

## INTRODUCTION

### R7RGS Protein Family

- RGS proteins are regulators of G protein signaling.
- R7 group of RGS proteins have been implicated in many neuronal processes such as vision, motor control and reward behavior.
- It has been reported that R7BP plays a part in:
  - catalytic activity
  - subcellular targeting
  - protein expression levels of R7 RGS complexes.
- R7BP is a R7 RGS family binding protein that has been reported to form trimeric complexes with RGS7 and Gβ5 subunits.

### Homer Family Proteins

- Thought to act as scaffolds that enable the linkage of various proteins in close proximity, thereby facilitating signal transduction.

### Yeast Two-Hybrid

- A technique used to discover protein-protein interactions by testing for physical interactions. A yeast two-hybrid screen with R7BP pulled out Homer1a as a potential interacting partner.
- In an attempt to confirm this interaction, an immunoprecipitation with R7BP and Homer was performed in CHO-K1 cells stably expressing FLAG-R7BP.

## Homer1a as a Potential Interacting Partner with R7BP.

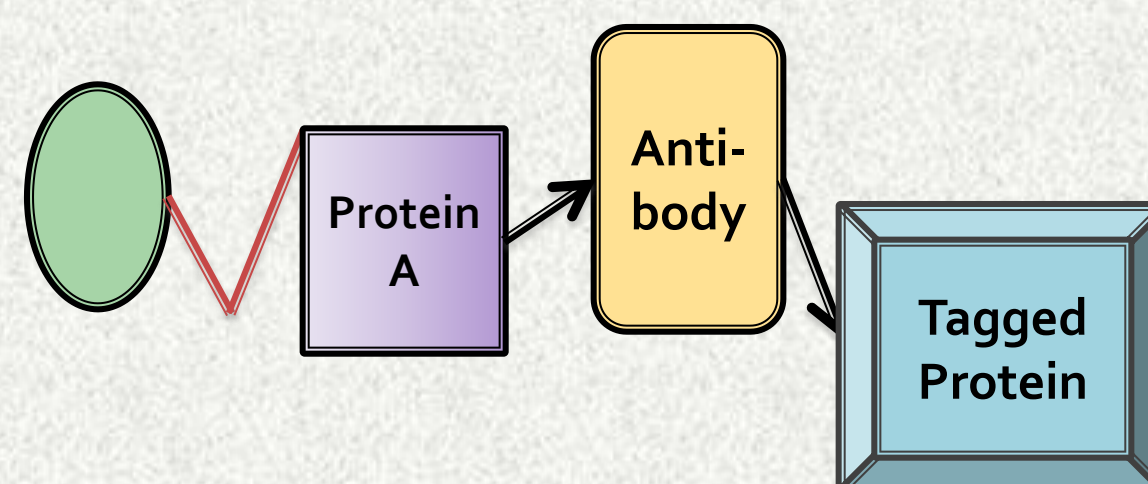
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## Immunoprecipitation

- A technique that precipitates a protein antigen out of solution using an antibody that specifically binds to that particular protein. This process is used to isolate a particular protein from a sample.



## METHODS

Grew CHO-K1 cells stably expressing FLAG-R7BP.

Transiently transfected myc-Homer, RGS7-YFP and Gβ5 plasmid DNA into CHO-K1 cells using Fugene 6 Reagent.

Performed immunoprecipitation experiment on lysed cells with: myc, FLAG and GFP antibodies, to determine if interaction with antibodies occurred and could be pulled out.

Ran protein gel of samples with SDS-PAGE and transferred to nitrocellulose for western blot.

Performed various western blots to test for protein interactions.

