PUBLISHABLE SUMMARY REPORT

The Scientist in Charge, Dr Aniko Varadi (AV) was away on maternity leave during the first half of the Fellowship (18th May 2008-18th May 2009) and then the Fellow, Dr Sophie Lajus (SL) took a 6 month maternity leave in October 2009. In addition, there were considerable technical challenges in the project which were hard to overcome in the absence of AV. Thus we made alterations to the project, however the general aim of the project remained the same which was to investigate the mechanisms that control large dense core vesicle (LDCV) mobilisation from the intracellular storage pool to the release sites at the beta cell plasma membrane.

***Identification of interacting partners of myosin Va that recruit it to insulin granules.*** The brain-spliced isoform of myosin Va (BR-MyoVa) is essential for the transport of secretory granules (SGs) to the plasma membrane in most hormone and neuropeptide-producing cells. The protein complex that recruits BR-MyoVa to SGs and regulates its function is not known. We have identified novel direct interaction between SG-associated proteins granuphilin-a/b (Gran-a/b), BR-MyoVa and Rab27a, a member of the Rab family of GTPases. Gran-a/b-BR-MyoVa interaction is direct; it occurs in the absence of Ca2+,but is significantly enhancedby the presence of Ca2+; involves regions downstream of the Rab27-binding domain and the C-terminal part of Gran-a determines exon-specificity. Disruption of Gran-a/b-BR-MyoVa binding leads to a perinuclear accumulation of SGs and enhanced nutrient-stimulated hormone secretion in pancreatic beta-cells. These results indicated the existence of another binding partner of BR-MyoVa that was identified as rabphilin-3A (Manuscript is under review in Traffic [2].)

Surprisingly, MyRIP (myosin VII- and Rab-interacting protein), which has been suggested to be a MyoVa receptor and been also implicated in insulin secretion, does not form a complex with BR-MyoVa and Rab27a under unstimulated conditions. However, when PKA is activated by IBMX and Forskolin or by Exendin-4 the long-acting Glucagon like peptide-1 (GLP-1) agonist MyRIP forms a protein complex with MyoVa. Under this condition Rph-3A becomes phosphorylated which is dependent on MyRIP association with MyoVa since siRNA knock down of MyRIP expression inhibited Rph-3A phosphorylation on secretory granules. Furthermore MyRIP seems to be also important for stabilising MyoVa. This study demonstrates that multiple novel binding partners of BR-MyoVa, universally used by hormone and neuropeptide-secreting cells, regulate SG transport. MyRIP rather than acting as a receptor for MyoVa functions as a scaffolding protein that links PKA to secretory granules (Manuscript will be submitted to Molecular Cell Biology in June 2011 [3]).

Furthermore, we identified another novel interacting partner, a well known metabolic enzymeATP-citrate lyase (ACL), for myosin Va using GST-pull down and Q-TOF analysis. ACL is known to be an important enzyme for glucose metabolism and been characterised as a cytosolic protein. The interaction with myosin Va has been confirmed by co-immunoprecipitation of endogenous proteins from pancreatic beta-cells. Using recombinant proteins, we demonstrated that the interaction with myosin Va is direct and by immunofluorescence that the two proteins partially co-localise on LDCVs. In addition, we demonstrated that ACL is able to translocate to LDCVs in a glucose-dependent manner. *To our knowledge this is the first clear direct physical link between metabolism and LDCV trafficking in beta-cells or in any other neuroendocrine cells.* Most patients with type 2 diabetes have defect in insulin secretion which likely to be associated with defects in LCDV trafficking. Therefore these findings will contribute to our understanding of the molecular mechanisms responsible for the impaired beta cell function seen in type 2 diabetes (Manuscript will be submitted to Diabetologia in September 2011 [4]).

***To investigate phosphorylation of myosin Va or its interaction partners in binding of the complex to LDCVs and insulin secretion.*** Our study revealed that the myosin Va protein complex phosphorylation on LDCVs is dependent on the presence of MyRIP that provide a scaffold for PKA. We reduced MyRIP protein level using siRNA which resulted in decreased phosphorylation of the myosin Va interacting partner Rph-3A. Rph-3A phosphorylation is stimulated by GLP-1 which increases its interaction with 14-3-3. We have now made the phosphorylation mutant of Rph-3A and will investigate its effect on hormone secretion (Manuscript will be submitted to Molecular Cell Biology in June 2011 [3]).

