Analysis of Interacting Proteins of Zinc Finger Protein 410

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Introduction

Based on our previous publication, we discovered that a transcription factor, Zfp410, was expressed in the brain and associated with genes that are controlled by folic acid. Although the expression of this transcription factor is not regulated by folate, this might suggest that it plays a role in exerting transcriptional effects in a diet-dependent manner. In order to further study Zfp410, we overexpressed the protein from *E. coli* and made a custom antibody. The antibody was then characterized and utilized in co-immunoprecipitation. This project utilizes a custom Zfp410 antibody in order to identify its interacting partners.

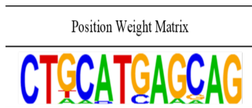
Position Weight Matrix	Type	*p*-Value	% Target	% BG	Fold	Match / Detail
	*de novo*	1e-12	5.54	0.31	17.9	MafB Homer (0.591)
	*de novo*	1e-12	6.27	0.49	12.8	Zfp410 Jaspar (0.643)

Figure 1. Sequence motifs enriched in folate-regulated genes.
Two sequences were enriched in the genes regulated by folic acid in the brain. These sequences are putative binding sites for Zfp410.

Past Work

During the fall of 2019, we successfully produced the recombinant protein Zfp410 by transforming a his-tagged form of its DNA sequence into E. coli cells. The protein was then purified with Ni-NTA resin, which binds the his-tagged protein. The result of the purification was successful demonstrated by SDS-PAGE and Coomassie blue stain. The protein was then sent to PRF&L for antibody generation.

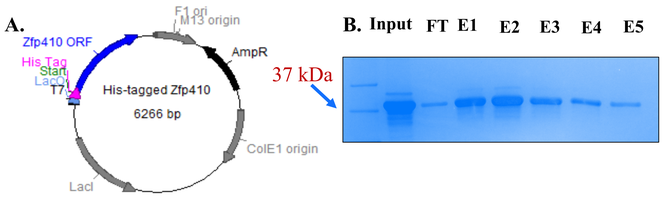


Figure 2. Zfp410 protein purification
(A) Genomic sequence that codes for Zfp410 was cloned into vector pET45b(+) with a His Tag resulting in a plasmid with a total of 6266bp; the total length of Zfp410 is about 1030bp. (B) Purification of denatured mouse Zfp410 using NTA resin.

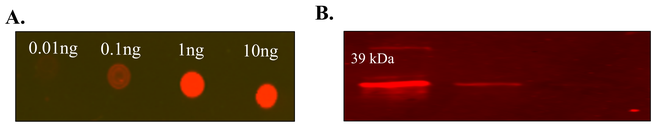


Figure 3. Characterization of Zfp410 antibody by dot blot and western
(A) The dot blot of antibody's affinity test using different concentrations of Zfp410 protein (0.01, 0.1, 1, and 10 ng). (B) SDS-PAGE analysis was used to test the same antibody as well within same four concentrations of pure Zfp410.

Experimental Plan/Aims

Experimental Plan
After successfully characterized the custom polyclonal antibody from PRFL, we were able to further study the function of ZFP410 by identifying its interacting protein partners using co-immunoprecipitation. By utilizing Zfp410 antibody, we were able to visualize all protein complexes that had been formed and then separated using SDS-PAGE and silver stain analysis. The proteins of interest were cut from the gel and are sent off for mass spectrometry analysis. The data was then analyzed and resulted four possible Zfp410 binding partners.

Aims
- Isolation of Zfp410 binding partners by co-immunoprecipitation, silver stain, and western blot.
- Identification of interacting partners of Zfp410 with the use of tandem mass spectrometry.
- Analysis of ultimate Zfp410's binding partners by their antibodies.

Isolation of Zfp410 binding partners

To isolate the Zfp410 binding proteins, we performed co-immunoprecipitation assay on two separate gels in which one was followed by silver stain analysis and the other was western blot. Based on comparison between each gel, there are two bands ~80 kDa, one band ~150 kDa, and the other band ~250 kDa that we believe to be **potential Zfp410 interacting proteins** that do not show up by western but do by silver stain. There is an additional band ~100 kDa that we believe to be a modified version of Zfp410 that appears by western blot.

