Processing of the Neuroligins proteins and autism-related mutations

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The α/β-hydrolase fold family is one of the largest families of proteins presenting significant structural homology with divergent functions: from catalytic hydrolysis to heterophilic cell adhesive interactions and hormone production. All the proteins of the family share a common three-dimensional core structure, containing the α/β-hydrolase fold domain that is crucial for proper protein localization and function. Mutations affecting the integrity of the α/β-hydrolase domain in conserved residues within of neuroligins, butyrylcholinesterase and thyroglobulin have been found associated with congenital disorders (De Jaco et al., Protein Pept Lett 2011). Characterization of the mutations found in the α/β-hydrolase fold domain shows that they mainly disrupt the architecture of the common structural domain, impairing trafficking along the secretory pathway and causing retention of the mutant protein in the endoplasmic reticulum (ER). This is indicating that the structural integrity of the α/β-hydrolase domain is required for the export of the protein from the ER (De Jaco et al., Protein Pept Lett 2011, De Jaco et al., submitted). The α/β-hydrolase fold domain of the postsynaptic adhesion molecules neuroligins (NLGNs) interacts directly with presynaptic ligands belonging to the neurexins (NRXNs) family. In the last years, studying processing of the NLGNs proteins has been particularly interesting in relation to autism spectrum disorders (ASDs). Molecular genetic studies on ASDs are focusing on alterations in the functioning of synapses with the synaptic proteins playing a central role. The NLGNs-NRXNs synaptic cell adhesion complex is the best-characterized pathway implicated in ASDs. Genetic alterations in the NLGNs genes have been identified in autistic patients, suggesting that impairments in synaptic adhesion might lead to deficits in brain development (Betancur et al., Trends Neurosci 2009). We have studied an autism-associated mutation, R451C in NLGN3. We have shown that the mutation induces local protein misfolding of the α/β-hydrolase fold domain of NLGN3 that ultimately results mostly retained in the ER. The mutation slows down protein processing in the HEK-293 cell line and trafficking in neurons with a small fraction still reaching the cell surface (De Jaco et al., JBC 2010). More recently we have been investigating whether the retention of the NLGN3 R451C mutant protein in the ER is determining the activation of an ER stress response. The Unfolded Protein Response (UPR) is the most important ER signaling pathway that regulates protein folding and processing capacity of the ER (Kaufman, Genes Dev 1999). UPR is initiated by three transmembrane ER proteins, PERK (PKR-like ER kinase), IRE1 (inositol requiring kinase 1), and ATF6 (activating transcription factor 6) that sense protein-misfolding and activate an ER-to-nucleus
signaling cascades in order to maintain homeostasis in the ER (Marciniak and Ron, *Physiol Rev* 2006). UPR activation has been detected in experimental models of neurodegenerative diseases (Matus *et al.*, *Curr Opin Cell Biol* 2011) but a UPR signaling pathway in ASDs has never been described. To evaluate whether the ER retention of the misfolded R451C NLGN3 mutant protein was determining the activation of the UPR, we have studied the ATF6 signaling pathway in the HEK-293 cell line. We have overexpressed in transient the NLGNs constructs together with a vector containing binding sites for ATF6, upstream of the luciferase reporter gene. Since these sequences are found in the promoters of most UPR target genes, the luciferase signal would indicate the activation of ATF6 and therefore and ER stress condition. Luciferase activity, measured at 24 and 48 hours after transfection shows that ATF6 activation is significantly higher for the mutant R451C NLGN3 compared to the wild type protein. We also studied IRE1 activation in response to accumulation of the R451C NLGN3 protein in the ER. Under ER stress condition, IRE1 activates the unconventional splicing of the mRNA of a transcriptional factor named XBP-1. We have studied the splicing of XBP1 mRNA by semiquantitative RT-PCR by and Real Time RT-PCR analysis, detecting both *unspliced* and *spliced* isoforms for XBP1. We have found that XBP1 splicing is enhanced in the presence of the R451C mutant compared to the wild type protein (manuscript in preparation).

From our results it is emerging that the autism-associated R451C mutation in NLGN3 is causing retention of the protein in the ER and this is determining an ER stress condition that results with the activation of ATF-6 and XBP-1, two of the UPR signaling pathways.

**Publications**


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**Collaborations**

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