## RESULTS

Figure 1: Optimization of anti-myc antibody

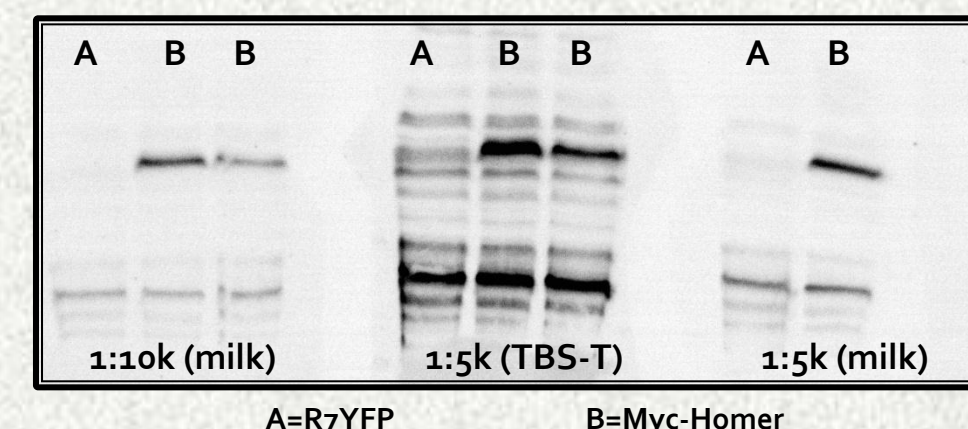
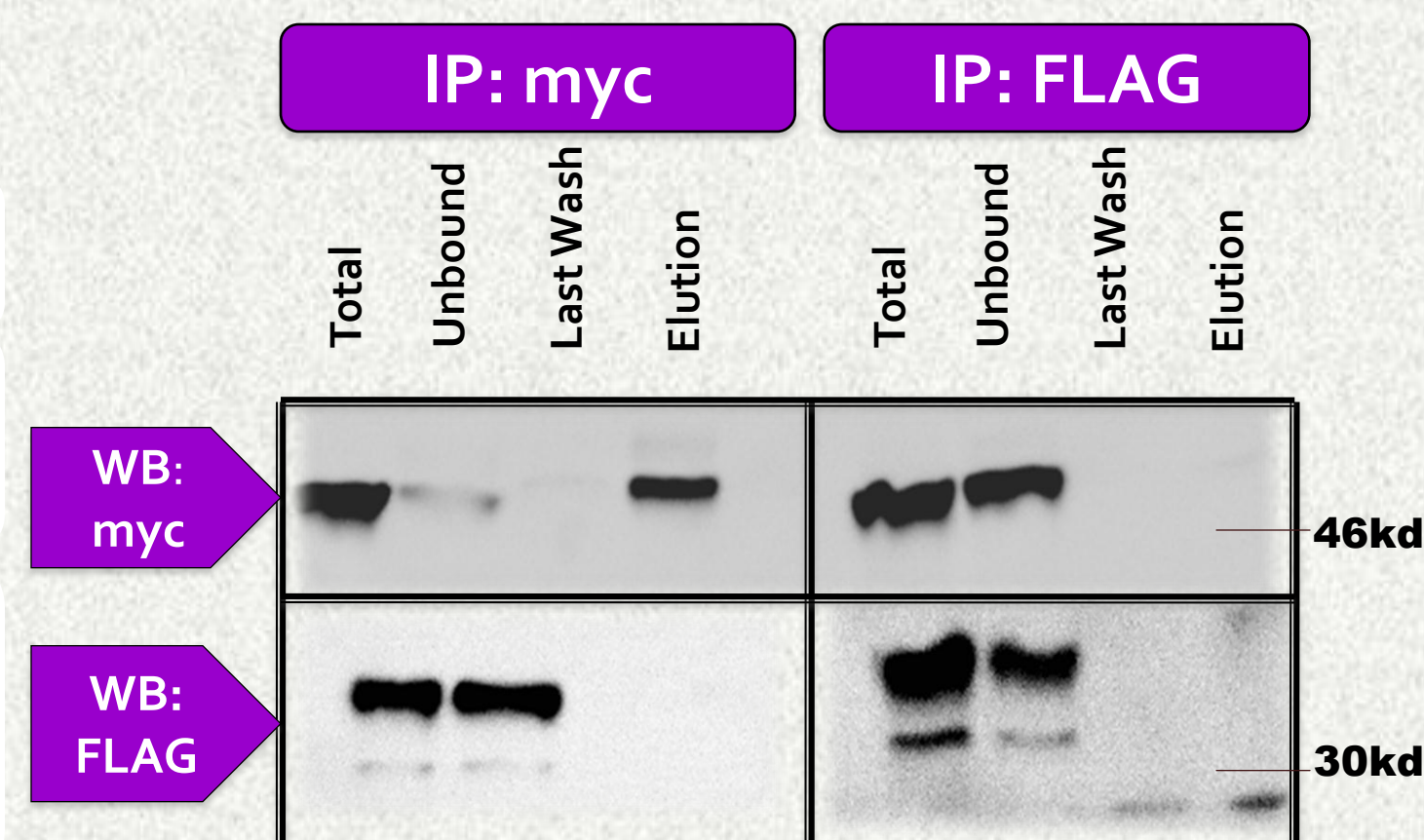


Figure 2: Co-immunoprecipitation of myc-Homer and FLAG-R7BP



Myc-Homer cDNA was transfected into CHO-K1 cells (stably expressing FLAG-R7BP). Cell lysates were immunoprecipitated with myc and FLAG antibodies.

## Future Plans

Repeat experiment with different cells. (HEK 293 cells)

Attempt experiment with different tags on proteins or using endogenous proteins.

## CONCLUSION

Flag IP didn't work despite the fact FLAG-R7BP is stably expressed in CHO-K1 cells. (Tag epitope may be hidden due to folding.)

Myc IP worked well. No interaction was detected between myc-Homer and FLAG-R7BP.

## REFERENCES

Shiraishi-Yamaguchi, Yoko, and Teiichi Furuichi "The Homer Family Proteins." *Genome Biology* 8.2 (2007): 206.1-206.9. Web. 25 May 2010. <<http://genomebiology.com/2007/8/2/206>>.

Anderson, Garret R., Ekaterina Posokhova, and Kirill A. Martemyanov "The R7 RGS Protein Family: Multi-subunit Regulators of Neuronal G Protein Signaling." *Humana Press* 54 (2009): 33-46. Print.