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REVIEW

The pathobiology of perturbed mutant huntingtin protein–protein interactions in Huntington's disease

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Abstract

Mutations are at the root of many human diseases. Still, we largely do not exactly understand how they trigger pathogenesis. One, more recent, hypothesis has been that they comprehensively perturb protein–protein interaction (PPI) networks and significantly alter key biological processes. Under this premise, many rare genetic disorders with Mendelian inheritance, like Huntington's disease and several spinocerebellar ataxias, are likely to be caused by complex genotype–phenotype relationships involving abnormal PPIs. These altered PPI networks and their effects on cellular pathways are poorly understood at the molecular level. In this review, we focus on PPIs that are perturbed by the expanded pathogenic polyglutamine tract in huntingtin (HTT), the protein which, in its mutated form, leads to the autosomal dominant, neurodegenerative Huntington's disease. One aspect of perturbed mutant HTT interactions is the formation of abnormal protein species such as fibrils or large neuronal inclusions as a result of homotypic and heterotypic aberrant molecular interactions. This review focuses on abnormal PPIs that are associated with the assembly of mutant HTT aggregates in cells and their potential relevance in disease. Furthermore, the mechanisms and pathobiological processes that may contribute to phenotype development, neuronal dysfunction and toxicity in Huntington's disease brains are also discussed.

Keywords: huntingtin, Huntington's disease, interactomics, pathobiology, perturbed protein–protein interactions, protein aggregation.

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Abnormal interactions are commonly found in genetic diseases including Huntington's disease

Proteins in cells do not function in isolation. Rather, they form either stable or transient protein assemblies to perform their cellular tasks (Gromiha *et al.* 2017; Ivarsson and Jemth 2018). Therefore, the detection and functional characterization of protein–protein interactions (PPIs) is of critical importance to gain a better understanding of cellular processes and disease phenotypes.

A large number of rare genetic disorders with Mendelian inheritance have been identified (Pogue *et al.* 2018;

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Abbreviations used: 26S, cytosolic 26S proteasome; CAG, trinucleotide; CBP, CREB-binding protein; cryo-EM, cryo-electron microscopy; CSE, cystathione γ-lyase; DHHC17, palmitoyltransferase; DRP1, dynamin-related protein-1; FUS, fused in sarcoma; GABA, gamma-aminobutyric acid; HAP1, huntingtin-associated protein 1; HD, Huntington's disease; HEAT, acronym for four proteins – huntingtin, elongation factor 3 (EF3), protein phosphatase 2A (PP2A) and the yeast kinase TOR1; HIP14, huntingtin interacting protein 14; HIP14L, HIP14-related protein; HTT, huntingtin; LCRs, low complexity regions; mHTT, mutant huntingtin; mHTTex1, mutant huntingtin exon-1 protein; mRNA, messenger RNA; MS, mass spectrometry; mTOR, mechanistic target of rapamycin; N17, 17 N-terminal HTT amino acids; N2a, mouse neuroblastoma cell line; NeuroD, neurogenic differentiation factor 1; NIIs, neuronal intranuclear inclusions; NRSF, neuron-restrictive silencer factor; PC12, rat pheochromocytoma cell line; polyQ, polyglutamine; PPI, protein–protein interaction; PRD, proline rich domain; REST, RE1-silencing transcription factor; Rhes, RAS-like mTOR inducer; RNA, ribonucleic acid; SNAP-25, synaptosomal nerve-associated protein 25; SP1, specificity protein 1; TBP, TATA-binding protein; TFIID, transcription factor II D; TFIIF, transcription factor II F; UPS, ubiquitin-proteasome system; VCP, valosin-containing protein; wtHTT, wild-type huntingtin; zQ175, knock-in mice expressing full-length mHTT.

Amberger *et al.* 2019). These diseases are generally caused by single mutations (missense point mutations or in-frame insertions or deletions, e.g.) located in specific genes that drive the pathogenic process (Fig. 1) and lead to the development of distinct phenotypes (Chen *et al.* 2018). This suggests that simple, linear genotype-to-phenotype relationships underlie the development of rare genetic diseases and that therapeutic strategies should be straightforward. However, experimental evidence increasingly points to complex genotype–phenotype relationships (Barabasi *et al.* 2011). This is easily explained: genetic mutations lead to the production of deviant proteins that are in direct contact with various other proteins in cells. A mutation in a single protein can therefore perturb the interactions of many associated proteins and their specific biological functions, and in this way cause disease (Fig. 1). Thus, it seems reasonable to regard the phenotypes of rare genetic diseases as perturbations of complex cellular networks rather than the result of the alteration in a single disease protein. Recent systematic profiling of human disease–protein interaction networks indicates that ~ 60% of previously identified disease-associated missense mutations perturb PPIs (Sahni *et al.* 2015). This means that the interaction network or 'edgotype' of a specific mutation represents the fundamental relationship between a specific genotype and a disease phenotype (Sahni *et al.* 2013).

Huntington's disease (HD) is a rare genetic neurodegenerative disorder that is caused by a trinucleotide (CAG) triplet repeat expansion located in exon-1 in the huntingtin (*HTT*) gene (Bates *et al.* 2015). A CAG sequence with 40 or more CAG repeats leads to HD, whereas the CAG sequence is repeated between 9 and 35 times in healthy individuals. This mutation results in the synthesis of an HTT protein with an elongated polyglutamine (polyQ) tract in neurons and various other cells (Saudou and Humbert 2016). This suggests that an abnormal polyQ expansion located at the N-terminus of HTT drives pathogenesis (Harjes and Wanker 2003). HTT is a large protein of ~ 350 kDa that plays critical functional roles in gene expression regulation (Valor 2015), vesicle transport (Harjes and Wanker 2003) and autophagy (Ochaba *et al.* 2014). It is modified post-translationally at multiple sites (Cariulo *et al.* 2017; Harding *et al.* 2019) and gets cleaved by various proteases (Sanchez Mejia and Friedlander 2001), leading to the release of N-terminal fragments with an expanded polyQ sequence, which have a high propensity to misfold and self-assemble into fibrillar aggregates (Scherzinger *et al.* 1997; Trepte *et al.* 2014). As a consequence, intranuclear inclusions are formed in neurons. They consist of aggregates of mutant HTT fragments as well as a variety of further proteins like ubiquitin, chaperones, and components of the ubiquitin-proteasome system (DiFiglia *et al.* 1997; Waelter *et al.* 2001) and are a pathological hallmark of HD (Fig. 2).

To date, more than 400 HTT associated proteins have been identified using biochemical and genetic PPI-detection methods (Shirasaki *et al.* 2012; Langfelder *et al.* 2016; Alanis-Lobato *et al.* 2017). These include proteins that function in gene regulation, RNA splicing, vesicle transport and protein degradation (Harjes and Wanker 2003; Saudou and Humbert 2016), supporting a functional role of the wild-type HTT (wtHTT) or its fragments in these processes. A substantial number of studies have provided experimental evidence that the expanded polyQ sequence in HTT perturbs PPIs in mammalian cells. Here, we aim to focus specifically on interactions of mutant HTT (mHTT) that may be

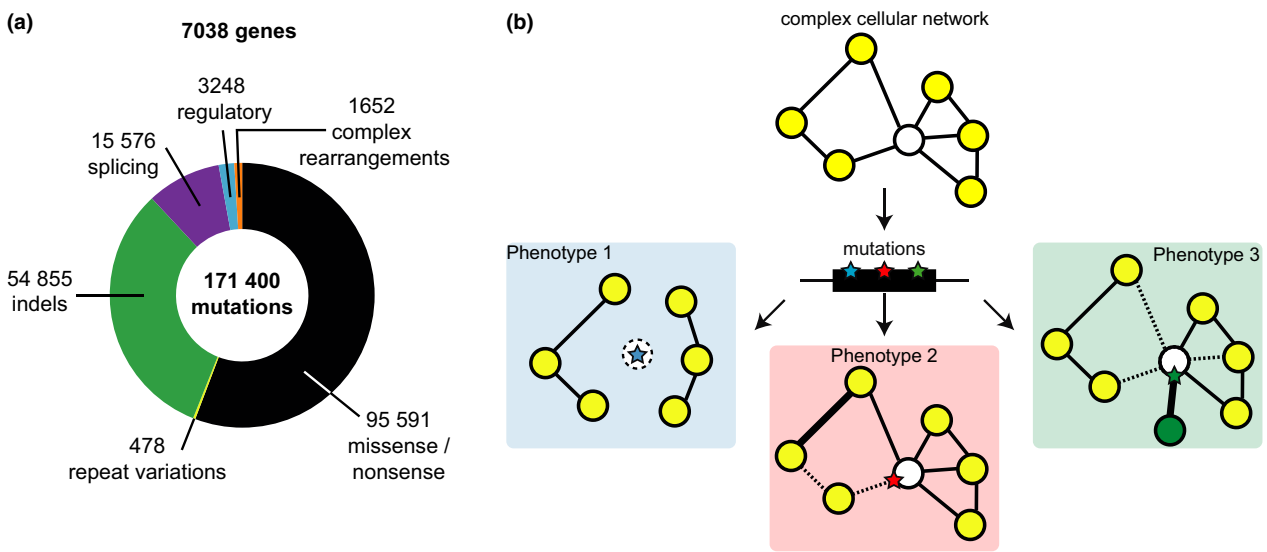

Fig. 1 Genetic mutations perturb protein–protein interaction networks. (a) The Human Gene Mutation Database currently lists more than 170 000 mutations of different types occurring in the human genome. (b) A fraction of these perturb protein–protein interaction networks and can lead to a gain or loss of interactions in cells. These changes manifest as different disease phenotypes.

