IMPLICATIONS OF ENDOPLASMIC RETICULUM DYSFUNCTION IN NEUROLOGICAL DISEASE

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ABSTRACT

Endoplasmic reticulum (ER) dysfunction is important in the pathogenesis of many neurological diseases. In this review, we examine the evidence for ER dysfunction in a range of neurological conditions including cerebral ischaemia, sleep apnoea, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, the prion diseases and Familial Encephalopathy with Neuroserpin Inclusion Bodies (FENIB). Protein misfolding in the endoplasmic reticulum initiates a well-studied 'Unfolded Protein Response' in energy-starved neurones during stroke that is relevant to the toxicity of reperfusion. The toxic peptide Aβ induces 'ER stress' in Alzheimer's disease leading to activation of similar pathways, while the accumulation of polymeric neuroserpin in the neuronal ER triggers a poorly understood 'ER overload response'. In other neurological disorders such as Parkinson's and Huntington's diseases ER dysfunction is well recognised but the mechanisms for this are less clear. By targeting components of these signalling responses, it may prove possible to ameliorate their toxic effects and treat a range of neurodegenerative conditions.

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Introduction

It can be argued that most neurodegenerative disorders arise from defective protein folding. This is clearly true for those rare disorders caused by the misfolding of mutated neuronal proteins such as Huntington's disease, but it is also the case for common disorders such as cerebral ischaemia where the lack of energy can impair normal protein folding [1] and sporadic Alzheimer's disease where protein aggregation causes cellular stress [2-4]. Even some infectious neurodegenerative disorders such as the prionopathies arise from abnormal protein folding [5-9]. Consequently, the processes for disposing of misfolded proteins are the focus of much research. Although the subcellular location of protein misfolding may differ between these disorders, it appears that the interdependence of protein folding throughout the cell results in ER dysfunction being the final common pathway for many, if not all, of these diseases (Table 1).

ER Dysfunction

The endoplasmic reticulum (ER) is required for folding of all secreted and membrane proteins and insults that impair its function induce a pathological state termed 'ER stress' [10]. This triggers an adaptive programme called the Unfolded Protein Response (UPR), which combines the early inhibition of protein synthesis with a later upregulation of genes that promote protein folding or disposal [10]. Both the translational and transcriptional components of the UPR protect the neurone from becoming overwhelmed by misfolded ER proteins; however, when the insult is too great, apoptotic cell death frequently ensues. While many cells

are relatively protected from accumulating misfolded protein through continued dilution of their ER by cell replication, this process is unavailable to post-mitotic neurones, which depend exclusively on the UPR. Even glial cells, which can replicate, are susceptible to ER stress owing to their highly developed secretory pathway.

It is important that the UPR be distinguished from the less well-understood ER Overload Response (EOR) [11]. The former is triggered by protein misfolding within the ER lumen, while the latter occurs when well-folded or misfolded proteins accumulate and distend the ER. In this review, we will discuss the relevance of these forms of ER dysfunction to neurological disease, since a better understanding of each is likely to identify novel therapeutic strategies (Panel 1). We will focus on ischaemic stroke, Alzheimer's disease and Parkinson's disease to discuss ER stress, while using Familial Encephalopathy with Neuroserpin Inclusion Bodies (FENIB) to illustrate ER overload, since these are the diseases for which the most evidence currently exists. In addition, we will discuss the evidence for ER dysfunction in multiple sclerosis, amyotrophic lateral sclerosis and the prion disorders. Some neurological disorders provoke ER stress signalling despite being caused by the accumulation of protein within the cytoplasm, for example Huntington's disease [12]. Such disorders emphasise the interdependence of protein folding networks in the neurone and so shall be discussed as examples of disordered protein homeostasis or 'proteostasis'.

ER stress signalling

The signalling pathways triggered by ER stress comprise the UPR [10](Figure 1). Although it may seem tiresome to dwell on individual molecular components, this is unavoidable when studying a phenomenon as complex as ER stress. Not least, because many of the measures commonly used to detect and quantify ER stress are themselves components of the UPR. Some of these are activated transiently and so their absence cannot be used as evidence for a lack of ER stress [10, 13]; conversely, certain components can be activated by stresses other than ER protein misfolding and so need to be interpreted with caution. We shall therefore begin with a review of the major mediators of ER stress signalling with an emphasis on their role in neurones before moving on to considering specific examples of neuropathology mediated by ER dysfunction.

- **PERK**

Whenever misfolded protein accumulates in the ER lumen, new protein synthesis is rapidly inhibited [14]. This occurs because ER stress activates the PKR-like Endoplasmic Reticulum eIF2α Kinase (PERK), also know as Pancreatic eIF2α kinase (Pek) because of the tissue in which it was first discovered [14]. This kinase is ubiquitously expressed but is most important in highly secretory tissues such as the brain where it functions to match protein synthesis with the efficiency of protein folding in the ER [15]. When protein folding is progressing well PERK is inactive, but when misfolded ER proteins accumulate the kinase is activated and phosphorylates eukaryotic translation initiation factor 2α (eIF2α) (Figure 1) [16]. This substrate is a subunit of the heterotrimeric GTP-binding complex eIF2 that

regulates the initiation of protein synthesis [14]. EIF2 recruits the methionyl-tRNA to the ribosome at the onset of protein translation, but when its eIF2α subunit is phosphorylated this activity is lost and translation ceases. The load of proteins entering the ER is thus reduced following PERK activation preventing the accumulation of more misfolded proteins.

- **ATF4**

Despite the inhibition of protein translation that accompanies phosphorylation of eIF2α, a small subset of mRNAs is translated more efficiently under these conditions [17]. The best characterised of these is Activating Transcription Factor 4 (ATF4), which triggers a transcriptional programme of stress-responsive genes [17]. Other members of the eIF2α kinase family can inhibit translation and activate these genes; for example PKR responds to viral infection, HRI to iron deficiency and GCN2 to amino acid starvation [18]. This ability of eIF2α phosphorylation to mediate a response to multiple stresses led to this pathway being named the Integrated Stress Response [19]. Surprisingly, within the brain this can provoke specific behavioural responses. A lack of essential dietary amino acids triggers the phosphorylation of eIF2α in the anterior piriform cortex by GCN2, which alters feeding behaviour in rodents [20, 21]. Thus, phosphorylation of eIF2α is not synonymous with ER stress and multiple lines of evidence should always be sought when attributing phosphorylation of eIF2α to ER dysfunction.

Phosphorylation of eIF2α is particularly important for the maintenance of axonal myelination in the human central nervous system (Table 1); indeed mutations that disrupt the regulation of eIF2α result in white matter hypomyelination disorders such as Childhood Ataxia with Central nervous system Hypomyelination (CACH syndrome) and Vanishing White Matter (VWM) [22]. This family of autosomal recessive conditions constitutes a spectrum of severity from congenital to adult-onset, involving the progressive loss of mental and motor function due to a loss of brain white matter. In all cases, the causative mutations are found within subunits of eIF2B, a guanine nucleotide exchange factor (GEF) responsible for recharging the eIF2 complex with GTP [23, 24] (Figure 1). These mutations interfere with the GEF activity of eIF2B and enhance the synthesis of ATF4. This deregulates many target genes including components of a negative feedback system, including CHOP and GADD34, that normally recover protein translation following its initial inhibition by PERK [13, 25].

