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INIMEC-CONICET-Universidad Nacional de Córdoba

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Report CAEN Category 1C Return Home Grant (Application - December 2012).

Project Title: Role of MAP6d1 in Golgi apparatus organization and neuronal morphogenesis.

Responsable: Mariano Bisbal, PhD

Laboratorio de Neurobiología, Instituto de Investigación Médica Mercedes y Martín Ferreyra, (INIMEC-CONICET, Universidad Nacional de Córdoba), Córdoba, Argentina.

To whom it may concern,

I am glad to report the activity carried out during my returning to my home country to develop my scientific career and setting my research laboratory. During this period the support provided by ISN under the CAEN Category 1C Return Home Grant has been very meaningful and had an appreciable impact on this difficult early career stage.

After receiving my PhD in Biological Sciences in 2009 in the Universidad Nacional de Córdoba, under the supervision of Dr. Alfredo Caceres, I moved to Grenoble, France where I worked as a postdoctoral fellow at the Grenoble Institute des Neuroscience. At the beginning (2009-2010) I participated in the NeuroFETs Project, a multi-disciplinary program directed by Dr. Catherine Villard. During this period I collaborated with physicists developing a non-specific poly-L-lysine micropatterns in order to manipulate neuronal shape, where we study how external geometrical constraints applied to neurons may modulate axonal polarization in vitro. Then, I made a second post-doc (2010-2012) under the direction of Dr. Annie Andrieux, with whom I maintain a close collaboration and also support me to come back to Argentina. During this postdoc, I studied the role of the microtubule associated protein MAP6 in dendritic development. During this period I obtained an extensive training in several techniques and proceedings, including microscopy, biochemistry, molecular and cellular biology. In 2012, finally I return home to Argentina, where I obtain a Return Home Postdoctoral Fellowship from National Research Council (CONICET) (Argentina) to start working in the laboratory of Dr A Caceres who kindly supports my position in Argentine and provides me significant mentoring. Then in 2014, I was appointed as an Assistant Investigator, which is a full-time research position from CONICET. With this position I am setting my laboratory in the Instituto Ferreyra (INIMEC-CONICET-UNC) which is a CONICET and National University of Cordoba University Institution, located in Cordoba, Argentina. I am also teaching cell biology at the Instituto Universitario de Ciencias Biomedicas of Cordoba (IUCBC). The financial support received from ISN was also very important during this period to settle up my own lab and to encourage continued doing research work at my home place. I also have to mention the support from the National Research Council (CONICET) for the funds to move me and my family back to Córdoba.

Once in Argentina I started my activities as a postdoctoral fellow, under the supervision of Dr Alfredo Caceres a world recognized Neurobiologist. He was kind to allow me to establish my independent lines of research and at the same time providing insights, mentoring and lab facilities and reagents to that end. During this period I also established some collaborations with researchers from local media as Dr. Unsain, a young researcher from our institute, Dr. Helguera (INIMEC-CONICET-UNC) and Dr. Srefani (CIBION, CONICET). I have recruited 1 undergraduate and 1 graduate student which are learning techniques and already conducting some experiments in my laboratory.

Below you will find a detailed report of the scientific activities performed during the funded period and the financial report.

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Scientific progress report

Research project: Role of MAP6d1 in Golgi apparatus organization and neuronal morphogenesis.

