Converging Pathways in the Occurrence of Endoplasmic Reticulum (ER) Stress in Huntington's Disease

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Abstract: A variety of neurological diseases including Huntington's disease (HD), Alzheimer's disease and Parkinson's disease share common neuropathology, primarily featuring the presence of abnormal protein inclusions containing specific misfolded proteins. Mutations leading to expansion of a poly-glutamine track in Huntingtin cause HD, and trigger its misfolding and aggregation. Recent evidence indicates that alterations in the secretory pathway, in particular the endoplasmic reticulum (ER), are emerging features of HD. Although it is not clear how cytoplasmic/nuclear located mutant Huntingtin alters the function of the ER, several reports indicate that mutant Huntingtin affects many essential processes related to the secretory pathway, including inhibition of ER-associated degradation, altered ER/Golgi vesicular trafficking and axonal transport, disrupted autophagy and abnormal ER calcium homeostasis. All these alterations are predicted to have a common pathological outcome associated to disturbance of protein folding and maturation pathways at the ER, generating chronic ER stress and neuronal dysfunction. Here, we review recent evidence involving ER stress in HD pathogenesis and discuss possible therapeutic strategies to target organelle function in the context of disease.

Keywords: Huntington's disease, ER stress, protein misfolding, Unfolded protein response, Huntingtin, endoplasmic reticulum.

INTRODUCTION

Huntington's disease (HD) is a late-onset autosomal dominant neurodegenerative disease causing progressive motor abnormalities and cognitive defects. The onset of psychiatric symptoms and dementia occur during early to mid-adult life, and continue in a relentless downhill course with death usually occurring twelve to fifteen years after the appearance of the disease symptoms [1, 2]. Currently, it is not clear what molecular events trigger the onset of HD and there is no effective treatment for this pathology.

HD is characterized by a widespread neuronal dysfunction and selective neurodegeneration in the central nervous system, particularly in the striatum [2]. An expansion of a polyglutamine stretch (poly(Q)) within the N-terminal region of Huntingtin (Htt) above ~40 repeats confers dominant toxic properties to the protein that are deleterious to neurons and possibly detrimental to normal Htt biological activities [2, 3]. HD represents one of a growing number of poly(Q)-related diseases that cause region-specific neuronal degeneration, including spinobulbar muscular atrophy, spinocerebellar ataxias, Machado-Joseph Disease [4, 5]. The human population exhibits an average poly(Q) of ~18 glutamines on the *Huntingtin* gene, which does not confer neurotoxic activity, yet expansions exceeding 35 glutamines result in disease development in most cases. A direct correlation is observed between the length of the poly(Q) and the average age of disease onset [6], where increased poly(Q) length accelerates disease onset, and individuals with more than 60 tandem glutamines usually develop the disease before the age of twenty [6].

Since identification of the *htt* gene mutations in HD patients, multiple murine genetic models have been generated to study the mechanisms involved in HD pathogenesis and to evaluate potential therapies in preclinical trials [7]. These models include the generation of transgenic mice expressing N-terminal fragments of mutant Htt, full length human Htt with artificial chromosomes or knock-in mouse models with an expanded poly(Q) track inserted into the mouse *htt* gene. Due to the complexity and high degree of variability in the phenotypes of these animal models in terms of survival, motor impairment and kinetics of histological alterations, it is difficult to consolidate most HD-related experimental findings. For a comprehensive understanding of the data discussed in this review, we summarize the fundamental characteristics of the HD animal models in Table **1**.

One of the major histopathological features observed in HD is the co-localization of Htt inclusions with ubiquitin [8-10]. In many cases, the formation of intracellular Htt inclusions precedes neuronal loss [11, 12] and increasing evidence suggests that abnormal Htt oligomerization (from small soluble oligomers to large aggregates) is one of the key events leading to neurotoxicity [11-13]. Nevertheless the pathological

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Table 1. Summary of the Most Common Animal Models for the Study of HD

HD Model	Transcript Length	Glutamine Repeats	Onset Date	Death Date	Striatum Characteristics	Motor Phenotypes	Neuronal Loss
R 6/2	Exon 1 (human)	148 - 153	5-6 weeks	12-14 weeks (premature death)	Early volume reduction. Rapid Htt aggregation.	Progressive abnormalities	Striatum, cortex and hipocampus
YAC128	Full length (human)	128	3 months	No lethality	15% volume reduction (9 months). Slow and progressive Htt aggregation. Inclusions evident after 10 months of age	Slight abnormalities from 3-4 months onwards	Striatum
HdhQ111 HdhQ92	knock-in	111 - 92	4 months	No lethality	Slight degeneration and increased gliosis. Intranuclear inclusions after 12 months of age	Gain deficits from 24 months of age	Striatum
Hdh(CAG)150	knock-in	150	4 months	No lethality	Increased gliosis (14 months) and nuclear inclusions	Gain and rotarod deficits, clasping, hypoactivity (4-10 months)	Striatum