- **CHOP**

The transcription factor CHOP is induced by ATF4 and has widely been thought to be pro-apoptotic during ER stress, since *Chop-/-* animals are resistant to ER stress-induced cell death [13]. However, this is inconsistent with observations made in neurological diseases. For example, CHOP expression is clearly anti-apoptotic in mouse models of the X-linked disorder Pelizaeus-Merzbacher leukodystrophy caused by abnormalities of the proteolipid protein 1 (*PLP1*) gene [26]. Indeed, identification of the target genes of CHOP failed to reveal a link with

apoptosis but instead identified genes that promote protein secretion [13]. This led us to suggest that in models of severe ER stress the promotion of protein secretion by CHOP might increase the intensity of stress and thus induce cell death [13]. By contrast, during conditions of milder ER stress, for example in Pelizaeus-Merzbacher leukodystrophy, it is likely that CHOP does not enhance ER stress sufficiently to trigger cell death [26]. These observations highlight the need to model not only the nature of the stress, but also its *intensity* and *duration* since differences in either can significantly affect the outcome and thus lead to beneficial or deleterious effects depending upon the context. There may also be cell type specific consequences of expressing CHOP. For example, while deletion of *Chop* worsens the central hypomyelination seen in Pelizaeus-Merzbacher leukodystrophy [26], *Chop*-/- animals are protected against the peripheral hypomyelination caused by mutant myelin protein zero (P0) in mouse models of Charcot Marie Tooth type 1B [27].

- **GADD34**

A critical target gene of CHOP encodes Growth Arrest and DNA Damage-inducible protein 34 (GADD34), which is a regulatory subunit of protein phosphatase 1 (PP1) [13]. This dephosphorylates eIF2α and thus brings about the recovery of protein translation after its initial inhibition. It has been shown in mice that inactivation of the *Gadd34* gene can protect animals from tissue damage during ER stress by reducing the accumulation of misfolded proteins [13]. Subsequently, a small molecule called salubrinal was identified that increases

the level of eIF2α phosphorylation and promotes survival of ER-stressed cells [28]. It has been suggested that salubrinal may be an inhibitor of the eIF2α phosphatases [28], but direct evidence for this is lacking. Recently, however, a selective inhibitor of GADD34, guanabenz, has been identified confirming the beneficial effects of GADD34 inhibition during ER stress at least in cultured cells [29]. It is therefore likely that future therapies involving the inhibition of GADD34 will be able to modulate ER stress.

- **ATF6**

In addition to the Integrated Stress Response genes regulated by PERK and ATF4, many additional genes are induced during the UPR by two further ER stress sensors, Inositol-Requiring Enzyme 1 (IRE1) and Activating Transcription Factor 6 (ATF6) [10] (Figure 1). Like PERK, both are ER membrane proteins that are held inactive under normal conditions by the binding of the ER chaperone Binding Immunoglobulin Protein (BiP; also known as GRP78). During stress, misfolded proteins sequester BiP, freeing ATF6 to traffic to the Golgi apparatus where it is cleaved to release a soluble transcription factor. Of note, astrocytes express an ATF6-related protein called OASIS (Old Astrocyte Specifically Induced Substance) whose functions appear to overlap partially with those of ATF6 [30]. This might enable astrocytes to respond to ER stress in a tissue-specific manner.

While cleavage of ATF6 is strong evidence of ER stress, this is difficult to detect. Consequently, many studies fail to measure this arm of the UPR, but the use of sensitive reporters that measure the binding of ATF6 to specific DNA sequences can circumvent this problem *in vitro* [11].

- **IRE1**

The activation of IRE1 closely resembles that of PERK but, unlike PERK, IRE1 triggers splicing of the mRNA encoding the transcription factor X-box Binding Protein 1 (XBP1) [31]. This induces a frame shift that generates the active form of this protein. Like phosphorylation of eIF2α, the splicing of *XBP1* mRNA is transient [13]. Following the alleviation of ER stress by the induction of UPR target genes, spliced *XBP1* mRNA is rapidly lost from the cell to be replaced by the unspliced form [13]. In laboratory studies, supra-physiological levels of ER stress can cause complete splicing of *XBP1* mRNA, but *in vivo* stresses are frequently subtler and so it is rare to observe complete splicing and careful quantification is therefore necessary [32].

If proteins fail to fold in the ER, they are prevented from progressing along the secretory pathway and eventually are targeted for degradation [33]. This 'ER-associated protein degradation' (or ERAD) returns terminally misfolded proteins to the cytosol where they are ubiquitinated by ER-associated ubiquitin ligases for degradation by the proteasome [34]. Many components of the ERAD machinery

are target genes of XBP1 and so the capacity for ERAD is increased by activation of IRE1 [35].

ER dysfunction in neurological disease

Cerebral hypoxia

In industrialized countries stroke is the third biggest killer and the leading cause of disability in adults. Acute ischaemia is the main cause of neuronal loss and, although its pathology is complex, appears to involve ER stress [1, 15, 36-38]. During cerebral ischaemia, neuronal depolarization due to energy depletion causes the uncontrolled release of glutamate. The consequent activation of NMDA receptors on nearby neurones generates further glutamate release and causes an ischaemic depolarization wave to spread outward from the initial site of damage leading to widespread disturbance of calcium homeostasis [39]. Unsurprisingly, many studies implicate ER calcium store release in the resulting excitotoxic death (reviewed in reference [39]).

The ER is the main site for calcium storage within the cell and so its chaperones have evolved to function efficiently in this high calcium environment; indeed many require high calcium to function [40]. Calcium is pumped into the ER by the sarcoendoplasmic reticular calcium-ATPase (SERCA) and released back into the cytosol by the inositol trisphosphate receptor (IP3R) in response to extracellular signals and by the ryanodine receptor during calcium-induced calcium release [39]. Inhibition of the SERCA pump by thapsigargin induces apoptosis in many cell

types including neuroblastoma cells and is often used to induce ER stress in the experimental setting [41]. During cerebral ischaemia, energy depletion leads to failure of the SERCA pump and thus redistribution of ER calcium into the cytosol. This delivers a double blow with combined toxicity from uncontrolled cytosolic calcium and ER stress due to chaperone dysfunction (Figure 2) [39].

The UPR is activated in many rodent models of cerebral ischaemia [1, 36]. In these models, protein synthesis is rapidly inhibited and accompanies the phosphorylation of eIF2α. Although the inhibition of translation has been attributed to aggregation of components of the translation machinery [42], the primary mechanism appears to be through phosphorylation of eIF2α [43], which can remain phosphorylated for up to twelve hours following ischaemia [44]. PERK is the sole eIF2α kinase activated by cerebral ischaemia and predictably *Perk-/-* mice fail to show eIF2α phosphorylation following transient cerebral ischaemia and fail to reduce protein translation during the reperfusion period [15]. Even with the far milder recurrent cerebral hypoxia seen in obstructive sleep apnoea, activation of PERK has been described and has been suggested to play a role in the loss of upper airway motor neurones in this condition [45].

IRE1 is also activated during cerebral ischaemia leading to the induction of UPR target genes [37, 38]. It appears to promote activation of pro-apoptotic caspase-12 in cultured rodent cells and this has been detected in ischaemic rodent brains [46]. However, caspase-12 is non-functional in the majority of humans, except those

from sub-Saharan Africa, owing to a common truncating mutation [47]. For this reason, it has been proposed that during ER stress in humans, caspase-4 might serve an analogous function to that of caspase-12 in rodents [48].

Due to the problems of detection referred to earlier, the role of ATF6 during ischaemia is unclear. ATF6 gene expression is elevated following transient middle cerebral artery occlusion in rats [36], but its activation has not been detected in rat brain following cardiac arrest and resuscitation [49]. OASIS is also upregulated and appears to play a neuroprotective role in response to kainate excitotoxicity [50].

CHOP is induced following forebrain ischaemia in a variety of rodent models including bilateral common carotid [51, 52] or middle cerebral artery occlusion in mice [44] and global cerebral ischaemia in rats [53]. The location within the brain of CHOP induction appears to correlate well with subsequent cell death [53, 54] and deletion of the *Chop* gene protects mice during bilateral common carotid artery occlusion [52]. Similarly, depletion of CHOP using RNA interference partially prevents the death of astrocyte cultures stressed by oxygen-glucose depletion [55]. As discussed, some of the toxicity attributable to CHOP reflects increased protein translation mediated by GADD34 [13]. Indeed, GADD34 induction is well-recognised to accompany cerebral ischaemia [56] being detected at the peri-infarct penumbra within two to twenty-four hours [57] and accounts for the transient nature of eIF2α phosphorylation during stroke [15]. Attenuation of translation protects the

cell from accumulating misfolded proteins, thus treatment with salubrinal (a drug that enhances eIF2α phosphorylation) reduces ER stress and promotes cellular survival in kainate-induced neurotoxicity [58]. Indeed, salubrinal has been shown to limit infarct size in a murine model of stroke [1].

