



Lincoln's Inn Fields

Cell Biophysics

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Membrane fusion is at the core of many essential cellular mechanisms including intracellular trafficking and mitotic organelle reconstitution. Without fusion, membrane traffic would cease and cellular compartments would shed vesicles until the compartments disappeared. To elucidate the mechanisms involved in membrane fusion, global lipid analysis of various membrane compartments is being performed and selective variation in species composition will be related to the physical properties that characterise membrane structure. Modulation of lipid species composition defines both the conformational changes of the membrane and the 'local signal'. Perturbations caused by changes in membrane curvature and phospholipid composition affect the affinity of proteins to be targeted to appropriate membrane compartments.

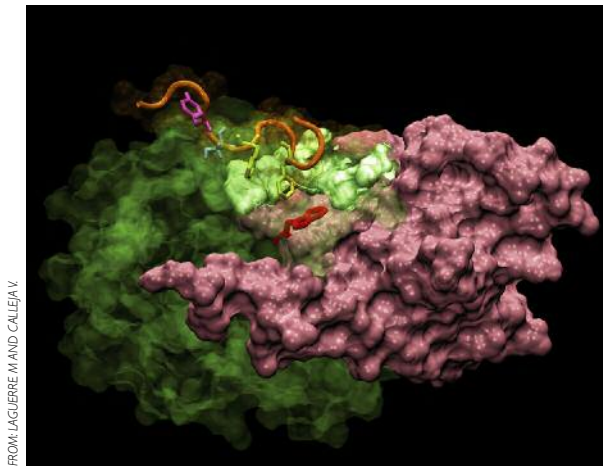
In addition we are investigating how the regulation of membrane protein-lipid and protein-protein interactions, both

inter- and intra-molecular, will be affected by the physical and compositional properties of the bilayer. Our main focus is to study and link, *structural* regulation and signalling during membrane fusion. Nano-analytical tools such as fluorescence lifetime imaging microscopy (**FLIM**) and other precise tools such as **NMR** spectroscopy and liquid chromatography tandem mass spectrometry (**LC-MS/MS**) are used to provide increased insight into molecular composition and associations in the cell and various sub-cellular compartments.

Proteo-lipid regulation in nuclear envelope assembly

Regulation of nuclear envelope dynamics is an important example of the universal phenomena of membrane fusion and fission. The nuclear envelope is disassembled and reassembled at each mitosis in typical animal cells. The processes of disassembly and reassembly may also occur in interphase and are usually but not always co-ordinate in nuclei sharing a common cytoplasm, for example in fertilised eggs. Male nuclear envelopes however are disassembled and reassembled in all cases. The study of the processes of male pronuclear membrane dynamics in fertilised sea urchin eggs investigated with cell free extracts has revealed several novel features, especially regarding the role of phospholipids during nuclear membrane formation. Our novel model combines and relates the study of membrane domains and regulation of membrane fusion. The fusion of chromatin-bound membrane vesicles is a process triggered by GTP, acting upstream of an endogenous PLC activity.

We have recently identified a sea urchin PLC γ isoform acting downstream of GTP. PLC γ is recruited to the nuclear envelope both *in vitro* and *in vivo* on vesicles (in collaboration with DL. Poccia, Amherst College, USA). The lipidome of these vesicles has been analysed with the HPLC-tandem mass spectrometer in Cell Biophysics and is rich in poly-phosphoinositides, including the PLC γ substrate PtdIns



FROM LAGUERRE M AND CALLEJA V

Figure 1. Complete model of the human PKB in the inactive PH-in conformer. The PH domain is in pink and the key PH domain residue W80 is in red sticks. The kinase domain is presented as a transparent green surface and the posterior half of the cavity is in bright translucent green. The C-terminal is in orange ribbon and the two Phe (F479 and F472) interacting with W80 are shown as yellow sticks. The S473 and Y474 residues from the C-terminal are shown as cyan and fuchsia sticks respectively.