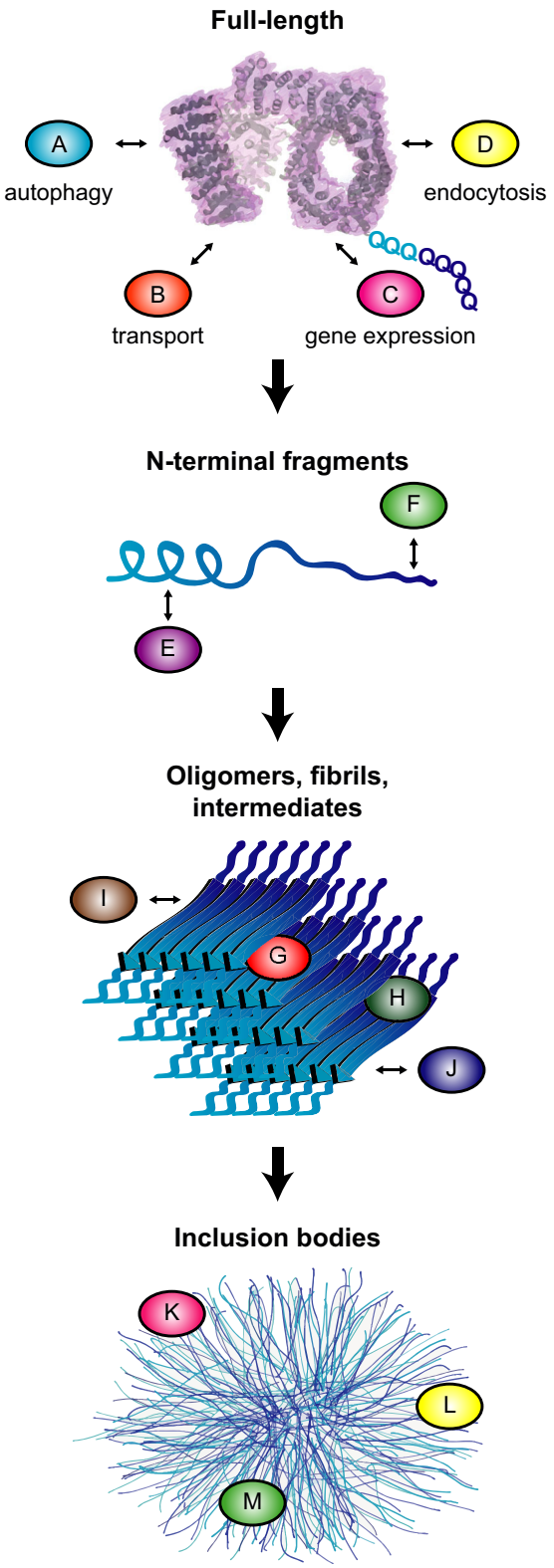

Fig. 2 Schematic representation of different mutant huntingtin species along the aggregation cascade. Each of these species can be expected to have different interactors and might therefore have different pathobiological roles in the disease.

perturbed in the human disease and discuss their putative impact on cellular processes and phenotypic changes in model systems.

Various putatively pathogenic mHTT protein species have been described

Besides full-length mHTT with a pathogenic polyQ tract and a largely α-helical conformation (Guo *et al.* 2018), a variety of other conformationally distinct mHTT protein species have been reported to be present in HD patient brains and disease models (Scherzinger *et al.* 1997; DiFiglia *et al.* 1997; Sathasivam *et al.* 2010). This includes monomers and oligomers of full-length mHTT (Sathasivam *et al.* 2010; Shirendeb *et al.* 2011) as well as protein species that are formed of truncated N-terminal mHTT fragments, such as oligomers or β-sheet-rich, ordered fibrils (Wagner *et al.* 2018). Several lines of evidence indicate efficient proteolytic cleavage of full-length mHTT resulting in the release of N-terminal polyQ-containing fragments (Lunkes *et al.* 2002; Landles *et al.* 2010). Furthermore, a truncated mutant HTT exon-1 (mHTTex1) fragment is continuously produced by messenger RNA splicing in addition to the full-length protein (Sathasivam *et al.* 2013; Neueder *et al.* 2018). It is important to note that monomeric, oligomeric and fibrillar forms of mHTT are dynamic structures that can interconvert into each other *in vitro* and *in vivo* (Hoffner and Djian 2014; Sahoo *et al.* 2016) and eventually are deposited into large inclusion bodies in cells (Waelter *et al.* 2001). A schematic representation of previously described, potentially pathogenic mHTT protein species and their assembly into inclusion bodies is shown in Fig. 2.

Recently, a high-resolution cryo-electron microscopy (cryo-EM) structure was obtained for soluble full-length wtHTT in complex with its interaction partner HAP40 (Guo *et al.* 2018). It revealed that wtHTT is predominantly a folded, α-helical protein that consists of three major domains. Two large domains containing multiple HEAT repeats (N-HEAT and C-HEAT) located at the N- and C-terminus of wtHTT are connected by a smaller bridge domain. The full-length protein is conformationally flexible and is stabilized upon binding to its interacting partner protein. The structural studies also support the view that full-length wtHTT is a multivalent hub that interacts with many different proteins in mammalian cells (Harjes and Wanker 2003; Li and Li 2004). Interestingly, no high-resolution secondary structure was obtained by cryo-EM for the N-terminal polyQ tract. This suggests that this region is highly flexible and likely to have limited influence on the overall α-helical architecture of full-length wtHTT (Fig. 2). Biochemical and biophysical studies with pathogenic and non-pathogenic full-length HTT proteins have shown that expanded polyQ tracts can increase the α-helical properties of HTT and alter the intramolecular interactions between N-

and C-terminal domains (Vijayvargia *et al.* 2016). Thus, it may be appropriate to describe wtHTT and mutant HTT as, at least in part, structurally distinct proteins, which may have important differences in their interaction profiles in mammalian cells.

Apart from full-length mHTT, truncated N-terminal polyQ-containing fragments may also play a critical role in HD (Davies *et al.* 1997; Ast *et al.* 2018). The mHTTex1 fragment has been reported to efficiently self-assemble into higher order structures such as spherical oligomers or amyloid fibrils *in vitro* and *in vivo* (Sathasivam *et al.* 2010; Sahoo *et al.* 2016; Wagner *et al.* 2018). It has been proposed that such protein assemblies might drive HD pathogenesis (Wanker 2000). Whether oligomers, fibrils or even misfolded mHTT monomers are the major proteotoxic species, however, is still a matter of debate (Diaz-Hernandez *et al.* 2005; Sahl *et al.* 2012; Leitman *et al.* 2013). Recent studies from different laboratories demonstrated that polyQ-expanded mHTTex1 fibrils, rather than oligomers or misfolded monomers exert toxicity in HD (Drombosky *et al.* 2018). Minute amounts of small self-propagating HTTex1Q97 fibrils in neurons were shown to be sufficient to dramatically shorten the life-span of HD transgenic flies (Ast *et al.* 2018). Here, it is important to note that misfolded mHTTex1 monomers and oligomers are structurally distinct from amyloid fibrils. While monomers and oligomers are dynamic structures and are assumed to have random coil or α-helical conformation *in vivo* (Kang *et al.* 2017a; Kotler *et al.* 2019), fibrillar mHTTex1 assemblies are rigid and predominantly consist of stable β-sheets (Hoop *et al.* 2016; Lin *et al.* 2017). Antibody binding studies demonstrated that polyQ tracts are exposed on the surface of mHTTex1 monomers and oligomers, but not on the surface of amyloid fibrils (Wagner *et al.* 2018). Being structurally and morphologically distinct, mHTT fibrils may have very different biological properties and interaction profiles in mammalian cells from those of non-β-sheet mHTT species.

Abnormal interactions between full-length mHTT and cellular proteins

Axonal transport

Previous studies have shown that a protein complex consisting of full-length HTT and its interaction partner huntingtin-associated protein 1 (HAP1) regulates the transport of organelles and various types of membrane vesicles along axons (Fig. 3), in both anterograde and retrograde directions (Gauthier *et al.* 2004; Wong and Holzbaur 2014). This happens because the HTT-HAP1 protein complex binds to molecular motors and to cargo vesicles such as synaptic precursor vesicles (Zala *et al.* 2013) or autophagosomes (Wong and Holzbaur 2014). The expression of full-length mHTT was shown to perturb the trafficking of vesicles with $GABA_A$ receptors to synapses with effects on neurotransmission, supporting a functional role of the HTT-HAP1 complex in the anterograde transport of membrane vesicles (Twelvetrees *et al.* 2010). In the same study, HAP1 stably associated with KIF5 motors, a critical interaction for the transport of $GABA_A$ receptors to synapses. HAP1 interacts more strongly with mutant than wtHTT in yeast two-hybrid assays (Li *et al.* 1995), suggesting that the expanded polyQ in full-length mHTT may also abnormally stabilize this interaction in neurons. This enhanced binding may in turn perturb the functionally important interaction between HAP1 and KIF5 motors, leading to a decrease in $GABA_A$-receptor trafficking in neurons. The transport of $GABA_A$ receptors to synapses is critical to brain excitability, and a significant reduction in their abundance in synapses may contribute to the development of HD (Garret *et al.* 2018).

