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Review

The Biology of Huntingtin

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Huntingtin (HTT) is now a famous protein because an abnormal expansion of a glutamine stretch (polyQ) in its N-terminal sequence leads to the devastating neurodegenerative disorder Huntington's disease (HD). The gene encoding huntingtin, *HTT*, and its dominantly inherited mutation were identified more than 20 years ago. Subsequently, in the hope of finding a cure for HD, there has been intense research aimed at understanding the molecular mechanisms underlying the deleterious effects of the presence of the abnormal polyQ expansion in HTT. Notwithstanding with the value of this approach, evidence has been emerging of a potential role of context and function of the HTT protein in the specificity and severity of the pathogenicity. HTT is ubiquitous both at the tissue and subcellular levels. It interacts with many partners and has long been considered having no clearly defined cellular function. Based on research over the past 20 years, specifically focused on the function of wild-type HTT, we reconsider the literature describing HTT-regulated molecular and cellular mechanisms that could be dysfunctional in HD and their possible physiological consequences for patients.

Introduction

Huntington's disease (HD) is a genetically inherited autosomal dominant neurodegenerative disorder, with a mean age at onset of 40 years (Ross and Tabrizi, 2011). It is a rare disorder with a prevalence of 5–10 individuals per 100,000 in the Caucasian population. Some juvenile forms exist but are rare, accounting for 5% of the cases. The symptoms vary between individuals but are usually characterized by a triad of motor, cognitive, and psychiatric symptoms. Motor symptoms can be divided into the choreiform movements with gait disturbances that tend to appear early in the course of the disease and motor impairments such as bradykinesia and rigidity that are observed in later stage patients. Cognitive symptoms can be detected up to a decade before diagnosis, and decline progresses as the disease progresses. The deficits include cognitive slowing and decreases in both attention and mental flexibility. Psychiatric symptoms and/or emotional deficits are also observed early in HD patients. HD patients are frequently depressed and show signs of apathy, irritability, impulsivity, and social disinhibition.

The neuropathology of HD is characterized by the dysfunction and death of specific neurons within the brain (Ross and Tabrizi, 2011). In particular, neurons of the striatum are the most susceptible to death. Interneurons are usually spared. The neurons that project from the cortex to the striatum are also particularly affected, and reduction of the striatum and thinning of the cortex start a decade before the appearance of the symptoms (Aylward et al., 2011; Rosas et al., 2008). Although alterations of the CNS are the most prominent clinical features of HD, patients also suffer from metabolic and immune disturbances, skeletal-muscle wasting, weight loss, cardiac failure, testicular atrophy, and osteoporosis (van der Burg et al., 2009). Patients usually die 20 years after onset, and, in many cases, death results from fatal aspiration pneumonia.

The mutation responsible for HD is an abnormal expansion of a CAG repeat in the *HTT* gene that encodes for huntingtin (HTT), a large protein of 3,144 amino acids. The CAG repeat in *HTT* codes for a polymorphic polyglutamine (polyQ) stretch. In the non-HD population, the CAG sequence is repeated 9 to 35 times, with an average median of between 17 and 20 repeats (Kremer et al., 1994). A CAG expansion exceeding 35 repeats results in HD. Rare carriers of 36 to 39 CAG repeats have lower penetrance and later onset of the disease than those with 40 or more CAG repeats. Indeed, the age at onset of the disease is inversely proportional to the length of the CAG expansion, with juvenile onset being associated with *HTT* carrying about 75 or more repeats. HD is autosomal dominant. Although rare, homozygous patients show the same age at onset as heterozygotes, but disease progression can be more severe (Lee et al., 2012).

Abnormal polyQ expansion has been found to be causative of eight other neurodegenerative disorders, including several spinocerebellar ataxias (Orr, 2012). Each of these diseases is associated with an expansion in a different protein and is characterized by the loss of specific neurons, with little overlap between the brain regions affected in these various diseases. This is a strong argument that, although polyQ expansion is the causative event that leads to disease in a dominant manner, the protein in which expansion occurs determines the specificity of the disease. In this review, we will focus on HTT protein, describe its native function(s) and physiological role(s), and discuss how a better knowledge of HTT biology may contribute to understanding HD pathogenesis.

Huntingtin Is a Multiple Conformation Protein

The *HTT* gene encodes a 348-kDa protein well conserved from flies to mammals, the highest identity being found between mammals. The very N-terminal region has been extensively studied, as it contains the expandable polyQ stretch. It is preceded

Table 1. HTT Post-translational Modifications

Residue	Modification	Enzyme	Function	Analysis	References
Thr 3	Phos			MS/Ab	Aiken et al. (2009), Huang et al. (2015)
Lys 6	Ubi/Sumo			IV	Steffan et al. (2004)
Lys 9	Acet/Ubi/Sumo			MS/IV	Cong et al. (2011), Steffan et al. (2004)
Ser 13	Phos	IKK	K9/Ubi/Sumo	MS/Ab	Thompson et al. (2009)
Lys 15	Ubi/Sumo			IV	Steffan et al. (2004)
Ser 16	Phos	IKK	K9/Ubi/Sumo	MS/Ab	Thompson et al. (2009)
Ser 116	Phos			MS	Watkin et al. (2014)
Ser 120	Phos			MS	Watkin et al. (2014)
Lys 178	Acet			MS	Cong et al. (2011)
Cys 214	Palm			MS/IV-Palm	Yanai et al. (2006)
Lys 236	Acet			MS/Ab	Cong et al. (2011)
Thr 271	Phos			MS	Watkin et al. (2014)
Lys 345	Acet			MS/Ab	Cong et al. (2011)
Ser 417	Phos	Akt/PKA?		MS	Huang et al. (2015), Watkin et al. (2014)
Ser 419	Phos	Akt/PKA?		MS	Huang et al. (2015), Moritz et al. (2010)
Ser 421	Phos	Akt/SGK1/PP2B	Axonal transport	MS/Ab	Huang et al. (2015), Humbert et al. (2002), Moritz et al. (2010), Pardo et al. (2006), Rangone et al. (2004), Schilling et al. (2006), Watkin et al. (2014)
Ser 431	Phos			MS/Ab	Dong et al. (2012), Huang et al. (2015), Watkin et al. (2014)
Ser 432	Phos			MS/Ab	Dong et al. (2012), Huang et al. (2015)
Ser 434	Phos	Cdk5	Casp-3 site 513	Ab/MS	Huang et al. (2015), Luo et al. (2005), Watkin et al. (2014)
Ser 438	Phos			MS	Huang et al. (2015)
Lys 444	Acet/Ubi	CBP/HDAC1	autophagy	MS/Ab	Cong et al. (2011), Jeong et al. (2009)
Ser 457	Phos			MS	Watkin et al. (2014)
Ser 459	Phos			MS	Watkin et al. (2014)
Ser 461	Phos			MS	Watkin et al. (2014)
Ser 464	Phos			MS	Watkin et al. (2014)
Ser 465	Phos			MS	Watkin et al. (2014)
Ser 466	Phos			MS	Watkin et al. (2014)
Ser 487	Phos			MS	Watkin et al. (2014)
Thr 488	Phos			MS	Watkin et al. (2014)
Ser 491	Phos			MS	Watkin et al. (2014)
Ser 536	Phos	PKC?	Calp 536 site	MS	Schilling et al. (2006)
Ser 642	Phos	Akt?		MS	Huang et al. (2015), Moritz et al. (2010)
Ser 644	Phos			MS	Huang et al. (2015)
Ser 645	Phos			MS	Huang et al. (2015)
Ser 1181	Phos	Cdk5	axonal transport	MS/Ab	Anne et al. (2007), Huang et al. (2015), Schilling et al. (2006)
Ser 1197	Phos			MS	Huang et al. (2015)
Ser 1201	Phos	Cdk5	axonal transport	MS/Ab	Anne et al. (2007), Huang et al. (2015), Schilling et al, (2006)
Ser 1351	Phos			MS	Huang et al. (2015)
Tyr 1357	Phos			MS	Huang et al. (2015)
Ser 1866	Phos			MS	Huang et al. (2015)
Ser 1868	Phos			MS	Huang et al. (2015)
Thr 1872	Phos			MS	Huang et al. (2015)
Ser 1876	Phos			MS	Huang et al. (2015)

(*Continued on next page*)

Table 1. *Continued*

Residue	Modification	Enzyme	Function	Analysis	References
Ser 2076	Phos	ERK1?		MS	Schilling et al. (2006)
Thr 2337	Phos			MS	Huang et al. (2015)
Ser 2550	Phos			MS	Huang et al. (2015)
Ser 2653	Phos	ERK1?		MS	Huang et al. (2015), Schilling et al. (2006)
Ser 2657	Phos	GSK3?		MS	Huang et al. (2015), Schilling et al. (2006)

The identification of the modifications was performed using mass spectrometry (MS), specific antibodies against the modified sites (Ab), and in vitro assays (IV; IV-Palm: in vitro palmitate assay) as indicated. Thr, threonine; Lys, lysine; Ser, serine; Cys, cysteine; Tyr, tyrosine; Phos, phosphorylation; Ubi, ubiquitination; Sumo, sumoylation; Acet, acetylation; Palm, palmitoylation.

by 17 amino acids and followed by a proline-rich domain (PRD). Both the polyQ stretch and the PRD are polymorphic in the human population. However, exon 1 has been poorly conserved during evolution, in contrast to other exons (e.g., exon 2) that are present in *Apis mellifera* or *Tribolium castaneum* and very similar to those in mammals.

