GPCRs Moonlighting as Scramblases: Mechanism Revealed

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Abstract:

Phospholipids can undergo transverse diffusion, changing leaflets in the bilayer via translocase or scramblase activity. In this issue of Structure, Morra et al. (2018) provide insight into the mechanism used by one scramblase, opsin, based on large-scale ensemble atomistic molecular dynamics simulations. Results support a proposed "credit card reader" model.

Keywords: scramblase | GPCR | phospholipid

Article:

The fluid mosaic model for lipid bilayers first proposed by Singer and Nicolson (1972) describes the cell membrane as a two-dimensional liquid that restricts the lateral diffusion of membrane components, such as proteins, glycoprotein complexes, and lipid rafts. We now know that in addition to lateral diffusion, phospholipids can also undergo transverse diffusion in which a lipid changes leaflets in the bilayer. This exchange is catalyzed by "flippases," which fall into two categories: "translocases" and "scramblases" (Williamson, 2011). A paper in this issue of *Structure* (Morra et al., 2018) provides insight into the mechanism used by one scramblase, opsin.

Translocases provide ATP-dependent unidirectional transport of specific phospholipids against a concentration gradient. One example is the translocation of phosphatidylserine in platelets from the inner to the outer leaflet by TMEM16F, one of the five transmembrane (TM) protein 6 members that support Ca^{2+}-dependent phospholipid scrambling. This process is critical for blood coagulation (Fujii et al., 2015). Scramblase activity, on the other hand, is bidirectional and relatively non-specific for phospholipid type, working for most lipids. For many years, however, the identities of the protein(s) responsible for scramblase activity were unknown. In 2011, Menon and colleagues reported that the class-A G protein-coupled receptor (GPCR) opsin is a phospholipid flippase (scramblase) (Menon et al., 2011). Since then, other class-A GPCRs, including the β2-adrenergic and the adenosine A2A receptor, have also been shown to scramble phospholipids when reconstituted into synthetic vesicles. (Goren et al., 2014) Each receptor facilitated rapid (> 10,000 phospholipids per protein per second) scrambling of phospholipid probes. In photoreceptor disk membranes, a transbilayer phospholipid imbalance is caused by the

translocase action of ATP-driven transporters. Rhodopsin or its retinal-free opsin form, acting as a scramblase, is critical for homeostasis of photoreceptor disc membranes because it can correct this transbilayer phospholipid imbalance via its scramblase activity.

The mechanism by which rhodopsin or opsin, facilitates phospholipid scrambling is not known. Various models have been proposed. One posits that the structural features needed for scrambling lie at the protein-protein interface of rhodopsin dimers (Mansoor et al., 2006). A second model posits that the receptor-lipid interface provides a hydrophilic pathway that connects the intracellular (IC) and extracellular (EC) sides of the membrane. Lipids are envisioned to insert their polar head groups in the hydrophilic space, while the lipid acyl chains remain in the non-polar region of the bilayer. This is reminiscent of a credit card (whose magnetic strip corresponds to the lipid head group, while the card body represents the lipid sn-1 and sn-2 acyl chains) swiping through a credit card reader (receptor protein) (Ernst and Menon, 2015, Quazi and Molday, 2014).

Morra and co-workers now explore the scramblase mechanism in opsin. They have carried out large-scale ensemble atomistic molecular dynamics (MD) simulations (totaling > 50 μs simulation time) of opsin embedded in an explicit phospholipid membrane. Analysis of the MD trajectories reveals a hydrophilic pathway for phospholipid translocation along the interface between transmembrane helices (TMHs) 6 and 7 of the protein. This pathway is enabled by a dynamically growing opening between the IC ends of TMHs 6 and 7 that is aided by disruption of the interaction between residues E249$^{6.32}$ and K311$^{7.58}$ and conformational switching of residue Y306$^{7.53}$ (see Figure 1, right). Also, the kinetics of the lipid translocation process were quantified using Markov State Model (MSM) analysis of the MD trajectories. Importantly, the kinetics of lipid translocation obtained in their detailed model is consistent with that measured in experiments.

