Kerry Zimdars
Specific Aims Final

Schizophrenia is a complex disease, meaning that the development of this disease is associated with multiple gene as well as environmental factors. Due to its complex nature, schizophrenia has a wide variety of symptoms, including communication and brain stress.[6,7] Although the exact cause(s) of schizophrenia remain unknown, mutations in the Topoisomerase 3 Beta (TOP3B) gene has been shown to increase the risk of schizophrenia.[2] The main function of topoisomerases, such as TOP3B, is to unwind highly condensed DNA to make small segments of DNA accessible so that processes such as DNA replication can occur.[3] Due to its important interactions with DNA, TOP3B is highly conserved in organisms ranging from mammals to plants. The TOP3β protein has also recently been found to directly bind RNA and associated proteins.[4] *However, the pathways that TOP3β influences through RNA and protein binding remains unknown.*

Here, we will test the hypothesis that wildtype TOP3β directly binds proteins and RNAs that are involved in stress pathways and that these are interrupted when TOP3B is mutated. This idea is supported by recent findings that TOP3β localizes to cytoplasmic compartments containing specific RNAs following cellular stress.[4] TOP3β was also directly binds to TDRD3, a protein that plays a role in the formation of RNA stress granules, aggregates of RNA formed when the cell in under stress.[4,8,9] In addition, TOP3β directly binds FMRP, a protein also moves to stress granules following stress.[4,1] These findings suggest the involvement of TOP3β in stress pathways, but the mechanism of such involvement remains unclear. The **primary goal** of this research is determine the function of TOP3β in schizophrenia related pathways, such as the stress pathway. The long-term goal is to improve our understanding the relationship between TOP3B and schizophrenia on a cellular level.

Specific Aim: To identify functional similarities among organisms with complex and basic nervous system. **Hypothesis:** There will be a functional similarity among these organisms correlated to important pathways related to schizophrenic symptoms that TOP3β affects. **Approach:** To perform a MEME motif search between Homo sapiens, C. elegans, Arabidopsis thaliana, and Oryza sativa. This group was chosen due to the varying degrees of neuronal development. The recovered motifs will then be analyzed for GO terms in GOMO.

Specific Aim: To identify novel protein interactions of TOP3β in the nervous system. **Hypothesis:** TOP3β will interact with proteins involved in stress pathways. **Approach:** To Perform Tandem Affinity Purification (TAP) with TOP3β as the protein of interest in C. elegans. Mass Spectrometry (MS) will be performed on individual bands after SDS-PAGE and in-gel trypsin digest. Brain-specific interacting proteins, found through cellular component Gene Ontology (GO) terms, will be analyzed and compared to the GO terms found in the first aim. C. elegans is an ideal organism for this proteomic study due to its sequenced genome and simple neural network.

Specific Aim: To determine if mutations in TOP3β affects pathways of interacting proteins identified in aim two and if a schizophrenia-like phenotype results. **Hypothesis:** Mutated TOP3β will result in increased cellular stress and RNA granule formation due to pathway interruption. **Approach:** TOP3β RNAi will be preformed on C. elegans; RNAi will be introduced through E. coli feeding. MS will be performed, compared to wildtype protein quantities, and changes grouped by GO terms. C. elegans will be stained for RCK1, a helicase found in stress granules, to quantify the granule amount, which will be compared to levels in worms without RNAi.[5] RNA-Seq will be performed between wildtype and RNAi C. elegans to quantify RNA changes within the stress granules.

A comprehensive study of the protein interaction of TOP3β as they relate to schizophrenia has not been performed to date. The outcomes of this research are expected to identify how TOP3β may lead to schizophrenia symptoms. This knowledge could aid in developing new, more effective treatments for schizophrenia.

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References

1. Soong, H. K, *et. al.* Fragile X Mental Retardation Protein Shifts between Polyribosomes and Stress Granules after Neuronal Injury by Arsenite Stress or In Vivo Hippocampal Electrode Insertion. JNEUROSCI. 2006, Mar; 26(9): 2413-2418; doi: 10.1523/JNEUROSCI.3680-05.
2. Stoll, G., Pietilainen, OP., Linder, B., *et. al.* Deletion of TOP3β, a component of FMRP-containing mRNPs, contributes to neurodevelopmental disorders. Nat. Neurosci. 2013 Sep;16(9):1228-37. doi: 10.1038/nn.3484. Epub 2013 Aug 4. PubMed PMID: 23912948.
3. McDowall, J. InterPro. (2014). *DNA Topoisomerase.* Retrieved Apr. 9, 2014 from http://www.ebi.ac.uk/interpro/potm/2006_1/Page1.htm
4. Nott, A. The TOP3B way to untangle RNA. Nat. Neurosci. 2013 Sep: 16(9):1163-64.
5. Anderson, P. and Kedersha, N. Stress Granules. 2009. Current Biology. Cell Press. Vol. 19 No. 10. Pp.R397-R398. http://anderson.bwh.harvard.edu/03-Lab%20Publications/03-pdf%20publication%20links/19_Curr_Biol09-May.pdf
6. Mayo Clinic. (2014). *Disease and Conditions: Schizophrenia.* Retrieved Feb. 5, 2014 from http://www.mayoclinic.org/diseases-conditions/schizophrenia/basics/definition/con-20021077
7. Fillman S.G. *et. al.* Markers of inflammation and stress distinguish subsets of individuals with schizophrenia and bipolar disorder. Transl Psychiatry. 2014 Feb 25; doi: 10.1038/tp.2014.8. http://www.ncbi.nlm.nih.gov/pubmed/?term=Fillman+markers+of+inflammation+and+stress+schizophrenia
8. Kayali F, Montie HL, Rafols JA, DeGracia DJ. Prolonged translation arrest in reperfused hippocampal cornu Ammonis 1 is mediated by stress granules. 2005. *Neuroscience* **134** (4): 1223–45. doi:10.1016/j.neuroscience.2005.05.047. PMID 16055272.
9. Genecard. Last updated Oct. 23, 2013. *Tudor Domain Containing 3*. Retrieved Apr. 9, 2014. http://www.genecards.org/cgi-bin/carddisp.pl?gene=TDRD3