The N-terminal 17 amino acids are conserved in vertebrates but less in protostomes (Tartari et al., 2008). This region consists of an amphipathic α-helix (Atwal et al., 2007), whose structure is important for retention in the endoplasmic reticulum (Atwal et al., 2007; Rockabrand et al., 2007). It functions as a nuclear export signal (NES) and is subject to post-translational modifications: acetylation, sumoylation, and ubiquitination at lysines 6, 9, and 15 and phosphorylation at serines 13 and 16 (S13 and S16, respectively) that affect the clearance of HTT and its subcellular localization (Table 1) (Atwal et al., 2007; Maiuri et al., 2013; Steffan et al., 2004; Thompson et al., 2009).

Ancestors at the base of the protostome-deuterostome divergence have either a single Q or no Q (Tartari et al., 2008). In contrast, the sea urchin (considered to be the most ancient deuterostome) has a NHQQ tract that appears to be the functional homolog of the 4Q repeat found in other vertebrates. The polyQ stretch is larger in mammals and especially in humans, where the stretch is polymorphic. The consequences of the variability of the polyQ stretch on normal HTT function are not well understood. However, consistent with the role of HTT in the regulation of autophagy, deletion of the polyQ stretch in mice enhances autophagy and results in increased longevity (Zheng et al., 2010).

The PRD is found only in mammals, suggesting recent evolution of HTT protein (Tartari et al., 2008). The PRD is variable in the non-HD population and is critical for interactions with proteins that contain tryptophans (WW-proteins) or Src homology 3 (SH3) domains (Harjes and Wanker, 2003). Although this domain appears to be important for mediating protein-protein interaction, its deletion in vivo has no profound effect on mouse behavior (Neveklovska et al., 2012).

The secondary structure of the N-terminal region of HTT containing 17Q has been resolved: the first N-17 amino acids form an α-helical structure, but the 17Q stretch is a flexible region that can adopt several conformations including α-helix, random coil, and extended loop (Kim et al., 2009). The PRD has a proline-proline (PP) helix, a relatively rigid structure that has a kinked or straight conformation. The PP helix formed in the PRD may be important for stabilizing the structure of the polyQ stretch; it may have an effect on the propensity of mutant HTT to aggregate.

The rest of the protein is less well characterized. This corresponds to 66 exons encoding amino acids 69 to 3,144 or 97.8% of the protein! The poor knowledge regarding this part of the protein is because the pathogenic mutation is in exon 1, resulting in most research being focused on this exon. The amino acid fragment between positions 69 and 3,144 contains several HEAT repeats that are important for protein-protein interaction (Palidwor et al., 2009). These repeats are found in HTT, Elongation factor 3, protein phosphatase 2A, and TOR1 and are formed of antiparallel α-helices separated by a non-helical region. Bioinformatic analyses of HTT report between 16 and 36 HEAT repeats clustered into three to five larger alpha-rod domains separated by disordered regions (Figure 1) (Palidwor et al., 2009; Takano and Gusella, 2002; Tartari et al., 2008; Warby et al., 2008).

The HEAT repeat domains may function as a solenoid-like structure that acts as a scaffold for numerous protein complexes and mediates inter- and intra-molecular interactions. The middle region of HTT (507–1,230) can bind to the N-terminal (1–506) and C-terminal (2,721–3,144) domains of HTT (Palidwor et al., 2009); the 507–1,230 domain can also self-associate to form homodimers of HTT. Similarly, N-terminal parts of HTT (1–416 and 1–586) bind to different C-terminal regions of HTT (1,725–2,800 and 2,416–3,144, respectively), and these intramolecular interactions are disrupted upon proteolysis (El-Daher et al., 2015; Ochaba et al., 2014). These observations suggest that HTT can adopt various three-dimensional (3D) conformations, depending on its intra-molecular interactions. These interactions may also involve other protein complexes, as HTT has numerous interacting partners. In agreement, purified HTT can adopt up to 100 structurally distinguishable conformations (Seong et al., 2010).

Finally, other functional motifs such as the NES identified at position 2,397–2,406 may regulate HTT function or localization (Xia et al., 2003).

Not Just One Huntingtin: Increasing Diversity and Fine-Tuning

From Canonical Huntingtin to Huntingtin Isoforms

HTT gene contains 67 exons and has two mRNA transcripts of 10,366 bp and of 13,711 bp (Lin et al., 1993). The second transcript differs by an additional 3′ UTR sequence of 3,360 bp and seems to be enriched in the brain. Only very recently, alternative splicing has been reported for HTT with the generation of HTT protein variants that could lack exons 10, 12, 29, and 46 or,

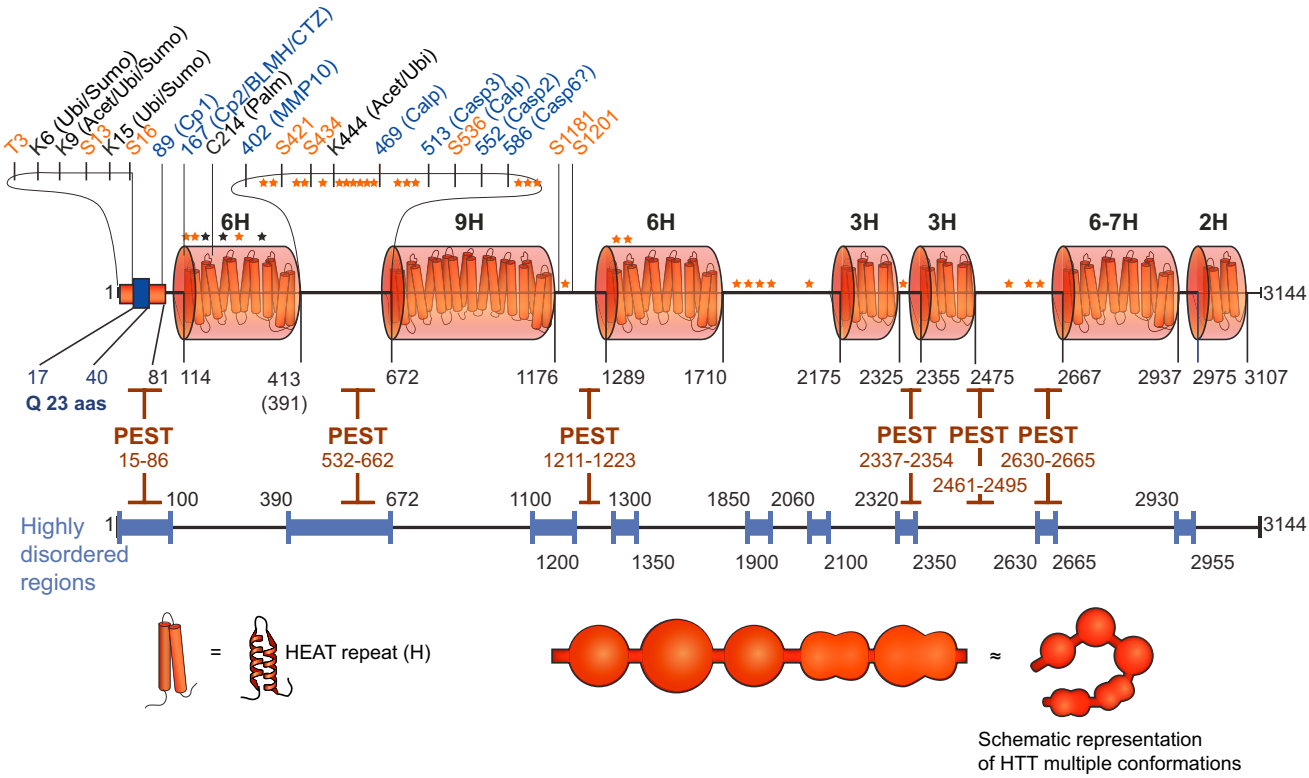

Figure 1. Cartoon of the Human Huntingtin Protein Sequence

Amino acid (aa) positions in orange: phosphorylation sites identified by mass spectrometry and further confirmed by other approaches. Amino acid positions in black: sites of indicated modifications. Amino acid positions in blue: cleavage sites. Cp1, cleavage site by unknown protease; Cp2/BLMH/CTZ, cleavage by Bleomycin hydrolase or cathepsin Z; Casp6? is a confirmed cleavage site: while initially identified as a caspase 6 site, caspase 6 may not be the cleaving protease in vivo. Orange and black stars indicate, respectively, phosphorylation and acetylation sites identified by mass spectrometry only with no further confirmations. Numbers arranged linearly on the 2D structure correspond to the limits of the indicated domains (HEAT, PEST, and Highly disordered regions). H indicates the number of predicted HEAT repeats organized in larger domains and PEST regions are proteolysis-sensitive domains. Highly disordered regions correspond to predicted disordered regions using PONDR, DisEMBL, and NORSp. Bottom: Schematic HTT with spheres that correspond to stretches of HEAT repeats. HTT could adopt multiple conformations according to (Seong et al., 2010). Ubi, ubiquitin; Sumo, sumoyl; Acet, acetyl; Palm, palmitoyl; MMP10, metalloproteinase 10; Calp, calpain; Casp3/2/6, caspase 3/2/6.

alternatively, retain a 57-bp portion of intron 28 or have an additional exon (41b) (Hughes et al., 2014; Ruzo et al., 2015). Although these variants are rare, some of them could be upregulated during development. Absence of these regions could modify the function of canonical HTT in protein-protein interaction, regulation by phosphorylation, and/or susceptibility to cleavage (Hughes et al., 2014; Ruzo et al., 2015).

