Review

Huntington's disease: underlying molecular mechanisms and emerging concepts

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Huntington's disease (HD) is a progressive neurodegenerative disorder for which no disease modifying treatments exist. Many molecular changes and cellular consequences that underlie HD are observed in other neurological disorders, suggesting that common pathological mechanisms and pathways may exist. Recent findings have enhanced our understanding of the way cells regulate and respond to expanded polyglutamine proteins such as mutant huntingtin. These studies demonstrate that in addition to effects on folding, aggregation, and clearance pathways, a general transcriptional mechanism also dictates the expression of polyglutamine proteins. Here, we summarize the key pathways and networks that are important in HD in the context of recent therapeutic advances and highlight how their interplay may be of relevance to other protein folding disorders.

HD: one gene many consequences

HD is an autosomal dominant neurodegenerative disorder that affects approximately 5–10 individuals per 100 000. Individuals typically suffer from progressive motor and cognitive impairments, loss of self and spatial awareness, depression, dementia, and increased anxiety over the course of 10–20 years before death. Currently, treatment is limited to suppressing chorea, the involuntary, irregular movements of the arms and legs that accompanies HD, and battling the mood altering aspects of the disorder with no disease modifying treatments available [1].

At the molecular level, HD is caused by a CAG trinucleotide repeat expansion within exon 1 of the *HTT* gene. In affected individuals, the number of CAG repeats expands from the normal population range (on average between 16 and 20 repeats) to >35 repeats [1,2]. This gives rise to an elongated polyglutamine tract at the amino terminus of the translated huntingtin (HTT) protein that is associated with protein aggregation and a gain-of-function toxicity [3].

Mutant huntingtin (mHTT) is highly aggregation prone and the formation of cytoplasmic aggregates and nuclear inclusions throughout the brain is one of the most striking hallmarks of HD [4,5]. Polyglutamine inclusions contain highly ordered amyloid fibers with high β-sheet content and low detergent solubility; they also sequester numerous other proteins, including factors important for transcription and protein quality control, suggesting that their presence is deleterious to cellular function and contributes to a complex loss-of-function phenotype [6]. Several lines of evidence implicate small oligomeric forms of mHTT as the most toxic species and propose that the formation of large inclusions may represent an alternative coping strategy in which mHTT is partitioned into a less pervasive structure [7]. Aggregate formation is a complex multistep process in which mHTT monomers assemble into a range of intermediate oligomeric species before inclusions are formed. This process is influenced by the amino acid sequences flanking the polyglutamine stretch, post-translational modifications of mHTT, and levels of molecular chaperones [8–12]. The spectrum of oligomeric conformations adopted by mHTT has made it challenging to understand the pathogenic role of each species as mHTT monomers, oligomers, and large inclusions can coexist and disrupt multiple cellular pathways and influence disease progression. Additionally, extracellular polyglutamine aggregates can be internalized by cells to promote polyglutamine aggregation. This raises the intriguing possibility of mHTT spreading between cells and regions during disease progression [13].

Despite its monogenic nature, HD pathogenesis is incredibly complex. The HTT interactome is composed of proteins involved in transcription, DNA maintenance, cell cycle regulation, cellular organization, protein transport, energy metabolism, cell signaling, and protein homeostasis (proteostasis) [14]. Given this diversity of molecular interactions, it is unsurprising that wide-scale destabilization of the proteome and subsequent disruption of multiple cellular processes occurs in the presence of mHTT (Figure 1).

Recent advances in our understanding of mHTT synthesis, processing, aggregation, and toxicity have suggested several therapeutic approaches, several of which have shown some promise against HD. Furthermore, despite being caused by unrelated proteins with distinct interactomes and unique expression patterns, other polyglutamine disorders, Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) all share characteristics with HD (Box 1), suggesting that common genetic modifiers of neurodegeneration exist and could be targeted as a potential panacea for neurological disorders [3,6,15]. Here, we highlight recent advances

