Clathrin Heavy Chain 22 (CHC22) controls biogenesis of the human Glucose Transporter 4 (GLUT4) storage compartment (GSC) from the early secretory pathway.

Stéphane M. Camus[1,2,3,4,10], Marine D. Camus[1,2,3,4,10], Carmen Figueras-Novoa[10], L. Amanda Sadacca[10] Christopher Esk[5], Gwyn W. Gould[6], Dimitrios Kioumourtzoglou[6,7], Franck Perez[8,9], Nia J. Bryant[7], Shaeri Mukherjee[3,4*] and Frances M. Brodsky[1,2,3,4,10,*]

[1]Department of Bioengineering and Therapeutic Sciences,
[2]Department of Pharmaceutical Chemistry,
[3]Department of Microbiology and Immunology,
[4]The G.W. Hooper Foundation, University of California San Francisco, San Francisco, CA 94143, USA
[5]IMBA Institute of Molecular Biotechnology GmbH, Vienna, Austria
[6]Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ. UK.
[7]Department of Biology, University of York, York, YO10 5DD. UK
[8] Institut Curie, 26 rue d'Ulm, 75248 Paris Cedex 05, France.
[9] CNRS UMR 144, 26 rue d'Ulm, 75248 Paris Cedex 05, France.
[10] Present address: Division of Biosciences, University College London, London WC1E 6BT, UK
[*]Co-corresponding authors

Aims: Intracellular GLUT4 trafficking to the GSC is critical for insulin-mediated GLUT4 translocation to the cell surface and glucose homeostasis, and its impairment contributes to pathogenesis of Type 2 diabetes. However, pathways leading to GSC biogenesis are unknown. We previously determined that formation of the perinuclear GSC relies on the non-canonical isoform of clathrin CHC22. In this work, we mapped the CHC22-mediated GLUT4 trafficking pathway leading to GSC biogenesis in humans. This will provide new insights into the pathophysiology of Type 2 diabetes and will foster novel therapeutic strategies.

Methods: We combined super-resolution fluorescence microscopy and insulin-mediated GLUT4 translocation experiments quantified by FACS in HeLa-GLUT4 and human skeletal muscle cells expressing human GLUT4 tagged with exofacial Haemagglutinin and cytosolic GFP. We also took advantage of the *Legionella pneumophila*'s property to hijack its host's early secretory trafficking machinery to probe for CHC22 function.

Results: Super resolution microscopy located CHC22 at the ER-to-Golgi Intermediate Compartment. Functional experiments with *L.p.* confirmed that CHC22 was operating in the early secretory pathway, trafficking core components of the GLUT4 pathway: IRAP, sortilin and GGA2. We showed by co-immunoprecipitation that CHC22 interacts with p115 and siRNA experiments indicated that CHC22 and p115 co-operate to establish insulin sensitivity by trafficking GLUT4 to the GSC via a GM130-independent manner.

Summary: Our data show that in humans, the formation of insulin sensitive GSC depends on a trafficking pathway where both CHC22 and p115 cooperate at the ERGIC to form GLUT4 vesicles and establish the GSC.