Degrade or degenerate

Glial protein degradation in Huntington’s disease

Jansen, A.H.P.

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Visualization of prion-like transfer in Huntington’s disease models

Anne H.P. Jansen\textsuperscript{1}, Kevin L. Batenburg\textsuperscript{1},
Eline Pecho-Vrieseling\textsuperscript{2}, Eric A. Reits\textsuperscript{1}

\textsuperscript{1}Department of Cell Biology & Histology, Academic Medical Center,
Amsterdam, The Netherlands
\textsuperscript{2}Department of Biomedicine, University of Basel and University hospital Basel,
Basel, Switzerland

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ABSTRACT
Most neurodegenerative diseases such as Alzheimer’s, Parkinson’s and Huntington’s disease are hallmarked by aggregate formation of disease-related proteins. In these diseases transfer of aggregation-prone proteins between neurons and between neurons and glial cells has been shown, thereby initiating aggregation in neighboring cells and so propagating the disease phenotype. Whereas this prion-like transfer is well studied in Alzheimer’s and Parkinson’s disease, only a few studies have addressed this potential mechanism in Huntington’s disease. Here, we present an overview of in vitro and in vivo methodologies to study release, intercellular transfer, and uptake of aggregation-prone protein fragments in Huntington’s disease models.

INTRODUCTION
Many neurodegenerative diseases are hallmarked by the presence of inclusion bodies (IBs) that are initiated by disease-related misfolded proteins. In general, most proteins adopt their distinct three-dimensional form shortly after being synthesized in order to perform their specific role in cellular processes. While most newly synthesized proteins fold correctly with or without help of molecular chaperones, others become misfolded and should either refold or become rapidly degraded by the proteasome and macroautophagy. Misfolded protein species that escape degradation will accumulate and clump together into protein aggregates. Here, soluble misfolded proteins tend to reversibly oligomerize and form small aggregates due to their exposed hydrophobic residues that would normally be shielded from the solvent (Hartl and Hayer-Hartl, 2009). These small aggregates subsequently serve as growth-competent surfaces (seeds) with which soluble monomers can associate into protofibrils (Kodali and Wetzel, 2007). Disease-related mutations in a protein can result in an increased sensitivity to become misfolded and aggregation-prone, as is for example observed in Huntington’s disease (HD). HD is one of nine polyglutamine (polyQ) diseases that is characterized by a progressive decline in cognitive and motor functions and chorea (Walker, 2007; Blum et al., 2013). The autosomal dominant inherited CAG expansion within the exon 1 of the HTT gene results in a mutant huntingtin (mHTT) protein with an aberrantly long polyQ tract, resulting in a toxic gain of function that causes neuronal dysfunction and death (Walker, 2007). Upon proteolytic cleavage of the full-length mHTT protein, the polyQ-containing N-terminal fragments form intracellular aggregates and eventually IBs, which can be observed in both the cytoplasm and the nucleus. The role of these IBs in the disease pathogenesis is still controversial. Being a pathological hallmark of many neurodegenerative diseases, IBs were long considered to be the pathological cause of disease. However, more recent data suggest that cellular toxicity is related with the initial stages of aggregation (the oligomeric stage) and that the subsequent formation of a single IB may even represent a protective mechanism to sequester oligomers (Arrasate et al., 2004; Nucifora et al., 2012).