mechanisms underlying neurodegeneration in HD still need further research. Different models have been put forward to explain the detrimental effects of mutant Htt expression. These models include (i) excitotoxicity [14-16], (ii) mitochondrial dysfunction/oxidative stress [17-21], (iii) transcriptional disturbances [22-27], (iv) proteasome dysfunction [28-30], and (v) altered axonal transport [31-34]. Besides, increasing evidence in different HD models suggests that alteration in the function of the secretory pathway and protein folding stress at the ER may contribute to the pathogenesis of HD. Of note, ER stress has also been suggested as a relevant factor in many other protein conformational disorders associated with abnormal protein aggregation (see examples in [35]). In this review we analyze the key mechanisms related to adaptation of ER stress or the elimination of irreversible damaged cells by apoptosis, and then summarize the specific evidence linking ER stress to HD pathogenesis. Possible therapeutic interventions to revert these subcellular/ molecular perturbations are discussed in the concluding remarks.

THE UNFOLDED PROTEIN RESPONSE (UPR)

Correctly folded proteins that pass the quality control are transported through the ER to reach their final destination including the ER itself, the Golgi apparatus, lysosomes, the endosomal system, the plasma membrane or the extracellular space. Perturbing ER function can trigger abnormal accumulation of unfolded proteins, a condition referred to as ER stress. ER stress activates the unfolded protein response (UPR), an integrated signal transduction pathway that relays information regarding the protein folding status at the ER lumen to the nucleus by controlling the expression of specialized transcription factors. Three distinct types of stress sensors are located at the ER membrane, namely double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK), activating transcription factor 6 (ATF6) α and β, and inositol requiring kinase 1 (IRE1α) (reviewed in [36]).

Activation of PERK leads to the phosphorylation and inhibition of eukaryotic translation initiation factor 2α (eIF2α), attenuating protein translation in the ER and thus decreasing unfolded protein load [37-39]. In addition, eIF2α phosphorylation augments the specific translation the mRNA of Activation of Transcription-4 (ATF4), a UPR transcription factor essential for the upregulation of many UPR-associated genes that function in amino acid metabolism and redox homeostasis [40]. IRE1α and its downstream target, X-Box-binding protein 1 (XBP-1), initiate the more conserved adaptive response of the UPR. IRE1α is a Serine/Threonine protein kinase and endoribonuclease that upon activation initiates the unconventional splicing of the mRNA encoding the transcription factor XBP-1 [41-43]. Unconventional splicing leads to the expression of a stable protein, XBP-1s (XBP-1 spliced), which is targeted to the nucleus and controls the upregulation of a subset of UPR-related genes, including genes linked to folding, protein quality control, folding, ER-associated degradation (ERAD) system, and ER/Golgi biogenesis [38]. Activation of ATF6 leads to its translocation from the ER to the Golgi where it is proteolytically processed. This event releases its cytosolic domain which is then translocated to the nucleus where it functions as a transcription factor that upregulates several ER chaperones and ERAD-related genes [44, 45]. In transcriptional control of ERAD genes, ATF6 heterodimerizes with XBP-1s to form an active transcription factor [46]. In addition to catalyzing XBP-1 mRNA processing, IRE1α has other functions in cell signaling. The cytosolic domain of activated IRE1α binds to the adaptor protein TRAF2 (TNFR-associated factor 2), triggering the activation of the Apoptosis Signal-regulating Kinase 1 (ASK1) and cJun-N terminal kinase (JNK) pathway [47-49]. The amplitude and kinetics of IRE1α signaling are modulated by the

formation of a protein complex referred as the *UPRosome* (reviewed in [36, 50, 51]). Thus, the UPR is a signaling mechanism that orchestrates adaptive processes against ER stress to recover cellular homeostasis, and it is mediated by specialized stress sensors and transcription factors that allow transcriptional reprogramming to maintain protein folding efficiency.

CHRONIC ER STRESS, THE APOPTOSIS PHASE

Under chronic ER stress, different pro-apoptotic factors trigger cell death by apoptosis [52]. At the mitochondria activation of pro-apoptotic BCL-2 family members, BAX and BAK, initiate intrinsic apoptosis through the release of cytochrome c and assembly of the apoptosome [53]. Engagement of BAX/BAK is mediated by the activation of a subgroup of pro-apoptotic BCL-2 family members termed BH3-only proteins [54]. Two BH3-only proteins, PUMA and NOXA, are strongly induced at the transcriptional level in cells undergoing prolonged ER stress [55, 56]. In addition, activation of BIM at the transcriptional and post-translational level is essential to trigger apoptosis under chronic ER stress in cellular and animal models [49, 57]. Activation of ASK1 and its downstream target JNK have been proposed to partially mediate mitochondrial-mediated apoptosis under irreversible ER stress in an analogous fashion to TNF receptor signaling [58, 59].

