Structural and Functional Exploration of GTPase-mediated Glucose Transporter Trafficking

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Project outline

Insulin-regulated uptake of glucose into adipocytes and myocytes is important for maintaining blood sugar homeostasis. Secretion of insulin activates a signalling pathway which leads to translocation of the GLUT4 sugar transporter to the plasma membrane thereby enabling cellular uptake of glucose molecules. This process requires an intricate interplay between a number of different proteins in a process termed vesicle trafficking whereby newly synthesised GLUT4 is stored in vesicles which upon insulin stimulation are recruited to and ultimately fused with the plasma membrane to release GLUT4. Trafficking of GLUT4 vesicles requires loading with the active form of the GTPase Rab10. Rab10 activity is controlled by GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs). GAPs stimulate GTP hydrolysis whereas GEFs facilitate the release of GDP from the GTPase. Several studies have shown that the key GEF for Rab10 is a protein called DENND4C.

DENND4C is a member of the DENN (differentially expressed in normal and neoplastic cells) domain containing protein family which are thought to act as GEFs for GTPases such as Rabs thereby playing important roles in vesicle trafficking. Notably, mutations in DENN family members have been linked to a variety of human pathologies including frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). A causal link has also been reported between mutations in DENND1A (connecdenn1) and women suffering from polycystic ovary syndrome (PCOS), which can lead to infertility and glucose intolerance. However, this protein family remains largely unexplored and specifically the mechanisms controlling DENND4C activity remain elusive. Our laboratory has recently determined the first crystal structure of human DENND1A alone and in complex with Rab35. Building on this effort, one of the aims of this proposal is to express and purify recombinant DENND4C (full-length protein and different domain constructs) and the Rab10 GAP TBC1D4 (AS160). We have already established expression constructs for several Rab family members including Rab10. Upon successful purification of DENND4C, Rab10, and TBC1D4 (AS160), a second aim will be to determine the structure of these proteins alone or in complex with each other using protein X-ray crystallography and cyoEM. In parallel and as a backup, efforts will be made to also express and purify the other two DENND4 family members DENND4A and –B. Finally, we will attempt to exploit the crystal structures by performing fragment screens to identify small molecules that can bind either of these proteins or the complex to reveal druggable pockets. Alternatively, biophysical binding and displacement assays such as SPR and TR-FRET will be explored.

In summary, this project will provide new insights into an unexplored yet important protein family and the regulation of glucose homeostasis and vesicle trafficking and eventually open up this pathway for drug discovery.

Aims

1. Establish a recombinant expression system for the DENND4 family and TBC1D4 (AS160) to investigate biochemistry and structural biology of this pathway alone and in complex with Rabs.
2. Attempt structure determination of Rab10 together with DENND4C or TBC1D4 (AS160) using protein crystallography or cryoEM.
3. Develop assays to enable interrogation of GLUT4 trafficking by small molecules by performing crystallographic fragment screens with DENND4C, TBC1D4 (AS160) or establishing functional and binding assays to identify regulators DENND4C and TBC1D4 (AS160).

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. GI Vladimer et al. Global survey of the immunomodulatory potential of common drugs. *Nature Chemical Biology* 2017; 13:681-690.
5. J Li et al. Artemisinins Target GABAA Receptor Signaling and Impair α Cell Identity. *Cell* 2017; 168:86-100.