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Commentary

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Mice deficient for ERAD machinery component Sel1L develop central diabetes insipidus

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Deficiency of the antidiuretic hormone arginine vasopressin (AVP) underlies diabetes insipidus, which is characterized by the excretion of abnormally large volumes of dilute urine and persistent thirst. In this issue of the *JCI*, Shi et al. report that Sel1L-Hrd1 ER-associated degradation (ERAD) is responsible for the clearance of misfolded pro-arginine vasopressin (proAVP) in the ER. Additionally, mice with Sel1L deficiency, either globally or specifically within AVP-expressing neurons, developed central diabetes insipidus. The results of this study demonstrate a role for ERAD in neuroendocrine cells and serve as a clinical example of the effect of misfolded ER proteins retrotranslocated through the membrane into the cytosol, where they are polyubiquitinated, extracted from the ER membrane, and degraded by the proteasome. Moreover, proAVP misfolding in hereditary central diabetes insipidus likely shares common physiopathological mechanisms with proinsulin misfolding in hereditary diabetes mellitus of youth.

In high demand: proAVP is prone to misfolding

Arginine vasopressin (AVP) is an antidiuretic hormone that is synthesized as part of a larger hormone precursor (1). The AVP gene is located on the short arm of chromosome 20 (20p13), and the three exons of AVP encode the four main segments of the prepro-hormone: a 19-amino acid signal peptide that directs the nascent protein to the ER, the AVP nonapeptide moiety, the 93-amino acid neurophysin II (NP) moiety, and a C-terminal 39-amino acid glycopeptide called copeptin. Upon cotranslational translocation into the ER lumen, the signal sequence is cleaved, and the prohormone undergoes N-linked glycosylation.

Folding of NP (Figure 1A) is challenging, because the protein consists of two β -sheet domains, with amino acid residues

11–37 and 59–82 forming two 4-stranded antiparallel β -sheets connected by a three-turn helix (residues 39–49) and a nine-residue (residues 50–58) loop that defines the back of the AVP-binding pocket (2). There are eight disulphide bridges, and the NP-AVP dimer is formed by the interaction of two β -sheet domains (Figure 1A). After folding and formation of the eight disulfide bonds, the prohormone exits the ER, passes through the Golgi apparatus, and is sorted into neurosecretory granules, where processing by prohormone convertases separates the AVP, NP, and copeptin moieties. The secretory granules reach nerve endings where AVP, NP, and copeptin are secreted into the circulation by regulated exocytosis. Thirst and AVP release are regulated not only by classical homeostatic, interosensory feedback (plasma osmolality, volume, and pres-

sure), but also by novel exterosensory, anticipatory signals (3).

AVP-producing cells are highly secretory, as AVP in the circulation is synthesized by only a few (approximately 10,000 in the rat and 100,000 in humans) large magnocellular neurons, whose cell bodies are located in the supraoptic and paraventricular nuclei of the hypothalamus. Each magnocellular neuron has one axon that projects to the posterior pituitary, where it gives rise to 10,000 neurosecretory endings that are packed with large dense-core vesicles (LDCVs) containing the products of AVP, AVP, NP, and copeptin. The rat hypothalamus contains 100 ng AVP, or 6×10^{13} molecules (AVP has a relative molecular mass of approximately 1,000 g/mol). As a single LDCV contains 1×10^4 molecules of AVP, the hypothalamic AVP content is equivalent to 6×10^9 LDCVs (4). The high level of proAVP generated in AVP-producing cells needs to be correctly folded, and any misfolded WT and/or mutant proAVP needs to be destroyed.

AVP, ERAD, and autosomal dominant central diabetes insipidus

Autosomal dominant central diabetes insipidus is secondary to AVP mutations that result in misfolding and aggregation of the protein in the ER (1). ER-associated degradation (ERAD) is a principal quality control mechanism in cells responsible for targeting misfolded ER proteins for cytosolic degradation (5). More than 70 human diseases, including hereditary central and nephrogenic diabetes insipidus, have been linked to ERAD dysfunction (6). In mammals, the most well-characterized ERAD machinery is the highly conserved complex of suppressor-enhancer of lin-12-like and hydroxymethylglutaryl-CoA reductase degradation protein 1 (SEL1L-HRD1), which consists of the E3 ubiquitin ligase HRD1 and its adaptor protein SEL1Lc (Figure 1B). In this issue Shi et al.