In addition to attenuating protein translation, the UPR leads to upregulation of ER molecular chaperones, the most abundant being BiP [10]. Following transient forebrain ischaemia, BiP expression in the hippocampus and cortex peaks between twelve to seventy-two hours after reperfusion [54, 59], but can remain elevated for up to two weeks [36]. This is protective, since suppression of BiP enhances apoptosis in hippocampal neurones exposed to excitotoxic and oxidative insults [60], while its overexpression in primary astrocyte cultures is protective against oxygen-glucose deprivation [61]. Indeed, higher levels of BiP induction correlate with neuronal protection [37, 62].

Induction of the ER chaperone ORP150 (150-kDa oxygen-regulated protein) is also seen in ischaemic neurones, and over-expression of ORP150 protects mice from cerebral ischaemia [63]. ORP150 modulates the activity of BiP, which exists in either an ATP- or ADP-bound form, each with different substrate-binding affinity. ORP150 functions as a nucleotide exchange factor for BiP to replace its bound ADP with ATP. Interesting, another BiP nucleotide exchange factor, SIL1, is mutated in the Marinesco-Sjogren syndrome of cerebellar ataxia in which homozygous loss of SIL1 leads to ER stress and selective loss of cerebellar

Purkinje cells [64]. When ATP-BiP binds its substrate protein, a co-chaperone of the DnaJ family is required to stimulate BiP's ATPase activity and so promote high affinity substrate binding. The relative levels of ADP-BiP and ATP-BiP appear to be important for neuronal survival, since the toxicity of SIL1 loss can be ameliorated by the deletion of an ER luminal DnaJ co-chaperone called $p58^{IPK}$ [65]. While loss of SIL1 would be predicted to reduce the level of ATP-BiP through impaired nucleotide exchange, loss of $p58^{IPK}$ is likely to restore balance by lessening BiP's ATPase activity.

When taken together, these observations suggest that ER stress is induced by cerebral ischaemia and that the UPR limits cerebral infarct size. There is good evidence to suggest that manipulation of ER stress signalling by increasing phosphorylation of eIF2α may have important therapeutic effects as an acute intervention for cerebral ischaemia.

Alzheimer's disease

Often presenting with failing memory, Alzheimer's disease (AD) is a relentlessly progressive neurodegenerative disorder and the most common cause of dementia in adults. The classic histopathological features consist of extracellular plaques of the β-amyloid (Aβ) peptide and intracellular neurofibrillary tangles of hyperphosphorylated aggregates of the microtubule-associated protein tau. Studies of familial AD demonstrate that the disorder is caused by overproduction of the more aggregation-prone $A\beta_{1-42}$ peptide [66] (Figure 3). Despite decades of

study, the molecular pathology of this disease remains unclear. However, increasing evidence suggests that ER stress may represent a new paradigm for understanding this condition.

Studies using post-mortem tissue from the brains of individuals with AD have provided evidence for ER dysfunction in this disease. But when looking at a single marker of ER stress, such studies can generate confusing results. For example, when BiP is examined in isolation it can show no change [3], a decrease [67] or even an increase [2, 68], although BiP expression does seem to be higher in brains with histologically more advanced disease [68, 69]. However, whilst often a useful indicator, the level of BiP expression is inadequate alone to assess the activation of UPR signalling, especially in a chronic disorder in which histological features evolve over time. It has therefore proved more useful to include additional markers of the UPR in such studies. For example, protein disulphide isomerase, another target of the UPR, is elevated in the temporal lobes of the brains of individuals with AD [3], associated with neurofibrillary tangles in the hippocampus and frontal lobes and in dystrophic neurites of senile plaques [4]. When one looks for more proximal markers of UPR signalling, there is activation of PERK in the hippocampus of AD brains, which appears to co-localise with staining for phosphorylated tau and correlates with histological staging [70, 71]. Yet phosphorylated PERK is barely detectable in tangle-bearing neurones themselves, suggesting that PERK is activated at the pre-tangle stage in neurones of the hippocampus [71]. In addition, there are elevated levels of spliced

XBP1 mRNA in the temporal cortex of AD patients when compared to age-matched controls [3]. Congruently, a marked increase in the number of neurones immunoreactive for phosphorylated (active) IRE1α has been detected in the hippocampus of individuals with AD [71].

There is good evidence for activation of PERK in laboratory models of AD. Increased BiP expression observed upon application of exogenous Aβ to primary cortical neurones suggests that accumulation of the peptide may directly activate ER stress signalling [72, 73]. Moreover, exogenous Aβ has been found to increase the phosphorylation of PERK and eIF2α in cultured neuronal cells [73, 74], while silencing of PERK by siRNA in $A\beta_{42}$-treated cells limits eIF2α phosphorylation and enhances cell death [73]. In addition, $A\beta_{42}$ treatment induces CHOP expression both in cultured cells and in rabbit hippocampus [75, 76], while prior treatment of cells with CHOP anti-sense RNA improves survival following exposure to the Aβ peptide, suggesting a role for CHOP in Aβ-mediated death [77]. In *Drosophila* neurones or mammalian PC-12 cells, exposure to Aβ induces *XBP1* mRNA splicing and in both models overexpression of spliced XBP1 is protective, while knockdown of XBP1 exacerbates Aβ toxicity [78].

PC-12 cells overexpressing mutant forms of presenilin 1 (PS1), a component of the γ-secretase, that has been linked to familial AD, show increased levels of phosphorylated eIF2α, as do hippocampal homogenates from PS1 mutant knock-in mice [79]. PC-12 cells expressing mutant PS1 have elevated levels of

CHOP, as do knock-in mice expressing mutant PS1 [79]. The nature of the interaction between PS1 and ER stress has yet to be fully elucidated, although it is known that PS1 and the SERCA calcium pump physically interact with one another [80]. As discussed, disordered calcium homeostasis is a potent cause of ER stress and much evidence suggests that calcium signalling is perturbed in Alzheimer's disease [81] (reviewed in reference [82]). Interestingly, ER stress induced by tunicamycin, a glycosylation inhibitor frequently used to induce ER stress *in vitro*, increases PS1 expression in multiple cell lines including neuroblastoma [80]. Recent evidence suggests that phosphorylation of eIF2α by PERK during ER stress can increase the level of β-site APP cleaving enzyme-1 (BACE) and thereby promoting amyloid formation [83]. This suggests that the interaction between AD and ER stress may prove to be bi-directional.

ER stress has been shown to alter APP localization, processing and degradation by the ERAD machinery [84] (Figure 4). During ER stress, immature forms of APP bind to BiP and when BiP is overexpressed Aβ generation is reduced as APP is retained in earlier compartments of the secretory pathway [84]. The ERAD ubiquitin ligase HRD1 promotes APP ubiquitination and degradation in HEK293 cells reducing the generation of both Aβ_{40} and Aβ_{42} [2]. In addition, an intronic polymorphism (IVS3-88A>G) of *SEL1L*, another component of the ERAD system, has been associated with AD in an Italian population [85]. These hints that ERAD is important in the pathogenesis of AD still require further validation. But there is accumulating evidence for elevated UPR signalling in AD and, combined with the

support from model systems, provides a potential mechanism by which the Aβ peptide and mutants of PS1 might induce ER stress. This offers the potential to identify new therapeutic targets in an otherwise incurable disease.