*Academic journal papers*

1. Diraison, F., Hayward, K., Sander, KL., Brozzi, F., **Lajus, S.,** Francis, J.E., Ainscow, E., Bummer, U.A., Hancock, J.,Molnar, E., Avent, N.D. and Varadi, A.(2011) Translationally controlled tumour protein (TCTP) is a novel glucose-regulated protein that is important for survival of pancreatic beta cells. *Diabetologia,* **54:**368-379.

2.Brozzi,F., **Lajus, S.,** Diraison, F., Regazzi, R., Molnar, E. and Varadi, A. (2011) Identification of a novel receptor of myosin Va in neuroendocrine cells. Under review with Traffic

3. **Lajus, S.,** Brozzi, F.,Diraison, F., Regazzi, R., Molnar, E. and Varadi, A. **(**2011) Role of MyRIP in hormone secretion (To be submitted to Molecular Cell Biology in June 2011)

4. **Lajus, S.,** Diraison, F.,Brozzi,F.,Molnar, E. and Varadi, A. (2011) Novel link between metabolism and vesicle trafficking in pancreatic beta cells. To be submitted to Diabetologia in September 2011.

*Preliminary communications/abstracts*

1.Brozzi, F., **Lajus, S.,** Diraison, F., Regazzi, R., Molnar, E and **Varadi, A** (2011) The Rab27 effector proteins granuphilin-a/b and rabphilin-3A bind the neuronal isoform of myosin Va to secretory granules. Joint Biochemical Society/Wellcome Trust Conference on Cellular cytoskeletal motor proteins, Cambridge, UK

2.Brozzi, F., **Lajus, S.,** Diraison, F., Regazzi, R., Molnar, E and **Varadi, A** (2011) MyRIP anchors PKA to the MyoVa-associated protein complex on secretory vesicles. Joint Biochemical Society/Wellcome Trust Conference on Cellular cytoskeletal motor proteins, Cambridge, UK

3.Brozzi, F., **Lajus, S.,** Regazzi, R. and **Varadi, A** (2010) Granuphilin-a/b and rabphilin-3A link myosin Va to LDCVs in pancreatic beta-cells. EASD Islet Study Group, Tallberg, Sweden

4. **Lajus, S** and **Varadi, A** (2008) Molecular mechanisms of nutrient stimulated insulin-containing vesicle transport in pancreatic beta cells - identification of secretory vesicle-specific motor protein receptors. *Diabetologia*51**,** S204-S204

5. **Lajus, S.** and **Varadi, A.** (2009) Large dense core vesicle (LDCV) transport in pancreatic beta-cells – identification of vesicle-specific motor protein receptor. *BSCB Spring Meeting**and Biochemical Society Focused Meeting. The Dynamic Cell.*

6. **Lajus, S.,** Brozzi, F. and **Varadi A.** (2009) Large dense core vesicle transport in pancreatic beta-cells – Myosin VIIa-Rab27-interacting protein (MyRIP) function in pancreatic beta-cells. Islet Study Group Meeting, Vienna.

*Invited talks:*

2011 **Molecular mechanism of the brain isoform of myosin Va (BR-MyoVa) recruitment to hormone and neuropeptide-containing secretory granules.** 16th International Symposium on Chromaffin Cell Biology. Beijing, China.(AV)

2010 ***MYRIP function in pancreatic β-cells: interaction with myosin Va and myosin VII or both?*** EASD,European Islet study Group Meeting, Tallberg, Sweden (SL)

2009 **Molecular mechanism of vesicle trafficking – Role of Myosin Va, MyRip and Rab27a.** EASD, European Islet study Group Meeting, Vienna, Austria (AV)

2010 **Life and death of the pancreatic beta-cell.** The Severnside Alliance for Translational Research(SARTRE), The First South West and South Wales Network Meeting, Translational Research in Diabetes (AV)

2010 **Regulation of pancreatic beta-cell function - survival and insulin vesicle transport**. Department of Clinical Science @ North Bristol , University of Bristol (AV)