**PhD PROPOSAL FOR THE**

**PASTEUR - PARIS UNIVERSITY INTERNATIONAL DOCTORAL PROGRAM**

Time for applicants to contact host laboratories: September 13 – November 2, 2017

Deadline for full application: November 13, 2017

Interviews: January 30, February 2, 2018

Start of the Ph.D.: October 1, 2018

**Title of the PhD project:** Combatting Alzheimer´s Disease by Activating Autophagy

**Keywords:** Autophagy, Initiation, ULK1 complex, ATG9, Alzheimer, Neurodegeneration

**Department:** Cell Biology and Infection

**Name of the lab:** Membrane Biochemistry and Transport

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***Doctoral school affiliation and University***: Ecole Doctorale BioSPC - Université Paris Diderot

Presentation of the laboratory and its research topics:

***Autopahgy*** is one of the most important recycling systems in cells. It confines cargo in a membrane sack that functions like waste bags by capturing unwanted or damaged cytoplasmic material. After the membrane sack has surrounded its cargo entirely, it is sealed to give rise to an autophagosome. The autophagosome eventually fuses with lysosomes where its cargo is being recycled. Failure of autophagy is involved in the onset of many human diseases, including cancer, autoimmune diseases and ***neurodegeneration***.

We are investigating the molecular mechanism of autophagy to reveal how reduced autophagy and neurodegenerative diseases like Alzheimer or Parkinson are interconnected. ***Our unique approach*** to investigate autophagy involves fascinating combinations of in vitro and in vivo approaches. We are reconstituting the formation of autophagic waste bags in the test tube using purified proteins and model membranes. Moreover, we are applying cutting edge cell-biology using human neuronal pluripotent stem (NPS) cells and cells derived from Parkinson patients to study autophagy in vivo. Methods applied in our laboratory involve CRISPR-Cas9 genome editing, high-resolution fluorescent microscopy of primary and NPS cells, recombinant protein expression, generation of model membranes including supported lipid bilayers and unilamellar liposomes, biophysical characterization of proteins in vivo and in vitro using fluorescent lifetime imaging, correlative light electron microscopy and single particle cryo-electron microscopy.

All projects in our laboratory involve this combination of techniques providing excellent training opportunities for our group members. Moreover, the projects are immediately related to human diseases allowing molecular mechanisms involved in the onset of such diseases to be identified.

Our team is truly international and highly interactive and enthusiastic. The vibrant and stimulating atmosphere in our laboratory creates a unique social and scientific environment.

Description of the project:

Neuronal cells strictly depend on functional cellular recycling systems to avoid accumulation of harmful material1. Therefore, any failure of these systems contributes significantly to the onset of neurodegenerative diseases. One such mechanism, termed autophagy, is of particular interest because is the most versatile recycling system that promotes degradation of damaged organelles such as mitochondria or peroxisomes, as well as protein aggregates or other cytoplasmic waste2.

The proposed project will focus on the initiation of autophagy. This key step decides how many autophagosomes are being formed, what type of cargo they select, and as a consequence how much of a given cargo is recycled3. Revealing its molecular mechanisms is therefore one of the most important open questions in the field. Initiation of autophagy involves collaboration of the multi-subunit ULK1-kinase complex and the transmembrane protein ATG94. Both are required to start the formation of autophagic membrane sacks that capture cytoplasmic cargo such as protein aggregates5.

We have previously studied the process in yeast and found that the yeast homolog of ULK1 recruits small membrane vesicles that contain the yeast homolog of ATG96,7. This step is crucial to initiate fusion of ATG9-vesicles that results in the formation of autophagic membrane sacks8. Our unique approach of combining in vitro reconstitutions and in vivo experiments revealed a dedicated regulatory mechanism that defines the nature of cargo captured by autophagic membranes and how the components of the yeast ULK1 complex are working together to initiate autophagy. However, although most components of the autophagic core machinery are highly conserved from yeast to humans, a significant difference in the molecular composition of yeast and human ULK1 exists. This suggests that autophagy initiation in yeast and humans are not immediately related. We thus aim to investigate, how autophagy is initiated in human cells and how this process can be modulated to treat neudegenerative disorders in patients.

In the proposed project, we will recombinantly express and purify the components of the ULK1 complex and ATG9 to reconstitute this key step in the test tube. We will produce these proteins by expression in insect cells using baculovirus expression systems or Schneider cells or by expression in HEK293 cells. Purification of the single components will be achieved by state of the art purification methods based on Äkta-HPLC, including affinity-, size exclusion- and ion exchange chromatography. The activity of single components, subcomplexes and the full ULK1-complex on model membranes will be studied in vitro. Therefore, ATG9 will also be expressed and purified and reconstituted in model membranes giving rise to ATG9-vesicles that are mimicking autophagic donor membranes in vivo. Combining ULK1 components with ATG9-vesicles in vitro will reveal whether the ULK1 complex functions as tethering complex to bring ATG9-vesicles in close proximity. This tethering step is thought to be required to stimulate fusion of ATG9-vesicles in order to generate autophagic membrane sacks that are capable of capturing cytoplasmic material.

Our in vivo approach will include genome editing methods (CRISPR-Cas9) to generate cells that are deficient for ULK1 complex components or ATG9 or that express fluorescent labeled protein variants. We will perform experiments using neuronal pluripotent stem (NPS) cell, which are obviously much better suited to study neuronal autophagy compared to e.g. HeLa cells. Furthermore, we will apply high-resolution fluorescent microscopy and biophysical methods involving fluorescent lifetime imaging (**FLIM**), fluorescent resonance energy transfer (**FRET**) and fluorescent recovery after photobleaching (**FRAP**) in human cells as well as in vitro using our reconstitution approaches. We will take characterization of autophagy-initiation to an even more complex level by initiating differentiation of NPS cells into different types of neuronal cells, including neurons, glia cells and astrocytes in order to investigate which cell type is most vulnerable to defects in autophagy initiation.

This unique combination9 of cutting edge methods will allow us to reveal how autophagy is initiated and why reduced autophagic flux as a result of reduced initiation of autophagy leads to the onset of neudegenerative disorders.

References:

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Expected profile of the candidate (optional):

Our approach is truly multidisciplinary and involve cutting edge in vitro and in vivo techniques. Our fluorescence microscope is equipped with FLIM-FRET and FRAP modules that allow for quantitative analysis of in vitro reconstituted reactions and in living cells. This quantitative approach requires interest in biophysics with a good understanding of mathematical evaluation of data. Moreover, many different techniques will be used, providing on the one hand excellent scientific training but requires on the other hand willingness to learn and apply new techniques.

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