Parkinson's disease

With age, 2% of the population suffer the loss of dopaminergic neurones in their nigrostriatal pathway and so develop Parkinson's disease (PD) characterised by hypokinesia, rigidity and tremor. Post-mortem studies identify characteristic Lewy body inclusions in many affected brains, suggesting aberrant protein disposal as a possible cause, while toxins that cause Parkinsonism, such as MPTP, rotenone and 6OHDA, impair mitochondrial function and promote ER stress [86-90].

Multiple lines of evidence point towards a role for ER stress in the pathogenesis of PD. For example, excess levels of dopamine are toxic to PC-12 cells, which correlates with their induction of ER chaperones [91]. The brains of patients suffering from sporadic PD, but not controls, have elevated levels of phosphorylated PERK and eIF2α [92]. *Perk-/-* sympathetic neurones are hypersensitive to 6OHDA [92], while overexpression of spliced XBP1 is protective both in cultured cells and animals treated with MMP+/MPTP [87]. CHOP expression is increased both by 6OHDA and MPTP, and knockout of the *Chop* gene is protective in mice treated with 6OHDA [93].

In Japanese and European families with the juvenile onset autosomal recessive variant of PD (ARPD), classical genetic mapping identified the disease-associated gene *PARK2* [94]. This encodes an ubiquitin ligase Parkin, whose activity is lost with disease-associated mutations [95]. Parkin was suggested to localize to the ER and to be upregulated during the UPR [96]. A major function of Parkin appears to involve targeting defective mitochondria for destruction by autophagy [97]. It is possible that impaired mitochondrial function in patients with *PARK2* mutations manifests as ER stress owing to defects in energy supply during ER protein folding. However, another component of Lewy bodies, α-synuclein, can be found throughout the cell including the ER lumen and may also be a target of Parkin [98]. Mutations of α-synuclein cause an autosomal dominant form of PD [99], while the glycosylated ER-form of α-synuclein has been shown to be ubiquitinated by Parkin and to accumulate in a non-ubiquitinated form in the brains of individuals with Parkin deficiency [98]. Overexpression of α-synuclein induces ER stress [100], perhaps through binding to BiP [101], while PDI appears to impair α-synuclein fibril formation [100]. Recently, oligomers of mutant α-synuclein were shown to accumulate within the ER in an animal model and to trigger the UPR, albeit with relatively little PERK activation [102]. When eIF2α phosphorylation was augmented with salubrinal, the accumulation of α-synuclein oligomers was ameliorated and disease onset was delayed. There is further evidence that modulating the levels of ER stress may be beneficial in PD, since 4-PBA, which has been shown to possess 'chemical chaperone activity' that can protect cells from ER stress, rescues cells from the neurotoxicity of rotenone [103] and 'pre-

conditioning' neuroblastoma cells with ER stress to up-regulate chaperone levels can protect the cells from subsequent challenge with 6OHDA [104].

Huntington disease

Huntington disease (HD) is an inherited autosomal dominant disorder characterised by motor dysfunction, psychiatric disturbances and intellectual decline. It is caused by expansions of CAG repeats within the *HTT* gene encoding huntingtin and so represents an archetypal poly-Q disease. In health, the normal *HTT* gene contains 6 to 35 repeats, while in patients affected by HD there may be more than 40, which generates a protein prone to aggregation. Although this aggregation takes place in the cytosol this has been shown to provoke ER stress [105].

It is now appreciated that perturbations of protein folding within one organelle can be propagated to another. This concept of cell-wide protein homeostasis, or 'proteostasis', may be especially relevant to HD and other poly-Q disorders wherein cytoplasmic aggregates trigger the UPR [106]. The precise mechanism linking aggregation in the cytosol with malfunction of the ER is poorly understood; however, expression of poly-Q proteins and mutant huntingtin in cells has been shown to bind components of the ERAD machinery and impair their function [12, 107]. It is possible that huntingtin is a target of the ERAD ubiquitin ligase HRD1, since HRD1 can ameliorate the toxicity of the poly-Q protein [107]. By sequestering HRD1 and other components, huntingtin might reduce the capacity of a neurone

to cope with misfolded proteins by ERAD, thereby rendering them more vulnerable to ER stress [12]. In addition, while chronic ER stress is known to induce cell death in some circumstances, it might also impair intracellular signalling in viable cells. Recent data suggest that ER calcium release via the IP3R1 channel is impaired by ER stress in HD owing to the loss of a stimulatory interaction between BiP and this channel [108].

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) involves the progressive loss of motor neurones causing an irreversible decline of muscle power and death usually within five years. The aetiology remains largely unknown, although 5-10% of cases display a dominant pattern of inheritance suggestive of a toxic-gain-of-function, so-called Familial ALS (FALS). Of these, a fifth are caused by mutations in the copper-zinc superoxide dismutase 1 (SOD1) that accumulates as fibrils in Lewy Body-like hyaline inclusions, the histological hallmark of FALS.

Post-mortem brains from ALS patients show elevated levels of markers of ER stress [109]. Furthermore, disease models of FALS implicate ER stress in its pathogenesis. Although SOD1 was thought to be an abundant cytosolic protein, it is also secreted by multiple cell types and co-localises with ER markers [110]. When expressed in cultured cells or in animal neuronal tissue, mutant SOD1 accumulates within the ER and upregulates targets of the UPR [111-113].

Activation of PERK can be detected in animals prior to the development of overt disease [114] and it has been shown in three models of FALS that a subset of motor neurones are more sensitive to the toxic effects of mutant SOD1 and show evidence of ER stress at a younger age [115]. When one allele of *Perk* is deleted in mice expressing a mutant form of SOD1, onset of disease is accelerated, suggesting that phosphorylation of eIF2α is protective in this disorder [116]. This is supported by the protection of vulnerable neurones in animals treated with salubrinal [115].

Surprisingly, deletion of neuronal XBP1 has been shown to delay the onset of disease extending the lives of FALS mice [117]. In cultured neurones depletion of XBP1 reduces SOD1 aggregates and toxicity through enhanced autophagy, which might plausibly explain this apparent neuroprotection through increased clearance of aggregated protein. Similarly, enhanced degradation of SOD1 by overexpression of ERAD protein derlin1 ameliorates SOD1-induced cell death [118]. Though mutant SOD1 has been shown to interact with derlin1 and so impair ERAD to promote ER stress [119].

Multiple sclerosis

Regulated phosphorylation of eIF2α is important for the health of myelinated neurones within the central nervous system as illustrated by the CACH/VWD syndrome and Pelizaeus-Merzbacher leukodystrophy as mentioned previously. This holds true in multiple sclerosis (MS), although the timing of eIF2α

phosphorylation relative to the onset of disease determines its consequences. In the experimental autoimmune encephalitis (EAE) model of MS, ER stress can hasten or delay disease progression if induced after of before disease onset respectively [120, 121]; a phenomenon previously seen with interferon treatment. Indeed, both EAE and interferon cause phosphorylation of eIF2α by PERK. Only when animals are exposed to interferon prior to EAE is a protective 'pre-conditioning' seen and, importantly, the protection is lost if one allele of *Perk* is deleted [120]. In contrast, interferon treatment of animals with established EAE exacerbates their ER stress and worsens demyelination through impaired oligodendrocyte survival.

FENIB

Ten years ago, the first cases of a new familial encephalopathy with inclusion bodies (FENIB) were described in two families living in the USA [122]. The primary symptom of this autosomal dominant neurodegenerative disease was pre-senile dementia. In each family, eosinophilic neuronal inclusions were observed in the deeper layers of the cerebral cortex and in the *substantia nigra* (Figure 5). After isolation and sequencing of these inclusions, it was demonstrated that they were composed of a single protein, neuroserpin. When expressed in neurones, the mutants of neuroserpin accumulate to form acid-Schiff-positive diastase-resistant inclusions in the ER, also called Collins' bodies [122].

