TMED10 for unconventional protein secretion cargoes translocation

Preview from "A Translocation Pathway for Vesicle-Mediated Unconventional Protein Secretion". Zhang, M., Liu, L., Lin, X., Wang, Y., Li, Y., Guo, Q., Li, S., Sun, Y., Tao, X., Zhang, D., Lv, X., Zheng, L., Ge, L. . Cell Apr. 4 2020

by Marzia Savini, Lang Ding and Max Gao

Introduction.

Proteins are synthesized as polypeptide chains on the ribosomes and bind the rough Endoplasmic Reticulum (ER) in a process referred to as co-translation process. Soluble proteins are located in the ER and subsequently transported into the appropriate destination outside or within the cell. Similarly, integral proteins are inserted into the rough ER membrane during their synthesis. However, while some proteins will not change the ER membrane location, many others will be targeted to distinct organelle or plasma membrane (PM) via secretion. Most of the cytosolic proteins entering the secretory pathway contain a predicted signal peptide located at the N-terminus which is recognized by a signal recognition particle (SRP) during the co-translational process. The nascent polypeptide will be subsequently inserted into the translocon, a membrane protein consisting of the Sec61 translocation complex in eukaryotes (Rapoport et al., 2017; Shan and Walter, 2005; Voorhees and Hegde, 2016). In secreted proteins, the signal peptide is immediately cleaved off from the nascent protein upon its translocation into the ER membrane. Within the ER, the protein interacts with chaperones that aid the proper folding. Misfolded proteins are recognized within the ER and retro-translocated by the ER-associated degradation (ERAD) complex to the cytosol to be degraded by the proteasome. Once folded correctly, the proteins will be transported into the Golgi Apparatus via transport vesicles known as cisterna coated by COPII proteins. From the Golgi, a subset of ER-localized proteins are retrieved to the ER via a different set of retrograde transport vesicles coated by COPI proteins. The ER-Golgi intermediate compartment (ERGIC) is a complex membrane system formed between those two organelles (Hauri et al. 2000, Schweizer et al. 1988). The newly synthesized folded proteins are exposed to post-translation modifications including glycosylation and further sorted into transport vesicles that move and fuse eventually with the plasma membrane. This overall process is termed as conventional secretion. However, eukaryotic cells also secrete cytoplasmic proteins that lack an N-terminal signal sequence by the unconventional protein secretion (UPS) pathway which includes two main mechanisms (Nickel and Rabouille, 2009; Rabouille et al., 2012). The first involves a direct insertion within the plasma membrane (type I), while the second is mediated by vesicle trafficking (type III) (Scha¨fer et al., 2004; Steringer and Nickel, 2018,

Duran et al., 2010; Malhotra, 2013). Specifically, the type I mediates secretions of leaderless cargoes are secreted directly out of cell through pore-mediated translocation across the plasma membrane, while the type III is a membrane-bound organelle (autophagosome/endosome)-based secretion (Duran et al., 2010, Kinseth et al. 2007, Ding et al. 2016, Zaherl et al. 2015) The main question asked by scientists is the underlying mechanism by which a lacking signal peptide protein can enter into the vesicle carrier. In this paper, Zhang *et al.* discovered a novel translocation pathway by identifying TMED10 as a protein channel for the vesicle entry and secretion of many proteins lacking the signal peptide.

Results

To test the hypothesis that TMED10 is a regulatory factor involved for the secretion of multiple UPS cargoes such as the mature IL-1β (mIL-1β, which is a model-like study object for UPS cargoes), the authors first verified that TMED10 was required for the secretion of mIL-1β. Specifically, TMED10 facilitated mIL-1β secretion via type III mechanism. Because protein unfolding or flexible conformation is usually required for protein translocation across a membrane, a previous study conducted by Sheckman and colleagues indicated that the translocon-like protein channel is involved in mIL-1β secretion (Zhang et al., 2015). Therefore, the authors fused mIL-1β protein to dihydrofolate reductase (DHFR), an enzyme whose three-dimensional structure can be stabilized by aminopterin to prevent the unfolding process, which is used for protein translocation study, allowing the identification of enriched translocation machinery of mIL-1β. Using semiquantitative mass spectrometry, they identified 11 transmembrane proteins as potential candidates related to the translocation machinery. After screening the candidates by silencing their expression, the authors found that only TMED10 was the key regulator required for mIL-1β secretion in both inflammatory and non-inflammatory cells. Specifically, TMED10 assists IL-1β secretion with its mature form rather than the precursor form. The authors also ruled out the possibilities of mIL-1β secretion via PM penetration and GSDMD cleavage-mediated pore formation.

