dynamin-catalyzed membrane fission prior to vesiculation. Our results highlight the general applicability of SUPER templates in analyzing several forms of vesicular and non-vesicular transport processes.

1942-Pos

Nanopore Formation in Cells Exposed to Nanosecond Electric Pulses

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Chemical and physical insults such as electroporation may compromise the membrane integrity and allow leak ion currents through *de-novo* formed lipid pores. These pores are thought to short-circuit the membrane and hinder ion channels' physiological function rather than to complement it. However, here we report that brief electric stimuli can trigger formation of membrane pores with specific behaviors that are traditionally considered to be unique for protein ion channels; still other behaviors of these pores distinguish them from both ''conventional'' electropores and any known ion channels. We found that a single electric shock (600-ns duration, 1 to 5 kV/cm) causes minutes-long increase of membrane electrical conductance due to formation of long-lived, voltage- and current-sensitive, rectifying, cation-selective, asymmetrical pores of nanometer diameter (''nanopores''). Once induced, nanopores oscillate between open and quasi-open (electrically silent) states, followed by either gradual resealing or abrupt breakdown into larger pores, with immediate loss of nanopore-specific behaviors. The formation and extended lifetime of nanopores were verified by non-electrophysiological methods, namely by fluorescent detection of Tl^+ uptake and of phosphatidylserine externalization. Apparently, nanopores are not unique to cell stimulation with nanosecond electric pulses, but may form under various physiological and pathological conditions. Nanopores appear adequately equipped for certain functions that are traditionally ascribed to ion channels. Clear distinction between nanopores- and ion channels-mediated currents may be critical for understanding how these currents are controlled.

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1943-Pos

Inhibition of Membrane Stretching Prevents Lipid Pore Enlargement in Cells Porated by Nanosecond Electric Pulses (NSEP)

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Recent studies established that nsEP cause formation of stable pores in plasma membrane of mammalian cells. Pores formed by a single nsEP of moderate intensity (e.g., one 600-ns pulse at 3-5 kV/cm) were permeable to alkali cations, but not to a larger propidium cation (van der Waals dimensions 1.6 x 1.4 x 0.8 nm), suggesting that the pore diameter does not exceed 1.4 nm. However, higher nsEP amplitudes and/or exposures to multiple pulses could cause minor propidium uptake. Meanwhile, the mechanism of transformation of initial ''nanopores'' into larger, propidium-permeable pores remains unclear.

One mechanism potentially responsible for nanopore enlargement could be membrane stretching caused by osmotic cell swelling following nsEP exposure. In isoosmotic and even hyperosmotic bath media, membrane permeabilization to small inorganic ions leads to equalization of their concentrations inside and outside of cells. However, larger molecules cannot escape the porated cell interior, creating additional osmotic pressure. To prevent nsEP-induced swelling, the bath buffer can be supplemented with a membrane-impermeable compound, which would prevent the increase of intracellular osmolarity.

We demonstrated that addition of sucrose (8-40 mM) to the bath buffer decreases or eliminates propidium uptake by nsEP-exposed cells (60-ns, 30 pulses at 30 kV/cm). However, addition of isosmotic amount of NaCl caused little or no protective effect. These data are indicative of the fact that membrane stretching by osmotic cell swelling could indeed be the cause of nanopores enlargement. This hypothesis is being additionally tested to exclude a potentially biasing effect of sucrose and NaCl addition on the efficiency of initial nanopore opening.

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1944-Pos

Lipid Raft and Arf6-GTPase Dependent Endocytosis of the hERG Potassium Channel

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The voltage-gated hERG potassium channel is critical for the repolarisation phase of the cardiac action potential. Genetic mutations leading to a decrease in the number of hERG channels at the cell surface lead to potentially fatal Long QT syndrome which is reflected by an elongated QT interval in the ECG. It is therefore important to understand the trafficking mechanisms that regulate the surface density of hERG. Here we have investigated the endocytic pathway of an epitope (hemagglutinin A) tagged hERG, expressed in HeLa cells, using techniques in molecular cell biology, electrophysiology and biochemistry. Our results demonstrate that the majority of the hERG channel is rapidly internalized from the plasma membrane in a dynamin and clathrin independent manner. Endocytosis of hERG is dependent on cholesterol rich lipid rafts and ADP-ribosylation factor 6 (Arf6). Depletion of cholesterol from cell membrane by treatment with methyl-β-cyclodextrin and disruption of Arf6 activity by over-expression of inactive Arf6 mutants or aluminum fluoride resulted in an inhibition of hERG endocytosis, leading to an increase in hERG currents. Majority of the internalized hERG channel was found in lipid rafts isolated by density gradient centrifugation, identified by the presence of raft marker proteins including flotillin and the co-expressed GFP-tagged GPI anchor protein. Raft associated caveolin, Rac-1 and RhoA-GTPase do not appear to be required for hERG endocytosis. A small fraction of hERG, however, appears to undergo endocytosis via clathrin mediated endocytosis. Following internalization the channel enters Rme1 positive recycling endosomes and also Lamp1-positive late endosome/lysosomal compartments. The significance of lipid-raft and Arf6 dependent endocytosis of hERG in cardiac physiology has yet to be understood. This work was funded by the British Heart Foundation.

1945-Pos

High-Resolution Frap of the Cilium-Localized Somatostatin Receptor 3 Reveals the Presence of a Lateral Diffusion Barrier at the Cilium Base

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It is well known that certain proteins localize to the cilia of cells where they perform various specialized functions, such as sensing the physical environment of the cell. Protein localization to subcellular compartments is important for normal cell function and mutations preventing protein localization to cilia are implicated in devastating human pathologies including blindness, deafness, infertility, obesity and many others.

While the mechanisms for transport of proteins to cilia are well-studied, the mechanisms for maintaining their localization to cilia are not understood. One proposed mechanism for the latter predicts the existence of a selective barrier at the base of the cilium that regulates the free movement of both water-soluble and membrane-bound proteins into and out of the ciliary compartment. We directly tested this hypothesis by examining the mobility of the murine somatostatin receptor 3 protein (SSTR3), a G-protein-coupled receptor that naturally localizes to the ciliary membrane of inner-medullary collecting duct (IMCD3) cells.

Using multiphoton fluorescence recovery after photobleaching (MPFRAP) we estimated the diffusion coefficient and measured the equilibration time of SSTR3-EGFP or SSTR3-PAGFP fusion proteins in IMCD3 cell cilia. We found that SSTR3 fusion proteins rapidly, equilibrate along the length of cilia without change in the total mass, indicating that the membrane-bound protein is highly mobile but remains confined inside the cilium. This finding is consistent with the hypothesis that lateral membrane diffusion at the base of the cilium is constrained.