Review

Regulation from within: the cytoskeleton in transmembrane signaling

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There is mounting evidence that the plasma membrane is highly dynamic and organized in a complex manner. The cortical cytoskeleton is proving to be a particularly important regulator of plasmalemmal organization, modulating the mobility of proteins and lipids in the membrane, facilitating their segregation, and influencing their clustering. This organization plays a critical role in receptor-mediated signaling, especially in the case of immunoreceptors, which require lateral clustering for their activation. Based on recent developments, we discuss the structures and mechanisms whereby the cortical cytoskeleton regulates membrane dynamics and organization, and how the nonuniform distribution of immunoreceptors and their self-association may affect activation and signaling.

The plasma membrane: highly dynamic, yet organized

Cells interact continuously with their environment. This is necessary for cell survival and for the development and functional coordination of multicellular organisms. To this end, myriad signaling cascades are initiated at the plasma membrane upon interaction of extracellular signals with plasmalemmal receptors.

The proteins and lipids that constitute the membrane are neither static nor homogeneously distributed along or across the membrane bilayer. Intermolecular interactions between membrane lipids and proteins generate inhomogeneities of varying size and stability. These can vary from dimers to multi-component domains – referred to hereafter as 'nanodomains', because their size is generally in the tens to hundreds of nanometers – and can last from microseconds to hours. Through interactions with various membrane components, the cortical cytoskeleton can restrict the diffusion of proteins and lipids, aid in their transport, and assist in the formation, segregation, or transport of nanodomains. As such, the cytoskeleton can potentially modulate signal transduction, coordinate events in distant parts of the cell, and couple mechanical signals to biochemical responses.

Here we examine the critical role of the cytoskeleton in regulating the spatiotemporal organization of the plasma membrane, highlighting new studies and technological advances. We discuss in depth the case of immunoreceptors, which often undergo long-range translocation to become clustered and activated and are therefore uniquely susceptible to cytoskeletal modulation.

The plasma membrane is more complex than a fluid mosaic

The 'fluid-mosaic' model, proposed by Singer and Nicolson 40 years ago, postulated that lipids form a bilayer that is effectively a two-dimensional fluid in which proteins are embedded, forming a lipid–protein mosaic [1]. Although the model captures many features of biological membranes, it makes two predictions that are at odds with experimental observations: first, it predicts that proteins and lipids undergo unrestricted diffusion in the membrane (Figure 1a); and second, as a result of this unrestricted diffusion, membrane proteins and lipids are anticipated to distribute randomly and homogeneously.

Evidence in conflict with the first prediction emerged shortly after the introduction of the Singer–Nicolson model, when several studies reported that the diffusion of membrane components is rather restricted [2,3]. The mobility of proteins in erythrocyte ghosts was found to be at least 20-fold slower than expected for a fluid lipid–protein mosaic, a reduction attributed to the presence of a cortical cytoskeleton meshwork (the 'membrane skeleton') [2]. Restricted diffusion of proteins and lipids has since been observed in the plasma membrane of many cell types, using various techniques including fluorescence recovery after photobleaching, single-particle tracking (SPT), and fluorescence correlation spectroscopy (FCS) (Table 1). These studies have found the reduction in molecular mobility to be caused not only by the cortical cytoskeleton [4,5], but also by membrane 'crowding' with proteins [6], interactions between membrane components [7], and lateral inhomogeneity in membrane composition and state [8].

The realization that membrane composition is laterally inhomogeneous is the second inconsistency between the predictions of the Singer–Nicolson model and experimental data. Convincing evidence to this end has stemmed from multiple approaches, including immuno-electron microscopy (immuno-EM) [9], FCS and its variants [10,11], atomic force microscopy [12], and the more recently developed super-resolution microscopy techniques