Sustained PERK signaling may also have pro-apoptotic effects under prolonged ER stress conditions [60]. Expression of ATF4 and possibly ATF6 regulate the induction of pro-apoptotic genes such as the CCAAT/enhancer binding protein (C/EBP) homologous (CHOP), also identified as a growth arrest and DNA damage-inducible gene (GADD153). The mechanism by which CHOP leads to cell death is not completely understood, but it may trigger apoptosis by down regulating anti-apoptotic BCL-2 [61], inducing the transcription of BIM [57], and by transcriptional control of GADD34, which interacts with protein phosphatase I to catalyze eIF2α dephosphorylation to promote the resumption of protein synthesis in a cell already burdened by unfolded proteins in the ER [62]. In murine cells, the proteolytic processing of the ER-resident caspase-12, and its human homologue caspase-4, are well accepted markers of ER stress, however their role in apoptosis is under debate [63, 64]. Recent evidence suggests that caspase-12 participates in inflammatory responses and may not operate as a pro-apoptotic protease like caspase-3 or caspase-9 [64, 65]. Other components involved in the ER stress-apoptosis response have been reviewed elsewhere [66, 67].

ER STRESS IN HD MODELS

Htt is expressed in most cell types, and experimental data suggest that it has essential functions in brain development in mice [68]. Current attempts to understand the function of wild type *htt* gene indicate that inhibition of its expression with small interfering RNAs drastically alters the structure of the ER network and ER trafficking [69], suggesting that the physiological function of Htt may be related to the morphogenesis of this organelle. The occurrence of UPR downstream responses was recently described in post-mortem brain samples from HD patients by observing the transcriptional upregulation of three UPR-responsive genes, *Chop*, *BiP* and *Herp* [70]. The 17 amino terminal region of Htt forms an amphipathic α-helical membrane-binding domain that can reversibly associate with the ER [3, 71]. The Htt/membrane interaction is dynamic because it is affected by ER stress [71, 72]. A single point mutation in Htt N-terminal region predicted to disrupt the α-helical structure displayed a striking phenotype of complete inhibition of poly(Q)-mediated aggregation. This phenotype was associated with increased Htt nuclear accumulation and higher mutant Htt toxicity in a striatal-derived mouse cell line [71, 72]. Atwal and co-workers proposed the hypothesis that Htt has a physiological function as an ER-associated protein that alternates between the nucleus and the ER in response to cellular/organelle stress [72].

An early report from Ichijo and co-workers demonstrated that ER stress activates ASK1 in models of HD, and cells lacking this protein are protected from the toxicity of poly(Q)79 peptides [47]. Similarly, the levels of ASK1 protein and ER stress markers are increased in the striatum and cortex in HD (R6/2) transgenic mice [73] (Fig. **1**). Remarkably, inhibition of ASK1 prevents the translocation of Htt fragments to the nucleus and improves motor dysfunction in mice. At the molecular level, a physical interaction between of ASK1 and mutant Htt fragments was detected, which prevented the translocation of the Htt fragments to the nucleus, correlating with improved motor function and reduced neuronal atrophy [73]. Therefore, experimental strategies to modulate the activity of ASK1 may have therapeutic benefits in HD patients.

Additional studies in cellular models of HD support the concept that chronic ER stress contributes to HD-related neurodegeneration. Expression of expanded poly(Q) peptides resembling the mutations observed in Htt triggers the activation of the stress sensors IRE1α and PERK, and activation of UPR downstream targets including JNK, ASK1, upregulation of Grp78/Bip, CHOP, and caspase-12 processing [47, 74, 75]. Induction of the proapoptotic protein BIM has been also linked to neuronal loss in cellular and animal models of HD [57, 76-78].

A recent study described the occurrence of spontaneous ER stress on a striatal cell line derived from Htt knock-in mice, showing increased basal expression of BiP, CHOP and PDI [79]. These cells are strongly sensitized to apoptosis triggered by ER stress-inducing agents [79]. SCAMP5 was recently identified as a novel regulator of the accumulation of mutant Htt. Expression of SCAMP5 is markedly increased in the