Huntingtin Proteolysis Generates Huntingtin Variants

HTT is subjected to proteolysis at several sites by a variety of proteases some of which remained to be identified. The proteolytic sites are in PEST—amino acids proline (P), glutamic acid (E) or aspartic acid (D), serine (S), and threonine (T)—domains that are mostly found in disordered regions (Figure 1) (Warby et al., 2008). Proteases reported to cleave HTT include several caspases, calpain, cathepsins, and the metalloproteinase MMP10 (Gafni and Ellerby, 2002; Goldberg et al., 1996; Hermel et al., 2004; Kim et al., 2001, 2006; Lunkes et al., 2002; Miller et al., 2010; Ratovitski et al., 2009; Tebbenkamp et al., 2012). These proteolytic sites are present on both wild-type and mutant polyQ-HTT, and wild-type HTT is as good as a substrate as the polyQ-HTT for cleavage in vitro (Goldberg et al., 1996). Importantly, in HD, there is a specific increase of the activity of these proteases in brains of patients. This disease-specific enhanced proteolysis is key, as it leads to the generation and accumulation of small N-terminal fragments that contain the polyQ stretch and that translocate into the nucleus where they are toxic (Benn et al., 2005; Graham et al., 2006; Saudou et al., 1998).

The physiological consequences of the cleavage of wild-type HTT remain elusive. Proteolysis of wild-type HTT has not been reported in normal individuals. Proteolysis of wild-type HTT may inactivate some of its functions (El-Daher et al., 2015). As toxic non-polyQ C-terminal fragments are generated by proteolysis of both wild-type and mutant polyQ-HTT, specific proteolytic events of wild-type HTT may also induce cell death. Consistent with this idea, artificial proteolysis of wild-type HTT causes toxicity in cells and in flies (El-Daher et al., 2015). When would cleavage of wild-type HTT occur? Such proteolysis may occur in disease conditions—other than HD—in which massive cell death and/or caspases are activated or may be associated to developmental apoptosis. Therefore, proteolysis of wild-type HTT by specific proteases may both inactivate specific HTT functions and activate cell death, as is the case for substrates of effector caspases, and, consequently, participate in the induction of cell death. As such, proteolysis of full-length

wild-type could be a switch to change the neuroprotective function(s) of wild-type HTT into a toxic one.

The plethora of proteolytic sites in HTT suggests that different cleavages elicit different physiological responses, depending on the coordinated proteolytic events. For example, the orchestrated cleavage of HTT at both positions 586 and 552 generates a 553–586 fragment that, when myristoylated, leads to autophagosome formation and an increase in autophagic flux (Martin et al., 2014).

Post-translational Modifications Regulate Huntingtin Function and Activity

HTT is subjected to multiple post-translational modifications. These include phosphorylation, acetylation, palmitoylation, ubiquitylation, and sumoylation (Figure 1; Table 1). The role of these modifications has been mostly studied in the context of the mutant polyQ protein. Several have a therapeutic relevance, as they modulate toxicity of mutant HTT. Acetylation of mutant HTT is important to mediate its clearance by the autophagic-lysosomal pathway, but wild-type HTT is comparatively acetylated at lower levels (Jeong et al., 2009). Phosphorylation of mutant HTT at S434 or S536 decreases HTT proteolysis by caspase 3 and calpain, respectively, and reduces polyQ-HTT toxicity (Luo et al., 2005; Schilling et al., 2006). Whether these mechanisms are relevant for the regulation of the normal functions of HTT is unknown. Similarly, phosphorylation at S13 and S16 promotes clearance of both wild-type and mutant polyQ-HTT and thereby reduces toxicity (Thompson et al., 2009). However, phosphorylation at S13 and S16 may be critical for mediating nuclear localization as the N-terminal domain sequence leucine 4 phenylalanine 11 S16 (L4F11S16) functions as an NES and a phospho-mimicking mutant at S16 blocks CRM1-dependent nuclear export (Maiuri et al., 2013). Phosphorylation/dephosphorylation at S421 and S1181/S1201 regulate MT-dependent intracellular transport of organelles. HTT phosphorylation at S1181/S1201 increases both anterograde and retrograde transport; phosphorylation at S421 selectively promotes transport in the anterograde direction (Ben M'Barek et al., 2013; Colin et al., 2008; Humbert et al., 2002).

HTT modifications could not only impact on HTT localization and/or function but also on the function of other proteins. HTT interacts with HIP14 and HIP14L (DHHC17 and DHHC13, respectively) that belong to the family of the palmitoyl-acyl transferases (PATs) (Yanai et al., 2006). HTT is itself palmitoylated at C214, and loss of HTT and polyQ expansion in HTT lead to a reduction in the enzymatic activity of HIP14 and of its auto-palmitoylation (Huang et al., 2011; Yanai et al., 2006). Consequently, this affects the PAT activity of HIP14 on other substrates. HTT via HIP14 and palmitoylation of proteins, including HTT and HIP14 themselves, could, therefore, regulate intracellular trafficking and synaptic localization of various proteins in neurons (Huang et al., 2011).

Huntingtin Is Ubiquitous

HTT transcripts and protein are found at different levels throughout most human and murine tissues (Marques Sousa and Humbert, 2013). Within the nervous system, its expression is not restricted to the brain regions that degenerate in cases of HD, as HTT is found in several types of striatal projection neurons and interneurons as well as in the cortex, hippocampus, and cerebellum. HTT expression is higher in the nervous system than in other tissues. However, an apparent scarcity of HTT in tissue may reflect heterogeneity in HTT levels, with expression restricted to certain cellular subtypes that contain large amounts of the protein. This might be the case for peripheral tissues. Peripheral cells expressing no or little HTT are mesenchymal cells, and epithelial cells in the same tissues have higher levels of HTT (Marques Sousa and Humbert, 2013).

The broad expression of HTT through time adds another layer of complexity. HD is characterized by clinical manifestations in adults, but HTT expression starts early during development and persists in adulthood. The most compelling proof of the early expression of HTT is that its knockout is lethal on embryonic day (E) 7.5 (Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995).

Thus, HTT is widely expressed in both space and time. However, most relevant studies report expression in lysates of whole tissues or fragmentary expression patterns, and when and where exactly HTT is present still requires clarification.

HTT Partners: The Story They Tell

The search for partners of HTT started quickly after the characterization of the gene and the protein. A first set of studies used yeast two-hybrid approaches and identified about 40 HTT interactors, including new uncharacterized proteins. The nature of some of these HTT-interacting proteins implicated HTT in diverse cellular functions, including transcription, RNA splicing, endocytosis, trafficking, and cellular homeostasis (Harjes and Wanker, 2003). Interestingly, the interaction of some of these partners and HTT is modified by the polyQ stretch. A systematic yeast two-hybrid screen unraveled an HTT protein-protein interacting network containing 186 protein-protein interactions and 15 additional new HTT interactors (Goehler et al., 2004). Kaltenbach and collaborators combined yeast-two hybrid screening with affinity pulldown followed by mass spectrometry and expanded the list by identifying 234 proteins interacting with HTT (Kaltenbach et al., 2007). Some of these HTT partners were further validated as genetic modifiers of mutant HTT-induced neurodegeneration in a *Drosophila* model of HD. Other sets of systematic analyses were designed to specifically compare or quantify the interactions of wild-type and mutant HTT, including quantitative proteomics coupled to tandem affinity purification (Culver et al., 2012; Ratovitski et al., 2012; Shirasaki et al., 2012). They led to the identification in striatal cells of 349 differential interactors; 200 were more abundant in mutant complexes and 149 were more abundant in wild-type HTT complexes in striatal cells (Ratovitski et al., 2012). Similarly, the analysis of affinity-purified complexes from wild-type and mutant mouse brains allowed the description of a total of 363 wild-type and 350 mutant differential HTT interactors (Culver et al., 2012). With these studies and several candidate-based investigations, more than 350 partners of wild-type HTT have now been identified. However, many of these HTT-interacting proteins remain to be further validated by binding assays and co-localization experiments. These proteins can be found using the Human Integrated Protein-Protein Interaction rEference (HIPPIE) website: http://cbdm-01.zdv.uni-mainz.de/~mschaefer/hippie/query.php?s=htt (Schaefer et al., 2012).

