

Lab Visit report- Dr Kim Jonas, Jan 08

The Society for Endocrinology lab visit grant was awarded to investigate the locality of the guanylyl cyclase-B (GC-B) receptor within cellular membrane compartments and to determine whether the GC-B receptor localised to membrane rafts in α T3-1 gonadotroph and GH3 somatotroph cell lines. In light of our recent data suggesting that C-type Natriuretic Peptide (CNP) activation of the GC-B receptor results in phosphorylation of MAPK family proteins [Jonas *et al.*, (2007) *Endocrine Abstracts* 13:P245; Jonas *et al.*, (2006) *Endocrine Abstracts* 12:OC20] and other recent studies, there is an increasing body of evidence suggesting that natriuretic peptide receptors signal through alternative non-cGMP dependent pathways. This lab visit aimed to determine whether the GC-B receptor localised to membrane rafts and potentially form signalling complexes with MAPK family proteins in GH3 and α T3-1 cell lines. The data obtained from this could provide key mechanisms of protein-protein interactions between receptor and downstream MAPK family proteins.

Initial studies conducted at Cornell University in conjunction with Professor Roberson's laboratory enabled me to learn the 2-day technical protocol of cellular fractionation using sucrose density gradients and ultra centrifugation methodology for generation of low density to high density fractions from whole cell lysates. Cholesterol depletion and repletion studies were also conducted using cyclodextrin to deplete cells of cholesterol, and water-soluble cholesterol to replete cellular cholesterol, to complement membrane raft fractionation studies

Preliminary data suggests that in α T3-1 cells, the GC-B receptor appears to be localised to low density cellular fractions, implying a membrane raft locality of the GC-B receptor in this cell type. Similar experiments conducted in GH3 cells suggest that refinement of cell lysis and homogenisation techniques would provide a clearer distinction between low density and higher density membrane fractions. Current methodology employed suggests that fractionation of the different density fractions is incomplete. This is work that is current on-going to optimise this procedure in GH3 cells.

Pilot studies have shown that depletion of cholesterol from cells with 2% cyclodextrin for 25 minutes resulted in approximately 50% decrease in CNP and ANP-stimulated cGMP accumulation in GH3 cells. However, cholesterol depletion over the same time point failed to alter the ability of CNP/GC-B to stimulate ERK phosphorylation in GH3 cells. Cholesterol repletion studies are currently underway dissect these effects and further studies into effects on signalling mechanisms of cholesterol depletion/repletion are underway.

These studies have continued since coming back to the UK and data generated using these protocols and samples generated whilst at Cornell University are being used as pilot data for a project grant that will be submitted to the BBSRC in the coming month. The project will see Professor Mark Roberson as an active collaborator and applicant on the grant and me as a recognised researcher and Dr Rob Fowkes as the primary applicant.

The funding received from the Society has not only benefited me scientifically in terms of learning new techniques, it has also enabled me to establish important links with a leading scientist in GnRH and membrane raft signalling in pituitary gonadotroph cells. This collaboration from a career development perspective is excellent, as it has given me a potential collaborator and mentor for future fellowship and young researcher grant applications. This collaboration will also benefit my current institution financially if the current BBSRC project grant application is successful.