Interestingly, an increasing number of misfolded proteins can spread across neural systems in a prion-like fashion. In the acceptor cells, many disease-related mutant proteins sequester also their wild-type (WT) counterparts into aggregates (self-templated amplification), including mHTT, polyQ-expanded ataxins, and α-synuclein (Busch et al., 2003; Haacke et al., 2006; Donaldson et al., 2003; Bernis et al., 2015). Initially this process has been described in prion diseases (Kanu et al., 2002; Schatzl et al., 1997; Gouset et al., 2009), however recently this cell-to-cell propagation of aggregate pathology has also been shown in models of Alzheimer’s disease (AD) (Pooler et al., 2013; Kfoury et al., 2012; Clavaguera et al., 2009; de Calignon et al., 2012; Frost et al., 2009), Parkinson’s disease (PD) (Kordower et al., 2008; Li et al., 2008; Emmanouilidou et al., 2010; Volpicelli-Daley et al., 2011; Luk et al., 2012a; Desplats et al., 2009), Amyotrophic Lateral Sclerosis (ALS) (Münch et al., 2011; Ayers et al., 2016), and HD (Pearce et al., 2015; Pecho-Vrieseling et al., 2014). Whereas it has been accepted that AD- and PD-associated proteins exhibit prion-like properties, only recently a similar intercellular
propagation of polyQ-expanded proteins in HD was proposed. Here, we describe various methods to visualize prion-like transfer in *in vitro* and *in vivo* models, and review published data and personal unpublished observations that provide evidence that mHTT may as well be able to spread between cells.

**STUDYING RELEASE OF MHTT BY DONOR CELLS**

The first step in the process of cell-to-cell transfer is the release of misfolded proteins or protein aggregates by donor cells. When studying the release of mHTT from donor cells it is possible to measure the amount of mHTT release in the medium by ELISA, as has been shown for tau (Pooler et al., 2013). To investigate whether mHTT expressing cells are able to release mHTT and whether released mHTT is already oligomerized, we developed an assay to concentrate conditioned medium from PC12 (rat adrenal gland pheochromocytoma) cells (medium taken from the cells after 48h of culturing) stably expressing HTT-exon1-23Q-GFP or HTT-exon1-74Q-GFP. The concentrated medium can be used for soluble–insoluble fractionation (Juenemann et al., 2015) and be analyzed by either SDS-PAGE followed by Western blot or AGERA (Agarose Gel Electrophoresis for Resolving Aggregates) (Weiss et al., 2008). We observed the release of both SDS-soluble and SDS-insoluble mHTT species (Figure 1A), which could subsequently be used in assays to study uptake by acceptor cells *in vitro*. However, no soluble HTT was detected in the supernatant of corticostraital organotypic brain slices (OTBS) derived from R6/2 HD mice (Mangiarini et al., 1996), even when the OTBS were kept in culture up to 6 weeks (Peche-Vrieseling et al. 2014). This discrepancy might be due to differences between *in vitro* and *in vivo* systems used. Besides, mHTT secreted by cells in the OTBSs, which mimics a complex cellular environment, might be efficiently cleared away by glia and neurons in the slice. Indeed, recent data showed that also glia can take up exogenous mHTT by phagocytosis (Pearce et al., 2015).

It remains unclear how mHTT is released by donor cells. Monomers, oligomers and larger aggregates may reach the extracellular space by active secretion following uptake by autophagosomes, lysosomes, multi-vesicular bodies or exosomes, and subsequently be released within vesicles or in non-encapsulated form (Fig 1B). Release can also take place by passive diffusion or transfer across the membrane, similarly as suggested for the uptake of polyQ peptides by HEK cells (Trevino et al., 2012). Studying neuronal release of mHTT via axonal transport can be studied using microfluidic devices where the soma and axon terminals are in different compartments. Recently, it was shown in cortical neurons that mHTT fibrils are able to travel via anterograde transport and are secreted in the medium while the axon stayed intact. When fluorescently labeled fibrils were added to the soma compartment, 24 hours later fluorescence could be measured in the axonal compartment (Brahic et al., 2016). Alternatively, these fragments may be released from necrotic cells due to membrane disruptions or from apoptotic bodies of cells driven into apoptosis (Fig 1D). To examine this, one should isolate the secreted aggregates and investigate the composition and the presence of membranes. Exosomes are isolated by ultracentrifugation, and these structures have been shown to play a role in α-synuclein transfer (Danzer et al., 2012). A similar approach was performed by Jeon and colleagues to search for mHTT-containing exosomes. They showed that mHTT is indeed present in exosomes-vesicles derived from patient fibroblasts and the human neuronal cell line SH-SYSY transfected with GFP-HTT exon 1 (Jeon et al., 2016). As a positive control for mHTT secretion by cells, we fused a secretion signal peptide to GFP-tagged mHTT exon 1. However, the fusion protein already accumulated in the secretion machinery of the cell, leading to cell death within a few days. When instead of GFP a smaller tetracysteine tag was used (Griffin et al., 1998), we could not observe an increased secretion of the signal peptide-HTT-exon 1 when compared to cells that expressed cytoplasmatic HTT without a signal peptide (data not shown), suggesting that mHTT is not efficiently released via the ER-Golgi pathway.

