Two-Hybrid Analysis of Pac1p Interactions With Binding Proteins

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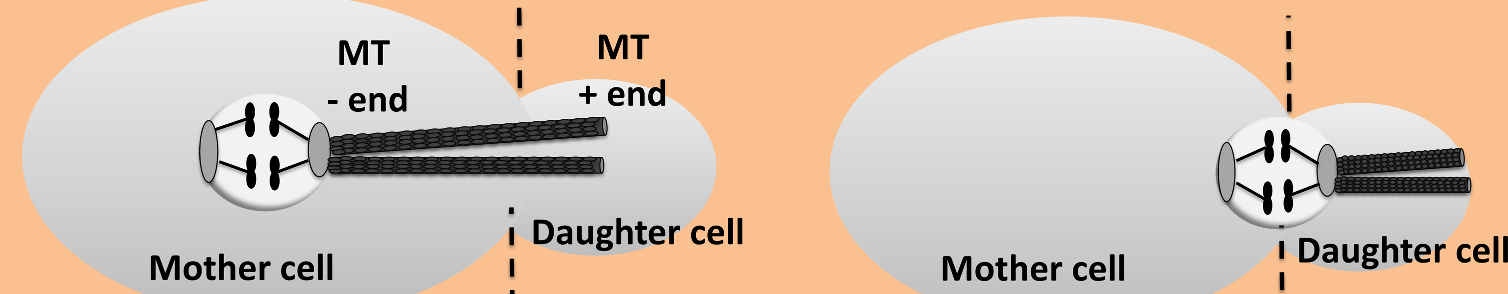
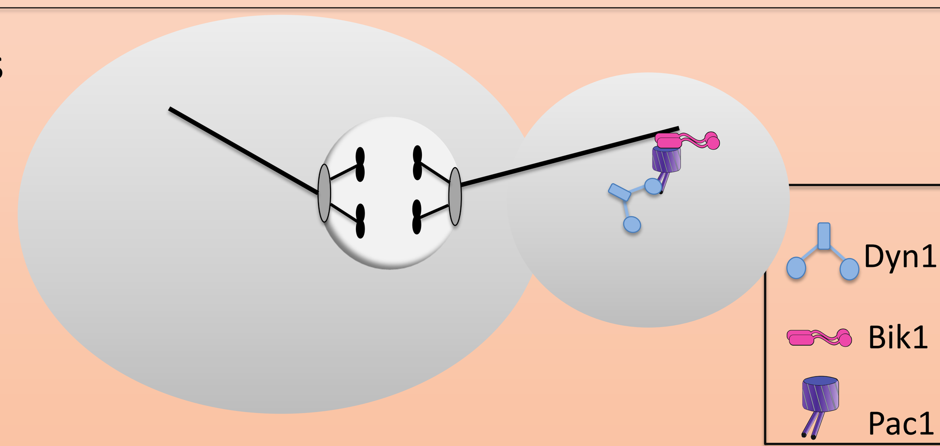
Abstract

The mitotic spindle is the cytoskeletal machinery that separates genetic information, stored in chromosomes, from the mother cell to the daughter cell during cell division. The positioning of the mitotic spindle is dependent on microtubule lengths and orientation. In the model organism *Saccharomyces cerevisiae,* the protein Pac1 helps in the localization of dynein to the plus end of the microtubule. The recruitment of dynein to the plus end of the microtubule is necessary for the sliding of microtubules along the bud cortex, and it has been shown that there is no microtubule sliding in cells lacking Pac1p. Pac1p interacts with the Small Ubiquitin-like MOdifier (SUMO), and ubiquitin itself. SUMO is an important post-translational modification of proteins in the cell that regulates many critical cellular processes including nuclear transport, transcription, chromosome segregation, and DNA repair. It is not known how the attachment of SUMO to Pac1p alters its function or the function of dynein. Our lab has identified two sites of Pac1p modification, *2K→R* mutants. Using two-hybrid analysis, we have identified changes in protein-protein interaction in the *2K→R* mutants compared to wild type.

