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Lessons from animal models of endocrine disorders caused by defects of protein folding in the secretory pathway

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Abstract

Most peptide hormones originate from secretory protein precursors synthesized within the endoplasmic reticulum (ER). In this specialized organelle, the newly-made prohormones must fold to their native state. Completion of prohormone folding usually occurs prior to migration through the secretory pathway, as unfolded/misfolded prohormones are retained by mechanisms collectively known as ER quality control. Not only do most monomeric prohormones fold properly, but many also dimerize or oligomerize within the ER. If oligomerization occurs before completion of monomer folding then when a poorly folded peptide prohormone is retained by quality control mechanisms, it may confer ER retention upon its oligomerization partners. Conversely, oligomerization between well-folded and improperly folded partners might help to override ER quality control, resulting in rescue of misfolded forms. Both scenarios appear to be possible in different animal models of endocrine disorders caused by genetic defects of protein folding in the secretory pathway. In this paper, we briefly review three such conditions, including familial neurohypophyseal diabetes insipidus, insulin-deficient diabetes mellitus, and hypothyroidism with defective thyroglobulin.

1. INTRODUCTION

Peptide hormone-secreting endocrine tissues have an especially highly developed protein secretion pathway designed for a high level of secretory protein synthesis, trafficking, processing, and storage of peptide hormones within secretory granules. These cells are among those considered as “professional secretory cells” (1), which have an extensive network of membrane-bound secretory organelles that carry their proteinaceous cargo progressively through increasingly mature stages of processing, packaging, and concentration. Throughout these stages, secretory proteins remain within the lumen of the membrane-bound organelles, and thus they communicate only indirectly with the packaging,

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processing, and signaling activities occurring on the cytosol side of the membrane, through proteins (and lipids) residing in the bilayer and on its cytosolic surface. The secretory cargo proteins themselves thus live two lives - one that takes place within the cell from which they are synthesized and secreted, and a second when they are finally allowed to explore the extracellular environment for which much of their biological function is encoded (2).

In the first life, within the secretory cell, the newly-made peptide prohormones and other secretory proteins must find a way to develop their native biological structure. While this is happening, there is strong reason to believe that the process of advancing through the series of intracellular compartments comprising the secretory pathway, is determined by “go / no-go” signals encoded within the structure of the young secretory proteins (3). Such proteins may need to expose anterograde transport signals that could lead to capture by a forward-going transport receptor, or they may expose structural information that triggers their retention within an individual compartment, preventing further forward transport.

The first hurdle for most newly-made secretory proteins occurs within the endoplasmic reticulum (ER). There, the majority of secretory preprohormones are injected across the ER membrane upon ribosome docking by virtue of the presence of a signal peptide (the “pre” piece) and its interaction with the signal recognition particle (SRP), followed by anchoring to the ER membrane via the SRP receptor, with guidance to the Sec61 translocon (4). This process usually begins when secretory preprohormone mRNAs have only begun to be translated; the bulk of the translocation of the growing polypeptide across the ER membrane tends to occur co-translationally (5). As it is being synthesized, the nascent secretory polypeptide chain finds itself in an entirely different environment from that of the cytosol, and it is in this new environment that preprohormones must first fold to their native conformation (6).

Many things may potentially go wrong during these early stages of peptide hormone biosynthesis. The preprohormone might not be properly or fully delivered across the ER membrane. The signal peptide (which is designed like a booster rocket, intended to be cleaved and jettisoned even as the first 80 amino acids of translation product are being injected across the ER membrane) – may not separate in a proper, timely way (7). Even if these events go well, the nascent prohormone may not find its way to the native state. The ER is a compartment filled with chaperones and processing enzymes (8). Many secretory proteins are designed to acquire N-linked glycosylation, which functions critically in ER protein folding. Additionally, the ER is a far more oxidizing milieu than the cytosol, promoting conversion of cysteine thiols into protein disulfide bonds. When those disulfide bonds are part of the native state of the mature peptide hormone then they are a stabilizing force, but they can wreak havoc if they result in intramolecular or intermolecular disulfide mispairing. There are numerous additional secretory protein structural modifications that can occur in the ER, including glucose and mannose trimming of N-linked carbohydrate side chains, proline isomerization (and hydroxylation), gamma carboxylation (of Glu residues), and many others (9).

Additionally, most peptide prohormones homo- or hetero-dimerize or oligomerize in the ER, with additional higher order assembly destined to occur in the Golgi complex or in secretory

granules (10). Within granules, mature peptide hormones may be stored at concentrations that exceed 100 mg/mL – the kinds of levels that cannot be achieved in a test tube. It is likely that many peptide hormones and prohormones have been evolutionarily selected for self-association to very high concentration (in addition to their ultimate biological function) and with this in mind, it is not surprising that misfolded prohormones may retain features that both permit and promote the formation of aggregates, fibrils, and non-native polymers.