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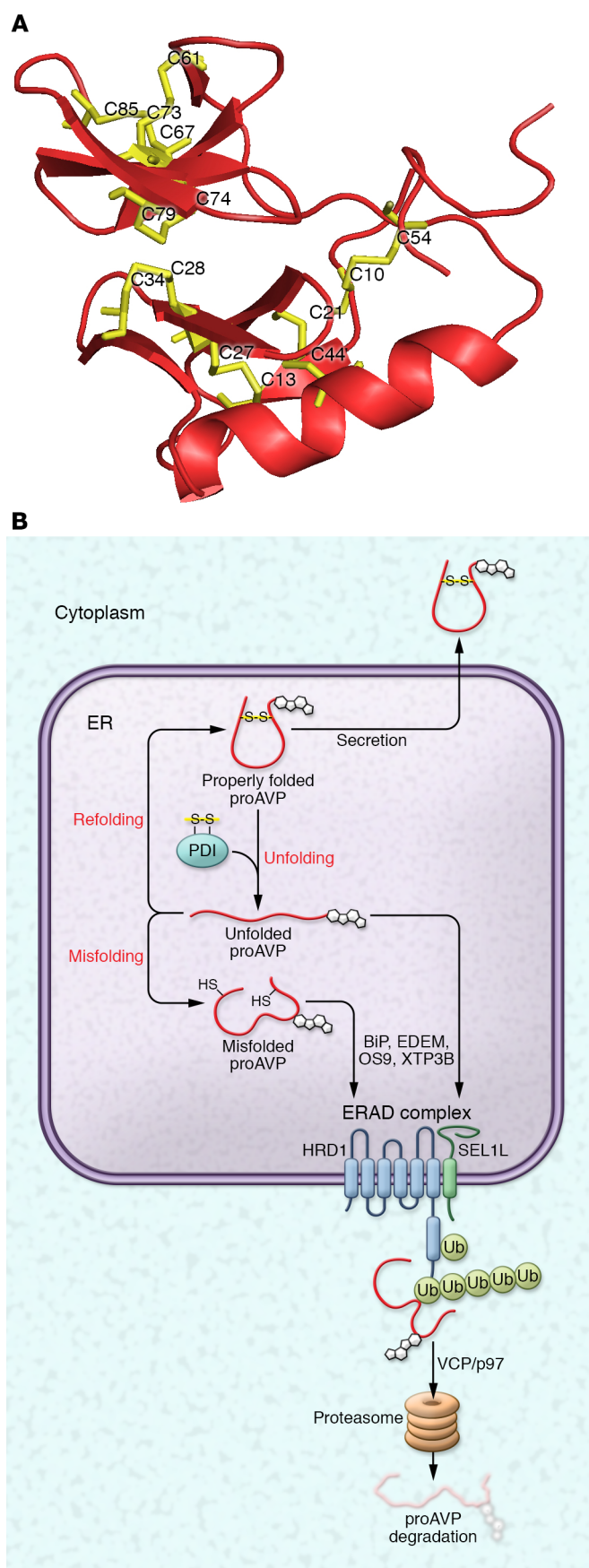


Figure 1. Misfolded and mutant proAVP are Sel1L substrates that are targeted for ER-association degradation. (A) Representation of a monomeric structure of proAVP with eight disulfide bridges. **(B)** The conformational maturation of the AVP precursor proAVP within the ER requires ERAD activity of the SEL1L-HRD1 protein complex. Misfolded proAVP is recruited to the ERAD complex via the activity of various ER chaperones such as binding Ig protein (BiP), ER degradation-enhancing α -mannosidase-like protein (EDE), osteosarcoma amplified 9 (OS9), and XTP3-transactivated gene B protein (XTP3B) for cytosolic degradation. SEL1L-HRD1 is part of an E3 ligase-coupled dislocation complex that integrates the coupled processes of substrate ubiquitination (Ub), membrane extraction via VCP/p97, and proteolytic destruction by the 26S proteasome (5, 13).

(7) report that mice with induced Sel1L deficiency, either globally or only within AVP-expressing neurons, develop central diabetes insipidus, with no magnocellular neuronal death or loss, as has been previously observed (8). These mice are polyuro-polydipsic, with low circulating AVP levels and increased urine osmolality after desmopressin (dDAVP) administration. The Sel1L-deficient experimental model of central diabetes insipidus is unlikely to be observed in humans, as Sel1L loss is embryonically lethal (9). Three-week-old mice with Sel1L deficiency specifically in AVP neurons (*Sel1L^{AVP}* mice) lacked AVP-positive transport vesicles in axons, a phenotype that explains the development of the central diabetes insipidus phenotype in these mice.