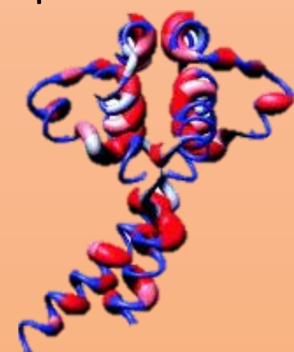
Introduction



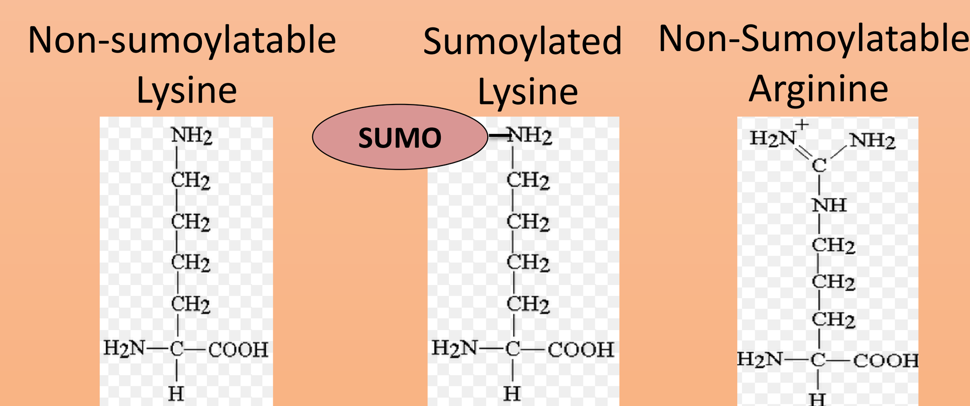
- It is important for the mitotic spindle to be positioned in the plane of cell division when the cell divides, so that the mother cell and the daughter cell each receive one copy of the duplicated chromosomes.
- Interactions between astral microtubules, microtubule associated proteins, the cell cortex, and anchor sites are the main source of information for positioning the mitotic spindle.



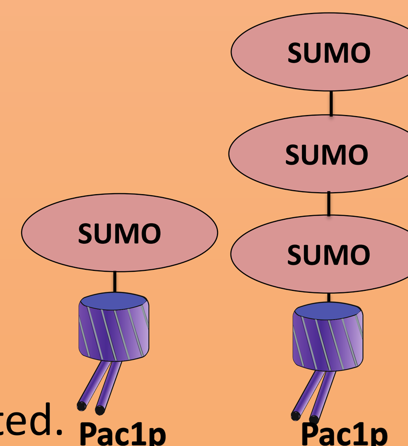
- In yeast, the motor protein dynein pulls on the cytoplasmic microtubules to position the mitotic spindle across the bud neck.
- Both Bik1p and Pacip help localize dynein to the plus end of the microtubule.
- Pac1p is homologous to the human protein Lis1, which is needed for development of the brain



Pac1 is sumoylated at Lysine sites



- SUMO (Small Ubiquitin like MOdifier) is a new regulatory molecule that has been shown to affect the stability, localization, and/or activity of a protein.
- SUMO can be crosslinked to target proteins. This can either occur as a monosumoylation or as a chain of polysumoylation.

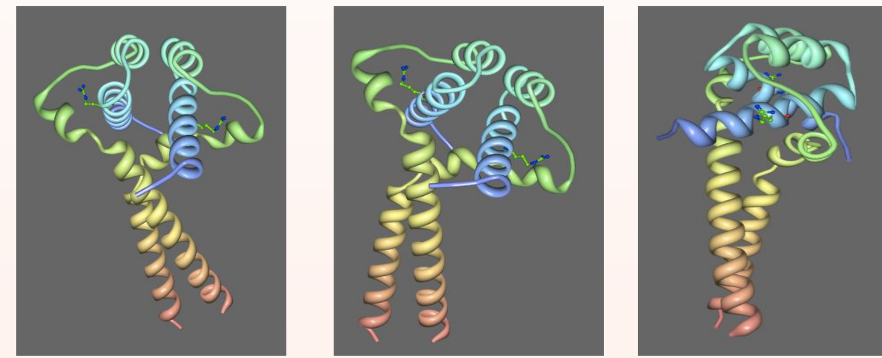


Hypothesis

Pac1p will lose interaction with SUMO or Ubc9 when lysines are mutated.

