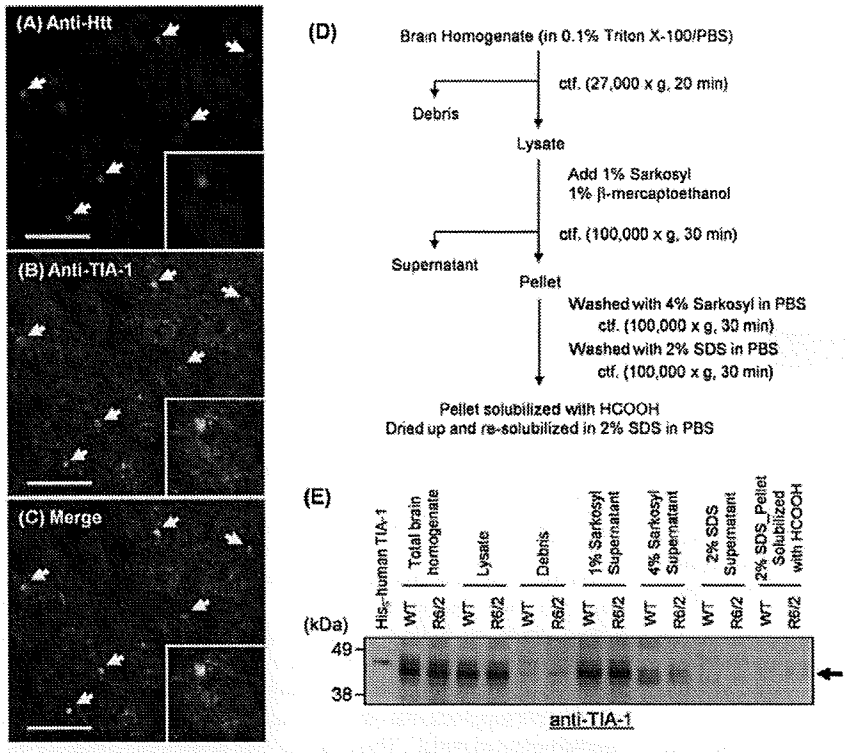

Figure 1. Endogenous TIA-1 is colocalized with Htt inclusions in R6/2 mouse brain. ***A–C***, Immunohistochemistry of hippocampus CA1 of an R6/2 brain was performed by double staining with mouse anti-Htt [EM48; red (***A***)] and goat anti-TIA-1 [C-20; green (***B***)] followed with Alexa 546-anti-mouse and Alexa 488-anti-goat antibodies (scale bar, 10 μm). Images were obtained by a confocal microscopy. A merged image is shown in ***C***, and colocalization of endogenous TIA-1 with Htt inclusions is shown by white arrows. Nuclei were counterstained with TOTO-3 (blue). A magnified image is also shown in the inset of each panel. Brain homogenates of R6/2 and WT mice at 12 weeks of age were prepared as shown in ***D***, and each fraction was analyzed by Western blot using anti-TIA-1 antibody (***E***). Recombinant 6× His-tagged human TIA-1 (molecular weight, 45,090) was loaded as a positive control and exhibits slower mobility than endogenous mouse TIA-1 (molecular weight, 42,800).

using a Speed-Vac, the pellet fraction was redissolved/sonicated in 150 μl of 100 mM Na-Pi/100 mM NaCl/2% SDS, pH 7.0 and saved as an RIPA-insoluble fraction. Protein concentration was determined by a BCA assay using BSA as a standard. Amounts of TIA-1 and Htt in both RIPA-soluble and -insoluble fractions were estimated by Western blotting.

Immunostaining of cultured cells and mouse tissues. *Neuro2a* cells seeded on a four-well chamber slide were cotransfected with 0.72 μg each of Htt60Q-GFP in pcDNA3.1 and TIA-1 variants C-terminally tagged with HA in pIRESneo3. After 4 h of transfection, cells were differentiated by exchanging the culture medium to DMEM containing 10% FBS and 5 mM dbcAMP. After 2 d incubation at 37°C, cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. The antibody dilutions were 1:300 for rabbit polyclonal anti-HA (Y-11) and 1:1000 for anti-rabbit IgG conjugated with Alexa 555 (Invitrogen). Nuclei were counterstained with Hoechst 33342. Images were obtained by using a fluorescence microscope (Olympus IX70, LCPlan Fl ×40).

For immunostaining of mouse brain tissues, mice were anesthetized and immediately fixed by perfusion through the left ventricle with 4% paraformaldehyde in PBS. A brain was collected and further fixed with the same fixative overnight, followed by treatment with 30% sucrose in PBS for 2 d and then with 20% sucrose solution overnight. The brains of R6/2 mice (12 weeks of age) mounted in Tissue mount (Chiba Medical) were cut into 10 μm sections with cryostat. For immunostaining, the primary antibody dilutions were used as follows: 1:500 for mouse monoclonal anti-Htt (EM48), 1:1000 for goat polyclonal anti-TIA-1 (C-20; Santa Cruz Biotechnology). The secondary antibody dilutions were 1:300 for goat anti-mouse IgG conjugated with Alexa 546 (Invitrogen) and donkey anti-goat IgG conjugated with Alexa 488 (Invitrogen). Nuclei were counterstained with TOTO-3. After being mounted using Immumount (Thermo Fisher Scientific), the tissues were observed under a confocal microscope (TCS SP2; Leica Microsystems).

Electrophoresis. Three or 10 μg of total proteins indicated in each experiment were boiled in an SDS-PAGE sample buffer containing β-mercaptoethanol and loaded on a 5–20% gradient or a 15% SDS-PAGE gel (PAGEL; ATTO). After electrophoresis, proteins were electroblotted on a 0.2 μm PVDF (polyvinylidene difluoride) membrane (Bio-Rad) and analyzed by Western blotting using either mouse monoclonal anti-poly(Q) (1C2; Millipore Bioscience Research Reagents; 1:1000), goat polyclonal anti-TIA-1 (C-20; 1:1000), mouse anti-GFP (Roche; 1:1000), or mouse monoclonal anti-cytochrome *c* (Cytc) (7H8.2C12; BD Biosciences Pharmingen; 1:1000) as a primary antibody. Corresponding secondary antibodies that are conjugated with HRP were used: anti-goat (1:3000) and anti-mouse (1:1000) antibodies. Blots were developed with the SuperSignal West Dura (Pierce), and images were obtained using LAS1000 (FUJI FILM).

Time-lapsed live-cell imaging. *Neuro2a* cells were cotransfected with a pair of Htt62Q-CFP and TIA-1^{FL}-YFP or Htt150Q-CFP and TIA-1^{C}-red fluorescent protein (RFP) on a glass-bottom dish (MatTeck). After 24 h of transfection, an observation of cells is started using a time-lapsed confocal microscopy. During the experiment, cells were maintained at 37°C with 5% CO_2 in a live-cell incubation chamber. Imaging was performed on an Olympus FV1000 confocal microscope equipped with a 60× UPlanSApo oil objective lens, a CO_2 live-cell incubator (MI-IBC-IF), a motorized *XY* stage, and an IR autofocus system (ZDC-IMAGE). Images at 10 different sites were acquired at intervals of 5 min for 10 h. For each site, five different *Z* planes (1 μm) in 110 μm pinhole were acquired to account for the aggregate location in the *Z* plane. CFP, YFP, and RFP were excited by 440 nm light-emitting diode, 515 nm argon, and 547 nm He–Ne laser, respectively. Fluorescence from CFP, YFP, and RFP is observed between 460 and 500 nm, 530 and 630 nm, and 600 and 700 nm emission wavelength, respectively.