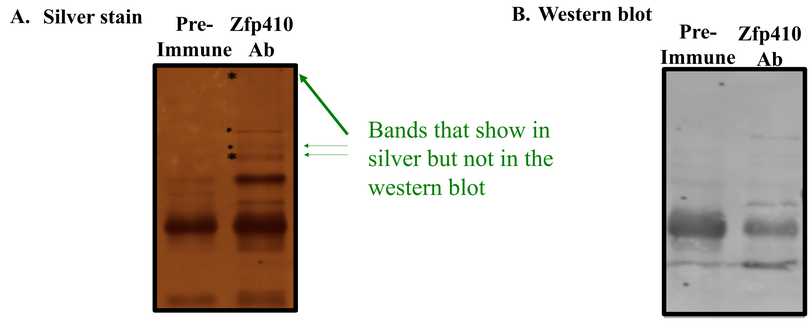


Figure 4. Silver stain and western blot analysis after co-IP assay on SDS-PAGE gels
(A) Silver stain on a SDS-PAGE gel from the co-IP assay that uses Zfp410 spiked Hela tissue extract. The custom Zfp410 antibody was used to precipitate out Zfp410's potential binding partners. (B) Western blot analysis from the same co-IP assay.

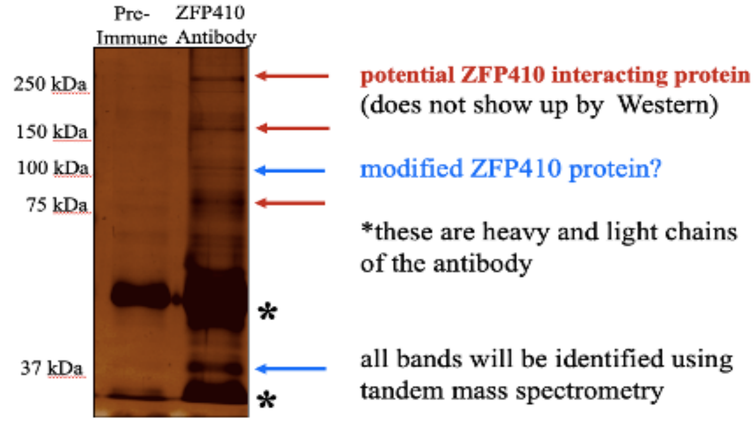


Figure 5. Silver stain of ZFP410 interacting proteins from HeLa extract
A co-immunoprecipitation assay was used to isolate potential ZFP410 interacting proteins. There are 4 bands that we believe to be ZFP410 interacting proteins and 1 that may be a modified version of ZFP410.

Identification of Zfp410 interacting partners via Mass Spectrometry

The co-IP assay demonstrated four distinctive bands that represent potential Zfp410 binding partners. All four bands were cut off from the gel and sent off for mass spectrometry in order to further identify proteins samples presented in the bands.

Band #	Protein name	Gene name	Mol. Weight(kDa)	Intensity	peptides
1	Heat shock protein 90-alpha	HSP90AA1	84.772	72835000	7
2	Heat shock protein 90-beta	HSP90AB1	83.466	11619000	6
3	Gephyrin	GPHN	79.748	4087500	2
4	Heat shock protein 70	HSP70	71.027	4592200	3

Figure 6. Mass spectrometry data of the four bands from co-IP analysis
Several proteins were identified in each band by the mass spectrometry. One distinctive protein that might be associated with cognition and unique was selected from each band as the major potential Zfp410 binding partner. The identity, size, and the intensity of each protein are listed in the chart above.

Analysis of ultimate Zfp410 binding proteins

The four proteins identified by the mass spectrometry needed to be tested in order to confirm interaction with Zfp410. We have purchased polyclonal antibodies that target each of these four proteins, and they will be used in western blot after the co-immunoprecipitation with Zfp410 antibody. Our expected result is shown below.

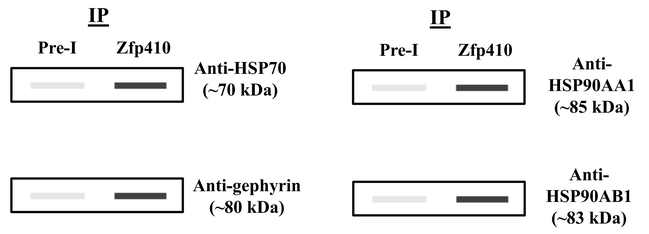


Figure 7. Possible co-IP result using each of the four antibodies
The co-IP of pre-immune vs. Hela tissue extract demonstrates enrichment of Zfp410 binding partners by their antibodies.

Future Work/References/Acknowledgements

Having identified the composition of ZFP410 complexes by its antibody, the next main goal is to examine these transcription factor complexes on DNA in order to identify Zfp410's binding site on DNA sequence using chromatin immunoprecipitation.

References

1. Abigail Lawton, C.M., Caleb Schreiner*, Chris Schreiner*, Jacqueline Bauman, Britton Upchurch, Feifan Xu, Michael S. Price, and Gary D. Isaacs, *Folate-dependent cognitive impairment associated with specific gene networks in hippocampus.* Journal of Nutrition. 2020.

Acknowledgements

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