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COMMENT

Lipid scrambling in immunology: why it is important

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Asymmetric lipid distribution in the plasma membrane (PM) is one of the fundamental features of living cells. Lipids not only serve as structural components of the biophysical structure of the PM that preserves cell integrity but also participate directly in signaling. Acidic phospholipids (PLs), such as phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphoinositol (PI), are preferentially located in the inner layer of the PM, creating electrostatic charges for the docking of membrane-associated and cytoplasmic proteins carrying polybasic stretches of amino acids. However, there is emerging evidence that reversible changes in the distribution of these lipids take place in the PM and that these changes are critical for normal cell signaling, functions and homeostasis [1–3]. They can also be implicated in the genesis of pathologies.

Randomization of lipids in membranes, commonly known as "lipid scrambling", is an active mechanism that involves specialized enzymatic machinery. Lipid scrambling is executed by lipid scramblases, which redistribute PLs vertically between the inner and outer leaflets of membranes. Unlike flippases and floppases, which unidirectionally transfer lipids across membranes and are dependent on ATP [4], scramblases bidirectionally convey lipids and are independent of ATP (Fig. 1a). The focus of this review will be the recent progress in our understanding of the physiological and pathological roles of lipid scramblases, particularly in immune cells. For discussions on flippases and floppases, the reader is referred to other published texts [4, 5].

Two major categories of PM scramblases exist: one is Ca^{2+}-activated and is exemplified by TMEM16F [also known as anoctamin 6 (ANO6)] and several other members of the TMEM16 family, and the other is activated via caspase cleavage or kinases and is represented by XKR8 (Fig. 1a). Several TMEM16 family members, including TMEM16F, are not only scramblases but also ion channels. As an ion channel, TMEM16F exhibits variable ion specificity depending on the context and intracellular Ca^{2+} level.

Since the identification of TMEM16F as the first bona fide scramblase, the resolution of the molecular structure of scramblases has been key for understanding their mechanisms of action. Current data indicate that TMEM16F possesses ten transmembrane domains with amino- and carboxy-terminal cytoplasmic domains, which assemble into homodimers to function. XKR8 also contains ten transmembrane domains but associate with the chaperone basigin to function [6]. Structural studies of TMEM16F and XKR8 have supported the idea that these enzymes have dissimilar mechanisms of action. In addition, recent work has provided an explanation for the two distinct roles of TMEM16F, i.e., lipid scramblase and ion channel. It was suggested that Ca^{2+}-activated TMEM16F forms an opening pore, which enables ion exchange [7]. This structural change allows the two functions of TMEM16F to occur in the same protein conformation.

There is firm evidence that TMEM16F is critical for several physiological processes in nonimmune cells, such as blood coagulation, trophoblast fusion, bone mineralization, and membrane repair. Several cases of TMEM16F loss-of-function mutations have been reported in humans, causing Scott syndrome. Due to a failure of PS exposure on platelets, these patients have abnormal coagulation. Germline ablation of TMEM16F in mice revealed that TMEM16F is also required for bone formation due to its role in osteoclast formation. In addition, TMEM16F is needed for embryonic viability owing to its involvement in trophoblast fusion, a key process in embryonic development. Recently, TMEM16F was found to be coupled to the TRPV4 calcium channel in this mechanism in human trophoblasts. Last, rapid PS exposure at the PM has long been known to be triggered by pathogenic pore-forming agents, such as streptolysin O. This process was reported to be mediated by TMEM16F and is essential for membrane repair, pore elimination and maintenance of cell viability [8].

Accumulating data have also implicated TMEM16F in the control of immune cell activation, although there is some contradictory evidence regarding the purpose served by TMEM16F in this setting. One study found that in response to T-cell activation, TMEM16F was activated by Ca^{2+} influx, thereby causing PM PLs to randomize vertically across the T-cell membrane [9]. This mechanism was reported to attenuate T-cell activation and to prevent T-cell exhaustion during chronic viral infection (Fig. 1b). Similarly, lipid scrambling was reported to occur during NK cell activation, where it may attenuate NK cell signaling and function [2]. In contrast, another group reported that in the T-cell line Jurkat, TMEM16F altered the electrostatic interactions between the inner side of the PM and the antigen receptor complex, thereby enhancing T-cell activation [1]. The changes in PM electrostatic potential induced by lipid scrambling were proposed to augment the ability of the antigen receptor to trigger T-cell activation.