Neuroserpin is a member of the serine protease inhibitor (serpin) superfamily. Serpins are capable of polymerisation under certain circumstances and many examples of naturally occurring mutations exist that promote polymerisation leading to disease [123, 124]. The retention of polymerised neuroserpin within the ER causes neurotoxicity through a toxic gain-of-function, while the lack of secretion causes the activation of proteolysis and hence disease through a loss-of-function [123].

Despite being retained in the ER, the polymerised mutants of neuroserpin fail to activate the UPR [11]. Instead, these polymers induce NFκB signalling in a calcium-dependent manner [11]. This is reminiscent of the ER overload response (EOR), which is activated by the accumulation of well-folded protein as happens, for example, in some viral infections [125]. The degradation of mutant neuroserpin involves the co-ordinated activity of both the ERAD and autophagic pathways [124]. Since these pathways have successfully been targeted in preclinical studies of a related serpinopathy using the drug carbamazepine [126], there is hope for pharmacological treatment of this and other ER overload disorders. Indeed, it is likely that similar strategies will prove beneficial in other diseases of protein accumulation, such as those described in the sections concerning ER stress.

Prion diseases

The nature of the ER dysfunction observed in prion diseases remains less clear. While ER stress pathways were activated in a cell model of Creutzfeldt-Jakob

disease (CJD), UPR signalling was not detected in a transgenic mouse model for prion disease [5]. Nevertheless, both primary neuronal cultures and PC-12 cells expressing PrP mutants develop dramatic ER swelling suggesting that ER homeostasis is perturbed [6]. The failure to detect splicing of *XBP1* mRNA or differences in BiP levels in prion disease models suggests that prion proteins might not elicit classical ER stress [5]. However, pharmacological induction of ER stress in neuronal cell lines expressing mutant PrP can decrease PrP levels [7]. Recently it was shown that prion replication causes prolonged repression of protein translation leading to synaptic failure of neuronal loss [9]. In contrast to many ER stress-related disorders in which enhanced phosphorylation of eIF2α is protective, prion-induced neurodegeneration is enhanced by salubrinal while overexpression of GADD34 is protective. The relationship between the prionopathies and ER dysfunction therefore requires further examination.

Concluding remarks and therapeutic potential

The evidence for activation of ER stress responsive pathways in a range of neurological conditions is strong. What remains to be determined is how successful strategies that target these responses will be in treating these diseases. The UPR signalling field has matured to a point at which small molecular inhibitors of its components are now being developed.

The muscle relaxant dantrolene targets IP3R channels of the ER to prevent the release of calcium. This provides neuroprotection in models of HD [127] and of

cerebral hypoxia-reperfusion injury, where its action is associated with reduced levels of ER stress [128]. Prolonging the phosphorylation of eIF2α and thus delaying the recovery of protein translation with agents such as salubrinal or guanabenz is another promising approach [28, 29]. Salubrinal has already been shown to reduce the accumulation of α-synuclein in models of ALS [102, 129], reduce the toxicity of Aβ to primary neurones [73], and lessen the ER stress associated with cerebral ischemia/reperfusion and models of HD [1, 130]. More recently, agents have been developed that inhibit IRE1, including quinotrierixin [131] and 4μ8C [132]. Both selectively inhibit the splicing of *XBP1* mRNA and the latter prevents expansion of the ER without obvious toxicity, which might have therapeutic utility to impede the formation of protein inclusions in neurones.

The list of neurological disorders with impaired ER protein folding is already long and appears set to grow further for some time to come (Table 1). It will therefore aid the neurologist and neuroscientist alike to appreciate the signalling pathways that communicate this failure of ER function to the rest of the cell, since it is likely these insights will inform research, diagnosis and ultimately therapy.

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Contributions and conflicts of interest

DBR and SJM planned and wrote the review. All authors commented on the final manuscript. DBR performed the literature search for and wrote the stroke, Parkinson's and Huntington's disease, multiple sclerosis and prionopathy sections and prepared figure 1. AJK performed the literature search for Alzheimer's disease, and contributed to drafting the Alzheimer's disease section and figure. EM helped draft the FENIB section. DCC helped draft the Alzheimer's section. DAL helped draft the FENIB section. SJM performed the literature search for ER stress and supervised the review project.

Panel 1

Search Strategy

We set out by searching the PubMed database with the terms "(endoplasmic reticulum) AND (brain OR neurological) AND disease" including only articles in English. These titles and abstracts were screened and relevant papers were obtained for detailed assessment. Their bibliographies were also screened for additional sources. Papers published prior to September 2012 are included. Where possible, emphasis is given to articles published within the last 5 years.

Figure legends

Figure 1. Endoplasmic reticulum stress signalling

In health, newly synthesised secretory proteins are translocated into the ER lumen co-translationally. They fold with the aid of chaperones, for example BiP. When recognised as correctly folded they exit the ER in COPII-coated vesicles and traffic along the secretory pathway. During ER stress, misfolded proteins accumulate in the ER lumen and sequester BiP from the sensor molecules PERK, IRE1 and ATF6. PERK phosphorylates eIF2α, which binds and inhibits its guanine nucleotide exchange factor eIF2B. This blocks new secretory protein synthesis and triggers the integrated stress response (ISR) through increased levels of ATF4. Activation of IRE1 leads to generation of active XBP1, while

cleavage of ATF6 generates ATF6c. These transcription factors co-operate to induce target genes of the unfolded protein response (UPR).

Figure 2. ER stress during cerebral ischaemia

During an ischaemic stroke, the lack of glucose and oxygen supply in neurones induces a dramatic fall in ATP production. Without energy, BiP binds to unfolded proteins less efficiently resulting in the accumulation of misfolded ER client proteins and subsequent activation of the UPR. In parallel, failure of the SERCA pump depletes ER calcium and worsens chaperone function still further. Extracellular tPA (tissue-type plasminogen activator) cleaves the NMDA receptor allowing calcium influx into the cytosol. The resultant elevation of cytosolic calcium triggers calcium-induced calcium release via ryanodine receptors located in the ER membrane leading to calcium depletion from the ER. In severely affected neurones, elevated cytosolic calcium ultimately induces cell death. In surviving neurones, induction of the eIF2α phosphatase GADD34 by ATF4 in a CHOP-dependent manner enables the eventual recovery of protein translation. This can contribute to reperfusion injury through increasing the level of ER protein load.

Figure 3. Alzheimer's disease and ER stress

(A) In health, amyloid precursor protein (APP) is cleaved sequentially by β-secretase followed by γ-secretase in the TGN and early endosomes mainly