**STUDYING UPTAKE OF PURIFIED MHTT BY ACCEPTOR CELLS**

The first reports to determine that polyQ-expanded protein fragments can transfer between cells were focusing on the induction of intracellular aggregates in acceptor cells. By visualizing the uptake of pure polyQ peptides by cells *in vitro* and *in vivo*, these experiments showed that these peptides were able to propagate aggregation in acceptor cells.

*Figure 1. Possible mechanisms of uptake and release of aggregation-prone polyQ fragments. A. Western blot of doxycycline-inducible PC12 cells expressing HTT-exon1-23/74Q-GFP that release soluble (mainly 24 hours after induction of expression) and insoluble (48 hours after expression) HTT fragments in the culture medium. Medium samples are made suitable for Western blotting by filter-mediated concentration and subsequent dialysis with a Tris-HCl (pH=7.4). HTT is also present in the cell lysate B. Release of these aggregates by donor cells may occur by enclosing cytoplasmatic aggregates in vesicles (like autophagosomes or exosomes) and the subsequent secretion of these vesicles. Alternatively, aggregates may pass the plasma membrane directly. C. Uptake of mHTT aggregates by acceptor cells may be mediated via endocytosis (e.g. receptor mediated endocytosis) or by direct passage over the plasma membrane. D. HTT aggregates can also be released due to cell death by necrosis (left) or apoptosis, resulting in apoptotic bodies containing aggregates (right).*
prion-like transfer of mHTT

Fluorescent synthetic peptides and recombinant proteins: In 2002, Yang et al. showed that aggregates composed of synthetic polyQ peptides can be taken up by cells when added to the culture medium (Yang et al., 2002). They showed that these FITC-labeled peptides, either non-encapsulated or in liposomes, can be efficiently introduced in PC12 and Cos-7 (monkey kidney fibroblast-like) cells. Interestingly, they observed that the cytotoxic properties of these peptides were dependent on nuclear localization. Using cyan fluorescent protein (CFP) and fluorescein isothiocyanate (FITC)-tagged polyQ peptides, Kopito and co-workers showed that synthetic polyQ and mHTT amyloids gain access to the cytoplasm of HEK293 (human embryonic kidney cells) and other mammalian cell types, resulting in the sequestration of fluorescently-labeled soluble WT HTT that was expressed in these cells (Ren et al., 2009). As the observed uptake could be mediated by either endocytosis or via direct transfer over the plasma membrane (Fig 1C), subsequent immunostaining was performed, which showed co-localization of internalized aggregation-prone proteins with cytoplasmic markers such as HSP70, but not with markers for endosomes, lysosomes or autophagosomes. This suggests that the proteins could pass the plasma membrane independent of endocytosis. By treatment of cells with trypsin to remove potentially involved receptors at the cell surface and bovine serum albumin (BSA) to block membrane surface proteins, the same group showed in a follow-up study that internalization of the extracellular polyQ fibrils is likely mediated by proteinaceous (protein-rich) sites on the cell surface (Trevino et al., 2012). The internalization and seeding propensity of fibrillar species is significantly enhanced compared to their non-fibrillar (i.e. amorphous) counterparts (the different species were distinguished by transmission electron microscopy). Recently, it was established that mHTT fibrils bind laterally to cell membranes and that this interaction depends on a minimum number of interaction sites, suggesting that longer fibrils are more easily taken up than shorter ones (Monsellier et al., 2016). This uptake of mHTT fibrils is mainly mediated by endocytosis, afterwards the fibrils are directed towards the lysosome. Via a currently unknown mechanism, a proportion of mHTT fibrils escapes these compartments and ends up in the cytosol (Ruiz-Arlandis et al., 2016). Many of the uptake experiments were performed with polyQ peptides with 44-glutamine repeat, just exceeding the disease-related threshold. Synthesis of long polyQ-expanded peptides by peptide chemistry is challenging, as these peptides are very aggregation-prone (unpublished observation). An alternative can be to express long polyQ-expanded peptides. This can be done by using e.g. ubiquitin-polyQ (Ub-polyQ) constructs. These constructs result in expression of pure polyQ, because the preceding Ub is cleaved off by Ub hydrolases (Raspe et al., 2009). Next to synthetic or expressed polyQ peptides it is also possible to purify mHTT following immunoprecipitation from cell lysates, and subsequently add the purified and aggregated mHTT to cultured cells. However, our own pilot experiments showed a high degree of toxicity when either purified WT HTT or mHTT was added to acceptor cells (data not shown), suggesting that co-immunoprecipitated material such as chaperones may induce stress and toxicity.