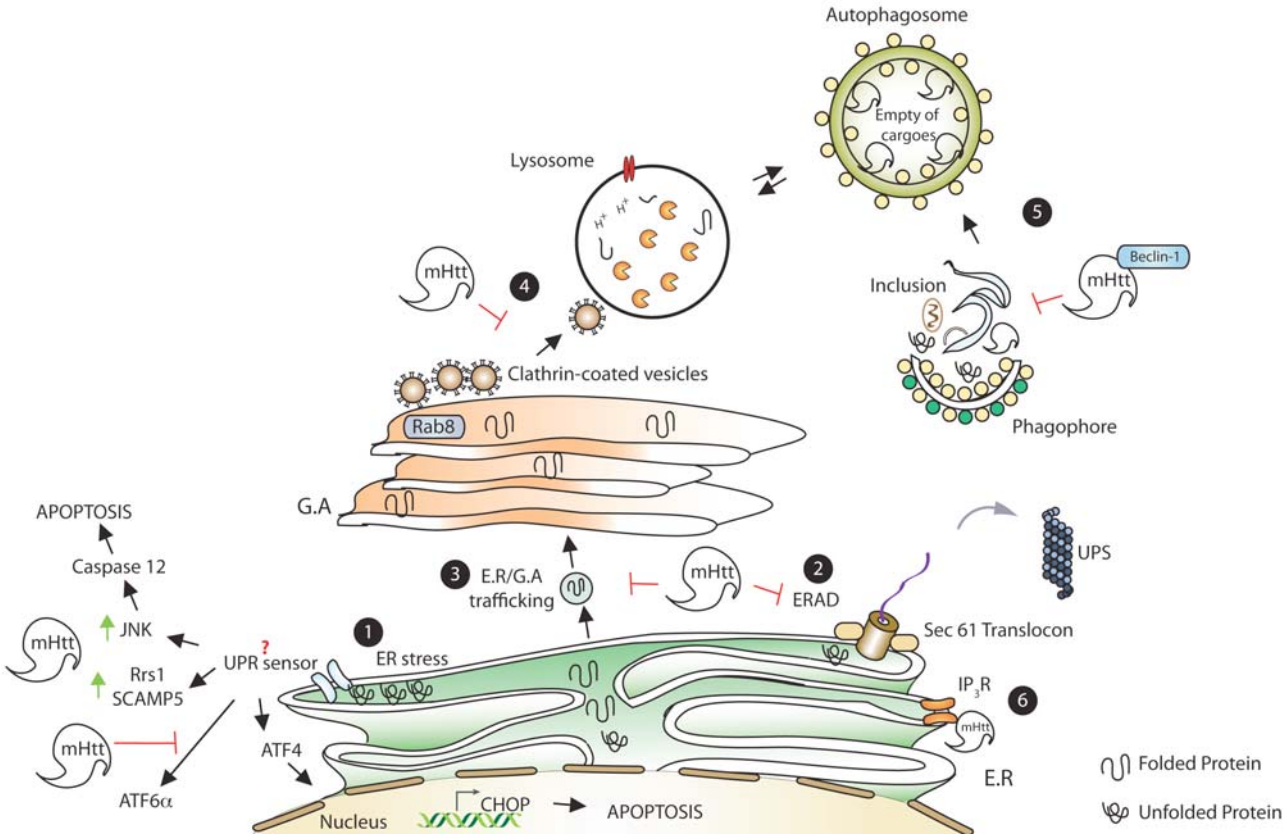

Fig. (1). Alterations of the secretory pathway function in HD: Mutant Htt (mHtt) alters the function of the secretary pathway at different stages, which may all led to a common alteration of the protein folding status at the ER lumen, triggering chronic ER stress and neuronal apoptosis. For example **(1)** mHtt expression increases the levels of ER stress associated with activation of UPR stress sensors PERK and IRE1α, leading to the upregulation of CHOP, ATF6α, Rrs1 and SCAMP5 and the activation of pro-apototic protein such as caspase-12 and JNK/ASK. ER stress induced by mHtt may be triggered by **(2)** ERAD dysfunction due to a direct interaction with components of this pathway, blocking ERAD activity and accumulation of abnormally folded protein at the ER. **(3)** mHtt also alters ER to Golgi apparatus trafficking, which may lead to accumulation in immature protein at the ER. **(4)** mHtt disrupts the exit of clathrin coated vesicles from the Golgi apparatus and lysosomal degradation and general trafficking of several proteins through the secretary pathway. **(5)** mHtt expression also impairs cargo recognition by autophagosomes and also the initiation of the autophagy pathway possibly by inhibiting Beclin-1 expression, leading to a general disturbance in protein homeostasis. **(6)** mHtt also affects the activity of the ER calcium channels IP_3R, which may lead to perturbed ER calcium homeostasis and abnormal chaperon activity at the ER lumen.

striatum of HD patients and it is induced in cultured striatal neurons by ER stress or by the expression of mutant Htt [80] (Fig. **1**). Moreover, down-regulation of SCAMP5 alleviates ER stress-induced by mutant Htt expression. Remarkably, intra striatum injection of the ER stress agent tunicamycin increases mutant Htt aggregation in two different HD mouse models [80]. Expression of Rrs1 is also involved in HD and ER stress [70]. The induction of *Rrs1* expression is an early event observed in knock-in HD mouse models, and persists over the course of the disease. Rrs1 is localized both in the nucleolus and the ER and its expression is induced by ER stress [70] (Fig. **1**). More importantly, increased expression of Rrs1 was reported in post mortem brain samples derived from HD patients [70].