Autophagosomes are known to form constitutively at axon tips and undergo robust retrograde axonal transport towards the cell body (Maday and Holzbaur 2012). Recent findings indicate that full-length wtHTT and its adaptor protein HAP1 control the retrograde transport of autophagosomes in neurons (Wong and Holzbaur 2014). Strikingly, the movement of autophagosomes to cell bodies was significantly impaired upon the expression of full-length mHTT in neurons (Fig. 3), leading to an accumulation of these structures with undegraded mitochondrial cargo in axons (Wong and Holzbaur 2014). Thus, an abnormal stabilization of the mHTT-HAP1 interaction through the expanded polyQ tract may disrupt autophagosome transport and lead to inefficient clearance of mitochondrial fragments in neurons. This, in turn, may contribute to neuronal dysfunction and cell death observed in HD patient brains.

Autophagy

Initial investigations have demonstrated that the activity of macroautophagy is significantly reduced in cells of HD patients compared to healthy controls, with a cargo-recognition failure likely to be responsible (Martinez-Vicente *et al.* 2010). More recent investigations in mammalian cells showed full-length wtHTT to interact directly with proteins that play a key role in selective autophagy, for example, ULK1, p62 and GABARAPL1 (Ochaba *et al.* 2014; Rui *et al.* 2015) and play a key role in the cargo recruitment process in cells (Bjorkoy *et al.* 2006). ULK1 is an autophagy-activating kinase that forms a complex with the cargo receptor p62, which recognizes polyubiquitin chains attached to protein aggregates (Rui *et al.* 2015). It is currently believed that, through the formation of a stable HTT-ULK1-p62 protein complex, misfolded, ubiquitinated protein aggregates are recognized in cells and subsequently incorporated in autophagosomes by interacting with LC3-associated phagophores (Fig. 3). The expanded polyQ tract could alter the functional HTT-UKL1-p62 cargo-recognition

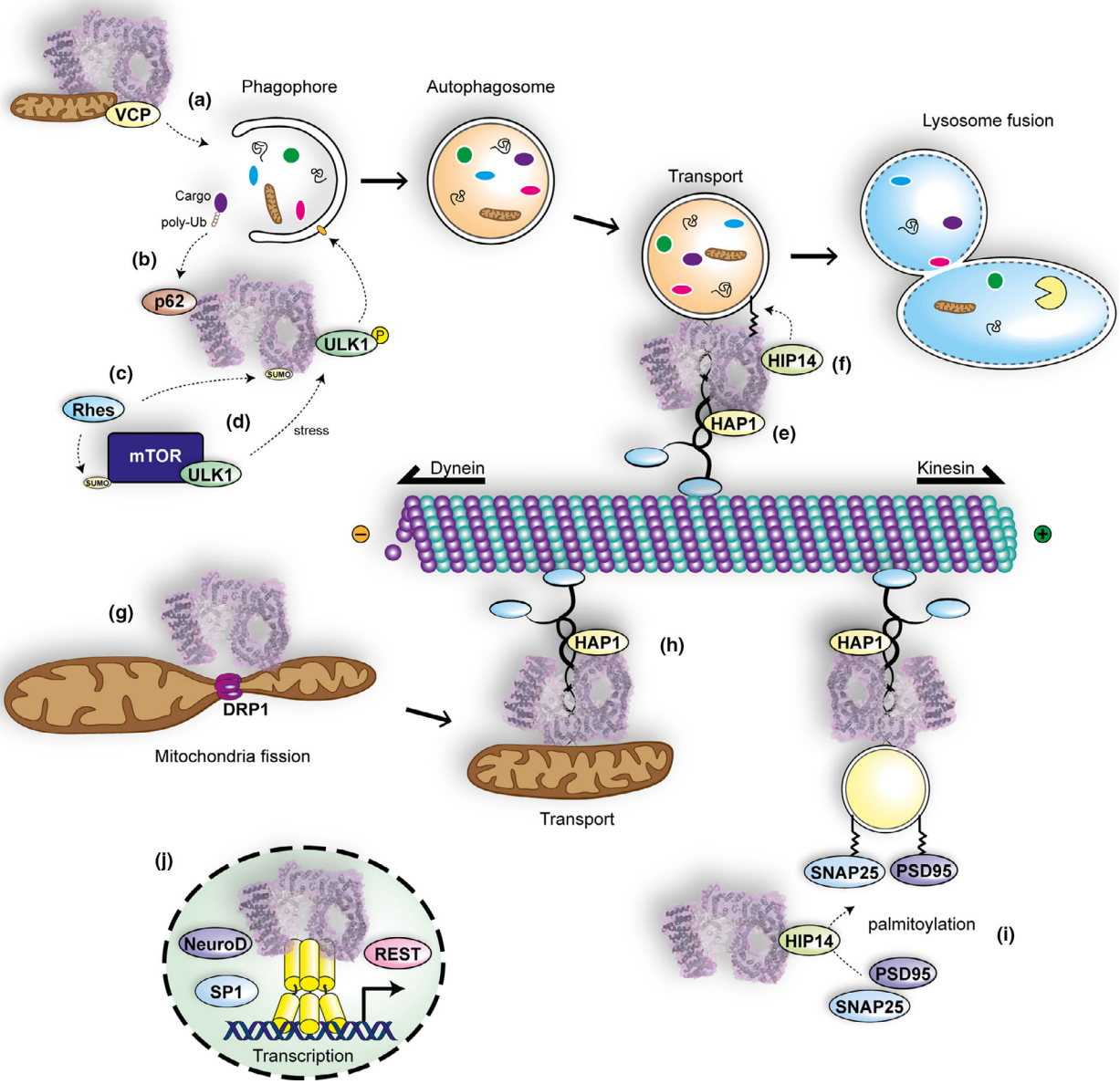

Fig. 3 A graphic summary of the abnormal interactions between full-length mutant huntingtin (mHTT) and proteins involved in various cellular processes. (a and b) Initiation of autophagy. (c and d) Mechanistic target of rapamycin (mTOR) signalling. (e and f) Vesicle transport along microtubuli. (g) Fission of mitochondria. (h) Transport of mitochondria along microtubules. (i) Palmitoylation of synaptic proteins. (j) Regulation of gene transcription.

complex and cause the observed malfunction of macroautophagy in HD (Martinez-Vicente *et al.* 2010). This view is supported by experiments with HD mice, in which a deletion in the polyQ tract in full-length HTT enhanced neuronal autophagy (Zheng *et al.* 2010). Finally, degradation of mutant but not wtHTT through selective autophagy was shown to decrease in cells (Fu *et al.* 2017), substantiating the relevance of this specific cellular protein degradation process in pathogenesis.

mTOR signalling

The mechanistic target of rapamycin (mTOR) is a serine-threonine kinase that regulates growth and metabolism of cells by activation or deactivation of protein translation (Laplante and Sabatini 2012). The mTOR protein assembles into two protein complexes, mTORC1 and mTORC2, which differ in their protein compositions and cellular activities (Saxton and Sabatini 2017). In the brain, mTORC1 controls

mitochondrial biogenesis and lipid homeostasis (Cunningham *et al.* 2007; Peterson *et al.* 2011). Additionally, it promotes myelination, axon growth and regeneration (Kim *et al.* 2012), making its continuous activity indispensable for neuronal function and survival. Recent results with HD knock-in and transgenic mice revealed that mTORC1 activity is significantly impaired in neuronal cells (Lee *et al.* 2015). In addition, expression of Rhes, a RAS-like mTOR inducer, which is enriched in the striatum (Subramaniam *et al.* 2009), was reduced in HD patient and mouse brains and exogenous expression of this protein alleviated motor symptoms and improved brain pathology in HD mice. All this evidence suggests that impairment of mTOR activity in neurons may drive development of HD (Fig. 3). Strikingly, independent investigations demonstrated that the GTPase Rhes binds more strongly to mutant than to wild-type full-length HTT (Subramaniam *et al.* 2009), suggesting that the observed impairment of mTOR activity in HD brains could be caused by this perturbed interaction. However, a potentially disease-relevant mTOR-independent function of Rhes has also been described later: Via an interaction with the autophagy regulator Beclin-1, Rhes robustly activated autophagy in PC12 cells (Mealer *et al.* 2014). However, this activity was blocked by co-expression of mHTT, suggesting that an inhibition of the abnormally strong interaction between mHTT and Rhes with therapeutic molecules might reactivate mTOR signalling and, independently of this, also lead to an activation of autophagy in HD neurons.

Palmitoylation

The reversible post-translational modification of cysteine residues by palmitate critically regulates the localization and cellular function of a large number of human proteins (el-Husseini Ael and Bredt 2002; Huang and El-Husseini 2005). Among other functions, it controls protein trafficking along axons or the release of neurotransmitters in synapses (Brigidi *et al.* 2014). Previous research has demonstrated that full-length wtHTT gets palmitoylated in cortical neurons (Yanai *et al.* 2006) and interacts directly with the palmitoyl transferase huntingtin interacting protein 14 (HIP14) (DHHC17). In turn, HIP14 interacts with many important synaptic proteins such as synaptosomal nerve-associated protein 25 or PSD-95 (Fig. 3) and thereby influences synapse function and neurotransmission (Huang *et al.* 2004). Mechanistic studies have shown that binding of wtHTT to HIP14 activates its palmitoyl transferase activity and promotes the palmitoylation of synaptosomal nerve-associated protein 25 and other synaptic proteins (Huang *et al.* 2011). This suggests that wtHTT functions as a palmitoylation facilitator for synaptic proteins and thereby regulates synapse function. In cells expressing mHTT, the palmitoylation of synaptic proteins was found to be reduced (Huang *et al.* 2011). The expanded polyQ tract appears to perturb the functional interaction with HIP14, leading to a decrease in its enzymatic activity. This is supported by yeast two-hybrid interaction studies, in which the binding of mHTT to HIP14 is decreased in comparison to the wild-type protein (Singaraja *et al.* 2002). Impairment of HIP14 palmitoyl transferase activity in specific neurons may contribute to the development of disease symptoms. The potential relevance of a loss of HIP14 function in HD is also supported by genetic knock-out studies in mice, indicating that mice deficient in HIP14 and HIP14L (a HIP14 related protein) both recapitulate important features of HD (Singaraja *et al.* 2011; Sutton *et al.* 2013).