Conditioned medium and cerebrospinal fluid (CSF): To study transfer of mHTT, conditioned medium of mHTT-expressing cells can be added to acceptor cells, as was done in early experiments with the prion protein PrP (Schatzl et al., 1997) and later in AD, PD and ALS models (Münch et al., 2011; Kfoury et al., 2012; Hansen et al., 2011; Lee et al., 2008; Ding et al., 2015; Danzer et al., 2011). WT mouse embryonic stem cell-derived neurons (m-neurons) showed intracellular mHTT aggregates when incubated with supernatant collected from mouse neuronal cultures expressing mHTT (unpublished observation). Similarly, Tan and colleagues recently observed that CSF of HD patients induced aggregation both in cultured acceptor cells as well as in cell-free lysates (Tan et al., 2015). Interestingly, several experiments have been conducted in the AD and PD field by injecting tissue homogenate of AD patients or transgenic mice in marmosets and mice (Baker et al., 1994; Kane et al., 2000; Morales et al., 2012; Clavaguera et al., 2009; Luk et al., 2012b), finding Aβ, tau and α-synuclein pathology in the accepting tissue. To date, no studies similar to these have been published in HD animal models with either (synthetic) mHTT peptides or tissue homogenates.

METHODS TO VISUALIZE PRION-LIKE CELL-TO-CELL TRANSFER AND CO-LOCALIZATION OF MHTT

Various approaches have been used to visualize prion-like transfer of disease-causing proteins, which can be separated in experiments to study either the release of aggregation-prone proteins from donor cells, the uptake of these proteins by acceptor cells, or the entire process of protein transfer from donor to acceptor cells. Often, visualization of the transferred protein from the donor cell by fluorescent microscopy relies on the sequesterization of fluorescently-tagged proteins expressed in the acceptor cell. When the fluorescently-tagged protein in the acceptor cell is by itself not aggregation-prone, such as the WT HTT protein, it can be used to act as a reporter protein for transfer due to its redistribution into aggregates initiated by the transferred mutant protein (Fig 2A). Alternatively, both the transferred protein and the reporter protein can be tagged with fluorescent proteins, and the transfer and subsequent co-aggregation results in co-localization as detected by fluorescence (Fig 2B). Instead of two different fluorophores, bimolecular fluorescence complementation (BiFC) can also be used to tag the transferred protein and reporter protein with the two different halves of the split fluorophore. Fluorescence will only be observed when the donor protein is actually transferred and interacts with the reporter protein (Fig 2C). Alternatively, Förster Resonance Energy Transfer (FRET) or Fluorescence Life-time Imaging Microscopy (FLIM) can be used to visualize the interaction between the two pools of proteins (Fig 2D).