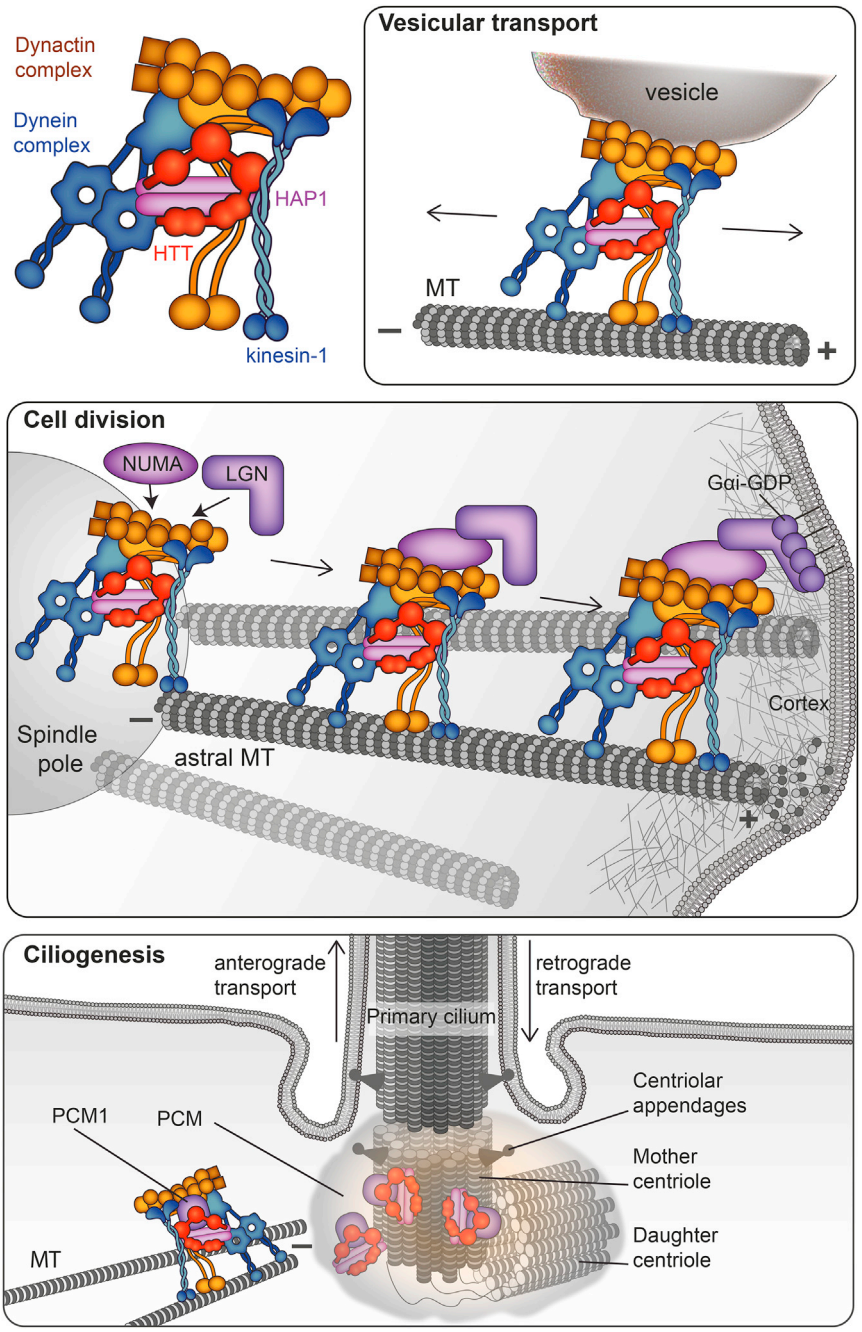

Figure 2. Huntingtin Scaffolds Dynein/Dynactin to Regulate Several Cellular Processes

Top: HTT controls the transport of organelles, in both anterograde and retrograde directions, and in axons and dendrites within neurons. Middle: During mitosis, HTT is important for spindle pole assembly and also regulates the kinesin 1-dependent trafficking of dynein/dynactin/NUMA/LGN to the cell cortex. Bottom: HTT mediates the dynein/dynactin/HAP1-dependent transport of proteins to the pericentriolar material, including PCM1 protein that is required for ciliogenesis. MT, microtubules; PCM, pericentriolar material.

The list of HTT interactors supports the idea that HTT is involved in several, but not all, cellular pathways. Most of the proteins found consistently in numerous studies belong to one of the following categories: cellular dynamics (cytoskeleton, endocytosis, trafficking, and adhesion), metabolism, protein turnover, and gene expression (transcription and RNA processing). Overlapping with these categories, the set of HTT-interacting partners is enriched in proteins involved in signal transduction. This restricted list of functional groups to which HTT partners can be assigned, as well as the large number of HTT partners, is consistent with HTT acting as a molecular scaffold. Other arguments in favor of HTT being a scaffold are its large size and its stability. HTT may serve as a hub, tethering multiple partners into complexes to coordinate cellular processes. The assembly and dis-assembly of the complexes may be tightly regulated in time and space by the presence on the HTT platform of proteins involved in signaling. For example (Figure 2), HTT regulates the assembly of dynein/dynactin complexes for several functions, and such dynein/dynactin scaffolding is modulated by HTT phosphorylation.

Very few proteins interacting with internal and C-terminal fragments of HTT have been identified, whether by using full-length HTT or HTT C-terminal regions as bait. This is coherent with the intramolecular interaction observed between the C-terminal and N-terminal regions of HTT (El-Daher et al., 2015; Li et al., 2006; Ochaba et al., 2014; Palidwor et al., 2009). HTT may adopt a closed conformation and establish only limited interactions between the C-terminal region and other proteins.

Finally, the nature of these HTT-containing protein complexes is likely to be cell type and time dependent, since the precise distribution of not only HTT but also its partners remains to be addressed. Therefore, a complete understanding of when and where these complexes function is required to precisely delineate the multiple roles of HTT and their relevance to HD pathogenesis.

HTT Functions: "One for All and All for One"

This section focuses on some of the functions that have been described for wild-type HTT at the molecular level, rather than those that have been attributed to HTT because they are affected when mutant HTT is expressed. The physiological outputs of the functions of HTT in the developing and mature organism are

numerous; however, these outputs at an integrated level (tissue or whole organism) may well correspond to a limited set of functions at the molecular level.

Huntingtin Traffics Vesicles

HTT interacts with the molecular motor machinery, either directly with dynein or indirectly through the Huntingtin-associated protein 1 (HAP1) with the $p150^{Glued}$ subunit of dynactin and the kinesin 1 member KIF5C (Caviston et al., 2007; Colin et al., 2008; Engelender et al., 1997; Gauthier et al., 2004; Gunawardena et al., 2003; Li et al., 1998; McGuire et al., 2006; Strehlow et al., 2007; Twelvetrees et al., 2010) (Figure 2). Through these interactions, HTT controls the transport of organelles, in both the anterograde and retrograde directions, and in axons and dendrites within neurons. Although the exhaustive list of cargo transported by HTT remains to be established, HTT transports a variety of organelles, including synaptic precursor vesicles (Zala et al., 2013a), vesicles that contain the v-SNARE VAMP7 protein (Colin et al., 2008), autophagosomes (Wong and Holzbaur, 2014), endosomes and lysosomes (Caviston et al., 2011; Liot et al., 2013), brain-derived neurotrophic factor (BDNF)-containing vesicles (Gauthier et al., 2004), APP (amyloid precursor protein)-positive vesicles (Colin et al., 2008; Her and Goldstein, 2008), and GABA-receptor-containing vesicles (Twelvetrees et al., 2010). This list is based on the observations that overexpression of wild-type HTT promotes transport efficacy, whereas HTT silencing decreases the motility of the organelles. Silencing and knockout of HTT both decrease transport in fly larvae (Gunawardena et al., 2003; Zala et al., 2013a), and expression of a fragment of the fly HTT rescues transport in mammalian neurons silenced for HTT (Zala et al., 2013a), showing that this function has been conserved during evolution. Whether wild-type HTT also contributes to the transport of mitochondria is less clear (Gauthier et al., 2004; Trushina et al., 2004). Indeed, most of the relevant described alterations in the dynamics of mitochondria have been observed with mutant HTT and could result from the association of mutant HTT to the fusion-fission machinery upon HTT proteolysis (Costa et al., 2010; Orr et al., 2008; Song et al., 2011) rather than from an altered function of HTT in transporting mitochondria when HTT is mutated.

