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Dynamic analysis and molecular regulation of protein transport and in iPSc-derived cardiomyocytes

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Research project:
Cardiomyocytes are the contractile units of the heart muscle. The alteration of the function of cardiomyocytes leads to cardiovascular diseases and/or to cardiac arrythmias, potentially fatal. Part of the functions ensured by cardiomyocytes depend on the correct targeting of proteins to the cell surface, such as ion channels (Blandin et al., 2021) and cardiomyokines (Chiba et al., 2018).
Proteins destined to be secreted enter the secretory pathway at the level of the endoplasmic reticulum. They are then exported to the Golgi apparatus where they are sorted and eventually processed before being exported to the cell surface (Boncompain and Weigel, 2018). Cells from differentiated tissues display distinct secretion needs and might adapt the organization and functional abilities of their Golgi apparatus to sustain them.
This project aims at understanding how protein transport and its regulation by Golgi apparatus components undergo adaptation to fulfil efficient trafficking in cardiomyocytes.
Induced pluripotent stem cells (iPSc) and iPSc-derived cardiomyocytes will be used.
The dynamic and quantitative analysis of transport of secretory proteins will be performed using the Retention Using Selective Hooks (RUSH) assay (Boncompain et al., 2012). This method allows the synchronization of the transport of a cargo of interest

by releasing it from the endoplasmic reticulum by simple addition of biotin. The transport of cardiomyocyte-specific cargos such as natriuretic peptides (atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) (Chiba et al., 2018)) and the ion channel Connexin-43 (Waxse et al., 2017) will be analyzed by real-time imaging in iPSc-derived cardiomyocytes and in undifferentiated iPSc.
To identify key molecular machineries associated to the Golgi apparatus in cardiomyocytes, proximity biotinylation (Branon et al., 2018) will be carried out comparatively in iPSc and in iPSC-derived cardiomyocytes. For this, iPSc stably expressing a Golgi-targeted TurboID construct will be used. After pull-down with streptavidin conjugated-beads, mass spectrometry analysis will identify the proteins whose levels is increased in cardiomyocytes compared to iPSc. Based on these results, we will employ genome editing using CRISPR-Cas9 technology to knockout or to degrade in a controlled way (Nabet et al., 2018; Nishimura et al., 2009) the protein of interest. In these conditions, the protein transport function will be analyzed using the RUSH assay as previously described.

Models and techniques:

- Culture of iPSc
- Differentiation of iPSC into cardiomyocytes
- Molecular biology
- Immunofluorescence
- Real-time imaging (by spinning disk and confocal microscopy)
- Biochemistry (proximity biotinylation, streptavidin pull-down and western blot)
- Genome editing (CRISPR/Cas9) in iPSc

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