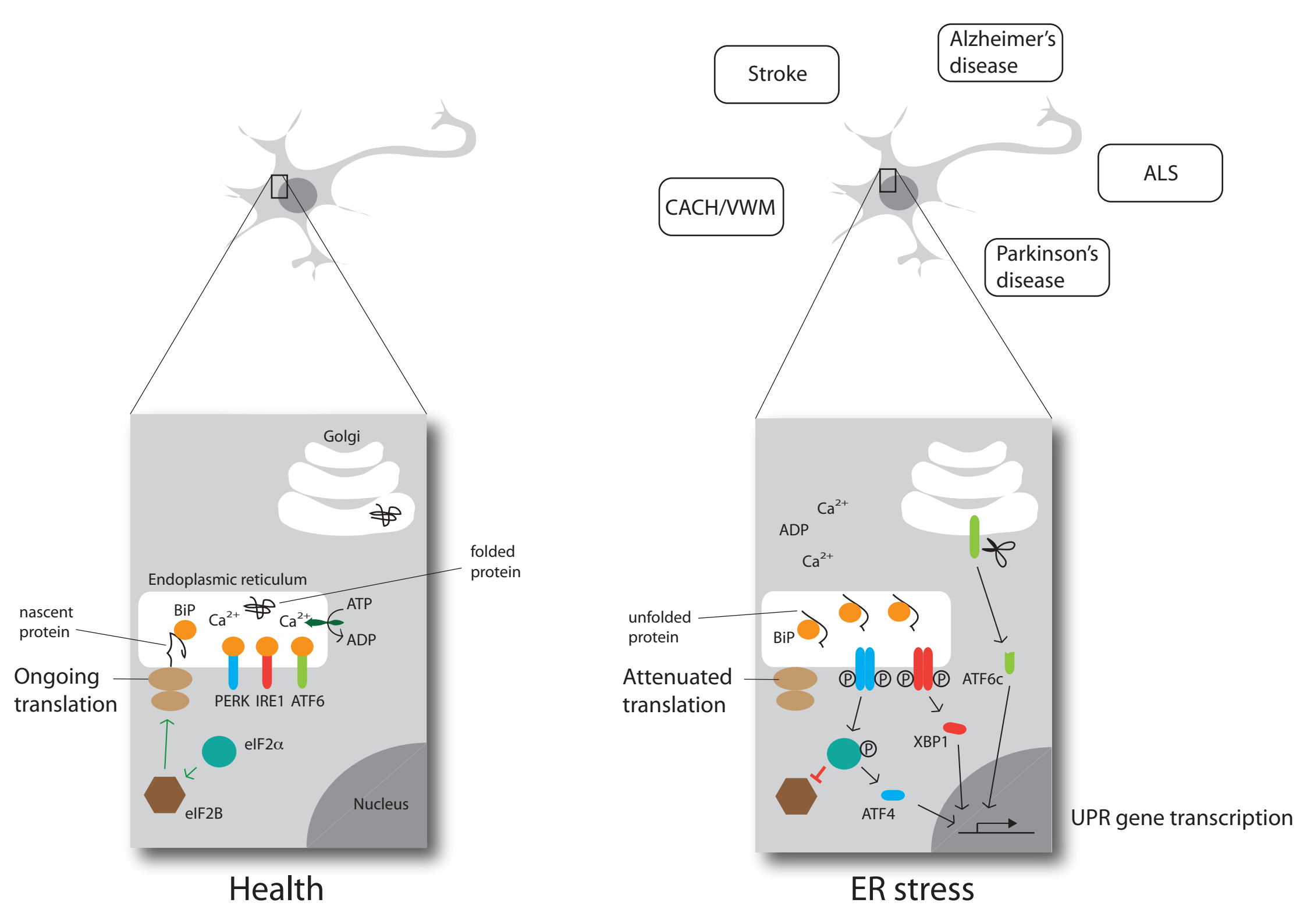
generating non-toxic $A\beta_{40}$. APP is recycled via the endosomal pathway. (B) In Alzheimer's disease, toxic $A\beta_{42}$ is generated and secreted. Exogenous $A\beta_{42}$ causes ER stress via a poorly understood mechanism. In some cases of familial Alzheimer's disease, mutations in PS1 impair trafficking of APP, thereby enhancing $A\beta_{42}$ secretion. Mutated PS1 may also lead to APP processing within the ER.

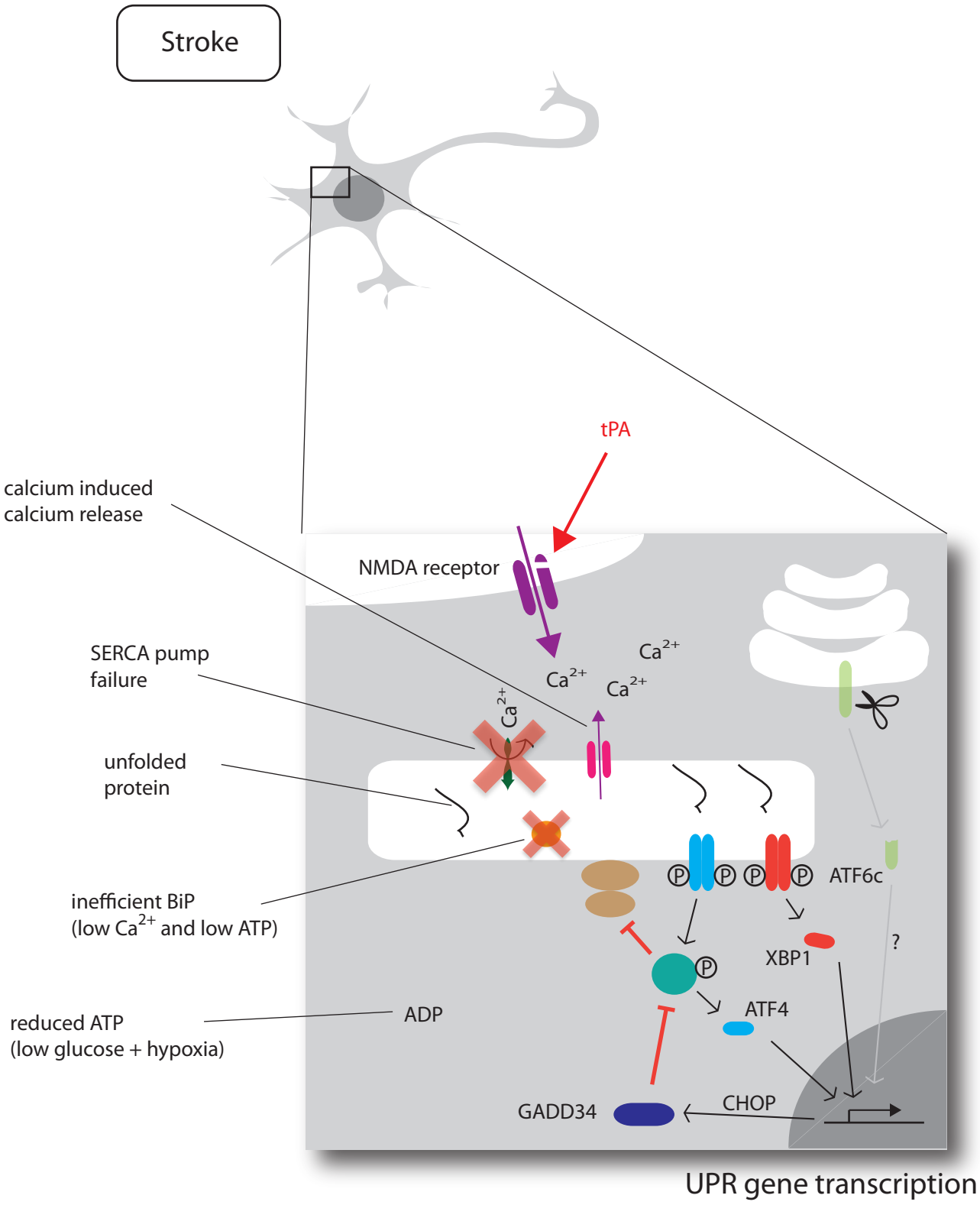
Figure 4: ERAD of amyloid precursor protein

Newly synthesised proteins, for example amyloid precursor protein (APP), interact with ER chaperones during multiple folding cycles until they reach their native folded state. Overexpression of BiP can increase retention of APP in the ER. If protein folding fails, the terminally misfolded client is targeted for degradation via ERAD. The interaction of glycoprotein substrates with both the folding machinery and the ERAD machinery is governed by cycles of de-mannosylation and re-mannosylation. Eventually, low mannose species can interact with EDEM and be targeted for dislocation from the ER lumen into the cytosol. This process is incompletely understood and displays substrate specific differences, but for APP involves a complex containing Hrd1 and Sel1L. Once in the cytosol, the ERAD substrate is ubiquitinated and eventually degraded by the proteasome.