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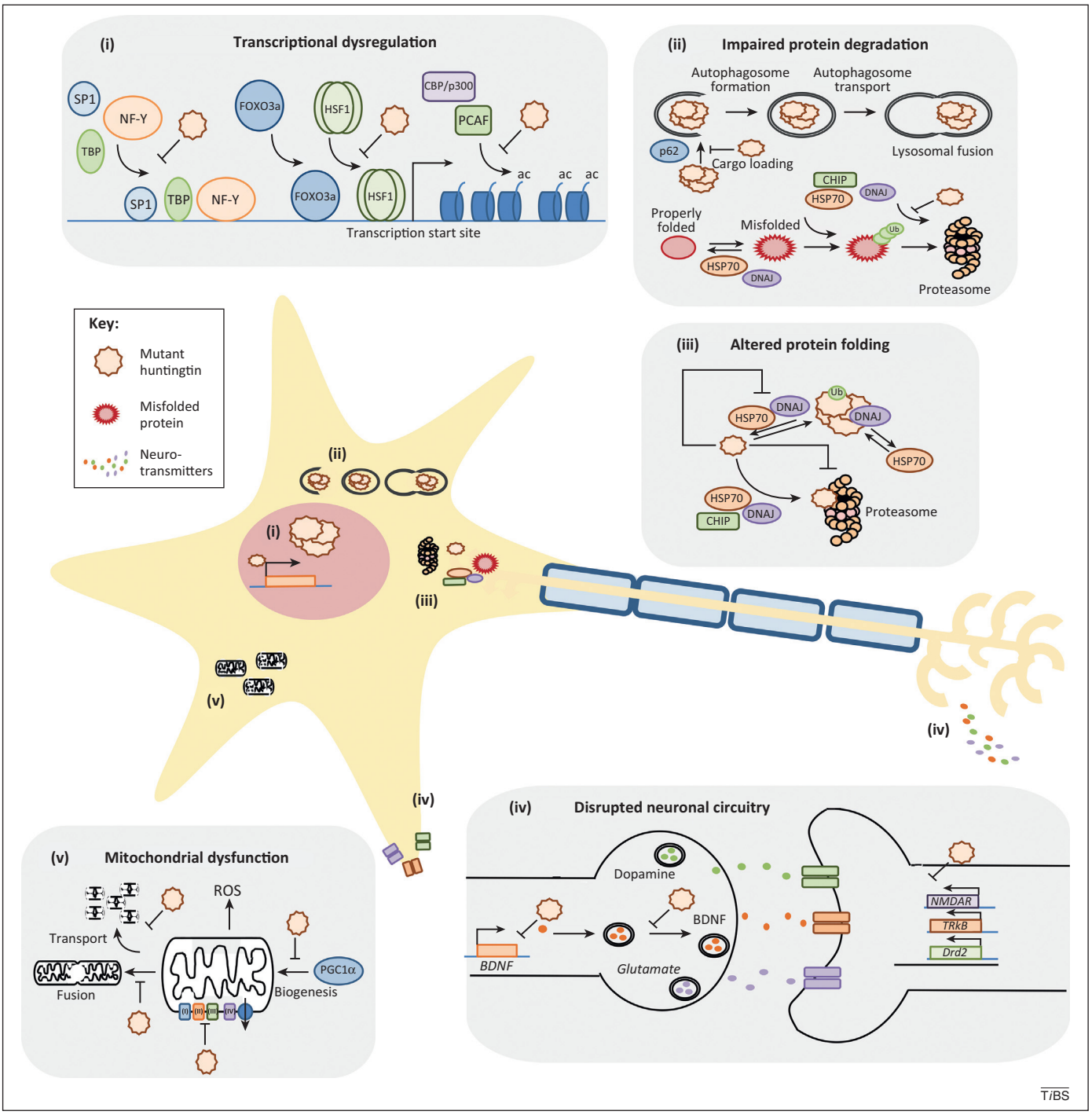

Figure 1. Major cellular pathways disrupted in Huntington's disease (HD). A diverse array of cellular processes is disturbed by the presence of mutant huntingtin (mHTT). Here, we depict a neuron and demonstrate the major sites of molecular disruption caused by the presence of mHTT. Roman numerals indicate which zoomed-in views (surrounding boxes) correspond to particular areas/processes in the cell. We provide a simplified overview of the pathogenic pathways in HD discussed in our review, specifically (i) transcriptional dysregulation of basal and inducible gene expression, (ii) impaired protein degradation, (iii) altered protein folding, (iv) disrupted synaptic signaling, and (v) perturbed energy metabolism through altered mitochondrial maintenance and localization. Pathway dysfunction can arise from direct or indirect interference of key components by soluble, oligomeric, and/or aggregated mHTT. Although we represent each pathway as an insular process, in reality, disruptions in one pathway probably influence HD pathogenesis (at least in part) by exacerbating dysfunction of other key events.

in HD research and address how these findings might further our understanding of other neurodegenerative diseases.