The physiological consequences of these various kinds of prohormone misfolding are as various as the biological activities of the peptide hormones themselves (11). In so-called “conformational diseases”, at a very minimum, a loss-of-function can be expected either from failure to secrete a peptide hormone, or from failure of a non-native secreted peptide hormone to fulfill its intended biological function. Beyond this, misfolded, unsecreted prohormones may accumulate in the cells from which they are synthesized, often clogging the ER and triggering a behavior known as the ER stress response (12). From there, the misfolded, unsecreted peptide prohormones may be cleared internally by protein degradative pathways such as ER-associated degradation (ERAD), or ER autophagy (ER-phagy), or other ER-to-lysosome (ERLAD) routes (13). Alternatively, the misfolded, unsecreted prohormones may accumulate at toxic levels, culminating in devastating biological consequences within the host cell, including de-differentiation, or cell death (14). In this review, we cite a few well-known examples of animal models of endocrine disorders caused by defects of protein folding in the secretory pathway, with defects that include loss-of-function, and gain-of-proteotoxic function, each leading to endocrine disease.

2.1. Familial Neurohypophyseal Diabetes Insipidus

Arginine vasopressin (AVP) is an antidiuretic hormone synthesized in magnocellular neurons of the supraoptic (SON) and paraventricular nuclei (PVN) of the hypothalamus, and delivered via axonal transport for storage in the posterior pituitary gland (15). The primary site of action of AVP is the collecting ducts of the kidney, where AVP promotes water reabsorption into the circulation. The subfornical organ (SFO) and the organum vasculosum laminae terminalis (OVLT) are osmosensors — when plasma osmolality rises, these hypothalamic nuclei send neural signals (via the nucleus medianus) to the SON and PVN, resulting in AVP secretion as well as AVP biosynthesis (16). This, along with an intact thirst mechanism, are essential to maintain water balance in the body.

Central diabetes insipidus (central DI) is caused by a deficiency of AVP secretion, and the affected patients excrete a large volume of hypotonic urine due to the insufficient water reabsorption in the renal collecting ducts. When AVP secretion is completely lost, urine excretion rates may reach up to 20 mL/min with a concentration as low as 50 mOsm/L (17). For long term management, desmopressin supplementation lowers urine volume with improved urine concentration (18).

The AVP gene encodes the signal peptide, AVP, neurophysin II (NPII) and glycopeptide (19). The prepro-AVP-NPII is converted to pro-AVP-NPII by excision of the signal peptide in the ER (20). In AVP neurons, in parallel with AVP mRNA up-regulation, the BiP mRNA (which encodes the major hsp70 family member of the ER and is highly expressed even under normal conditions) is further elevated under conditions of water-deprivation (21).

Independent of pharmacologic causes, familial neurohypophyseal diabetes insipidus (FNDI) is an autosomal dominant central DI caused by AVP gene mutations — most of which reside in the NPII region (22,23). A limited number of autopsy reports have shown that AVP-immunostained cells were reduced in the hypothalamus of patients with FNDI (24–26). From this it has been surmised that FNDI may lead to cell death of hypothalamic neurons that synthesize the protein (Figure 1). Indeed, a mouse model of FNDI was created with a C98X heterozygous nonsense mutation at the NPII locus, which is known to cause FNDI in humans (27). In these mice, accompanying the progressive polyuria was the finding that BiP protein levels were highly elevated in the AVP neurons (28). The mutant was postulated to have a high toxicity on neuronal cells in culture as demonstrated by the observation that the number of NPII immunostained cells progressively decreased over time (29).

However, another group independently created FNDI model mice with the same mutation and found that AVP neurons survived until late stages of DI. Using *in situ* hybridization and ultrastructural analysis in AVP producing cells, protein aggregates were observed in limited regions of the ER, which were called “ER Associated Component (ERAC)” (30,31). Aggregate formation was ameliorated by treating the mice with exogenous desmopressin, which decreases endogenous AVP expression (32). Similarly, administration of 4-phenylbutylate (4-PBA), considered to be a “chemical chaperone”, also reduced protein aggregation in the ER of AVP neurons in FNDI mice and helped to restore endogenous AVP release, ameliorating the DI phenotype (33). On the other hand, a high salt (2.0 %) diet accelerated formation of AVP protein aggregates as well as central DI (32). Interestingly, under intermittent water deprivation, protein aggregates were scattered all over the ER (34,35), accompanied by autophagy activation and autophagic cell death (Figure 1). However, in yet another related model, transgenic rats expressing the mutant AVP-C98X in their hypothalamic neurons showed an expanded ER with trapping of wild-type and mutant AVP that was targeted for lysosomal degradation by activated autophagy, but the rat model did not exhibit cell death or atrophy (36). Therefore, there remains an element of uncertainty whether the diminished hypothalamic AVP immunostaining in FNDI reflects downregulated protein expression (i.e., diminished synthesis ± enhanced degradation) or truly reflects hypothalamic cell death.

