Project N - Supervisor: Carlos M Farinha, BioISI | Co-supervisor: Manuela Zaccolo, Univ Oxford

Title: Linking cyclic AMP to the cytoskeleton to regulate CFTR trafficking

Objectives: CFTR seems to be at a crossing between the cytoskeleton and signalling pathways, particularly cAMP signalling. Both CFTR functional activation and trafficking (particularly, plasma membrane stability) rely on correct cytoskeletal organization that enables generation of properly localized cAMP pools and brings the correct interacting partners to CFTR proximity.

We have shown recently that the effect of the cAMP sensor EPAC1 on CFTR PM levels involves association with multiple cytoskeleton components, among which the capping protein CAPZA2 and the inverted formin INF2 (Santos et al (2020) *Biochem J*). These two regulators compete for binding to CFTR, with CAPZA2 having a dominant effect at the PM. Interestingly, data also suggests a possible role for these modulators in regulating the disposal of mutant endoplasmic reticulum (ER)-retained CFTR to degradation - a role that is in line with reports of INF2 localizing to the ER, a unique feature among inverted formins.

The main objective is to study these novel connections between the cytoskeleton and cAMP signalling in regulating CFTR trafficking (both at the ER and the PM), particularly to characterize the role of the cytoskeleton regulators CAPZA2 and INF2 in orchestrating this cross-link. For this, three specific aims/tasks are proposed:

i) To characterize the role of CAPZA2 and INF2 in regulating cAMP signalling.

ii) To dissect the formation of CFTR PM complexes involving CAPZA2.

iii) To characterize the role of INF2 in regulating CFTR ER exit and degradation.

Achieving these goals will elucidate basic mechanisms for a protein connected to a relevant human health problem.

Methodology:

To accomplish task 1, we will assess cAMP compartmentalization with genetically encoded molecular probes that allow direct imaging of this second messenger in living cells. This will greatly rely on the expertise available at the University of Oxford in studying cAMP signalling using live cell imaging (*Oxford Anatomy and Physiology ranked #1 the QS World University Rankings by subject in 2021*).

To accomplish task 2, we will mainly use co-immunoprecipitation approaches and cell surface biotinylation to assess the kinetics of the formation of PM complexes involving CFTR and the cytoskeleton modulators CAPZA2 and INF2, as well as its dependence on the key players EPAC1, INF2, NHERF1 and ezrin.

To accomplish task 3, we will focus mainly on the impact of INF2 upon mutant CFTR exit from the ER and its targeting to degradation. For this, we will combine the use of different INF2 variants (modulating its intracellular localization) with proximity-dependent biotinylation, towards a proteomics/interactomics characterization of INF2 interactions, providing for the first time a mechanistical insight into the role of this formin in the ER.

Supervisor: Carlos M Farinha (BioISI, FCUL)
Co-Supervisor: Manuela Zaccolo (Dep Anatomy, Physiology and Genetics, Univ Oxford)

Type of fellowship (select the correct option)
☐ National
☒ Mixed (Portugal and abroad: Univ Oxford)