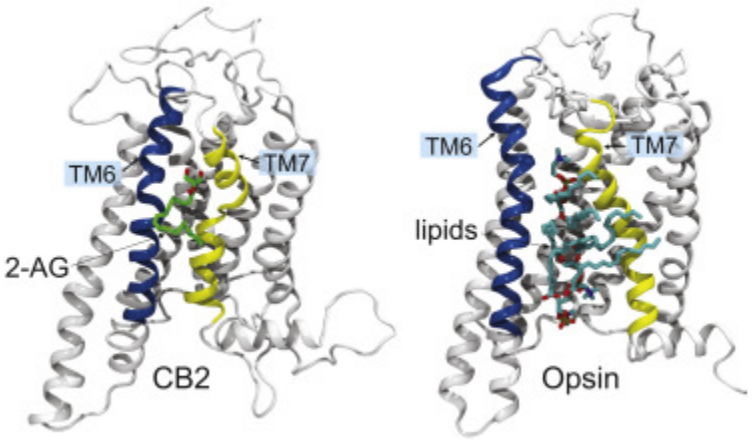

Figure 1. Importance of the TMH6-TMH7 Interface in Class-A GPCRs
(Left) The figure illustrates the interface between transmembrane helices (TMH) 6 and 7 during endogenous cannabinoid entry into and activation of the cannabinoid CB2 receptor. (Right) Opsin/MD trajectories reveal a hydrophilic pathway for phospholipid translocation along the TMH6 and TMH7 interface of opsin. This pathway is enabled by a dynamically growing opening between the intracellular ends of TMHs 6 and 7 that is aided by disruption of the interaction between residues E249$^{6.32}$ and K311$^{7.58}$ and conformational switching of residue Y306$^{7.53}$.

Results reported by Morra et al. (2018) support the "credit card" mechanistic model of scramblase action discussed above. Furthermore, several experimental observations can be interpreted based upon these results, First, both opsin monomers and dimers have been shown to scramble phospholipids at the same rate. The GPCR dimer interfaces suggested from both computational and experimental studies involve TMH4-TMH5 as well as TMH1-Hx8. Given that the TMH6-TMH7 interface would not be occluded by receptor dimerization at either or both of these interfaces, scramblase activity would be unaffected by oligomerization. Second, scrambling has been shown to be a constitutive activity of both the ligand-bound (inactive) and the ligand-free (active) forms of opsin. The opsin conformational dynamics reported by Morra and colleagues that produce the TMH6-TMH7 opening for scrambling are distinct from the major rearrangements associated with GPCR activation. And finally, the TMH6-TMH7 lipid translocation pathway in opsin revealed by the Morra MD simulations (see Figure 1, right) is very similar to the entry pathway for the lipid-derived endogenous ligand sn-2-arachidonoylglycerol (2-AG) into the cannabinoid CB2 receptor, a class-A GPCR (see Figure 1, left) (Hurst et al., 2010).

The scramblase activity of rhodopsin and other GPCRs appears to be silent at the plasma membrane (Ernst and Menon, 2015), which brings up a few further questions: why does GPCR location play a role in the ability to scramble? How does the presence of cholesterol and saturated lipids impact the dynamics of the system? Does this directly lead to loss of scramblase activity? Future MD studies combined with mutation studies should be able to answer these questions and fully illuminate how certain GPCRs become scramblases under certain conditions.

References

Ernst, O.P., and Menon, A.K. (2015). Phospholipid scrambling by rhodopsin. Photochem. Photobiol. Sci. 14, 1922–1931.

Fujii, T., Sakata, A., Nishimura, S., Eto, K., and Nagata, S. (2015). TMEM16F is required for phosphatidylserine exposure and microparticle release in activated mouse platelets. Proc. Natl. Acad. Sci. USA 112, 12800–12805.

Goren, M.A., Morizumi, T., Menon, I., Joseph, J.S., Dittman, J.S., Cherezov, V., Stevens, R.C., Ernst, O.P., and Menon, A.K. (2014). Constitutive phospholipid scramblase activity of a G protein-coupled receptor. Nat. Commun. 5, 5115.

Hurst, D.P., Grossfield, A., Lynch, D.L., Feller, S., Romo, T.D., Gawrisch, K., Pitman, M.C., and Reggio, P.H. (2010). A lipid pathway for ligand binding is necessary for a cannabinoid G protein-coupled receptor. J. Biol. Chem. 285, 17954–17964.

Mansoor, S.E., Palczewski, K., and Farrens, D.L. (2006). Rhodopsin self-associates in azolectin liposomes. Proc. Natl. Acad. Sci. USA 103, 3060–3065.

Menon, I., Huber, T., Sanyal, S., Banerjee, S., Barre´ , P., Canis, S., Warren, J.D., Hwa, J., Sakmar, T.P., and Menon, A.K. (2011). Opsin is a phospholipid flippase. Curr. Biol. 21, 149–153.

Morra, G., Razavi, A.M., Pandey, K., Weinstein, H., Menon, A.K., and Khelashvili, G. (2018). Mechanisms of lipid scrambling by the G protein-coupled receptor opsin. Structure 26, this issue, 356–367.

Quazi, F., and Molday, R.S. (2014). ATP-binding cassette transporter ABCA4 and chemical isomerization protect photoreceptor cells from the toxic accumulation of excess 11-cis-retinal. Proc. Natl. Acad. Sci. USA 111, 5024–5029.

Singer, S.J., and Nicolson, G.L. (1972). The fluid mosaic model of the structure of cell membranes. Science 175, 720–731.

Williamson, P. (2011). Phospholipid transport: sighting a new face of an old friend. Curr. Biol. 21, R168–R169.