

Identification of Aha1 and SUMO Interaction by Immunoprecipitation and Western Blotting of Yeast Cell Lysates



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ABSTRACT

Heat shock protein 90 (Hsp90) is an ATP dependent molecular chaperone that assists in the folding and function of many client proteins including hormone receptors and protein kinases, as well as proteins which are necessary for the function of the hallmarks of cancer. Aha1 is a co-chaperone of Hsp90, which activates the ATPase cycle of Hsp90, making it an important subject of research to inhibit cancer cells. A post translational modification protein known as SUMO has been shown to encourage binding of Aha1 and Hsp90. This project focuses upon investigating the interaction of Aha1 and SUMO. Through immunoprecipitation and western blotting this project has found correctible issues that could lead to greater understanding of the Aha1 and SUMO interaction. Future research will likely be able to indicate functions of Aha1 and SUMO which could be applied to chemotherapeutic treatments of diseases such as cancer and cystic fibrosis.

INTRODUCTION

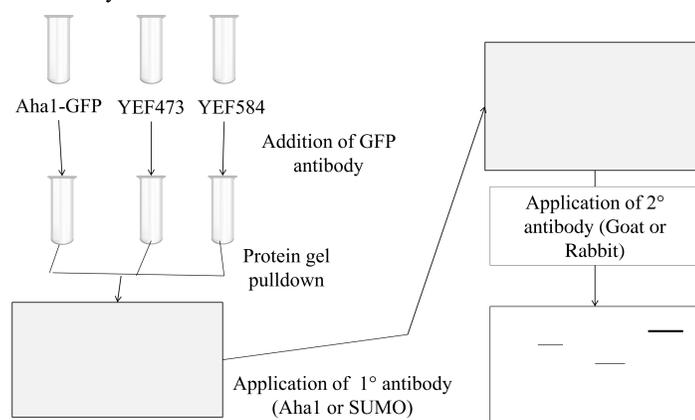
Heat shock protein 90 (Hsp90) is a dimeric, ATP-driven molecular chaperone that assists in the folding and function of numerous proteins throughout eukaryotic cells such as protein kinases, steroid receptors, and proteins which are necessary in the function of the six hallmarks of cancer. Aha1 is an important co-chaperone of Hsp90, which activates its ATPase cycle (Lotz et al, 2003). Aha1 has also been demonstrated to have chaperone activity outside of its interaction to Hsp90 (Sun et al, 2014). SUMO is a post translational modification protein that when bound to Hsp90 has been shown to encourage Aha1 binding (Mollapour et al, 2014). However, there has not been published information discussing whether or not Aha1 directly binds to SUMO. It has been demonstrated that when Hsp90 is sumoylated (bound to SUMO), it is more susceptible to attack from Hsp90 inhibitors (Mollapour et al, 2014). This project focuses on the interaction of Aha1 and SUMO in order to better understand why SUMO causes an increase in Aha1 affinity and to gain knowledge over the currently limited view of Aha1 protein interaction.

MATERIALS AND METHODS

Yeast Strains used in this Project:

- Aha1-GFP (Mat a ura 3-52 lys2-801 leu2Δ-1 his3-Δ200 trp1Δ-63 Aha1-GFP:KanR)
- YEF473 (Mat a ura3-52 lys2-801 leu2Δ-1 his3-Δ200 trp1Δ-63)
- YEF584 (Mat alpha ura3-52 lys2-801 leu2Δ-1 his3-Δ200 trp1Δ-63)

Lysed Cells



RESULTS

Immunoprecipitation and Pulldown of GFP with Aha1 Blot

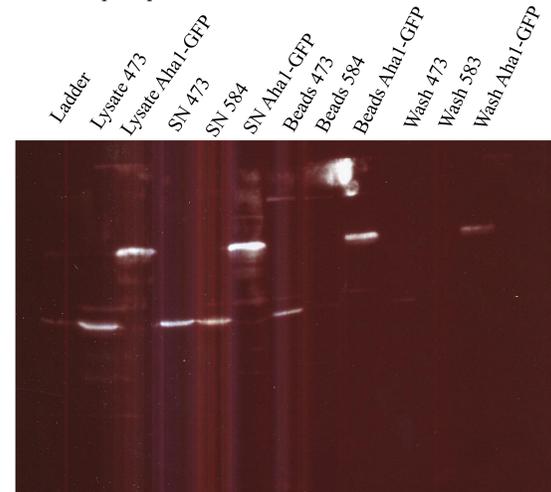


Figure 1. Treated with anti-Aha1 antibody after pulldown.