Ablation of the ATF6α gene in FNDI mice [by crossing them with *ATF6*$\alpha^{-/-}$ mice (37,38)] resulted in diminished ERAC formation while exacerbating apparent AVP neuronal loss. These results seem to suggest that ERAC formation may serve a protective function, perhaps by isolating the aggregated protein within a subcompartment of the ER, resulting in decreased ER stress and its adverse downstream consequences.

ER-associated degradation (ERAD) is one of the main mechanisms of clearance of misfolded ER proteins, which involves degradation via cytosolic proteasomes (39). Indeed, both wild-type and mutant AVP-G57S were found to be substrates of Sel1L-Hrd1 ERAD (40,41). The core ERAD components include the E3 ubiquitin ligase HRD1 and its adaptor protein SEL1L, which are regulated by the ATF6 pathway (42). Recently, both whole-body and AVP neuron-specific SEL1L knock-out mice were found to exhibit a central DI phenotype. These results seem generally consistent with findings noted above in ATF6α deficient FNDI (C98X) mice. Interestingly, poly(A) tail length, a possible regulator of

mRNA stability and translational efficacy (43–46), was shortened for the AVP mRNA in FNDI mice, and this occurred concomitant with a decreased AVP mRNA level. This decrease in mRNA level did not appear to occur as a result of nonsense-mediated mRNA decay specific to the C98X mutation, because the wild-type AVP mRNA level was also decreased (31,47). In contrast, longer poly(A) tail lengths of certain mRNAs, including known UPR genes (e.g., XBP1, CHOP and BiP) has been described under ER stress conditions (48).

2.2. Insulin-Deficient Diabetes Mellitus

Diabetes mellitus is characterized by abnormally high blood glucose level accompanied by absolute or relative insulin deficiency. A limited physiological ER stress response in pancreatic ß-cells that is concomitant with normal insulin production may be positively adaptive and may even help to support ß-cell proliferation (49), but overall, the intracellular and physiological feedback loops resulting from *less* insulin biosynthesis with *less* ER stress response may actually be even more beneficial as a stimulus to ß-cell proliferation and survival (50). Moreover, when highly increased ER stress in ß-cells is imposed by various pathological situations [such as mutant proinsulin production, ER dysfunction, or insulin resistance], these can often culminate in a profound loss of functional pancreatic ß-cell mass (51).

One of the most famous animal models of a secretory protein folding defect causing diabetes is the *Akita* mouse, which exhibits heterozygous expression of a missense mutant proinsulin-C96Y from one *Ins2* allele leading to a defect in proinsulin disulfide bond formation (52). These animals actually express two wild-type copies of the *Ins1* gene as well as one wild-type Ins2 allele; nevertheless, the animals develop insulin-deficient diabetes with a high degree of penetrance (53). In the pancreatic ß-cells of *Akita* mice, the misfolded mutant proinsulin is retained within the ER (54,55). The two interchain disulfide bonds of proinsulin, Cys(B19)-Cys(A20) and Cys(B7)-Cys(A7) are known to be required for proinsulin export from the ER (56). Thus, ER retention of the product of the *Akita* mutant proinsulin allele, which can never form the Cys(B7)-Cys(A7) disulfide bond, is expected. However, what is more remarkable is that the products of the remaining three wild-type *Ins* alleles are also defective for ER export in *Akita* islets (57). One possibility is that the misfolded mutant proinsulin could cause generalized ER dysfunction, blocking all other secretory protein traffic (58). At least in the early life of the animal, this does not seem to be the case, but rather, the misfolded mutant proinsulin directly engages innocent wild-type proinsulin “bystander” molecules in inappropriate intermolecular disulfide bonds (59), blocking the ER export of the wild-type proinsulin (60).

The problem in *Akita* mice has also been found in humans, with the syndrome termed Mutant Ins-gene induced Diabetes of Youth, or MIDY (59). Roughly 30 different human MIDY mutations have been reported, including an identical substitution to that found in the *Akita* mouse (61). The dominant-negative effect of the misfolded proinsulin on the trafficking of wild-type proinsulin is directly related to the relative expression levels of the mutant and wild-type molecules (62,63). These aberrant interactions are initiated within the ER compartment (64). Wild-type bystander proinsulin that does escape the ER and reaches

the Golgi complex can still be processed to insulin and packaged in secretory granules (65,66); however, there are relatively few “successful” molecules, and there is insufficient insulin to maintain normoglycemia (67).

The *Akita* mutant proinsulin protein is predisposed to form disulfide-linked dimers, trimers, tetramers, and higher order complexes (58). Some of the misfolded *Akita* mutant proinsulin appears to be dissociated from higher order complexes with the help of the ER oxidoreductase known as PDI (68) and the atypical hsp70/helper protein known as GRP170 (69). Failure of these proteins to prevent aggregation of *Akita* mutant proinsulin can result in the misfolded forms accruing to enormous size; in such case, large misfolded proinsulin aggregates may be routed to lysosomal degradation by ER-phagy (70).