Since the whole-body TMED10-KO mice are not viable, to further investigate the role of TMED10 in mIL-1β secretion, the authors generated myeloid-cell lineage specific conditional knockout mice model which resulted in mIL-1β increased levels in the serum and subsequent reduction of IL-6 and IL-1β release. These results suggested that TMED10 also regulates inflammation physiologically. TMED10 is a non-glycosylated multiple-domain transmembrane protein consisting of a luminal domain containing the signal peptide (SS), a GOLD domain, a coil-coil (CC) domain and a C-terminal tail (CT) facing the cytoplasm. The next question the authors asked was about which domain is required for mIL-1β association and secretion? By conducting domain deletion mutants analysis, the authors determined that the CT domain was directly interacting with mIL-1β by co-IP. Then, a question about the specificity of

TMED10-mediated UPS mechanism raised up. Not surprisingly, TMED10 has a broader role in distinct UPS cargoes tested by the authors, resulting in the secretion decrease for mature forms of IL-1 family members, HSPB5, galectin-1/3, annexin A1 and Tau, but not high mobility group box 1 (HMGB1), α-synuclein, type I UPS cargoes or conventional secretion cargoes. According to the previous knowledge about cargoes' translocation research, it has been demonstrated that the chaperone-mediated autophagy (CMA) translocation process relies on a KFERQ motif in the cytosolic cargo recognized by Hsc70. How about the TMED10 mediated translocation process? Similarly, the authors identified common motifs (motif-1 and motif-2) for the UPS cargoes using the MEME-Suite website (Bailey and Elkan, 1994). The molecular dynamics simulation assay showed that the motif-1 rather than motif-2 had strong binding with TMED10-CT.

Since the authors demonstrated that TMED10 is a sufficient and direct factor for membrane translocation of UPS cargoes, what is the membrane compartment that allows the cargoes to enter? They found that TMED10 directly promotes the leaderless cargo's entry into the ER-Golgi intermediate compartment (ERGIC) based on the GFP complementation assay in which the fluorescence complementation specifically increased in the co-expressed pIL-1β-GFP11 with TMED10 containing a luminal GFP(1–10) tag (GFP(1–10)-TMED10. During the process, the unfolding state of the UPS cargo is required for its translocation, the authors confirmed that in the cytosolic side, HSP90A facilitated the unfolding of the cargo, while in the lumen side, HSP90B1 helped the cargo's entry. There were interactions between both HSP90A and HSP90B1 with mIL-1β, indicated by the GST pull-down experiment. In addition, the crosslink assay data shows that TMED10 forms high molecular weight complexes, suggesting that the TMED10 oligomers form a channel to traffic mIL-1β. Moreover, the formation of TMED10 oligomers is triggered by both the motif-1 sequence in the UPS cargoes and their binding to TMED10-CT, while the GOLD domain is required for TMED10 oligomerization.

Overall, the paper proposed a model for the UPS cargo translocation process. The cargo undergoes the unfolding process with the help of cytosolic HSP90A, then interacts with TMED10 by inducing its oligomerization to form a protein channel on the ERGIC enabling the UPS cargoes to translocate into vesicles. On the lumen side of ERGIC, HSP90B1 assists the whole mechanism by facilitating the cargo translocation into the lumen of ERGIC through the channel.

Discussion

The role of the ER and the Golgi apparatus in the canonical transport and release of secreted proteins has been established for decades. However, researchers have been exploring the secreting machinery that regulates leaderless cargoes including the soluble inflammatory

cytokine interleukin-1b (IL-1b) since 1984 (Auron et al,, 1984)Zhang *et al.*'s work provides the full revelation of this unconventional protein secretion (UPS) pathway. They not only identified the hypothetical "translocon" TMED10 for the autophagy-mediated secretion of IL-1b and all other IL family members (Zhang et al., 2015), but also proposed a potential motif present on various IL1 family members and other leaderless secreting proteins that is self-sufficient to initiate the unconventional route of secretion. The *in vivo* experiments demonstrating the myeloid-specific disruption of TMED10 enhances the serum levels of IL-1b and subsequent reduction of the effector IL-6. The distal organ damage in CLP model is also an informative support for the physiological function of this pathway.

