Positive zip coding in small protein translocation

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Most newly synthesized proteins destined for the secretory pathway contain a signal peptide (SP) that triggers cotranslational translocation into the endoplasmic reticulum (ER). However, how small polypeptides undergo ER translocation is not fully understood. In this issue of JBC, Guo et al. describe a mechanism for posttranslational translocation of small secretory proteins featuring a positive charge within the SP N-terminal region. Defects in this element disrupt proper secretion and explain the effects of genetic mutations associated with one type of diabetes.

Proteins directed to the secretory pathway can be secreted from the cell or targeted to various membrane compartments, including the endoplasmic reticulum (ER); nearly 40% of human proteins are predicted to go through this pathway (1). The first decisive step of this journey is the translocation of a nascent chain across the ER membrane. In mammalian cells, translocation of most secretory proteins occurs cotranslationally, a process in which the membrane insertion of a nascent chain initiates before the protein translation completes by the ribosome. Secretory proteins in bacteria and yeast occur primarily via posttranslational ER translocation, although this process is increasingly acknowledged to be active in mammalian cells (2). Although various cellular machinery components have been linked to posttranslational ER translocation (3–5), it is not well understood whether there are any structural elements within the secretory cargo proteins themselves that facilitate posttranslational ER translocation. Guo et al. (6) now demonstrate that positive charge within the signal peptide (SP) N-terminal region of small secretory proteins plays an essential role in their posttranslational translocation. This finding broadens our understanding of protein-trafficking mechanisms and provides a new molecular explanation for the impact of genetic mutations without obvious functional defects, such as those in the insulin gene that lead to one type of diabetes, maturity onset diabetes of youth (MODY) (7).

Secretory proteins commonly bear SPs at their N terminus, which typically contain a stretch of hydrophobic residues that may be preceded by one or two positively charged residues called the n-region (8). In most secretory proteins, SPs are recognized by the signal recognition particle (SRP), whereas chain elongation is still ongoing on the ribosome. SRP binding causes a translational pause until the complex delivers the nascent chain–ribosome complex to the ER membrane by binding the SRP receptor. After the complex docks on the translocon channel Sec61, translation resumes and the nascent polypeptide passes through the channel, where the SP is cleaved by the signal peptidase (Fig. 1) (4). In the case of small proteins, this process is expected to be inefficient, because protein synthesis will likely terminate before the SP can be bound by the SRP. Indeed, previous studies have revealed that precursor chain length determines whether a protein will traffic via SRP-independent posttranslational translocation. In addition, postranslational translocation uses distinct cellular components such as Sec62 and Sec63, which are in complex with the Sec61 channel (Fig. 1) (4–6). However, it has not been known whether there are intrinsic signals of small secretory proteins that contribute to the efficiency of their postranslational translocation. Guo and colleagues (9) had previously found one clue to this mystery: In studying the R6C and R6H preproinsulin mutations associated with MODY, they observed that these constructs were not fully translocated and that the untranslocated preproinsulin proteins remained associated with the ER membrane and accumulated intracellularly, which promoted β-cell death. Because Arg-6 is found in the n-region of preproinsulin, the authors suspected the positive charge played a particularly important role in postranslational translocation.

In the current issue, Guo et al. (6) now test the generality of this hypothesis, that the n-region positive charge is required for the postranslational translocation of small secretory proteins. Throughout the study, ER translocation was assessed by SP cleavage, an approach that was justified by the fact that only the SP-cleaved forms were solubilized by sodium carbonate and also glycosylated upon introduction of an artificial N-glycosylation site (6). The authors first examined the effect of the R6C mutation on the translocation of preproinsulin–GFP fusion proteins with varying C-terminal truncations. In contrast to the R6C preproinsulin alone, the full-length GFP–fused preproinsulin (348 residues) was successfully translocated. However, when the polypeptide length was shortened to 160 residues, the translocation defect caused by the R6C mutation became increasingly apparent; nearly 70% of the preprotein failed to be translocated at a length of 110 residues. The translocation...
defect was not due to problems in the delivery of nascent protein to the ER membrane, suggesting the n-region positive charge may specifically contribute to the translocation process.

The authors examined six more secretory preproteins with different lengths to confirm the role of the n-region–positive charge. Their data showed impaired translocation by the loss of n-region–positive charge for four proteins ranging from 143 to 77 residues but not for two larger proteins of 275 and 306 residues. Importantly, when protein translation was slowed down by a low dose of the ribosome inhibitor cyclohexamide, the translocation defect of the R6C preproinsulin mutant was alleviated. In combination, these results suggest that small secretory proteins are quickly released to the cytosol before they can engage SRP and thus have to go through posttranslational translocation and that this pathway depends more on the SP n-region–positive charge than for larger proteins. Informatics provide further support for this conclusion, as the authors’ analysis of the eukaryotic secretome revealed that 69.3% of the 1877 secretory preproteins had positive charge in the n-region of the SP, with the frequency of proteins with n-region–positive charge increasing as the polypeptide length decreases, reaching ≥80% for proteins smaller than 130 residues. Hence, small secretory proteins might be evolutionarily constrained to preserve the SP n-region–positive charge to maximize posttranslational translocation efficiency.