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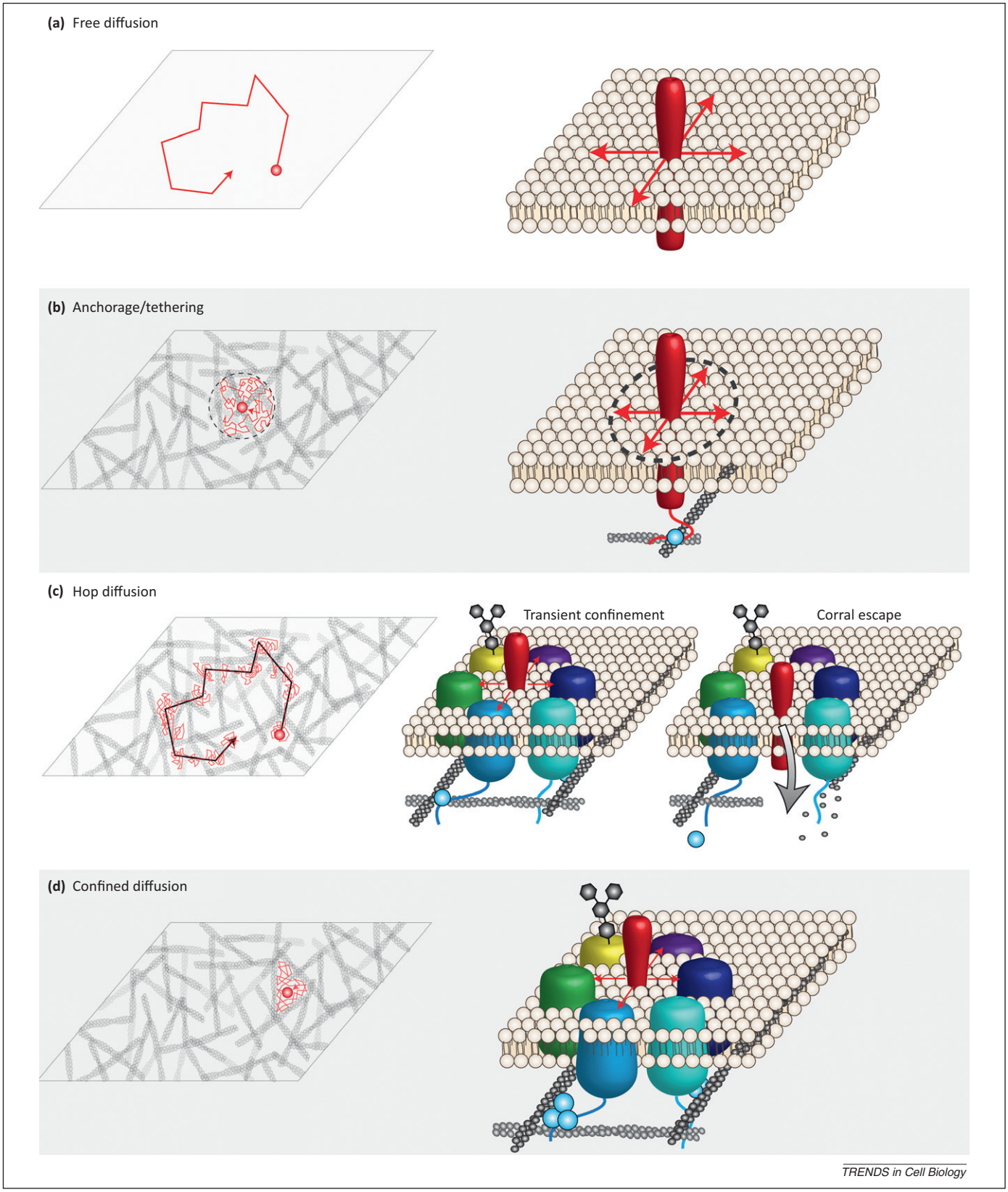

Figure 1. Types of motion of membrane proteins. (**a**) Free diffusion, as experienced by a protein in a lipid bilayer. Left panel: 'biophysical' view illustrating the trajectory of a molecule. Each segment of the line indicates the displacement recorded using a fast rate of image acquisition. Right panel: molecular view illustrating the lipids constituting the bilayer (beige; in all panels) and a transmembrane protein (red; in all panels). The arrows indicate the ability of the protein to diffuse in any direction. (**b**) Anchorage/tethering, as experienced by a protein while directly or indirectly associated with the cortical cytoskeleton. Left panel: 'biophysical' view illustrating that the mobility of a transmembrane protein is restricted to a limited area (broken circle) as a result of its attachment to cytoskeletal filaments (gray rods; in all panels). Right panel: molecular view illustrating attachment of the transmembrane protein to the cytoskeleton, in this illustration by association of its cytoplasmic tail to an adaptor linker molecule (blue). Shorter arrows imply restricted mobility. (**c**) Hop-diffusion, as experienced by a protein temporarily trapped within corrals formed by picket-fences that form on the cytoskeleton. Left panel: 'biophysical' view illustrating that the protein diffuses rapidly within the corral (red lines) but only occasionally escapes from one corral to the next, resulting in eventual long-range displacement, observable at slower rates of image acquisition (black line). Middle panel: illustrates the confinement of a transmembrane