Methods for Resolving Post-Translational Modifications of Giant Ankyrin G, the Master Organizer of the Axon Initial Segment

Michael Liou, [1]Kathryn Walder, [1,2]Vann Bennett
[1]Department of Cell Biology, Duke University, Durham, NC, [2]Cell Biology, Howard Hughes Medical Institute

Abstract

The axon initial segment is a site of signal integration and modulation in vertebrate neurons. Giant Ankyrin G (AnkG) is the master organizer of the axon initial segment. AnkG contains a vertebrate specific giant 7.8-kb exon and is necessary for assembly of the axon initial segment. Genetic variations in AnkG have been implicated in major psychiatric disorders. Giant AnkG has been heretofore uncharacterized in terms of post-translational modifications due to technical challenges. Our lab has developed techniques to isolate the giant 480 kDa isoform Ankyrin G in sufficient quantities to analyze by mass spectrometry. Using a novel transgenic mouse that tags endogenous Ankyrin G with GFP under control of Cre-inducible recombination, we can now isolate giant AnkG from defined neuronal populations. This sets the stage for the first study of AnkG regulation by phosphorylation and potentially other post-translational modifications (PTMs).

Challenges

1 - Low Abundance
2 - Highly Protease Sensitive
3 - Difficult to Solubilize
4 - Many Ankyrin G Isoforms

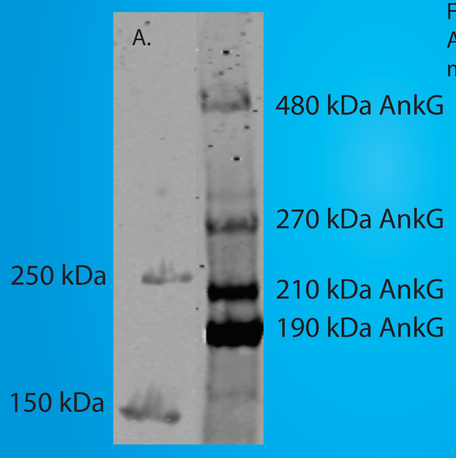


Figure A: Western blot image of Ankyrin G isoforms from PND 100 mouse brain.

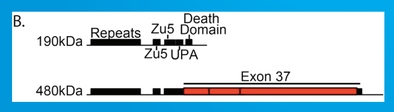


Figure B: Schematic of 190kDa and 480kDa Ankyrin G including giant exon 37.

