The Biophysical Society of Japan General Incorporated Association

S3B2

Direct Force Transduction by Integrins and the Cytoskeleton

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Force is a critical signal in cellular motile events. Cell spreading,migration, wound healing, and organ development involve coordination offorce generation and force sensing, which goes awry in transformed cells. Recent findings demonstrate two molecular pathways of force transduction: 1) in early adhesion, a receptor-like protein tyrosine phosphatase alpha transduces force on the avb3 integrin into the strengthening of the cytoskeletal connection and activation of Src family kinases, which then weaken the connections (Felsenfeld et al., Nat. Cell Biol. 1:200-206 1999), and 2) in established contacts, Triton X-100 cytoskeletons directly transduce stretching force from the collagen matrix into the binding of focal contact proteins to the cytoskeleton (paxillin, FAK, and p130cas but not vinculin bind to stretched but not control cytoskeletons) (Sawada and Sheetz, J. Cell Biol. 156:609-615 2002). In the initial events of cell spreading, we suggest that avb3 integrin and RPTPa form a force-transduction complex, and the application of force to avb3 causes the direct activation of RPTPa. In mature fibrillar collagen contacts, an established trans-cellular cytoskeleton network that remains after Triton extraction is altered by collagen stretch and will preferentially bind several cytoplasmic focal contact proteins at the sites of greatest stress. We suggest that force on the intact cytoskeleton exposes binding sites for focal contact and heat shock proteins at the sites of maximal stress.

M. P. Sheetz : Direct Force Transduction by Integrins and the Cytoskeleton

S3B3

Single molecule scanning in-plane force imaging of the membrane of live cells

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Single molecule techniques have shown that the slow diffusion of individual membrane proteins in the cell membrane of living cells results from slow hops over barriers between neighboring free diffusion regions. We have performed a mean first passage time calculation to determine the escape frequency over such barriers to diffusion. The consequences of applying a force to aid in passage results in a logarithmic dependence of the force required to pass a barrier on the rate of force application. To verify this prediction we have employed laser tweezers to repeatedly drag a single freely diffusing, colloidal gold-labeled CD44 transmembrane protein in the plasma membrane of normal rat kidney cells (NRK). The force required to pass the barriers was always less than 0.3 pN under our conditions (scanning rate 0.5-2 μm/s, trap stiffness 1-5 pN/μm) and was dependent on the rate of scanning and the spring constant of the trap. Further, the technique has been extended to use the single CD44 molecule probe to force-image the membrane compartmental structure by scanning the probe across a defined area of the cell. A network of lines of high resistance to dragging of the probe was found in the NRK plasma membrane with a mesh size of about 0.5-1 μm, consistent with the compartment size as determined from diffusion studies. As such, we have successfully imaged the cortical layer of actin under the plasma membrane of live cells that is in close enough proximity to control the movements of membrane molecules.

K. Ritchie and A. Kusumi : Single molecule scanning in-plane force imaging of the membrane of live cells

S3B4

Structure and function of GPI-anchored protein membrane rafts in living cells

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Cholesterol and sphingolipid -enriched regions of cellular membranes, termed 'rafts', have been envisaged as platforms for the lateral segregation of lipids, proteins and lipid-anchored proteins. Glycosylphosphatidylinositol-anchored proteins (GPI-APs) have served as markers of rafts since many of the GPI-anchor specific properties such as signalling across the plasma membrane, and sorting in endosomal compartments are dependent on cholesterol and sphingolipid levels in membranes of living cells. Using a combination of fluorescence resonance energy transfer microscopy and theoretical modelling, we show that instead of the expected large-sized raft assemblies, cell surface GPI-APs occur as nanometer sized (less than 10 nm) clusters, composed of multiple GPI-AP species. The extent of clustering is independent of the concentration of the GPI-AP. but, reduction in cholesterol levels decreases the proportion of clusters. Sphingolipid-depletion does not change the proportion of clusters, instead makes the GPI-AP clusters easier to disrupt upon cholesterol removal. These results suggest that GPI-APs form complexes with cholesterol, potentially stabilised in sphingolipid-rich environments. The ability of GPI-anchoring to promote nanometer proximity between diverse proteins has implications for biological function.

S. Mayor : Structure and function of GPI-anchored protein membrane rafts in living cells

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Stimulation-induced formation of transient but stabilized rafts.

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Raft domains have been drawing extensive attention as a signaling platform in the cell membrane. However, the structure, stability, and function of rafts have remained to be unknown. To investigate this issue, we observed the movement of a raft marker, a GPI-anchored protein CD59 using 40 nm-gold particles on the cell membranes at 25 μs-resolution. Because the hop rate of CD59 between the compartments made of membrane skeleton was exactly the same as that of DOPE, rafts that CD59 is associated with might consist of only several molecules and/or their lifetimes could be on the order of a millisecond or less in the steady state without extracellular stimulation. Meanwhile, upon liganding or crosslinking, activated CD59 repeatedly stayed in transient confinement zones (TCZs) of 90 nm for about 0.7 s once every 3 s on average. TCZs were found to be actin-stabilized, cholesterol-enriched rafts induced by the src family kinases (SFK) activities where intracellular signals are triggered. In SFK-deficient SYF cells, TCZs are depleted, but that transfecting the SYF cells with SFKs cDNA recovered TCZs. Furthermore, by simultaneously observing crosslinked CD59 and single molecules of Gα i-YFP, whose effector is SFK, we found that Gα i-YFP became colocalized with crosslinked CD59 slightly before or at very early stages of TCZ formation for 0.1 s on average. These results support our working hypothesis in which activated CD59 forms, with the aid of SFK, transiently immobilized raft domains, where it triggers intracellular signals.

K. Suzuki, F. Sanematsu, T. Fujiwara, M. Edidin and A. Kusumi : Stimulation-induced formation of transient but stabilized rafts.

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