Results

TIA-1 colocalizes with Htt inclusions in transgenic mice expressing Htt exon I with abnormal length of poly(Q)

Htt constitutes intranuclear inclusions when the length of consecutive glutamine residues in exon I exceeds a pathogenic threshold (~36 glutamines). An R6/2 transgenic mouse, in which human Htt exon I with ~130 glutamines is expressed, mimics pathological processes of HD including the formation of neuronal intranuclear inclusions (Davies et al., 1997). In R6/2 mice, mutant Htt starts to form inclusions at an early stage of the disease (4 weeks), and accumulates until the disease endstage (12 weeks). Although TIA-1 has been implied to colocalize with inclusions of mutant Htt in a cultured cell model (Waelter et al., 2001), we first examined whether TIA-1 is a constituent of pathological inclusions in an R6/2 mouse brain. Double immunostaining of a brain section of an R6/2 mouse at 12 weeks of age has been performed using anti-Htt (EM48) and anti-TIA-1 (C-20) antibodies. We confirmed intranuclear Htt-positive inclusions in cortex, hippocampus, and striatum (data not shown) and found that extensive colocalization of TIA-1 with Htt-positive inclu-

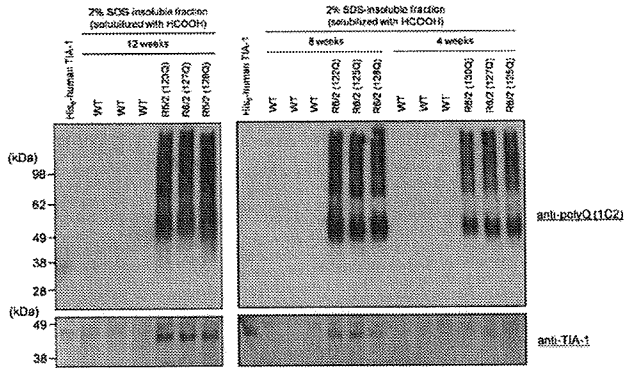

Figure 2. Htt aggregation precedes TIA-1 insolubilization in R6/2 mouse brain. The SDS-insoluble fraction redissolved with HCOOH treatment was prepared from WT and R6/2 mouse brains at 4, 8, and 12 weeks of age. Ten micrograms of total proteins were loaded on a gradient gel and analyzed by Western blot using anti-poly(Q) (1C2) or anti-TIA-1 (C-20) antibodies. Calculated molecular weight of exon I of Htt with 130Q is 24,075 Da, but it is known that a long poly(Q) tract retards the electrophoretic mobility and increases apparent molecular weight. In addition, redissolved Htt aggregates exhibit smear signals in the high molecular weight region probably because of the SDS-resistant interactions between long poly(Q) tracts in Htt proteins.

sions especially in hippocampus (Fig. 1*A–C*). These immunohistochemical observations suggest that TIA-1 is recruited into pathological HD inclusions *in vivo*.

Aggregates of mutant Htt remain insoluble in 2% SDS but can be solubilized after treatment with 100% formic acid (Hazeki et al., 2000). To examine biochemical alterations of TIA-1 on its recruitment into pathological inclusions, a fractionation was performed using homogenates of wild-type (WT) and R6/2 mouse brain at 12 weeks of age (Díaz-Hernández et al., 2004) (Fig. 1*D*). As expected, Htt aggregates solubilized with formic acid are detected in a 2% SDS-resistant fraction of an R6/2 mouse brain homogenate (Fig. 2). Although current experimental conditions do not detect difference in Sarkosyl-soluble amounts of endogenous TIA-1 between WT and R6/2 mice at 12 weeks of age, TIA-1 remains in a final SDS-resistant pellet fraction in R6/2 but not WT mice (Fig. 1*D,E*). Furthermore, the insolubilization of TIA-1 associated with the inclusion formation is found to be age dependent (Fig. 2). At 4 weeks of age, significant amounts of Htt aggregates are already found in an SDS-resistant pellet, whereas insoluble TIA-1 is not yet detected. TIA-1 starts to accumulate in SDS-resistant pellets between 4 and 8 weeks of age, and amounts of SDS-insoluble TIA-1 increase at 12 weeks of age in R6/2 mice. Together, a recruitment of TIA-1 into pathological inclusions occurs after Htt aggregation and leads to decrease the solubility of a TIA-1 protein.

C-terminal domain of TIA-1 is responsible for its recruitment into Htt inclusions

TIA-1 is comprised of three RNA recognition motifs at its N terminus and a Q/N-rich auxiliary domain at its C terminus (Tian et al., 1991) (Fig. 3*A*). To test which region(s) of TIA-1 is responsible for the recruitment into inclusions containing mutant Htt, exon I of Htt with a pathological (60Q) length of a poly(Q) tract (Htt60Q) was coexpressed with either a full-length (TIA-1^{FL}, 1–406), N-terminal RNA recognition motifs (TIA-1^{N}, 1–309), or a C-terminal Q/N-rich domain (TIA-1^{C}, 310–406) of human TIA-1 in mouse neuroblastoma cells, *Neuro2a*. Htt60Q and TIA-1 variants were C-terminally fused with GFP and HA, respectively. After incubation for 2 d, Htt60Q-GFP exhibits cytoplasmic inclusions, with which TIA-1^{FL}-HA and TIA-1^{C}-HA are colocalized (Fig. 3*B,D*). In contrast, TIA-1^{N}-HA is segregated from Htt60Q-GFP inclusions (Fig. 3*C*). We also attempted to test specificity of the recruitment of TIA-1^{FL}-HA or TIA-1^{C}-HA into Htt inclusions; for that purpose, we note Cu,Zn-superoxide dismutase (SOD1) with a G85R mutation, which is causative for familial form of amyotrophic lateral sclerosis (Rosen et al., 1993). Overexpression of SOD1^{G85R} C-terminally tagged with GFP (SOD1^{G85R}-GFP) in *Neuro2a* cells leads to formation of cytoplasmic inclusions (Furukawa et al., 2008); however, TIA-1^{FL}-HA or TIA-1^{C}-HA was not recruited into the SOD1 inclusions (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). These immunocytochemical results thus show that interaction of TIA-1 with Htt inclusions does not appear to be nonspecific and that TIA-1 interacts with Htt inclusions through a C-terminal Q/N-rich domain.

TIA-1 itself is fibrillogenic and integrated into Htt fibrillar aggregates

We next sought to decipher a molecular mechanism of how TIA-1 is recruited into inclusions. Htt exon I with 42 glutamines (Htt42Q) was prepared as a fusion protein with GST (GST-Htt42Q) (Scherzinger et al., 1997). Treatment of GST-Htt42Q with a protease, HRV3C, specifically cleaves the site between Htt42Q and a GST-tag within 10 min (data not shown), and additional incubation at 20°C for 6 h results in the formation of fibrillar aggregates (supplemental Fig. 2*A*, available at www.jneurosci.org as supplemental material). These *in vitro* Htt fibrils well reproduce the pathological Htt aggregates purified from inclusions in patients and rodent models of HD (Díaz-Hernández et al., 2004).

Interestingly, overnight agitation of 10 μM TIA-1^{FL} at 37°C increases the intensity of thioflavin T fluorescence (Fig. 4*A*). Thioflavin T fluoresces on specific binding to β-sheet rich protein fibrils (Naiki et al., 1989). In addition, the insoluble TIA-1^{FL} aggregates red-shift the absorption peak of Congo red (Fig. 4*B*). These are typical tinctorial changes on formation of amyloid-like fibrillar aggregates (Harper and Lansbury, 1997). To identify the fibrillogenic region of TIA-1, a soluble form of either TIA-1^{N} or TIA-1^{C} was agitated overnight at 37°C. We found that TIA-1^{C} but not TIA-1^{N} forms the thioflavin T and Congo red-positive aggregates (Fig. 4*A,B*). Fibrillar morphology of TIA-1^{C} aggregates was further confirmed by an atomic force microscopy (Fig. 4*C*). Given a high propensity of TIA-1 itself for fibrillation, therefore, we hypothesize that mutant Htt can form a mixed fibril with TIA-1.

To test this hypothesis in *in vitro* conditions, 5 μM GST-Htt42Q was incubated with 0.5 μM of a GST-TIA-1^{C} protein that is further C-terminally fused with a triple HA tag (GST-TIA-1^{C}-HA$_3$). A GST tag in both proteins was liberated by adding a specific protease, HRV3C, during incubation for aggregation. Htt42Q/TIA-1^{C} mixed aggregates were then observed by an electron microscopy after immunodecoration by anti-Htt and anti-HA antibodies. As shown in Figure 4*D*, we found that TIA-1^{C}-HA$_3$ is integrated in Htt42Q fibrils. Because TIA-1 remains SDS-soluble in the absence of Htt inclusion formation (Figs. 1*E*, 2), mutant Htt/TIA-1 mixed fibrils would form in the pathological inclusions found in HD.