HTT may enhance the velocity of vesicles along MTs through its capacity to bind and to maintain the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) on vesicles that act locally to provide energy for the fast axonal transport of vesicles (Zala et al., 2013b). Indeed, reducing the amount of HTT both in vitro and in mouse in vivo detaches GAPDH from vesicles and reduces transport efficiency. Therefore, HTT, through the scaffolding of both GAPDH and the molecular motors, could couple energy production to energy consumption.

Not only is HTT a facilitator of transport by increasing vesicle velocity, but it also coordinates the directionality of transport through its phosphorylation at S421 (Colin et al., 2008). S421 phosphorylation is mediated by the kinases Akt/PKB and the serum- and glucocorticoid-induced kinase SGK (Humbert et al., 2002; Rangone et al., 2004), and dephosphorylation occurs upon calcineurin activation (Pardo et al., 2006).

Huntingtin Coordinates Cell Division

HTT expression is not restricted to differentiated neurons and is abundant in dividing cells, where it is localized at the spindle poles, mitotic spindles, and astral microtubules (Elias et al., 2014; Godin et al., 2010; Gutekunst et al., 1995). During the mitosis of neuronal and non-neuronal cells, HTT is targeted to the spindle poles through its interaction with dynein, where it promotes the accumulation of NUMA and LGN (Elias et al., 2014; Godin et al., 2010) (Figure 2). In cells from the mammary gland, HTT also regulates the kinesin-1-dependent trafficking of dynein, dynactin, NUMA, and LGN along astral microtubules to the cell cortex (Elias et al., 2014). This cortical localization of dynein/dynactin/NUMA/LGN is required to generate pulling forces on astral microtubules for mitotic spindle positioning. Thus, loss of HTT function during mitosis leads to spindle misorientation.

As for transport, the function of HTT in mediating spindle orientation is evolutionarily conserved: silencing *Drosophila* HTT leads to spindle misorientation in fly neuroblasts (Godin et al., 2010). Conversely, expression of *Drosophila* HTT rescued spindle misorientation defects in HTT-depleted mammalian cells.

Huntingtin Regulates Ciliogenesis

HTT is required for ciliogenesis (Haremaki et al., 2015; Keryer et al., 2011) (Figure 2). HTT is found at the base of the cilia in primary cilia in neurons, photoreceptor cilia, and cilia in multiciliated cells (Haremaki et al., 2015; Karam et al., 2015; Keryer et al., 2011). In the photoreceptor cilium, HTT is particularly abundant in the axoneme (Karam et al., 2015). HTT may have several roles in cilium function. HTT associates with HAP1 and pericentriolar material 1 protein (PCM1) at the centrosome in interphasic cells in a microtubule-dependent manner (Keryer et al., 2011). HTT, thereby, mediates the dynein/dynactin/HAP1-dependent transport of proteins to the pericentriolar material, including PCM1, which is absolutely required for ciliogenesis. Absence of HTT from mouse cells impairs the retrograde trafficking of PCM1, and the primary cilium is not formed. Similarly, the absence of HTT from multiciliated cells, such as ependymal cells in mouse or skin epithelial cells in *Xenopus*, leads to reduced ciliogenesis (Haremaki et al., 2015; Keryer et al., 2011). The localization of HTT in the axomeme could be regulated by the phosphorylation of the NES motif present on the N-terminal domain (Maiuri et al., 2013). Also, its interaction with HIP1—which itself interacts with Hippi, the human homolog of the intraflagellar protein IFT57—provides a potential pathway by which HTT could regulate intraflagellar transport and ciliogenesis (Badano et al., 2005).

Huntingtin Mediates Endocytosis, Vesicle Recycling, and Endosomal Trafficking

HTT interacts with several proteins involved in endocytosis, including huntingtin-interacting protein 1 (HIP1) and HIP1R (HIP12); these proteins participate in clathrin-mediated endocytosis by supporting membrane invagination and the assembly of the clathrin coating (Engqvist-Goldstein et al., 2001; Legendre-Guillemin et al., 2002; Waelter et al., 2001). In addition to possibly regulating the first step of endocytosis though membrane coating and invagination, HTT interacts with dynamin 1 through both its N- and C-terminal domains, suggesting a scaffolding role of HTT in dynamin 1 activation (El-Daher et al., 2015; Kaltenbach et al., 2007; Moreira Sousa et al., 2013). Support for such a role for HTT in the fission step of endocytosis is the observation that artificially induced proteolysis of HTT leads to the inactivation of dynamin 1 and inhibition of endocytosis, as measured

by transferrin uptake (El-Daher et al., 2015). HTT may be part of a larger complex: HTT is present in a complex containing SH3GL3/endophilin-A3, endophilin-B1, amphiphysin, and dynamin and possibly acting during both endocytosis and vesicle recycling (Modregger et al., 2003; Sittler et al., 1998). Consistent with this, HTT also interacts with and activates the GTPase Rab11 that participates in vesicle recycling during endocytosis (Li et al., 2008). Loss of HTT in HTT knockout embryonic stem cells reduces Rab11 attachment to membranes and its activity (Li et al., 2008). In 3D Madin-Darby canine kidney (MDCK) cultures and cysts, HTT activates Rab11 to coordinate the apical vesicular trafficking of PAR3-PAR6-aPKC, a mechanism that is involved in the establishment of epithelial polarity (Elias et al., 2015).

HTT also regulates endosomal trafficking via the protein HAP40 that binds with Rab5 (Pal et al., 2006) to form a complex that is recruited onto early endosomes. When associated with HAP40, HTT interacts preferentially with actin, reducing endosome motility and allowing the switch of endosomes from MTs to actin filaments. Another mechanism by which HTT may mediate transition of cargo between MTs and actin cytoskeletons is through its interaction with the Rab8/optineurin/myosinVI complex (Faber et al., 1998; Hattula and Peränen, 2000; Sahlender et al., 2005) and the HAP1/dynactin complex (Engelender et al., 1997; Gauthier et al., 2004; Li et al., 1998). This transition could occur both at the plasma membrane and at the *trans*-Golgi network, thereby ensuring proper trafficking between the two compartments.

Huntingtin Is an Autophagy-Related Protein

Evidence for the involvement of HTT in autophagy has emerged following a series of studies highlighting autophagy defects in HD (Martin et al., 2014; Steffan, 2010). PolyQ-HTT abnormally activates the autophagy pathway in various HD models through inactivation of mammalian target of rapamycin (mTOR) kinase (Kegel et al., 2000; Ravikumar et al., 2004). However, although the formation of autophagosomes is abnormally high in HD, a defect in loading of the autophagosomes is observed, leading to a reduced capacity of cells to degrade aggregated proteins and organelles (Martin et al., 2014).

HTT may directly regulate selective autophagy via several and complementary mechanisms. First, through its scaffolding function of the dynein/dynactin/HAP1 complex, HTT regulates the retrograde transport of autophagosomes along axons (Wong and Holzbaur, 2014). HTT silencing reduces localization of optineurin at the Golgi apparatus, so HTT may also regulate the dynamics of autophagosome/lysosomes through its interaction with optineurin/Rab8 (del Toro et al., 2009). Second, domains of HTT are very similar to the yeast autophagy proteins Atg23, Vac8 (Vacuolar protein 8), and Atg11; these domains are arranged in an N-to-C linear manner in the HTT two-dimensional (2D) structure (Ochaba et al., 2014). In particular, the C-terminal part of HTT that is similar to yeast Atg11 interacts with the mammalian homologs of the Atg1/ULK1 kinase complex, whereas the more central region of HTT, similar to Vac8, interacts with Beclin-1.

The presence of 11 LC3-interacting repeats (LIRs) within HTT provides further support for a role of HTT in selective autophagy. Such LIR motifs are found in Atg8 family-interacting proteins, including p62 and optineurin, and are key to the capacity of the autophagy receptor proteins to link cargos to LC3 and/or GABA-receptor-associated protein (GABARAP) (Ochaba et al., 2014). The identification of the p62-interaction domain in HTT provided clues to the mechanisms by which HTT regulates both cargo recognition and autophagy induction (Rui et al., 2015). HTT binds to p62 and thereby facilitates the recognition of ubiquitinylated proteins at Lys 63 by p62; this facilitates cargo loading into autophagosomes. In addition, HTT binding to ULK1 releases it from its interaction with mTOR, leading to the induction of autophagy. This autophagy regulatory function of HTT is conserved in flies and may be fine-tuned through changes in HTT 3D conformation. Indeed, the C-terminal domain of HTT similar to Atg11 interacts with a more N-terminal domain of HTT similar to Atg23, recapitulating the Atg11-Atg23 interaction observed in yeast (Ochaba et al., 2014). Disordered regions in between contain several modification and cleavage sites that may regulate this function (Martin et al., 2014). In addition, the C-terminal fragments generated upon proteolysis may be toxic by dysregulating autophagy (Ochaba et al., 2014). Finally, HTT function in autophagy may also be regulated by variations in the number of polyQ within the normal range: deleting the normal Q stretch in mice promotes autophagy and enhances longevity (Zheng et al., 2010).