Additionally, TMEM16F has been implicated in pathological processes. It was reported that the spike protein of SARS-CoV-2 triggered TMEM16F-mediated PS exposure, leading to syncytium formation by pneumocytes [10]. Ca^{2+} inhibitors had an anti-syncytial effect by blocking TMEM16F-dependent PS exposure. TMEM16F-mediated PS exposure was also reported to be essential for SARS-CoV-2 entry and COVID-19 pathogenesis [10]. Similarly, cellular binding of human immunodeficiency virus (HIV) elicited

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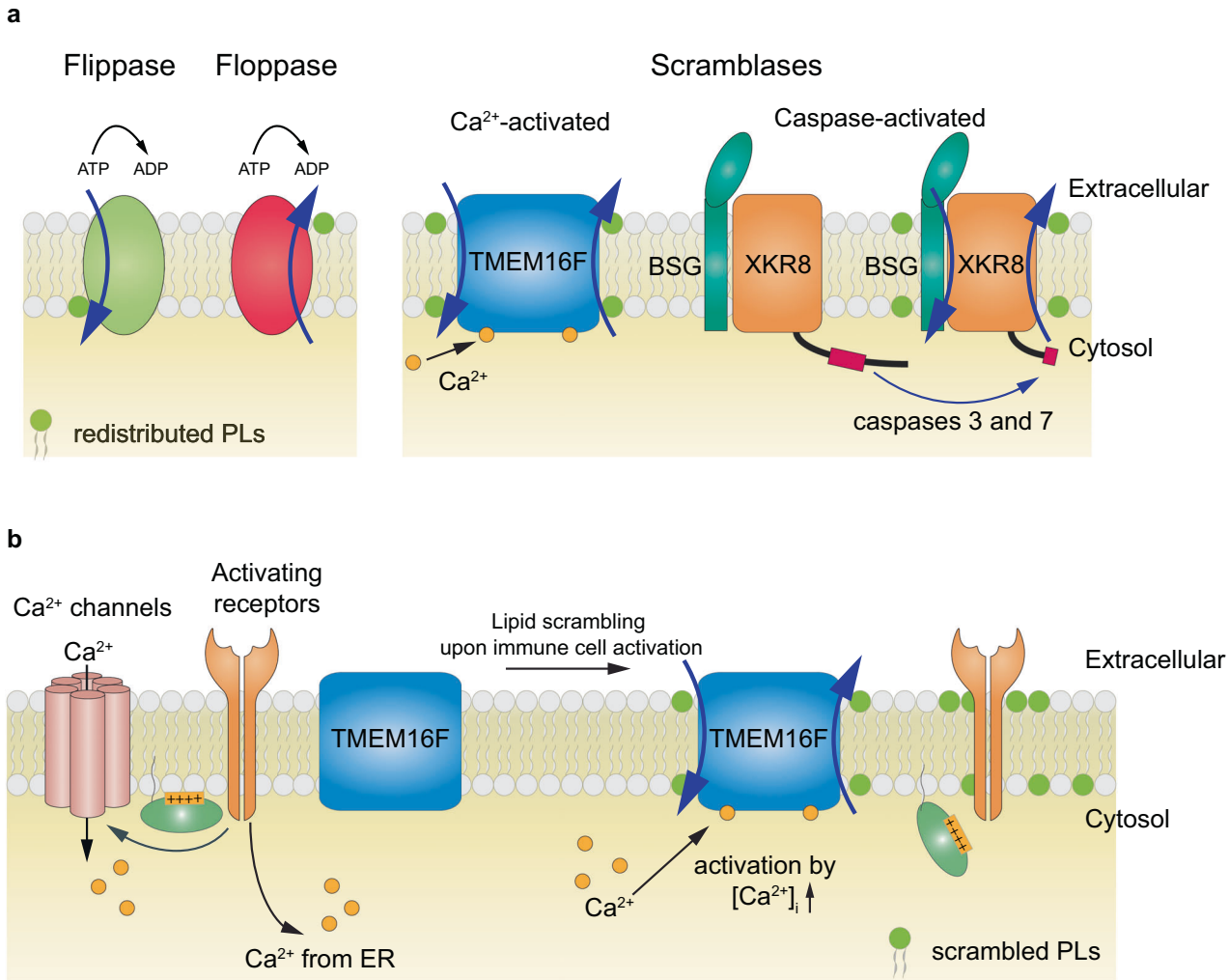