This TMED10-channeled unconventional protein secretion (THU), opportunistically named after the famed institution where the authors reside, opens a nascent subfield for the secretory regulation. As the authors mentioned in the discussion, it would be essential to test if THU acts independently with the auto/endolysosomal compartments. Given that the lysosomal transporter for UPS is pending for discovery, it is tempting to speculate that THU might function directly to generate UPS cargos. Another future direction is the regulating mechanism of this newly identified pathway. The translocation of the cargo into ERGIC is dependent on the oligomerization which is tightly temporal controlled. How does TMED10 "sense" the board range of cargos and initiate oligomerization? Structural analysis might help to understand this dynamic change. The regulation of TMED10 itself also might raise interesting questions for the immune response regulation, as TMED10 has been linked to inflammation in the brain (Gong et al,. 2011). Future research on the THU pathway will likely shed light on the mechanistic understanding of immune response regulation.

References.

Bailey, T.L., and Elkan, C. (1994). Fitting a mixture model by expectation maximization to discover motifs in biopolymers. Proc. Int. Conf. Intell. Syst. Mol. Biol. 2, 28–36.

Ding, J., Wang, K., Liu, W. *et al.* Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature* 535, 111–116 (2016). https://doi.org/10.1038/nature18590

Duran, J. M., Anjard, C., Stefan, C., Loomis, W. F. and Malhotra, V. (2010). Unconventional secretion of Acb1 is mediated by autophagosomes. J. Cell Biol. 188, 527-536. doi:10.1083/jcb.200911154

Duran, J.M., Anjard, C., Stefan, C., Loomis, W.F., and Malhotra, V. (2010). Un- conventional secretion of Acb1 is mediated by autophagosomes. J. Cell Biol. *188*, 527–536.

Hauri, H. P., Kappeler, F., Andersson, H. and Appenzeller, C. (2000). ERGIC-53 and traffic in the secretory pathway. *J. Cell Sci.* 113, 587-596.

Kinseth, M. A., Anjard, C., Fuller, D., Guizzunti, G., Loomis, W. F. and Malhotra, V. (2007). The Golgi-associated protein GRASP is required for unconventional protein secretion during development. Cell 130, 524-534. doi:10.1016/j.cell.2007.06.029
Malhotra, V. (2013). Unconventional protein secretion: an evolving mecha- nism. EMBO J. *32*, 1660–1664.
Nickel, W., and Rabouille, C. (2009). Mechanisms of regulated unconventional protein secretion. Nat. Rev. Mol. Cell Biol. *10*, 148–155.
Rabouille, C., Malhotra, V., and Nickel, W. (2012). Diversity in unconventional protein secretion. J. Cell Sci. *125*, 5251–5255.
Rapoport, T.A., Li, L., and Park, E. (2017). Structural and Mechanistic Insights into Protein Translocation. Annu. Rev. Cell Dev. Biol. *33*, 369–390.
Scha ¨ fer, T., Zentgraf, H., Zehe, C., Bru ¨ gger, B., Bernhagen, J., and Nickel, W. (2004). Unconventional secretion of fibroblast growth factor 2 is mediated by direct translocation across the plasma membrane of mammalian cells. J. Biol. Chem. *279*, 6244–6251.
Schweizer, A., Fransen, J. A., Bachi, T., Ginsel, L. and Hauri, H. P. (1988). Identification, by a monoclonal antibody, of a 53-kD protein associated with a tubulo-vesicular compartment at the cis-side of the Golgi apparatus. *J. Cell Biol.* 107, 1643-1653.
Shan, S.O., and Walter, P. (2005). Co-translational protein targeting by the signal recognition particle. FEBS Lett. *579*, 921–926.
Steringer, J.P., and Nickel, W. (2018). A direct gateway into the extracellular space: Unconventional secretion of FGF2 through self-sustained plasma membrane pores. Semin. Cell Dev. Biol. *83*, 3–7.
Voorhees, R.M., and Hegde, R.S. (2016). Toward a structural understanding of co-translational protein translocation. Curr. Opin. Cell Biol. *41*, 91–99.
Zacherl, S., La Venuta, G., Müller, H.-M., Wegehingel, S., Dimou, E., Sehr, P., Lewis, J. D., Erfle, H., Pepperkok, R. and Nickel, W. (2015). A direct role for ATP1A1 in unconventional secretion of fibroblast growth factor 2. J. Biol. Chem. 290, 3654-3665. doi:10.1074/jbc.M114.59006
Zhang, M., Kenny, S.J., Ge, L., Xu, K., and Schekman, R. (2015). Translocation of interleukin-1b into a vesicle intermediate in autophagy-mediated secretion. eLife 4, e11205.