Huntingtin Regulates Transcription

Transcriptional dysregulation has been repeatedly observed in postmortem human HD brains and in diverse samples from transgenic mouse models (reviewed in Valor, 2015). Numerous mechanisms could account for this altered gene expression, including the loss of a transcriptional function of HTT. HTT is largely cytoplasmic, but it is also present in the nucleus. HTT contains a polyQ tract: such motifs are found in transcription factors and act as transcriptional regulating domains through mediating binding between transcription factors and transcriptional regulators. Wild-type HTT binds numerous transcription factors, including the cAMP-response element (CREB)-binding protein (CBP) (Steffan et al., 2000), NeuroD (Marcora et al., 2003), the specificity protein-1 (SP1) (Dunah et al., 2002; Li et al., 2002), the nuclear factor-kB (NF-kB) (Takano and Gusella, 2002), and the tumor suppressor protein 53 (p53) (Steffan et al., 2000). HTT also interacts with transcriptional activators and repressors: the Gln-Ala repeat transcriptional activator CA150 (Holbert et al., 2001), the co-activator TAFII130 (Dunah et al., 2002), the nuclear co-repressor (NCOR) (Yohrling et al., 2003), the repressor element-1 transcription factor/neuron restrictive silencer factor (REST/NRSF) (Zuccato et al., 2003), and the transcriptional corepressor C-terminal-binding protein (CtBP) (Kegel et al., 2002). Finally, HTT binds nuclear receptors, including LXRα, peroxisome proliferator-activated receptor-γ (PPARγ), vitamin D receptor (VDR), and thyroid hormone receptor-α1 (TRα1) (Futter et al., 2009). Through these interactions, HTT can potentiate transcription factors and inhibit repressors (and vice versa) and thereby promote and repress gene transcription with a wide range of cellular outcomes. For instance, p53 interacts with HTT (Bae et al., 2005), and consequently, HTT potentially influences the transcription of p53 target genes, genes that are involved in cell-cycle control, apoptosis, cellular stress responses, and DNA repair. Consistent with this idea, HTT also activates the transcription of genes containing a conserved 21- to

23-bp DNA repressor element 1 sequence (RE1, also known as the neuron-restrictive silencer element; NRSE) (Zuccato et al., 2003). The NRSE is recognized by REST, a transcriptional regulator that acts as a transcriptional silencer. Thus, HTT may act as a positive transcriptional regulator for NRSF-regulated genes (Zuccato et al., 2003). NRSF-regulated genes are essential for neuronal development and maintenance. An example is the brain-derived neurotrophic factor (BDNF) gene, the promoter II of which contains an NRSE. Wild-type HTT promotes *BDNF* transcription through the sequestration of the available REST/NRSF in the cytoplasm, thereby preventing it from forming the nuclear co-repressor complex at the RE1/NRSE nuclear site (Zuccato et al., 2001).

While there have been many studies describing mutant HTT interference with the components of transcriptional machinery, exactly how wild-type HTT regulates transcription in each pathway remains unclear. In the nucleus, HTT might act as a scaffold for transcriptional complexes. This possibility is illustrated by the reported binding of HTT to both SP1 and TAFII130 (Dunah et al., 2002): HTT may link the DNA-bound SP1 and core components of the basal transcription machinery. HTT may also serve as a transcriptional cofactor itself (Benn et al., 2008): HTT associates with gene promoters in mouse and human brain samples. Wild-type HTT also regulates chromatin remodeling. HTT interacts with Ezh2 (enhancer of zeste 2) and Suz12 (Suppressor of zeste 12), two components of the polycomb repressive complex 2 (PRC2) and thereby facilitates the histone H3K27me3 catalytic activity of PRC2 both in vitro and in vivo (Seong et al., 2010). The cytoplasmic enrichment of HTT and its involvement in regulating intracellular dynamics may also have consequences for transcription: HTT may bind transcription factors and regulators and mediate their transport to the nucleus. Supporting this possibility, HTT binds NeuroD through HAP1, facilitating the activation of NeuroD by phosphorylation by the mixed-lineage kinase 2 (MLK2) (Marcora et al., 2003). Given the role of HAP1 in intracellular trafficking, it is plausible that the HTT-HAP1 complex participates in the nuclear translocation of NeuroD. Analogously, the role of HTT in NRSF-regulated transcription may also be a consequence of HTT function as a regulator of intracellular dynamics: REST forms a complex with $p150^{Glued}$, HAP1, HTT, and the REST/NRSF-interacting LIM domain protein (RILP), and this complex is involved in the trafficking of REST to the nucleus (Shimojo, 2008).

Translating Huntingtin Molecular Function into Physiology

Embryonic Development

Several studies have shown that the gene is essential for embryonic development: the inactivation of the *HTT* gene by targeting exon 1 or 5 is lethal in mice at E7.5 (Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995); knockout embryos display defects in gastrulation. These effects may be the consequences of an altered nutritive function of the extraembryonic tissues in the absence of HTT. Coherent with this idea, extra-embryonic tissues lacking HTT show defects in iron transport (Dragatsis et al., 1998). These findings are supported by studies in zebrafish in which HTT knockdown produces symptoms of cellular iron deficiency (Lumsden et al., 2007).

HTT was found to be important for the formation of the nervous system. Indeed, to bypass the lethality due to the absence of HTT and analyze the role of HTT after gastrulation, mice expressing less than 50% of the normal of the protein were generated (White et al., 1997). These mice present defects in the formation of the precursor of the epiblast and malformations of the cortex and striatum and die shortly after birth. In particular, these mice have masses of ectopic differentiated neurons in the striatum (White et al., 1997). Furthermore, analysis of chimeric embryos, in which a limited number of cells are depleted of HTT, showed that the protein is essential for the differentiation of neuroblasts in the striatum, cortex, and thalamus (Reiner et al., 2001). Thus, HTT is essential for embryogenesis and development. What are the HTT molecular functions involved?

During embryonic cortical neurogenesis, invalidation of HTT, specifically in proliferating radial glial progenitors (RGPs) in the ventricular zone (VZ), promotes neuronal differentiation at the expense of their maintenance in a proliferative status (Godin et al., 2010). The underlying molecular mechanisms involve HTT regulation of the dynein complex during mitosis and, consequently, the mitotic spindle orientation of the dividing cortical progenitors (Godin et al., 2010). Another study suggested that HTT is involved in the migration of newborn cortical neurons from the VZ to the cortical plate (CP) (Tong et al., 2011). In this study, however, HTT was knocked down in all neuroepithelial cells in the neocortex by short hairpin RNA (shRNA). Therefore, how HTT regulates migration needs to be determined, because the migration defects observed might have been related to HTT deficiency in dividing cortical progenitors.

Another mechanism by which HTT impacts on brain development and homeostasis is the control of motile cilium biogenesis (Keryer et al., 2011). HTT inactivation in Wnt1 lineages reduces ciliation of ependymal cells, leading to altered circulation of the cerebrospinal fluid and, as a result, closure of the Sylvius aqueduct and hydrocephalus (Dietrich et al., 2009; Keryer et al., 2011).

Tissue Maintenance and Cell Morphology

In parallel to the role of HTT during brain development, recent studies imply that HTT is a regulator of tissue maintenance. This is the case in the mammary epithelium; this epithelium consists of two major layers of cells, the myoepithelial basal cells and the luminal cells surrounding the lumen. HTT is much more abundant in luminal cells than in basal cells and increases as differentiation progresses (Elias et al., 2014, 2015). In vivo depletion of HTT from the basal compartment decreases the epithelial content and alters the self-renewal properties of the basal and luminal progenitors (Elias et al., 2014). HTT is also important for the establishment of apical polarity; consequently, the specific depletion of HTT from luminal cells alters ductal morphogenesis and lumen formation (Elias et al., 2014).

Further supporting a role for HTT as a regulator of epithelial morphogenesis, HTT null embryonic stem cells and HTT morpholino zebrafish embryos show alterations in N-cadherin-mediated adhesions, with aberrant distribution of the tight junction protein zona occludens 1, ZO1 (Lo Sardo et al., 2012). The amounts of mRNAs of adherence proteins are also reduced in embryonic stem cells and neurons with lowered HTT levels (Strehlow et al., 2007). Such mechanisms could underlie the

spermatogenesis defects observed in mice with reduced HTT expression in testis that results in disorganized seminiferous tubules with fewer spermatocytes and round spermatids (Dragatsis et al., 2000). Consistent with these observations, mammary tumor cells with reduced amounts of HTT lose tight junctions and undergo a greater epithelial-to-mesenchymal transition than in the wild-type situation (Thion et al., 2015).

HTT may also influence histogenesis and organogenesis by regulating metabolism. Mice overexpressing wild-type HTT show increased body weight and increased weights for heart, liver, kidneys, lungs, and spleen. These effects are mediated by the role of HTT as a modulator of IGF-1 expression in a dose-dependent fashion (Pouladi et al., 2010; Van Raamsdonk et al., 2006).

