**Background**

- Anaphase onset is triggered by the activation of Esp1/Separase, a protease that cleaves Mcd1/Scc1 subunit of the Cohesion ring complex that physically holds sister chromatids together. Separase is regulated by the anaphase inhibitor Pds1/Securin.
- Anaphase onset can be monitored by the lengthening of the spindle fibers as the spindle pole bodies move apart from each other after ~20 minutes in metaphase:

  ![Spc42-GFP to measure progression through mitosis](image)

- Wild-type cells: rapid anaphase lengthening of the spindle occurs after a pause in metaphase (95% of cells).
- slk19Δ and pds1Δ cells display similar spindle elongation pattern as wild-type cells (graph A).
- Many slk19Δ pds1Δ cells display immediate spindle lengthening (40%) (graph B) or immediate spindle lengthening with short periods of shortening (40%) (graph C). No cells behave like wild-type.

**Methodology**

- Wild-type (GAL-FLAG-ESP1) or mutant (GAL-FLAG-esp1-C1551A) yeast
- Cut with Bam1 & Sal1
- Induce with 2% galactose
- Arrest in benomyl + isolate mitotic nuclei
- Cells with over-expressed Esp1
- Isolate supernatant
- PGEX6P-1
- Rosetta Bacteria
- IPTG induction + Purification
- In vitro cleavage of partially purified nuclear Mcd1
- In vitro cleavage of bacterially purified Slk19

**Results**

**Conclusions**

- The results have shown that Esp1/Separase cleaves Mcd1/Scc1 and Slk19 separately as expected.
- Future steps:
  2. Inhibition with Slk19 mutants to compare phosphorylated vs unphosphorylated Slk19, and cleavage site mutants that suppress pds1Δ cells.

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