VISUALIZING TRANSFER OF HTT BETWEEN DONOR AND ACCEPTOR CELLS

To identify mechanisms involved in the transfer of mHTT between cells, one can visualize mHTT release by donor cells or uptake by acceptor cells. However, the ultimate experimental setup is the visualization of the actual transfer and propagation between donor and acceptor cells. Here we describe various approaches that were performed in cell and tissue culture models and in in vivo models.

In vitro techniques: Co-culturing of donor and acceptor cells is a well-established method to study transfer of disease-causing proteins, as demonstrated for scrapie and PD (Kanu et al.,...
It would be interesting to validate these results in intact primary neuronal networks, as has been done for tau transfer (Stancu et al., 2015). In addition, transfer of mHTT between different cell types can be studied. While most researchers focus on transfer of aggregation-prone proteins between neurons, mHTT but also α-synuclein aggregates can transfer to non-neuronal cells such as astrocytes and microglia, as was recently demonstrated in vitro and in vivo (Pearce et al., 2015; Lee et al., 2010; Bae et al., 2012).

In vivo techniques: Visualizing transfer in brain tissue is much more complicated when compared to cultured cells or in vitro neuronal networks. Still in vivo methods are essential to validate the in vitro findings. The Drosophila model is often used to study HD in vivo since flies are easy to modify genetically. Transgenic Drosophila expressing a 588aa long N-terminal HHT fragment fused with RFP specifically in the olfactory receptor neurons (ORN) showed that HHT can spread to neighboring neurons. By labeling the ORN synaptic terminals with synaptotagmin-GFP, Babcock et al. (2015) could show that mHTT-RFP aggregates spread beyond this specific neuronal population towards the large posterior neurons. The mechanism of this spreading was discovered by blocking endocytosis with a shibire (dynamin-like protein) mutant, suggesting that posterior neurons take up mHTT via dynamin-mediated endocytosis (Babcock and Ganetzky, 2015). Also in Drosophila, Pearce et al. used FRET experiments to reveal that upon phagocytic uptake, mHTT-91Q acts as a seed for intracellular aggregation in WT HHT-25Q expressing glia. HHT-25Q normally does not aggregate but since glia showed fluorescent puncta indicating sequestered WT HHT, and FRET analysis showed that the two fluorophores of WT and mHTT were indeed in close proximity (Pearce et al., 2015). Spreading of aggregates has also been studied in mammals, as we have shown for OTBS of R6/2 mice co-cultured with healthy human embryonic stem cell (hESC)- or healthy human induced pluripotent stem cell (hiPSCs)-derived neurons that expressed GFP (hGFP) and functionally integrate into the mouse neuronal network. The presence of intracellular mHTT aggregates in the hESCs and hiPSC-derived neurons, which were observed after four weeks, could only be the result of mHTT transfer from neighboring R6/2 cells that were present in the original OTBS (Pecho-Vrieseling et al., 2014). Interestingly, mHTT propagation from mouse to human cells takes primarily place within a short timeframe, only between three and four weeks of coculture, which could be due to altered intracellular localization of mHTT in donor cells, as discussed below. This might have consequences in particular for in vitro studies and as it could explain the different results concerning transcellular mHTT propagation in these studies. The results obtained with the hESCs and hiPSCs were confirmed with mixed-genotype corticostriatal brain slice cultures (R6/2 cortical slices combined with WT striatum slices or vice versa). These cultures revealed that only in slices composed of R6/2 cortex and WT striatum a corticostriatal pathway is established and mHTT is propagated from R6/2 cortex to WT striatum. When R6/2 striatum is combined with a WT cortical slide, the corticostriatal pathway is not efficiently formed and no mHTT is found in the WT cortex. Together, these mixed-genotype culture experiments revealed that functional connectivity between neurons is essential for transfer of mHTT. Interestingly, the transneuronal propagation was dependent on synaptic contacts in vivo, as was established using co-expression of viral vectors coding for Alexa594-tagged mHTT.
and the synapse marker synaptophysin, tagged with GFP in neurons. mHTT was predominantly found in neurons which were in close proximity to synaptophysin positive synaptic terminals (Pecho-Vrieseling et al., 2014).