Until now, only three reports are available describing the engagement of ER stress responses *in vivo* in animal models of HD [70, 73, 80]. Besides, the majority of these studies are correlative and no functional data exists to demonstrate a functional role of ER stress/UPR signaling in the progression of disease *in vivo*. Genetic or pharmacological manipulation of the UPR will contribute to understand how ER stress is regulated in HD and the cellular consequences of this process.

WHAT CAUSES ER STRESS IN HD?

Although different research groups have provided evidence for the occurrence of ER stress in HD, the actual causes of disturbances on the homeostasis of the ER remain poorly understood. Solving this issue is particularly relevant since mutant Htt has not been described inside the ER lumen, although it interacts with the cytosolic surface of organelle membranes. This contrasts with observations in other neurological diseases where mutant misfolded proteins directly

Table 2. Summary of the Evidence Describing a Perturbation on the Secretory Pathway Function in Different HD Models

Secretory Stage	Cellular	Animal model	Human Postmortem
UPR-ER stress	✔	✔	✔
Apoptosis	✔	✔	
ERAD	✔		
ER-Golgi Trafficking	✔		
Endocytosis	✔	✔	
Axonal Transport	✔	✔	✔
Autophagy	✔	✔	✔
ER calcium homeostasis	✔	✔	

accumulate and interact with ER components (see examples in [35, 81, 82]). Converging evidence highlights the relevance of the secretory pathway in HD models, including perturbations at the level of ERAD/protein quality control mechanisms, ER/Golgi trafficking, endocytosis, vesicular trafficking, ER calcium homeostasis, and autophagy/lysosomal-mediated protein degradation (Table **2**). All these defects are predicted to impact the protein folding status at the ER, generating ER stress. In the following sections we summarize emerging alternatives to explain the causes of protein folding stress at the ER in HD.

i. Altered ERAD Generates ER Stress

ERAD is a major mechanism employed by the ER protein quality control system and the calnexin cycle to eliminate misfolded or unassembled proteins generated during the folding process at the ER lumen and alterations on this process are predicted to trigger ER stress [83]. The ERAD machinery includes chaperones, transmembrane proteins and ubiquitin-associated enzymes that select, target, and retrotranslocate misfolded proteins to the cytoplasm for degradation by the proteasome system [39, 84, 85]. Susan Lindquist's laboratory reported that expression of mutant Htt leads to a fast defect in ERAD in yeast and mammalian models of HD [79] (Fig. **1**). This was associated with an entrapment of essential ERAD proteins by mutant Htt in yeast, including Npl4, Ufd1, and p97. Ectopic expression of ERAD components ameliorates mutant Htt pathogenesis, and significantly reduced the induction of ER stress in the model [79]. This is the first report that provides a mechanism to explain the occurrence of ER stress in HD. The role of ERAD impartment on Htt pathogenesis has been recently confirmed [86]. Mutant Htt interacts with gp78 in mammalian cells. Gp78 is an ER membrane-anchored ubiquitin ligase (E3) involved in ERAD. This physical interaction negatively alters the function of gp78, inhibiting ERAD and resulting in ER stress [86]. Besides, mutant Htt inhibits proteasome function [87, 88], which also precludes the degradation of ERAD substrates. Currently, ERAD is the most direct mechanism described to cause ER stress in HD cellular models. Of note, another report suggests that a similar mechanism of disease pathogenesis and ER stress induction might operate in models of familial amyotrophic lateral sclerosis [89, 90].

ii. Impairment of Vesicular Trafficking Leads to Accumulation of Immature Proteins at the ER

The disruption of vesicular trafficking at different stages, especially between the ER and the Golgi apparatus, causes the accumulation of cargo vesicles and may directly affect ER function. Vesicle trafficking alterations are predicted to trigger the accumulation of immature proteins at the ER, generating a traffic jam in the secretory pathway [36, 91]. In fact, a classical experimental paradigm of ER stress is the treatment of cells with brefeldin A, which interferes with the trafficking between the ER and the Golgi apparatus.

