Comprehensive Proteomic Profiling of Vesicle-mediated Glucose Transporter Trafficking in Human Cells

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Project outline

GTPase-mediated vesicle trafficking plays a fundament role in maintaining glucose homeostasis. A key step controlling the uptake of glucose into adipocytes is the recruitment of GLUT4-containing storage vesicles to the plasma membrane. Fusion of the vesicles is followed by insertion of the GLUT4 transporter into the membrane allowing for glucose to be taken up by the cell. Previous reports have established the GTPase Rab10 as well as its corresponding GTPase-activating protein (GAP) TBC1D4 (AS160) and guanine nucleotide exchange factor (GEF) DENND4C as critical nodes in this pathway. However, their regulation is not well understood and dysfunctional signalling in this pathway has been linked to diabetes. For example, it is thought that DENND4C is constitutively active but it is likely that DENND4C activity is controlled by posttranslational modifications and protein-protein interactions.

Our laboratory has established a suite of proteomic techniques to study intracellular signalling and protein-protein interactions (PPI). These approaches include tandem affinity purifications, proximity-based labelling (BioID), phosphoproteomics, and thermal profiling. Thermal profiling takes advantage of the observation that the thermal stability of a protein can be modulated by binding of a ligand or binding partner. Such an interaction often leads to a conformational change in the protein which, in the case of a small molecule or drug, tends to induce the formation of a more rigidized structure, thus leading to an increase in the thermal energy required to unfold the protein. This concept has been used in structural biology for many years to identify stabilising ligands to support crystallisation. The finding that this approach can also be applied to intact living cells (CETSA) in combination with proteomics has opened up a completely new way to investigate protein-protein, protein-metabolite, or protein-drug interactions in physiologically relevant contexts whilst removing the need for tagged proteins or compounds. This so-called meltome analysis can reveal new insights into protein complex composition and cellular signalling as protein-protein interactions can be captured in intact cells before lysis.

One principal aim of this proposal is to combine tandem AP-MS, BioID, phosphoproteomics, and thermal profiling to precisely map the network regulating GLUT4 vesicle trafficking. To this end, individual stable cell lines expressing the respective key components DENND4C, Rab10, Rab14, and

TBC1D4 (AS160) will be established. Due to the known crosstalk between phosphorylation and glycosylation in the regulation of vesicle trafficking, glycoproteomics will be considered as well. This substantial dataset will require the development of new bioinformatic tools including methods to analyse raw thermal profiling data and refinement of existing approaches to exploit meltome data for protein complex analysis (thermal co-aggregation). Furthermore, the various datasets generated by the project will have to be intersected with publicly available data including, but not limited to, protein-protein networks and functional genomic and phenotypic data to establish causality and directionality to filter and prioritise candidate genes for follow-up studies. Due to the special expertise required for this part of the project a close collaboration with the NNRCO Discovery Bioinformatics team is anticipated. Selected protein interactions will be validated by classical cell and molecular biology approaches or live cell PPI monitoring using BRET technology. In case of phosphorylation changes, in-house kinase chemogenomic compound sets will be used for orthogonal validation and evaluation of potential options for therapeutic interventions.

In summary, this project aims to establish a comprehensive map of insulin-regulated vesicle trafficking using a complementary set of proteomic approaches and to provide tools for bioinformatics data analysis with potential applications in future studies.

Aims

1. Define the cellular interactome of Rab10, Rab14, DENND4C and TBC1D4 (AS160) using tandem AP-MS and thermal profiling.
2. Together with NNRCO develop an analysis pipeline to establish a multiomics network of vesicle trafficking and evaluate new approaches to analyse and apply thermal profiling of raw MS data in this context.
3. Validate interactions using classical cell biology approaches (antibody immunoprecipitation, crosslinking, KD/KO), chemogenomic compound libraries, and novel live cell assays like NanoBRET.

Supervisor's recent relevant publications:

1. **KVM Huber** et al. Proteome-wide drug and metabolite interaction mapping by thermal-stability profiling. *Nature Methods* 2015; 12:1055-1057.
2. **KVM Huber**, G Superti-Furga. Profiling of Small Molecules by Chemical Proteomics, Proteomics in Systems Biology: Methods and Protocols. *Springer New York* 2016; 211-218.
3. JD Vasta et al. Quantitative, Wide-Spectrum Kinase Profiling in Live Cells for Assessing the Effect of Cellular ATP on Target Engagement. *Cell Chemical Biology* 2018; 25:206-214.
4. P Giansanti et al. Evaluating the Promiscuous Nature of Tyrosine Kinase Inhibitors Assessed in A431 Epidermoid Carcinoma Cells by Both Chemical- and Phosphoproteomics. *ACS Chem Biol* 2014; 9:1490-1498.
5. J Li et al. Artemisinins Target GABAA Receptor Signaling and Impair α Cell Identity. *Cell* 2017; 168:86-100.