Interestingly, mHTT spreading in human brains was demonstrated in post-mortem material from genetically normal tissue that was grafted into the striatum of three HD patients. These patients received the transplants with fetal striatal tissue in an attempt to slow disease progression. The grafted healthy tissue harbored mHTT inclusions a decade post-transplantation (Cicchetti et al., 2014). Using a variety of techniques, including immunofluorescence, Western immunoblotting, and infrared spectroscopy, it was demonstrated that the mHTT was localized in the extracellular matrix of the transplants, whereas mHTT within the non-grafted tissue was primarily localized within neurons and neuropil. While the study did not therefore provide direct proof of cell-to-cell transfer in human patients, these results do suggest that mHTT is released from donor cells, possibly as a result of mHTT-induced toxicity resulting in the release of mHTT content in the extracellular matrix.

MECHANISMS OF MHTT SPREADING

While in vitro and in vivo experiments showed mHTT transfer between cells, the questions remain when and how the actual transfer of mHTT from donor to acceptor cells occurs. Several observations indicate that mHTT transfer is not a continuous process. Acceptor WT m-neurons only showed internalization of mHTT when exposed to supernatant collected from 1-2 week old cultures of mHTT expressing m-neurons, but not when exposed to supernatant collected from 4-week-old cultures of the same mHTT expressing m-neurons. Similarly, mHTT propagation from R6/2 mouse brain slices to hESC- or hiPSC-derived neurons mainly takes place between 3-4 weeks of co-culture, as described above (Pecho-Vrieseling et al., 2014). Changes in the intracellular localization of mHTT may affect the efficiency of mHTT release. For example, mHTT aggregates in mouse cells appear first in the cytoplasm before ending up in the nucleus, which is also observed for mHTT in the human embryonic stem cell-derived neurons (h-neurons). While the cytoplasmic localization of mHTT may facilitate release from the cytoplasm (Fig 1B), the nuclear localization may result in toxicity and necrosis or apoptosis (Fig 1D). A different re-localization was observed by Costanzo et al. 2013, who found that transfer of fluorescently labelled mHTT aggregates through tunneling nanotubes (TNTs) merely takes place within the first 12 hours of co-culture of mHTT expressing mouse catecholaminergic neuronal (CAD) cells and non-transfected CAD cells. After 24 hours the aggregates were widely distributed in the cytoplasm of acceptor cells, but after 36 hours the aggregates were larger and were re-located to the perinuclear region. Here, the early distribution of mHTT throughout the cytoplasm may facilitate release, while clustering of mHTT aggregates in the perinuclear region may prevent release of mHTT from these cells. Taken together, these data suggest that there might be a critical time window for mHTT propagation, which is set by the presence of mHTT in the cytoplasm and/or the size of aggregates. Several mechanisms of intercellular propagation have been proposed, such as TNTs, transfer via synaptic vesicles, and phagocytosis by glial cells or exosomes. Although spreading via the exocytosis-endocytosis pathway is described in PD and AD (Frost et al., 2009; Lee et al., 2008; Lee et al., 2010), to date there are no studies reporting this phenomenon in HD independent of synaptic transfer.

