Regulation of membrane protein function in human blood vessel formation

Vas Ponnambalam, Lorna Ewan, Gareth Howell, Shweta Mittar,
Mudassir Mohammed and Jane Murphy

Background

The major interest in the laboratory is how membrane proteins interact with different factors to programme cell and organ development in human health and disease. Our current model system is the human endothelial cell that lines the inside of all blood vessels and regulates angiogenesis i.e. the sprouting of new blood vessels from pre-existing blood vessels. Understanding this process is critical in treating diseases such as atherosclerosis and cancer. Importantly, many diabetic patients suffer from atherosclerosis and eye diseases due to dysregulation of endothelial cell function.

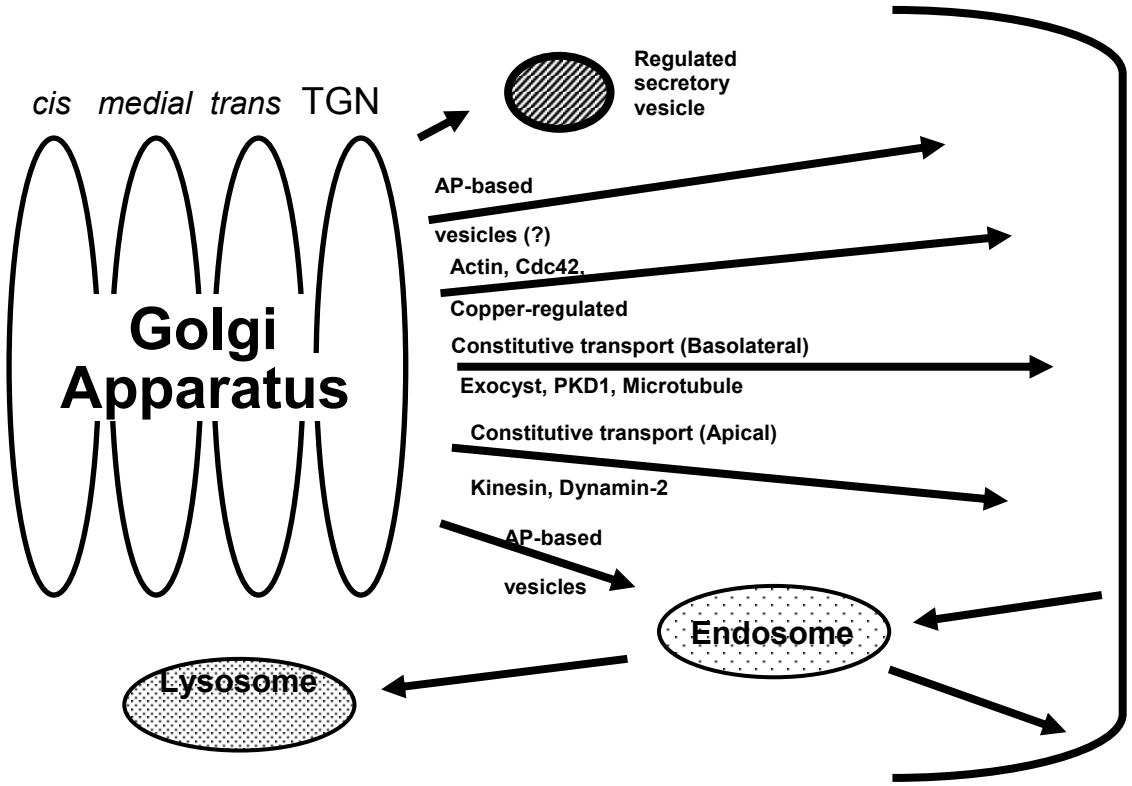

Fig. 1 Steps in membrane traffic to and from the Golgi apparatus, plasma membrane and endosome-lysosome system are under study using different model systems with a particular emphasis on human endothelial cells.