Altered neural circuitry underlies cognitive and molecular abnormalities in HD

Perturbed neuronal activity underlies the cognitive and physical decline observed in HD patients [16,17]. The expression of genes important for calcium homeostasis, neuronal differentiation, neuronal survival, and neurotransmission are reduced early in HD [17]. In addition, mHTT dramatically impairs neurotransmitter release at presynaptic junctions by physically impeding axonal transport and by reducing the efficiency with which synapse-bound cargo can be loaded onto microtubules [18,19].

Box 1. Protein conformational disease

HD is one of nine inherited neurodegenerative disorders caused by an expansion of glutamine residues in the causative protein, the others being spinocerebellar ataxias (SCAs) 1, 2, 3, 6, 7, and 17, spinobulbar muscular atrophy (SBMA), and dentatorubral-pallidoluysian atrophy (DRPLA) [3]. Toxicity in these disorders stems primarily from a gain-of-function conferred by the polyglutamine stretch, the pathogenic length of which is disease-specific. All nine disorders arise from aberrant protein folding as a result of polyglutamine expansion and can therefore be thought of as protein conformational diseases [3]. Interestingly, other neurodegenerative diseases such as AD, PD, and ALS are also characterized by the presence of misfolded and aggregated proteins, specifically extracellular Aβ plaques and intracellular tau tangles in AD, α-synuclein Lewy bodies in PD, and SOD-1 or TDP-43 aggregates in ALS [3,6]. Despite significant differences in pathology, these disorders share similarities with HD such as late onset of disease, neuronal dysregulation, altered energy metabolism, and global changes in gene expression. This suggests that the chronic expression of misfolded proteins may cause progressive neuronal toxicity through common mechanisms and pathways.

Box 2. Glutamatergic excitotoxicity

Glutamate is the most abundant excitatory neurotransmitter in the central nervous system and is associated with synaptic plasticity and learning. Glutamate binds to and activates NMDARs, which results in calcium influx to the cell. Normally this interaction is transient; however, an excess of extracellular glutamate can lead to continuous stimulation of NMDARs and neuronal death in a process termed excitotoxicity [16,83]. Excitotoxic cell death was one of the first mechanisms proposed to explain the selective vulnerability of MSNs in HD [83]. The first evidence supporting this claim came from the development of similar biochemical and behavioral symptoms in animals injected with the NMDAR agonist quinolinic acid [83]. In contrast to the relatively spared interneurons, MSNs express high levels of NMDARs, perhaps providing some explanation for the increased susceptibility of these cells to mHTT [83]. Given that excitotoxicity has also been described as a feature of AD, PD, and ALS, it is possible that excitotoxicity is central to many neurological disorders.

Although the presence of mHTT is deleterious to many neuronal subtypes, medium spiny neurons (MSNs) of the striatum exhibit enhanced vulnerability. This observation has been attributed to several factors, including reduced neurotrophin availability, MSN-specific SUMOylation of mHTT mediated by the small GTP binding protein Rhes, and glutamate receptor mediated excitotoxic cell death (Box 2) [20–22].

Glutamatergic excitotoxicity of MSNs through aberrant *N*-methyl-D-aspartate receptor (NMDAR) activity is proposed to be a major component of HD. NMDAR activity can be modified by neuroactive metabolites of the tryptophan degradation pathway, particularly kynurenic acid (KA) (an NMDA antagonist) and quinolinic acid (QA) (an NMDA agonist) [16,23]. The abundance of these molecules in the bloodstream is regulated by the enzyme kynurenine-3-monoxoygenase (KMO), the inhibition of which leads to elevated levels of the neuroprotective metabolite KA in the brain. Manipulation of the tryptophan pathway or KMO activity has been shown to abrogate disease in diverse models of HD, AD, and PD [23–25]. Importantly, the novel KMO inhibitor JM6 successfully suppresses disease in rodent models of HD and AD without entering the brain, thereby providing support that modifying metabolites in the blood can markedly influence neurological dysfunction and that brain permeability may not be a prerequisite for small molecule treatments of HD [23].