Indeed, recruitment of TIA-1 in *in vivo* Htt fibrils was supported by immunoelectron micrograms of SDS-insoluble pellets that were prepared from *Neuro2a* cells doubly transfected with Htt60Q-GFP and TIA-1$^{FL\ or\ C}$-HA. Htt60Q-GFP forms fibrillar aggregates, which were reacted with an anti-Htt (EM48) antibody, and an anti-HA antibody detects TIA-1^{FL}-HA or TIA-1^{C}-HA in SDS-insoluble Htt fibrils (Fig. 4*E,F*; supplemental Fig. 1*B*, available at www.jneurosci.org as supplemental material).

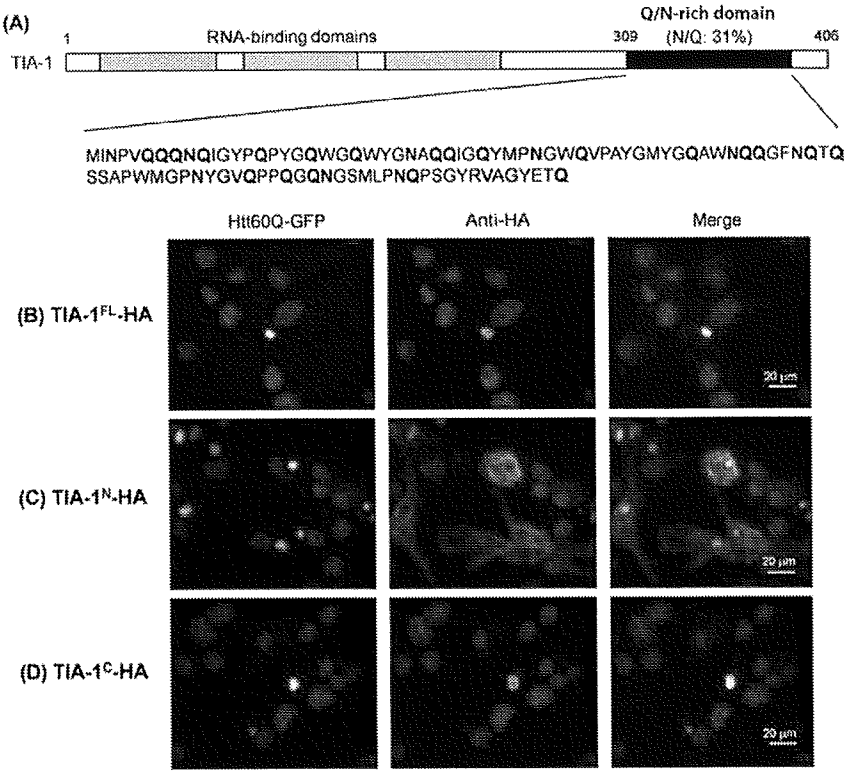

Figure 3. C-terminal Q/N-rich domain of TIA-1 is responsible for coaggregation with Htt. *A*, Schematic representation of domain structure of TIA-1. Thirty-one percent of a C-terminal domain corresponds to glutamine (Q) or asparagine (N) residues. *Neuro2a* cells were doubly transfected with Htt60Q-GFP and TIA-1^{FL}-HA (*B*), TIA-1^{N}-HA (*C*), or TIA-1^{C}-HA (*D*). Two days after transfection, cells were fixed, stained with rabbit anti-HA antibody and Alexa 555-anti-rabit antibodies (shown in red), and observed using a fluorescence microscope. Nuclei were counterstained using Hoechst 33342 (blue). Htt60Q-GFP forms cytoplasmic inclusions in all three cases (shown in green), and colocalization was observed in TIA-1^{FL}-HA and TIA-1^{C}-HA, but not in TIA-1^{N}-HA.

Such fibrillar structures were not found in the SDS-insoluble fraction of cells transfected with TIA-1$^{FL\ or\ C}$-HA alone (data not shown). These results hence suggest that Htt aggregates induce the conformational transition of TIA-1 from a soluble state into insoluble aggregates *in vitro* and *in vivo*.

Htt fibril functions as an efficient seed for *in vitro* fibrillation of TIA-1^{C}

In *in vitro* mutant Htt fibrillation, a nucleation step precedes a fibril elongation step, which was monitored by fluorescence increase of thioflavin T after a lag time (Fig. 5*A*, blue curve). Interestingly, addition of soluble 0.5 μM TIA-1^{C} to the solution containing 5 μM Htt42Q extended the lag time of Htt fibrillation (from ~100 to ~400 min) (Fig. 5*A*, blue and black curves), implying that soluble TIA-1 decelerates fibrillation of mutant Htt by protein–protein interactions. Once mutant Htt starts to fibrillate, however, TIA-1 becomes intertwined in a fibrillar structure of mutant Htt aggregates as observed in Figure 4*D*.

As a possible mechanism of the recruitment of TIA-1 into Htt fibrils, furthermore, we have noted a seeding phenomenon of protein fibrils, in which existing fibrils induce subsequent fibrillation of a soluble protein (Harper and Lansbury, 1997). Effective concentrations of fibrillar species can be increased by shearing fibrils with ultrasonication, and sheared fibrils are used as "seeds" (Harper and Lansbury, 1997). When Htt42Q seeds are added to a soluble form of Htt42Q, thioflavin T fluorescence increases without a lag time, supporting a seeding reaction (Fig. 5*A*, blue and red curves). TIA-1^{C} also exhibits a seeding reaction, in which addition of TIA-1^{C} seeds diminishes the lag time in TIA-1^{C} fibrillation (Fig. 5*B*, blue and red curves). Interestingly, albeit with less efficiency compared with the self-seeding of TIA-1^{C}, Htt42Q seeds also accelerate fibrillation of TIA-1^{C} by shortening the lag time (Fig. 5*B*, blue and green curves), whereas addition of soluble Htt42Q does not alter the fibrillation kinetics of TIA-1^{C} (Fig. 5*B*, blue and black curves). Such a "cross-seeding" reaction between heterologous proteins was not, however, observed in the fibrillation of Htt42Q with TIA-1^{C} seeds (Fig. 5*A*, blue and green curves). Htt aggregate can thus act as a nucleus (or seed) to accelerate fibrillation of TIA-1^{C}.

To further confirm the cross-seeding reaction of TIA-1^{C} by Htt42Q aggregates, we performed direct observation of protein fibrils using a fluorescence microscope. Alexa 555-modified Htt42Q and Alexa 488-modified TIA-1^{C} were first prepared and then incubated for aggregation. When Alexa 555-Htt42Q seeds were incubated with soluble Alexa 488-TIA-1^{C} on a glass slide, it is clearly observed that a green TIA-1^{C} fibril grows from a red Alexa 555-Htt42Q seed (Fig. 5*C–E*). A cross-seeding activity of Htt aggregates in TIA-1 fibrillation is, therefore, proposed to be one of molecular origins to describe the recruitment of TIA-1 into Htt fibrillar aggregates.

Htt inclusions induce recruitment of TIA-1 in a cultured cell, but not vice versa

A seeding role of Htt aggregates in the *in vitro* recruitment of TIA-1 is tested by the time-lapse live-cell imaging experiments. First, *Neuro2a* cells are cotransfected with Htt62Q-CFP and TIA-1^{FL}-YFP. Htt62Q-CFP inclusions first appear, but no colocalization of TIA-1^{FL}-YFP occurs in an early phase (Fig. 6*A*, 0–50 min). TIA-1^{FL}-YFP becomes sequestered into Htt62Q-CFP inclusions in the later phase of observation (Fig. 6*A*, 75–125 min). These results appear to reproduce the observations that Htt inclusion formation precedes insolubilization of TIA-1 in a mouse brain (Fig. 2).