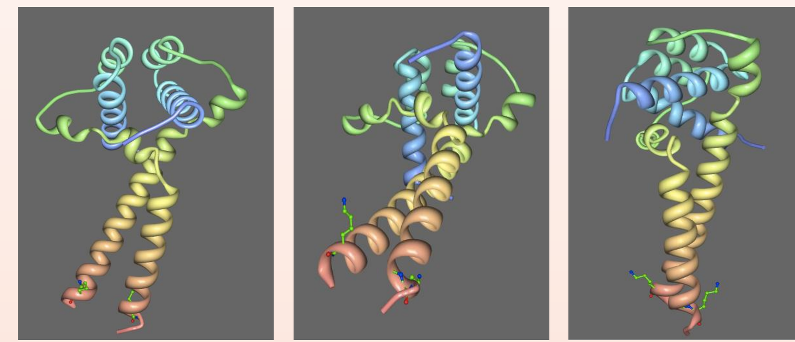
Methods

- PCR was used to mutate Pac1p at hypothesized SUMO attachment sites.
- Lysines were changed to non-sumolaytable arginines.
- The two-hybrid assay was used to analyze changes in the protein-protein interactions



N-terminal crystal structure of Pac1p homologue Lis1 with K20 Mapping

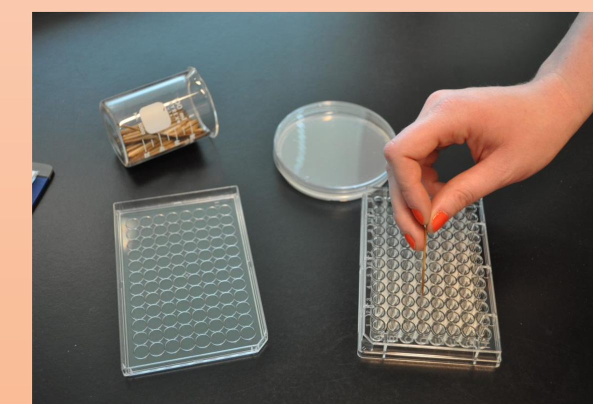
N-terminal crystal structure of Pac1p homologue Lis1 with K114 Mapping

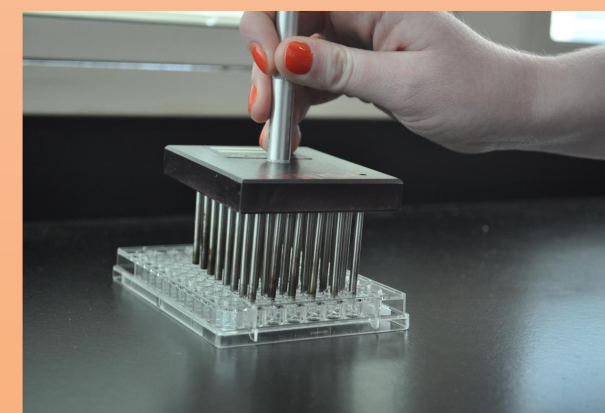


- The mutated Pac1p was transformed into bacteria.
- The bacterial colonies were isolated and plasmids were sequenced to confirm single mutation sites.
- The two-hybrid plasmid was transformed into a Pac1p yeast strain.

Photo Credit:Sarah Fridaus

- A multi-channel pippetman was used to inoculate a 96 well plate with water.
- Using a toothpick, the wells were inoculated with the yeast strains of interest.