Alternatively, excitotoxicity can be directly suppressed with small molecule NMDAR antagonists. Treatment with one such molecule, memantine, has contrasting effects on HD progression in mice [20]. At high doses, memantine inhibits both synaptic and extrasynaptic NMDAR activity and exacerbates disease progression, whereas at low doses that selectively inhibit extrasynaptic NMDAR activity it can suppress mHTT toxicity [20]. The suppression of mHTT toxicity via low-dose treatment with memantine is proposed to occur through alterations to the cellular protein quality control machinery and supports observations from *Caenorhabditis elegans* that neuronal signaling regulates proteostasis and polyglutamine toxicity throughout the organism [20,26–28].

Together, these findings highlight the opposing roles of synaptic and extrasynaptic NMDAR activity in HD and suggest that changes in neuronal circuitry could result in non-autonomous cellular dysfunction in neuronal and non-neuronal cells as a result of altered protein homeostasis. This could explain recent reports that striatal projection neurons present in neural grafts die when transplanted into HD patients even though the grafted cells do not express mHTT. However, other cell non-autonomous mechanisms may also explain these observations [29]. Whereas it is clear that changes in brain physiology and neuronal circuitry underlie behavioral abnormalities in HD, the precise molecular mechanisms responsible for cellular dysregulation are more nebulous. Here, we attempt to summarize the major molecular pathways linked to neuronal dysfunction in HD.

Mitochondrial dysfunction and impaired energy metabolism

Numerous observations support a role for mitochondrial dysregulation in HD pathogenesis. For example, mHTT associates with the mitochondrial outer membrane and leads to an impairment of electron transport chain (ETC) complexes II and III, an observation that correlates with depletion of the intracellular ATP pool and increased reactive oxygen species (ROS) [30]. The mitochondrial tricarboxylic acid (TCA) cycle enzyme aconitase is particularly susceptible to superoxide-mediated inactivation, suggesting that generation of ROS through disruption of the ETC may further restrict ATP production through inhibition of the TCA cycle [30].

Another example of mitochondrial dysregulation in HD involves mitochondrial trafficking. Retrograde and anterograde mitochondrial trafficking along axons is impeded by mHTT leading to disruption of mitochondrial maintenance and reduced deposition of mitochondria at sites with high energy demand such as synapses [31]. Furthermore, mHTT has been shown to impair peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator-1α (PGC-1α) mediated expression of genes that regulate mitochondrial biogenesis [32–34]. Early mitochondrial fragmentation has also been reported in HD and has recently been proposed to occur through GTPase dynamin related protein-1 (DRP-1).

In support of this, reducing DRP-1 GTPase activity restores aberrant mitochondrial fission, mitochondrial transport, and improves phenotype in HD mice [35]. These observations highlight the potential significance of perturbed mitochondrial function in HD and suggest a mechanism by which mHTT causes neuronal dysfunction by disrupting energy metabolism and promoting oxidative damage.

Perturbations in mitochondrial maintenance, localization, and activity have also been reported for ALS, AD, and PD [36,37]. This is particularly intriguing as a deficit in cellular energy could have far-reaching consequences, not only in terms of neuronal signaling but also for maintenance of a functional proteome in general, as a progressive depletion of ATP levels could impede core activities of proteostasis and transcriptional networks.

Transcriptional dysregulation in HD

The expression of mHTT has global effects on the transcriptome suggesting that transcriptional dysregulation is a key feature of HD pathogenesis [38]. mHTT interacts with, and disrupts, major components of the general transcriptional machinery, affecting both general promoter accessibility and recruitment of RNA polymerase II [38]. Studies in presymptomatic HD brains have shown that soluble mHTT oligomers interact with and impede the function of specificity protein 1 (SP1), TATA box binding protein (TBP), the TFIID subunit TAFII130, the RAP30 subunit of the TFIIF complex, and the CAAT box transcription factor NF-Y, all of which are important mediators of general promoter accessibility and transcription initiation [39–43].

