Master de Sciences et Technologies
Mention Biologie Intégrative et Physiologie
Parcours : Neurosciences
Responsable : Professeur Régis Lambert

Internship Proposal
Academic Year 2018-2019

1. Host team :

Research Unit (e.g. Department or Institute) : Institute for Psychiatry and Neurosciences of Paris, Inserm U894
Research Unit Director : Thierry GALLI
Research Team Director : Cyril HANUS
Team name : Biogenesis and Dynamics of Dendritic Proteins

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2. Internship project title:

Atypical N-glycosylation of the neuronal surface and its impact on protein turnover and synaptic plasticity .

3. Internship Description :

Key words: *cell-biology, neurons, synaptic plasticity, local protein synthesis, unconventional secretion, live-cell imaging, super-resolution microscopy, CLICK-chemistry, mass-spectrometry*

For us to learn and form memories, our neurons must selectively modify the composition and properties of a few selected synapses among the tens of thousands synapses that they maintain with other neurons. To achieve this daunting task, neurons have evolved novel means to regulate the core cellular machinery and, for example, locally synthesize proteins to directly functionalize specific synapses during synaptic plasticity.

In the past ten years, we and others have characterized multiple mechanisms that enable neurons to locally process *membrane* and *secreted proteins* and traffic them to the specific segments of dendrites and synapses where they are needed. Intriguingly in doing so, we found that while dendrites contain all the machinery that is needed for the biogenesis of secretory proteins, dendrites are devoid of generic Golgi membranes. Because a key function of the Golgi apparatus is to glycosylate proteins, this led us to investigate how this unique organization of the neuronal secretory pathway impacts N-glycosylation and hence the dynamics and functional properties of synaptic proteins.

We hence discovered that, as a result of Golgi-independent secretory trafficking, most *surface expressed* neurotransmitter receptors and virtually all the key proteins of the neuronal surface display N-glycosylation profiles that are typically only found on immature intracellular proteins in the endoplasmic reticulum. This atypical N-glycosylation regulates synaptic transmission and, as seen for candidate receptors, accelerates protein turnover, thus revealing a previously unrecognized mechanism that controls the sensing properties and plasticity of the neuronal membrane.

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We use advanced live-cell and super-resolution imaging and biochemical approaches in cultured neurons and brain slices to investigate how unconventional secretory trafficking and N-glycosylation regulate the dynamics of surface proteins during neuronal development and synaptic plasticity. *We are looking for driven and skilled students whose work would contribute to the following 3 non-alternative projects:*

1) *Our previous work shows that the vast majority of neuronal dendrites contain early secretory membranes (rough endoplasmic reticulum and intermediate compartments) but are devoid of Golgi membranes. Yet, we cannot formally exclude that glycosylation steps typically associated with the Golgi in other cells are not arranged according to distinct principles in neurons. To address this, we will combine super-resolution imaging and protein and sugar metabolic labeling approaches to generate high-resolution maps of secretory organelles and N-glycosylation in dendrites in basal conditions and after induction of synaptic plasticity.*

2) *Our previous on candidate proteins shows that the atypical or standard glycosylation profile of surface synaptic proteins correlates with the sensitivity of these proteins to Golgi-disruption. Here we aim to use unbiased omics approaches to generalize this finding. To do so, we will combine protein metabolic labeling, sequential protein purifications and protein and sugar mass spectrometry to provide an in-depth characterization of the neuronal (atypical) surface glycoproteome and its regulation by synaptic activity.*

3*) Our core-hypothesis is that the synthesis of synaptic receptors in the soma or dendrites enables neurons to use Golgi-dependent (soma) and Golgi-independent pathways (dendrites) to diversify the glycosylation status and hence the function of synaptic proteins. A key objective in this context will be to demonstrate that local protein synthesis in dendrites is sufficient to determine the secretory itinerary of candidate proteins and hence regulates their glycosylation status and dynamics. To do so, we developed a versatile artificial system to target specific mRNAs to dendrites, to hence favor the local synthesis of reporter proteins. We will now combine this system with live-cell and super-resolution imaging to determine whether local protein synthesis regulates protein glycosylation, accelerates protein exocytosis and recruitment to synapses, and modulates how these proteins respond to changes in synaptic activity.*

Selected references

- B. Alvarez-Castelao, C.T. Schanzenbächer*, C. Hanus*, C. Glock, S. tom Dieck, A.R. Doerrbaum, I. Bartnik, B. Nassim-Assir, E. Ciirdaeva, A. Mueller, D. Dieterich, D.A. Tirrell, J.D. Langer, and E.M. Schuman. Genetically-Restricted Protein Metabolic Labeling in the Mouse Brain *in vivo* (2017). Nature Biotechnology *(*Nov 6. doi: 10.1038/nbt.401*).*

- A.B. Bowen, A.M. Bourke, B.G. Hiester, C. Hanus, M.J. Kennedy. Golgi-independent secretory trafficking through recycling endosomes in neuronal dendrites and spines (2017). eLife *doi:10.7554/eLife.27362.*

- C. Hanus[#], H. Geptin, G. Tushev, S. Garg, B.Alvarez-Castelao, S. Sambandan, L. Kochen, A-S. Hafner, J. Langer and E.M. Schuman[#] (2016). Unconventional secretory processing diversifies neuronal ion channel properties. eLife *doi:10.7554/eLife.20609.*

- C. Hanus[#] and M.D. Ehlers[#] (2016). Neuronal adaptation to biosynthetic membrane trafficking: secretion in developing and mature dendrites. Current Opinion in Neurobiology. 39:8-16.

- S. tom Dieck*, L. Kochen*, C. Hanus, M. Heumüller, I. Bartnik, B. Nassim-Assir, K. Merk, T. Mosler, S. Garg, S. Bunse, D.A. Tirrell, E.M. Schuman (2015). Direct visualization of newly synthesized target proteins in situ. Nature Methods*, doi: 10.1038/nmeth.3319.*

- C. Hanus[#], L. Kochen, S. tom Dieck, V. Racine, J.B. Sibarita, E.M. Schuman and M.D. Ehlers[#] (2014). Synaptic Control of Early Secretory Trafficking in Dendrites. Cell Reports, 7(6):1771-8.

- C. Hanus and E.M. Schuman (2013). Proteostasis in Complex Dendrites. Nature Reviews Neuroscience, 14(9):638-48.

- T. Cui-Wang*, C. Hanus*, T. Cui, T.D. Helton, J. Bourne, D.J. Watson, K.M. Harris and M.D. Ehlers (2012). Local Zones of ER Complexity Confine Cargo in Neuronal Dendrites. **Cell**, 148(1-2):309-21.