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SPOTLIGHT

Cholesterol: Enhancing FGF2 translocation in unconventional secretion

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Fibroblast growth factor (FGF2) is a potent mitogen that is secreted through an unconventional secretory pathway by crossing the plasma membrane directly. In this current issue, Lolicato et al. (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202106123) find that the secretion process is promoted by cholesterol, which enhances PI(4,5)P_2 accessibility to FGF2 binding and alters membrane property to increase FGF2 translocation.

The ER-Golgi secretory pathway is a well-defined route for the secretion of proteins containing signal peptides. Emerging evidence indicates that some proteins lacking signal peptides can be released through alternative pathways collectively termed unconventional protein secretion (UPS; 1–3). In UPS, two major pathways have been proposed. One is the non-vesicle trafficking route in which proteins are directly translocated across the plasma membrane (PM; e.g., FGF2 secretion and Gasdermin D-mediated pore formation; 4,5). The other is the vesicle-mediated route in which UPS cargoes are secreted through multiple routes of vesicle trafficking (6–10).

FGF2 is one of the most systematically investigated UPS cargoes and is secreted through direct translocation across the plasma membrane. It is recruited to the inner PM leaflet through interaction with Na/K-ATPase, Tec kinase, and PI(4,5)P_2. In this step, binding to PI(4,5)P_2 triggers the oligomerization of FGF2 into a toroidal structure across the PM, which forms the membrane pore. Once FGF2 reaches the outer leaflet of PM, the oligomers are captured and disassembled by heparan sulfate proteoglycans (HSPGs) such as Glypican-1 (2). A key question left unresolved by this model is what drives the pore formation on the plasma membrane for FGF2 translocation?

In this issue of *JCB*, Lolicato et al. (11) identify cholesterol as a potent regulator of FGF2 secretion and provide an answer to the above question. The authors first performed a liposome binding assay, through which they found that increasing amounts of cholesterol can enhance FGF2 binding to PI(4,5)P_2-containing liposomes. This result is echoed by several elegant cellular experiments in which they found that cholesterol enhances the recruitment of FGF2 to the plasma membrane. Interestingly, the extent of cholesterol-promoted FGF2 translocation exceeds that of FGF2 binding to the plasma membrane, indicating that cholesterol may increase FGF2 secretion at both the membrane binding and translocation steps. With super-resolution microscopy, the authors identify colocalization between FGF2, PI(4,5)P_2, and cholesterol on the plasma membrane, indicating that cholesterol may directly affect FGF2 translocation and that this process occurs in a cholesterol-enriched microdomain on the plasma membrane.

Cholesterol is a lipid with unique biophysical properties. It plays an important role in cell membrane composition and fluidity, modulation of membrane trafficking, and signal transduction (12). To understand how cholesterol regulates FGF2 secretion, the authors employed a series of fully atomistic molecular dynamics simulations. Their calculations suggested that the average charge density on the headgroup regions of the membrane became more negative with increasing concentration of cholesterol. This in turn enhances headgroup visibility of PI(4,5)P_2, making it more accessable to binding factors. In addition, transient trimers or tetramers of PI(4,5)P_2 clusters are formed in the presence of cholesterol. Therefore, the increase of PI(4,5)P_2 head group and formation of PI(4,5)P_2 clusters dramatically affect FGF2 binding to PI(4,5)P_2 and may subsequently enhance FGF2 oligomerization as an initial step for membrane translocation (11). To determine how cholesterol promotes the translocation step, the authors combined microfluidic technology with drop shape analysis and found that cholesterol increases membrane tension. Prior work has proposed that this would favor membrane pore formation and therefore facilitate FGF2 translocation (11,13; Fig. 1).

Taken together, the study identified cholesterol as an important lipid for enhancing PI(4,5)P_2-dependent unconventional secretion of FGF2 via three means. Firstly, cholesterol enhances the visibility of PI(4,5)P_2, likely by diluting the positively charged headgroup of phosphotidylcholine (PC), to facilitate FGF2 recognition. Secondly, cholesterol leads to PI(4,5)P_2