The expression of mHTT also disrupts the activity of histone acetyl transferases (HATs), such as CBP/p300 and p300/CBP associated factor (PCAF), which results in histone hypoacetylation and increased heterochromatin formation [44]. Strategies that utilize histone deacetylase (HDAC) inhibitors to correct transcriptional dysregulation by restoring or enhancing histone acetylation have been shown to ameliorate mHTT toxicity in flies and mice, thereby supporting a central role for transcriptional dysregulation in HD [44]. However, because of the broad action of many HDAC inhibitors and the promiscuous nature of HDAC activity, the precise mechanisms by which these molecules influence mHTT toxicity remain unclear [45]. Yet, genetic studies in flies and worms suggest that HDACs 1 and 3 are required for mHTT toxicity and could be the primary targets of HDAC inhibitors [46,47].

Impaired protein homeostasis in neurodegenerative disease

Although aberrant neuronal signaling, energy production, and gene expression underlie the molecular basis of HD, ultimately cell function is dictated by the functional properties of the proteome. Therefore, to fully describe HD it is essential that we understand and integrate how the dynamic properties of the proteome are reorganized upon expression of mHTT.

Under normal conditions, proteome integrity is maintained by the proteostasis network (PN), the main effectors of which are molecular chaperones and clearance machineries (Box 3) [48]. Intriguingly, chronic expression of expanded polyglutamine peptides results in an age-dependent collapse of proteostasis as evidenced by increased aggregation and mislocalization of metastable proteins [49,50]. Recent proteomic analysis of the mHTT interactome has revealed that members of the heat shock protein 90 kDa (HSP90), TCP-1 ring complex (TRiC), HSP70, and DNAJ chaperone families all associate with mHTT [14]. Moreover, levels of HSP70 and DNAJ chaperones are progressively reduced in brain tissues of HD mice through a combination of sequestration and transcriptional dysregulation [43,51]. Proteostasis collapse also occurs in the presence of mutant superoxide dismutase 1 (SOD1), an aggregation-prone protein that is the primary cause of familial ALS, and expression of synthetic amyloid forming peptides, suggesting that proteostasis collapse may be a general feature of protein-folding disorders [52,53]. These observations support a model where the chronic expression of aggregation-prone proteins, such as mHTT, titrates chaperones away from clients and leads to global disruption of the proteome.

In support of this hypothesis, restoration or enhancement of protein-folding capacity through chaperone

Box 3. The proteostasis network

The transition from nascent polypeptide to functional tertiary structure is an incredible challenge in the context of the intracellular milieu. Errors in translation coupled with the disordered structure of newly synthesized peptides promote inappropriate intra- and intermolecular interactions within the cell that can lead to protein mislocalization, aggregation, cell dysfunction, and death; therefore, the ability to efficiently maintain proteostasis is essential [84]. Proteome integrity is maintained through the concerted action of molecular chaperones, which are proteins that facilitate the folding of nascent polypeptide chains, recognize and refold misfolded proteins, disassemble protein aggregates, and direct clients to distinct subcellular locations [84]. In addition, irrevocably damaged or misfolded proteins must be selectively degraded as and when required. This clearance is achieved via two major pathways, the UPS and autophagy. Old or irreversibly damaged or misfolded proteins are recognized by chaperone/co-chaperone complexes, polyubiquitylated by E3 ligases, and transported to the proteasome, a large multisubunit complex that proteolytically degrades ubiquitylated substrates [84]. By contrast, bulkier cargo, such as protein aggregates or organelles, are too large to pass through the proteasomal pore. Instead, they are sequestered by the formation of an autophagosome, which is then transported along microtubules and fused with the lysosome where the cargo is degraded [62,84]. Through these mechanisms, cells successfully balance protein synthesis, folding, trafficking, and degradation to maintain proteostasis. The network of proteins involved in this is collectively referred to as the PN [48,84].

