the plasma membrane (Marsden and DeSimone, 2001). Dsh localization to the plasma membrane has also been associated with the activation of noncanonical Wnt signaling (Rothbacher et al., 2000), while interfering with noncanonical Wnt signaling through the Wnt receptor Frizzled 7 leads to defects in both cadherin-mediated cell adhesion and separation of the ectodermal and mesodermal germ layers at the onset of Xenopus gastrulation (Winklbauer et al., 2001, and the references therein). It is therefore likely that integrin and noncanonical Wnt signaling cooperate in the regulation of cadherin-mediated cell adhesion, which controls tissue separation and CE movements during gastrulation.

Understanding the crosstalk, not only between different adhesion molecules, but also between secreted signals, such as Wnts and adhesion molecules, will be extremely helpful in elucidating the molecular and cellular mechanisms underlying gastrulation movements. The study by Marsden and DeSimone provides a first step in this direction.

Juan-Antonio Montero and Carl-Philipp Heisenberg
Max-Planck Institute of Molecular Cell Biology and Genetics
Pfotenhauerstr. 108
01307 Dresden
Germany

Selected Reading


Picket and Other Fences in Biological Membranes

Nakada et al. revisit the controversial question of whether membrane lipids are able to diffuse from the axon of a neuron into the soma. Using single molecule imaging of a fluorescent phospholipid, the authors show that a diffusion barrier in the axon initial segment blocks the diffusion of lipids.

Neurons are highly compartmentalized, with distinct domains such as synapses, nodes of Ranvier, axons, and dendrites. The membrane proteins present in these domains are frequently distinct. How is the differential distribution of membrane proteins established and maintained? The initial segment of the axon (AIS) has been reported to act as a diffusion barrier impeding the lateral mobility of several membrane proteins, largely immobilizing them (Winckler et al., 1999). The actin-based membrane skeleton containing special ankyrin G (Kordeli et al., 1993) and spectrin isoforms (Berghs et al., 2000) has been implicated in the integrity of the AIS diffusion barrier based on the effect of actin-disrupting drugs. An ankyrin binding membrane protein and a GPI-linked protein, which does not bind the ankyrin/spectrin network, are both immobilized in the AIS in an actin-dependent manner, suggesting that ankyrin binding proteins enriched in the AIS might act as immobile obstacles that reduce the long-range diffusibility even of proteins not themselves bound to cytoskeletal elements (Winckler et al., 1999).

A recent paper in Nature Cell Biology by Akihiro Kumai’s group now investigates the diffusion of membrane lipids in the AIS (Nakada et al., 2003). The paper makes two significant contributions. First, stunningly, even the phospholipid phosphatidylethanolamine cannot escape the diffusion barrier and is immobile in the AIS. Second, the progressive immobilization of lipids in the AIS correlates in time and space with the progressive assembly of ankyrin G and the voltage-gated sodium channel (VGNC) in the AIS, and the immobilization of both lipids and VGNCs is actin dependent. Importantly, a diffusion barrier to lipids at the AIS could provide a mechanism for maintaining lipid asymmetry between the axon and the soma.
Whether or not the AIS constitutes a diffusion barrier to lipids has been controversial (Kobayashi et al., 1992; Winckler and Poo, 1996), awaiting more quantitative and sensitive assays. The most common assays for diffusion measurements are fluorescence recovery after photobleaching (FRAP) in which the long-range diffusion of a large number of fluorescently labeled molecules can be measured, or single particle tracking (SPT), where the short-range diffusion of a small number of molecules attached to beads can be measured. The drawback of SPT is that the observed diffusion might not reflect the true diffusional behavior of the attached tracer but rather that of the bead, which could get slowed down by extracellular components near the membrane. Only assays without attached beads can ultimately demonstrate a diffusion barrier to a lipid. This paper achieves a major technical advance by using fluorescently labeled phospholipids to monitor directly the movement of single lipid molecules, as well as using bead-attached tracers. The major limitation of the single fluorescence imaging approach is weak and short-lived fluorescence, leading to low signal-to-noise and thereby to low spatial and temporal resolution. Notably, however, the authors obtain very similar results using either fluorescence imaging of fluorescent phospholipids or SPT of lipids attached to gold nanoparticles.

How would a lipid diffusion barrier work? Kusumi and coworkers propose a “picket fence model” in which membrane proteins bound to the actin-based membrane skeleton are immobilized and thereby provide obstacles or “pickets” to the diffusion of other molecules. VGNC is an ankyrin binding protein itself and likely constitutes an immobilized obstacle in the AIS. Theoretical modeling of diffusion through immobile obstacles predicts that the diffusion coefficients of tracer molecules are highly nonlinearly dependent on the density of immobilized obstacles (Sheetz, 1993; Saxton, 1990). At a critical density of obstacles (as low as 25% coverage), the diffusion can abruptly slow down or stop. This is exactly what is observed: the diffusion of the lipid tracer is indistinguishable from the nonmobile tracer on the coverslip. Disruption of actin filaments is predicted by the model to lead to unetheting of the obstructions thereby lowering their effect on the diffusion of other molecules, even of lipids present in the outer leaflet of the plasma membrane.

Nakada’s innovative study opens several questions: For instance, it is well established that lipid diffusion in plasma membranes is significantly slower than that in artificial lipid bilayers when measured by conventional techniques. Kusumi’s group (Fujiwara et al., 2002) previously measured the diffusion coefficients of gold-tagged lipids at 25 μs temporal resolution (as opposed to the usual 33 ms for video rate) in nonneuronal cells. At this resolution, lipids in the plasma membrane are not slower and diffuse close to the diffusion rate observed in lipid bilayers. Therefore, diffusion per se is not slow. Rather, the diffusion coefficients observed on a 33 ms scale are slower because lipids show restricted diffusion in small confinement zones from which they can hop into adjacent confinement zones every so often. A similar actin-based picket fence model as for the AIS has been proposed to explain these observations. If the fibroblast membrane constitutes a picket fence, then the AIS apparently is a maximum-security prison that completely immobilizes lipids when observed at video rate (Nakada et al., 2003). This raises important questions: do the lipids show confined diffusion in the AIS at 25 μs resolution? How big are potential confinement zones and is there an appreciable hop rate to adjacent compartments? What unique molecular features of the AIS (of the obstacles, or the cytoskeleton, or even extracellular components) make it a maximum-security prison rather than a picket fence?

A second question arises from the observation that the diffusion of the lipid and the putative obstacle (i.e., VGNC) is quantitatively very similar (Nakada et al., 2003). The lipid does not show higher diffusion rates than the VGNC even when the barrier is disrupted. Is the lipid physically coordinated with the VGNC such that they diffuse as a unit of sorts? One wonders then if there are any molecules not affected by the AIS diffusion barrier. An earlier study had observed spreading across the axon/soma boundary of the relatively short-tailed artificial fluorescent lipid DiIC12 (Winckler and Poo, 1996). It might be informative to revisit the Dils using the more sophisticated methods available now. Therefore, we can look forward to additional exciting work, probing how multiple extracellular and cytoplasmic interactions give rise to a “high-security picket fence” in the AIS. Last but not least, perhaps the maintenance of cellular polarity and functional compartmentalization elsewhere (such as pre- and postsynaptic domains, nodes of Ranvier, yeast buds, Drosophila septate junctions, epithelial tight junctions, and mammalian sperm) may involve picket and other fences as well.