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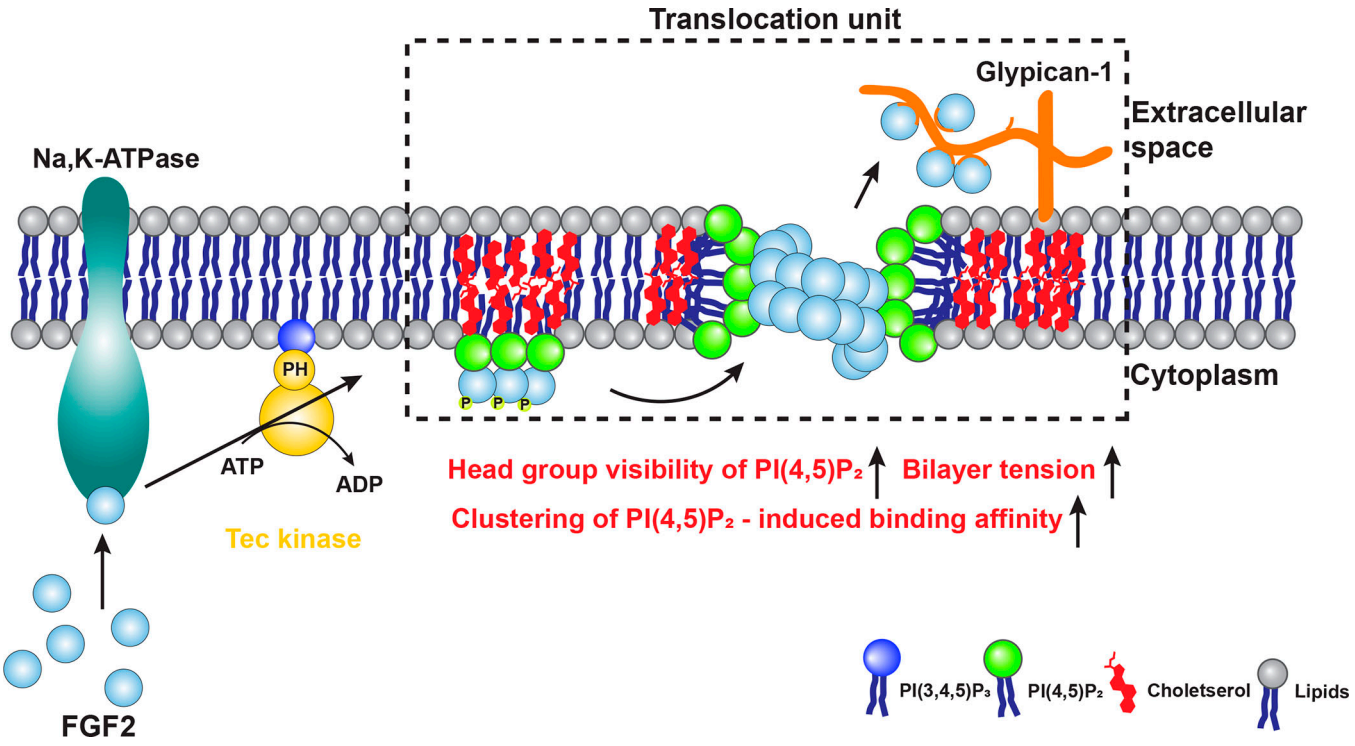

Figure 1. **Cholesterol-organized formation of translocation unit in FGF2 UPS.** FGF2 is recruited to the inner PM leaflet through interaction with Na, K-ATPase, Tec kinase, and PI(4,5)P_2. PI(4,5)P_2 binding and translocation occurs in a cholesterol-enriched microdomain (translocation unit), in which cholesterol increases the head group visibility of PI(4,5)P_2 and induces PI(4,5)P_2 multimer formation, therefore enhancing the binding affinity between FGF2 and PI(4,5)P_2 and oligomerization of FGF2. In addition, cholesterol increases the membrane tension, which together with the membrane deformation likely caused by the clustering of cone-shaped PI(4,5)P_2 faciliates pore formation and translocation. Enrichment of HSPGs such as Glypican-1 in the cholesterol-enriched translocation unit may promote quick transfer of membrane-inserted FGF2 to the cell surface to minimize membrane damage.

clustering, which increases binding avidity to FGF2 and increases FGF2 oligomerization. In addition to FGF2 binding, the local enrichment of PI(4,5)P_2 is likely to cause stress to the membrane due to its conical shape. This membrane stress would in turn facilitate lipid pore formation. Thirdly, cholesterol increases membrane bilayer tension, which lowers the activation energy of pore formation to facilitate FGF2 translocation. Therefore, the effect of cholesterol on headgroup charge, PI(4,5)P_2 clustering, and membrane tension provides an answer for the initial driving force of FGF2 membrane translocation (Fig. 1).

The current work provides important insights into the contradiction between pore formation and membrane integrity maintenance during FGF2 translocation. It is easy to speculate that FGF2 translocation must be completed in a swift manner in a rigid region of the plasma membrane to minimize membrane damage. Indeed, previous work has found that the translocation period is as short as 200 ms (14). To accomplish this, the handover of FGF2 from PI(4,5)P_2 to the extracellular HSPG must be tightly coupled. The current work indicates that the translocation of FGF2 occurs in a cholesterol-enriched microdomain in which proteins with HSPGs, such as Glypican 1, are enriched (15). Therefore, it is likely that cholesterol organizes a translocation unit on the plasma membrane where FGF2 binding, pore formation, and delivery to HSPGs are sufficiently linked to speed up translocation. In addition to increasing the rate of translocation, cholesterol forms liquid-ordered phases to prevent membrane damage via elevated membrane rigidity, which constrains the damaged region and prevents membrane disintegration (16; Fig. 1).

It is worth mentioning that besides FGF2, the authors also found that cholesterol recruited another PI(4,5)P_2 binding protein, PH-PLC-δ1, to the membrane (11). Therefore, the principle of cholesterol-regulated protein-phospholipid binding identified in the study may explain the reported effect of cholesterol on diverse cellular processes, such as membrane trafficking and organelle dynamics (12). The current work provides an excellent example for future efforts on dissecting the regulatory role of lipids not only in UPS but also in other essential cellular processes.

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