Investigating the Role of Csm1 in Regulating Ulp2 Activity and Cell Cycle Progression in *Saccharomyces cerevisiae*

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Introduction

SUMOylation is a biological process where a protein called SUMO, a Small Ubiquitin-like Modifier, is attached to target proteins and regulates their function, location, or abundance in the cell (Gutierrez-Morton, 2024). This involves activation of the SUMO protein by an E1 enzyme, transfer by an E2 enzyme which brings the SUMO protein closer to its target protein, and attachment of the SUMO protein to the target protein by an E3 ligase (Gutierrez-Morton, 2024). In some cases, a chain of SUMO proteins is attached to a target protein, polySUMOylation, this chain can influence the target protein's behavior (Gutierrez-Morton, 2024). It is important to study SUMOylation as it is essential for multiple cellular activities, like DNA replication and mitotic progression, and since disruptions in SUMOylation are linked to diseases like cancer and neurodegenerative disorders (Gutierrez-Morton, 2024). Furthermore, monoSUMOylation helps in forming large molecular structures through interactions between SUMO and SUMO-interacting motifs (SIMs), while polySUMOylation promotes the separation of these complexes by inducing downstream processes (Gutierrez-Morton, 2024).

Additionally, mitotic exit is the final phase of cell division. One thing which characterizes mitotic exit is the breakdown of the mitotic spindle. In *Saccharomyces cerevisiae* the SUMO protease Ulp2 is essential for restarting the cell cycle after DNA damage has caused a stop, as it counters signals from the Rad53 checkpoint pathway to help the cell cycle resume (Schwartz, 2007). It was also found that Cells lacking Ulp2 often escape the metaphase arrest but cannot complete division due to defective mitotic spindles (Schwartz, 2007). This suggests that Ulp2 is critical not only for restarting the cell cycle after DNA damage but also for ensuring proper spindle function during this recovery, also that the absence of Ulp2 would cause early mitotic exit, since this SUMO protease is necessary for the cell cycle continuation and completion. Moreover, Ulp2 is a SUMO protease that breaks down polySUMO chains in the nucleus (Gutierrez-Morton, 2024). Without Ulp2, these chains accumulate, disrupting the cell cycle (GutierrezMorton, 2024). This can cause early mitotic exit or a cell cycle delay (Gutierrez-Morton, 2024). In Upl2 deletion cells Pds1 accumulation was observed, indicating a cell cycle delay, given that Pds1 degradation indicates anaphase entry (Gutierrez-Morton, 2024).

In addition, in Saccharomyces cerevisiae, Ulp2 is recruited to the nucleolus by the Csm1-binding domain (de Albuquerque, 2018). It is found at the rDNA region because of its interaction with the Cohibin complex, Csm1-Lrs4, which connects the nuclear envelope and rDNA (de Albuquerque, 2018). Moreover, The SIM (SUMO-interacting motif) found in the C-terminal part of Ulp2 works with Csm1 to ensure it targets specific nucleolar substrates and enhances its ability to act as a SUMO protease (de Albuquerque, 2018). Both the Csm1-binding domain and the SIM are important for deSUMOylating the RENT complex and Tof2 in the nucleolus (de Albuquerque, 2018). However, mutations that prevent Csm1 from binding to Ulp2 do not significantly affect SUMOylation in areas like the inner kinetochore or MCM subunits, which implies that while Csm1 is essential for nucleolar activity, Ulp2 also rely on other mechanisms (de Albuquerque, 2018).

Furthermore, we know that csm1 is an essential protein for nucleolar activity; therefore, we expect to see lower ulp2 activity and premature mitotic exit in yeast cells with csm1Δ compared to the wild-type.

Results

In the experiment, we did not see a significant increase in ulp2 phosphorylation over the course of the cell cycle for the mutant compared to the wildtype. However, we did see premature ulp2 phosphorylation of *S. cerevisiae*, for Csm1Δ (Figure 1a-b). For the budding index performed, the wild-type strain progresses through the cell cycle in a synchronized and controlled manner, peaking around 60 to 80 minutes with large-budded cells and returning to G1 at 120 minutes (Figure 1c). However, the mutant strain (csm1Δ) shows premature exit from mitosis, suggesting that CSM1 is required for proper mitotic progression (figure 1c). This aligns with the western blot data, which indicated premature phosphorylation in the mutant strain, likely contributing to differences in cell cycle progression.

Conclusion

Finally, the protein Ulp2 within *Saccharomyces cerevisiae* is responsible for cleaving Smt3/SUMO-1 peptides from proteins, it contributes to chromosome cohesion at centromeric regions, recovery from checkpoint arrest caused by DNA damage or DNA replication defect, and RNA splicing (Engel, 2024). Additionally, it is recruited to the nucleolus by the csm1-binding domain (de Albuquerque, 2018). Csm1 essential for nucleolar activity, therefore, we hypothesised that *S. cerevisiae* cells with csm1Δ would display lower ulp2 activity and premature mitotic exit compared to the wild-type. To test this, a csm1Δ ulp2-myc phosphorylation western blot (G1 arrest) and budding index were performed. The cells were synchronized in the first cell cycle stage using alpha factor, released, then time points were taken from 0 to 140 minutes. In the experiment, we expected to see a slight increase in ulp2 phosphorylation over the course of the cell cycle as well as premature ulp2 phosphorylation for the mutant cell. In the experiment, we did not see different levels of phosphorylation between the mutant and wild-type, however we did observe premature phosphorylation for the mutant. This indicates that for csm1Δ, Ulp2 is inactivated prematurely which would lead to premature polySUMOylation in *S. cerevisiae* cells. Moreover, this research is significant because it builds upon the foundational knowledge of biochemical pathways relevant to human disease such as chromosome nondisjunction and cancer.