Cell Survival

Several early studies highlighted the prosurvival properties of wild-type HTT. A first set of experiments showed that expression of wild-type HTT in cell lines and primary cultures of neurons protects against cell death induced by several stimuli, including mutant HTT itself (Ho et al., 2001; Leavitt et al., 2006; Rigamonti et al., 2001). Conversely, depletion of HTT renders cells more vulnerable to cell death (Zhang et al., 2006). These in vitro studies were supported by in vivo observations in mouse, showing neuroprotection conferred by overexpression of HTT against excitotoxicity or ischemic injuries and cell death when HTT levels are lowered (Dragatsis et al., 1998, 2000; Leavitt et al., 2006; Nasir et al., 1995; O'Kusky et al., 1999; Zhang et al., 2003). In support, a non-coding SNP variant in the *HTT* promoter downregulates the expression of the wild-type and mutant alleles and proteins and, thereby, is associated with earlier or delayed age of onset in HD patients (Bečanović et al., 2015).

This idea of HTT having antiapoptotic/prosurvival properties is particularly pertinent to HD. Indeed, one of the striking histopathological features of HD brain samples post-mortem is the massive neuronal atrophy of several brain regions, such that brain weight may be reduced by up to 30% (Rosas et al., 2008). The cell death associated with the mutant HTT is recapitulated in cellular and mouse models (Gray et al., 2008; Saudou et al., 1998).

The first attempts to decipher the molecular details of how wild-type HTT promotes survival focused on the apoptotic machinery. It was found that HTT blocks the activation of caspase-3 and -9 (Rigamonti et al., 2001; Zhang et al., 2003). Another mechanism by which HTT promotes cell survival is exemplified by the nature of the cortico-striatal connection (Figure 3). The striatum does not produce BDNF and depends almost exclusively on the BDNF delivered by cortico-striatal afferences (Baquet et al., 2004). First, HTT favors the transcription of BDNF (Zuccato et al., 2001); second, HTT promotes the axonal transport and delivery of vesicles containing BDNF to the cortico-striatal synapse (Gauthier et al., 2004). Upon BDNF release at the cortico-striatal synapse, activated TrkB receptors are endocytosed and transported retrogradely to striatal cell bodies, where they activate survival signaling (Liot et al., 2013). This retrograde transport of TrkB-signaling endosomes is under the control of HTT and is mediated by an HTT-dynein IC-1B complex (Liot et al., 2013). Therefore, HTT ensures both the anterograde delivery of BDNF to cortico-striatal synapses and also the retrograde transport of the BDNF-TrkB endosomes along striatal dendrites. It is, therefore, not surprising, given the crucial role of HTT in this coordinated process and the strict dependence of striatal neurons on the cortically produced and transported BDNF, that these neurons are the first to degenerate in cases of HD.

From Huntingtin Dysfunction to HD

A Cascade of Events Leading to Neuronal Death

Historically, and for obvious reasons, most studies have focused on identifying the cellular consequences of expressing mutant HTT in cell lines, primary cultures of neurons, and animal models. This led to the description of a cascade of cellular events that ultimately lead to neurodegeneration (Borrell-Pagès et al., 2006). One major pathway has been the center of attention for the scientific community. Mutant HTT is cleaved by proteases, generating N-terminal fragments containing the abnormal polyQ stretch. These fragments translocate to the nucleus, where they cause neuronal cell death by interfering with transcription (Ross and Tabrizi, 2011; Saudou et al., 1998; Valor, 2015). Mutant HTT also forms aggregates that were initially described as being the toxic species in HD: it was suggested that these aggregates interfere with cellular functions, thereby causing cell death (Davies et al., 1997). However, inclusions of mutant HTT were also described as being protective, as they reduce the level of the toxic soluble protein (Miller et al., 2010; Saudou et al., 1998). The toxicity of aggregates may depend on the stage of the disease and on their nature and subcellular location.

The canonical pathway leads to the generation of polyQ-containing fragments that are more toxic the shorter they are, and that may act through gain of function mechanisms in the nucleus (Ross and Tabrizi, 2011). HTT proteolysis also leads to the generation of the corresponding C-terminal fragments that are toxic because they dysregulate the activity of dynamin 1 (El-Daher et al., 2015). Therefore, not only may HTT proteolysis cause the loss of the function of HTT by inducing its cleavage, but it also generates fragments, some of which interfere with the transcriptional and autophagic machineries and others of which affect the homeostasis of the endoplasmic reticulum in the cytoplasm (El-Daher et al., 2015; Martin et al., 2014; Steffan, 2010; Valor, 2015). To further complicate the situation, other effects of mutant HTT have been described, including (but not restricted to): proteasome and autophagy inhibition; mitochondrial abnormalities and related metabolic impairments; alteration in endocytosis and microtubule-based transport; synaptic activity deficiencies; impaired calcium signaling; and excitotoxicity caused by increased glutamate release and dysfunction of several neurotransmitters involved in signaling (glutamate receptor, adenosine A2, cannabinoid, and GLT-1 receptors) (Ross and Tabrizi, 2011). However, most of these data were obtained through the overexpression of mutant HTT fragments in vitro and/or in vivo but whether the altered mechanisms identified are also under the control of wild-type HTT has been overlooked.

HD Mutation Changes HTT Function

Although few biological functions have been attributed to HTT, all those described so far are altered by the presence of the

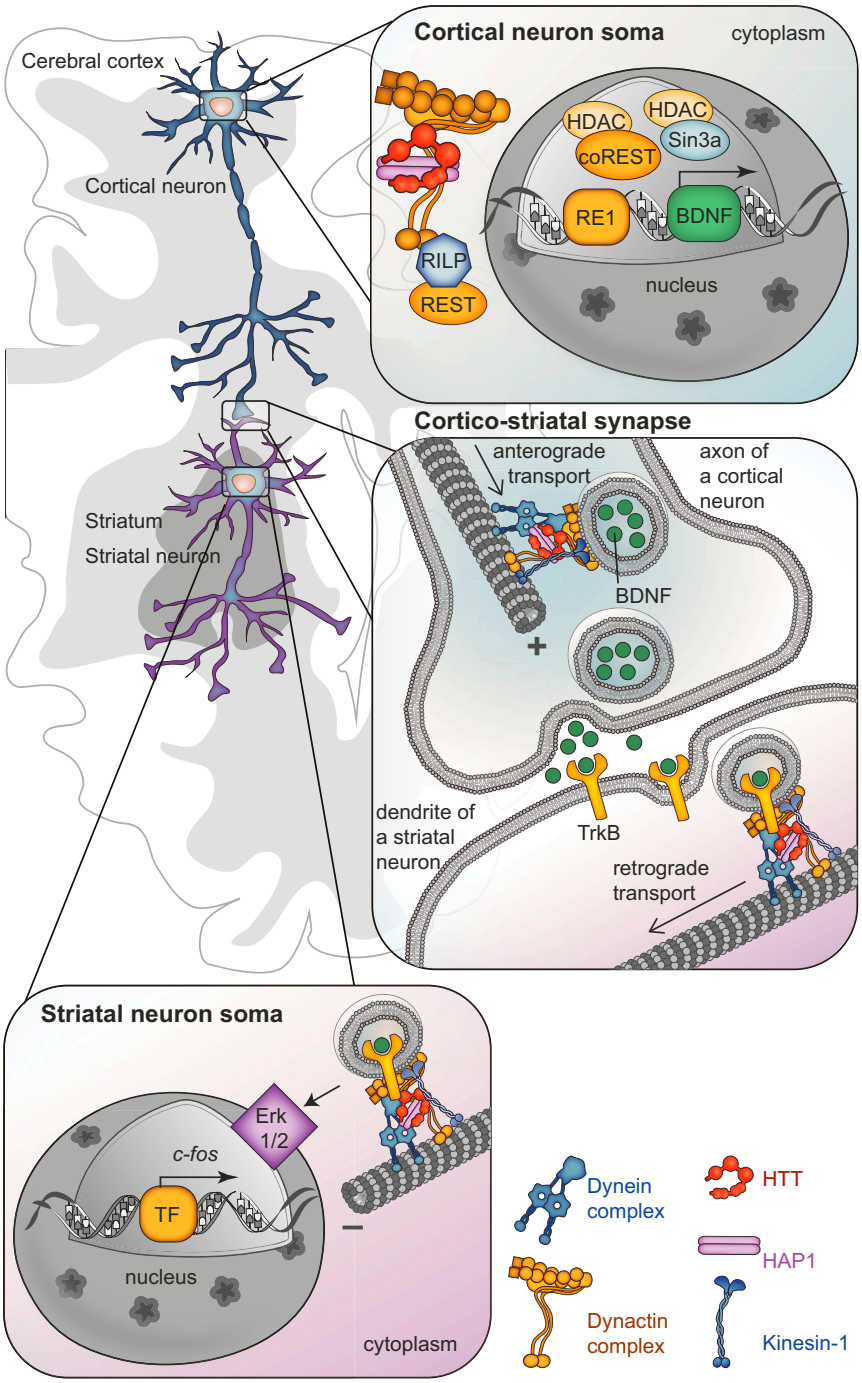

Figure 3. HTT Regulates the Function of the Cortico-Striatal Connection

HTT favors the transcription of BDNF and promotes the axonal transport and delivery of vesicles containing BDNF to the cortico-striatal synapse. Upon BDNF release at the cortico-striatal synapse, activated TrkB receptors are endocytosed and transported retrogradely to striatal cell bodies, where they activate survival signaling.