To examine whether preexisting TIA-1 aggregates affect the formation of Htt inclusions in the cellular environment, we also performed time-lapse imaging of *Neuro2a* cells transfected with Htt150Q-CFP and TIA-1^{C}-RFP. Unlike TIA-1^{FL}-YFP, TIA-1^{C}-RFP forms cytoplasmic inclusions before Htt150Q-CFP starts to aggregate. Htt inclusion formation occurs at the site at which TIA-1^{C}-RFP inclusions are not evident (Fig. 6*B*, 0–60 min). At a later stage of the observation (Fig. 6*B*, 80–100 min), however, TIA-1^{C}-RFP is eventually colocalized with Htt inclusions.

Inclusion formation observed in cultured cells is supposed to be a distinct process from protein fibrillation in a molecular level and has been known as a complex cellular process involving ubiquitination, active protein transport, and interactions with molecular chaperones (Kopito, 2000). For example, a proteasomal inhibition caused by insoluble Htt aggregates (Jana et al., 2001) may also be a possible mechanism of Htt/TIA-1 colocalization in the pathological inclusions. In *Neuro2a* cells, Htt62Q-YFP and TIA-1^{FL}-HA form coaggregates that are trapped on a 0.2 μm cellulose

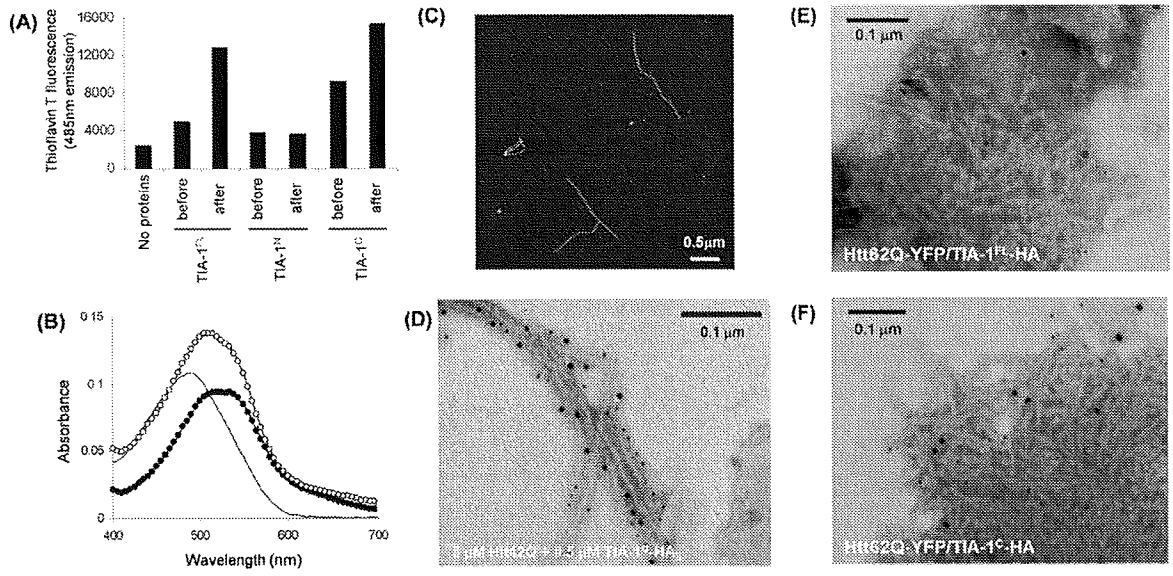

Figure 4. TIA-1 forms amyloid-like fibrils and is integrated in Htt42Q fibril. ***A***, Fluorescence intensity from thioflavin T before and after overnight agitation of 10 μM TIA-1^{FL}, TIA-1^{N}, and TIA-1^{C} at 37°C, 1200 rpm. Excitation and emission wavelengths were 442 and 485 nm, respectively. TIA-1^{C} was prepared by adding an HRV3C protease to GST-TIA-1^{C}. ***B***, Electronic absorption spectra of Congo red in the presence of either TIA-1^{FL} aggregates (curve with open circles) or TIA-1^{C} aggregates (curve with filled circles). A plain curve shows an absorption spectrum of Congo red alone. ***C***, Fibrillar morphologies of TIA-1^{C} aggregates were observed by an atomic force microscopy. ***D***, An immunoelectron microscopic image of Htt42Q aggregated in the presence of TIA-1^{C}-HA$_3$. Htt is decorated with smaller gold particles (5 nm diameter), whereas larger gold particles (10 nm diameter) detect TIA-1^{C}-HA$_3$. ***E***, ***F***, Immunoelectron microscopic images of SDS-insoluble aggregates prepared from *Neuro2a* cells transfected with Htt62Q-YFP and TIA-1^{FL}-HA (***E***) or Htt62Q-YFP and TIA-1^{C}-HA (***F***). Anti-Htt, Smaller gold particles (5 nm diameter); anti-HA, larger gold particles (10 nm diameter).

acetate membrane even after a wash with 2% SDS (supplemental Fig. 3*A*, available at www.jneurosci.org as supplemental material). When *Neuro2a* cells were transiently transfected with TIA-1^{FL}-HA alone and treated with a specific proteasomal inhibitor, epoxomycin, we could not find any SDS-insoluble TIA-1 aggregates trapped on the membrane (supplemental Fig. 3, available at www.jneurosci.org as supplemental material). Although other cellular processes might regulate colocalization of Htt and TIA-1 in pathological inclusions, it is unlikely that proteasomal inhibition by Htt aggregates plays major roles in decreasing the solubility of a TIA-1 protein. As shown in *in vitro*/*in vivo* experiments, instead, we suppose that a cross-seeding reaction can be appended as one of molecular mechanisms for the recruitment of TIA-1 into SDS-insoluble Htt aggregates. Based on these results, our next question is whether the recruitment of TIA-1 into Htt inclusions is associated with dysregulation of its physiological function.

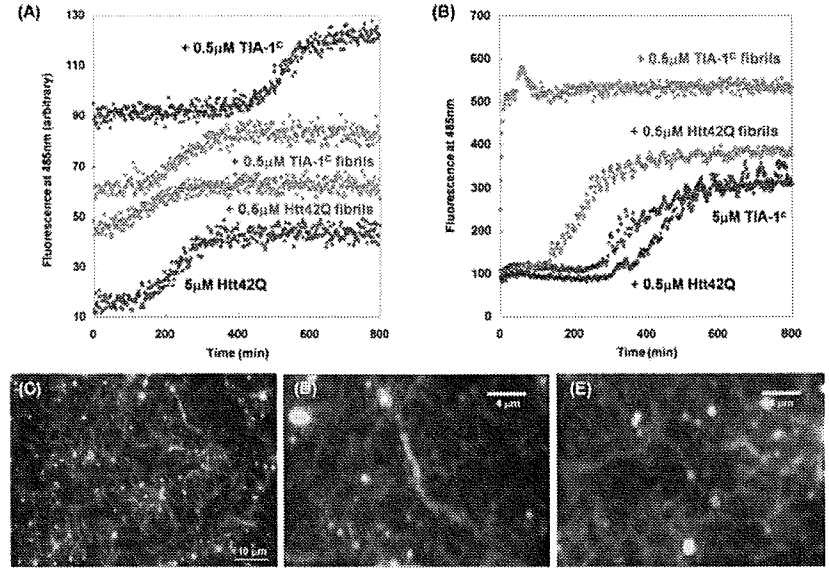

Figure 5. Htt aggregates function as an efficient seed for accelerating TIA-1^{C} fibrillation. ***A***, ***B***, A cross-seeding activity of TIA-1^{C} and Htt42Q aggregates toward Htt42Q and TIA-1^{C} fibrillation, respectively. ***A***, GST-Htt42Q (5 μM) was incubated with 0.5 μM of either soluble GST-TIA-1^{C}, Htt42Q aggregates, or TIA-1^{C} aggregates at 20°C in 100 mM Na-Pi/100 mM NaCl/1 mM EDTA/16.7 μM thioflavin T/2 U of HRV3C, pH 8.0. ***B***, GST-TIA-1^{C} (5 μM) was incubated with 0.5 μM of either soluble GST-Htt42Q, Htt42Q aggregates, or TIA-1^{C} aggregates in the same experimental conditions. Fibrillation was monitored by increase of thioflavin T fluorescence (442 nm excitation and 485 nm emission wavelength). ***C–E***, Direct observation of TIA-1^{C} fibrils (shown in green) seeded with Htt42Q aggregates (shown in red) by a fluorescence microscope (Olympus IX70, UPLAPO100x013). Soluble TIA-1^{C} modified with Alexa 488 was incubated with seeds of Alexa 555-modified Htt42Q. A cross-seeding reaction proceeds on a glass slide covered with a cover glass at 37°C overnight. Magnified images are shown in ***D*** and ***E***.