By studying multiple small secretory proteins, including the natural disease mutants of preproinsulin, this work provides new insights for the physiological and pathological significance of the SP n-region–positive charge in the posttranslational translocation. However, how exactly the n-region–positive charge contributes to this process is still unclear. Does it help correct orientation of the SP by following a “positive-inside” rule (10) during the translocation through the Sec61 channel, or does it also help to promote an interaction with any of the recently identified translocation machinery (e.g. Sec62/63) to facilitate translocation? How are the small proteins delivered to the ER without being recognized by SRP? How are the small secretory proteins that do not possess positive charge in the n-region still translocated? Addressing such questions would further our understanding of the posttranslational translocation of small secretory proteins.

**References**

1. Uhlen, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, Å., Kampf, C., Sjöstedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C. A., Odeberg, I., Djureinovic, D., Takane, J. O., Hober, S., Alm, T., Edqvist, P. H., Berling, H., Tegel, H., Mulder, J., Rockberg, J., Nilsson, P., Schwenk, J. M., Hamsten, M., von Felitzen, K., Forsberg, M., Persson, L., Johansson, F., Zwahlen, M., von Heijne, G., Nielsen, J., and Pontén, F. (2015) Proteomics: Tissue-based map of the human proteome. *Science* **347**, 1260419 CrossRef Medline

2. Zimmermann, R., Eyrisch, S., Ahmad, M., and Helms, V. (2011) Protein translocation across the ER membrane. *Biochim. Biophys. Acta* **1808**, 912–924 CrossRef Medline

3. Johnson, N., Powis, K., and High, S. (2013) Post-translational translocation into the endoplasmic reticulum. *Biochim. Biophys. Acta* **1833**, 2403–2409 CrossRef Medline

4. Johnson, N., Haßdentüfel, S., Theis, M., Paton, A. W., Paton, J. C., Zimmermann, R., and High, S. (2013) The signal sequence influences post-translational ER translocation at distinct stages. *PLoS One* **8**, e75394 CrossRef Medline

5. Shao, S., and Hegde, R. S. (2011) A calmodulin-dependent translocation pathway for small secretory proteins. *Cell* **147**, 1576–1588 CrossRef Medline

6. Guo, H., Sun, J., Li, X., Xiong, Y., Wang, H., Shu, H., Zhu, R., Liu, Q., Huang, Y., Madley, R., Wang, Y., Cui, J., Arvan, P., and Liu, M. (2017) Positive charge in the n-region of the signal peptide contributes to efficient post-translational translocation of small secretory proteins. *J. Biol. Chem.* **293**, 1899–1907 CrossRef Medline

7. Johnson, S. T., Boulé, N. G., Bell, G. J., and Bell, R. C. (2008) Walking: a matter of quantity and quality physical activity for type 2 diabetes management. *Appl. Physiol. Nutr. Metab.* **33**, 797–801 CrossRef Medline

8. Nothwehr, S. F., and Gordon, J. I. (1989) Eukaryotic signal peptide structure/function relationships. Identification of conformational features which influence the site and efficiency of co-translational proteolytic processing by site- directed mutagenesis of human pre(delta pro)apollipoprotein A-II. *J. Biol. Chem.* **264**, 3979–3987 Medline

9. Guo, H., Xiong, Y., Witkowski, P., Cui, J., Wang, I. J., Sun, J., Lara-Lemus, R., Haataja, L., Hutchison, K., Shan, S. O., Arvan, P., and Liu, M. (2014) Inefficient translocation of preproinsulin contributes to pancreatic beta cell failure and late-onset diabetes. *J. Biol. Chem.* **289**, 16290–16302 CrossRef Medline

10. Gonder, V., Junne, T., and Spiess, M. (2004) Sec61p contributes to signal sequence orientation according to the positive-inside rule. *Mol. Biol. Cell* **15**, 1470–1478 CrossRef Medline

**EDITORS’ PICK HIGHLIGHT:** Translocation of small proteins

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**Figure 1. Posttranslational translocation of small secretory proteins.** A, most secretory proteins carrying an SP undergo cotranslational ER translocation mediated by recognition of the SP by the SRP and interaction of the ribosome–nascent chain–SRP complex with the SRP receptor (SRPR) and the Sec61 channel. B, small proteins (e.g. <160 amino acids) will be released from the ribosome before SRP recognition of the SP occurs and therefore must be postranslationally translocated. Guo et al. (6) establish a critical role for positive charge in the SP n-region in this process, plausibly by helping correct the orientation of the SP during insertion through Sec61. C, when this positive charge is genetically lost in preproinsulin (MODY-associated R6C), the ER translocation of nascent chain is blocked and the uncleaved protein accumulates intracellularly.