(4,5)P₂. Our data reveal that PLC γ , when activated in response to GTP, controls the fusion of nuclear envelope precursor membrane vesicles, by the generation of the fusogenic lipid diacylglycerol (DAG) from PtdIns(4,5)P₂. We show the intersection of tyrosine kinase and phosphoinositide signalling pathways during membrane fusion. We have identified a sea urchin src family kinase (SFK) colocalised with PLC γ *in vivo* and *in vitro*. The site of this colocalisation is on MVI, a cortical vesicle population of unprecedented lipid composition, being 60% phosphoinositides (of which one-fifth is the PLC γ substrate PtdIns(4,5)P₂). MVI is absolutely required for the formation of the nuclear envelope. Moreover, in collaboration with EJ Dufourc (CNRS-European Institute of Chemistry and Biology, (IECB) Bordeaux, France), solid-state nuclear magnetic resonance spectroscopy (ssNMR) was used to define the structure of the natural membrane domains in our model. Our future work in the regulation of NE assembly encompasses both somatic and non-somatic cells.

Protein kinase B (PKB/Akt) activation mechanism: from structure to function

The serine/threonine kinase PKB/Akt has received considerable attention over recent years and has become the focus of drug targeting for cancer therapy since a major role for this protein in tumour progression has emerged. We have tackled a very challenging problem of defining *in vivo* how a process works, leading to a different view of the molecular process.

Our results provide novel insights into the processes involved in the *in vivo* conformational change of the protein kinase and its association with PDK1, namely that it

precomplexes in the cytoplasm with PDK1. The full-length structure of PKB/Akt has not been resolved and the key aim of our most recent investigation was to elucidate PKB/Akt three-dimensional structure in relation to its function.

By using a multi-disciplinary approach including molecular dynamic simulations, classical biochemical assays and FRET/two-photon FLIM, essential insights into the quaternary structure of PKB/Akt, in its inactive conformation was demonstrated. The use of molecular dynamic simulations challenged by mutagenesis experiments revealed a novel structure at the interface of the PH and kinase domains in the PKBa inactive conformation (a PH-domain induced cavity). The critical role of this novel structure in the regulation of the PKB inactive state was substantiated by its connection to the C-terminal regulatory domain of PKB: the hydrophobic motif.

These findings have led to an understanding of the molecular mechanism of action of a new class of highly selective allosteric PKB inhibitors. It elucidates at the molecular level its selectivity towards the different PKB/Akt isoforms that had remained so far elusive. We anticipate this will facilitate the optimisation of a new generation of more selective allosteric inhibitors.

Prognostic value of an activation state marker for EGFR and its downstream pathways in tissue microarrays of breast tumours

Current method for assessing patient's entry for treatment of signal transduction inhibitor is limited. About 30% of breast cancer patients overexpress the ErbB2 receptor but of those patients, only 35% with 3+ over-expression respond to Herceptin. This indicates the limited capacity of the immunohistochemistry alone in predicting treatment success of these patients as the over expression of the ErbB2 may not correlate with the functional status of these receptors. A methodology to improve assessment of not just concentration of signal transduction target but their status is required. We have developed an innovative analytical approach to measure functional status of these signal transductions using FRET efficiency by high throughput fluorescence lifetime microscopy (FLIM) in tumour arrays. We have validated this FRET method to assess ErbB1 functional status in cells and head and neck tumour and various breast cancer cell lines. The FRET efficiency by FLIM has great potential to be a prognostic marker molecular therapy. Current work is being done on breast tumours in collaboration with Peter Parker's laboratory (Protein phosphorylation laboratory). The samples are available through collaboration with Anthony Kong and Adrian Harris's laboratory (Oxford-CRUK). The high throughput screening system has been implemented for tumour array analysis in collaboration with Pierre Leboucher (College de France-CNRS – Paris).

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