Functional inactivation of TIA-1 on recruitment into Htt inclusions

One of the physiological functions of TIA-1 is to suppress a translation of a target mRNA by binding its 3′-untranslated region (3′-UTR) (López de Silanes et al., 2005). It has been shown that translation of Cytc is suppressed by binding of TIA-1 to 3′-UTR of Cytc mRNA (Kawai et al., 2006). To test whether intracellular amounts of TIA-1 modulate expression of a Cytc protein, endogenous TIA-1 was decreased in human embryonic kidney cells, HEK293T, by using miRNA. Our miRNA targeting a human TIA-1 gene reduced amounts of an endogenous TIA-1 protein (~50% reduction), resulting in the increase in expression level of Cytc (~50% increase) (Fig. 7*A*,*B*). These results confirm

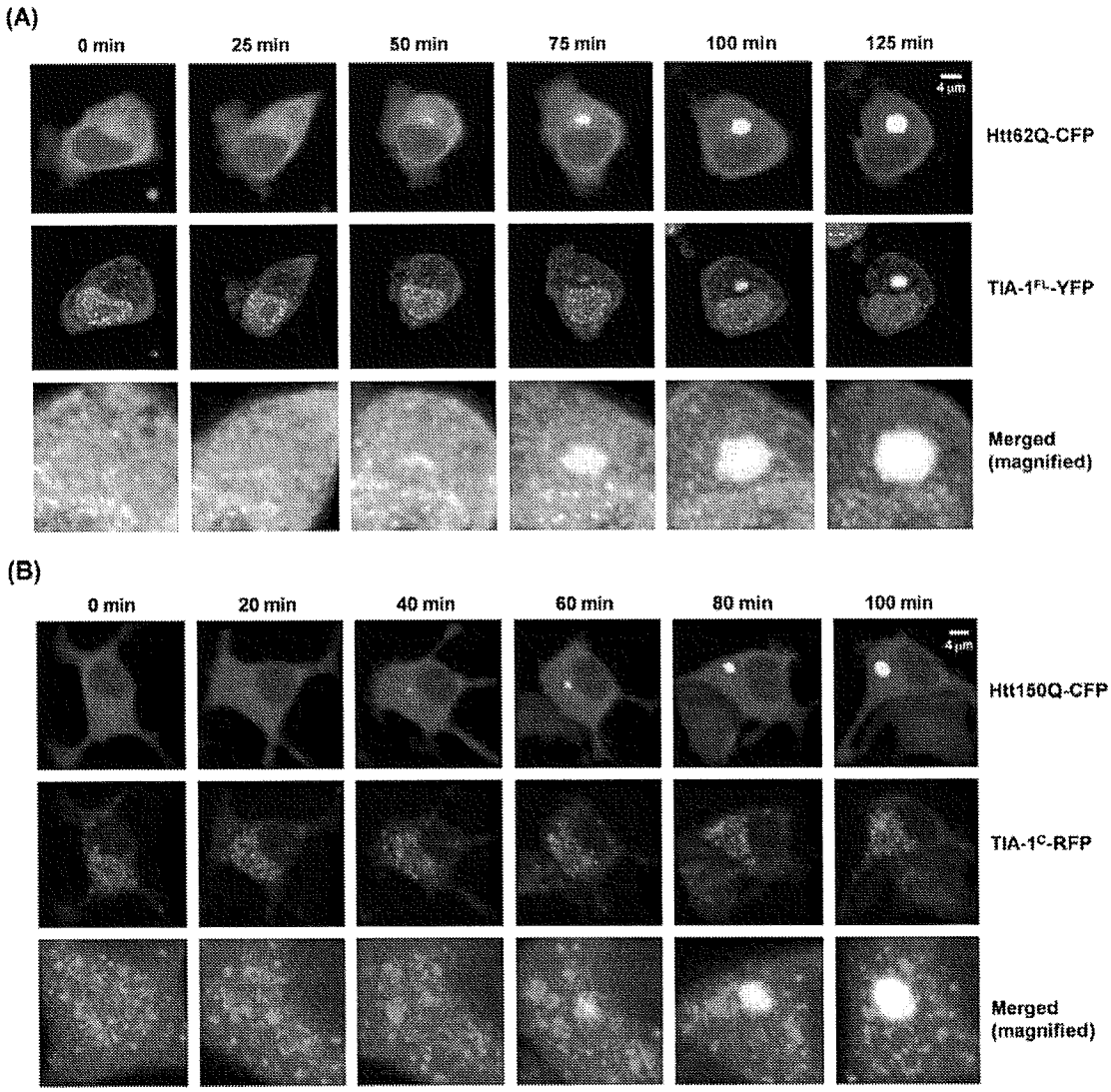

Figure 6. Htt inclusions sequester TIA-1 in a *Neuro2a* cell, but not vice versa. ***A***, Representative time-lapsed images of *Neuro2a* cells cotransfected with Htt62Q-CFP and TIA-1^{FL}-YFP. After overnight transfection, the confocal live-cell imaging was started and performed at intervals of 5 min. A time point when the cell contains no aggregates was arbitrarily set as 0 min. Scale bar, 4 μm. ***B***, Time-lapse confocal imaging of a live *Neuro2a* cell cotransfected with Htt150Q-CFP and TIA-1^{C}-RFP. Most of the cells expressing TIA-1^{C}-RFP form inclusions after overnight transfection, but those TIA-1^{C}-RFP inclusions do not induce Htt150Q-CFP aggregation. Scale bar, 4 μm.

the suppressive role of TIA-1 on expression of a Cyt*c* protein (Kawai et al., 2006).

We next attempt to reveal functional dysregulation of TIA-1 on its recruitment into Htt inclusions. Either Htt18Q-YFP or Htt62Q-YFP was transiently transfected with HEK293T cells together with a pPUR plasmid that contains a puromycin-resistant gene; thereby, contributions from untransfected cells can be reduced by treatment with puromycin. After 3 d of incubation, Htt62Q-YFP but not Htt18Q-YFP has accumulated in a RIPA-insoluble fraction (Fig. 7*C*, bottom), which agrees with the fact that abnormal length of a poly(Q) tract (62Q) causes Htt aggregation. Aggregation of Htt62Q-YFP also associates with insolubilization of endogenous TIA-1 and concomitantly reduces amounts of RIPA-soluble TIA-1 compared with those in the cells with an aggregation-free Htt18Q-YFP (~30% decrease) (Fig. 7*C*, top, Fig. 7*D*). Furthermore, significant increase (~30%) in the amount of Cyt*c* was observed in Htt62Q-YFP compared with Htt18Q-YFP, but its increase was suppressed when TIA-1^{FL}-HA was coexpressed (Fig. 7*E*,*F*). Even though significant fractions (~70%) of TIA-1^{FL}-HA becomes RIPA-insoluble on coexpression of TIA-1^{FL}-HA with Htt62Q-YFP (Fig. 7*C*,*D*), remaining amounts of soluble TIA-1^{FL}-HA are ~2.5-fold higher than those of endogenous TIA-1 in the cells transfected with Htt18Q-YFP alone (data not shown) and appear to be enough to complement the function of recruited endogenous TIA-1. These results are thus consistent with the idea that coaggregation with mutant Htt reduces intracellular soluble amounts of TIA-1 and represses a physiological function of TIA-1.

