Published in final edited form as:

Trends Biochem Sci. 2015 December ; 40(12): 715–717. doi:10.1016/j.tibs.2015.10.001.

Flipping a Lipid-Linked Oligosaccharide? *You Must Whip It!*

Mark A. Lehrman^{1,*}

¹Dept. Pharmacology, UT-Southwestern Medical Center at Dallas, Dallas, TX USA

Abstract

The mechanism for flipping large lipid-linked oligosaccharides across membranes has remained a paradox. Perez *et al.* now report the structure of the PglK protein of *C. jejuni*, a flippase for a bacterial lipid-linked oligosaccharide, and reveal an unexpected whip-like mechanism.

Keywords

polyisoprenol; undecaprenol; dolichol; lipid-linked oligosaccharide; flippase

Prokaryotes, eukaryotes, and most likely archaea all use similar strategies to attach large oligosaccharides onto proteins [1, 2]. Cytoplasmically-oriented oligosaccharides are assembled, via pyrophosphate (double-phosphate) linkage, onto long polyisoprenol membrane anchor lipids, forming lipid-linked oligosaccharides (LLOs). Then, by an energetically challenging process, the polar pyrophosphate-oligosaccharide head groups flip across a membrane bilayer into the periplasm (prokaryotes) or endoplasmic reticulum lumen (eukaryotes) while still anchored to polyisoprenol. Flipped oligosaccharides are then transferred to proteins. Perez *et al.* [3] report X-ray crystal studies of an LLO flippase, the *Campylobacter jejuni* PglK protein. Their results provide our best view into LLO flipping, and reveal satisfying explanations for nature's choices of pyrophosphate for the linker and polyisoprenol for the membrane-bound head-group carrier. Unlike more common switch-like lipid flippases [4], PglK flips LLO by a whip-like mechanism (Figure 1).

All lipid flippases mediate passage, across a membrane bilayer (with a hydrophobic core), of polar cargo covalently attached to non-polar groups tightly embedded in the membrane [4]. Flippases for oligosaccharides attached to polyisoprenols are among the least understood, and are perplexing because of the large polar groups (typically heptasaccharides). In a landmark study, Perez *et al.* [3] crystallized and conducted mechanistic studies of the flipping reaction of *C. jejuni* membrane protein PglK, which directionally flips a LLO consisting of a heptasaccharide in pyrophosphate linkage to undecaprenol (a C55 polyisoprenol) from a cytoplasmic to a periplasmic orientation.

*Correspondence: mark.lehrman@utsouthwestern.edu, (M. A. Lehrman).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Given that flippases do not chemically alter their substrates, Perez *et al.* used a secondary reaction to detect flipping. To begin, LLO and recombinant PglK were both randomly oriented in proteoliposomes. PglK is an ATP binding cassette protein with a defined nucleotide binding domain (NBD), and so external addition of ATP to proteoliposomes selectively activates PglKs with externally-oriented NPDs, enabling inward flipping. To quantify the flippase reaction, loss of external LLO head groups due to inward flipping was then measured. This measurement required some clever biochemical trickery using a substitute PglK substrate that can also be recognized by another sugar transferase. Instead of its typical cargo, a smaller trisaccharide form of *C. jejuni* LLO was used because it is well tolerated by PglK, and the trisaccharide headgroup is an excellent acceptor for the *C. jejuni* enzyme PglH, a GalNAc transferase. Added to the outside of proteoliposomes with its donor UDP-[³H]GalNAc, PglH can mark unflipped LLO by attaching radioactive GalNAc (Figure 1). Controls ensured that this system detected authentic LLO flipping and not modulation of PglH-dependent catalysis by PglK.

With an assay in hand, Perez *et al.* then crystallized PglK. Two forms of PglK were identified by crystallization and modeling: both membrane-associated homodimers, but dramatically different in orientation. Nucleotides are only available in the cytoplasm, therefore the NBD domains allowed biological orientations of structures to be deduced. One form of PglK has the NBD domain (an apo-form lacking nucleotide) participating in formation of a broad cytoplasmic-facing pocket. Although this pocket suggests docking of the oligosaccharide head group, experimental and theoretical considerations led Perez *et al.* to rule out a functional role of the cytoplasmic-facing pocket in LLO flipping. This is significant because other lipid flippases with similar predicted structures are thought to employ ‘alternating’ or reciprocating toggle switch-like mechanisms, in which polar head groups reversibly shuttle between inner- and outer-facing pockets [4].

How then might PglK flip LLO, if not through a switch-like mechanism? The second form of PglK homodimer has a periplasmic (outer) facing pocket, and importantly exists in two states: an ADP-bound state with a narrow and shallow (occluded) pocket; and an ATP-bound state with the pocket wide open and so deep that it almost spans the entire membrane bilayer. By assaying wild-type and mutagenized PglK for flippase activity with the trisaccharide version of LLO (as well as LLO analogues with modified lipids and/or missing saccharides to determine specificity for the polyisoprenol), Perez unexpectedly deduced a whip-like mechanism for outward-facing PglK and concluded that this is the biologically relevant conformation. The key to this discovery was the identification of a novel hydrophobic extended helix (EH) along the periplasmic membrane-water interface. An EH is not found in switch-like flippases, and abuts two fully embedded transmembrane helices (TM1 and TM2) forming a hydrophobic groove. In the occluded form, this groove engages the undecaprenol end farthest from the oligosaccharide, keeping it near the periplasmic face of the bilayer (Figure 1). This interaction begins the flipping process.