abnormal polyQ expansion. Overexpression of wild-type HTT stimulates BDNF vesicular transport along microtubules, but mutant HTT does not (Gauthier et al., 2004). Conversely, downregulating wild-type HTT reduces transport, further arguing for a loss of HTT function in BDNF transport in HD cells. How do we integrate such a loss-of-function mechanism into the genetic characteristic of HD patients, as one mutant allele is sufficient to cause disease? It is noteworthy that BDNF transport is reduced to the same extent in cells derived from knockin animals being heterozygous or homozygous for mutant HTT (Gauthier et al., 2004). Similarly, neurons derived from human embryonic stem cells (hESCs) from heterozygote HD patients also show a reduced BDNF transport. Interestingly, specific silencing of the mutant allele restores BDNF transport up to values of control hESCs; this indicates that the selective silencing of mutant HTT can alleviate the dominant-negative effect of mutant HTT on wild-type HTT on vesicular function (Drouet et al., 2014). Thus, in HD, mutant HTT has lost the ability to stimulate BDNF vesicular transport but acts in a dominant manner on wild-type HTT to alter transport.

The situation during ciliogenesis is different. HTT traffics PCM1 protein to and from the PCM that is at the base of the cilium. This bidirectional transport is necessary for proper ciliogenesis. In absence of HTT, PCM1 is not transported to the PCM, resulting in short or absent cilia. When HTT contains the polyQ expansion, PCM1 is not transported from the PCM and accumulates at the base of the cilium, causing the formation of abnormally long cilia (Keryer et al., 2011). In both cases, abnormally short or long cilia are dysfunctional, but the exact underlying molecular mechanisms are different. Nevertheless, they both involve a modification of HTT function in the transport of PCM1 protein.

During the autophagic process, HTT facilitates p62-mediated cargo recognition, at least in part, by enhancing the affinity between p62 and ubiquitylated cargos and LC3 (Ochaba et al., 2014; Rui et al., 2015). This function is altered in HD with the formation of empty autophagosomes due to failure in the cargo recognition (Martinez-Vicente et al., 2010). In this case, loss of HTT or polyQ expansion in HTT both lead to the reduced function of HTT in autophagosome formation.

These examples illustrate that an abnormal polyQ expansion may have diverse consequences on HTT functions.

Gain of Function versus Loss of Function: Do We Need to Choose?

The debate opposing gain of function versus loss of function started with the observations that HD has a pattern of dominant inheritance and that one mutant allele of the gene is sufficient to cause the disorder (Ross and Tabrizi, 2011). This was supported by the embryonic lethality of HTT knockout mice at E7.5 (Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995). In contrast, mice with brain-specific HTT knockout at adulthood show progressive neurodegeneration that is reminiscent of HD (Dragatsis et al., 2000), arguing that loss of HTT function could participate to neurodegenerative process.

HD patients express one mutant allele and one wild-type allele, so that the wild-type HTT is present but at about half the normal concentrations. We have seen how HTT has beneficial activities in the developing and mature brain: the presence of only low levels of HTT will undoubtedly have consequences on these properties. Apart from the Dragatsis et al. (2000) study, there have been few studies of the effects of reducing wild-type HTT levels in the long term, either in a normal or a pathological context. These few studies suggest that reducing the amount of wild-type HTT may have substantial consequences both in the wild-type context (Nasir et al., 1995; O'Kusky et al., 1999) and also when one allele of HTT contains the polyQ expansion (Van Raamsdonk et al., 2005). In particular, heterozygous HTT knockout mice show impaired motor activity and cognitive deficits (Nasir et al., 1995; O'Kusky et al., 1999). In addition, there are signs of neurodegeneration in the subthalamic nucleus and globus pallidus. It is worthwhile to note that the extent of the phenotypes observed is milder than those developed by HD mice overexpressing short polyQ-HTT fragments but comparable to those observed in HD knockin mice (Menalled et al., 2009). These studies strongly support the hypothesis that part of the HD pathological events may be attributable to the loss or modifications of the normal functions of wild-type HTT.

Conclusions and Outlook

In conclusion, several co-existing mechanisms may participate to HD pathogenesis. The abnormal polyQ stretch may induce the gain of toxic function that is independent of the protein context. This type of effect would be common to all polyQ disorders and may explain some overlapping phenotypes in patients with different disorders but with a very long polyQ stretch. Other gain of toxic functions may be specific to the polyQ HTT protein but unrelated to the function of normal HTT. Finally, some pathological mechanisms may be caused by the modification of HTT function that may manifest dominantly.

As we just mentioned, one of the benefits of studying the alteration in HTT function in the disease is that it may reveal pathological mechanisms that are specific to HD, whether these are due to modifications of HTT function or to HTT-specific gain-of-function effects. It is stating the obvious to say that HTT is unique and that, because the mutation is in HTT, patients will develop HD with its characteristic clinical manifestations. Studying HTT function and how HTT function is impaired by the polyQ expansion may thus open the door to understanding the specificity of HD. A good example is the selectivity of the alteration of the cortico-striatal connection that is very likely to result—at least in part—from the dysfunction of HTT in the regulation of the BDNF-TrkB signaling through transcription and transport. In support of this view, loss of BDNF signaling in the cortex is sufficient to induce striatal degeneration (Baquet et al., 2004).

The second argument for further investigating HTT function and its alterations in disease is that it may give access to pathological steps occurring early during disease progression. Early dysfunctions are of major importance in HD, because preventive treatment strategies targeting the presymptomatic stages may be possible given the monogenetic nature of HD. Early dysfunctions, including the extreme example of developmental defects, may underlie the prodromal neurological signs of HD (Tabrizi et al., 2009). Such understanding may eventually lead to the identification of markers of disease progression that could be used to validate early neuroprotective therapies.

The third argument is based on the emerging therapeutic strategies aiming at lowering HTT levels in HD patients. The strategy is to reduce the amount of mutant HTT, and this approach has the advantage of being possibly efficacious, regardless of any of the many downstream toxic events elicited by mutant HTT. However, the first clinical trials, currently underway, may not be able to discriminate between wild-type and mutant HTT. Thus, it is of utmost importance to elucidate HTT function and thereby identify how loss of HTT affects cell physiology and in which conditions—in time and space—low levels of both wild-type and mutant HTT can be tolerated. Indeed, reduced wild-type *HTT* transcriptional activity is associated with earlier age of onset in HD patients (Bečanović et al., 2015). Furthermore, loss of HTT is associated with increased metastasis in the context of breast cancer (Thion et al., 2015).

Since the identification of the *HTT* gene in 1993, numerous advances have been made in identifying the molecular mechanisms that control cell death in HD. However, there is still no treatment available to delay appearance or progression of symptoms in patients. This may be due to the complexity of the protein that regulates several mechanisms in the cell. We may need to revisit the basics of HTT. We are still in the early stages of HTT characterization with respect to its promoter regulation, the identity of its splice isoforms and their role, its spatial and temporal expression at cellular levels during development and in adult, and whether and when HTT expression is regulated by miRNA. Also, HTT is subject to many post-translational modifications, but few have been extensively studied to determine their roles in HTT function and disease progression. Similarly, given the large number of proteins that interact with HTT, an understanding of how HTT regulates these proteins linked to specific functions may be extremely informative.

It is tempting to use overexpression of short fragments that produce strong phenotypes in vitro and/or in vivo to demonstrate the importance of downstream events to the presence of mutant pathogenic HTT; however, with this approach, some of the effects may be exaggerated, compared to those associated with the presence of mutant HTT in HD cases, and may mask more deleterious effects of the mutant protein. Indeed, given the durable, but slowly progressive, nature of the disease, it is likely that there are subtle alterations at the cellular and organism levels that accumulate to become detrimental at later stages. Therefore, future studies in the field should consider, when possible,

the analysis of the pathogenic cascade in model systems in which the mutant allele is endogenously expressed and replace one wild-type allele to faithfully recapitulate the genetic situation of HD patients.

Another important challenge is to understand HTT function and dysfunction linked to specific neuronal networks within the brain and how they relate to specific neurological, psychiatric, or cognitive symptoms. Finally, although brain-related symptoms are the most visible and dramatic, it is important that we address the functions of HTT outside the nervous system. The alteration of extra-nervous-system functions may provoke other major symptoms in HD patients. Possible examples are the possibly increased metastasis in HD patients susceptible to cancers, muscle wasting, and cardiac failure. Increasing our knowledge about the multifaceted protein that is HTT is certainly not a short or easy path, but it may contribute significantly to curative approaches for this complex disease.

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