1853-Pos Board B697

Using Thiol-Reactivity to Identify Proteins Involved in the Ca^{2+}-Triggering Steps of Native Membrane Fusion

Kendra L. Furber[1], **Jens R. Coorssen[2,1]**.
[1]Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada, [2]School of Medicine, University of Western Sydney, Penrith South DC, NSW, Australia.

Ca^{2+}-triggered membrane fusion, the defining step of exocytosis, enables temporal/spatial control over the release of biologically active compounds. The mechanism by which Ca^{2+} triggers and modulates native membrane fusion is still poorly understood. As an unbiased approach to investigating this process, the effects of several thiol-reactive reagents on the homotypic fusion of isolated cortical vesicles (a stage-specific preparation for analyses of native Ca^{2+}-triggered fusion) have been characterized. Such reagents have been consistently shown to inhibit the Ca^{2+}-sensitivity, rate and extent of triggered fusion. However, we recently showed that iodoacetamide can also potentiate the Ca^{2+}-sensitivity and rate of release [1]. This implicates two distinct thiol sites in the fusion process - one involved in the ability of vesicles to fuse (extent) and one that modulates fusion efficiency (Ca^{2+}-sensitivity and kinetics). Capitalizing on this potentiating effect, we have now identified other fluorescent thiol-reactive reagents with similar effects: treatment with Lucifer yellow iodoacetamide, monobromobimane or dibromobimane resulted in an average leftward shift in EC_{50} from $17.2 \pm 1.6\mu M$ to $8.9 \pm 1.9\mu M$ $[Ca^{2+}]_{free}$. These fluorescent reagents can be used to enhance fusion and label proteins involved in the Ca^{2+}-sensing mechanism. The lipid matrix at or near the fusion site can also modulate the fusion process, specifically via cholesterol- and sphingomyelin-enrichment that is thought to regulate the Ca^{2+}-sensitivity and rate of fusion through spatial organization of critical lipids and proteins [2,3]. Proteins involved in Ca^{2+}-sensing are thus likely to be situated within such areas of the membrane. Isolation of fluorescently labeled proteins from cholesterol-enriched vesicle membrane fractions by 2-dimesional electrophoresis is now being used to identify proteins potentially involved in the Ca^{2+}-triggering steps of membrane fusion.

1) J. Chem. Biol., Epub 3/10/08.
2) J. Cell Sci. 2005, 118:4833.
3) J. Cell Sci. 2006, 119:2688.

1854-Pos Board B698

Control by Calcium of mammalian cell membrane electropermeabilization

Muriel Golzio[1], Florin Ciobanu[2], Eugenia Kovacs[2], **Justin Teissie[1]**.
[1]CNRS, Toulouse, France, [2]University Carol Davila, Bucarest, Romania.

Electric pulses, when applied to a cell suspension, induce a reversible permeabilization of the plasma membrane. This permeabilized state is long lived (minutes). The biophysical molecular mechanisms supporting the membrane reorganization associated to its permeabilization remain poorly understood. Modeling them by toroidal lipidic pores cannot explain why they are long lived and why their resealing is under the control of the ATP level. Our results describe the effect of the level of free Calcium ions. Permeabilization induces a Ca^{2+} burst as shown by imaging of cells loaded with Fluo3. But this sharp increase is reversible even when Calcium is present at a mM concentration. Viability is preserved to a larger extend when submillimolar concentrations are used. The effect of Calcium is during the resealing step not during the creation of permeabilization as the same effect is observed if Ca^{2+} is added in the few seconds following the pulses. The resealing time is faster when Ca^{2+} is present. Mg^{2+} is observed to play a competitive role. These observations suggest that Ca^{2+} is acting not on the external leaflet of the plasma membrane but due to its increase concentration in the cytoplasm. Exocytosis will be enhanced by this Ca^{2+} burst (but hindered by Mg^{2+}) and occurs in the electropermeabilized part of the cell surface. This description is supported by previous theoretical and experimental results The associated fusion of vesicles will be the support of resealing.