Mitochondrial fission and mitophagy

Cycles of mitochondrial fusion and fission are essential for neuronal function. Imbalance of these opposing processes initiates neurodegeneration (Knott *et al.* 2008). Evidence was presented that the GTPase dynamin-related protein-1 (DRP1), which binds to mitochondria and controls mitochondrial fission, interacts more strongly with mutant than with wild-type full-length HTT in co-immunoprecipitation experiments (Song *et al.* 2011). In cells expressing mHTT, a significant increase in DRP1-mediated fission activity was observed (Fig. 3), indicating that the mutation affects the interaction with functional consequences (Song *et al.* 2011). As it may trigger downstream apoptosis and cell death, the fragmentation of mitochondria is tightly controlled in neuronal cells (Costa *et al.* 2010). Thus, mHTT-induced mitochondrial fission may lead to neuronal dysfunction and toxicity in HD patient brains. This hypothesis is supported by investigations in zQ175 HD mice, which showed that inhibition of DRP1 hyperactivity with a peptide reduces behavioural deficits (Zhao *et al.* 2018). An abnormal interaction between mHTT and DRP1 was also detected in postmortem brains of HD patients (Shirendeb *et al.* 2012).

The relevance of mitochondrial dysfunction in HD is substantiated further by recent findings that mHTT promotes the recruitment of the AAA ATPase valosin-containing protein (VCP) to mitochondria and that this translocation is associated with enhanced mitophagy in models of HD (Guo *et al.* 2016). Hence, an abnormal mHTT–VCP interaction at the surface of mitochondria may also contribute to the induction of cell death in brains of HD patients. The disease relevance of these results is supported by the fact that a peptide inhibitor which blocks the interaction between VCP and mHTT reduces the behavioural and neuropathological phenotypes in HD mice.

Gene transcription

Numerous studies have reported that wtHTT binds to transcription factors (Steffan *et al.* 2000; Valor 2015) suggesting that it functions as gene expression regulator in mammalian cells. This involves the binding to important transcriptional activators such as specificity protein 1 (SP1)

(Dunah *et al.* 2002) and neurogenic differentiation factor 1 (Marcora *et al.* 2003) to transcriptional repressors such as RE1-silencing transcription factor/neuron-restrictive silencer factor (Zuccato *et al.* 2003) or to components of the core transcription apparatus such as transcription factor II D or transcription factor II F (Zhai *et al.* 2005). Because wtHTT interacts with many partners belonging to the transcription machinery in the nucleus, it is regarded as a relevant influence on the expression of a wide range of target genes (Fig. 3). This critical molecular function is perturbed in cells upon expression of mHTT with an expanded polyQ tract: in co-immunoprecipitation experiments, for instance, mHTT was shown to bind more strongly to the transcription activator SP1 than the wild-type protein (Dunah *et al.* 2002). More detailed studies revealed that mHTT specifically repressed SP1-dependent transcription in cell-free assays (Zhai *et al.* 2005), underlining the perturbatory role of aberrant interactions between mHTT and transcription factors on gene expression. The disease relevance of a stronger mHTT-SP1 binding was supported by an independent study demonstrating that the expression of the metabolic enzyme cystathione γ-lyase is significantly down-regulated in HD models at the transcript level and that this effect is mediated by an abnormal interaction between mHTT and SP1 (Paul *et al.* 2014). Consistent with the notion of a loss of cystathione γ-lyase as a pathogenic mechanism, supplementation with cysteine reversed abnormalities in HD tissue cultures and mouse models (Sbodio *et al.* 2016). Interfering with the enhanced binding of mHTT to SP1 may be a valid therapeutic strategy for HD.

More recent investigations indicate that the peroxisome proliferator-activated receptor delta (PPAR-δ) binds more strongly to mHTT than to the wild-type protein and that PPAR-δ transactivation is repressed by this abnormal PPI (Dickey *et al.* 2016). PPAR-δ is a ligand-activated transcription factor that belongs to a nuclear hormone receptor family. It serves as a lipid sensor that is activated in response to increasing energy requirements and regulates mitochondrial energy production in the skeletal muscles and brain (Berger and Moller 2002). The abnormal interaction might contribute to the mitochondrial dysfunction observed in HD patient brains (Vonsattel *et al.* 2011). This view is supported by findings in HD mouse models that PPAR-δ activation by bexarotene restores mitochondrial function and promotes neuroprotection (Dickey *et al.* 2017). The dysfunction of mitochondria in HD is linked to transcriptional dysregulation of PGC-1α (peroxisome proliferator-activated receptor gamma co-activaotor-1 alpha) that coordinates the transcriptional profiles responsible for mitochondrial biogenesis (Finck and Kelly 2006). The importance of PGC-1α in HD is underscored by the observation that its levels are decreased in mHTT-expressing striatal cells and that its overexpression is sufficient to rescue motor dysfunction and to reduce neurodegeneration in HD mice (Cui *et al.* 2006). Thus, abnormal interactions between PPAR-δ or its related proteins PPAR-α and PPAR-γ (Jin *et al.* 2012; Chandra *et al.* 2016) with mHTT and PGC-1α may be responsible for mitochondrial dysfunction as well as the reduced production of ATP observed in HD patients and model systems (Intihar *et al.* 2019).

Abnormal interactions among mHTT fragments and with other polyQ-containing proteins

The HTTex1 fragment contains the expandable polyQ stretch which is preceded by 17 amino acids (N17) and followed by a proline-rich domain. It is conformationally highly flexible and therefore considered an intrinsically disordered protein (Kim *et al.* 2009; Giorgini 2013; Baias *et al.* 2017). Homotypic interaction of HTTex1 fragments leads to the spontaneous self-assembly of HTTex1 fibrils in aqueous solution (Scherzinger *et al.* 1997; Scherzinger *et al.* 1999; Wanker *et al.* 1999; Sahoo *et al.* 2014). The expansion of the polyglutamine stretch greatly facilitates this process and leads to the rapid formation of insoluble amyloidogenic fibrillar aggregates with cross-β-sheet architecture (Scherzinger *et al.* 1999; Sahoo *et al.* 2014). Fibril formation follows a nucleated growth mechanism (Bhattacharyya *et al.* 2005; Wagner *et al.* 2018). The initial association between HTTex1 molecules is believed to be mediated by coiled-coil interactions of multiple N17 domains (Thakur *et al.* 2009; Jayaraman *et al.* 2012). This association may bring polyQ stretches of individual molecules into close proximity, enhancing the polyQ–polyQ interaction and facilitating the transition to a β-hairpin conformation (Hoop *et al.* 2016; Kang *et al.* 2017b). These initial conformational changes (primary nucleation) are slow and rare to occur and therefore manifest in a lag phase, when looking at the aggregation process from a kinetic point of view. Then, fibril formation proceeds to a rapid elongation phase in which additional HTTex1 monomers are recruited to the primary nucleus and adapt a β-hairpin conformation (Wagner *et al.* 2018). Within the mature fibre, multiple molecules with a β-hairpin conformation are stacked atop each other, stabilized by intra- and intermolecular hydrogen bonds, thereby forming tightly interconnected β-sheets (Hoop *et al.* 2016).

Pathogenic HTTex1 fragments also interact in cells and efficiently form fibrillar aggregates *in vivo* (Davies *et al.* 1997; DiFiglia *et al.* 1997; Scherzinger *et al.* 1997), demonstrating that abnormal homotypic mHTT interaction leads to the formation of aggregates which may perturb cellular functions and thereby drive pathogenesis. Interactions between pathogenic and non-pathogenic HTTex1 fragments have been detected in cell-free assays, leading to the co-assembly of mixed HTTex1 fibrils with high stability (Busch *et al.* 2003). Such structures may also form *in vivo*, suggesting that loss of normal HTT function through incorporation of wild-type protein molecules into mutant

HTT fibrils may contribute to HD development. In addition, the disease relevance of fibrillar mutant HTTex1 aggregates is supported by cell-based assays indicating that such structures are rapidly taken up into cells (Ren *et al.* 2009; Holmes *et al.* 2013), possibly inducing toxicity. Furthermore, there is accumulating evidence that HTTex1 fibrils can self-propagate and efficiently spread from cell to cell (Costanzo *et al.* 2013; Babcock and Ganetzky 2015; Jeon *et al.* 2016; Masnata *et al.* 2019), suggesting that the active uptake and release of mHTT fibrils are important elements of pathogenesis and disease progression. The discovery that mutant HTTex1 seeding activity in fly neurons correlates with neurotoxicity and reduced survival supports this view (Ast *et al.* 2018).

