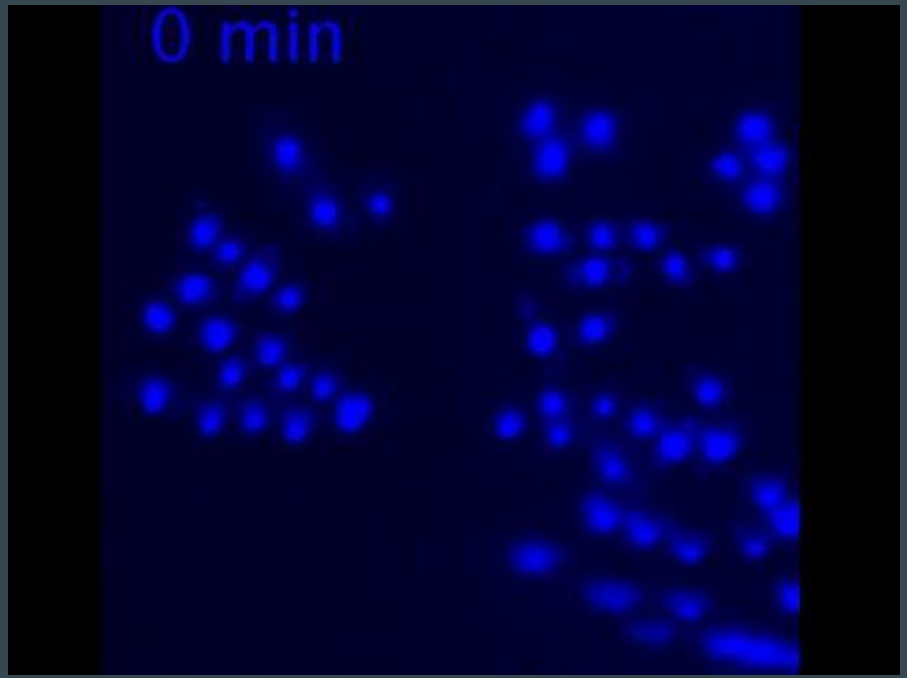
Yeast strain with microtubules tagged with GFP with the nucleus stained with hoechst



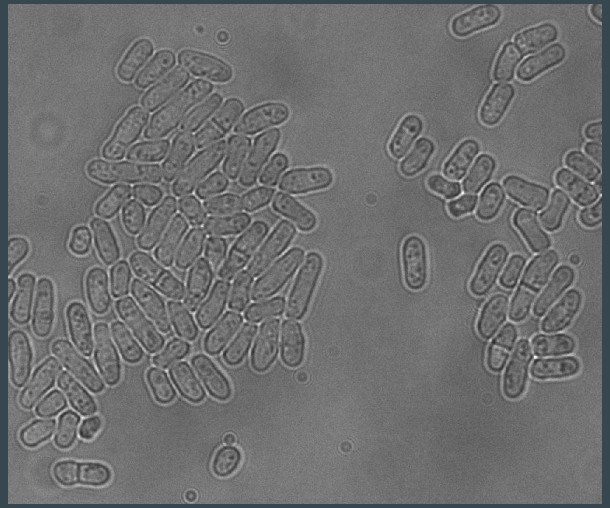


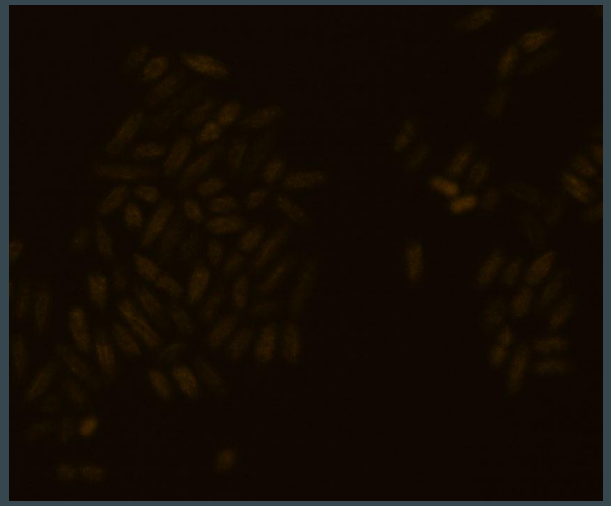
Methods

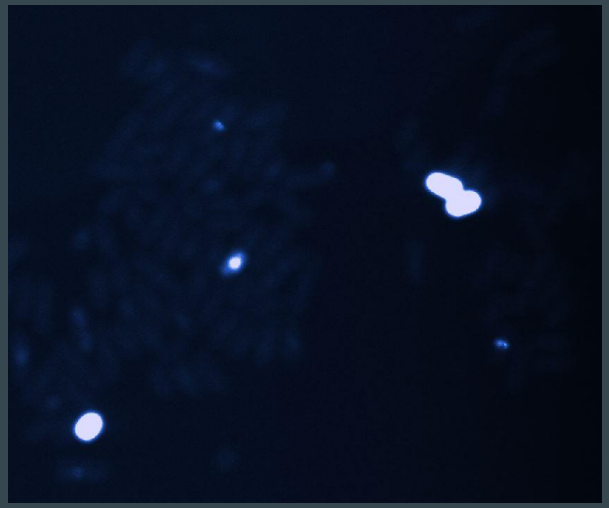
Yeast Strain with microtubules tagged with mcherry with the nucleus stained with hoechst



Weak mcherry signal in stained cells







Attempted Protocols

- Stain concentration: 2, 5, and 10 microgram/mL hoechst stain
- Time: 15 and 20 minutes of staining
- Location: staining in the hood, staining in the incubator
- Imaging: confocal dishes, slides, and slides with agarose pads
- Imaging media: EMM+N, EMM+N and amino acids, hoechst stain
- Cells: grown overnight in YEA, taken directly from plate

Optimized protocol: 2 microgram/mL stain for 15 minutes in the incubator, image on slides with agarose pads in EMM+N and amino acids, with cells taken directly from the plate.

Methods

Unable to visualize microtubules and nucleus simultaneously

Future possibility:

Yeast strain with the microtubules tagged with mcherry and the ER tagged with GFP

- Requires cross

The Lab

Exploring Bangalore

Acknowledgements

Thank you to Vaishnavi, Leeba, all the lab, and the SN Bose Program!