

ISN symposium on “Membrane Contact sites in neurodegenerative disorders”

Dr. Alessandra d'Azzo, Chair and Organizer

The ISN Symposium was held in Milano on August 31st as part of the scientific program of the Glyco 25, International Symposium on Glycoconjugates that was attended by 504 participants from 44 countries. Although Saturday 31st was the last day of the meeting and a number of attendees had already left, the ISN symposium was attended by approximately 150-200 participants. Dr. Alessandra d'Azzo invited three prominent investigators, Drs. Benoît Kornmann, Marianna Leonzino and Thomas Simmen, who have a long-standing expertise in this field of research. She was pleased to ascertain that the subject of the symposium raised great interest and some of the attendees considered it one of the best sessions of the entire meeting.

The symposium started with an introduction to interorganellar membrane contact sites (MCS) given by Alessandra d'Azzo. She talked about the dynamic interplay among intracellular organelles that occurs at specific membrane tethering sites, where two organellar membranes come in close apposition but do not fuse. Such membrane microdomains allow for rapid and efficient interorganellar communication that contributes to the maintenance of cell physiology. MCS have now been identified that tether the extensive network of the endoplasmic reticulum (ER) membranes with the mitochondria, the plasma membrane (PM), the Golgi and the endosomes/lysosomes. The most studied are the MAMs, or mitochondria associated ER membranes, and the ER-PM junctions (PAMs) that share functional properties and crosstalk to one another. Specific molecular components that define these microdomains have been shown to promote the interaction in trans between these intracellular compartments and the transfer or exchange of Ca^{2+} ions, lipids, including glycosphingolipids (GSL), and metabolic signaling molecules that determine the fate of the cell. Given the multiple functions already assigned to MCS, d'Azzo emphasized that it is not surprising that pathological conditions interfering with the proper lipid and protein composition, number, and physical vicinity of the opposing membranes of the MCS can initiate a cascade of events resulting in loss of cells homeostasis and cell death.

She went on describing the major functions so far ascribed to the MCS. These include lipid trafficking and synthesis, Ca^{2+} and organelle dynamics. In brief, MCS provide a non-vesicular transport pathway for lipids and sterols, and enzymes for lipid modification reactions. Several conserved lipid-binding proteins were shown to regulate the ER-PM contact sites (PAMs), for example, in yeast and animal cells. Furthermore, the biogenesis of the mitochondrial membranes relies on precursor lipids synthesized at the ER and transferred via the MAMs into the outer and inner mitochondrial membranes. The best-known function of the MCS is Ca^{2+} flux. Several Ca^{2+} release and uptake channels at apposing membranes of contact sites have been identified; Ca^{2+} transfer across MCS is essential for cell signaling in animal cells, especially in the muscle and nervous system. The MCS formed between the ER and mitochondria (MAMs) provide a preferential way for the influx of Ca^{2+} into the mitochondrial matrix, which under physiological conditions regulates energy production, but under pathological conditions triggers cell death pathways. The PAMs regulate Ca^{2+} -dependent muscle contraction and depolarization, and store-operated-calcium entry in muscle and neurons. Lastly, d'Azzo reported on the recently discovered function of the MCS in regulating the assembly and disassembly of molecular motors at the ER-endosomes, ER-mitochondria, and lysosomes-mitochondria contact sites. Thus, MCS are also emerging as crucial regulators of endosome trafficking, mitochondrial and endosome fission and endosome maturation.

The meeting continued with a keynote lecture by d'Azzo, who talked about the involvement of MCS in the pathogenesis of the lysosomal storage disease GM1 gangliosidosis. The monosialylated

ganglioside GM1 is a major sialoglycan of neuronal PMs, present abundantly in membrane microdomains or rafts. Owing to its interaction with Ca^{2+} binding proteins and Ca^{2+} channels, GM1 modulates Ca^{2+} flux across the PM and interorganellar membranes, a process vital for neuronal communication, particularly at the synapses. Catabolism of GM1 is driven by the lysosomal β -galactosidase (β -GAL), a ubiquitous but differentially expressed glycosidase that targets with high affinity this ganglioside, one of its primary natural substrates. β -GAL deficiency in humans results in the progressive accumulation of undegraded GM1 in the nervous system, leading to the generalized neurodegenerative disease, GM1-gangliosidosis. In β -Gal^{-/-} mice, a faithful model of the disease, impaired lysosomal turnover of GM1 leads to its massive buildup at neuronal PMs and other intracellular membranes, particularly those of the ER. The d'Azzo lab previously demonstrated that GM1 clusters at the MAMs, altering Ca^{2+} flux between the opposing organelles, ultimately leading to UPR- and mitochondria-mediated apoptosis. Currently, her lab is investigating the downstream effects of GM1 accumulation at the PM, focusing on the PAMs. Evidence accumulated so far indicates that GM1 accumulation at the PAMs induces changes in several Ca^{2+} binding proteins that govern Ca^{2+} flux and Ca^{2+} -dependent signaling pathways. These studies have begun to address the need to maintain homeostatic levels of GM1 at organellar contact sites in order to ensure proper neuronal functions and prevent the sequence of events leading to neurodegeneration in GM1-gangliosidosis.