Fig. 1 Regulation of lipid distribution in the plasma membrane. **a** Flippases and floppases mediate unidirectional redistribution of phospholipids (PLs) across the PM, a process that involves ATP. Scramblases, including Ca^{2+}-activated TMEM16F and caspase-activated XKR8, transport PLs bidirectionally and are activated by conformational change. They do not require ATP. BSG, basigin. Green circles represent the redistributed PLs. **b** Potential impact of lipid scrambling by TMEM16F on immune cell activation. Activating signals triggered by the T-cell antigen receptor (TCR) in T cells or the activating receptor 2B4 on NK cells elicit cytosolic Ca^{2+} elevation, thereby activating TMEM16F. Activated TMEM16F then redistributes PLs in the PM, resulting in loss of acidic PLs at the inner side of the PM and dissociation of polybasic stretch-containing signaling molecules (such as Src kinases) from the PM. As a consequence, immune cell activation is attenuated

Ca^{2+} influx, thereby activating TMEM16F to externalize PS [11]. Suppression of PS exposure impeded HIV cell membrane fusion and infection. Thus, lipid scrambling by TMEM16F and perhaps other scramblases may be a vital step utilized by several viruses for infection [11].

Unlike Ca^{2+}-activated scramblases, XKR8 is activated following cleavage by caspases 3 and 7, which are proapoptotic caspases. As a result, XKR8 plays a crucial role in the externalization of PS during apoptosis. Exposed PS is then recognized by phagocytes to eliminate dead or dying cells and is known as an "eat-me" signal. XKR8 is also activated by kinases, thereby potentially extending its involvement to processes other than dead cell clearance. Threonine or serine phosphorylation of XKR8 at specific sites augmented its activity. Some forms of XKR8 may be constitutively active: an analog of XKR8 in *Drosophila* was found to scramble PLs constitutively to control the flexibility of insect cell membranes [12].

Whereas PS externalization serves as an eat-me signal for phagocytes, it may paradoxically provide an immunosuppressive signal that promotes tumor growth. For instance, XKR8-deficient tumor cells, which had defective PS exposure, grew slower, not faster, in immunocompetent mice [13]. Such tumor growth-promoting effects of lipid scrambling were proposed to be due to the activation of immunosuppressive tumor-associated macrophages (TAMs) by tumor cell-exposed PS. Hence, in addition to being prophagocytic, PS on the outer leaflet of tumor cells may be a mechanism of immunosuppression. Attempts to manipulate PS exposure on tumor cells may be a new strategy to control tumor-associated immunosuppression [14].

In addition to the PM, other intracellular membrane systems also carry lipid scramblases. For instance, over the last few years, significant advances have been made in identifying several lipid scramblases on the ER, such as TMEM41B, VMP1, CLPTM1L, and ATG9A. As PL biosynthesis occurs in the ER, newly generated PLs need to be transported from the ER membrane to other organelles, including the PM, lipid droplets, and mitochondria. These processes likely involve lipid scramblases. In general, studies of internal organelle scramblases have revealed at least three aspects distinct from those of PM scramblases: first, organelle scramblases such as TMEM41B and VMP1 are constitutively active for lipid scrambling, and as a consequence, they are vital for maintaining lipid homeostasis in response to constant lipid

biosynthesis; second, organelle scramblases often require other lipid transporters to execute lipid translocation among organelles; third, organelle scramblases have different lipid selectivity, including cholesterol. Further structural information on these newly defined scramblases will provide helpful clues to understand their molecular mechanisms.

The emergence of lipid scrambling as a key molecular behavior unveils a novel dimension in the regulation of immune signaling and functions. To date, lipid scrambling has been shown to affect cell-to-cell communication, leading to phagocytosis by exposing PS at the cell surface; to regulate signaling by diminishing acidic PLs on the inner side of the plasma membrane; and to enable membrane repair. It may also be implicated in viral infections and tumor-mediated immunosuppression. Thus, scrambling reveals lipids, an ancient cellular component present across evolution, as a core player in various physiological and pathological conditions of immune cells. Considering the broad existence of scramblases and lipids, as well as the consequences of lipid reshuffling in the PM and intracellular organelles, future studies are warranted to better understand the roles of scramblases in immune cells and will likely lead to novel insights in immunology.

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ADDITIONAL INFORMATION

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