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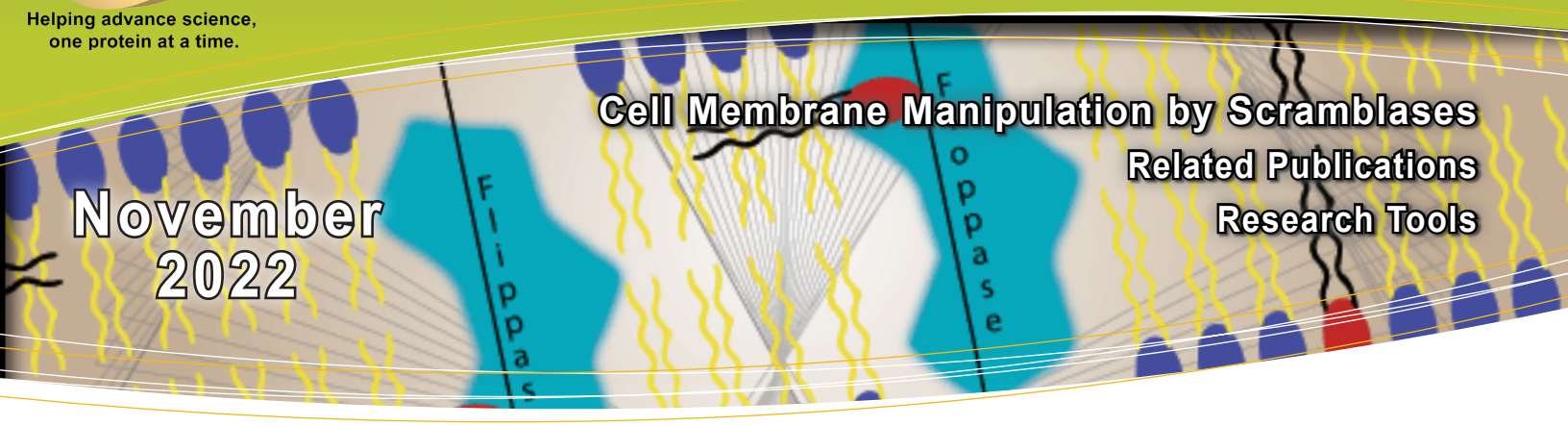

November 2022

Cell Membrane Manipulation by Scramblases
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Cell Membrane Manipulation by Scramblases

Introduction

Since the seminal paper describing the cell membranes via the fluid-mosaic model[1], there has been continued interest in membrane composition and its physiological importance. Soon after, it was first hypothesized that the lipids within the membranes existed in an asymmetric state, which was shown using intact vs compromised red blood cells[2]. Importantly, it was shown that phospholipid composition, in particular, phosphatidylserine (PS) and phosphatidyl ethanolamine (PE) localize primarily on the cytoplasmic leaflet of the plasma membrane. Conversely, other phospholipids such as phosphatidylcholine (PC) and sphingomyelin (SP) are concentrated on the outer leaflet. Passive, transverse movement of phospholipids occurs on the scale of many hours, and it is unlikely the distinct asymmetric pattern of phospholipids could be achieved without a catalytic mediator (reviewed in [3]). This led to the postulation that active transporters likely existed, and soon thereafter ATP-dependent lipid pumps like flippases and floppases were discovered. Additionally, ATP-independent but Ca^{2+}-dependent lipid channels were also identified, and unlike the lipid pumps which move specific phospholipids in a specific direction[4], scramblases move phospholipids bidirectionally (see figure 1). Lipid asymmetry is critical for many physiologic processes, so one may wonder what the benefit of diminishing asymmetry via scramblases is. Below we discuss the physiologic role of scramblases and their structural features.

Structural Analysis of TMEM Scramblases

The TMEM16 family is comprised of Ca^{2+}-activated chloride channels and scramblase channels that share a homodimeric architecture; within each protomer there are 10 transmembrane helices (reviewed in [5]). Brunner *et al.* showed that in nhTMEM16, helices 3-7 form a unique groove-like structure that is important for the transfer of lipids across membranes [6]. Interestingly, this groove is open to the membrane core but is hydrophilic alluding to the possibility as the pathway for lipid permeation. Several studies have confirmed that this groove is important for lipid scrambling whereby lipid headgroups enter and diffuse through this hydrophilic groove while their tails remain within the hydrophobic core of the membrane (reviewed in [5]). More recently, it was shown that Ca^{2+} binding in nhTMEM16 induced a stepwise transition that transitions the catalytic groove from a closed state (shielded from the membrane) to an open state (accessible to lipid headgroups) [7]. Previous studies with mTMEM16F showed that it existed in a closed state even under maximal scramblase activity; however, a recent study suggested that massive molecular dynamic examination was needed to observe its true state. In this study, they found that distal Ca^{2+} binding allosterically affected the groove region and promoted an open hydrophilic structure during scrambling activity [8]. Another recent study by Falzone *et al.* showed that an open hydrophilic groove is not a requirement for scrambling by the family member, afTMEM16; rather, this scramblase performs membrane thinning as a mechanism to enable phospholipid scrambling [9]. The authors suggest that this mechanism may be relevant to other scramblases that do not have an open groove structure. Advancements in cryo-EM have enabled investigators to study these complex scramblase proteins to better understand how their discrete structural differences affect their functions.