Neurons are highly polarized cells typically extending several short, thick tapering dendrites and one functionally distinct long, thin axon. It is currently accepted that the development and maintenance of neuronal polarity involve several basic and interrelated cellular events, such as the correct assembly of components of the cytoskeleton; the selection of membrane proteins at the Golgi Apparatus (GA), the transport of membrane to sites of active growth and the insertion of organelles in specialized regions of the neuronal plasma membrane. During the last decade several studies have shown that GA is important in the development and maintenance of neuronal polarity, through the maintenance of an active and polarized membrane traffic towards the dendritic compartment. In neurons, the GA not only consists in the pericentriolar membrane array, but also comprises discrete structures named "Golgi outpost" (GOPs), dispersed in dendrites and excluded from the axon, which have a vital role in the synaptic plasticity and in dendritic development. In pyramidal neurons, Golgi outposts are enriched in the apical dendrite, similar to the somatic GA that is positioned polarized towards this apical neurite. The main interest of my laboratory is to study the cellular and molecular mechanisms that regulate the formation and maintenance of neuronal polarity. In recent years we have studied some of the cellular and molecular events that regulate the biosynthetic pathway, more specifically the Golgi apparatus and the associated cytoskeleton (Bisbal et al 2008, Quassollo et al 2015).

As part of my research project, I am interested in elucidating the mechanisms that regulate the polarized positioning of the GA in differentiated neurons, and how this process regulates the morphology of the dendritic compartment. For this, we studied the role of MAP6d1 (MAP6 domain-containing protein 1), a protein that is expressed exclusively in differentiated neurons. This protein has two domains highly homologous to the microtubule associated protein MAP6. MAP6 has the property of stabilizing microtubules against depolymerizing stimuli and has been implicated in synaptic functions (Bisbal et al., Manuscript in preparation). The MAP6 knockdown mouse presents anomalies in synaptic plasticity, associated with behavioral problems related to diseases such as schizophrenia (Deloulme et al 2015). The N-terminal domain of MAP6d1 has a high homology with the N-terminal domain of the MAP6 protein. MAP6d1 has a second domain with high homology with the Mn3 module of MAP6, which has the ability to interact and stabilize microtubules (Gory-Faure et al 2006). My results obtained in collaboration with the laboratory of Dr. Andrieux (Grenoble, France), with whom I maintain a collaboration from my postdoctoral stay, demonstrate that the MAP6d1 protein is located specifically in the perinuclear region of differentiated neurons colocalizing with GA markers as GM130 and TGN38. We have also observed, throughout the dendritic compartment, that MAP6d1 is enriched in the "Golgi outpost". The location of the MAP6d1 protein in the AG is regulated by the amino terminal domain, which contains three cysteine amino acids which could be palmitolayted. In overexpression studies in cell lines we demonstrated that MAP6d1 localization in GA is dependent on this palmitoylation state since in the presence of 2-bromopalmitate (2-BP), a strong inhibitor of palmitoylation, MAP6d1 protein loses its normal location in the AG and it is redistributed in the microtubular cytoskeleton. Likewise, the ectopic expression of MAP6d1-3G (a mutant unable to be palmitolayted), also presents a distribution along the microtubules and absent from the AG. Using the double hybrid technique and co-immunoprecipitation experiments, we found that MAP6d1 interacts, through the Mn domain, with the Tctex protein, a light chain of dynein which interacts with the cargo of the microtubular motor, and it has been described that this protein is important for the positioning of AG, at least in non-neuronal cells.

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Our studies suggest that MAP6d1 regulates the morphology, distribution and polarization of AG. The inhibition of MAP6d1 expression, using a plasmid that expresses small hairpins of interfering RNA (shRNAi,) against a target sequence of MAP6d1, produces significant changes in different morphological parameters of somatic GA in mature neurons, which presents continuous GA with a loss of the typical polarity towards the apical dendrite. As previously mentioned, the neuronal GA presents a polarized distribution with an extension of the cisterns towards the apical dendrite (referred as deployment) and the presence of GOPs in the dendritic compartment. Likewise, neurons treated with shMAP6d1 also present GA with continuous deployment and a significant decrease in the number of cells that present GOPs. Even more, overexpression experiments of mutants unable to bind Tctex or GA lead to similar conclusions.

During neuronal development, GA polarization occurs during dendritic differentiation and the secretory transport of vesicles that exit from the GA is predominantly directed towards the main dendrite, suggesting that this spatial organization of the secretory pathway is important for neuronal morphogenesis and the asymmetric development of the dendritic tree. Our experiments also suggest that MAP6d1 is important to regulate the development of the dendritic tree since the inhibition of MAP6d1 produces a significant increase in the length and complexity of the dendritic compartment in mature neuronal cultures.