Using the CRISPR/Cas9 system, Shi et al. (7) generated SEL1L- and HRD1-deficient human HEK293T cells and murine neuroblastoma Neuro2A (N2a) cells. N2a cells have been regularly used to express AVP mutants responsible for hereditary autosomal dominant central diabetes insipidus in humans (10). Both WT and some human proAVP mutants were shown to be substrates of Sel1L-Hrd1 ERAD. In Sel1L-null N2a cells, both WT and mutant proAVP appeared to accumulate in amyloid-like fibrillar structures, similar to what has been previously observed for human AVP mutants (10). Together, these results demonstrate that Sel1L-Hrd1 ERAD helps to limit ER retention and aggregation of WT proAVP.

Shi et al. (7) further characterized WT proAVP and a human proAVP mutant that

underlies autosomal dominant central diabetes insipidus (proAVP-G57S). In WT cells, WT proAVP predominantly formed monomers, while proAVP-G57S exclusively formed high-molecular-weight aggregates. When expressed together, the presence of proAVP-G57S resulted in the recruitment of WT proAVP into high-molecular-weight complexes. These data are consistent with an autosomal dominant effect of proAVP-G57S, in that expression of the normal allele in AVP-producing cells will be hampered by the formation of high-molecular-weight complexes destined for ubiquitination. This model helps explain previously characterized diabetes insipidus mouse models carrying human proAVP mutations, in which AVP neuronal cell death was not observed at the time of disease onset and thus not thought to play a role in disease initiation.

Protein disulfide isomerase (PDI) is an enzyme in the ER that catalyzes the oxidation (formation), reduction (break down), and isomerization (rearrangement) of disulfide bonds between cysteine residues within proteins as they fold (Figure 1B). This allows proteins like proAVP with eight cysteine bridges per monomer to quickly find the correct arrangement of disulfide bonds in a fully folded state. Oxidative protein folding is prone to error, and incorrectly folded proteins arise as part of normal physiology. Hence, non-native disulfide bonds need to be corrected via isomerization or reduced to produce the native conformation. Shi et al. (7) used PDI-C56A, a so-called “trap mutant” that favors persistent mixed disulfide-crosslinking with substrates. In ERAD-deficient cells, the PDI trap mutant sequestered proAVP predominantly in lower-molecular-weight binary complexes. Also, siRNA knockdown of endogenous PDI attenuated the formation of disulfide bond-mediated high-molecular-weight complexes and aggregates in both Sel1L- and Hrd1-deficient cells.

Misfolding in other forms of autosomal dominant diabetes

The diabetic syndrome mutant insulin (*INS*) gene-induced diabetes of youth (MIDY) is characterized by decreased insulin secretion in patients heterozygous for MIDY-associated *INS* mutations (11). Expression of mutant MIDY proinsulin prevents WT proinsulin from exiting the ER, which is essential for insulin production in pancreatic β cells. Retention of WT insulin decreases overall insulin production and is toxic, thereby decreasing β cell numbers, which suggests that blockade of WT proinsulin by mutant proinsulin in MIDY is a triggering event in the onset of insulin deficiency (12).

Conclusions

The data from Shi et al. (7) provide a pathophysiological understanding of autosomal dominant central diabetes insipidus. Specifically, this study shows that WT proAVP aggregates with mutant proAVP to form complexes that are retrotranslocated and destroyed (Figure 1B). Moreover, WT proAVP and mutant proAVP were shown to be substrates for Sel1L, which links these proAVPs to Hrd1 and ERAD-mediated destruction and eliminates expression of the normal allele. Additionally, PDI, which is so important for the formation of disulfide bridge formation within proAVP, promotes the formation of proAVP aggregates. Similar mechanisms have also been described in autosomal dominant insulin deficiency. Individual variations in the many different components of this WT proAVP aggregation and destruction with mutant proAVP may explain the variability observed in the onset of signs and symptoms in humans with autosomal dominant central diabetes insipidus. Finally, this ER stress response described by Shi et al. (7) might be a general mechanism, as a similar response has been observed in other highly secretory cells such as plasma B lymphocytes, salivary glands, and pancreatic β cells (5).

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