An important checkpoint in the secretory pathway is the vesicular trafficking between the ER and the Golgi apparatus. Cellular studies have demonstrated that mutant Htt expression perturbs ER/Golgi trafficking [92]. Alterations of ER/Golgi trafficking is observed in Parkinson's disease models where mutant α-Synuclein blocks the exit of vesicles from the ER through interactions with Rab1, triggering ER stress [86, 93]. Of note, mutant Htt expression diminishes the ER/Golgi trafficking of Val-BNDF in striatal mutant Htt knock-in cell lines [92]. Mutant Htt also perturbs the post-Golgi trafficking of epidermal growth factor receptor and atrial natriuretic factor [92] (Fig. **1**). Furthermore, the post-Golgi trafficking of clathrin-coat vesicles to lysosomes is impaired in cells expressing mutant Htt [94] (Fig. **1**). In addition, alterations in the intracellular trafficking and distribution of the excitatory neurotransmitter receptors of N-methyl-D-aspartate receptor (NMDAR) subunit 2B (NR2B) has been reported in models of HD, which may reflect a general disturbance in secretory pathway function (Fig. **2**) [95-101]. Moreover, evidence for abnormal trafficking of inhibitory neurotransmitter receptors also is available in HD models (Fig. **2**). A key mediator of pathological alterations in protein trafficking

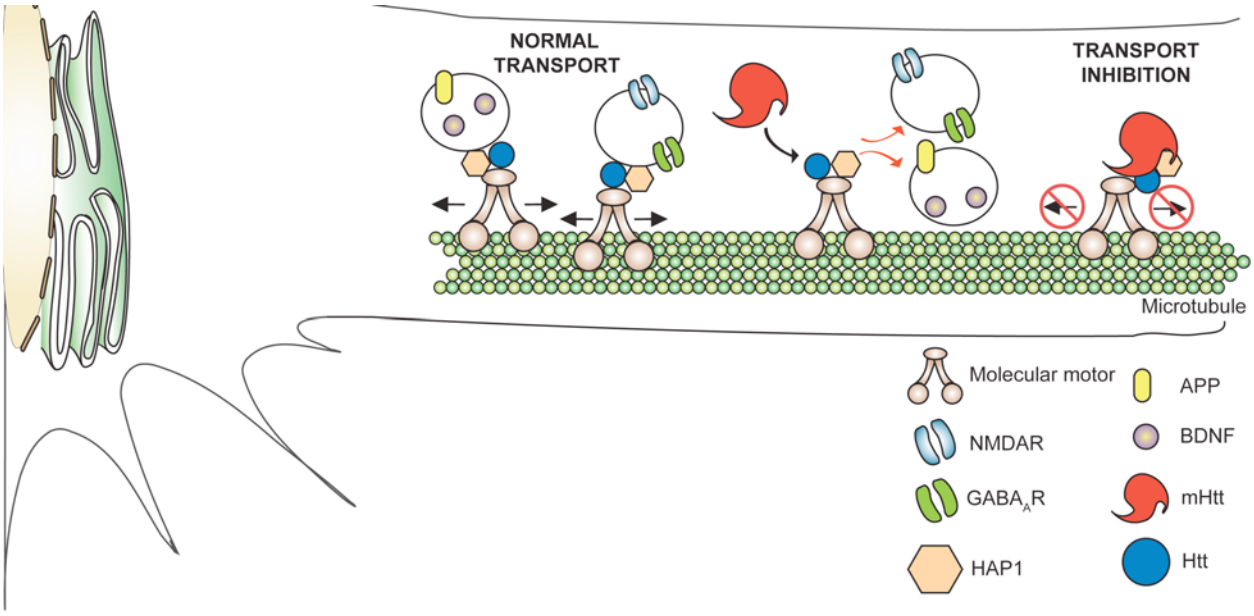

Fig. (2). Vesicle trafficking impairment in HD. mHtt disrupts the protein complex formation between the molecular motors kinesin and dynein, adaptor proteins and cargo vesicles, inhibiting transport. This alteration in cargo trafficking may generate a traffic jam during early steps of the secretary pathway triggering ER stress. Altered trafficking of protein such as APP, NMDAR, BDNF, and GABAR has been reported in HD models.

produced by mutant Htt is the inhibition of HAP1 [102-106]. HAP1 operates as an adaptor that links $GABA_A$Rs to the KIF5 kinesin motor, forming a motor protein complex for rapid delivery of $GABA_A$Rs to synapses.

Htt also interacts with Rab5, an early endosomal protein [107]. Similarly, one of the Htt interacting partners, Htt-interacting protein 1 (HIP1), functions as an endocytic adaptor protein that plays a role in clathrin-mediated endocytosis and the ligand-induced internalization of AMPA receptors (AMPARs) [108, 109]. As mentioned before, SCAMP5 is induced in HD models, and regulates the accumulation of mutant Htt aggregates [80]. Additionally, SCAMP5 expression impairs endocytosis, and knocking down SCAMP5 recovers endocytic levels and alleviates ER stress-induced by mutant Htt. This data suggest a functional role of endocytosis impairment on the occurrence of ER stress in HD. It remains to be determined whether or not reversion of the trafficking defects observed in HD models attenuates ER stress as shown for Parkinson's disease [93].