A number of studies have shown that other proteins with glutamine-rich domains can interact with mHTT fragments and might be at the root of cellular toxicity by being sequestered into HTT aggregates. These proteins are mainly transcription factors such as TATA-binding protein (TBP) and CREB-binding protein (CBP). The TBP that contains a polymorphic polyQ domain in the N-terminus is recruited into aggregates formed of N-terminal mHTT fragments *in vitro* and in cells and is also present in HTT aggregates from post-mortem HD brains (Huang *et al.* 1998; Schaffar *et al.* 2004). The nuclear transcriptional coactivator CBP, which contains a glutamine-rich C-terminus, interacts with mHTTex1 *in vitro* (Steffan *et al.* 2000) as well as with N-terminal mHTT (Nucifora *et al.* 2001). Immunoprecipitation experiments with N2a cells confirmed a direct interaction between CBP and an mHTT fragment (Nucifora *et al.* 2001). The interactions of both CBP and TBP with mHTT are highly dependent on the polyQ domains, as they do not take place when the polyQ regions are deleted in the two proteins (Nucifora *et al.* 2001; Schaffar *et al.* 2004). CBP localizes to intranuclear inclusions in striatal sections of R6/2 transgenic HTT mice (Steffan *et al.* 2000), cortical neurons of Htt-N171-82Q transgenic mice and human post-mortem cortex (Kazantsev *et al.* 1999). These studies provide strong evidence that CBP and TBP are sequestered into mHTT aggregates leading to decreased CBP and TBP-mediated transcription (Nucifora *et al.* 2001; Schaffar *et al.* 2004). The resulting cellular toxicity could be compensated by the overexpression of TBP or CBP respectively (Nucifora *et al.* 2001; Schaffar *et al.* 2004). Another glutamine-rich transcription factor, SP1, has been shown to directly interact with wild-type and mutant HTTex1 as well as other N-terminal HTT fragments (Li *et al.* 2002). The polyQ expansion enhances the interaction of mHTT and the SP1 protein and thereby reduces its transcriptional activity in cultured cells. In R6/2 mice, which express mHTTex1, SP1 interacts with soluble mHTT, but not with mHTT aggregates (Li *et al.* 2002). Finally, studies in yeast showed a co-localization of mHTT fragments with several polyQ-rich proteins, such as Cyc8, Snf5 (Duennwald *et al.* 2006) or Gts1p, a direct interactor (Ripaud *et al.* 2014), supporting a role of abnormal polyQ–protein interactions in disease.

Abnormal interactions between mHTT aggregates and cellular proteins – formation of inclusion bodies

The deposition of insoluble fibrillar mHTT aggregates in neuronal intranuclear inclusions (NIIs) in the cortex and striatum is a pathological hallmark of HD patient brains (DiFiglia *et al.* 1997). Also, NIIs with fibrillar mHTTex1 aggregates were detected in the brains of HD transgenic mice prior to the development of neuronal symptoms (Davies *et al.* 1997; Scherzinger *et al.* 1997). The formation of such structures in neurons is therefore associated with disease development or might even drive the pathogenic process. However, experimental evidence from cell culture studies also pointed to inclusion body formation as reducing levels of soluble mHTT in the cytoplasm and decreasing the risk of neuronal death. This suggests that the formation of such structures in neurons might be a homeostatic coping mechanism rather than a process that drives pathogenesis (Arrasate *et al.* 2004; Miller *et al.* 2010).

Histological investigation of HD patient and mouse brains revealed that NIIs are complex structures that contain a large number of cellular proteins besides insoluble mHTT aggregates. These include, for example, ubiquitin, molecular chaperones or components of the ubiquitin-proteasome system (Davies *et al.* 1997; DiFiglia *et al.* 1997). As a consequence, recruitment of important cellular proteins into inclusion bodies and abnormal interactions between mHTT aggregates and components of the protein quality control machinery have to be regarded as linked to each other and possibly to the progressive neuronal dysfunction observed in HD transgenic mice.

Cell-based studies confirmed that N-terminal mHTTex1 fragments with pathogenic polyQ tracts rapidly self-assemble into insoluble, fibrillar protein aggregates and sequester quality control proteins such as ubiquitin, chaperones or components of the 26S proteasome into inclusion bodies (Waelter *et al.* 2001). Dramatic ultrastructural changes in cells with mHTTex1 inclusion bodies, such as the disruption of the nuclear and ER membranes, have been established formerly (Waelter *et al.* 2001). Recent high-resolution cryo-EM studies in primary neurons and immortalized cells confirm these initial observations (Fig. 2), indicating that complex networks of mHTTex1 fibrils in inclusion bodies lead to aberrant interactions with endomembranes and impair the ER organization (Bauerlein *et al.* 2017).

Mass spectrometry (MS)-based quantitative proteomics of neuronal inclusions with mHTTex1 aggregates have detected many other proteins to be enriched, in addition to chaperones and components of the ubiquitin-proteasome system, including RNA-binding proteins, myelin components and proteins

involved in neuronal plasticity (Hosp *et al.* 2017). This extends the range of neuronal functions impaired by protein sequestration into NIIs in the disease. A similar result had been obtained when the composition of insoluble mHTT inclusions formed in a neuroblastoma cell line was assessed with quantitative mass spectrometry-based proteomics methods (Kim *et al.* 2016). Interestingly, these studies also demonstrated that smaller, soluble mHTTex1 assemblies have more abnormal interactions with cellular proteins than fibrillar aggregates deposited in inclusion bodies. This indicates that the binding of chaperones and proteins of the quality control machinery to mHTTex1 fibrils in inclusion bodies, at least partly, results in surface shielding, while the cellular interactions of diffusible mHTT species remain manyfold. They are thought to perturb multiple critical processes such as ribosome biogenesis, translation, transcription and vesicle transport (Kim *et al.* 2016).

Bioinformatic analysis revealed that predominantly unstructured low complexity regions (LCRs) associate with soluble or insoluble mHTTex1 assemblies (Kim *et al.* 2016). Such domains have an important physiological role in RNA-binding proteins and recently have been found to mediate their reversible functional polymerization into hydrogels, which leads to an increased concentration of binding partners in cells and is crucial for the formation of RNA granules (Han *et al.* 2012; Murakami *et al.* 2015). However, under conditions of prolonged stress or when mutated, the LCR-containing proteins can undergo a phase transition, leading to the formation of potentially toxic fibrillar aggregates (Mompean and Laurents 2017). Thus, it seems reasonable to speculate that the fibrillogenesis of RNA-binding proteins with LCRs gets stimulated by abnormal interactions with aggregated mHTT species. Experimental evidence that RNA-binding proteins with LCRs such as FUS (fused in sarcoma) co-aggregate with mHTTex1 fragments into SDS-stable, high-molecular weight aggregates was provided previously (Wear *et al.* 2015). However, a liquid-to-solid phase transition for polyQ-containing HTTex1 proteins has been recently described, indicating that also the mHTT aggregation process in neurons may involve liquid-like assemblies prior to the formation of fibrillar structures (Peskett *et al.* 2018).

Future perspectives

While the scientific community agrees that the expanded polyQ tract in HTT, directly or indirectly, perturbs multiple PPIs in cells and various other disease models, it remains debated which of these alterations are detectable under endogenous conditions in patient neurons and at what point they occur in the pathogenic process. It will be of critical importance to determine whether changes in PPIs that can be detected and quantified in patient cells eventually contribute to pathogenesis. In HD, selective striatal neuronal loss is observed in patient brains (Ross and Tabrizi 2011). Mutant and wtHTT, however, are expressed uniformly throughout the brain and the rest of the body. Striatal selectivity of HD may be explained by binding of mHTT to proteins that are predominantly expressed in this brain region such as the small guanine nucleotide-binding protein Rhes, which, together with proteins such as ACBD3 (Sbodio *et al.* 2013), mediates mHTT toxicity in various model systems (Subramaniam *et al.* 2009). Experimental evidence was presented that Rhes binds more strongly to mutant than to wtHTT in knock-in striatal neuronal cells (Subramaniam *et al.* 2009). Whether an abnormal mHTT : Rhes interaction in brains of HD patients or knock-in mice is responsible for the observed selective striatal vulnerability, however, remains unclear. Further specific *in vivo* studies with Rhes mutants, which weakly bind mHTT, are necessary to elucidate whether an abnormal association between Rhes and mHTT is indeed responsible for the selective destruction of the corpus striatum in HD.

In systematic future studies perturbed PPIs need to be prioritized in terms of their relevance to disease and experimental strategies to rescue such interactions with therapeutic molecules in model systems need to be developed. Complex disease phenotypes triggered by multiple changes in the interactome network might only be treated successfully with a polypharmacological approach that involves more than one therapeutic molecule to target and correct the perturbed network. In the context of HD, a successful therapeutic strategy could require the targeting of abnormal cellular interactions of full-length α-helical mHTT as well as the targeting of the interactions of mHTTex1 and β-sheet-rich mHTT aggregates. At the same time, an inhibition of homotypic interactions between mHTT fragments, leading to the assembly of stable, self-propagating fibrillar structures, may be crucial for therapeutic success.

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References

Alanis-Lobato G., Andrade-Navarro M. A. and Schaefer M. H. (2017) HIPPIE v2.0: enhancing meaningfulness and reliability of protein-protein interaction networks. *Nucleic Acids Res.* **45**, D408–D414.

Amberger J. S., Bocchini C. A., Scott A. F. and Hamosh A. (2019) OMIM.org: leveraging knowledge across phenotype-gene relationships. *Nucleic Acids Res.* **47**, D1038–D1043.

Arrasate M., Mitra S., Schweitzer E. S., Segal M. R. and Finkbeiner S. (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* **431**, 805–810.

Ast A., Buntru A., Schindler F., *et al*. (2018) mHTT Seeding activity: a marker of disease progression and neurotoxicity in models of Huntington's disease. *Mol. Cell* **71**, e676.