Figure 1

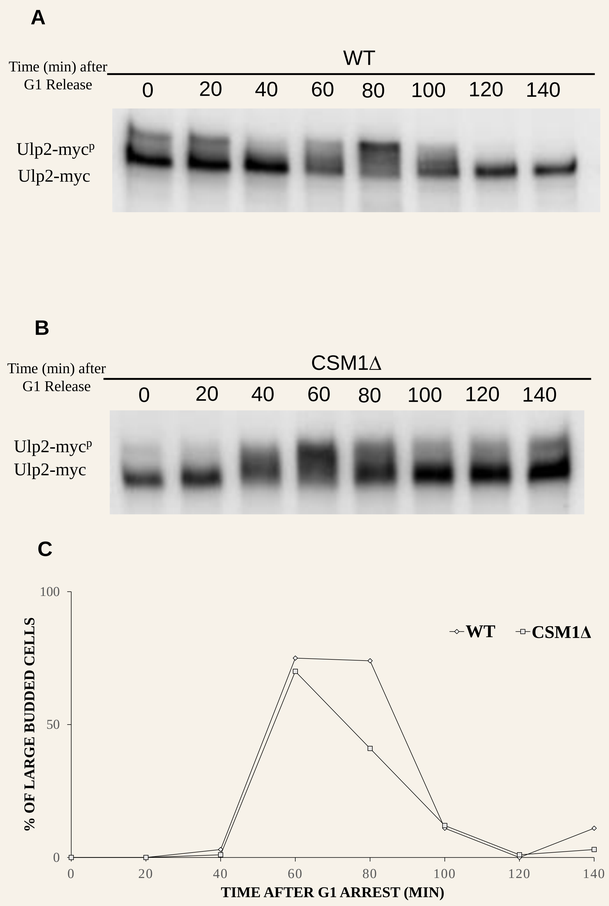

Figure 1.(A, B, C). Phosphorylation during the cell cycle in WT and ulp2-myc with csm1Δ cells. G1-arrested WT (4786-3-1) and ulp2-myc cells with csm1Δ (4980-1-2) were released. Cells were collected every 20 min for western blotting and budding index. Cry 1 as the negative control.

Methods

Standard protocols for mating and tetrad dissection were used to construct a *Saccharomyces cerevisiae ulp2-myc* strain containing *csm1Δ*. Cells were grown in YPD medium at 30°C. A two-hour G1 arrest was then performed to synchronize the cell cycle. For the G1 arrest, nine microliters of wild-type and mutant cell cultures were used, both at an optical density of 0.4, and incubated in a 30°C water bath. Initially, alpha factor was added to the cultures at a 1:1000 concentration, followed by a second addition after one hour. Following G1 arrest the cells were released and later used for western blotting and budding index analysis.

Cell Release:

After the two-hour G1 arrest, the cell cultures were spun down for one minute at 4000 RCF, the YPD medium was discarded, and the cells were washed with 1 mL of Millipore water. The mixtures were then spun down in Eppendorf tubes for ten seconds using a tabletop centrifuge. The water was aspirated and replaced with 1 mL of fresh YPD medium, which was then transferred to Erlenmeyer flasks containing an additional 8 mL of YPD medium.

Samples were collected every 20 minutes. At each time point, 800 microliters of each cell culture were transferred into labeled Eppendorf tubes for western blot analysis, while 180 microliters of each cell culture were transferred into Eppendorf tubes containing 20 microliters of formaldehyde for budding index analysis. At 40 and 100 minutes, after collecting time points, alpha factor was added at a 1:500 concentration. For each western blot time point, cells were centrifuged, and the media was aspirated, leaving only the cell pellets.

Western Blot Procedure:

For western blot analysis, 200 microliters of 0.1 M NaOH were added to each time point tube. The tubes were vortexed to dissolve the pellets and incubated at room temperature for five minutes. After incubation, the samples were spun down at 10,000 RCF for 30 seconds, and the liquid was aspirated, leaving only the pellet. Then, 100 microliters of 1X loading buffer were added to each sample. The samples were sonicated for four seconds and then boiled at 90°C for five minutes. Next, the tubes were spun down at 13,000 RCF for five minutes. The prepared samples were loaded into the wells of a 6% gel and run in running buffer at 40 mA for 90 minutes. Following electrophoresis, the proteins were transferred to membrane paper in transfer buffer at 250 mA for 60 minutes.

Membrane Blocking and Antibody Incubation:

After the transfer, the membrane was incubated with 5% milk in 5 mL of TBST for 30 minutes on a rocker. Next, it was incubated overnight with the primary antibody Anti-myc at a 1:1000 concentration in 2% milk in 5 mL of TBST. The following day, the primary antibody was discarded, and the membrane was rinsed three times with TBST, each for five minutes. After the third rinse, the secondary antibody was added at a 1:2500 concentration in 2% milk in 5 mL of TBST. The wild-type and mutant membranes were incubated with the secondary antibody for 40 minutes on a rocker. After incubation, the secondary antibody was removed, and the membrane was washed three more times with TBST. Next, 1 mL of Western Lighting Oxidizing Reagent Plus and 1 mL of enhanced Luminol Reagent Plus were added to each sample for imaging. The membrane was then imaged using a Western Blot Imager.

Budding Index Analysis:

For the budding index, 100 cells from each time point were counted from the formaldehyde-fixed samples. The cells were examined under a light microscope and categorized into three groups: no-budded, small-budded, and large-budded cells. These counts were recorded in an Excel document and used to generate a line graph.

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Resources

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