Alteration in axonal transport has been consistently reported in models of HD [110-112], which may also lead to traffic jam in earlier secretory compartments (Fig. **2**). For example, several studies suggest that wild-type Htt plays a role in axonal transport and that disease associated mutations interfere with this function [103, 113-122]. Loss of Htt expression or the expression of mutant Htt affect the axonal transport of BDNF and the amyloid precursor protein (APP) [123]. Interestingly, Htt and its interacting partner HAP1 have been reported to physically associate and alter the function of components of both the anterograde and retrograde transport machinery [105, 124-126]. Alternatively, mutant Htt may inhibit fast axonal transport through a mechanism involving activation of JNK3 and phosphorylation of kinesin-1 [127]. A direct contribution of axonal transport defects to the engagement of ER stress responses in HD has not been provided yet.

iii. Defects in Autophagosomal Vesicular Compartments Lead to Accumulation of Abnormal Protein Aggregates

Lysosomal-mediated degradation can be viewed as the final stage of the secretory pathway, responsible for removing proteins that traffic through the secretory and endocytic pathways [128-130]. Macroautophagy, here referred as autophagy, is a major mechanism for the lysosomal-mediated catabolism of cytoplasmic components including damaged or superfluous organelles, toxic protein aggregates and intracellular pathogens, and also operates as a survival pathway against ER stress [36]. Alterations in autophagy are predicted to trigger the accumulation of misfolded proteins and ER stress as recently shown *in vivo* [131]. Autophagy is characterized by the encapsulation of cargo on a double-membrane vesicle to form the autophagosome, in a process controlled by a large family of autophagy-related genes (termed *ATGs*). Autophagy was initially described as an adaptive cellular mechanism triggered during metabolic stress conditions, providing nutrients by recycling cellular components. Recent studies, however, indicate a crucial role of autophagy as a protein quality control mechanism in the brain, based on the fact that selective genetic inactivation of autophagy in the

nervous system triggers spontaneous neurodegeneration associated with the accumulation of abnormal protein inclusions in the brain [132, 133].

Yuan and co-workers demonstrated that mutant Htt inclusions recruit an essential autophagy regulator, Beclin-1, possibly impairing Beclin-1 pro-autophagy activity. Beclin-1 exhibits inclusion-like distribution in HD-derived post-mortem brain samples, co-localizing with Htt [134] (Fig. **1**). The authors speculated that this event might lead to enhancement of mutant Htt accumulation and general alterations of protein homeostasis. Interestingly, Ana Maria Cuervo's group recently reported that mutant Htt expression leads to a defect in the recognition of cargo by macroautophagy in cellular and mouse models of HD [135] (Fig. **1**). This data was confirmed in lymphoblasts from patients affected with HD and also in post mortem striatal samples from HD-affected individuals [135], suggesting a general alteration in protein degradation in the disease.

Another recent report indicated that the expression of full-length Htt lacking its poly(Q) stretch region in a knock-in mouse model for HD reduces significantly mutant Htt aggregates, ameliorates motor deficits and extends lifespan in comparison to an HD mouse model [136]. This phenotype correlates with enhanced autophagy by the expression Htt lacking the polyQ region. Moreover, mice lacking the Htt polyQ region live significantly longer than wild-type mice [136], suggesting that autophagy upregulation may be beneficial both in diseases caused by toxic intracellular aggregate-prone proteins and also as a lifespan extender in normal mammals. Finally, in addition to accumulation of abnormally folded proteins by inhibiting autophagy, damaged or non-functional organelles, including ER [137], may also accumulate in HD due to impaired autophagy-mediated degradation. In summary, increasing evidence suggests that inefficient autophagy in HD may lead to abnormal accumulation of substrates, leading to altered protein homeostasis and possibly to secretory pathway stress.

iv. Deregulation of ER Calcium Homeostasis Alters ER Protein Folding

Sustained calcium release from the ER negatively affect the activity of different ER-resident chaperons, leading to ER stress due to deficiency of protein folding (reviewed in [138, 139]). ER calcium homeostasis is primary controlled by different components including inositol 1,4,5-triphosphate receptors (IP_3R), ryanodine receptors (RyR), the sarco-endoplasmic reticulum calcium ATPase pump (SERCA) and components of BCL-2 family proteins [140, 141]. It is interesting to mention that one of the classical experimental paradigms of ER stress is the treatment of cells with thapsigargin, a SERCA inhibitor, which leads to decreased ER luminal calcium levels.

