Regulation of membrane protein localisation in eukaryote systems

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Protein localisation is an essential feature in biological systems from bacteria to man. How does a cell control the localisation of proteins to different parts of the cell? In eukaryotes, intracellular biological membranes around organelles such as lysosomes, Golgi apparatus and endoplasmic reticulum (ER) form boundaries that enclose a specific mixture of proteins and accessory molecules. Additionally, specific factors can also be recruited to the surface of each biological membrane to produce unique biochemical and functional properties.

Cell biology and membrane traffic

Endocytosis and exocytosis are two key routes by which a eukaryote cell transports proteins to different locations. Integral membrane proteins at different steps can recruit specific protein complexes to membrane surfaces to trigger steps in vesicle biogenesis, fission, movement and fusion. Cell biological projects in the laboratory are using different model proteins to identify key molecules, factors and mechanisms along each route. Integral membrane proteins that are constitutively secreted are convenient markers for studying the last step in protein secretion: *trans*-Golgi network (TGN) to plasma membrane. An atypical protein kinase C called PKD, diacylglycerol, microtubules and cytosolic factors are key players in this key membrane traffic step. Collaborations with Prof. Vivek Malhotra (Univ. California at San Diego) and Prof. Steve Baldwin (Leeds University) are in progress to elucidate key factors needed for this final step in constitutive protein secretion.

A key theme that is emerging in my laboratory is on the link between pathways for intracellular signalling and membrane traffic in cancer and heart disease. A programme of work (in collaboration with Dr. John Walker) is under way to study the cell biology of endothelial cells. These cells regulate angiogenesis, the process by which new blood vessels sprout from existing blood vessels. Thus damaged blood vessel repair after heart disease and strokes is critically dependent on endothelial cells. Importantly, new blood vessel formation is vital for cancerous tumour and metastatic growth. Manipulation of endothelial cell function by regulating responses to specific growth factors would allow treatment of such diseases. Projects include regulated secretion of key hormones such as von Willebrand factor and the endocytosis and degradation of vascular endothelial growth factors. The localisation of phospholipase A2-α in endothelial and non-endothelial cells in response to growth factor and other stimuli is also the focus of a collaborative effort.

A collaboration with Prof. Tony Monaco and Dr. Christian Cobbold (Oxford University) is ongoing to study the cell biology of the Menkes (MNK) protein. This ATPase is a multiple transmembrane protein and copper transporter that cycles between the Golgi apparatus and the cell surface in response to copper ions. Our lab is providing help and expertise in membrane trafficking in these studies on factors needed for the copper-regulated exocytosis and endocytosis of the human MNK protein.

Biophysics of membrane-protein interactions

A key aspect of biological membranes is lipids. These can come in a variety of shapes and 'flavours' including phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, sphingomyelin and cholesterol. A minor, but key element of biological membranes, are the phosphoinositides which contain the inositol sugar moiety. These phosphoinositides can be phosphorylated at different positions on the inositol ring by a vast array of lipid kinases. These different phosphoinositides are recognised by different cellular proteins and act as signals that regulate membrane attachment, localisation and/or activity of different classes of proteins and thus cell function. This allows a cell to regulate many proteins along a specific pathway in response to specific signals e.g. expression of a specific set of genes. Membrane traffic routes can also be regulated by phosphoinositides that mediate the recruitment of one or more factors.

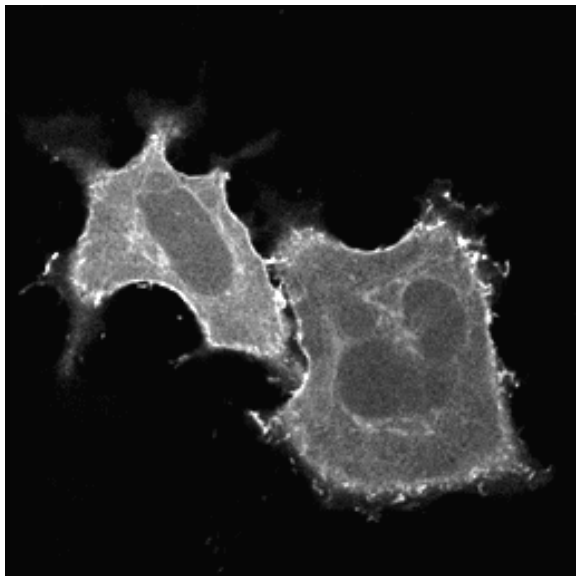

Figure 1. HeLa cells expressing a lipid-binding protein that is recruited to the plasma membrane on binding a specific phospholipid at the cytosolic face of the membrane bilayer. Note that plasma membrane ruffles in both cells are clearly defined by the localisation of this protein.

We have shown that a phosphatidylinositol 4-phosphate can act as a signal to mediate the targeting of oxysterol-binding protein to the Golgi apparatus. Other phosphoinositides such as phosphatidylinositol 4,5-bisphosphate which is enriched at the plasma membrane can similarly mediate the recruitment of different enzymes and proteins. Biophysical projects will (1) determine the basis for lipid specificity in different proteins with phosphoinositide-binding domains, (2) test the influence of different lipids and complex biological membrane systems for such protein recruitment, and (3) test lipid analogues and competitive inhibitors for their ability to modulate membrane binding and cell function.

Structural projects are also on-going to determine the three-dimensional structure of protein-lipid complexes using nuclear magnetic resonance and crystallography. These will take advantage of the state-of-art facilities and expertise in the Astbury Building.

Collaborators

Prof. Steve Baldwin (Leeds University)
Prof. Peter Downes (Dundee University)
Prof. Vivek Malhotra (Univ. California San Diego, USA)
Prof. Tony Monaco (Oxford University)
Dr. Ewan Morrison (St. James' Hospital, Leeds University)
Prof. Sheena Radford (Leeds University)
Dr. John Walker (Leeds University)

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