Misfolded *Akita* proinsulin accumulation in the ER provokes ER stress (59) with activation of ATF6α and Ire1α-XBP1 pathways (71). CHOP expression is also elevated in the islets of *Akita* mice, and when crossed with mice null for the CHOP portion of the integrated stress response pathway, *Akita* mice exhibited less pancreatic ß-cell death, [although the animals still proceeded to insulin-deficient diabetes (72)]. Additionally, genetic ablation of the transcription factor C/EBP in ß-cells of *Akita* mice resulted in improved glycemic control with an increase of ß-cell mass (73).

Independent of genetic mutations in the *INS* gene, a loss of function in one or more branches of the tripartite ER stress response can itself cause diabetes mellitus (74). One such branch of the ER stress response involves Perk, which is one of four kinases (and the only ER-localized kinase) that phosphorylates eIF2α to suppress general protein translation (75). Mice with global Perk-KO display early-onset diabetes mellitus (at 4 weeks of age). The exocrine and endocrine pancreas develop normally before birth and in very early postnatal life, and glucose-induced proinsulin biosynthesis is actually robust in the islets of *Perk*$^{-/-}$ mice. However, ER expansion rapidly ensues in both the acinar and islet cells of *Perk*$^{-/-}$ mice, progressing to both exocrine pancreatic insufficiency and insulin-deficient diabetes mellitus (76), along with significant skeletal disorders (77). Perk activity in these tissues seems to reflect a cell-autonomous requirement both for development and adult function (78–83). Similarly, mice with ß-cell-specific expression of a mutation at the eIF2α phosphorylation site (Ser51Ala) were also predisposed to insulin deficiency and diabetes, especially under conditions of high metabolic demand (84). In the case of both ß-cell Perk insufficiency or insufficiency of ß-cell eIF2α phosphorylation, there is strong evidence that these conditions lead, directly or indirectly, to increased proinsulin misfolding (81,84–86).

A second branch of the ER stress response involve ATF6α. Global *Atf6*α$^{-/-}$ mice exhibit essentially normal glucose tolerance on a normal chow diet. However, on a high fat diet, ATF6α-deficient mice exhibit less insulin secretion and a swollen ER in ß-cells, which accompanies higher insulin resistance with hepatic steatosis and ER stress response. Not surprisingly, when *Atf6*α$^{-/-}$ were crossed with *Akita* mice, the double-mutant progeny showed accelerated loss of pancreatic insulin (87).

The third and most evolutionarily conserved branch of the ER stress response is mediated by Ire1α. Mice with tamoxifen-inducible ß-cell-specific deletion of Ire1α develop

hyperglycemia and hypoinsulinemia that is especially apparent upon feeding, or acute glucose stimulation (88). In this mouse model, insulin mRNA translation was reduced primarily because of defective induction of genes related to proinsulin biosynthesis, including SRP, SRP receptor, the ER translocon, signal peptidase complex, as well as many other genes associated with the function and development of the secretory pathway. These findings are supported by the work of others who reported that ß-cell-specific deletion of Ire1α impairs insulin biosynthesis leading to hyperglycemia (89). Interestingly, such mice also exhibit decreased proinsulin folding that could be attributed to lower expression of several ER oxidoreductases, including PDI, PDIR, P5, ERp44, and ERp46 (90). Ire1α works primarily through the unconventional splicing of the mRNA encoding the XBP1 transcription factor (91,92). ß-cell-specific XBPI-deficient mice exhibit modest hyperglycemia due to the decreased number of insulin granules, impaired proinsulin processing with an increase in serum proinsulin : insulin ratio, blunted glucose-stimulated insulin secretion, and inhibited ß-cell proliferation. These phenotypes might possibly also be related to defects of proinsulin folding in the secretory pathway but more likely, these ß-cells may suffer from diminished mRNA expression of many ß-cell differentiation genes as a consequence of constitutive Ire1α hyperactivation that can result in more widespread mRNA degradation by Ire1α endonuclease activity (93).

Finally, defective proinsulin folding also occurs (to a lesser degree) in ß-cells in the absence of any MIDY or known genetic defect of ß-cell ER stress response (Figure 2). Interestingly, in *db/db* mice, aberrant disulfide-linked proinsulin complexes – not unlike to those seen in *Akita* mouse islets – have been observed as one of the earliest molecular defects during the prediabetic progression to type 2 diabetes (86). The *db/db* animals have a global leptin receptor defect, which is also expressed in pancreatic ß-cells, but a similar mouse model bearing leptin receptor deficiency limited to the hypothalamus but not the pancreatic islets, still yields early onset proinsulin misfolding with aberrant disulfide-linked complexes (86). These recent findings support the notion that pancreatic ß-cell stress and dysfunction during the development of type 2 diabetes may represent an endocrine disorder promoted by defective folding (of proinsulin) in the secretory pathway (Figure 2).