Babcock D. T. and Ganetzky B. (2015) Transcellular spreading of huntingtin aggregates in the Drosophila brain. *Proc. Natl Acad. Sci. USA* **112**, E5427–5433.

Baias M., Smith P. E., Shen K., Joachimiak L. A., Zerko S., Kozminski W., Frydman J. and Frydman L. (2017) Structure and dynamics of the huntingtin exon-1 N-terminus: a solution NMR perspective. *J. Am. Chem. Soc.* **139**, 1168–1176.

Barabasi A. L., Gulbahce N. and Loscalzo J. (2011) Network medicine: a network-based approach to human disease. *Nat. Rev. Genet.* **12**, 56–68.

Bates G. P., Dorsey R., Gusella J. F., *et al*. (2015) Huntington disease. *Nat. Rev. Dis. Primers* **1**, 15005.

Bauerlein F. J. B., Saha I., Mishra A., *et al*. (2017) In Situ architecture and cellular interactions of PolyQ inclusions. *Cell* **171**, e110.

Berger J. and Moller D. E. (2002) The mechanisms of action of PPARs. *Annu. Rev. Med.* **53**, 409–435.

Bhattacharyya A. M., Thakur A. K. and Wetzel R. (2005) Polyglutamine aggregation nucleation: thermodynamics of a highly unfavorable protein folding reaction. *Proc. Natl Acad. Sci. USA* **102**, 15400–15405.

Bjorkoy G., Lamark T. and Johansen T. (2006) p62/SQSTM1: a missing link between protein aggregates and the autophagy machinery. *Autophagy* **2**, 138–139.

Brigidi G. S., Sun Y., Beccano-Kelly D., Pitman K., Mobasser M., Borgland S. L., Milnerwood A. J. and Bamji S. X. (2014) Palmitoylation of delta-catenin by DHHC5 mediates activity-induced synapse plasticity. *Nat. Neurosci.* **17**, 522–532.

Busch A., Engemann S., Lurz R., Okazawa H., Lehrach H. and Wanker E. E. (2003) Mutant huntingtin promotes the fibrillogenesis of wild-type huntingtin: a potential mechanism for loss of huntingtin function in Huntington's disease. *J. Biol. Chem.* **278**, 41452–41461.

Cariulo C., Azzollini L., Verani M., *et al*. (2017) Phosphorylation of huntingtin at residue T3 is decreased in Huntington's disease and modulates mutant huntingtin protein conformation. *Proc. Natl Acad. Sci. USA* **114**, E10809–E10818.

Chandra A., Sharma A., Calingasan N. Y., White J. M., Shurubor Y., Yang X. W., Beal M. F. and Johri A. (2016) Enhanced mitochondrial biogenesis ameliorates disease phenotype in a full-length mouse model of Huntington's disease. *Hum. Mol. Genet.* **25**, 2269–2282.

Chen S., Fragoza R., Klei L., Liu Y., Wang J., Roeder K., Devlin B. and Yu H. (2018) An interactome perturbation framework prioritizes damaging missense mutations for developmental disorders. *Nat. Genet.* **50**, 1032–1040.

Costa V., Giacomello M., Hudec R., *et al*. (2010) Mitochondrial fission and cristae disruption increase the response of cell models of Huntington's disease to apoptotic stimuli. *EMBO Mol. Med.* **2**, 490–503.

Costanzo M., Abounit S., Marzo L., Danckaert A., Chamoun Z., Roux P. and Zurzolo C. (2013) Transfer of polyglutamine aggregates in neuronal cells occurs in tunneling nanotubes. *J. Cell Sci.* **126**, 3678–3685.

Cui L., Jeong H., Borovecki F., Parkhurst C. N., Tanese N. and Krainc D. (2006) Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. *Cell* **127**, 59–69.

Cunningham J. T., Rodgers J. T., Arlow D. H., Vazquez F., Mootha V. K. and Puigserver P. (2007) mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature* **450**, 736–740.

Davies S. W., Turmaine M., Cozens B. A., *et al*. (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* **90**, 537–548.

Diaz-Hernandez M., Torres-Peraza J., Salvatori-Abarca A., Moran M. A., Gomez-Ramos P., Alberch J. and Lucas J. J. (2005) Full motor recovery despite striatal neuron loss and formation of irreversible amyloid-like inclusions in a conditional mouse model of Huntington's disease. *J. Neurosci.* **25**, 9773–9781.

Dickey A. S., Pineda V. V., Tsunemi T., *et al*. (2016) PPAR-delta is repressed in Huntington's disease, is required for normal neuronal function and can be targeted therapeutically. *Nat. Med.* **22**, 37–45.

Dickey A. S., Sanchez D. N., Arreola M., *et al*. (2017) PPARdelta activation by bexarotene promotes neuroprotection by restoring bioenergetic and quality control homeostasis. *Sci. Transl. Med.* **9**, eaal2332.

DiFiglia M., Sapp E., Chase K. O., Davies S. W., Bates G. P., Vonsattel J. P. and Aronin N. (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science* **277**, 1990–1993.

Drombosky K. W., Rode S., Kodali R., Jacob T. C., Palladino M. J. and Wetzel R. (2018) Mutational analysis implicates the amyloid fibril as the toxic entity in Huntington's disease. *Neurobiol. Dis.* **120**, 126–138.

Duennwald M. L., Jagadish S., Giorgini F., Muchowski P. J. and Lindquist S. (2006) A network of protein interactions determines polyglutamine toxicity. *Proc. Natl Acad. Sci. USA* **103**, 11051–11056.

Dunah A. W., Jeong H., Griffin A., *et al*. (2002) Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. *Science* **296**, 2238–2243.

el-Husseini Ael D. and Bredt D. S. (2002) Protein palmitoylation: a regulator of neuronal development and function. *Nat. Rev. Neurosci.* **3**, 791–802.

Finck B. N. and Kelly D. P. (2006) PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J. Clin. Invest.* **116**, 615–622.

Fu Y., Wu P., Pan Y., Sun X., Yang H., Difiglia M. and Lu B. (2017) A toxic mutant huntingtin species is resistant to selective autophagy. *Nat. Chem. Biol.* **13**, 1152–1154.

Garret M., Du Z., Chazalon M., Cho Y. H. and Baufreton J. (2018) Alteration of GABAergic neurotransmission in Huntington's disease. *CNS Neurosci. Ther.* **24**, 292–300.

Gauthier L. R., Charrin B. C., Borrell-Pages M., *et al*. (2004) Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell* **118**, 127–138.

Giorgini F. (2013) A flexible polyglutamine hinge opens new doors for understanding huntingtin function. *Proc. Natl Acad. Sci. USA* **110**, 14516–14517.

Gromiha M. M., Yugandhar K. and Jemimah S. (2017) Protein-protein interactions: scoring schemes and binding affinity. *Curr. Opin. Struct. Biol.* **44**, 31–38.

Guo X., Sun X., Hu D., *et al*. (2016) VCP recruitment to mitochondria causes mitophagy impairment and neurodegeneration in models of Huntington's disease. *Nat. Commun.* **7**, 12646.

Guo Q., Bin H., Cheng J., *et al*. (2018) The cryo-electron microscopy structure of huntingtin. *Nature* **555**, 117–120.

Han T. W., Kato M., Xie S., *et al*. (2012) Cell-free formation of RNA granules: bound RNAs identify features and components of cellular assemblies. *Cell* **149**, 768–779.

Harding R. J., Loppnau P., Ackloo S., *et al*. (2019) Design and characterization of mutant and wild-type huntingtin proteins produced from a toolkit of scalable eukaryotic expression systems. *J. Biol. Chem.* **294**, 6986–7001.

Harjes P. and Wanker E. E. (2003) The hunt for huntingtin function: interaction partners tell many different stories. *Trends Biochem. Sci.* **28**, 425–433.

Hoffner G. and Djian P. (2014) Monomeric, oligomeric and polymeric proteins in huntington disease and other diseases of polyglutamine expansion. *Brain Sci.* **4**, 91–122.

Holmes B. B., DeVos S. L., Kfoury N., *et al*. (2013) Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proc. Natl Acad. Sci. USA* **110**, E3138–3147.

Hoop C. L., Lin H. K., Kar K., *et al*. (2016) Huntingtin exon 1 fibrils feature an interdigitated beta-hairpin-based polyglutamine core. *Proc. Natl Acad. Sci. USA* **113**, 1546–1551.

Hosp F., Gutierrez-Angel S., Schaefer M. H., *et al*. (2017) Spatiotemporal proteomic profiling of Huntington's disease inclusions reveals widespread loss of protein function. *Cell Rep.* **21**, 2291–2303.

Huang K. and El-Husseini A. (2005) Modulation of neuronal protein trafficking and function by palmitoylation. *Curr. Opin. Neurobiol.* **15**, 527–535.

Huang C. C., Faber P. W., Persichetti F., Mittal V., Vonsattel J. P., MacDonald M. E. and Gusella J. F. (1998) Amyloid formation by mutant huntingtin: threshold, progressivity and recruitment of normal polyglutamine proteins. *Somat. Cell Mol. Genet.* **24**, 217–233.

Huang K., Yanai A., Kang R., *et al*. (2004) Huntingtin-interacting protein HIP14 is a palmitoyl transferase involved in palmitoylation and trafficking of multiple neuronal proteins. *Neuron* **44**, 977–986.

Huang K., Sanders S. S., Kang R., *et al*. (2011) Wild-type HTT modulates the enzymatic activity of the neuronal palmitoyl transferase HIP14. *Hum. Mol. Genet.* **20**, 3356–3365.

