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CENTRO DE INVESTIGACIONES EN QUÍMICA BIOLÓGICA DE CÓRDOBA - CIQUIBIC
DEPARTAMENTO DE QUÍMICA BIOLÓGICA, FACULTAD DE CIENCIAS QUÍMICAS
UNIVERSIDAD NACIONAL DE CÓRDOBA

Córdoba, June 24th 2015

First year progress report CAEN Category 1C Return Home Grant.

Project Title**: Regulation of amyloid precursor protein (APP) traffic by specific rabs proteins and its implication in neuronal migration during brain development.**

Responsable: **Lucas J. Sosa, M.D.-PhD.**
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Dear Dr. **Roberto Cappai**

I am glad to write about my first year of adaptation in Argentina after I have lived for almost 6 years in USA where I worked as a postdoc at the beginning in the Dr. Karl Pfenninger lab (Linda Crnic Institute for Down Syndrome and Department of Pediatrics, University Colorado School of Medicine, Aurora, CO, USA.) and then in the Jorge Di Paola lab (Dept. of Pediatrics - Hematology/Oncology, University of Colorado School of Medicine, Aurora, CO, USA). I currently hold a Faculty position as an Assistant Professor at the School of Chemical Science (Facultad de Ciencias Químicas) of the National University of Cordoba, and a Research position as Assistant Investigator at CIQUIBIC (Centro de Investigaciones en Química Biológica de Cordoba)-CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), the National Research Council. These positions offer me the stability necessary to set down and installed with my family properly in Argentina and focus in my project as a researcher.

Once back to the bench, I started to work in my project related to the study of the **regulation of amyloid precursor protein (APP) traffic by specific rabs proteins and its implication in neuronal migration during brain development**. At the beginning my goals were acquired the tools necessary to carry on the planned experiments. In these context, in order to knockdown the

expression of rab21 protein (one of the rabs candidate to be involved in APP traffic) in cell lines and hippocampal neurons, I developed in our lab two shRNA target to rab21, and also one scramble sequence as a control. All of these constructs were checked by nucleotide sequencing. Then, I received the rab21 GFP fusion protein and its mutant variants from a generosity gift from Dr. Johanna Ivaska (Turku Centre for Biotechnology, University of Turku, Turku, Finland). At the same time, I got the Rab35 GFP fusion protein and its mutant variants from generosity gift from Dr. Mikael Simons (Department of Psychiatry and Psychotherapy, University Medicine Goettingen, Goettingen, Germany).

Then, I started to test the transfection conditions for the constructs in the SH-SY5Y and Neuro-2a cell line where they work successfully. Also I set conditions for the neuronal hippocampal cultures from embryonic mice brain. The grant form CAEN was crucial to acquire the surgical instruments necessary for the dissection of the hippocampus from the mice brain. The primary culture of hippocampal neurons do not showed any trouble. However, the transfections of the neurons shortly after seeding has shown some difficulties related to viability, which were improved after changing the timing of transfection. Thus, in general terms the culturing conditions are now optimized in our laboratory. In order to study the distribution and co-localization of the proteins in the cell lines and the neuronal hippocampal cultures, we required the rab21, rab35 and APP antibodies which arrived to Argentina with some delay. I began to test the antibodies and set the concentration of them in the cell line and primary culture of hippocampal neurons. I scheduled to start doing immunoprecipitation experiments of APP containing vesicles from embryonic brain microsomal preparation, followed by “western blot” analysis and identification of rab35 and rab21 proteins using the specific antibodies in next month. In addition, I have made some experiments where I am planning to analyze the phenotype and neurite length in cell lines and primary hippocampal cultures transfected with the different rab21 constructs.

References:

- Sosa, L.J., et al., Amyloid precursor protein is an autonomous growth cone adhesion molecule engaged in contact guidance. PLoS One, 2013. 8(5): p. e64521.
- Villarroel-Campos, D., et al., Rab-mediated trafficking role in neurite formation. J Neurochem, 2014. 129(2): p. 240-8.
- Pellinen, T., et al., Small GTPase Rab21 regulates cell adhesion and controls endosomal traffic of beta1-integrins. J Cell Biol, 2006. 173(5): p. 767-80.

In addition, I am collaborating with Dr. Santiago Quiroga (Universidad Nacional de Cordoba) studying the participation of the IGF1 receptor in the neuronal migration during neurodevelopment.

Also I am preparing a manuscript in collaboration with Dr. James Malter (University of Texas Southwestern Medical Center.USA.) regarding the participation of the protein Pin1 (protein interacting with NIMA-1) in the adhesion and advance of the axonal growth cone.
I participated of the Sociedad Argentina de Investigación en Neurociencias (SAN) meeting in October of 2014, were I gave an oral presentation in the Young Investigator Symposium. Also, I am co-author of one publication which is in submission: (Diego Grassi, Mariana Oksdath, Florentyna Bustos Plonka, Alvaro Nieto Gil, Lucas Sosa and Santiago Quiroga. *Selected SNARE proteins are essential for the polarized membrane insertion of IGF-1 receptor and the regulation of initial axonal outgrowth in neurons. 2015. Cell Discovery. CELLDISC-00031-T).*

Financial Status of CAEN award:

- Surgical instruments: $ 700.
- Laboratory Reagents (cell culture reagents and media, antibodies, molecular biology reagents, etc.): $ 2550.
- Animal Facility Room maintenance (filtered air supply, irradiated food, bedding supply): $1400.-
- Office supply (Desktop computer, external hard drivers, desktop chair): $ 1600
- Amount left: $ 1750

It would be a pleasure for me submit a review article to the Journal of Neurochemistry as part of the CAEN Return Home grant conditions of ISN. I would like to know which are the specifications and whom should I contact in order to get the conditions requested by the Journal of Neurochemistry to submit a review. In this way I can send whom correspond a brief description about how I plan to organize this review, including the remarks and future directions of this issue, directly related to my projects.
The review that I am working and planning to submit in the JNC is focus in the physiological role of the Amyloid Precursor Protein (APP) as an adhesion molecule in the developing nervous system.

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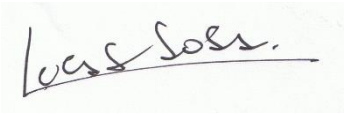
Although APP is best known for its role in the pathogenesis of Alzheimer disease as the source of the amyloid beta fragment, these review shift the angle of address of APP from the aging to the developing nervous system. In such a way the review will contemplate previous neurochemical studies that describe APP as an adhesion protein and it is role in axonal growth cone spreading, contact guidance and neuronal migration. In addition, I would discuss how a perturbation in the (neuronal adhesion properties) function of APP during early brain development may alter neuronal wiring and be involved in the pathogenesis of Down syndrome associated intellectual disability and also in the neurodegenerative process of Alzheimer disease.
I hope the scope of the review would be of interests for the JNC.

After my arrival to Argentina it was no easy to set up the lab since the accessibility to the different resource is complicated and very often we are dealing with delays of all source. For the described and other reasons the overall adaptation take time in Argentina. However day by day I feel the confidence that I made the right decision for me and my family to return to our home country. I feel proud to help the improvement of the science in Argentina. Again, I want to be grateful to the ISN's support to establish my research in Argentina, through the CAEN grant. Also I would like to acknowledge the ISN for the help that offer to the young scientist in developing countries. I have a commitment to do my best effort and hopefully contribute with the scientist community.

Sincerely,



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