**Num1p interaction with SUMO**

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**Abstract**

During cell division, the daughter centrosome is pulled to the center of the bud cortex by Num1p and dynein. Num1 is a protein that works as a cortical anchor for the dynein motor. Dynein pulls the microtubules to move the mitotic spindle from the mother cell to the bud. Num1 is not essential for nuclear migration, but it is important for efficiency. It is suspected that the Num1 protein is sumoylated. Sumoylation is the post-translational modification of proteins by Small Ubiquitin like modifiers (SUMO). SUMO proteins regulate various cellular processes including nuclear transport, transcription, chromosome segregation, and DNA repair. Sumoylation can affect the stability, localization, or activity of a protein. A temperature sensitive strain of Ubiquitin- Like Protein-1 (Ulp1), that inactivates the protease activity of Ulp1 at 37°C and cleaves Smt3p from any proteins sumoylated to it, will be used to identify whether Num1p is sumoylated. The localization and activity of Num1p is important because of this protein’s role in the nuclear migration and placement of the bud. These findings will lead to a better understanding of how Num1p functions and interacts with other proteins.

**Introduction**

Microtubule lengths and placement are an important factor in determining the orientation of the mitotic spindle. • 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.

**Num1 protein**

• Num1p anchors dynein to the cell wall of the bud.
• Dynein is a powerful motor protein that walks toward the minus-end of the microtubules.
• Dynein-Num1p pulls the centrosome across the bud neck before the cell divides

**Hypothesis**

Num1p is sumoylated

- 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.

**Methods**

- PCR was used to tag Num1p with the his6 epitope.
- The PCR product was inserted into a His3+ vector.
- The ligated product was transformed into DH5α competent bacteria using blue white selection on LB-AMP plates.

**Integration of Num1p-his6 into yeast by homologous recombination**

- A restriction enzyme was used to cut and linearize the plasmid.
- The linear plasmid was integrated at the endogenous NUM1 locus by homologous recombination in the yeast genome.

**Expected Results**

- A+B= 1.2kb band
- A+C= no band
- 3000 bp
- 1000 bp
- 37°C
- 30°C

**Future Directions**

Will Num1p interact with SUMO?

Ulp1 is a protease which cleaves SUMO from target proteins. This Ulp1 allele inhibits the protease from cleaving SUMO at 37°C and permits the cleaving of SUMO at 30°C

**Expected Results:**

If Num1p is conjugated with SUMO, we predict that there will be a shift in size of Num1p at 37°C when the Ulp1 protease is inactivated.

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