Structure of membrane proteins

Receptors on the surface of human endothelial cells bind a variety of substances including nutrients, lipoproteins and growth factors. Depending on the substance that is bound, a series of intracellular processes are triggered including sorting of receptor-ligand complexes, activation of signalling pathways, internalisation of receptor-ligand complexes, processing, sorting and receptor recycling. The goal of the laboratory is to study how such biochemical reactions can be linked to membrane remodelling in the endothelial cell in terms of blood vessel formation. Protein domains of such receptors expressed as single modules or linked to each other in bacterial and eukaryote systems will be tested using the excellent crystallisation facilities and technical support within the Astbury Centre. Large protein assemblies purified from mammalian

cells and tissues can be subjected to electron microscopy analyses, sophisticated and computerised image reconstruction to obtain a 3-D image. The information obtained from such studies will help us to better test our hypotheses for blood vessel development using site-directed mutagenesis and expression in human endothelial cells.

Analysis of conformational changes in membrane proteins

Detecting and understanding conformational changes in endothelial membrane receptors is an important at many levels including potential clinical assays for human disease situations. Increasingly, conformational changes in membrane proteins can be detected using sophisticated biophysical techniques such as NMR and fluorescence spectroscopy. NMR is a powerful and sensitive tool for detecting conformational changes of protein domains in solution. This is especially important where many membrane proteins are difficult to crystallise and solution-based interactions are critical in programming such interactions. The Astbury Centre has a state-of-art NMR facility including 500 MHz and 600 MHz NMR machines and provide excellent opportunities for such studies. The recently upgraded state-of-art spectroscopic and spectrometry facilities include Q-ToF, SELDI/MALDI/Protein Chip Reader, FT-IR, circular dichroism and fluorescence machines is also available for the study of macromolecular assemblies. In addition, atomic force microscope (AFM) systems are also being developed for detection of single molecule events in biological systems.

Collaborators

Prof. Steve Baldwin (BMB Leeds)
Dr. Francis Barr (Max-Planck Institute for Biochemistry, Munich, Germany)
Prof. Grahame Hardie (Univ. of Dundee, UK)
Dr. John Walker (BMB Leeds)
Prof. Vivek Malhotra (UC San Diego, USA)
Prof. Tony Monaco (Wellcome Trust Centre for Human Genetics, Oxford)
Dr. Ewan Morrison (Leeds Cancer Research UK Clinical Centre)
Dr. Michelle Peckham (BMS Leeds)
Dr. Asipu Sivaprasadarao (BMS Leeds)
Prof. Liz Smythe (University of Sheffield, UK)
Prof. Tony Turner (BMB Leeds)
Dr. Ian Zachary (Centre for Cardiovascular Biology and Medicine, University College London)

Publications

Cobbold, C., Coventry, J., Ponnambalam, S. & Monaco AP (2003).The Menkes disease ATPase (ATP7A) is internalized via a Rac1-regulated, clathrin- and caveolae-independent pathway. *Hum. Mol. Genet.* In Press.

Cobbold, C., Ponnambalam, S., Francis, M.J. & Monaco, A.P. (2002) Novel membrane traffic steps regulate the exocytosis of the Menkes disease ATPase. *Hum. Mo.l Genet.,* **11**, 2855-2866.

Grewal, S., Morrrison, E.E., Ponnambalam, S. & Walker, J.H. (2002) Nuclear localisation of cytosolic phospholipase $A_2\alpha$ in the EA.hy.926 human endothelial cell line is proliferation dependent and modulated by phosphorylation**.** *J. Cell Sci.,* **115**, 4533-4543.

Grewal, S., Ponnambalam, S. & Walker, J.H. (2003) Golgi localisation of cytosolic phospholipase A2α in the human A549 epithelial cell line. *J. Cell Sci*., **116**, 2303-2110.

Ponnambalam, S., Baldwin, S.A. (2003) Constitutive protein secretion from the *trans*-Golgi network to the plasma membrane. *Membr. Mol. Biol.,* In press.

Funding
We acknowledge funding by the British Heart Foundation, The Wellcome Trust and the White Rose Consortium.