Quality Control in the ER: Misfolded Prohormones Get a Checkup

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In Mutant *INS*-gene-induced Diabetes of Youth (MIDY) syndrome, mutant proinsulin aggregates interfere with the folding of wild-type proinsulin in the endoplasmic reticulum, ultimately decreasing insulin secretion. In this issue of *Molecular Cell*, Cunningham et al. (2019) identify two mechanisms by which prohormone aggregation is prevented and cleared.

Protein misfolding and consequent aggregation underpin numerous disorders including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, familial amyloidogenic cardiomyopathy, and diabetes. To better understand these disorders, it is essential to establish how these proteins misfold and how the aggregates overwhelm the cell. The misfolding of two proteins, namely proinsulin and islet amyloid polypeptide (IAPP), is implicated in certain forms of diabetes. Proinsulin is the inactive precursor of insulin and is stabilized by three conserved disulfide bonds formed during its folding in the endoplasmic reticulum (ER) lumen (Figure 1A) (Mukherjee et al., 2015). Folded proinsulin then exits the ER and is processed to form bioactive insulin, which is secreted by pancreatic β cells into the bloodstream to control blood glucose levels. IAPP is co-secreted with insulin and can misfold to form amyloid deposits in pancreatic islets (Mukherjee et al., 2015). In MIDY, mutations to proinsulin disrupt its folding and processing. MIDY patients are heterozygous, thus half of their proinsulin harbors mutations that cause proinsulin to misfold and become trapped in the ER (Liu et al., 2010). While over 30 missense mutations have been identified in the insulin gene, the best-characterized one is the *Akita* proinsulin mutant, in which a conserved cysteine is replaced by tyrosine (Liu et al., 2010). Loss of this intramolecular disulfide bond results in the formation of non-native intermolecular disulfide bonds with other mutant and wild-type (WT) proinsulin molecules (Figure 1A) (Liu et al., 2010). By engaging other proinsulin molecules, high molecular weight complexes form that entrap mutant and WT proinsulin, inhibiting the exit of insulin from the ER. This decrease in circulating insulin upregulates proinsulin biosynthesis, which ultimately overloads the ER and leads to β cell failure (Liu et al., 2010). To combat protein misfolding, cells have evolved a network of chaperones that preserves protein homeostasis. In the ER, the ER-associated degradation (ERAD) pathway targets misfolded proteins for retrotranslocation to the cytosol and proteasomal degradation (Guerriero and Brodsky, 2012). While ERAD can process soluble *Akita*, large insoluble *Akita* accumulations are resistant to ERAD. In this issue of *Molecular Cell*, Cunningham et al. (2019) identify two key ER quality control mechanisms that regulate the accumulation and clearance of proinsulin aggregates. Furthermore, they establish that these same pathways function to broadly clear mutant prohormone aggregates composed of other proteins.

The ER-resident chaperone Grp170 is an atypical Hsp70 ATPase that targets *Akita* for ERAD (Cunningham et al., 2017). However, its mechanism has remained elusive and it is unclear whether Grp170 prevents *Akita* aggregation, or alternatively, disaggregates *Akita* accumulations to generate smaller species for ERAD. Cunningham and colleagues determine that Grp170 interacts with soluble, high molecular weight *Akita* complexes (Figure 1B) while BiP, the canonical Hsp70 ER-resident chaperone, interacts with mid molecular weight species. Because *Akita* has been shown to recruit WT proinsulin into aggregates, the key next question was if Grp170 could prevent this recruitment. Indeed, via experiments in cell extracts, in HEK293T cells, and in pancreatic β cells, Grp170 was found to prevent recruitment of WT proinsulin by *Akita* (Cunningham et al., 2019). Cunningham et al. propose that high molecular weight species are dynamic complexes that can extend and shrink via crosslinking or reduction of disulfide bonds, respectively, and that Grp170 might block growth of the complex. While this is a plausible mechanism, it will be important to acquire more insights into the structure and dynamics of high molecular weight proinsulin complexes, as well as their interaction with Grp170. Another ER-resident chaperone, GRP94 of the Hsp90 family, was recently implicated in proinsulin handling. Here, inhibition of GRP94 function led to decreased proinsulin levels, decreased insulin secretion, and a shorter proinsulin half-life (Ghiasi et al., 2019). Whether GRP94 and Grp170 have redundant or complementary roles in proinsulin regulation remains an important question.