2.3. Congenital Hypothyroidism with Deficient Thyroglobulin Transport

Thyroglobulin (Tg), the thyroid hormone precursor encoded by the *TG* gene, is the most highly expressed gene product in the thyroid gland (94). The Tg protein acquires N-linked glycosylation, conformational maturation, and homodimerization in the ER. The primary functions of Tg in all vertebrates are iodide storage and thyroid hormonogenesis (95). The N-terminal two-thirds of the protein contains three regions known collectively as Tg region I-II-III (96) that include multiple cysteine-rich repeat motifs. The carboxyl-terminal region of Tg has homology with acetylcholinesterase (AChE) and is known as the Cholinesterase-Like (ChEL) domain (97–99).

Mutations of the *TG* gene, especially in homozygotes or compound heterozygotes, can cause congenital hypothyroidism (95,100). Curiously, however, two rodent models of congenital hypothyroidism bearing homozygous Tg missense mutations in the ChEL domain show very similar thyroid ultrastructure, but ultimately lead to interesting and important pathological

differences in thyroid anatomy. The L2263P mutation in the ChEL domain causes congenital goitrous hypothyroidism in homozygous *cog/cog* mice (101). As a result of primary hypothyroidism, the mice are exposed to chronic TSH stimulation leading to the formation of a grossly large goiter [indeed the name *"cog"* stands for congenital goiter (102)]. The *cog/cog* mice have a highly distended thyrocyte ER with massive accumulation of mutant Tg protein within the ER lumen (103). This is accompanied by induction of the ER stress response that dramatically upregulates the protein levels of ER molecular chaperones including GRP94, BiP, ERp72, ERp57, and calreticulin (104). Importantly, despite the defect, as the mice grow to full adulthood, they gradually achieve near-normal serum T_4 levels, which parallels the growth of the large goiter (102).

The G2300R mutation in the ChEL domain of rat Tg (numbering based on the length of the mature protein — just 35 residues from the site of the mutated amino acid encoded by the *cog* mutation) also results in congenital hypothyroidism in *rdw/rdw* rats (105,106) with a highly dilated thyrocyte ER filled with mutant Tg, accompanied by absence of Tg secretion into the thyroid follicle lumen (107). Once again, a marked elevation of ER molecular chaperones GRP94, BiP and hsp70 is observed (108). However, despite highly elevated levels of circulating TSH (as a result of primary hypothyroidism), these rats develop a hypoplastic thyroid gland (109). Thus, the two single missense mutations fall closely within the same domain of Tg, yet the latter mutation has been shown to be associated with thyroid cell death suggesting a toxic gain-of-function (110), which could provide a pathophysiologic mechanism to explain the absence of goiter (Figure 3).

Currently, 167 hypothyroidism-inducing mutations have been identified in the human *TG* gene (111). Although most of the patients coming to medical attention with homozygous or compound heterozygous *TG* mutations develop goiter, patients homozygous for G2300D (i.e., mutating the same residue as that found in *rdw/rdw* rats) also lack goiter development (112). These data suggest that that goitrous/non-goitrous phenotype might be linked to the biochemical or biophysical properties of the protein encoded by the particular mutant allele(s). Two human mutations replacing cysteine by either arginine or serine (C1245R and C1977S) have also been found to be retained in the ER (113). *TG* mutations are reported not only in human and rodents but also in Afrikander cattle (R697X) (114–117), Dutch goats (Y296X) (118–120), and other species. It is highly likely that mutant Tg retention in the ER is a common feature in most if not all forms of congenital hypothyroidism with deficient Tg.

3. DISCUSSION

In this review, we have discussed three representative protein conformational diseases of the endocrine system, including FNDI, insulin-deficient diabetes mellitus, and congenital hypothyroidism with deficient Tg. Here, we wish to highlight some of the interesting potential differences between the various conformational diseases.

There is a single AVP gene in both humans and mice (two alleles; with oxytocin encoded by a separate gene), and heterozygosity is sufficient to bring about the FNDI phenotype (22,23). There is one *INS* gene in humans (two alleles) but two such genes in mice (four alleles), and heterozygosity in the *Ins2* locus alone is sufficient to bring about insulin-deficient diabetes

mellitus in *Akita* mice or *Munich* mice (86). Although the pathogenesis of some autosomal dominant conformational diseases have been hypothetically attributed to haploinsufficiency, this idea can be excluded for either FNDI or MIDY, because other AVP and insulin heterozygous alleles that cause pure loss-of-function, lack any disease phenotype (121,122). This leaves at least two possibilities. In one case, the gene product of the mutant allele could directly associate with product of the wild-type allele, resulting in a protein complex that together is incompetent for exit from the ER, which leads to insufficient successful prohormone to move through the secretory pathway to become mature hormone. In a second case, the product of the mutant allele could form a species that brings about cytotoxicity, with either cell death or de-differentiation of the specialized secretory cells, including diminished expression of the normal allele through mRNA destabilization (123,124)(47) under ER stress — all resulting in hormonal insufficiency in the heterozygous state.