Scramblases Regulate Critical Cellular Events

The kinetics by which flippases/floppases and scramblases function is vastly different. Due to the active movement of phospholipids against an equilibrium gradient the necessary energy requirements and conformational changes results in a slower movement of phospholipids by flippases/floppases relative to scramblases (reviewed in [3]). Scramblases allow for the rapid change in phospholipids across its channels that can quickly abolish asymmetry. These types of drastic changes are necessary during critical cellular events. For

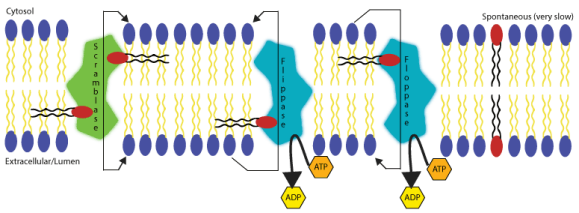

Figure Legend: The three types of phospholipid translocases and their directional movement of phospholipids

Membrane Tension PRODUCTS

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example, Suzuki *et al*. identified that the Xkr8 scramblase plays a critical role in apoptosis, a form of highly regulated programmed cell death [10]. When Xkr8 is activated through caspase cleavage it results in the rapid translocation of PS to the outer leaflet that functions as an "eat me" signal by macrophages. This event is a hallmark of apoptosis and is important for programmed death as engulfment by macrophages minimizes the release of toxic cytoplasmic substances that would be harmful to neighboring cells. Xkr8 was recently shown to be activated in a caspase-independent mechanism that utilizes phosphorylation and targets critical sites near the caspase cleavage site on the protein [11]. Another well-known role for scramblases is their effect on blood coagulation[12]. During secondary hemostasis, a scramblase known as transmembrane protein 16F (TMEM16F) promotes loss of lipid asymmetry through PS exposure on the outer leaflet. Abundant PS exposure on the outer leaflet on platelets recruits clotting factors that lead to the activation of thrombin and the formation of an insoluble fibrin clot [13]. More recently, TMEM16F was shown to promote PS exposure during trophoblast fusion in a calcium-dependent fashion, and if the PS signal was suppressed, cell fusion was blocked [14]. Knockout of TMEM16F using CRISPR-Cas9 methods completely blocked trophoblast fusion, which was rescued with the reintroduction of TMEM16F. Other TEMEM scramblases have been identified in several genetic diseases, neurological disorders, and even cancer (reviewed in [5]).

Summary and Future Directions

Asymmetric composition of phospholipids in the cell membrane is important for normal physiologic function, and acute changes by scramblases are observed in critical cellular events. In another example, a recent study showed how the SARS-CoV-2 promotes an increase in Ca^{2+} levels, activation of TMEM16F (ANO6), exposure of PS, and enhanced fusion of the viral and cell membranes [15]. The group identified a compound A6-001 with high potency and selectivity to TMEM16F, and treatment with the compound blunted SARS-CoV-2 replication. Furthermore, novel scramblases continue to be identified, such as, the protein CLPTM1L that functions to translocate glucosaminylphatidylinositols from the cytosol to the lumen of ER and is critical for biosynthesis of GPIs which function as membrane anchors [16]. Clearly, there is much to learn about this critical family of lipid regulating proteins, their impact on membrane composition, and how they affect critical cellular processes. Cytoskeleton has an array of live cell membrane probes to aide in cell membrane investigation; such as, the novel Flipper-TR membrane tension probes, the MemGlow polarity probes, and the fluorogenic MemGlow membrane labeling probes.

Flipper-TR Membrane Tension Probes

Product	Ex / Em	Cat #
Flipper TR Kit **Measure Plasma-Membrane Tension**	Ex 488 / Em 600	CY-SC020
ER Flipper-TR Kit **Specifically Target the Endoplasmic Reticulum**	Ex 480 / Em 600	CY-SC021
Lyso Flipper-TR Kit **Localizes to Lysosome Membrane Cells**	Ex 480 / Em 600	CY-SC022
Mito Flipper-TR Kit **Localizes to the Mitochondrial Membrane**	Ex 480 / Em 600	CY-SC023

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MemGlow™ Plasma Membrane Probes

Product	Ex/Em	Cat #
MemGlow 488 - Fluorogenic Plasma Membrane probe **(A MEMBRIGHT Family Probe)**	Ex 499 / Em 507	MG01
MemGlow 560 - Fluorogenic Plasma Membrane probe **(A MEMBRIGHT Family Probe)**	Ex 555 / Em 570	MG02
MemGlow 590 - Fluorogenic Plasma Membrane probe **(A MEMBRIGHT Family Probe)**	Ex 580 / Em 620	MG03
MemGlow 640 - Fluorogenic Plasma Membrane probe **(A MEMBRIGHT Family Probe)**	Ex 650 / Em 673	MG04
MemGlow 700 - Fluorogenic Plasma Membrane probe **(A MEMBRIGHT Family Probe)**	Ex 650 / Em 720	MG04

MemGlow™ Membrane Polarity Probes

Product	Ex/Em	Cat #
MemGlow™ NR4A Membrane Polarity Probe **(A MEMBRIGHT Family Probe)**	Ex 553 / Em 637	MG06
MemGlow™ NR12A Membrane Polarity Probe **(A MEMBRIGHT Family Probe)**	Ex 555 / Em 637	MG07
MemGlow™ NR12 S Membrane Polarity Probe **(A MEMBRIGHT Family Probe)**	Ex 550 / Em 633	MG08