Exchange of two molecules of ADP for ATP per PglK dimer then opens the outward-facing pocket, exposing a positively-charged segment (arginines 86, 260, 302, and 309) that was occluded. The polar head group, from its position at the inner (cytoplasmic) membrane-water interface, executes a whip-like motion to penetrate the PglK dimer into the wide-open

pocket. However, it remains unclear how the head group can navigate through the membrane environment surrounding PglK. Strikingly, reorientation of the head group is driven by electrostatic interaction of the pyrophosphate with the arginine-rich region deep within the pocket. No specific PglK-oligosaccharide contacts were detected, helping to explain why PglK can flip LLOs with varied oligosaccharides.

LLO binding stimulates ATP hydrolysis, allowing the pocket to squeeze back into its narrower conformation and break electrostatic interactions with the pyrophosphate. This change ejects the pyrophosphate-oligosaccharide unit into the aqueous phase while PglK continues to tether the undecaprenol chain which is still engaged by the EH. Presumably, disengagement of the undecaprenol chain follows.

The unexpected flipping mechanism of PglK has additional important implications. First, monophosphate linkages can energize catalysis of other polyisoprenol-linked saccharides, so why does LLO need pyrophosphate? Apparently, the pyrophosphate acts like a tag to drive docking to the arginine rich surface in the outer pocket, as well as provide energy for later protein transfer. Second, short aliphatic chains of glycosylphosphatidylinositols and C15-20 polyisoprenes can anchor large polar cargo (proteins) to membranes, so why does LLO need C55 undecaprenol? Perhaps the long polyisoprenol provides flexibility, a broad range of motion, and the bilayer-spanning length needed for whip-like LLO flipping, whereas the shorter chains cannot. The PglK mechanism could also apply to eukaryotic LLO flipping, which still a poorly understood process [5-8]. However, eukaryotic LLO flipping does not seem to require ATP, and eukaryotic LLOs have longer polyisoprenols (dolichols, C75-95), so some differences with the PglK mechanism may exist. Regardless, Perez *et al.* have answered questions that have long confounded bacteriologists and glycobiologists.

Acknowledgments

Work in the author's laboratory is supported by NIH grant R01-GM038545. Charles "Skip" Waechter and Anant Menon provided excellent editorial comments. The italicized portion of the title is quoted from "Whip It" by Devo (written by Mark Mothersbaugh and Gerald Casale), © Sony/ATV Music Publishing LLC, BMG Rights Management US, LLC.

References

1. Schenk B, Fernandez F, Waechter CJ. The ins(ide) and outs(ide) of dolichyl phosphate biosynthesis and recycling in the endoplasmic reticulum. *Glycobiology*. 2001; 11:61R–70R.
2. Helenius A, Aebi M. Roles of N-linked glycans in the endoplasmic reticulum. *Annu. Rev. Biochem.* 2004; 73:1019–1049. [PubMed: 15189166]
3. Perez C, et al. Structure and mechanism of an active lipid-linked oligosaccharide flippase. *Nature*. 2015; 524:433–8. [PubMed: 26266984]
4. Sanyal S, Menon AK. Flipping lipids: why an' what's the reason for? *ACS Chem Biol*. 2009; 4:895–909. [PubMed: 19689162]
5. Helenius J, et al. Translocation of lipid-linked oligosaccharides across the ER membrane requires Rft1 protein. *Nature*. 2002; 415:447–450. [PubMed: 11807558]
6. Jelk J, et al. Glycoprotein biosynthesis in a eukaryote lacking the membrane protein rft1. *J. Biol. Chem.* 2013; 288:20616–20623. [PubMed: 23720757]
7. Snider MD, Rogers OC. Transmembrane movement of oligosaccharide-lipids during glycoprotein synthesis. *Cell*. 1984; 36:753–761. [PubMed: 6697396]

8. Sanyal S, Menon AK. Specific transbilayer translocation of dolichol-linked oligosaccharides by an endoplasmic reticulum flippase. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106:767–72. [PubMed: 19129492]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1. Proposed Mechanism of Lipid-Linked Oligosaccharide (LLO) Flipping by PglK
The cytoplasmic (inner) and periplasmic (outer) sides of the *Campylobacter jejuni* periplasmic membrane (gray) are indicated. The black oval and thick line represent the oligosaccharide and polyisoprenol chain, respectively, of LLO coupled by pyrophosphate (-P-P-). (A) Perez *et al.* showed that the oligosaccharide structure is not critical for flipping by PglK (a dimer, indicated by dashed vertical lines) in the case of large linear oligosaccharide chains, while not all highly branched oligosaccharides are expected to fit. (B) The polyisoprenol straightens sufficiently to reach the hydrophobic groove formed by the extended helix (orange cylinder) and transmembrane segments 1/2, with the outer pocket occluded. (C) Exchange of 2 ADP for 2 ATP opens the pocket, accommodating the large oligosaccharide as it moves forward toward the periplasmic space. The arginine-rich region (red polygon) is exposed to dock the pyrophosphate. (D) ATP hydrolysis returns the outer pocket to an occluded state, dislodging the pyrophosphate and ejecting the polar head group as it moves ahead into the aqueous milieu. (E) The final step of whip-like flipping presumably requires the polyisoprenol chain to disengage from the hydrophobic groove. (F) After flipping, LLO cannot be detected by PglH-dependent transfer of GalNAc onto the oligosaccharide.