European Cells and Materials Vol. 10. Suppl. 5, 2005 (page BS18) ISSN 1473-2262

Supported Cell Membrane Sheets for Functional Imaging of GPCRs

J-B.Perez[1], K.L.Martinez[2], J-M.Segura[1], H.Vogel[1]

[1] *EPF Lausanne, Lausanne, Switzerland.*

[2] *University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark.*

INTRODUCTION:

G protein-coupled receptors (GPCRs) are the largest family of membrane receptors and a major target for therapeutic compounds. They transduce external signals (light, hormones, odorants, drugs) inthe activation of intracellular heterotrimeric G-proteins. After activation, G proteins dissociate and induce the production of second messengers by interacting with enzymes or ion channels.

Many unresolved questions remain about the way GPCRs function. For instance, these membrane proteins are able to rapidly and sensitively transduce extracellular stimuli although the physiological concentrations of the proteins involved are usually low. Elucidating these complex processes will require the development of in-vitro systems allowing the selective investigation of particular aspects of the signaling cascade.

We present here the use of cell membrane sheets suitable for *in-vitro* functional fluorescence studies, prepared by direct detachment from cell membranes using poly-L-lysine coated glass slides (Fig 1).

METHODS:

Cell membrane sheets were prepared from HEK-293 cells expressing various proteins of interest. A glass coverslip coated with PLL was pressed to the apical parts of the cells. After several minutes of contact, the coverslip was removed, ripping off from the cells large regions of the apical native plasma membrane.

RESULTS: Cell membrane sheets suitable for *in-vitro* functional fluorescence studies were prepared by direct detachment from cell membranes using poly-L-lysine coated glass slides. The resulting transferred planar membranes conserved the composition as well as most properties of the original plasma membrane, in particular both leaflets remained fluid allowing the investigation of diffusion properties of different cellular membrane components. Measurements on membrane sheets offer several advantages as compared to living cells. First, access to the intracellular leaflet is obtained, in particular to the intracellular part of membrane proteins and to cytoplasmic, membrane-associated proteins, opening the possibility to label them and to modulate their properties with non membrane permeable compounds. Second, the cytosolic autofluorescence of the cells is absent allowing ultra-sensitive measurements to be performed down to the single-molecule level. Third, the complexity of cellular processes occurring at the plasma membrane can be reduced allowing the sequential investigation of selected events from complex biochemical networks. These advantages were used to perform ligand-binding studies on a representative GPCR.

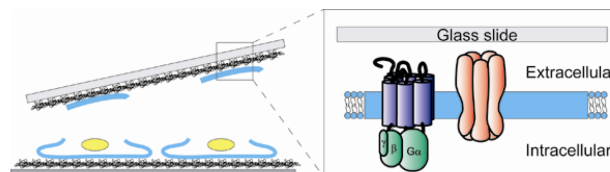

Fig. 1: Formation of cell membrane sheets containing membrane proteins.

DISCUSSION & CONCLUSIONS:

Our results show that supported membrane sheets might find a broad application as ideal *in-vitro* system for the elucidation of complex signaling pathways. We applied for instance this methodology in recent experiments onthe localization and diffusion of the G proteins investigated using single-molecule microscopy (SMM).