The PN is able to maintain proteome integrity in response to fluctuating intra- and extracellular conditions. However, more extreme conditions that cause acute, wide-scale disruption of protein folding (e.g., elevated temperature, altered pH, increased ROS) can place demands on the PN that cannot be met. In response to these insults, the composition of the PN is dramatically altered by the activation of stress response pathways such as the HSR, unfolded protein response (UPR), and oxidative stress response (OSR) [84]. These pathways are regulated by the transcription factors HSF1 (HSR), DAF-16/FOXO3a (HSR and OSR), SKN-1/NRF2 (OSR), ATF-6, PERK, and XBP1 (UPR). When activated, these stress transcription factors act in a concerted manner and lead to the upregulation of molecular chaperones and other pro-survival genes, thereby enhancing protein folding capacity and promoting cell survival.

overexpression or enhancement of chaperone gene regulatory pathways suppresses mHTT toxicity in multiple models of HD [54]. These effects have been attributed to the suppression of aggregate formation, enhanced mHTT degradation, and the partitioning of mHTT into less toxic structures [54]. Further support comes from a screen of HSP70 and DNAJ chaperones for suppressors of polyglutamine aggregation in mammalian cells that identified the DNAJB subclass of molecular chaperones (particularly DNAJB2a, B6b, and B8) as potent inhibitors of mHTT aggregation, with DNAJB2a overexpression also found to suppress aggregation in HD mice [55,56]. DNAJ chaperones are generally considered to be co-chaperones for the main effector of protein refolding, HSP70; however, DNJB6b and DNAJB8 appear to suppress polyglutamine aggregation independent of HSP70, suggesting that mHTT can be targeted by several different molecular chaperone machines, each of which could have distinct effects on the folding and aggregation state of mHTT.

The notion that novel-folding pathways can influence polyglutamine toxicity is further supported by recent findings that the gene *moag-4* (encoding a small protein of unknown function), identified in a *C. elegans* RNAi screen for modifiers of aggregation (MOAG), influences polyglutamine aggregation independently of the proteasome or autophagy and without activation of stress response pathways or upregulation of molecular chaperones [57]. Although a precise role for moag-4 in the absence of disease-causing proteins is unknown, the presence of moag-4 appears to promote a conformational change in polyglutamine monomers that facilitates their assembly into large aggregate species via the formation of compact oligomeric structures [57]. The involvement of moag-4 in polyglutamine toxicity is conserved across species, as RNAi of the human orthologs of moag-4, SERF1A, and SERF2 suppresses mHTT aggregation and toxicity in neuronal cells. Furthermore, *moag-4* deletion in worms also suppresses the toxicity of amyloid-β (Aβ) and α-synuclein, two aggregation-prone proteins central to AD and PD pathogenesis, respectively, suggesting that moag-4 could have a role in multiple neurodegenerative disorders [57].

These observations demonstrate that numerous protein quality control pathways can suppress mHTT toxicity and that several novel protein quality control mechanisms can be targeted for therapeutic gain.

Impaired protein degradation pathways in HD

The ubiquitin proteasome system (UPS) (Box 3) has been a focus of study in HD since mHTT inclusions were first identified as ubiquitin-positive [58]. An accumulation of ubiquitin chains occurs in brain tissue from HD patients and HD mice [59]; however, the mechanism by which mHTT causes disruption of the UPS is unclear. Recent findings suggest that the accumulation of ubiquitin chains in HD is not a result of direct proteasome inhibition by mHTT oligomers. Rather, it appears that imbalance of the UPS arises due to an overwhelming of the PN by mHTT, which in turn leads to increased levels of improperly folded clients. As a consequence, the abundance of polyubiquitylated proteins within the cell increases causing a 'queue' of polyubiquitylated proteins that overloads the proteasome. This is further enhanced by reduced trafficking of ubiquitylated clients as a consequence of molecular chaperone sequestration [60].

In addition, whereas autophagosome formation and lysosomal fusion appear to be unaffected by the expression of mHTT, recent evidence suggests that the engulfment of cytosolic cargo (particularly organelles) by autophagosomes is inefficient in HD, possibly due to aberrant interactions among p62, ubiquitin chains, and mHTT [61]. UPS impairment and defects in autophagy are thought to contribute to AD, PD, and ALS, suggesting that a loss of protein degradation pathways could also be a central feature of neurodegeneration [58,62].

Aberrant activation of stress responses in polyglutamine disease

Cytoprotective stress responses (Box 3) are crucial determinants of lifespan and healthspan that must be controlled with exquisite precision to maintain cellular health and prevent disease. Recent evidence suggests that the dysregulation of stress response transcription factors could contribute to neurodegeneration.