To better understand how insoluble *Akita* is disposed, Cunningham et al. compared the effects of impairing the ERAD pathway and impairing the recently identified ER-phagy pathway by knocking down Hrd1 and Beclin1, respectively. Knockdown of Beclin1, but not Hrd1, prevented *Akita* degradation, suggesting that ER-phagy is crucial for clearance of large complexes of insoluble *Akita*. Further, they found that reticulon3 (RTN3) is the specific ER membrane protein that mediates ER-phagy of *Akita* (Figure 1C) (Cunningham et al., 2019). RTN3 has been linked to decreased β-secretase activity, leading to decreased amyloid deposition in Alzheimer's disease models (Shi et al., 2014). Overexpression of RTN3 partially

restores the secretion of WT proinsulin in *Akita* models, presenting a potential therapeutic target for MIDY (Cunningham et al., 2019). Further, RTN3 knockdown prevented clearance of detergent-insoluble aggregates composed of mutant pro-opiomelanocortin and pro-arginine-vasopressin, suggesting that RTN3-mediated ER-phagy has a broad role in the clearance of mutant prohormones (Cunningham et al., 2019). This pathway appears analogous to that which clears NPC1 and procollagen via FAM134B-dependent ER-phagy (Forrester et al., 2019; Omari et al., 2018; Schultz et al., 2018). These new findings regarding ER quality control may have broad implications on our understanding of diverse protein-misfolding disorders. Elucidating the structural features of RTN3 and other receptors that promote ER-phagy is an important next step.

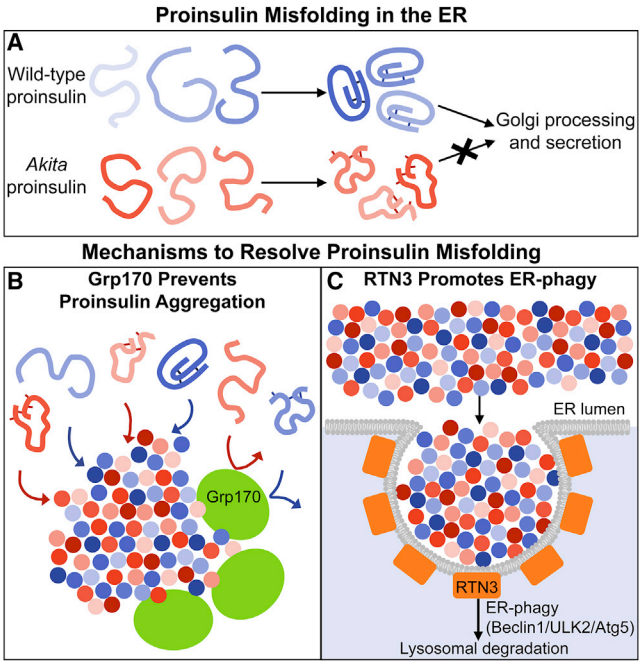

Figure 1. Mechanisms for Countering Proinsulin Misfolding in the ER

(A) The *Akita* proinsulin mutant harbors a cysteine to tyrosine mutation that results in loss of an intramolecular disulfide bond and misfolding. *Akita* dominantly interferes with the folding WT proinsulin, decreasing insulin secretion.

(B) Grp170 is deployed to prevent proinsulin aggregation and promote its degradation via ERAD.

(C) Large insoluble proinsulin aggregates are cleared from the ER via RTN3-mediated ER-phagy.

Cunningham et al. advance our mechanistic understanding of Grp170 regulation of *Akita* proinsulin and establish RTN3 as an ER-phagy receptor. Grp170 interacts with high molecular weight *Akita* to prevent further multimerization and aggregation. Complementary to Grp170, RTN3 mediates degradation of aggregated insoluble proinsulin via the ER-phagy pathway (Cunningham et al., 2019). Increased clearance of aggregates restores secretion of mature insulin, alleviating disease pathology. This two-pronged model provides new therapeutic targets for diabetes and suggests that these pathways may be broadly functional in countering misfolded prohormones. To further pursue these therapeutic avenues, it is important to confirm these findings in *Akita* rodent models. Moving forward, it will be imperative to comprehensively understand the structural basis for Grp170 and RTN3 activity. Determining whether these pathways process diverse proteins or are primarily employed in prohormone quality control will also be important. Additionally, it will be interesting to mechanistically compare the ER and cytoplasmic protein quality control systems. Finally, it will be important to better understand the delicate balance of ER protein quality control and precisely how this system is insufficient or becomes overwhelmed in MIDY patients.

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