The two possibilities stated above are not mutually exclusive. In MIDY, not only is there direct evidence that misfolded mutant proinsulin directly associates with wild-type proinsulin (57), but there is also evidence that the eventual consequence of this behavior is the loss of functioning pancreatic beta cells, suggesting a state of proteotoxicity (60,125). In fact, the recruitment of wild-type proinsulin into misfolded protein complexes with mutant proinsulin implies that the ER-retained wild-type proinsulin, despite absence of any mutation, itself may be a significant contributor to beta cell proteotoxicity (65). A very similar confluence of circumstances appears to occur in FNDI, with both association between mutant and wild-type forms of pro-AVP (41) as well as an ultimate deficiency of AVP-replete neurons (126). Thus, we believe that a dominant-negative effect of the mutant protein by oligomerization with the wild-type could be a general mechanism in a variety of autosomal dominant conformational diseases (63).

Nevertheless, not all degenerative diseases caused by a mutant misfolded protein are inherited in an autosomal dominant fashion. Autosomal recessive retinitis pigmentosa (a non-endocrine conformational disease) results in progressive loss of photoreceptor cells that may lead to blindness, and several mutant genes linked to this phenotype encode proteins that traverse the secretory pathway [e.g., CRB1 (127)]. Similarly, alpha-1-antirypsin deficiency in patients bearing the Z-allele exhibits recessive inheritance such that most heterozygotes are generally healthy, yet homozygous ZZ patients commonly develop hepatocyte cell death with ultimate progression to cirrhosis (128). Thus, we in the endocrine research community should remain aware of the possibility of discovering non-dominant degenerative endocrinopathies.

The thyroglobulin (Tg) mutations described in this review highlight that a dominant versus recessive phenotype may be very much dependent upon the specific misfolding encoded by the mutant allele. Both the *cog/cog* mouse and the *rdw/rdw* rat have loss of Tg function with dramatic intracellular accumulation of Tg in the ER (129,130). Nevertheless, hypothyroidism in both cases is a recessive trait, as with most other *TG* mutations (95). Amazingly, in the homozygous *cog/cog* mouse, the thyroid gland continues to grow (129) even as evidence of chronic, continuous ER stress persists (131). In many ways, this is more remarkable than the *rdw/rdw* rat thyroid, which develops loss of the specialized secretory cells (132) [with a complete loss of circulating endogenous thyroxine (133)] as would be

predicted from most models of cytotoxic failure from chronic continuous ER stress (134). In heterozygous *rdw/+* rats, cross-dimerization between the wild-type and mutant gene products might lead to rescue of the latter (135)(63). Alternatively, in these and other endocrine proteinopathies, clean-up mechanism(s) for misfolded secretory proteins might be the critical contributor to cell survival (136) despite hormone deficiency from loss of function.

Finally, we must consider that endocrine disorders caused by defects of protein folding in the secretory pathway may yield insights not only into the pathogenesis of rare diseases, but also may provide clues about secretory cell dysfunction in common, polygenic endocrine diseases that lead to inadequate peptide hormone secretion.

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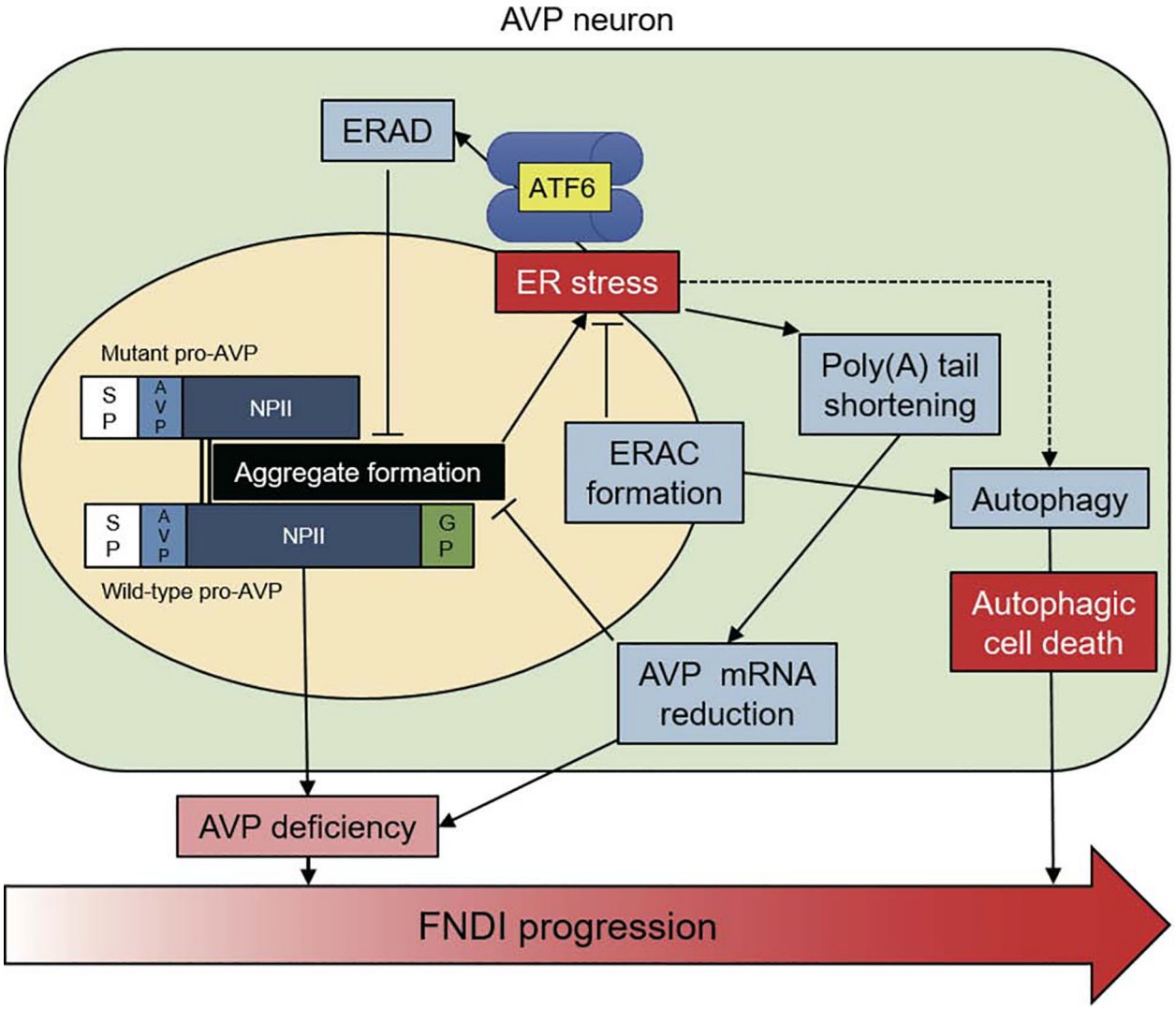

