Phosphorylation of Orb2 by Wnd

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Abstract

Wallenda (Wnd) is an evolutionarily conserved protein kinase that is essential for neural development including axon branch development, axon regeneration and degeneration. However, the molecular mechanisms underlying Wnd action is not fully understood. Kim lab recently has found Orb2 as a novel Wnd pathway. Orb2 is one of the cytoplasmic polyadenylation factors that control the length of poly (A) tail in its target mRNAs. This suggests that Wnd controls neural development through Orb2 phosphorylating. To study the biological function of Orb2 phosphorylation by Wnd, it is essential to map the phosphorylated amino acid residues in Orb2 by Wnd kinase. We have mapped the phosphorylated Serine and Threonine residues in Orb2 upon Wnd co-expression in cultured Drosophila S2 cells using phospho-proteomics. Subsequently, we generated the mutant Orb2 that are defective in Wnd-mediated phosphorylation as well as the mutant Orb2 that mimics the phosphorylated version of Orb2. Wnd regulates the expression of Dscam. The expression regulation of Dscam by Orb2, and the effect of Orb2 phosphorylation by Wnd in this process will be discussed.

Introduction

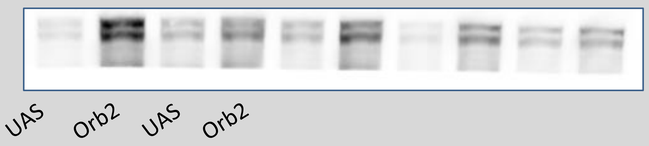


Figure 1. Western blot of Dendra2-SV40 (modified 3' UTR) transfected with either UAS (empty vector) or Orb2. Dendra is a marker that is used to detect Dscam (Down Syndrome Adhesion Molecule) expression in Drosophila S2 cells. Cells transfected with Orb2 appear to exhibit negative regulation compared to cells transfected with UAS. This suggests that Orb2 may play a role in regulating Dscam expression.

Orb2 may play a role in the regulation of Dscam. As discussed previously, Orb2 acts on the 3' UTR of its target RNAs thus making them translationally silent until reaching their target destination (Richter, 2007). Its exact mechanism, however, is largely unknown. Kim lab hypothesizes that it is through this 3' UTR modifying mechanism that allows Orb2 to negatively regulate Dscam. The role of Orb2 in axonal development is believed, by Kim Lab, to be facilitated by a protein kinase called Wallenda. The process of testing this relationship between Wallenda and Orb2 is discussed here.

Methodology

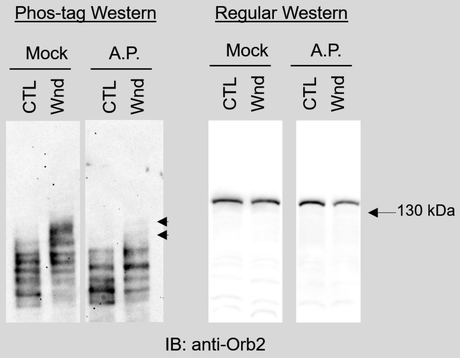
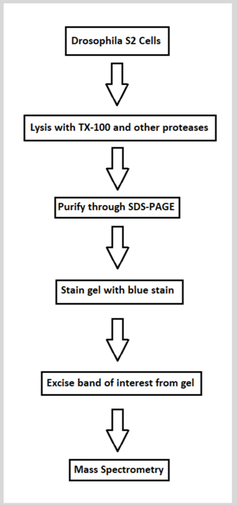


Figure 1. Orb2 is phosphorylated by Wnd. A phos-tag western blot of Orb2 (left) and a conventional western blot of Orb2 (right) in the presence of Wnd (Wnd) or the absence of Wnd (CTL). The same lysates were treated with alkaline phosphatase (AP) to reverse the phosphorylation on Orb2 (Lane 3 and 4). Orb2::tdTomato proteins were detected with anti-RFP antibody.



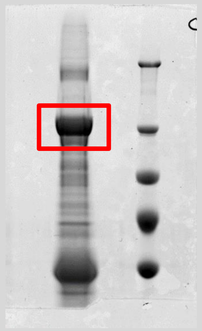
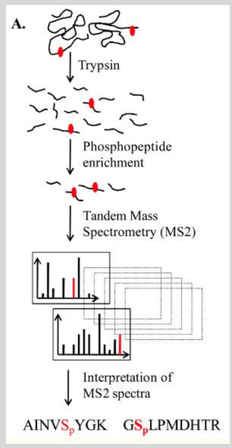


Figure 3. Immunoprecipitation of Orn2 transfected with a UASattB plasmid using an anti-RFP antibody. Protein purification was performed through SDS PAGE before blue staining and excising. This gel excision was then sent to Harvard's Mass Spectrometry and Proteomics Resource Laboratory in order to undergo mass spectrometry.



Mayya and Han, 2010

Figure 4. General outline of the main steps in the phosphomapping mechanism. The protein is first digested by a protease before the peptides are enriched. This is done so that the Tandem Mass Spectrometry can easier detect possible phosphorylation sites.

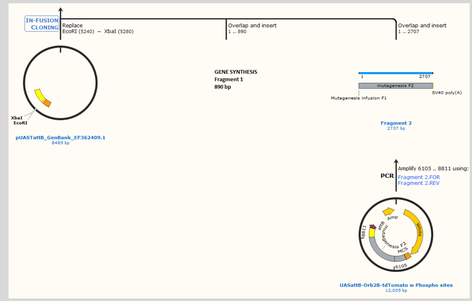
Results

MDSLKLPKANSATSSASGSNSNLSGSTSASASAATSPTSSGTAVGGIL
SGAPKPPGLGSSTPISVRFNANEESLDDILQSFHHSKHSPSGGASGGG
DASPTSNLLGMKNNGLGLVVGNCDSLSSSPSQPQMHAGSASLFGN
DEVSLRNNFMQAGGFFNRSKCGGLPNLNLNKPPQLHQQQHQQHQ
QHQQQQQLHQHQQQLSPNLSALHHHHQQQQQLRESGGSHSPSS
PGGGGGGSPYNGSQAGCSSGGISPIPPQMGVSPKYRRSISFPIKGNS
PTAIYGNMHMDGMGSGHMNI

Figure 5. Orb2 amino acid sequence with possible phosphorylation sites marked in red. The 5 serine and threonine sites were identified through Harvard's Mass Spectrometry and Proteomics Resource Laboratory using mass spectrometry. These 5 phosphorylation sites were isolated through comparison with a phosphorylation site analysis of an empty vector. The goal will be to mutate all possible phosphorylation sites into alanine (Phospho-defective mutant) or aspartate (Phospho-mimic mutant).

Future Direction

PCR will be performed on Orb2b::tdtomato to amplify a fragment containing all identified possible phosphorylation sites. Gene synthesis will used to create another sequence that will also be amplified through PCR. These two sequences will be added to a plasmid cut with restriction enzymes. In-Fusion will be used. This will allow us to mutate all possible phosphorylation sites into alanine (Phospho-defective mutant) or aspartate (Phospho-mimic mutant). The SDS-PAGE and Western blot results of these constructs will give us a better understanding of the relationship between Orb2 and Wallenda.



References/Acknowledgements

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