FINAL REPORT

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Project acronym: AXOGLIA

Project title: Understanding the axon-glial functional unit in myelination and remyelination

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[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement .

[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

Summary

Myelination of nerve fibres by glial cells, oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS), gives rise to the axon/glia functional unit with its unique properties, e.g. axon protection, and increased resistance and decreased capacitance across large calibre axons for fast saltatory conduction of action potentials. The importance of the myelination process is highlighted by the fact that loss or damage of myelin is one of the major mechanisms underlying the pathology of devastating neurological disorders including leukodystrophies, central and peripheral neuropathies, and inflammatory demyelinating diseases such as multiple sclerosis (MS). These diseases present a high incidence, severe symptoms and a poor treatment outcome. Thus, they represent a serious health as well as economic and social burden at European level (Gustavsson et al., 2011). Remyelination, the process by which demyelinated axons are reinvested with new myelin sheath following demyelination, is required to ensure recovery of physiological activity of the nerves (Bruce et al., 2007). Spontaneous remyelination in human demyelinating diseases can occur, but it is an uneven process often insufficient to preserve axon integrity and, ultimately, proper physiological activity. It is possible that during demyelination and remyelination expression of key signaling molecules is altered. Long-term axon protection could therefore be achieved only through the re-establishment of an efficient axon/myelin functional unit cross-talk. According to this hypothesis, only a “correct” remyelination consisting in the preservation of the original axonal and myelin expressed proteome could potentially reduce axonal loss. Over the past few years it has been realized that the interaction of myelinating glia with axons is bidirectional and recent data suggest that the myelinating glia plays an active role in the maintenance of axon integrity, whose preservation could be the key to prevent chronic progressive disability (Edgar and Garbern, 2004; Lubetzki et al., 2005; Zawadzka and Franklin, 2007). Despite the importance of these processes, no clear understanding of the key molecules and signalling pathways involved and altered in demyelination and remyelination is presently available (Bruce et al., 2007).

Our lack of a clear understanding of the general mechanisms underlying myelination and remyelination arises in part from the fact that most of the studies have been relying on conventional molecular, biochemical and cellular methodologies which permit to successfully address one or a few identified molecules of interest but cannot provide a complete profile of the complex events underlying these processes.

This fellowship aimed to combine this classical approach to systems biology approach. Conventional molecular, biochemical and cellular methodologies have been applied to study a previously identified interesting candidate (i.e. Profilin 1, see 1 below) for its role in PNS and CNS myelination. A system biology approach has been applied to identify new potential candidates likely to play a role in CNS myelination/remyelination (or its failure) (see 2 below).

1) Few cytoskeletal molecules have been previously reported to play a role in myelination (References). The formation and compacting of myelin is likely to require highly regulated cytoskeletal reorganization, pointing to cytoskeletal and in particular to actin-polymerization regulators as potential candidate for a role in the regulation of myelination/remyelination. During the first phase of the project the fellow addressed the role of Profilin1, an actin binding protein, and showed that Profilin1 is indeed essential for proper axon/glia interaction during PNS development, but dispensable for CNS development. During the second phase of the project, the cell biology mechanisms and molecular pathways involved have been further studied and elucidated. The produced results have permitted us to further clarify the basic cellular mechanisms through which the PNS axon/glia functional unit is established to allow early myelination. The project has been completed and generated data have been prepared in the form of a manuscript for submission to a high-impact international peer-reviewed scientific journal.

2) With the aim of identifying potential candidates likely to play a role in myelination/remyelination, in the second year of the grant, the fellow has applied forefront proteomics techniques and profiled membrane proteins expressed at the axon/myelin interface, quantifying their regulation following experimental demyelination. The proteome of the developmentally formed intact axon/glia functional unit has been screened by mass-spectrometry and bioinformatics analysis in collaboration with Dr. Bernd Wollscheid (Institute of Molecular Systems Biology, ETH Zurich, Switzerland). The same

experimental approach has been in parallel applied to address how remyelination differs from developmental myelination. These experiments have been conducted in a toxin-induced demyelination animal model, for which the fellow received specific training in the group of Prof. Robin J.M. Franklin (Cambridge University, UK). Interesting potential candidates have been identified, selected and validated by western-blot and immunohistochemistry. In order to broaden our knowledge of the basic molecular processes underlying myelination/remyelination in the CNS, and to identify potential therapeutical targets, selected candidates have been depleted by shRNA in oligodendrocytes. The identification of new players in oligodendrocyte differentiation and myelination processes has permitted to open follow-up research projects aiming to address the specific functions and roles of the identified candidates in both CNS and PNS during developmental myelination and remyelination. A manuscript draft of the obtained data has been prepared for submission to a high-impact international peer-reviewed scientific journal.

3) During the proteomic approach, the fellow has also identified for the first time a constitutively active – atypical - Rho-GTPase, RhoE, as being expressed in adult CNS myelin. The role of atypical RhoGTPases, i.e. RhoE, in CNS and PNS signaling is far from clear. Being the study of Rho-GTPases signaling in myelination one of the main focus of the host lab, the fellow has started a side-project aiming to study the role of this signal transducer protein by analyzing its interaction network using a SILAC proteomic approach (Harsha et al., 2008), in collaboration with Dr. Bernd Wollscheid (Institute of Molecular Systems Biology, ETH Zurich, Switzerland) and Dr. Rosa Guasch (Department of Cellular Pathology, Centro de Investigacion Principe Felipe, Valencia, Spain). A comprehensive determination of Rnd3 interacting proteins will provide a framework for understanding Rnd3 signaling and allow the identification of modulated biological functions. A list of candidates has been generated which will need to be validated by co-immunoprecipitation and co-localization studies in a follow-up project in the host lab. The functional role of Rnd3 and of the validated interacting partners in nervous system development, e.g. in myelination, could be then addressed *in vitro* in neuronal and myelinating co-cultures and *in vivo* in Knockout/Cre conditional mice in follow-up projects. The used method to study atypical Rho-GTPases interactomes is currently in press as a book chapter, and it will be further applied in the host lab in new studies aiming to address the role of other atypical Rho-GTPases (i.e. RhoBTBs) in CNS and PNS myelination/signaling.

The fellowship has allowed to further strength ongoing and creating new European research collaborations. It has permitted knowledge transfer by introducing several state-of-the-art techniques, therefore increasing competitiveness of Portuguese Research. The fellowship has also allowed increasing European networking and competitiveness in the field of Systems Biology/Proteomics research and in the field of multiple sclerosis and demyelinating-diseases research. This is particularly important considered the huge economical and societal burden that these diseases represent, in particular due to the lack of efficient therapies. The carried out projects have resulted in new important results, i.e. the identification of a new signaling pathway key for early myelination events in the peripheral nervous system, and the identification of new potential therapeutical targets for remyelination therapies, which have been presented and discussed in national and international meetings. Two manuscripts have been prepared for submission to high-impact international peer-reviewed scientific journals and an additional manuscript will be published in the form of book chapter for dissemination of an established method. The resulting publications will allow increasing competitiveness of European research in the field of myelination/remyelination, as well as increase the competitiveness of the fellow and of the host group. In addition, the fellowship has successfully allowed to implement the professional career developmental plan of the fellow by acquisition of new technical skills, promoting multidisciplinarity in the fields of interest, organization/people management/communication skills, grant writing, independent thinking and leadership qualities. This has allowed the fellow to obtain a postdoctoral position at a prestigious institute in another European country, where she will be able to further explore signaling pathways involved in myelination/remyelination of the PNS and CNS and their misregulation in demyelinating diseases.