Figure 1. Conceptual diagram of FNDI progression based on misfolding of the AVP precursor protein in the ER.

In the ER, mutated pro-AVP (truncated at the NPII region) causes ER stress by forming aggregates that also encompass the non-mutant (wild-type) precursor (black box). At an early stage of the disease, ERAD, ERAC formation, autophagy, and reduction of AVP mRNA (light blue boxes) each help to prevent loss of functional AVP neurons, but ultimately, FNDI ensues as a consequence of limited AVP secretion (pink box). At the end stage of the disease, there is a net loss of immunostainable AVP neurons, which may make the central DI phenotype irreversible (large arrow at bottom).

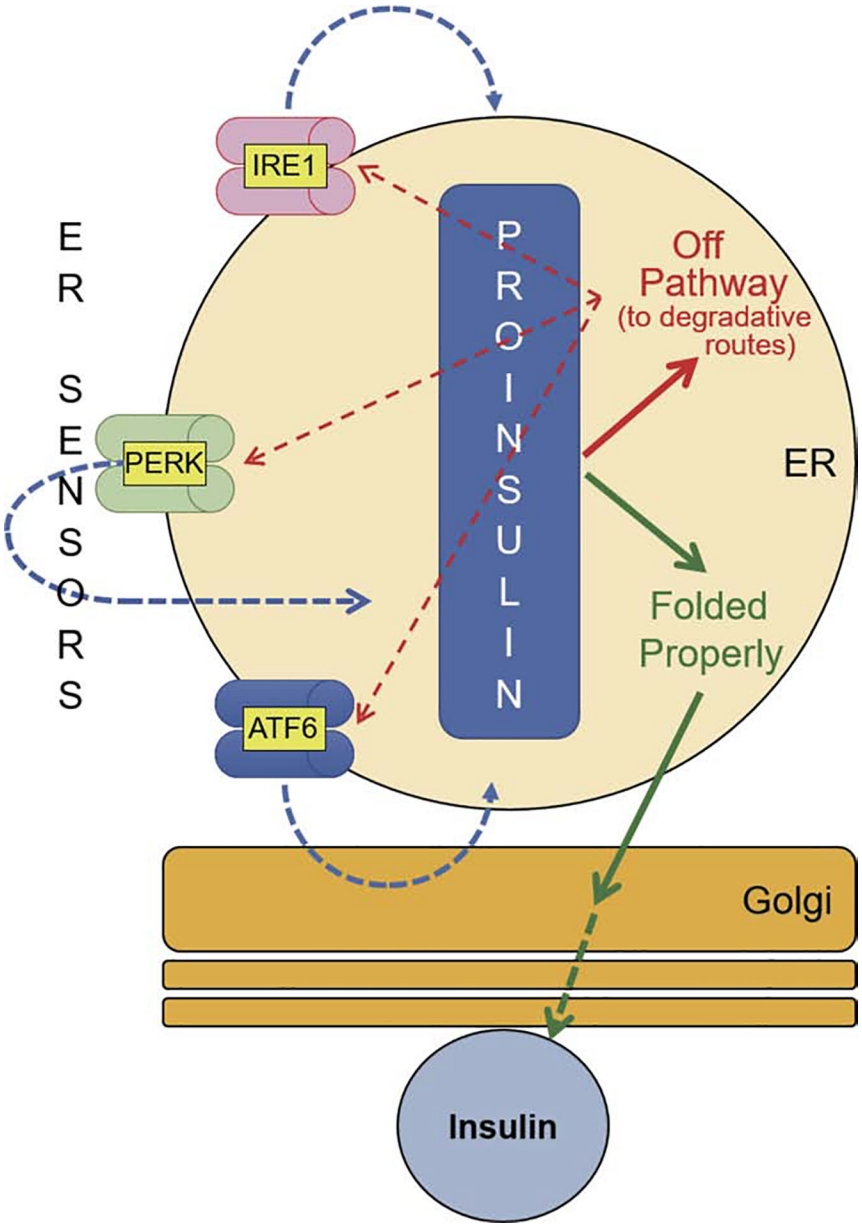

Figure 2. Conceptual diagram of the fate and sensing of proinsulin folding in pancreatic ß-cells.

Newly-synthesized proinsulin may achieve the native state (in green), and when it meets all ER quality control requirements, the native proinsulin is allowed anterograde transport through the Golgi complex to secretory granules, where insulin is made and stored for release in response to glucose challenge. Newly-synthesized proinsulin may misfold into forms bearing non-native intramolecular or intermolecular disulfide bonds. These forms are "off pathway" (in red) and it is currently unknown if they can be returned to proper folding. Additionally, similar to that seen for mutant pro-AVP (see Figure 1), misfolded proinsulin can recruit innocent bystander (wild-type) proinsulin into