Discussion

Our studies show that an RNA-binding protein, TIA-1, is a member of the proteins recruited into insoluble Htt inclusions. *In vivo* and *in vitro* experimental results suggest that Htt aggregates function as an efficient seed for the conversion of a soluble TIA-1 protein into an insoluble structure. On recruitment into Htt inclusions, furthermore, we have shown in a cultured cell model

that intracellular amounts of soluble TIA-1 are decreased and that the physiological function of TIA-1 is repressed. A cross-seeding activity of Htt aggregates could hence be one of molecular mechanisms to describe how mutant Htt causes functional dysregulation of coaggregated proteins. Based on the results presented here, we propose that cross-seeded fibrillation of Q/N-rich proteins leads to a pathological diversity in a poly(Q) disease (Fig. 8).

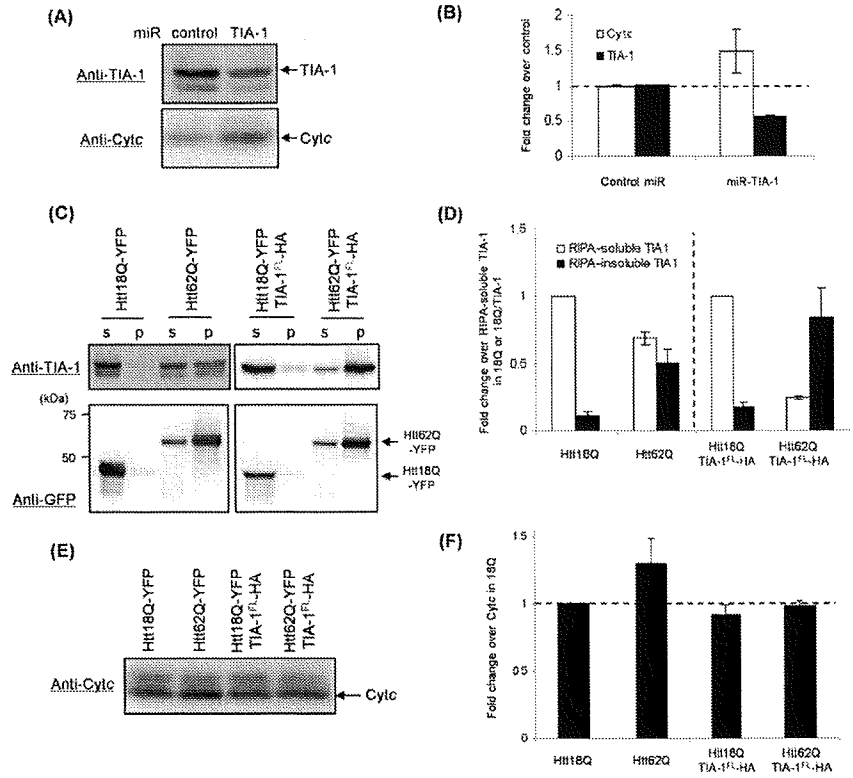

Figure 7. Functional repression of TIA-1 as a translational silencer on coaggregation with mutant Htt. ***A***, ***B***, HEK293T cells transfected with negative control or miR-TIA-1 were lysed in a RIPA buffer. After ultracentrifugation, a supernatant containing 3 μg (for TIA-1) or 10 μg (for Cytc) of total proteins was analyzed by Western blotting (***A***) and densitometric quantification of band intensity (***B***). ***C–F***, HEK293T cells transfected with Htt18/62Q-YFP and pPUR were lysed in a RIPA buffer. For coexpression of TIA-1^{FL}-HA, Htt18Q-YFP or Htt62Q-YFP in pIRES-TIA-1 was used. After ultracentrifugation, supernatant (s, RIPA-soluble fraction) and pellet (p, RIPA-insoluble fraction) were analyzed by Western blotting: TIA-1 and GFP (***C***), Cytc (***E***). ***D***, Amounts of TIA-1 in each sample were quantified and normalized by those of RIPA-soluble TIA-1 in either the sample transfected with Htt18Q-YFP or the sample with Htt18Q-YFP plus TIA-1^{FL}-HA. ***F***, Amounts of Cytc were quantified and normalized by those of the sample transfected with Htt18Q-YFP. Three independent experiments were performed to estimate errors (***B***, ***D***, ***F***).

Precedents of a coaggregation mechanism: proteins with a benign length of poly(Q) in Htt fibrils

So far, coaggregation with a mutant Htt protein has been reported in several proteins containing a nonpathogenic length of a poly(Q) tract. Important examples include the TATA box binding protein (TBP) and the transcriptional coactivator CBP (CREB binding protein), which possesses 38 and 19 consecutive glutamine residues, respectively (Huang et al., 1998; Nucifora et al., 2001; Schaffar et al., 2004). In addition, Htt with a normal length of poly(Q) (Q20, Q32, and Q37) is coaggregated with mutant Htt *in vitro* (Busch et al., 2003). These benign poly(Q)-containing proteins themselves are not fibrillogenic but become an integral component of fibrils on coaggregation with mutant Htt (Busch et al., 2003; Schaffar et al., 2004). Although Htt aggregates do not trigger fibrillation of these benign poly(Q) proteins *in vitro* (Busch et al., 2003), poly(Q)–poly(Q) interactions in the soluble state would be enough to intertwine benign poly(Q) proteins into Htt fibrils. In a human proteome, 64 proteins have more than five consecutive glutamines in their amino acid sequences (Butland et al., 2007), although not all of those poly(Q) proteins are necessarily sequestered in HD inclusions (Busch et al., 2003). Compared with poly(Q) proteins, the total number of Q/N-rich domains in a human proteome has been estimated to be ~200 (Harrison and Gerstein, 2003). As evidenced in TIA-1 as a model of Q/N-rich proteins, we suppose that more numbers of Q/N-rich proteins are potentially involved in HD inclusions by adopting a fibrillar structure (Fig. 8).

Cross-seeding activity of Htt aggregates leads to recruitment of Q/N-rich proteins in HD inclusions

TIA-1 has no poly(Q) tract (at most three consecutive glutamines), but its C-terminal domain is rich in Q/N residues (31% abundance) (Fig. 3*A*), given that the average abundance of Q and N for the eukaryotic proteomes is ~5% each per open reading frame (Michelitsch and Weissman, 2000). Fibrillation of a Q/N-rich protein sequence has been shown in several yeast prion proteins (Ross et al., 2005) such as Sup35 and New1. Q/N richness of the prion domain of Sup35 and New1 is higher than (48.8%) or comparable with (26.1%) that of TIA-1 C-terminal domain (31.0%), respectively. Sup35 exhibits weak sequence homology with TIA-1 at the Q/N-rich domain; TIA-1^{C} (97 aa) and Sup35 N-terminal NM domain (124 aa) share 26.1% identity and 53.4% similarity in the overlapped 88 aa. TIA-1^{C} also exhibits a limited homology with exon I of Htt with 42Q (112 aa): 25.0% identity and 36.0% similarity in the overlapped 72 aa. As implied in the species barrier of the prion transmission, a seeding specificity does not always correlate with the primary sequence similarities; rather, conformational roles of fibrils are more evident in a seeding reaction (Vanik et al., 2004). A structure of poly(Q) fibrils has been described by a "polar zipper" model, in which hydrogen bonding interactions among both side and main chains of glutamine residues constitute an ordered β-sheet structure (Perutz et al., 1994). Although it remains unclear how many glutamines can sustain a polar zipper interaction in the poly(Q)-Q/N mixed fibrils, our results imply that ~30% abundance of Q/N residues as found in TIA-1 is sufficient for being zipped with a poly(Q) sequence. Although a Q/N-rich sequence with low complexity would play a role in interacting with another protein molecule including a hydrogen-bonding interaction (Wright and Dyson, 1999), there is a caveat that presence of Q/N-rich region(s) in a certain protein molecule does not necessarily mean the ability to interact with poly(Q) aggregates. Nonetheless, it is worth noting that the coaggregated proteins our group has recently reported contain Q/N-rich sequences; an N-terminal part (1–162) of an RNA-binding protein, TLS, exhibits 26% in the Q/N abundance, and 33 and 34% of an N-terminal part of a transcription factor,