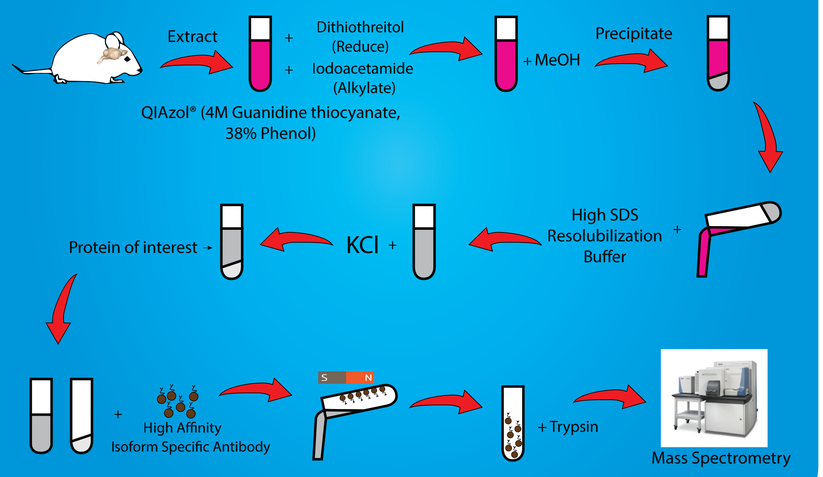
Approach

1. High affinity, isoform specific antibody

2. RNA isolation inspired conditions coupled with dithiothreitol and iodoacetamide to reduce and alkylate thioproteases.

Methods

High Quantity Isolation of Protease-Sensitive Protein for Mass Spectrometry



Results

Successful Isolation of Giant AnkG in High Quantity

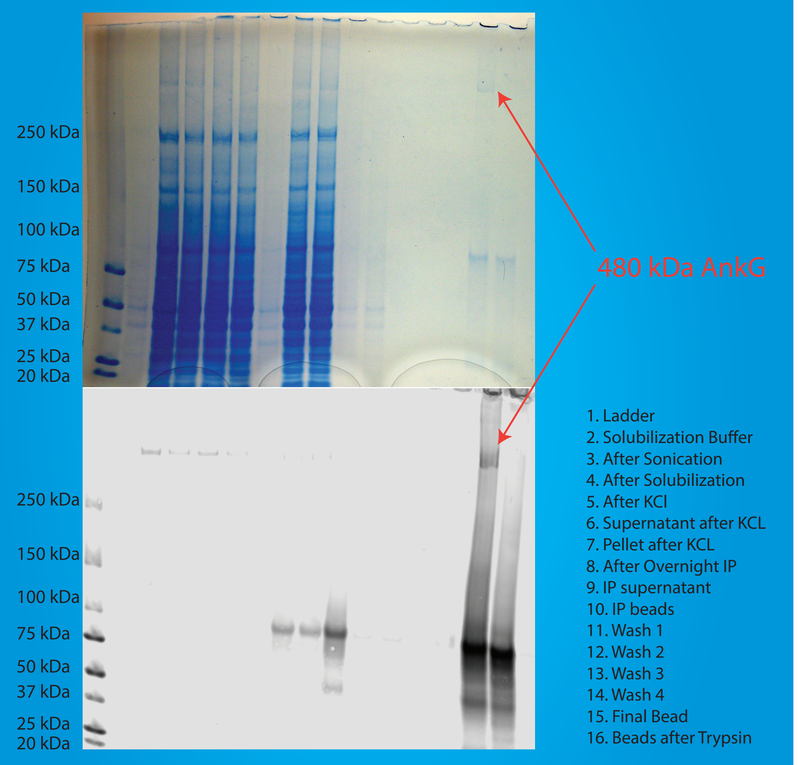


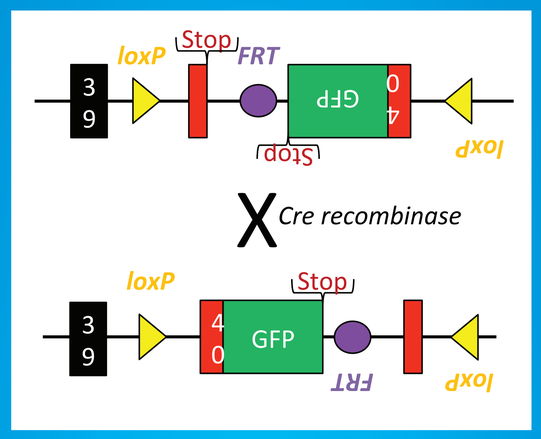
Figure: Colloidal Coomassie Blue staining of 480 kDa and Western Blot probed with Giant AnkG specific antibody of AnkG Isolation protocol including steps leading up to final purification. Estimated quantity is ~100 ng of purified protein.

Conclusions

• Isolating large quantity of isoform specific protein allows further analysis of PTMs by mass spectrometry. An estimated 1 picomol of protein was purified which is sufficient for mass spectrometry.

Future Directions

Conditional Expression of GFP-tagged Ankyrin G in Transgenic Mouse



Transgenic Mouse Cre-Lox recombination inversion design of AnkG with loxP site between exons 39 and 40. GFP can now be turned on endogenously by Cre recombinase.

Characterization of Neuronal Ankryin G Tagged with GFP

Using different promotors for Cre-Recombinase GFP tagged Ankyrin G can be targeted to specific neural populations.

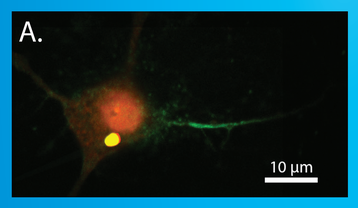


Figure A - Localization of GFP tagged Ankyrin G to axon initial segment in cultured hippocampal neuron transfected with Cre.

General Post-Translational Modifications Detection

Previous work in our lab has characterized the phosphorylation profile of AnkG. Reliable repetition of these techniques allow us to study other PTMs such as:

- Ubiquitination
- Acetylation
- Methionine Oxidation

Behavioral and Environmental Effects on Post-Translational Modifications

Potential future studies include effects of the following on PTMs:

- Sleep Deprivation
- High Stress
- Ethanol
- Valium

Acknowledgment

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References

Jenkins PM, Kim N, Jones SL, Tseng WC, Svitkina TM, Yin HH, Bennett V. Giant ankyrin-G: a critical innovation in vertebrate evolution of fast and integrated neuronal signaling. Proc Natl Acad Sci U S A. 2015 Jan 27;112(4):957-64. doi: 10.1073/pnas.1416544112. Epub 2014 Dec 31. PubMed PMID: 25552556; PubMed Central PMCID: PMC4313853.