

TorsinA, a Dystonia Linked ATPase, & Its Mutant, TorsinA Δ E

Forrest Kohl

Copyright May, 2015, Forrest Kohl & Koni Stone

Dystonia, the third most common movement disorder, is a disorder that causes involuntary movement in the affected person. Type one dystonia, also referred to as DYT1 and early onset dystonia, is the most common form of dystonia.¹ It severely limits the affected person's ability to perform day to day activities through larynx muscle contraction, arm & leg contractions or other contractions that would normally be voluntary. This can also lead to the person feeling fatigued if the contractions last an extended period of time and, in tandem, can lead to poor muscle development. This condition is caused by the deletion of a GAG codon in the TOR1A gene which holds the information for creating torsinA, an ATPase that is known to interact near the endoplasmic reticulum (ER). The GAG deletion causes the torsinA protein to lack a glutamate which severely limits its ability to bind with its binding partners & causes other problems related to the binding. This mutant is named torsinA Δ E and it, along with its wild type, are being investigated to learn more about torsinA's function and its link to dystonia.

Because dystonia is a movement disorder it would be expected that torsinA should play some role in the nervous system or have an interactions within neural cells. By using computer modeling, immunofluorescence, and immunoblotting of torsinA wild type and torsinA Δ E in neural cells the mutation's impact on neural cell function was investigated.² Lentivirus vector plasmids were used to transfect cells with both the wild type and mutant torsinA variants. The cells were also transfected to produce gaussia luciferase and firefly luciferase to analyze torsinA Δ E's affect on protein production. Two sets of cells, differentiated (neuronal) and undifferentiated, were then immunostained in order to detect torsinA, PDI, and β -tubulin.

The results show that the cells with torsinA Δ E have strange spacing between the inner and outer nuclear membrane that is much larger than the spacing between membranes on the wild type cell. The analysis of neurite extension, which is essential for neuron function, shows that torsinA Δ E cells are barely above the starting length while the wild type grew two and a half times longer than their starting point. When the amounts of gaussia (G) and firefly (F) luciferase are compared in each cell, the wild type has around 40 units G:F while the mutant has around 25 units G:F. This indicates that the mutant torsinA has an impact on protein synthesis within neuronal cells. Overall, the mutant has a negative impact on several essential neuronal cell functions which could be causing dystonia.

LULL1 and LAP1C are both membrane proteins that are known to normally interact with torsinA. LAP1C is also known to interact with torsinA in neuronal cells. To test for the impact of the torsinA Δ E mutation, cells are transfected with plasmids in order to synthesize LULL1, LAP1C and torsinA.³ Two sets of cells are used: 1 with the mutant and 1 with the wild type. Some mutant varieties also had more than the Δ E mutation to observe the effects multiple mutations had on the cells. Before lysis, some cells undergo immunofluorescence to detect the distribution of each protein in the cells. These cells are then lysed and separated out via an SDS-PAGE and the identity of each protein is verified by mass

spectroscopy. An immunoblot is performed using three samples for both binding partners: the unseparated lysate, unbound protein and bound protein. (Six sets in total)

The immunoblot tests show a clear problem with torsinA Δ E's binding capabilities. The blots for torsinA have almost no unbound protein for LULL1 and only a small amount for LAP1C. This is in stark contrast to the mutant which has almost no bound for LULL1 and no bound for LAP1C. It was also observed that the loss of a glutamate does not trap torsinA in its ATP bound state which makes sense because torsinA prefers to interact with its binding partners in its ATP bound state. Other mutations of torsinA often have the potential to trap torsinA in its ATP bound state and it was shown that the loss of a glutamate will supersede these mutations. As seen from the results, the glutamate deletion causes torsinA to become unstable and changes it enough to render any other mutation meaningless.

TorsinA Δ E is not the only mutant form of torsinA that has adverse effects. Another mutant which removes cysteines from the torsinA can impact its binding. The cysteines in question operate as redox sensors in the protein.⁴ Tests using LAP1C & LULL1 analyze the binding with different variants of torsinA: the wild type, torsinA Δ E, torsinACS (torsinA without the redox sensing cysteines), and torsinA Δ E/CS (a combination of both mutants). Immunofluorescence is used to observe the location of each torsinA in the cells. Immunoblots are subjected to a pulse-chase analysis to measure the amount of glycosylated protein over time for each torsinA variant. Separate immunoblots test for torsinA binding to LAP1 and LULL1.

It is deduced once again that the torsinA Δ E has impaired binding capabilities when compared to the wild type as is indicated by the lack of a bound blot for torsinA Δ E. It is also shown, via the pulse chase analyses, that the lack of the cysteines does impact torsinA's redox sensing capabilities. More importantly, the torsinA Δ E mutant also has redox sensing problems even if the cysteines were present. This means that the torsinA Δ E mutation most likely causes a structural or conformational change which

impacts its function. It is also deduced from this that the C-terminal portion of torsinA Δ E most likely plays an important role in the binding process.

TorsinA is known to interact around the endoplasmic reticulum and is found to be distributed in the brain around the same regions as printor.⁵ In order to analyze the binding capabilities of torsinA with printor, torsinA/printor transfected cells (wild type and Δ E) are immunoprecipitated via bead antibody complexes. The precipitated antigen-antibody complexes are then purified via SDS-PAGE, after which pre SDS-PAGE aliquots are used in an immunoblot. A separate analysis is prepared via subcellular fractionation to determine where printor and torsinA interact. In this test, calnexin is used to identify the fraction that contains the smooth and rough endoplasmic reticulum. Immunofluorescence tests are also conducted to monitor printor and torsinA locations within the cells as well as a separate test to observe torsinA's positioning from the ER for different variants compared to KDEL, a protein used to compare distances. KDEL is a sequence of amino acids commonly found on ER interacting proteins which draws them towards the ER or keeps them from secreting out. KDEL proteins tend to stay close to the ER, therefore they make for good markers when determining a proteins localization patterns.

