

Dual-PhD program between Sussex and HKUST

Regulation of P2X7-purinoreceptor ER export and trafficking to the plasma membrane

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Summary

We are looking for a student interested in participating in a joint PhD project between Hong Kong University of Science and Technology (HKUST; Guo laboratory) and the University of Sussex (UoS; Murrell-Lagnado laboratory) to explore the regulation of P2X7 receptor export from the endoplasmic reticulum (ER). Enhanced cell surface expression of the P2X7 receptor has been linked to a wide variety of inflammatory diseases including chronic pain hypersensitivity. The project will involve an in vitro assay to reconstitute vesicular release of proteins from the ER and the identification and characterization of cytosolic factors and proteins that regulate vesicular release of P2X7 from the ER (expertise of the Guo lab). It will also involve the subsequent analysis of the role of these factors in regulating P2X7 plasma membrane expression using cell biological assays including confocal and TIRF microscopy and patch clamp electrophysiology (expertise of the Murrell-Lagnado lab).

Scientific background

P2X receptors are extracellular ATP-gated cation channels. One of the P2X subtypes, P2X7, is highly expressed in immune cells in the CNS and periphery and plays an important role in inflammation and protection against infectious disease. Within the P2X family, P2X7 has a uniquely low affinity for ATP and is thought to be the mediator of pathologically high concentrations of extracellular ATP. Enhanced P2X7 receptor signaling has been linked to various diseases, including chronic pain hypersensitivity, inflammatory bowel syndrome, retinal degeneration and traumatic brain injury. The Murrell-Lagnado lab has a long-standing interest in the trafficking behavior of this family of receptors and has shown that regulation of trafficking is an important determinant of their plasma membrane expression and activity. Surface expression of P2X7 is highly variable and cell-type dependent, with considerable retention of the protein within the ER. As newly synthesized P2X7 receptors need to be delivered from the ER to the plasma membrane to perform their functions, we hypothesize that regulating ER export of P2X7 is an effective way to regulate P2X7 signaling, providing a novel therapeutic strategy to treat P2X7-related diseases.

Project

We propose a collaborative approach to use biochemical and cell biological approaches to identify regulators of P2X7 trafficking. The Guo lab at HKUST has developed an in vitro assay to reconstitute vesicular release of proteins from the ER. They will use this biochemical approach to reveal how P2X7 is exported out of the ER. **During the first 18-24 months** of the PhD the student will be located at HKUST and will focus on the following three aims: 1) to reconstitute vesicular release of P2X7 from the ER in undifferentiated and differentiated THP-

1 monocytic cells; 2) to identify and characterize cytosolic factors that regulate vesicular release of P2X7 from the ER; 3) to reveal and characterize proteins that specifically co-enriched with P2X7 in ER-derived vesicles. Alongside these experiments a complimentary approach will be utilized which takes advantage of differences in trafficking behavior of the human and mouse P2X7 receptor isoforms, identified by the M-L lab. When heterologously expressed in HeLa cells, human P2X7 receptor is predominantly retained in the ER whereas the mouse version is efficiently delivered to the cell surface. Human and mouse P2X7 share 80% sequence identity and mutagenesis analysis will be used to identify the motifs that are important for ER export of P2X7.

During the second half of their PhD the student will be located within the M-L lab at the University of Sussex and will perform cell biological assays to analyse the role of these cytosolic and protein factors in regulating P2X7 expression to and from the plasma membrane. The M-L lab have already developed the required molecular tools for imaging the trafficking of P2X7 using confocal and TIRF microscopy, including different fluorescent protein tags, constructs with extracellular epitopes and antibodies to the endogenous receptors. They also have the expertise for analysing P2X7R function by patch clamp.

Proposed time-line:

Summer of 2018-Fall of 2020: the PG student in the joint program will take courses at HKUST and conduct in vitro biochemical experiments in the lab of Dr Guo.

Spring of 2020 – Summer of 2022: the student will conduct imaging and electrophysiological experiments in the lab of Dr Murrell-Lagnado. Preparation for thesis and publication papers.

Summer of 2022: Defence of PHD thesis either at University of Sussex or HKUST.