Targeting distinct autophagic proteins and their interactome in central and peripheral myelination relevant for human diseases.

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Recent evidence highlighted the essential role of autophagy in regulating myelin production, as well as the relevance of alterations and dysfunctions in myelinating glia in different neurodegenerative diseases and in peripheral neuropathies. Moreover, a number of evidence point at supportive function of myelinating glia for neurons in a myelin-independent manner. Indeed, oligodendrocytes sustain CNS function, regulating the structural and electrical properties of axons, defining their diameter, as well as nodes and paranodes. In the PNS, Schwann cells are essential for the regeneration of axons and in the formation and function of neuromuscular junctions; in addition, they provide trophic support to neurons, sustaining the long-term integrity of axons independently of myelin itself. Myelin abnormalities are causative for a number of neurological diseases and can also contribute to complex neuropsychiatric disorders. The emerging role for autophagy in regulating the maturation and homeostasis of myelinating glia opens new perspectives for the development of therapies for combating myelin-related diseases.
Within this project, the PhD student will investigate the role of key proteins involved in the fine regulation of autophagy in oligodendrocytes and Schwann cells, by carrying out a range of *in vivo* studies in selected mutant mice and zebrafish models, already available in the lab and *ad hoc* generated by our team. *Ex vivo* and *in vitro* experimental approaches will parallel the *in vivo* studies, in order to reveal how these autophagy mediators, as well as their dynamic interaction with other protein complexes and signaling pathways, impact on the physiology and homeostasis of myelinating glia.

Exploring the dopamine-induced toxicity in striatal astrocytes to enhance neuroinflammation and neurodegeneration in Parkinson's Disease.

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Parkinson's disease is an age-related and severe neurodegenerative disorder. The main pathological hallmarks are the loss of the dopaminergic neurons in the *Substantia Nigra pars compacta* and the presence of cytoplasmic inclusions. Along with the neurodegenerative process, the associated neuroinflammation plays a relevant role in exacerbating the progression of the pathology. Among the driving factors of the neurodegeneration process, dopamine dyshomeostasis is known to play a relevant role. Previous data from our lab demonstrated that the increased concentration of the dopamine toxic catabolite DOPAL strongly affects neuronal proteostasis, triggers aS oligomerization and leads to synapse dysfunction.
Under physiological conditions, striatal astrocytes participate in the dopamine clearance from the neuronal synaptic cleft, via dopamine uptake and degradation by MAO-B and ALDH enzymes. Epidemiological studies revealed an increased risk to develop PD following the exposure to drugs and pesticides that are potent ALDH inhibitors. At the same time, MAO-B elevation in mouse brain astrocytes was shown to induce astrogliosis and microglia activation, mitochondrial dysfunction, and progressive dopaminergic neuron loss. Nevertheless, little is known about the consequences of an impaired dopamine degradation in astrocytic physiological functions.
The proposed project aims to dissect the molecular mechanisms triggered by astrocytic DOPAL buildup, with focus on astrocytes activation, inflammatory responses, and the repercussions on the neuronal electrophysiological activity. The candidate will setup protocols for primary mouse co-cultures of dopaminergic mesencephalic neurons and striatal astrocytes, as well as nigrostriatal organotypic slices exposed to ALDH inhibitors, as an advance and integrated approach to unravel the role of astrocytes in handling pathological DOPAL accumulation, neuroinflammation outbreak and neuronal dysfunction. Moreover, a translational aspect will be explored, by repurposing MAO inhibitors and ALDH activators to rescue the DOPAL-induced toxicity as therapeutic strategy for PD.

Role of VPS13C in neuronal physiology and in Parkinson's disease.

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Parkinson's disease (PD) is a common neurodegenerative disorder involving a complex interaction of genetic and environmental factors. Besides identifying as many as 90 risk loci for sporadic PD, large population-based GWAS and meta-analyses have also established a number of pleomorphic risk loci,

harboring both rare large effect and common smaller effect variants, including *SNCA*, *LRRK2*, *GBA* and *VPS13C*. This wealth of genetic information together with the rapidly growing knowledge on the cellular functions of these genes clearly point to endo-lysosomal pathways and vesicular trafficking as central processes in PD etiology. Mutations in *VPS13C* cause a severe form of autosomal recessive PD and more common variants in *VPS13C* locus increase lifetime risk for PD. While the function of VPS13C has been poorly explored, some recent studies suggest a role in tethering late endosomes/lysosomes to the endoplasmic reticulum (ER) for the direct transfer of lipids between the two organelles. The aim of this PhD project is to explore the neuronal role of VPS13C in regulating vesicle dynamics using state-of-the art imaging and cellular assays as well as proteomic-based approaches. CRISPR-mediated knockout or tag-knockin in neuronal lines and iPSCs will allow to define the role of VPS13C in neuronal organelle physiology and explore its functional interaction with other PD genes implicated in endo-lysosomal pathways and vesicular trafficking. The project will be carried out in collaboration with international research groups, providing the candidate with the opportunity to spend part of her/his training abroad.