Printor is found to bind with torsinA around the endoplasmic reticulum when it was in an ATP free state which would indicate it as a cofactor rather than a substrate. Immunoblot analyses from the subcellular fractionation indicate that torsinA and printor do interact around the ER as was suspected. The mutant torsinA Δ E is also tested with an immunoblot and it is once again observed to have trouble binding, this time with printor. The immunofluorescence tests for torsinA's position indicate that mutant torsinA had a tendency to dislocate from the ER to the nuclear membrane (NE) which may further disable torsinA from binding to printor. The reason for torsinA's movement from ER to NE cannot be determined with the data collected.

From these examples of torsinA binding it can be seen that the mutant most likely suffers from an issue where it can not bind to its cofactors or its substrate. The potential reasons given rely on a structural change caused by the loss of the glutamate residue which also causes redox-sensing cysteine residues to become inert. Another possible explanation is the formation of a dimer which is not formed by normal torsinA.² Using immunoblots with unbound and bound forms of torsinA as well as a computer model of torsinA's structure and molecular dynamics it is believed that the mutant forms tested most likely form a dimer.

This formation has the potential to block the binding site of torsinA making it unable to bind. However, this is most likely not the case, because in the same study different mutants of torsinA were found to dimerize and most of them were not linked to dystonia in any way meaning that dimerization is most likely not the cause of torsinA Δ E's problems. This strengthens the hypothesis that the glutamate deletion causes a shape change which limits torsinA's binding and may indicate a dimer structure isn't actually formed.

The issues with torsinA Δ E may not be limited to its binding with substrate. It is possible that the generation and synthesis of torsinA Δ E is somehow flawed.⁶ Bip, a folding chaperone known to reside around the ER, could be used in the folding of torsinA during or after torsinA synthesis. TorsinA and torsinA Δ E are generated in cells via a plasmid. A pulse-chase analysis, in which the amount of torsinA was measured, is done in order to test for the difference in stability between a protein with Bip present and one where Bip was not available; similar tests were performed with known cofactors of Bip. The cells were pulsed with methionine & cysteine and were chased by DMEM, a medium with amino acids and vitamins used to culture cells.

Results of the pulse-chase analysis indicate that both forms of torsinA have their stability damaged when no Bip is available during or after synthesis with only 60% of torsinA remaining after 90 minutes. Pulse-chase tests with cofactors have the same result. Interestingly, torsinA and torsinA Δ E are observed to have similar stabilities which indicates that torsinA Δ E's binding issues are not caused by torsinA Δ E being unstable.

From these results it is clear that Bip plays a role in torsinA folding. Bip is associated with the treatment of other neurological disorders such as Parkinson's disease. This means that Bip could be used as a therapeutic treatment for dystonia. The results surrounding torsinA's interaction with LULL1, LAP1, and Printor could lead to more treatment options if safe methods of restoring their binding capabilities are found and it is shown that they were the cause of the involuntary movements.

Overall torsinA remains a somewhat unknown entity whose exact function isn't clear. A lot of progress has been made in the last ten years surrounding which proteins torsinA interacts with and what effects the glutamate deletion has on those interactions but a clear path has not been found that links the inability to bind with involuntary movements. Further investigation into the full chemical interaction with torsinA and the neuronal proteins LAP1 seem to have the most promise as it is the most direct link that torsinA has to the nervous system.

Bibliography

- 1 Kustedjo K, Bracey M H, Cravatt B F TorsinA and its torsion dystonia-associated mutant forms are luminal glycoproteins that exhibit distinct subcellular localizations, *The Journal of Biological Chemistry*, (2000) **275**, 27933-27939
- 2 Hettich J, Ryan S, Norberto de Souza O, Timmers L, Tsai S, Atai N, da Hora C, Zhang X, Kothary R, Snapp E, Ericsson M, Grundmann K, Breakefield X, Nery F Biochemical and Cellular Analysis of Human Variants of the DYT1 Dystonia Protein, TorsinA/TOR1A, *Human Mutation*, (2014) **35**, 1101-1113. Molecular Neurogenetics Unit, Department of Neurology and Center for Molecular Imaging Research Department of Radiology, Massachusetts General Hospital and Program in Neuroscience, Harvard Medical School, Boston, Massachusetts
- 3 Naismith T, Dalal S, Hanson P Interaction of TorsinA with Its Major Binding Partners Is Impaired by the Dystonia-associated Δ GAG Deletion, *The Journal of Biological Chemistry*, (2009) **284**:41, 27866-27874. Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis Missouri 63110.
- 4 Zhu L, Millen L, Mendoza J, Thomas P A Unique Redox-sensing Sensor II Motif in TorsinA Plays a Critical Role in Nucleotide and Partner Binding, *The Journal of Biological Chemistry*, (2010) **285**:48, 37271-37280. Department of Physiology, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75390
- 5 Giles L, Li L, Chin L Printor, A Novel TorsinA-interacting Protein Implicated in Dystonia Pathogenesis, *The Journal of Biological Chemistry*, (2009) **284**:32, 21765-21775. Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia 30322
- 6 Zacchi L, Wu H, Bell S, Millen L, Paton A, Paton J, Thomas P, Zoiewski M, Brodsky J The BiP Molecular Chaperon Plays Multiple Roles during the Biogenesis for TorsinA, and AAA+ ATPase Associated with the Neurological Disease Early-onset Torsion Dystonia, *The Journal of Biological Chemistry*, (2014) **289**:18, 12727-12747. Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260