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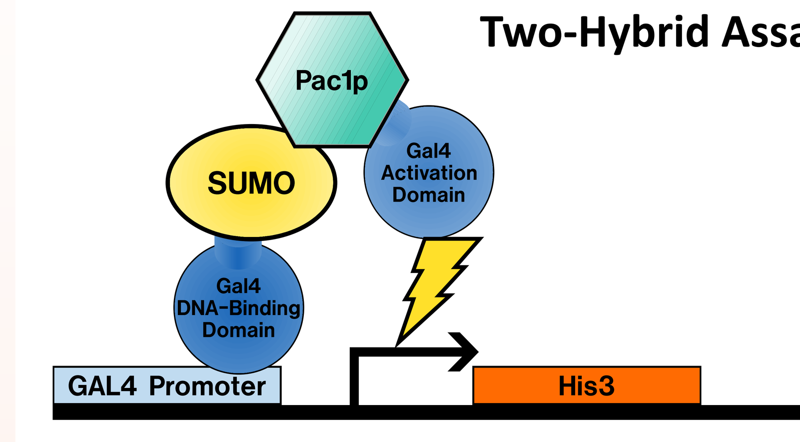
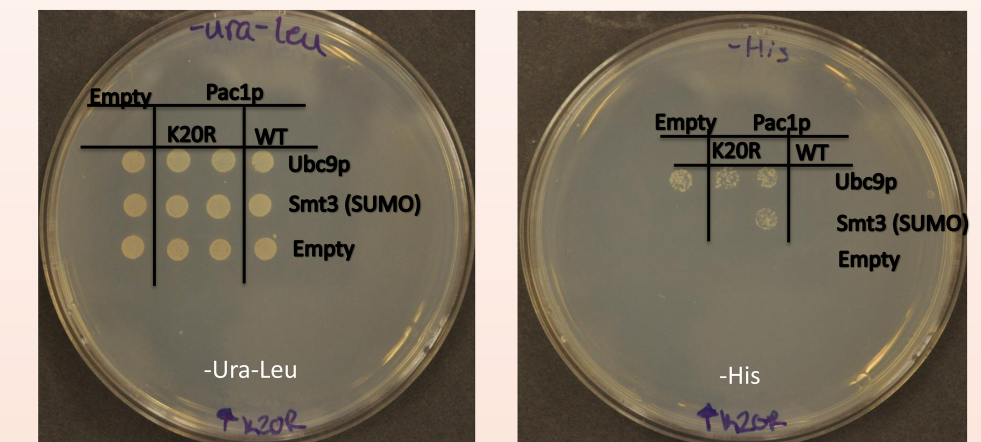
- A sterilized multipronged transfer device was used to transfer the resuspended cells in the wells onto a media plate.
- Interactions were assayed by growth of the cells on SC plates lacking uracil and leucine (-Ura-Leu), or histidine (−His).