Expression of mutant Htt also drastically affects calcium homeostasis both at the level of the cytosol/plasma membrane and the ER [142]. The N-terminal membrane targeting sequence of mutant Htt disrupts cytosolic calcium levels in glutamate-challenged cell cultures [3]. In addition, cultured neurons expressing mutant Htt show increased susceptibility to apoptosis triggered by P2X7-receptor stimulation [143]. P2X7 are ATP-gated cation channels known to modulate neurotransmitter release from neuronal presynaptic terminals. Cultured striatal neurons derived from full-length mutant Htt transgenic mice lead to altered calcium signaling and apoptosis [144, 145]. A yeast two-hybrid screen revealed that mutant Htt interacts with the IP_3R. Further studies confirmed this interaction *in vivo*, and showed enhanced IP_3R activity upon interaction with mutant Htt [146]. These examples, which constitute a small sample of a large body of literature relating mutant Htt expression with calcium homeostasis disturbances [147, 148], suggest a relevant role of disrupted calcium homeostasis in HD models. Of note, a recent report indicated that modulation of calcium homeostasis actually has a clear impact on protein homeostasis on the context of diseases [149].

v. Other Possible Mechanisms of ER Stress in HD

In addition to the mechanisms described in the previous sections, other interesting possibilities remain to be tested to explain the occurrence of ER stress in HD. One of the well-documented pathological effects of mutant Htt in the nucleus is the entrapment of transcription factors, altering gene expression patterns [22, 23, 150, 151]. It remains to be determined whether or not mutant Htt interacts with UPR transcription factors (i.e. XBP-1, ATF4, ATF6, CHOP). Remarkably, a recent report suggested that processing of ATF6α is impaired in both animal models and HD patients [152], which may diminish the ability of neurons to adapt to ER stress. Besides, wild type Htt may operate as a stress sensor at the ER membrane since its distribution is modulated by ER stress [71]. It may be also interesting to test the possibility that wild type and/or mutant Htt interacts with UPR stress sensors regulating their activity. Of note, this mechanism has been shown to operate in familial ALS models [153]. Another interesting hypothesis to explore could be related with the observation that knocking down wild type Htt specifically disrupts the ER network pattern [69, 154]. Mutant Htt may lead to loss of function of wild-type Htt, altering the morphogenesis of this organelle and its broad physiological functions.

Taken together the evidence reviewed above suggests that mutations in Htt lead to impairment of protein transport and processing at different stages of the secretory pathway, possibly resulting in accumulation of immature proteins at the ER. Therefore, multiple abnormal activities of mutant Htt may converge to generate ER stress, a common pathological outcome in the disease process.

CONCLUDING REMARKS: THERAPEUTIC STRATEGIES TO DECREASE PROTEIN-FOLDING STRESS

HD is a fatal neurodegenerative disease with no effective treatment. Most clinical trials of drugs designed and validated in HD mouse models have failed to alleviate disease progression in HD patients. This may be explained because they have often focused in targeting truncated forms of mutant Htt with high levels of expression or have been tested in experimental mouse models with a pure genetic background, a condition far removed from the scenario observed in humans. The literature addressing the molecular mechanism of HD is complex and diverse in terms of possible targets and mechanisms of the pathology. The key strategy toward designing new therapeutic strategies may rest on molecular events that are transversal to different cellular and animal HD models, with a clear correlate in human HD-derived samples.

In this review, we have attempted to perform a systematic analysis to uncover a common molecular feature observed in different cellular and animal HD models. The data discussed here support an emerging concept suggesting that secretory pathway-related processes are major cellular events affected in HD. Defects in HD neurons are observed almost at every stage of the secretory pathway, including chaperone-mediated protein folding, ERAD and related quality control mechanisms, vesicular transport, ER network patterning and lysosome-mediated degradation. Most of these events may generate alterations in the protein folding process, leading to chronic ER stress. Surely *in vivo* and human post-mortem studies are needed to help define the impact of secretory pathway stress in HD. However, taken together these data suggest that experimental strategies to alleviate ER stress or improve secretory pathway function may benefit HD patients.

Therapeutic strategies to alleviate ER stress may be achieved by the use of pharmacological approaches that include treatments with chemical chaperons, small molecules to activate UPR components, or gene therapy approaches to deliver key folding mediators (i.e. chaperones and foldases) to express modulators of the UPR or quality control mechanisms. In this line, administration of chemical chaperones, including 4-PBA and TUDCA [155, 156], delay HD progression in animal models, and both drugs are efficacious in decreasing ER stress levels in other disease models [157-160]. Secondly, secretory pathway stress may be reduced by targeting degradation or clearance pathways of misfolded proteins such as ERAD or autophagy, alleviating the load of unfolded proteins at diverse stages or sub-compartments of the secretory pathway. Increasing evidence indicates that mutant Htt aggregates have a high dependency on autophagy for their clearance, while wild-type species do not rely on autophagy for their degradation [161], and different pharmacological manipulations to enhance autophagy increases the clearance of Htt aggregates and delays the progression of HD in cellular and animal models [134, 161-166]. Since protein misfolding and ER stress is an emerging feature of diverse neurological disorders with high incidence in the human population including Parkinson and Alzheimer's disease, it is predicted that interesting new drug candidates will emerge to improve progression of disease in HD patients.

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