aberrant protein complexes. The misfolding of proinsulin in pancreatic ß-cells can be detected by ER stress sensor proteins (red dotted lines). The activities of all three ER sensors (IRE1, PERK and ATF6) contribute to a proper ER folding environment for proinsulin (blue dotted lines). When the misfolded

proinsulin accumulates in the ER (as has been observed for MIDY mutants, and in early type 2 diabetes) some of the misfolded molecules are likely to be degraded via ERAD and ER-phagy, but excessive accumulation of misfolded proinsulin is likely to trigger ß-cell failure.

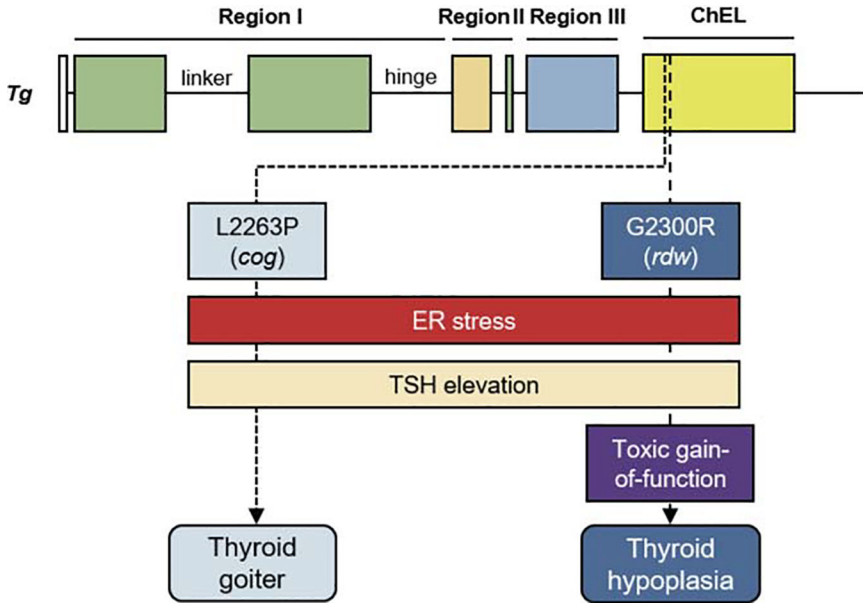

Figure 3. Conceptual diagram of the fate of the thyroid gland in rodents with homozygous expression of one of two distinct misfolded mutant Tg gene products: the *cog/cog* mouse, *or the rdw/rdw* rat.

The Tg-L2263P mutation *(cog* mouse) is only 35 residues away from that of the *rdw* rat (Tg-G2300R). Both point mutations are located in the amino-terminal portion of the ChEL domain (yellow box), and both cause severe thyroidal ER stress (red rectangle) and primary hypothyroidism with TSH elevation (beige rectangle). However, the adult *cog/cog* mice exhibit a significant goiter (sky blue) whereas the adult *rdw/rdw* rats exhibit a hypoplastic thyroid gland (dark blue). Published data to date suggest thyrocyte proliferation in *cog/cog* mice and thyroid cell death in *rdw/rdw* rats, suggesting the possibility that the Tg-G2300R mutant protein may confer a toxic gain-of-function (purple box).