Intihar T. A., Martinez E. A. and Gomez-Pastor R. (2019) Mitochondrial dysfunction in Huntington's disease; interplay between HSF1, p53 and PGC-1alpha transcription factors. *Front. Cell. Neurosci.* **13**, 103.

Ivarsson Y. and Jemth P. (2018) Affinity and specificity of motif-based protein-protein interactions. *Curr. Opin. Struct. Biol.* **54**, 26–33.

Jayaraman M., Kodali R., Sahoo B., Thakur A. K., Mayasundari A., Mishra R., Peterson C. B. and Wetzel R. (2012) Slow amyloid nucleation via α-helix-rich oligomeric intermediates in short polyglutamine-containing huntingtin fragments. *J. Mol. Biol.* **415**, 881–899.

Jeon I., Cicchetti F., Cisbani G., *et al*. (2016) Human-to-mouse prion-like propagation of mutant huntingtin protein. *Acta Neuropathol.* **132**, 577–592.

Jin Y. N., Hwang W. Y., Jo C. and Johnson G. V. (2012) Metabolic state determines sensitivity to cellular stress in Huntington disease: normalization by activation of PPARgamma. *PLoS ONE* **7**, e30406.

Kang H., Vazquez F. X., Zhang L., Das P., Toledo-Sherman L., Luan B., Levitt M. and Zhou R. (2017a) Emerging beta-sheet rich conformations in supercompact huntingtin exon-1 mutant structures. *J. Am. Chem. Soc.* **139**, 8820–8827.

Kang H., Vázquez F. X., Zhang L., Das P., Toledo-Sherman L., Luan B., Levitt M. and Zhou R. (2017b) Emerging β-sheet rich conformations in supercompact huntingtin exon-1 mutant structures. *J. Am. Chem. Soc.* **139**, 8820–8827.

Kazantsev A., Preisinger E., Dranovsky A., Goldgaber D. and Housman D. (1999) Insoluble detergent-resistant aggregates form between pathological and nonpathological lengths of polyglutamine in mammalian cells. *Proc. Natl Acad. Sci. USA* **96**, 11404–11409.

Kim M. W., Chelliah Y., Kim S. W., Otwinowski Z. and Bezprozvanny I. (2009) Secondary structure of Huntingtin amino-terminal region. *Structure* **17**, 1205–1212.

Kim S. R., Kareva T., Yarygina O., Kholodilov N. and Burke R. E. (2012) AAV transduction of dopamine neurons with constitutively active Rheb protects from neurodegeneration and mediates axon regrowth. *Mol. Ther.* **20**, 275–286.

Kim Y. E., Hosp F., Frottin F., Ge H., Mann M., Hayer-Hartl M. and Hartl F. U. (2016) Soluble oligomers of PolyQ-expanded huntingtin target a multiplicity of key cellular factors. *Mol. Cell* **63**, 951–964.

Knott A. B., Perkins G., Schwarzenbacher R. and Bossy-Wetzel E. (2008) Mitochondrial fragmentation in neurodegeneration. *Nat. Rev. Neurosci.* **9**, 505–518.

Kotler S. A., Tugarinov V., Schmidt T., Ceccon A., Libich D. S., Ghirlando R., Schwieters C. D. and Clore G. M. (2019) Probing initial transient oligomerization events facilitating Huntingtin fibril nucleation at atomic resolution by relaxation-based NMR. *Proc. Natl Acad. Sci. USA* **116**, 3562–3571.

Landles C., Sathasivam K., Weiss A., *et al*. (2010) Proteolysis of mutant huntingtin produces an exon 1 fragment that accumulates as an aggregated protein in neuronal nuclei in Huntington disease. *J. Biol. Chem.* **285**, 8808–8823.

Langfelder P., Cantle J. P., Chatzopoulou D., *et al*. (2016) Integrated genomics and proteomics define huntingtin CAG length-dependent networks in mice. *Nat. Neurosci.* **19**, 623–633.

Laplante M. and Sabatini D. M. (2012) mTOR signaling in growth control and disease. *Cell* **149**, 274–293.

Lee J. H., Tecedor L., Chen Y. H., Monteys A. M., Sowada M. J., Thompson L. M. and Davidson B. L. (2015) Reinstating aberrant mTORC1 activity in Huntington's disease mice improves disease phenotypes. *Neuron* **85**, 303–315.

Leitman J., Ulrich Hartl F. and Lederkremer G. Z. (2013) Soluble forms of polyQ-expanded huntingtin rather than large aggregates cause endoplasmic reticulum stress. *Nat. Commun.* **4**, 2753.

Li S. H. and Li X. J. (2004) Huntingtin-protein interactions and the pathogenesis of Huntington's disease. *Trends Genet.* **20**, 146–154.

Li X. J., Li S. H., Sharp A. H., Nucifora F. C., Jr, Schilling G., Lanahan A., Worley P., Snyder S. H. and Ross C. A. (1995) A huntingtin-associated protein enriched in brain with implications for pathology. *Nature* **378**, 398–402.

Li S. H., Cheng A. L., Zhou H., Lam S., Rao M., Li H. and Li X. J. (2002) Interaction of Huntington disease protein with transcriptional activator Sp1. *Mol. Cell. Biol.* **22**, 1277–1287.

Lin H. K., Boatz J. C., Krabbendam I. E., Kodali R., Hou Z., Wetzel R., Dolga A. M., Poirier M. A. and van der Wel P. C. A. (2017) Fibril polymorphism affects immobilized non-amyloid flanking domains of huntingtin exon1 rather than its polyglutamine core. *Nat. Commun.* **8**, 15462.

Lunkes A., Lindenberg K. S., Ben-Haiem L., Weber C., Devys D., Landwehrmeyer G. B., Mandel J. L. and Trottier Y. (2002) Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions. *Mol. Cell* **10**, 259–269.

Maday S. and Holzbaur E. L. (2012) Autophagosome assembly and cargo capture in the distal axon. *Autophagy* **8**, 858–860.

Marcora E., Gowan K. and Lee J. E. (2003) Stimulation of NeuroD activity by huntingtin and huntingtin-associated proteins HAP1 and MLK2. *Proc. Natl Acad. Sci. USA* **100**, 9578–9583.

Martinez-Vicente M., Talloczy Z., Wong E., *et al*. (2010) Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. *Nat. Neurosci.* **13**, 567–576.

Masnata M., Sciacca G., Maxan A., *et al*. (2019) Demonstration of prion-like properties of mutant huntingtin fibrils in both in vitro and in vivo paradigms. *Acta Neuropathol.* **137**, 981–1001.

Mealer R. G., Murray A. J., Shahani N., Subramaniam S. and Snyder S. H. (2014) Rhes, a striatal-selective protein implicated in Huntington disease, binds beclin-1 and activates autophagy. *J. Biol. Chem.* **289**, 3547–3554.

Miller J., Arrasate M., Shaby B. A., Mitra S., Masliah E. and Finkbeiner S. (2010) Quantitative relationships between huntingtin levels, polyglutamine length, inclusion body formation, and neuronal death provide novel insight into huntington's disease molecular pathogenesis. *J. Neurosci.* **30**, 10541–10550.

Mompean M. and Laurents D. V. (2017) Intrinsically disordered domains, amyloids and protein liquid phases: evolving concepts and open questions. *Protein Pept. Lett.* **24**, 281–293.

Murakami T., Qamar S., Lin J. Q., *et al*. (2015) ALS/FTD Mutation-induced phase transition of FUS liquid droplets and reversible hydrogels into irreversible hydrogels impairs RNP granule function. *Neuron* **88**, 678–690.

Neueder A., Dumas A. A., Benjamin A. C. and Bates G. P. (2018) Regulatory mechanisms of incomplete huntingtin mRNA splicing. *Nat. Commun.* **9**, 3955.

Nucifora F. C., Jr, Sasaki M., Peters M. F., *et al*. (2001) Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. *Science* **291**, 2423–2428.

Ochaba J., Lukacsovich T., Csikos G., *et al*. (2014) Potential function for the Huntingtin protein as a scaffold for selective autophagy. *Proc. Natl Acad. Sci. USA* **111**, 16889–16894.

Paul B. D., Sbodio J. I., Xu R., Vandiver M. S., Cha J. Y., Snowman A. M. and Snyder S. H. (2014) Cystathionine gamma-lyase deficiency mediates neurodegeneration in Huntington's disease. *Nature* **509**, 96–100.

Peskett T. R., Rau F., O'Driscoll J., Patani R., Lowe A. R. and Saibil H. R. (2018) A liquid to solid phase transition underlying pathological huntingtin exon1 aggregation. *Mol. Cell* **70**(588–601), e586.

Peterson T. R., Sengupta S. S., Harris T. E., *et al*. (2011) mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. *Cell* **146**, 408–420.

Pogue R. E., Cavalcanti D. P., Shanker S., Andrade R. V., Aguiar L. R., de Carvalho J. L. and Costa F. F. (2018) Rare genetic diseases: update on diagnosis, treatment and online resources. *Drug Discov. Today* **23**, 187–195.

Ren P. H., Lauckner J. E., Kachirskaia I., Heuser J. E., Melki R. and Kopito R. R. (2009) Cytoplasmic penetration and persistent infection of mammalian cells by polyglutamine aggregates. *Nat. Cell Biol.* **11**, 219–225.

Ripaud L., Chumakova V., Antonin M., *et al*. (2014) Overexpression of Q-rich prion-like proteins suppresses polyQ cytotoxicity and alters the polyQ interactome. *Proc. Natl Acad. Sci. USA* **111**, 18219–18224.

