A TRiP to the plasma membrane

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TRP ion channels are ubiquitously present in the mammalian body and take part in numerous key physiological functions, including temperature sensing, taste perception, osmo-regulation, cardiac function, renal function, development, and glucose homeostasis. The mechanisms whereby TRP channels are transported to the plasma membrane, where most of them exert their physiological actions, remains a poorly understood aspect of TRP channel biology.

In a recent study,1 we were able to visualize for the first time the fusion of TRP channel-containing vesicles with the plasma membrane. Here, in this article, we highlight the main findings of that study and discuss their potential implications.

The broad physiological importance of TRP channels is evident through numerous pathophysiological conditions associated with their malfunctioning.2 The physiological actions of TRP channels depend on the transmembrane currents mediated by them, which is a function of the open probability of the channels (which depends on the channel gating properties), the conductance through a single channel (which depends on the properties of the channel pore), and also on the total number of functional channels present at the site of its action. Whereas channel gating and pore function have been widely studied for many TRP channels, very little is known about the mechanisms that regulate and modulate the number of functional channels.3

For TRP channels localized at the plasma membrane, the regulation of the total number of functional channels critically depends on the transport mechanisms that deliver the channels to the cell surface. However, very little is known about the fundamentals of intracellular trafficking of TRP channels. In fact, the actual process of TRP channel incorporation into the plasma membrane has escaped visualization, and the lateral diffusion of TRP channel in the plasma membrane has not been well characterized.

TIRFM (Total Internal Reflection Fluorescence Microscopy) represents an invaluable tool of choice for studying near-membrane events.4 The technique is based on the creation of an evanescent wave, created by the total reflection of a laser beam at the coverslip-water interface, whose intensity decays exponentially as the wave moves in z direction from the coverslip. The length constant (i.e. the distance in the z direction over which the intensity of the evanescent wave decreases e-fold) depends on the incidence angle, the wavelength of the laser, and on the refractive index of the coverslip and medium. With a length constant of ~80 nm, fluorophores within 100–200 nm from the coverslip surface can be readily observed, whereas fluorophores that are further away are not or only weakly excited, giving an unparalleled signal to noise ratio and an unparalleled spatial resolution in the z-axis.4

TIRFM had been used earlier to investigate plasma membrane trafficking of TRP channels.5 However, under typical overexpression conditions, fluorescently labeled TRP channels are present in such high densities at the cell surface that it obscures subtle new events (such as vesicle fusion) that may take place. A way to circumvent this problem is the use of the TIR-FRAP (Total Internal Reflection – Fluorescence Recovery after Photobleaching) technique, where a high intensity evanescent wave bleaches all near-membrane fluorophores, while leaving the fluorescence in the rest of the cell intact. This allows selective visualization of proteins coming from inside the cell to the cell surface during the post-bleach recovery phase.

In our recent article,1 we illustrated the power of TIR-FRAP to study the dynamics of TRP channels near the membrane with greater clarity. In particular, this approach enabled us to visualize for the first time the approach and full fusion of vesicles containing the TRP channel TRPM4 with the plasma membrane, and to measure the subsequent lateral diffusion of the channels. A typical example of such a fusion event is illustrated in Figure 1. During the approach phase, a TRPM4-containing vesicle first becomes visible as it enters the evanescent wave, and becomes gradually brighter as it comes closer to the plasma membrane. We found that these vesicles have a diameter of approximately 200 nm, and rapidly approach the

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Abbreviations: Transient receptor potential, (TRP); Total Internal Reflection Fluorescence Microscopy, (TIRFM); Total Internal Reflection – Fluorescence Recovery after Photo-bleaching, (TIR-FRAP); root-mean-square, (rms); green fluorescent protein, (GFP); square root of time following fusion, (√t).

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membrane at a speed of 275 ± 25 nm/s. Then, the vesicles remain (linger) at short distance of the membrane (approximately 30 nm) for on average 7.6 ± 1.2 s, before undergoing full fusion with the plasma membrane. The fusion event itself is observed as a rapid increase in fluorescence, due to the flattening out of the vesicle close to the coverslip as it merges with the plasma membrane, followed by a dispersion of the fluorescence signal due to lateral diffusion of the channel protein in the plasma membrane. The lateral diffusion, which can be quantified as the 2-dimensional root-mean-square (rms) displacement of TRPM4-GFP fluorescence from the site of fusion, showed a linear relation to the square root of time following fusion (√t), from which a diffusion coefficient of approximately 0.1 μm²/s could be calculated.

These results represent, to our knowledge, the first visualization of the full fusion of a TRP channel-carrying vesicle with the plasma membrane. However, it seems that there is no general mechanism of TRP channel trafficking to and fusion with the plasma membrane, but rather that different TRP channels are differentially targeted to distinct cellular trafficking pathways. For instance, earlier studies on TRPC5 and other TRP channels provided evidence that the TRP channel-containing transport vesicles undergo “kiss and linger” fusion with plasma membrane, where the channels remain trapped within a non-collapsed vesicle. In our study, we also failed to detect fusion of transport vesicles containing TRPV2-GFP. In particular, following bleaching of the near-membrane zone, TRPV2-GFP fluorescence was found to recover mainly through lateral diffusion from non-bleached areas of the plasma membrane, with a diffusion coefficient approximately 0.3 μm²/s. An important challenge for the future will therefore be to identify the molecular and cellular processes and specific targeting sequences that determine the transport of TRP channels to their destination, as well as how these processes are regulated by various cellular signaling pathways. There is increasing evidence that dysregulation of TRP channel activation may be the prime cause of human diseases. For instance, mutations in TRPM4 cause cardiac conduction disease, which has been attributed to an increased channel expression at the plasma membrane. Likewise, increased plasma membrane expression of sensory TRP channels may result in (thermal) hypersensitivity and pain. Therefore, targeting the process of TRP channel trafficking represents an interesting alternative to classical channel antagonists for treatment of such conditions.

References
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