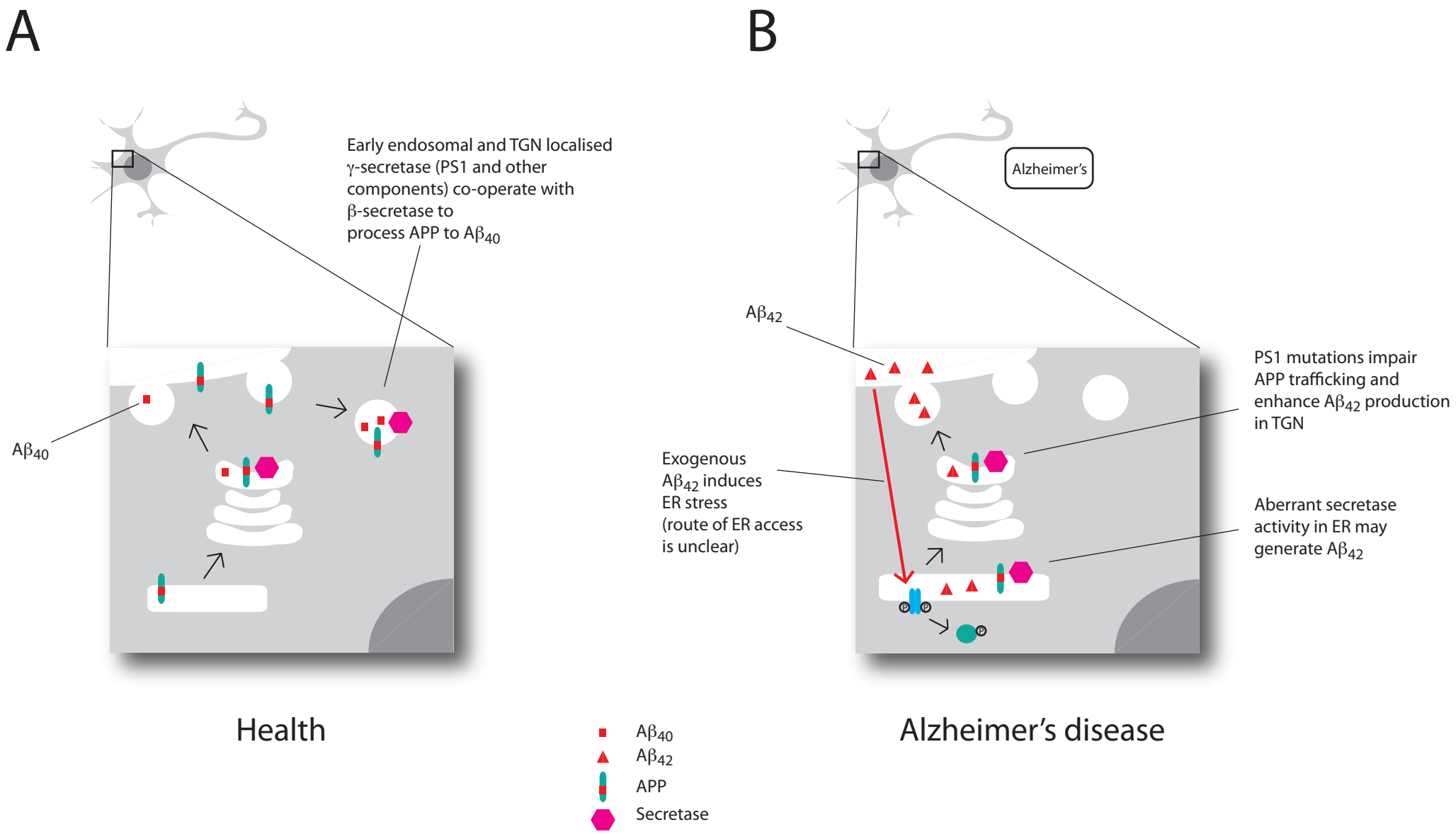
Figure 5: FENIB and the ER overload response

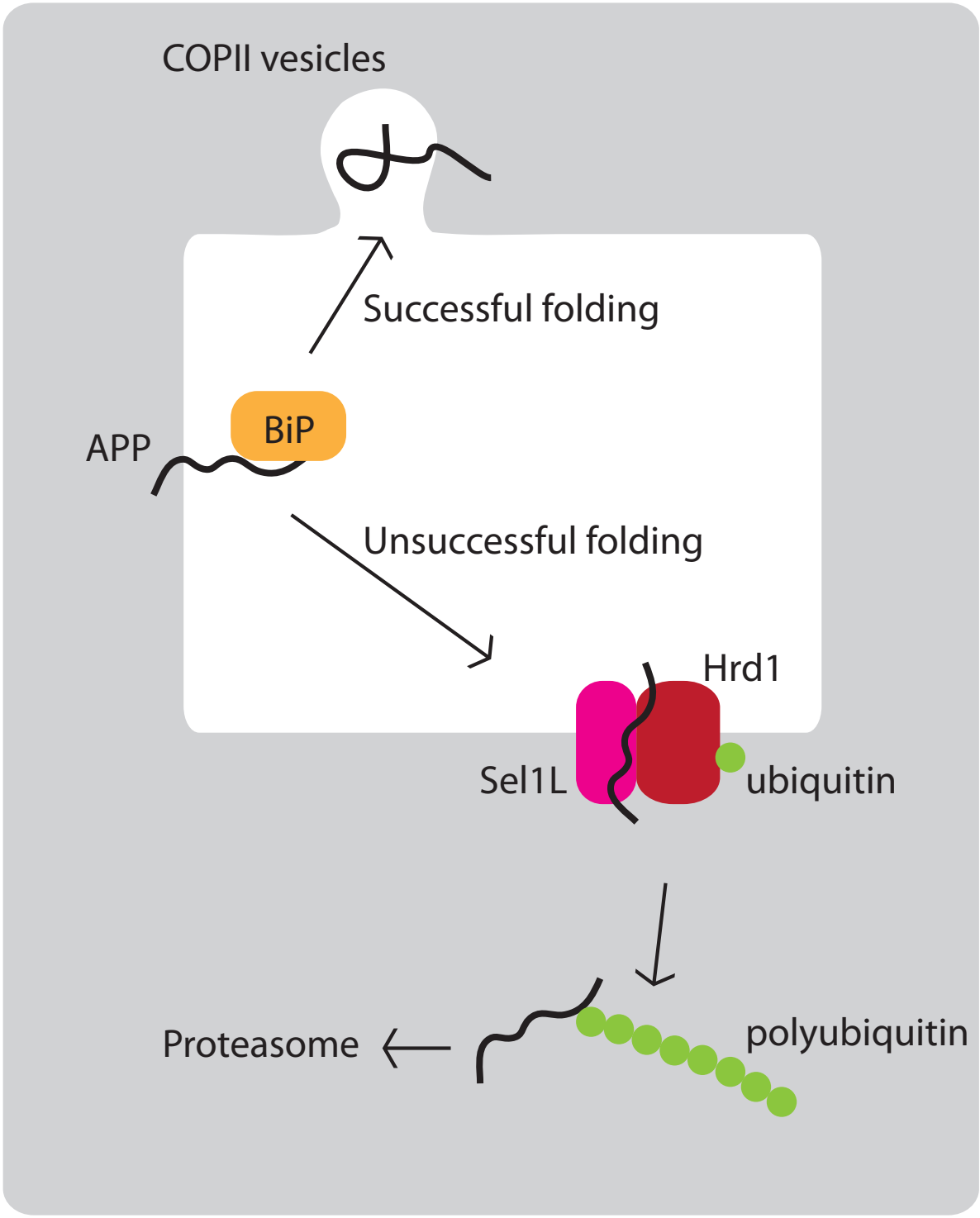
(A) Eosin staining of cerebral cortex from individuals with wild-type neuroserpin (WT) and the S52R or G392E mutations. Mutant protein is retained as polymers within the ER to form Collins bodies (red). The rate of polymer formation correlates with the age at which symptoms develop and the amount of Collins' bodies that can be detected. (B) Monomeric serpins, for example neuroserpin, present a reactive centre loop (red) that functions as a pseudosubstrate for their target protease. However, point mutations in the shutter domain (purple circle) open β-sheet A (green) to allow the insertion of the reactive centre loop belonging to a second serpin molecule. If allowed to propagate, this loop-sheet linkage generates large stable pathological polymers that are retained in the ER to form Collins' bodies. (C) These polymers are formed of folded neuroserpin and therefore fail to activate the UPR. Instead they cause the release of ER calcium into the cytosol, which causes NFκB activation and target gene induction.


