

P008 Using a proteomics strategy to identify GRIF-1 associated proteins

Kieran Brickley, Yuqin Wang, William J Griffiths and F Anne Stephenson

*School of Pharmacy, University of London,
London WC1N 1AX, UK.*

γ -Aminobutyric acid_A receptor interacting factor (GRIF-1) is a member of a coiled/coil family of proteins thought to function as adaptor proteins in the anterograde trafficking utilizing molecular motor proteins of organelles to synapses in excitable tissues. To obtain further insight into the function of GRIF-1, a proteomics strategy was used to identify GRIF-1 associated proteins. Detergent extracts were prepared from rat brain and immunoprecipitations carried out using either anti-GRIF-1₈₇₄₋₈₈₉ antibodies or non-immune Ig each directionally, covalently coupled to protein G Sepharose. Precipitated proteins were identified by one dimensional SDS-PAGE, followed by *in situ* tryptic digestion of gel slices and electrospray tandem mass spectrometry. Peptides corresponding to GRIF-1 were found in immune but not in control pellets thus confirming the specificity of the anti-GRIF-1₈₇₄₋₈₈₉ antibodies. The enzyme, OGT, a known GRIF-1 interacting protein was also present in immune but not control pellets confirming proof of principle for this strategy. Other proteins (> 30) specifically precipitated were rank ordered according to the number of times they were detected in immune pellets, the number of peptide matches and respective amino acid sequence coverage. High ranking proteins included the glutamate transporter, EAAT1, syntaxin binding protein and α or β subunits of the adaptor related protein complex. These results substantiate a role for GRIF-1 in organelle trafficking. Supported by the BBSRC (UK).