The ability to effectively initiate the heat shock response (HSR) (Box 3) is compromised in mouse and cell models of HD. This correlates with reduced occupancy of HSF1 at the promoters of chaperone genes following stress, but also reflects genome-wide changes in HSF1 DNA binding, probably due to changes in HSF1 expression, and/or altered chromatin architecture [63–65]. Dysregulation of the HSR has also recently been described in models of other polyglutamine/amyloid disorders [42,53], whereas activation of HSF1 has been shown to suppress mHTT, mutant ataxin-3 [another polyglutamine disease protein that is the cause of spinocerebellar ataxia type 3 (SCA-3)], and Aβ aggregation and toxicity in cells, flies, worms, and mice [54].

Likewise, the activity of the metabolic stress factor, DAF-16/FOXO3a, is also impaired in HD mice, possibly through dysregulation of the deacetylase SIRT1 [66]. Overexpression of SIRT1 increases DAF-16/FOXO3a activity and improves phenotype in HD mice, possibly by reducing oxidative stress [66,67]. The activity of SIRT1 is also involved in the regulation of HSF1 by enhancing DNA-binding activity; therefore, it may be of interest to ascertain whether increased HSF1 activity also contributes to SIRT1-mediated neuroprotection in HD mice [68]. Furthermore, reduced insulin signaling (which reduces polyglutamine toxicity in *C. elegans* in an HSF1- and DAF-16-dependent manner), through reduced levels of insulin receptor substrate 2 (IRS2) or insulin-like growth factor 1 (IGF-1), improves disease phenotypes in mouse models of HD and AD, respectively [69,70].

These observations, coupled with our existing knowledge that stress transcription factors are prominent modifiers of lifespan and proteostasis, support a model in which the progressive loss or compromise of stress response pathways renders neurons increasingly vulnerable to transient environmental insults and to the chronic presence of mHTT or other aggregation-prone proteins. Early activation of these pathways has been shown to ameliorate disease progression in multiple models of neurodegenerative disease, suggesting that small molecules that can activate stress transcription

factors may be an effective strategy for the treatment of HD. However, it remains unclear whether disease progression could stymie the long-term efficacy of stress pathway activation [64].

Small molecule regulators of proteostasis as therapeutics for neurodegenerative disease

Although genetic approaches have proven invaluable to identify the pathways that modify HD, translation of these findings to the clinic will probably require small molecule pharmacological agents that can selectively modify disease progression. For example, small molecules that reduce excitotoxicity or enhance histone acetylation have shown promise in mouse models of HD [20,23,45]. However, these approaches attempt to rectify the harmful consequences of mHTT rather than targeting the early events associated with mHTT expression. A more effective approach will probably target the causative agent itself through reduced expression, enhanced refolding, and/or increased degradation of mHTT.

One promising approach is the pharmacological activation of HSF1 leading to increased protein-folding capacity through upregulation of multiple chaperones. This has been achieved with molecules that inhibit HSP90 (a negative regulator of HSF1) and suppress mHTT aggregation and toxicity in a variety of disease models [71]. Although these studies represent an important proof-of-principle, long-term inhibition of HSP90 is likely to be detrimental. Recent screens have identified new classes of HSF1-activating molecules that act independently of HSP90. For example, the small molecule HSF1A was identified using a yeast strain engineered to express human HSF1 and was found to suppress toxicity in cell and fly models of HD and SCA-3 [72].

More recently, a ~1 000 000 compound screen for novel small molecule activators of HSF1 identified a barbituric acid-like compound (F1) that restores proteostasis in cell and worm models of protein conformational disease [73]. Intriguingly, suppression of polyglutamine toxicity was achieved despite only modest induction of gene expression, suggesting that subtle changes to the PN may be sufficient to achieve therapeutic benefit in HD [73]. Pharmacological activation of HSF1 suppresses toxicity in numerous models of neurodegenerative disease, suggesting that augmenting the PN via activation of HSF1 may be a common strategy for treatment of neurological disorders [71].

Suppressing the generation of huntingtin fragments could significantly influence HD pathogenesis

Although approaches for refolding or clearance of mHTT have proven successful in multiple disease models, the ability to specifically reduce intracellular levels of mHTT could be of great benefit, either alone or in combination with other approaches.