This work was supported by grants from the region Midi Pyrénées, the ANR « Cemirbio », the DGA and the Université P Sabatier

1855-Pos Board B699

Asymmetric Role of Negative Curvature Lipids on the SNARE Mediated Vesicle Docking and Fusion

Yuji Ishitsuka[1,2], Jiansong Tong[3], Bin Lu[3], Yeon-Kyun Shin[3], Taekjip Ha[4,2].
[1]Department of Physics, University of Illinois Urbana-Champaign, Urbana, IL, USA, [2]Howard Hughes Medical Institute, Urbana, IL, USA, [3]Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA, USA, [4]Department of Physics and Center for Biophysics and Computational Biology, University of Illinois Urbana-Champaign, Urbana, IL, USA.

N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) are fundamental machinery in the cellular membrane fusion process. Much of focuses of studies have been placed on the formation of core ternary SNARE complex and key accessory proteins, but the role of membrane environment in SNARE complex assembly is still unclear. We have used neuronal SNARE proteins (syntaxin-1HT, soluble SNAP-25 and VAMP-2) reconstituted proteoliposomes to study the effect of the cholesterol on the membrane fusion. Cholesterol is one of the most abundant components of eukaryotic plasma membrane. Bulk lipid mixing assay revealed that the addition of cholesterol to both acceptor and donor vesicles promote lipid mixing. However, when cholesterol was added to only one of the vesicle, VAMP-2 containing vesicle was found to contribute more towards the overall effect. Surprisingly, a similar result was observed using another negative curvature lipid POPE. Single vesicle fusion assay further revealed that this asymmetry comes from the fact that the vesicle docking and fusion of syntaxin-1HT/SNAP-25 and VAMP-2 containing vesicles are promoted differently in the presence of negative curvature lipids. A molecular mechanism behind this effect is discussed in the context of protein organization in different membrane environment. This study demonstrates the importance of membrane context in which SNARE proteins are being studied in in vitro system.

1856-Pos Board B700

MG53 Nucleates Assembly Of Cell Membrane Repair Machinery

Chuanxi Cai[1], Haruko Masumiya[2], Noah Weisleder[1], Noriyuki Matsuda[3], Miyuki Nishi[4], Moonsun Hwang[1], Jae-Kyun Ko[1], Peihui Lin[1], Angela Thornton[1], Xiaoli Zhao[1], Zui Pan[1], Shinji Komazaki[5], Marco Brotto[1], Hiroshi Takeshima[6], Jianjie Ma[1].
[1]UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA, [2]Department of Medical Chemistry, Graduate School of Medicine, Tohoku University, Tohoku, Japan, [3]Center Laboratory of Frontier Science, The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan, [4]Department of Biological Chemistry, Kyoto University Graduate School of Pharmaceutical Sciences, Kyoto, Japan, [5]Department of Anatomy, Saitama Medical School, Saitama, Japan, [6]Department of Biological Chemistry, Kyoto University Graduate School of Pharmaceutical Sciences, Kyoto, NJ, USA.

Dynamic membrane repair is essential not only for long-term maintenance of cellular integrity but also for recovery from acute cell injury. While compromised membrane repair contributes to various pathological states, including muscular dystrophy, heart failure and neurodegeneration, the associated molecular machinery is largely unknown. We have recently found MG53, a muscle-specific tri-partite motif family protein (TRIM72), is a component of the sarcolemmal membrane-repair machinery. Mice null for MG53 exhibit progressive myopathy, reduced exercise capability and defective membrane-repair capacity. MG53 interacts with phosphatidylserine to associate with intracellular vesicles that display trafficking to and fusion with sarcolemmal membranes. Injury of the sarcolemmal membrane leads to MG53 oligomerization in an oxidation-dependent manner that results in recruitment of MG53-containing vesicles to the injury site. A conserved cysteine residue (C242) is involved in oxidation-mediated oligomerization of MG53, and is critical for MG53 function in membrane repair. The response of MG53-mediated membrane patching is rapid, occurring on the order of seconds after injury, indicating that MG53 mediates the acute repair process following cellular damage. While MG53-mediated vesicle accumulation at the injury site does not require entry of extracellular Ca, Ca entry does facilitate vesicle fusion with the plasma membrane to complete the formation of a repair patch. Our data indicate that intracellular vesicle translocation and Ca-dependent membrane fusion are distinct steps involved in repair of membrane damage, and that MG53 may act as a sensor for oxidation to nucleate the assembly of the membrane repair machinery.