Tunneling nanotubes. In their co-culture experiments using CAD cells, Costanzo et al. showed that fluorescently-labeled mHTT aggregates were transferred to neighboring cells through TNTs (Costanzo et al. 2013) (Fig 3A). No aggregate transfer could be observed when additional experiments were conducted with conditioned medium of mHTT-expressing cells, or with donor and acceptor cells that were separated by filters that allow passage of secretory vesicles and exosomes but do not allow cell-cell contact. Therefore, they suggest that secretion is not the predominant mode of transfer. Indeed, this result confirms the demonstration that mHTT propagation in the above described mixed-genotype cultures, solely happens in the presence of functional corticostriatal connectivity. Moreover, several studies demonstrated that prion conversion depends on close cell-cell contact (Kanu et al., 2002) and TNT-formation (Gouset et al., 2009). Nevertheless, these findings are in contradiction with other experiments showing that conditioned medium from prion-infected or mHTT-transfected cells enables the transfer of prions/mHTT to uninfected cells (Schatzl et al., 1997) and that the same holds true for CSF from HD patients (Tan et al., 2015). Currently, it remains to be established whether TNT are contributing to transfer in vivo in (neurodegenerative) disease pathogenesis and aggregate propagation. Since Costanzo et al. had to ensure an optimal cell density for TNT-formation to occur (Costanzo et al., 2013), this may indicate that TNTs are not similarly involved in intercellular communication in the human brain. However, this important concern does not conveniently rule out the TNTs existence nor its importance in intercellular propagation of mHTT in the human brain.

Synaptic transfer. Transneuronal propagation of protein aggregates has been demonstrated between synthetically connected neurons. Release of synaptic vesicles might therefore not only be essential for synaptic signaling, but could conversely aid in the trans-synaptic spreading of mHTT. Similar to what has been shown for tau (Pooler et al., 2013; Frost et al., 2009; Guo and Lee, 2011; Liu et al., 2012) and α-synuclein (Lak et al., 2012a; Ulusoy et al., 2013), our experimental data showed that mHTT is transferred between synthetically connected neurons in vivo. We demonstrated that transneuronal propagation of mHTT occurs in the corticostriatal network in vitro and in vivo in the mouse brain ((Pecho-Vrieseling et al., 2014), Fig 3B). By blocking synaptic vesicle release in vitro in the OTBS/h-neuron co-culture system by pharmacological application of tetanus toxins, it was shown that trans-synaptic transfer of mHTT involves synaptic vesicle exocytosis (Pecho-Vrieseling et al., 2014). Similarly, Babcock et al. showed by RNAi-techniques that mHTT transfer depends on SNARE-mediated vesicle fusion in Drosophila. Interestingly, also blocking endocytic uptake of the receiving neuron with the Shibire-mutant leads to a failure in aggregate transmission (Babcock and Ganetzky, 2015). Altogether, both studies clearly highlight the in vivo importance of synaptic transmission of mHTT in mice and Drosophila, but its relevance and contribution in mHTT spreading in the human brain is yet to be elucidated.
Glial phagocytosis. Recent findings on intercellular propagation of mHTT highlight the importance of glial cells in aggregate uptake by phagocytosis of neurons undergoing degeneration. Pearce et al. demonstrated a prion-like spreading of mHTT into phagocytic glia, and subsequent conversion of soluble into aggregation-prone WT HTT in a Drosophila model (Pearce et al., 2015) (Fig 3C). Although no axonal degeneration and apoptosis is visible upon expression of an aggregation-prone mHTT in ORNs, WT HTT sequesters into aggregates in neighboring glia, suggesting that either close proximity, or direct physical contact is required for aggregate transfer and acceleration of aggregation of soluble HTT. Although differences exist between vertebrate and Drosophila glial cells, there are also many functional similarities between Drosophila and vertebrate glia, including their ability to phagocytose (Freeman and Doherty, 2006). Indeed, upon induction of glial phagocytosis by antennal axotomy, prion-like propagation of mHTT from neurons to glial cells is induced, as cytoplasmic aggregates in glial cells are readily observed within days after induction of phagocytosis. By glial-specific knockout of the scavenger receptor Draper, the engulfment and degradation of ORN debris by phagocytic glia is inhibited and mHTT propagation is diminished (Pearce et al., 2015). Interestingly, Draper’s mammalian homolog MEGF10 is also in astrocytes responsible for synaptic pruning (Iram et al., 2016), which would result in a similar take-up of mHTT aggregates from neurons by astrocytes.

