

P027 Trafficking of a novel GPI-anchored lipid-raft-associated protein
Clare Hamilton^{*}, Ruth Rollason^{*}, Viktor Korolchuk⁺
and George Banting^{*}

^{}University of Bristol, Department of Biochemistry,*

⁺University of Cambridge, Department of Genetics

Bst2/HM1.24 has a novel topology with an N-terminal transmembrane domain and a C-terminal glycosyl phosphatidyl inositol (GPI) anchor and is localised to lipid rafts in the plasma membrane. Bst2/HM1.24 cycles between the cell surface and an intracellular pool and was found in purified clathrin coated vesicles. Pull-down assays have shown an interaction between the cytosolic domain of Bst2/HM1.24 and both μ 2 and μ 1 subunits of the clathrin adaptor proteins. This suggests a role for clathrin-mediated endocytosis in Bst2/HM1.24 trafficking and clathrin-coated vesicles in intracellular trafficking of Bst2/HM1.24. In order to investigate the trafficking and function of Bst2/HM1.24 we are currently identifying and characterising candidate proteins that interact with its cytosolic N-terminus. The N-terminus of Bst2/HM1.24 was used to screen a T7-bacteriophage based cDNA library. Using this technique along with pull-down assays ADP ribosylation factor 1 (ARF1) was identified as a putative Bst2/HM1.24 interacting protein. In conjunction with AP1, ARF1 may have a role in vesicle formation at the TGN. T7-bacteriophage cDNA screen and pull down assays have also identified RICH2, a Rac1/Cdc42 RhoGAP protein as a possible Bst2/HM1.24 interactor. Antibodies are being raised to RICH2 to determine its intracellular localisation and investigate its interaction with Bst2/HM1.24.