This keynote lecture was followed by the presentation by Dr. Benoît Kornmann. The Kornmann laboratory studies the ultrastructural organization of the cell and the biology of organelles. Eukaryotic cells are densely packed with macromolecular complexes and intertwining organelles, continually transported and reshaped. Kornmann discussed the intriguing features of organelles that avoid clashing and entangling with each other in such limited space. Mitochondria form extensive networks constantly remodeled by fission and fusion. His lab has shown that mitochondrial fission is triggered by mechanical forces. Mechano-stimulation of mitochondria - via encounter with motile intracellular pathogens, via external pressure applied by an atomic force microscope, or via cell migration across uneven microsurfaces results in the recruitment of the mitochondrial fission machinery, and subsequent division. The hypothesis is that the mitochondrial fission factor, owing to affinity for narrow mitochondria, acts as a membrane-bound force sensor to recruit the fission machinery to mechanically strained sites. Thus, mitochondria adapt to the environment by sensing and responding to biomechanical cues. The notion that mechanical triggers can be coupled to biochemical responses in membrane dynamics may explain how organelles cohabit the crowded cytoplasm in an orderly way.

The lecture of Dr. Marianna Leonzino, a postdoctoral research associate in the laboratory of Dr. Pietro de Camilli (Yale University, USA), focused on the role of lipid transporters localized at MCS, specifically on a class of lipid transporters called VPS13 proteins. VPS13 is an evolutionarily conserved, very large protein encoded by one gene in yeast and four genes in mammals. Mutations in each of the four human proteins cause severe neurodegenerative or neurodevelopmental diseases, but the localization and function of this family of proteins remained elusive until very recently. In yeast, Vps13 was shown to be localized at contacts between the vacuole and either mitochondria or the ER, and to have a partially redundant function with the ERMES, a protein complex with lipid transport properties that bridges the ER and the mitochondria. Her lab has recently shown that the N-terminal portion of Vps13 has lipid harboring and transport abilities, and that it contains a large hydrophobic cavity that can accommodate multiple lipids and thus account for these properties. Furthermore, VPS13A and VPS13C, whose mutations result in Neuroacanthocytosis and Parkinson's disease, two severe neurodegenerative conditions, bind to the ER via an interaction with VAP (an intrinsic membrane protein of the ER) and tether it to

other organelles. These findings identify VPS13A and VPS13C as lipid transporters at contact sites, implicating disruption of intracellular lipid homeostasis in neurodegenerative disorders resulting from their mutations. Interestingly, the N-terminal portion of Vps 13 shares homology with the N-terminal portion of the autophagy protein ATG2 and, accordingly, ATG2 has now been reported to also have lipid transport properties. The peculiar rod-like shape of the lipid transport portion of these proteins suggest that they may act as hydrophobic channels connecting two membranes.

The symposium concluded with the lecture of Dr. Thomas Simmen, which discussed the connection between ER-stress and the MAMs. An important property of MAMs is their functional connection with ER stress. This condition leads to tightening of the MAMs and an alteration of their proteome. Within this proteome, ER chaperones constitute an important group of proteins. ER chaperones often interact with calcium-handling proteins, but the significance of this interaction is not yet fully understood. The Simmen's laboratory has now discovered how the ER folding enzymes calnexin and TMX1 interact with the SERCA calcium pumps and how this interaction controls mitochondria metabolism and ER-mitochondria tethering. Simmen also discussed how other ER folding enzymes form protein complexes and determine ER-mitochondria signaling and metabolism, dependent on the functioning of multiple ER-mitochondria tethers.

The symposium was well received by all those who attended. Each invited speaker had an exceptional discussion following their presentations.

From the discussion it became apparent that many exciting questions remain to be addressed in this quickly evolving, interesting field:

1. Are there more contact sites that have not yet been described?
2. What are all the molecular tethers, spacers, and other contact residents?
3. What is the repertoire of functions carried out at contact sites?
4. How are contact sites regulated and co-regulated to maintain cellular homeostasis?
5. Are there other genetic defects of lysosomal catabolism that influence the function and structural characteristics of the MAMs or other MCS?
6. Is deregulation of signaling pathways at the MCS caused by their altered lipid composition, especially with regard to neurodegeneration?
7. Are there differences in the lipid and protein makeup of the MCS depending on the cell types or physiological state of the cells?
8. How can we monitor and assess lipid distribution at MCS?