P-170

Nuclear transport receptors play a role in maintaining the RanGTP/RanGDP gradient

Larisa E. Kapinos, Alexandra Feng, Suncica Barabato, Chantal Rencurel, Roderick Y. H. Lim
Biozentrum and the Swiss Nanoscience Institute, University of Basel, CH-4056 Basel, Switzerland;

Nucleocytoplasmic transport (NCT) is a fundamental biological process in eukaryotic cells. This is facilitated by nuclear transport receptors known as importins and exportins that traffic specific cargoes through nuclear pore complexes (NPCs). Recently, we found that a key importin, Kapβ1, is highly enriched in NPCs and in doing so functionally reinforces the NPC selective barrier [1-3]. The bound-fraction of NTRs at the NPC is governed by their affinity towards FG Nups and cellular concentration. Here, we show now that cellular apoptosis susceptibility protein (CAS, known also as Xpo2 or Exportin2), interacts differently with the phenylalanine-glycine nucleoporins (FG Nups) and consequently is less enriched at NPCs. Immunostaining reveals that CAS accumulates mostly inside the nucleus in contrast to Kapβ1. In addition to its interaction with the FG Nups, this suggests that an additional mechanism is required to regulate the nuclear localization of CAS. Indeed, CAS functions to export Kapα, an adaptor protein required for the specific cargo import. Furthermore, import and export processes are dependent on the RanGTP/RanGDP gradient that is established at the NPC being generated by RanGAP/RanBP1 and RanGEF [4]. We show here that an NPC transport barrier can be also selective for such small cargos. As in the present case, RanGTP or RanGDP interact with transport receptors within the NPC and this in turn can play a role in maintaining the RanGTP/RanGDP gradient.

RanGTP/RanGDP gradient

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P-171

A potent partial agonist of PKC orients in membranes like the biological activator diacylglycerol

S. Lautala[1], A. Koivuniemi[1], W. Kulig[2], T. Rog[2], V. Talman[3], R. Tuominen[1], A. Bunker[1].
[1]University of Helsinki, Faculty of Pharmacy, Helsinki, Finland; [2]University of Helsinki, Faculty of Physics, Helsinki, Finland; [3]Imperial College London, National Heart and Lung Institute and University of Helsinki, Faculty of Pharmacy, Helsinki, Finland.

The enzyme protein kinase C (PKC) has a plethora of roles in human physiology, and thus plays also a significant role in pathologies of many diseases, such as cancer and Alzheimer's disease [1, 2]. This enzyme is activated when its C1 domain interacts with an intracellular membrane that incorporates the lipid second messenger diacylglycerol (DAG). In cancer research PKC was previously thought to be an oncoprotein, however, upon discovering that PKC associated cancer activity is in fact associated with loss-of-function mutations in PKC, moderate activation could actually be tumour suppressing [2]. The PKC enzyme's activation is also involved in learning and memory formation, and therefore drugs that modulate PKC could additionally be used to treat Alzheimer's disease.

In studies performed by R. Provenzani et al. isophthalate derivatives and pyrimidine analogs, respectively, were tested to target PKC [3]. Both sets of compounds were predicted to bind well to the C1 domain based on their structure, however, only isophthalate derivates showed in vitro binding in a standard assay with phosphatidylserine membrane present.

These behaviors are not completely explained by just the fit of the molecule to the binding site of the PKC C1 domain, and thus we hypothesized that the interaction of the drug molecules and the respective inner plasma membrane is of importance for activation. To answer this, we simulated one isophthalate and a respective pyrimidine compound in the PS binding assay environment.

Analysis of the molecular dynamics simulations indicate that isophthalate compound quickly equilibrates to DAG-like orientation, but the pyrimidine compound fails to do so, while also sinking deeper into the membrane. This results to the pyrimidine compound being inaccessible for the enzyme at the interface. These factors begin to explain the difference in experiments, and highlight the importance of environment effects in drug design.

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P-172

Dynamic recruitment of BAR domain proteins by Fluid-FM combined with fast-scanning confocal microscopy

C. Lo Giudice[1], H.F. Renard[2], F. Tickaert[2], P. Morsomme[2], D. Alsteens[1].
[1]Louvain Institute of Biomolecular Science and Technology (LIBST), NanoBiophysics Lab (nBio), Université catholique de Louvain, Louvain-La-Neuve, Belgium; [2]Louvain Institute of Biomolecular Science and Technology, Group of Molecular Physiology, Université catholique de Louvain, Louvain-La-Neuve, Belgium.

Cell responses to external stimuli and many vital processes, such as nutrient uptake and receptor turnover, are mediated by cell membrane and endocytosis. Endocytosis occurs through a variety of pathways, broadly classified into clathrin-mediated and non clathrin-mediated[1-2]. While clathrin-mediated endocytosis is well characterized, most of non-clathrin mediated endocytic pathways are poorly understood from a mechanistic point of view. In this context, BAR (Bin/amphiphysin/Rvs)-domain proteins play a key role in membrane curvature induction/recognition, and some of them, particularly the endophilin subclass, have been found to be involved in non-clathrin dependent endocytosis of several cargoes, such as β-adrenergic receptors, IL-2 receptor or bacterial toxins[3-5]. Nevertheless, the dynamics of endophilins participation in receptor-endocytosis is still not clear, as well as many aspects of the molecular mechanisms.

We present an original single-cell approach, using the combination of Fluidic Force microscopy (Fluid-FM) and fast scanning confocal microscopy. This set-up allows the dynamic imaging of endophilin recruitment to be followed directly at the cell membrane in response to local extracellular stimulation. Fluid-FM combines microfluidics to accurate force control by using microchanneled cantilevers connected in parallel to a vacuum pump and to an atomic force microscope (AFM). In our approach, we tested the induction of endophilin-mediated endocytosis from several ligands that have been immobilized on the surface of sub-micron beads and trapped at the aperture of Fluid-FM cantilevers. By approaching the cantilevers to cells expressing fluorescently-labeled endophilins we achieved spatially and temporally resolved extracellular stimulation at controlled force, while monitoring simultaneously the dynamic movement of the endophilins in response to the local extracellular stimulus via fast-scanning confocal microscopy. Although at its infancy, this new approach holds the potential of monitoring, *in situ* and with high spatial and temporal resolution, variations of cell mechanical properties and intracellular trafficking in response to extracellular signalling, while measuring simultaneously interaction forces, thus enabling to tackle a broad range of unsolved biological questions.

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P-173

Gag polyprotein of HIV-1 shows preference to membrane curvature

Z. Denieva, T. Galimzyanov, P. Kuzmin, O. Batishchev.
A.N. Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences, Moscow, Russian Federation.

Enveloped viruses presents a large family of pathogens including such dangerous ones like HIV, Ebola, Zika, etc. These viruses have their genome wrapped in a protein scaffold, which, in turn, is surrounded by the bilayer lipid membrane. Viral membrane is formed by lipid molecules captured by the virus during its budding from the plasma membrane of the infected cell. It is of great interest to investigate, which of the viral proteins is responsible for the formation of the curvature required for bud initiation and whether viral proteins have sensitivity to membrane curvature. Using a system of lipid nanotubes, which allows the creation of highly curved lipid surfaces, we investigated the self-organization of the human immunodeficiency virus (HIV-1) polyprotein Gag and showed that this protein tends to self-organize in curved sections of the membrane.