Full-length mHTT is processed into an array of fragments that exhibit toxicity when expressed; this is particularly well demonstrated with small N-terminal fragments of mHTT containing the polyglutamine stretch [74–76]. Therefore, the ability to prevent the generation of these fragments could prevent disease progression. Initial efforts suggested that mHTT fragments generated by caspase-6 are the primary pathogenic species in HD [75]. However, these results have been inconsistently observed, with evidence that caspase-6-derived mHTT fragments may undergo further proteolysis to smaller, more toxic, N-terminal fragments, perhaps through the action of matrix metalloproteinases [76,77]. Other recent findings have added a new perspective by demonstrating that N-terminal mHTT fragments may also be generated by aberrant splicing [78]. This suggests that N-terminal fragments may not be generated solely through proteolysis and that understanding the mechanistic basis of these observations could be of great relevance to treating HD.

Targeting mutant huntingtin expression for therapeutic gain in HD

Seminal observations using a conditional mouse model of HD demonstrated that mHTT aggregation and toxicity can be reversed when mHTT expression is arrested [79]. These observations revealed that the intrinsic protein quality control mechanisms of cells can reverse the toxic effects of polyglutamine expression and that mHTT inclusions and oligomers can be cleared. More recent work now demonstrates that antisense oligonucleotides (ASOs) or single-stranded small interfering RNAs (ss-siRNAs) (Box 4) delivered to the central nervous system of HD mice can reduce levels of mHTT protein with little to no effect on the levels of wild type huntingtin [80,81]. Furthermore, ASO treatment of HD mice results in a pronounced reduction in aggregate load with a concomitant improvement in motor coordination and survival [81]. These RNAi based silencing approaches have also been shown to be effective in mouse models of SCA, ALS, and PD.

Although these findings are encouraging, they also represent treatment protocols at an early stage of development, as they could have significantly debilitating effects on the lifestyles of affected individuals. In particular, the transient nature of these effects would demand patients to suffer repeat infusions throughout their life. Therefore, the ultimate aim must be to derive less invasive and less time-consuming methods of treatment through the administration of drugs that can selectively reduce mHTT expression. Although this goal is ambitious, recent findings suggest that this may be possible [82].

Box 4. Methods for silencing toxic proteins

Oligonucleotides that reduce the expression of target genes are used extensively in studies of gene function. Two prominent classes of gene silencing molecules are ASOs and siRNAs [85]. ASOs are single-stranded oligonucleotides that engage target RNA sequences and prevent message translation through RNase H-dependent and -independent pathways. By contrast, siRNAs are generally expressed as double-stranded RNAs that silence target mRNAs through the RNA-induced silencing complex (RISC) [85]. Although siRNAs can be expressed as single-stranded forms (ss-siRNA), these have significantly reduced potency compared with their double-stranded counterparts. However, recent studies to deduce the impact of chemical modifications on ss-siRNA stability and potency have revealed that a 5′-(*E*)-vinylphosphate modification of the 5′-phosphate group in the oligonucleotide results in enhanced ss-siRNA activity [85]. This has numerous advantages for treatment of diseases as ss-siRNAs are suggested to have reduced off-target effects and lower toxicity [85].

As well as general mechanisms of folding and clearance, new findings suggest that polyglutamine proteins also share a common mode of expression. A genetic screen for modifiers of polyglutamine toxicity in yeast has revealed that the conserved transcription elongation factor Spt4 is required for the selective expression of long polyglutamine stretches [82]. Remarkably, a loss of Spt4 results in a dramatic reduction in mHTT expression, aggregation, and toxicity without obvious gross changes to the transcriptome. These findings suggest that it may be possible to pharmacologically target SPT4 or other factors and selectively reduce the expression of polyglutamine disease proteins [82].

Concluding remarks

In this review, we have highlighted the plethora of genetic pathways that underlie pathogenesis in HD and probably also modulate disease progression in other neurodegenerative disorders. We propose that the chronic exposure of cells to misfolded and aggregation-prone proteins exerts a demand on the PN that cannot be met, in particular as the system ages, resulting in wide-scale cellular disruption and disease. If this hypothesis is correct, an attractive therapeutic approach for HD may be to develop small molecule cocktails that enhance the cellular protein folding capacity whilst simultaneously reducing mHTT expression. As we continue to understand the precise mechanisms that govern neurodegeneration, it may be possible to develop strategies that act as a panacea for protein conformational disorders.

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