Ross C. A. and Tabrizi S. J. (2011) Huntington's disease: from molecular pathogenesis to clinical treatment. *Lancet Neurol.* **10**, 83–98.

Rui Y. N., Xu Z., Patel B., *et al*. (2015) Huntingtin functions as a scaffold for selective macroautophagy. *Nat. Cell Biol.* **17**, 262–275.

Sahl S. J., Weiss L. E., Duim W. C., Frydman J. and Moerner W. E. (2012) Cellular inclusion bodies of mutant huntingtin exon 1 obscure small fibrillar aggregate species. *Sci. Rep.* **2**, 895.

Sahni N., Yi S., Zhong Q., Jailkhani N., Charloteaux B., Cusick M. E. and Vidal M. (2013) Edgotype: a fundamental link between genotype and phenotype. *Curr. Opin. Genet. Dev.* **23**, 649–657.

Sahni N., Yi S., Taipale M., *et al*. (2015) Widespread macromolecular interaction perturbations in human genetic disorders. *Cell* **161**, 647–660.

Sahoo B., Singer D., Kodali R., Zuchner T. and Wetzel R. (2014) Aggregation behavior of chemically synthesized, full-length huntingtin exon1. *Biochemistry* **53**, 3897–3907.

Sahoo B., Arduini I., Drombosky K. W., Kodali R., Sanders L. H., Greenamyre J. T. and Wetzel R. (2016) Folding landscape of mutant huntingtin exon1: diffusible multimers, oligomers and fibrils, and no detectable monomer. *PLoS ONE* **11**, e0155747.

Sanchez Mejia R. O. and Friedlander R. M. (2001) Caspases in Huntington's disease. *Neuroscientist* **7**, 480–489.

Sathasivam K., Lane A., Legleiter J., *et al*. (2010) Identical oligomeric and fibrillar structures captured from the brains of R6/2 and knock-in mouse models of Huntington's disease. *Hum. Mol. Genet.* **19**, 65–78.

Sathasivam K., Neueder A., Gipson T. A., *et al*. (2013) Aberrant splicing of HTT generates the pathogenic exon 1 protein in Huntington disease. *Proc. Natl Acad. Sci. USA* **110**, 2366–2370.

Saudou F. and Humbert S. (2016) The biology of huntingtin. *Neuron* **89**, 910–926.

Saxton R. A. and Sabatini D. M. (2017) mTOR signaling in growth, metabolism, and disease. *Cell* **168**, 960–976.

Sbodio J. I., Paul B. D., Machamer C. E. and Snyder S. H. (2013) Golgi protein ACBD3 mediates neurotoxicity associated with Huntington's disease. *Cell Rep.* **4**, 890–897.

Sbodio J. I., Snyder S. H. and Paul B. D. (2016) Transcriptional control of amino acid homeostasis is disrupted in Huntington's disease. *Proc. Natl Acad. Sci. USA* **113**, 8843–8848.

Schaffar G., Breuer P., Boteva R., *et al*. (2004) Cellular toxicity of polyglutamine expansion proteins: mechanism of transcription factor deactivation. *Mol. Cell* **15**, 95–105.

Scherzinger E., Lurz R., Turmaine M., *et al*. (1997) Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. *Cell* **90**, 549–558.

Scherzinger E., Sittler A., Schweiger K., Heiser V., Lurz R., Hasenbank R., Bates G. P., Lehrach H. and Wanker E. E. (1999) Self-assembly of polyglutamine-containing huntingtin fragments into amyloid-like fibrils: implications for Huntington's disease pathology. *Proc. Natl Acad. Sci. USA* **96**, 4604.

Shirasaki D. I., Greiner E. R., Al-Ramahi I., *et al*. (2012) Network organization of the huntingtin proteomic interactome in mammalian brain. *Neuron* **75**, 41–57.

Shirendeb U., Reddy A. P., Manczak M., Calkins M. J., Mao P., Tagle D. A. and Reddy P. H. (2011) Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: implications for selective neuronal damage. *Hum. Mol. Genet.* **20**, 1438–1455.

Shirendeb U. P., Calkins M. J., Manczak M., Anekonda V., Dufour B., McBride J. L., Mao P. and Reddy P. H. (2012) Mutant huntingtin's interaction with mitochondrial protein Drp1 impairs mitochondrial biogenesis and causes defective axonal transport and synaptic degeneration in Huntington's disease. *Hum. Mol. Genet.* **21**, 406–420.

Singaraja R. R., Hadano S., Metzler M., *et al*. (2002) HIP14, a novel ankyrin domain-containing protein, links huntingtin to intracellular trafficking and endocytosis. *Hum. Mol. Genet.* **11**, 2815–2828.

Singaraja R. R., Huang K., Sanders S. S., *et al*. (2011) Altered palmitoylation and neuropathological deficits in mice lacking HIP14. *Hum. Mol. Genet.* **20**, 3899–3909.

Song W., Chen J., Petrilli A., *et al*. (2011) Mutant huntingtin binds the mitochondrial fission GTPase dynamin-related protein-1 and increases its enzymatic activity. *Nat. Med.* **17**, 377–382.

Steffan J. S., Kazantsev A., Spasic-Boskovic O., *et al*. (2000) The Huntington's disease protein interacts with p53 and CREB-binding protein and represses transcription. *Proc. Natl Acad. Sci. USA* **97**, 6763–6768.

Subramaniam S., Sixt K. M., Barrow R. and Snyder S. H. (2009) Rhes, a striatal specific protein, mediates mutant-huntingtin cytotoxicity. *Science* **324**, 1327–1330.

Sutton L. M., Sanders S. S., Butland S. L., *et al*. (2013) Hip14l-deficient mice develop neuropathological and behavioural features of Huntington disease. *Hum. Mol. Genet.* **22**, 452–465.

Thakur A. K., Jayaraman M., Mishra R., *et al*. (2009) Polyglutamine disruption of the huntingtin exon 1 N terminus triggers a complex aggregation mechanism. *Nat. Struct. Mol. Biol.* **16**, 380.

Trepte P., Strempel N. and Wanker E. E. (2014) Spontaneous self-assembly of pathogenic huntingtin exon 1 protein into amyloid structures. *Essays Biochem.* **56**, 167–180.

Twelvetrees A. E., Yuen E. Y., Arancibia-Carcamo I. L., *et al*. (2010) Delivery of GABAARs to synapses is mediated by HAP1-KIF5 and disrupted by mutant huntingtin. *Neuron* **65**, 53–65.

Valor L. M. (2015) Transcription, epigenetics and ameliorative strategies in Huntington's disease: a genome-wide perspective. *Mol. Neurobiol.* **51**, 406–423.

Vijayvargia R., Epand R., Leitner A., *et al*. (2016) Huntingtin's spherical solenoid structure enables polyglutamine tract-dependent modulation of its structure and function. *eLife* **5**, e11184.

Vonsattel J. P., Keller C. and Cortes Ramirez E. P. (2011) Huntington's disease – neuropathology. *Handb. Clin. Neurol.* **100**, 83–100.

Waelter S., Boeddrich A., Lurz R., Scherzinger E., Lueder G., Lehrach H. and Wanker E. E. (2001) Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. *Mol. Biol. Cell* **12**, 1393–1407.

Wagner A. S., Politi A. Z., Ast A., *et al*. (2018) Self-assembly of mutant huntingtin exon-1 fragments into large complex fibrillar structures involves nucleated branching. *J. Mol. Biol.* **430**, 1725–1744.

Wanker E. E. (2000) Protein aggregation and pathogenesis of Huntington's disease: mechanisms and correlations. *Biol. Chem.* **381**, 937–942.

Wanker E. E., Scherzinger E., Heiser V., Sittler A., Eickhoff H. and Lehrach H. (1999) Membrane filter assay for detection of amyloid-like polyglutamine-containing protein aggregates. *Methods Enzymol.* **309**, 375–386.

Wear M. P., Kryndushkin D., O'Meally R., Sonnenberg J. L., Cole R. N. and Shewmaker F. P. (2015) Proteins with Intrinsically disordered domains are preferentially recruited to polyglutamine aggregates. *PLoS ONE* **10**, e0136362.

Wong Y. C. and Holzbaur E. L. (2014) The regulation of autophagosome dynamics by huntingtin and HAP1 is disrupted by expression of mutant huntingtin, leading to defective cargo degradation. *J. Neurosci.* **34**, 1293–1305.

Yanai A., Huang K., Kang R., *et al*. (2006) Palmitoylation of huntingtin by HIP14 is essential for its trafficking and function. *Nat. Neurosci.* **9**, 824–831.

Zala D., Hinckelmann M. V. and Saudou F. (2013) Huntingtin's function in axonal transport is conserved in Drosophila melanogaster. *PLoS ONE* **8**, e60162.

Zhai W., Jeong H., Cui L., Krainc D. and Tjian R. (2005) In vitro analysis of huntingtin-mediated transcriptional repression reveals multiple transcription factor targets. *Cell* **123**, 1241–1253.

Zhao Y., Sun X. and Qi X. (2018) Inhibition of Drp1 hyperactivation reduces neuropathology and behavioral deficits in zQ175 knock-in mouse model of Huntington's disease. *Biochem. Biophys. Res. Comm.* **507**, 319–323.

Zheng S., Clabough E. B., Sarkar S., Futter M., Rubinsztein D. C. and Zeitlin S. O. (2010) Deletion of the huntingtin polyglutamine stretch enhances neuronal autophagy and longevity in mice. *PLoS Genet.* **6**, e1000838.

Zuccato C., Tartari M., Crotti A., *et al*. (2003) Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nat. Genet.* **35**, 76–83.