Immunoprecipitation and Pulldown of GFP with SUMO Goat blot

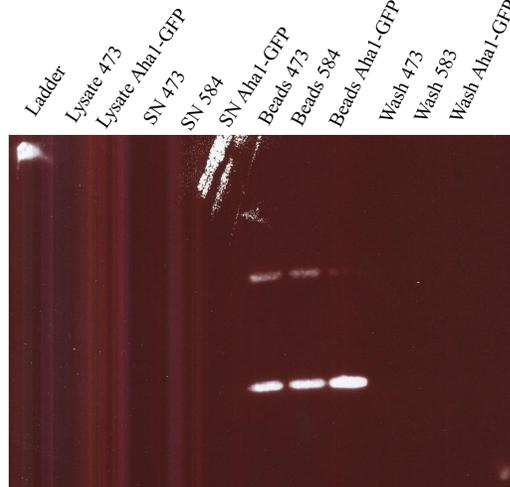


Figure 2. Treated with goat anti-SUMO after pulldown.

Immunoprecipitation and Pulldown of GFP with SUMO Rabbit blot

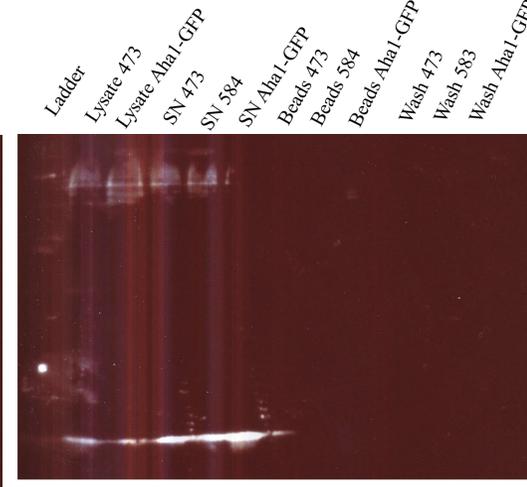


Figure 3. Treated with rabbit anti-SUMO after pulldown.

DISCUSSION

As indicated in the images it was somewhat difficult to determine a pulldown method that could successfully view Aha1 and SUMO. As indicated in figure 1 a method for easily isolating and viewing Aha1 was determined. The pulldown of green fluorescent protein (GFP) for a yeast strain that has Aha1 fused with GFP proved successful. A blot with anti-Aha1 antibody after the pulldown clearly shows distinct bands of Aha1 in the lysate and supernatant (SN) lanes as well as the lane of the Aha1-GFP strain that was treated with the anti-Aha1 antibody. This model allowed for clear isolation of Aha1 as desired. In future testing it will likely be used to determine Aha1 interactions through western blotting.

Figure 2 indicates the first, unsuccessful attempt to view SUMO for possible correlation to Aha1. Distinct bands appeared in all lanes treated with the anti-GFP antibody. These chains likely occur due to the beads having a goat antibody attached to them that allows them to pull out the GFP antibody that holds the Aha1-GFP fused protein to prevent it from washing out. When separating the beads it is likely that some of these antibodies came off with them. Since the blotting anti-SUMO antibody was from a goat, to view a secondary anti-goat antibody is needed which likely attached to the antibodies from the beads. The bands seen are likely the heavy and light chains from the goat bead antibodies. This result indicates that the goat anti-SUMO antibody should be avoided due to the large interference given by it.

Figure 3 indicates a possibly unsuccessful attempt to view SUMO. All bands appear in the lysate and SN lanes which may indicate that the hypothesis was incorrect. This would be because when Aha1-GFP was removed by the anti-GFP antibody SUMO was not bound to Aha1 and thus not pulled out as well. Another possibility is that rabbit anti-SUMO may not have been sensitive enough since it often isn't in non-enriched cultures. However, difficulty has occurred in the past with trying to view correlational data of SUMO with other proteins. Dr. Rita Miller found a method using a temperature sensitive yeast strain grown at specific temperature that was able to view SUMO in western blotting. For future investigation, a new strain may need to be developed which crosses the Aha1-GFP strain with the temperature sensitive strain.

The information in this project will be vitally helpful for understanding the approach necessary to study interaction of Aha1 and SUMO. By investigating Aha1 and SUMO interactions, links to diseases and cellular processes of importance may be discovered. Also since Aha1 is linked to diseases such as cystic fibrosis and cancer, inferring interactions of Aha1 may lead to additional pathways to fight disease (Mollapour et al, 2014). Aha1 may prove to be an important chemotherapeutic target in future treatments.

REFERENCES

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