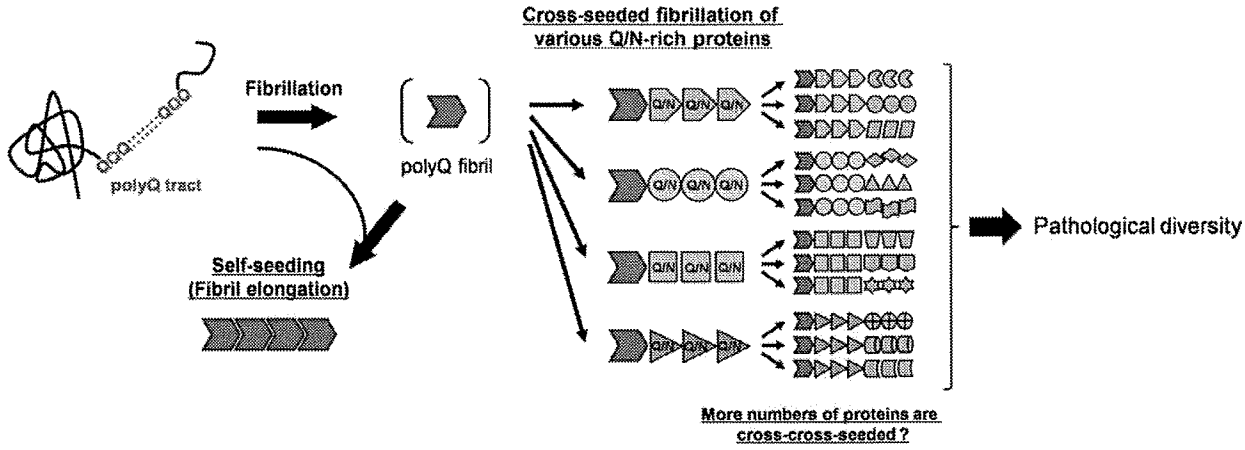

Figure 8. Our concept of pathological diversity based on a cross-seeding mechanism. An abnormally expanded poly(Q) tract forms fibrillar aggregates, which can induce fibrillation of Q/N-rich proteins by acting as a seed (cross-seeding). Various kinds of Q/N-rich proteins (designated as different shapes and/or colors) can be potentially recruited into the fibrillar aggregates through a cross-seeding reaction. Cross-seeded fibrils of Q/N-rich proteins may further facilitate fibrillation of the other proteins that cannot be directly seeded by poly(Q) aggregates (cross-cross-seeding). Given that physiological functions of recruited proteins are repressed, cross-seeding reactions will describe diverse pathologies observed in the poly(Q) diseases.

NF-YA (1–153), and a C-terminal part of NF-YC (150–186) is composed of Q/N residues, respectively (Doi et al., 2008; Yamanaka et al., 2008). In contrast, it has been reported that fibrillation of Aβ(1–40), which contains only one each of glutamine and asparagine residues, are not efficiently seeded with poly(Q) aggregates (O'Nuallain et al., 2004). A poly(Q) fibrillar structure could, therefore, provide a seeding scaffold to induce fibrillation of some Q/N-rich proteins.

Pathological outcome of cross-seeding reactions in neurodegenerative diseases

Compared with a transient protein–protein association reaction, one of the kinetic features of a cross-seeding reaction is to explosively promote protein aggregation once the seeding starts. Pathological inclusion bodies in HD contain many kinds of protein molecules such as chaperones and proteasome components (Jana et al., 2001), which are possibly recruited through a cellular process distinct from cross-seeding fibrillation. A molecular chaperone, Hsp70, for example, is one of the proteins found in the HD inclusions, but its interaction with inclusions is transient and highly dynamic (Kim et al., 2002). Unlike TIA-1 and the other Q/N-rich proteins such as TBP, NF-Y, and TLS, Hsc70 as well as Hsp84 do not contain a Q/N-rich region in its primary sequence and are easily dissociated from Htt inclusions in the presence of SDS (Mitsui et al., 2002). Although it remains unknown how functional repression of TIA-1 is related to HD pathologies, a cross-seeding behavior of mutant Htt bears some analogy with phenotypic changes in HD; diseases rapidly progress after the onset, and neurological symptoms often follow appearance of inclusions in the affected tissues (Davies et al., 1997).

In addition to a translational silencing of specific targeted genes including Cyt*c*, TIA-1 has been known to play an essential role in forming stress granules (SGs) under stressed conditions (Kedersha et al., 1999) and is also a constituent of intracytoplasmic basophilic inclusions found in the patients with adult-onset motor neuron disease (Fujita et al., 2008). TIA-1 recruits untranslated mRNAs into SGs without apparent specificity and self-aggregates within SGs (Gilks et al., 2004). Formation of SGs promotes disassembly of a functional polysome and arrests the translation of housekeeping genes. A cross-seeded aggregation of TIA-1 by Htt fibrils may lead to global suppression of translation by converging SGs and protein aggregates. As is the case with TIA-1, many DNA/RNA-binding proteins possess a Q/N-rich domain, which has been proposed to support protein–protein interactions (Michelitsch and Weissman, 2000). We thus suppose that cross-seeded fibrillation of a Q/N-rich domain with mutant Htt disrupts a canonical interaction in the transcriptional/translational complexes in particular and contributes to produce pathological phenotypes (Fig. 8).

Moreover, pathological phenotypes could become more diverse when a cross-seeded product further induces fibrillation of proteins that are not directly seeded with Htt aggregates (Fig. 8). Such a "cross-cross-seeding" initiated by Htt aggregates may affect physiological functions of more numbers of proteins (Fig. 8). Given a role of Htt aggregates as a structural template toward fibrillation of certain Q/N-rich sequences, therefore, we propose that a cross-seeding reaction by mutant Htt is one of molecular origins of diverse pathological phenotypes in HD patients.

References

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

Butland SL, Devon RS, Huang Y, Mead CL, Meynert AM, Neal SJ, Lee SS, Wilkinson A, Yang GS, Yuen MM, Hayden MR, Holt RA, Leavitt BR, Ouellette BF (2007) CAG-encoded polyglutamine length polymorphism in the human genome. BMC Genomics 8:126.

Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, Bates GP (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 90:537–548.

Díaz-Hernández M, Moreno-Herrero F, Gómez-Ramos P, Morán MA, Ferrer I, Baró AM, Avila J, Hernández F, Lucas JJ (2004) Biochemical, ultrastructural, and reversibility studies on huntingtin filaments isolated from mouse and human brain. J Neurosci 24:9361–9371.

Doi H, Okamura K, Bauer PO, Furukawa Y, Shimizu H, Kurosawa M, Machida Y, Miyazaki H, Mitsui K, Kuroiwa Y, Nukina N (2008) RNA-binding protein TLS is a major nuclear aggregate-interacting protein in huntingtin exon 1 with expanded polyglutamine-expressing cells. J Biol Chem 283:6489–6500.