Taken together, the results presented here suggest a novel and important role of MAP6d1 not only to maintain the morphology and positioning of the neuronal GA, but also to regulate the polarized distribution of neuronal AG structures such as the elongation and formation of cisterns that form the deployment in the apical dendrite and in the training/maintenance of GOPs. We also show that the normal MAP6d1 activity is important to regulate the growth and complexity of the dendritic compartment.

Publications during ISN funding

*Bisbal M, Quassollo G, Caceres A. (2016). Imaging Golgi outposts in fixed and living neurons. Methods Mol Biol. 1496:31-9.

*Quassollo, G., Wojnacki, J., Salas, D.A., Gastaldi, L., Marzolo, M.P., Conde, C., Bisbal, M., Couve, A., Caceres, A. (2015) A RhoA Signaling Pathway Regulates Dendritic Golgi Outpost Formation. Current biology. 25(8):971-82.

Daoust, A., Saoudi, Y., Brocard, J., Collomb, N., Batandier, C., Bisbal M., Salome, M., Andrieux, A., Bohic, S., and Barbier, E.L. (2014). Impact of manganese on primary hippocampal neurons from rodents. Hippocampus. 24(5):598-610

The ISN-CAEN is acknowledged in this paper as some of the funds were allocated to these experiments and performed in my laboratory.

Participation in Scientific Meetings during ISN funding

Oral presentation

"Microtubule-associated protein 6 (MAP6/STOP) is required for the formation and maturation of dendritic spines". EMBO Workshop: Emerging Concepts on Neuronal Cytoskeleton. Puerto Varas, Chile.

Posters presentation

"Role of cofilin in nucleus accumbens core during the cross sensitization between chronic stress and

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cocaine". Rigoni D, Avalos MP, Bisbal M, Guzman AS, Cancela ML, Bollati F. Sociedad Argentina de Investigación en Neurociencias (SAN) XXX Anual Meeting. Mar del Plata, Argentina. 2015.

"Characterizing cytoskeleton changes during axonal degeneration". Barabas F, Remedi M, Bisbal M, Barker P, Stefani F, Cáceres A, Unsain N. Sociedad Argentina de Investigación en Neurociencias (SAN) XXX Anual Meeting. Mar del Plata, Argentina. 2015.

"A Novel CLN8 Missense Mutation Underlies Variant Late Infantile Neuronal Ceroid Lipofuscinosis In South America" Pesaola F, Cismondi I, Guelbert N, Kohan R, Carabelos Mn, Alonso G, Pons P, Oller-Ramirez Am, Bisbal M, Noher De Halac I. 14th International Conference on Neuronal Ceroid Lipofuscinoses (Batten Disease). Córdoba, Argentina. 2014.

"Microtubule-associated protein 6 (MAP6/STOP) is required for the formation and maturation of dendritic spines" Bisbal M., Peris, Andrieux A. EMBO Workshop: Current advances in membrane trafficking: Implications for polarity and diseases, Puerto Natales, Chile. 2014

Financial report of CAEN award:

Item	U$D
Laboratory supplies (consumables, reagents and disposables for cell culture, molecular biology, antibodies, plasmid DNA purification kit, etc.)	3550
Small Equipment (micropipettes, vortex, shaker, mixer, etc.)	2000
Animal Facility	750
Microscopy Facility	500
Travel costs for EMBO Workshop: Current advances in membrane trafficking: Implications for polarity and diseases, Puerto Natales, Chile. 2014	500
Office supply (Desktop computer, external hard drivers, office consumables)	700
TOTAL	**8,000**

Finally, I want to point out that I am really grateful to the ISN for giving me this strong support to establish my research laboratory in Argentina, through the ISN CAEN Category 1C Return Home Grant.