Localization and Function of ULP2 in the event of CSM1 Deletion

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Introduction

In the model organism of *saccharomyces cerevisiae*, commonly known as budding yeast, there are many proteins, with one of interest being Ulp2. Ulp2 is described as "Peptidase that deconjugates Smt3/SUMO-1 peptides from proteins; plays a role in chromosome cohesion at centromeric regions, recovery from checkpoint arrest induced by DNA damage or DNA replication defect, and RNA splicing; potential Cdc28p substrate; human homolog PML implicated in promyelocytic leukemia can partially complement yeast null mutant". This protein plays a role in the chromosome cohesion at the centromeric regions, notably allowing the sister chromatids to properly separate at the correct time by deconjugating the SUMO chains holding them together. There is evidence that Csm1, an essential protein for nucleolar activity, may play a role in the localization of Ulp2 in the nucleolus except during anaphase of cellular division where Ulp2 is instead localized onto the kinetochore. Using the kinetochore protein Nuf2 with the fluorescent protein tag MCH, we can track the localization of Ulp2 using the fluorescent protein tag NG in the event of Csm1Δ. This is further tested by performing a NOC arrest, providing analysis on the effects in cell phase synchronization in Csm1Δ. The temperature dependent protein Cdc13 provides an additional means of arrest qualifying data found from the synchronized cells in NOC arrest. In these experiments, we expect to see Ulp2 localized throughout the cytosol in the event of Csm1Δ as it is no longer available to play a role in Ulp2 localization. This research is significant as studying Ulp2 furthers knowledge in known biochemical processes and pathways relevant to human disease such as cancer and chromosome nondisjunction.

Methods

Strains of *S. cerevisiae* were crossed to create cell cultures with Ulp2 NG and Nuf2 MCH as a wild type and cell cultures with Ulp2 NG and Nuf2 MCH with Csm1Δ. The cells were inoculated overnight and prepared once reading an optical density (OD) of 0.2-0.3 when compared to YPD solution as a base. The cells were then imaged using a Keyence microscope after removal of YPD, washing with water, and treatment with Phosphate-Buffered Saline (PBS). These images provided data on Csm1Δ in asynchronous cells. Next, cells from stock were similarly prepared for a NOC arrest by overnight inoculation. Cells were then treated with NOC and left to spin in 30°C for 2 hours to fully arrest in early anaphase. Cells were then similarly prepared for imaging by removal of solution, washing with water, and treatment with PBS. These images provided data on Csm1Δ in synchronous cells (early anaphase). Following imaging, a quantitative analysis was performed to identify ratio of kinetochore binding and non-kinetochore binding cells in anaphase in both experiments.

Acknowledgments

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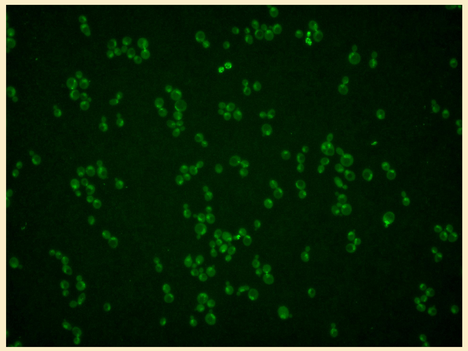
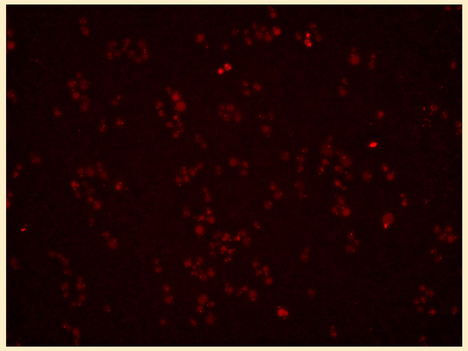
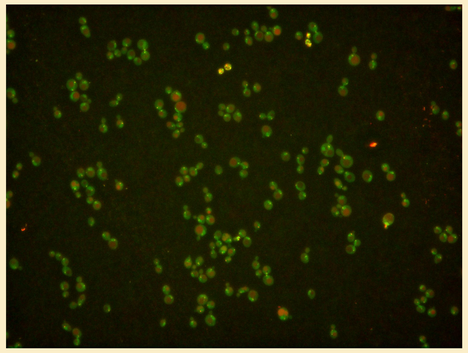


Ulp2 NG Nuf2 MCH GFP Keyence Image



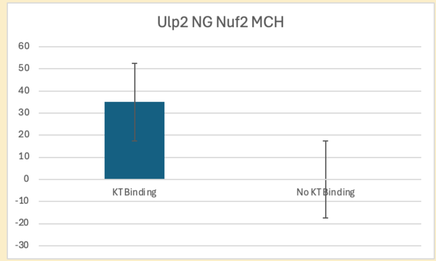
Ulp2 NG Nuf2 MCH RFP Keyence Image

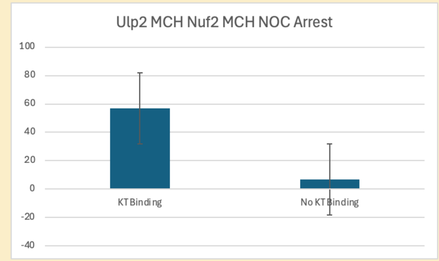


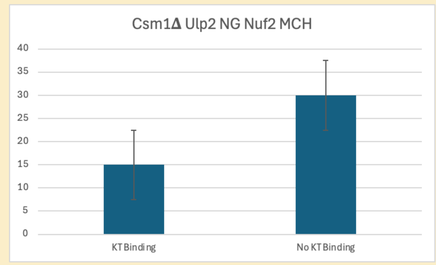
Ulp2 NG Nuf2 MCH Overlay Keyence Image

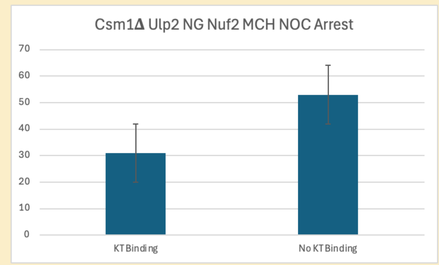
Results

The Keyence images displayed a clear different in wildtype and mutant cells. Wild type cells displayed high ratios of kinetochore binding (KT), consistent with data from previous research. Mutant cells with the Csm1**Δ** displayed very low ratios of KT binding. These conclusions were quantified through quantitative analysis with Ulp2 NG Nuf2 MCH having a KT binding ratio of 35:0, Ulp2 NG Nuf2 MCH (NOC arrest) KT binding ratio of 57:7, Csm1**Δ** Ulp2 NG Nuf2 MCH KT binding ratio of 15:30, and Csm1**Δ** Ulp2 NG Nuf2 MCH (NOC Arrest) KT binding ratio of 31:53.









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