Photo Credit:Sarah Fridaus

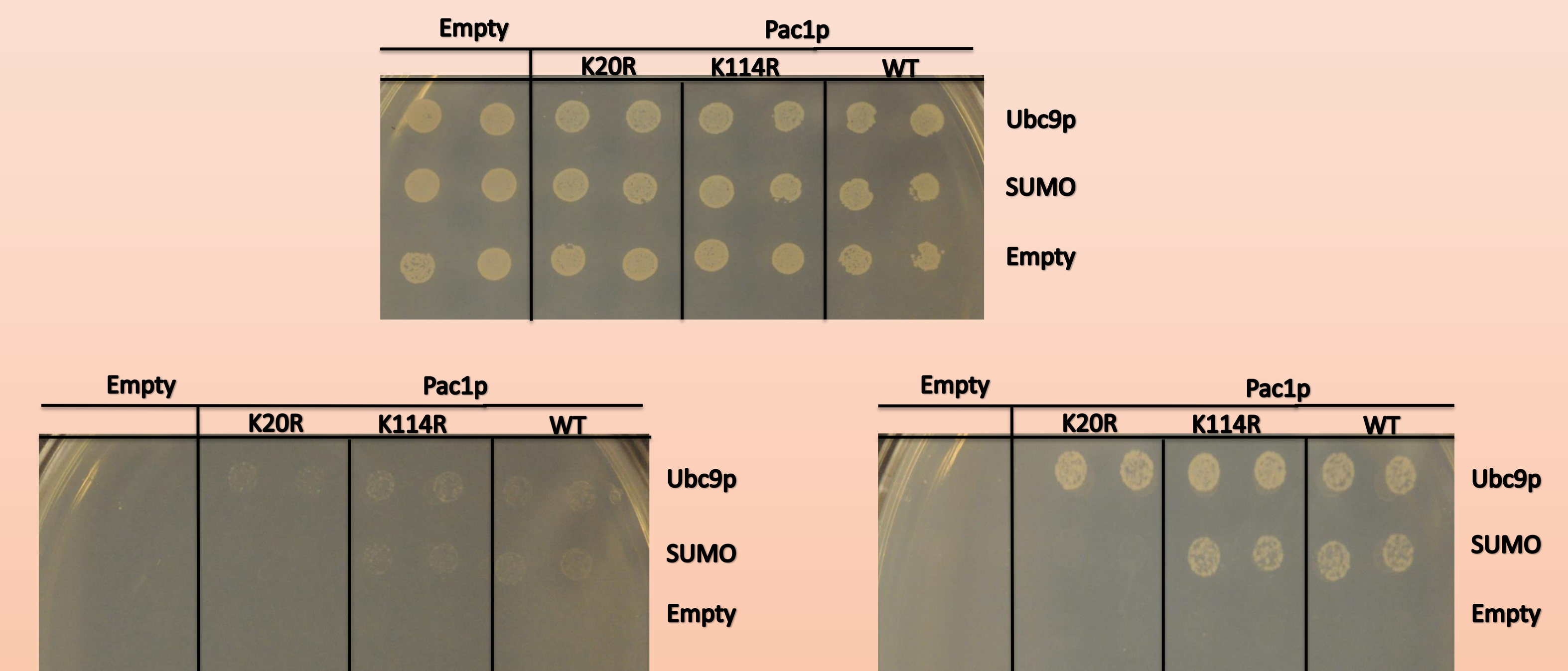


- When proteins of interest interact with each other it activates the Gal4 promoter causing the synthesis of histidine. Therefore if there is protein-protein interaction, growth can be observed on plates lacking the amino acid histidine.
- Protein-protein interaction was analyzed from two-hybrid analysis results on SC plates lacking uracil and leucine, and histidine (−His) after 2 and 3 days growth at 30°C.

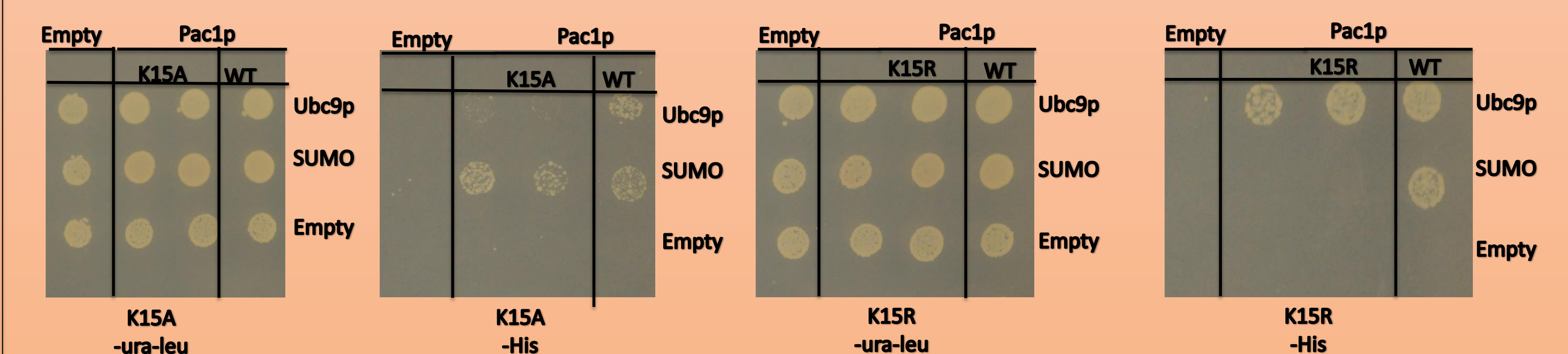


Results

Mutation of Pac1p's K20 site inhibits interaction with SUMO but Pac1p's K114 site does not



Mutation of Pac1p's K15 site inhibits interaction with SUMO and UBC9



- It is important to know how SUMO modification affects protein-protein interaction to gain a better understanding of how SUMO functions in cell division and which protein-protein interactions alter cell division. This contributes to our knowledge of dynein regulation and mitosis control.

Acknowledgements