Golgi
Endoplasmic reticulum
folded protein
nascent protein
BiP
Ca2+
Ca2+
ATP
ADP
Ongoing translation
PERK IRE1 ATF6
eIF2α
eIF2B
Nucleus
Health
Stroke
Alzheimer's disease
CACH/VWM
ALS
Parkinson's disease
Ca2+
ADP
Ca2+
unfolded protein
BiP
Attenuated translation
P P P P
ATF6c
XBP1
P
ATF4
UPR gene transcription
ER stress

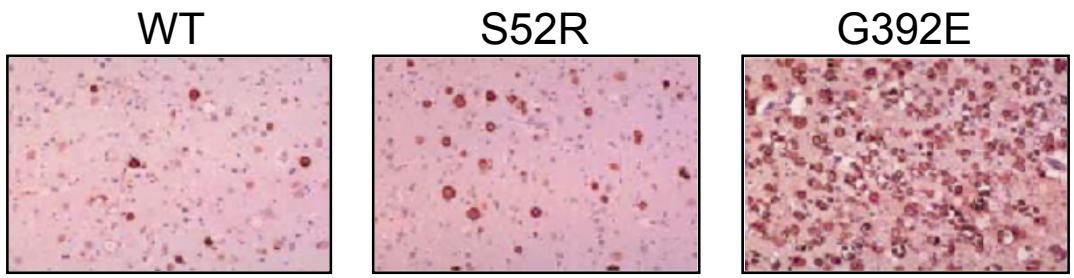

Stroke
tPA
calcium induced
calcium release
NMDA receptor
SERCA pump
failure
unfolded
protein
inefficient BiP
(low Ca2+ and low ATP)
reduced ATP
(low glucose + hypoxia)
ADP
Ca2+
Ca2+
Ca2+
Ca2+
ATF6c
XBP1
?
ATF4
GADD34
CHOP
UPR gene transcription

Stroke

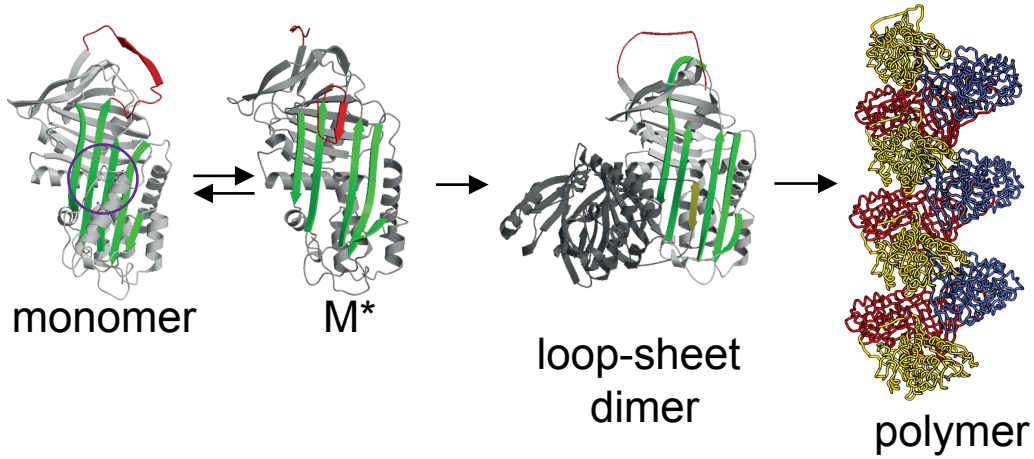

A
Early endosomal and TGN localised γ-secretase (PS1 and other components) co-operate with β-secretase to process APP to Aβ40
Aβ40
Health
B
Alzheimer's
Aβ42
PS1 mutations impair APP trafficking and enhance Aβ42 production in TGN
Exogenous Aβ42 induces ER stress (route of ER access is unclear)
Aberrant secretase activity in ER may generate Aβ42
Alzheimer's disease
Aβ40
Aβ42
APP
Secretase


COPII vesicles
Successful folding
APP
BiP
Unsuccessful folding
Hrd1
Sel1L
ubiquitin
Proteasome
polyubiquitin

A

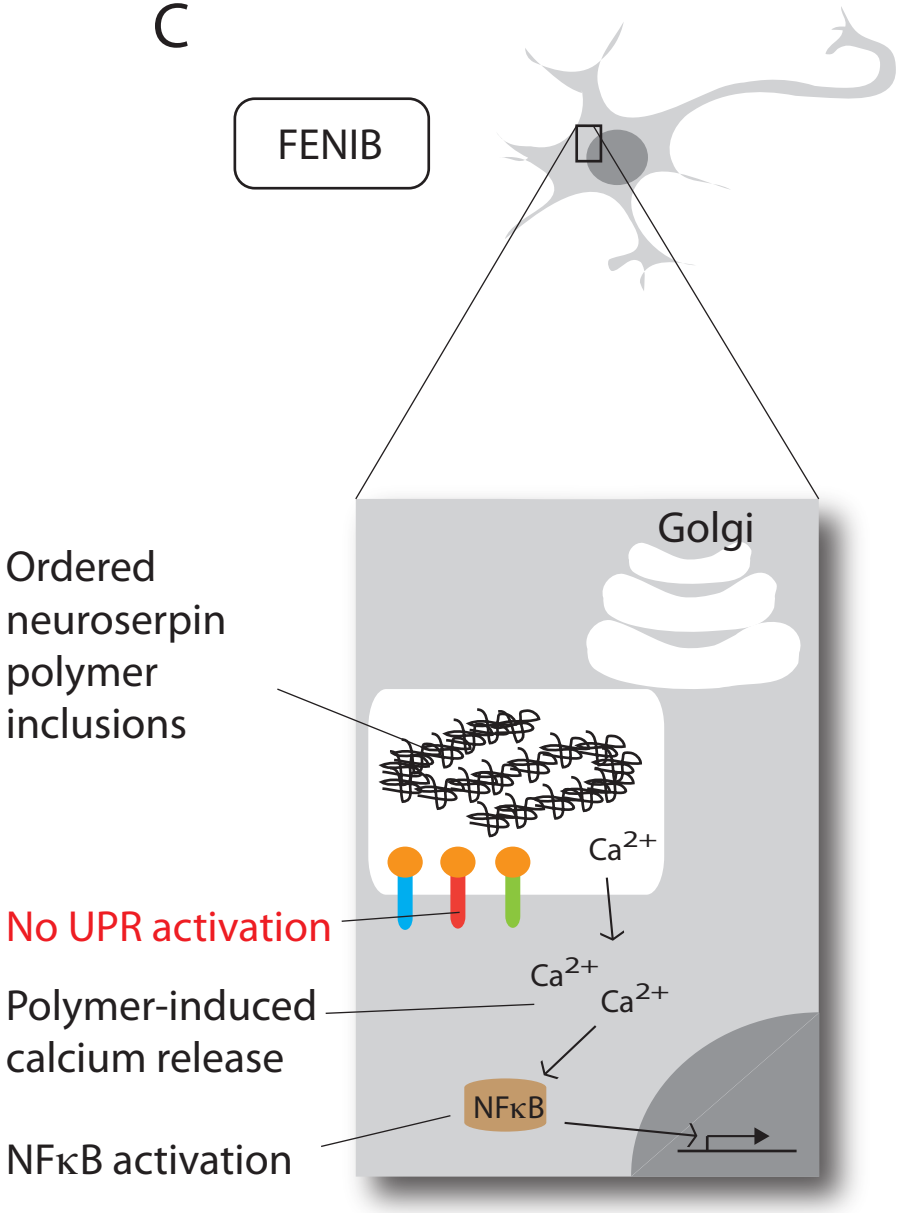


B



Pathway to serpin polymerisation

C



ER overload response