Surprisingly, whereas mHTT aggregates in ORNs are heterogeneous in size (~0.3-1.8 µm), newly formed aggregates in the cytoplasm of glial cells are distinctly smaller (0.3-0.5 µm), which could be due to an upper size limit for efficient phagocytosis and subsequent cytoplasmic entry. Indeed, reactive astrocytes and microglia are abundantly found in HD-affected human brains, especially in the cortex and striatum, and nuclear inclusions are also present in glial cells (Sapp et al., 2001; Faideau et al., 2010; Jansen et al., 2016). Furthermore, synapse dysfunction is well described in HD cell cultures and mouse models (Deng et al., 2013; Buren et al., 2016; Sepers and Raymond, 2014) and a decrease in synaptic activity leads to phagocytosis of synapses by both astrocytes and microglia (Iram et al., 2016; Kettenmann et al., 2013). Given their ability to phagocytose inactive synapses and degenerative mHTT-containing neurons and since glia become activated in HD and contain nuclear inclusions, spreading of mHTT via glia is a plausible mechanism.

Exosomes. It has been shown that exosomes are able to transfer their content from one cell to another without direct cell-to-cell contact, and can assist in the propagation of disease-associated protein aggregates such as tau (Saman et al., 2012; Asai et al., 2015), α-synuclein (Danzier et al., 2012; Emmanouilidou et al., 2010), and PrP (Alais et al., 2008; Coleman et al., 2012). Given their small size, the carriage of a myriad of molecules and the fact that some of them even contain organelles like mitochondria and travel long distances, it is possible that exosomes contribute to the intercellular propagation of mHTT throughout the brain (Fig. 3D). Exosomes are released from a variety of cells in the brain, including microglia (Potolicchio et al., 2005), developing neurons (Faure et al., 2006), astrocytes (Taylor et al., 2007) and oligodendrocytes (Kramer-Albers et al., 2007), which could potentially contribute to mHTT spreading between multiple cell types. mHTT accumulation in endosomal-lysosomal organelles and multivesicular bodies (MVBs) has been demonstrated (Sapp et al., 1997). In fact, for efficient clearance of intracellular mHTT aggregates through autophagy, proper MVB function is required (Filimonenko et al., 2007). These cellular compartments are connected to the formation of exosome secretion (Fevrier and Raposo, 2004), and therefore exosome-mediated cell-to-cell delivery of mHTT might conversely constitute a Trojan horse in disease propagation. Indeed, recently it was shown that mHTT is present in exosomes in the cell culture media from iPSCs of HD patient fibroblasts and SH-SY5Y cells expressing HTT-exon1-103Q-GFP. Furthermore, it was shown that mHTT is transmitted to other cells, both in vitro and in vivo. The presence of mHTT was established in the acceptor cells, and in addition a change in cell morphology was observed. In WT mice, transfer of mHTT from transplanted HD patient-derived fibroblasts leads to gliosis and a full-blown HD-like behavioral phenotype (Jeon et al., 2016).

Altogether, several mechanisms have been described which cover either neuron-to-neuron or neuron-to-glia propagation of mHTT aggregates in vitro and in vivo. Various mechanisms may contribute to local or distant transfer of mHTT and are therefore interesting for further exploration. For cell-to-cell transfer via TNTs, more evidence is needed to understand its
prion-like transfer of mHTT

relevance and contribution to cell-to-cell transfer in vivo, which is already there for other mechanisms including synaptic transmission, glial phagocytosis and exosomal transfer. There is a possibility that all mechanisms can occur in the HD brain, at different stages of disease or between different cell types. Hence, the predominant mechanisms of intercellular propagation as well as its relevance for progression of HD remain yet to be elucidated.

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