Stem cells in budding and regeneration in the ascidian *Botryllus schlosseri.*
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Tunicates are invertebrate marine species, considered the sister group of vertebrates. The ascidian *Botryllus schlosseri* is a colonial tunicate, showing cyclical asexual reproduction (budding) and regenerative capabilities (such as whole body regeneration) mediated by multipotent stem cells. Stem cell populations are morphologically recognizable and home in specific niches of adult individuals. These niches are transient since, during the colonial life, buds cyclically substitute in the filtering activities the adult individuals that undergo regression. The molecular pathways driving potency and differentiation of stem cells, and their ability to home in their transient niches are still enigmatic. This project aims to shed light on the role of stem cells in budding and in regeneration in *B. schlosseri*. Regeneration will be induced by removing all the individuals from a colony, promoting the development of atrophied buds or partially ablated buds. All these processes potentially involve the activation of early development molecular programs and the differentiation of stem cells. Attention will be paid to developmental factors (such as *soxB1*, *pou2*, *pou3*, *myc*, *vasa*, *piwi*) with top reprogramming activity in vertebrates, focusing on the mechanisms controlling cell fate stability and renewal. The work plan will embrace cell sorting, gene expression (realtime PCR, ISH), functional (RNA interference), behavioral, and morphological (confocal, TEM microscopy) analyses.

Role of cellular metabolism in angiogenesis and cancer.
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Endothelial cells (ECs) and cancer exhibit a remarkable and unique plasticity in terms of redox biology and metabolism. By using advanced redox and metabolic imaging platforms, and innovative molecular and genetic approaches in different in vivo animal models, we aim to shed light on the role of novel metabolic pathways in health and disease. The ultimate objective is to open the way for the development of innovative therapeutic strategies and complement the existing ones based on genetic and pharmacological manipulation of redox and metabolic state in angiogenic and tumor processes.

Mitochondrial dynamics in cellular signaling networks.
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Mitochondria, the central metabolic hubs of the cell, undergo continuous fusion/fission cycles that ultimately control their shape. These processes are regulated by core mitochondria-shaping proteins and impact on virtually every aspect of mitochondrial and cellular biology (Giacomello et al., Nature Reviews Molecular & Cell Biology 2020). Our lab investigates the key biological question of the form-function relationship at the molecular level. We pioneered mitochondrial dynamics, contributing to our understanding of the basic tenets of the regulation of mitochondrial shape (e.g., Cipolat et al, PNAS 2004; Frezza et al., Cell 2006; Cipolat et al., Cell 2006; de Brito&Scorrano, Nature 2008; Pyakurel et al., Molecular Cell 2015, Quintana-Cabrera et al., Nature Communications 2018) and on its role in mitochondrial as well as in cellular physiology and pathology (e.g., Cogliati et al., Cell 2013; Kasahara et al., Science 2013; Varanita et al., Cell Metabolism 2018; Herkenne et al., Cell Metabolism 2020, Zaninello et al Nature Communications 2020). We are seeking a talented, motivated graduate student who would join a multinational lab of 20

colleagues from 14 different countries of 3 continents, tackling all aspects of mitochondrial dynamics and mitochondrial membrane contact sites. The successful candidate will develop a project at the interface between mitochondrial dynamics and signaling, with a particular outlook on how changes in mitochondrial shape affect sterile inflammation, metabolism, retrograde signal to the nucleus and ultimately cancer.

Mitochondrial quality control in neurodegenerative diseases.

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Mitochondrial dysfunction and quality control has become a central theme in neurodegenerative diseases, Parkinson's Disease (PD) in particular. Mitochondrial stress can lead to the release of reactive oxygen species (ROS), which triggers inflammation and cell death. Thus, approaches that boost mitochondrial autophagy (mitophagy) have the potential to clear damaged mitochondria as sources of ROS, and prevents neuroinflammation and neuronal cell death. Mitophagy can be triggered by ubiquitination of mitochondrial proteins. Stimulation of mitophagy can be achieved via inhibition of specific de-ubiquitination enzymes (DUBs) that remove ubiquitin from mitochondrial proteins. We are particularly interested in the regulation of the activity of DUBs Usp8 and Usp14, which inhibition promotes mitochondrial quality control via enhancement of mitochondrial autophagy. To address the effects of DUBs inhibition in mitochondrial physiology and quality control, we use primary neurons of human origin generated from PD patients, and established *drosophila melanogaster* models of PD. In summary, our research is focused on understanding the molecular mechanisms that regulate macroautophagy and mitophagy in neurodegenerative conditions, and it will explore the potential of enhancing basal mitophagy as therapeutic strategy to treat neurodegenerative diseases.