Fujita K, Ito H, Nakano S, Kinoshita Y, Wate R, Kusaka H (2008) Immunohistochemical identification of messenger RNA-related proteins in baso-

philic inclusions of adult-onset atypical motor neuron disease. Acta Neuropathol 116:439–445.
Furukawa Y, Kaneko K, Yamanaka K, O'Halloran TV, Nukina N (2008) Complete loss of post-translational modifications triggers fibrillar aggregation of SOD1 in familial form of ALS. J Biol Chem 283:24167–24176.
Gilks N, Kedersha N, Ayodele M, Shen L, Stoecklin G, Dember LM, Anderson P (2004) Stress granule assembly is mediated by prion-like aggregation of TIA-1. Mol Biol Cell 15:5383–5398.
Harper JD, Lansbury PT Jr (1997) Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins. Annu Rev Biochem 66:385–407.
Harrison PM, Gerstein M (2003) A method to assess compositional bias in biological sequences and its application to prion-like glutamine/asparagine-rich domains in eukaryotic proteomes. Genome Biol 4:R40.
Hazeki N, Tukamoto T, Goto J, Kanazawa I (2000) Formic acid dissolves aggregates of an N-terminal huntingtin fragment containing an expanded polyglutamine tract: applying to quantification of protein components of the aggregates. Biochem Biophys Res Commun 277:386–393.
Huang CC, Faber PW, Persichetti F, Mittal V, Vonsattel JP, MacDonald ME, Gusella JF (1998) Amyloid formation by mutant huntingtin: threshold, progressivity and recruitment of normal polyglutamine proteins. Somat Cell Mol Genet 24:217–233.
Jana NR, Zemskov EA, Wang Gh, Nukina N (2001) Altered proteasomal function due to the expression of polyglutamine-expanded truncated N-terminal huntingtin induces apoptosis by caspase activation through mitochondrial cytochrome *c* release. Hum Mol Genet 10:1049–1059.
Kawai T, Lal A, Yang X, Galban S, Mazan-Mamczarz K, Gorospe M (2006) Translational control of cytochrome *c* by RNA-binding proteins TIA-1 and HuR. Mol Cell Biol 26:3295–3307.
Kedersha NL, Gupta M, Li W, Miller I, Anderson P (1999) RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2 alpha to the assembly of mammalian stress granules. J Cell Biol 147:1431–1442.
Kim S, Nollen EA, Kitagawa K, Bindokas VP, Morimoto RI (2002) Polyglutamine protein aggregates are dynamic. Nat Cell Biol 4:826–831.
Kopito RR (2000) Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 10:524–530.
López de Silanes I, Galbán S, Martindale JL, Yang X, Mazan-Mamczarz K, Indig FE, Falco G, Zhan M, Gorospe M (2005) Identification and functional outcome of mRNAs associated with RNA-binding protein TIA-1. Mol Cell Biol 25:9520–9531.
Michelitsch MD, Weissman JS (2000) A census of glutamine/asparagine-rich regions: implications for their conserved function and the prediction of novel prions. Proc Natl Acad Sci U S A 97:11910–11915.
Mitsui K, Nakayama H, Akagi T, Nekooki M, Ohtawa K, Takio K, Hashikawa T, Nukina N (2002) Purification of polyglutamine aggregates and identification of elongation factor-1α and heat shock protein 84 as aggregate-interacting proteins. J Neurosci 22:9267–9277.
Nagai T, Ibata K, Park ES, Kubota M, Mikoshiba K, Miyawaki A (2002) A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. Nat Biotechnol 20:87–90.
Naiki H, Higuchi K, Hosokawa M, Takeda T (1989) Fluorometric determination of amyloid fibrils in vitro using the fluorescent dye, thioflavin T1. Anal Biochem 177:244–249.
Nucifora FC Jr, Sasaki M, Peters MF, Huang H, Cooper JK, Yamada M, Takahashi H, Tsuji S, Troncoso J, Dawson VL, Dawson TM, Ross CA (2001) Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. Science 291:2423–2428.
O'Nuallain B, Williams AD, Westermark P, Wetzel R (2004) Seeding specificity in amyloid growth induced by heterologous fibrils. J Biol Chem 279:17490–17499.
Perutz MF, Johnson T, Suzuki M, Finch JT (1994) Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. Proc Natl Acad Sci U S A 91:5355–5358.
Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX, Rahmani Z, Krizus A, McKenna-Yasek D, Cayabyab A, Gaston SM, Berger R, Tanzi RE, Halperin JJ, Herzfeldt B, Van den Bergh R, et al. (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362:59–62.
Ross ED, Minton A, Wickner RB (2005) Prion domains: sequences, structures and interactions. Nat Cell Biol 7:1039–1044.
Schaffar G, Breuer P, Boteva R, Behrends C, Tzvetkov N, Strippel N, Sakahira H, Siegers K, Hayer-Hartl M, Hartl FU (2004) Cellular toxicity of polyglutamine expansion proteins: mechanism of transcription factor deactivation. Mol Cell 15:95–105.
Scherzinger E, Lurz R, Turmaine M, Mangiarini L, Hollenbach B, Hasenbank R, Bates GP, Davies SW, Lehrach H, Wanker EE (1997) Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. Cell 90:549–558.
Tian Q, Streuli M, Saito H, Schlossman SF, Anderson P (1991) A polyadenylate binding protein localized to the granules of cytolytic lymphocytes induces DNA fragmentation in target cells. Cell 67:629–639.
Vanik DL, Surewicz KA, Surewicz WK (2004) Molecular basis of barriers for interspecies transmissibility of mammalian prions. Mol Cell 14:139–145.
Waelter S, Boeddrich A, Lurz R, Scherzinger E, Lueder G, Lehrach H, Wanker EE (2001) Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. Mol Biol Cell 12:1393–1407.
Wright PE, Dyson HJ (1999) Intrinsically unstructured proteins: reassessing the protein structure-function paradigm. J Mol Biol 293:321–331.
Yamanaka T, Miyazaki H, Oyama F, Kurosawa M, Washizu C, Doi H, Nukina N (2008) Mutant huntingtin reduces HSP70 expression through the sequestration of NF-Y transcription factor. EMBO J 27:827–839.
Zoghbi HY, Orr HT (2000) Glutamine repeats and neurodegeneration. Annu Rev Neurosci 23:217–247.

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REVIEW

The pathogenic mechanisms of polyglutamine diseases and current therapeutic strategies

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Abstract

Expansion of CAG trinucleotide repeat within the coding region of several genes results in the production of proteins with expanded polyglutamine (PolyQ) stretch. The expression of these pathogenic proteins leads to PolyQ diseases, such as Huntington's disease or several types of spinocerebellar ataxias. This family of neurodegenerative disorders is characterized by constant progression of the symptoms and molecularly, by the accumulation of mutant proteins inside neurons causing their dysfunction and eventually death. So far, no effective therapy actually preventing the physical and/or mental decline has been developed. Experimental therapeutic strategies either target the levels or processing of mutant proteins in an attempt to prevent cellular deterioration, or they are aimed at the downstream pathologic effects to reverse or ameliorate the caused damages. Certain pathomechanistic aspects of PolyQ disorders are discussed here. Relevance of disease models and recent knowledge of therapeutic possibilities is reviewed and updated.

Keywords: CAG repeat, huntingtin, Huntington's disease, pathogenesis, polyglutamine, therapy.

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Polyglutamine (polyQ) diseases comprise one of the most common groups of inherited neurodegenerative conditions. This category of diseases is characterized by the pathological expansion of CAG trinucleotide repeat in the translated regions of unrelated genes. There are at least nine polyQ-related disorders known to date. The first disease connected with the expansion of CAG repeat and causing progressive motor neuron degeneration, spinal bulbar muscular atrophy (SBMA), was reported in 1991 (La Spada *et al.* 1991). Eight other disorders including Huntington's disease (HD), dentatorubropallidoluysian atrophy (DRPLA), and six types of spinocerebellar ataxia (SCA1, 2, 3, 6, 7, and 17) (Gardian *et al.* 2005) have since been identified, as associated with expanded polyQ (Table 1). The most common polyQ diseases worldwide are HD and SCA3 (Schols *et al.* 2004), but the incidence of these disorders differs between nations. For example, SCA3 accounts for vast majority of SCA in the Portuguese/Brazilian population but appears rarely in the Italian or is absent in the Czech population (Schols *et al.* 2004; Bauer *et al.* 2005b).

The amounts of mRNA and protein produced seem to be relatively unaffected in these conditions although *de novo* allele-specific DNA methylation has been proposed to affect the expression of mutant ataxin-2 in SCA2 patients (Bauer *et al.* 2004b). The disease phenotypes are usually observed

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Abbreviations used: 17-AAG, 17-(allylamino)-17-demethoxygeldanamycin; AR, androgen receptor; BAC, bacterial artificial chromosome; BDNF, brain-derived neurotrophic factor; CBP, CREB-binding protein; CHIP, C-terminal Hsp70-interacting protein; CK, casein kinase; CRE, cAMP response element; CREB, cAMP response element-binding protein; DRPLA, dentatorubropallidoluysian atrophy; ER, endoplasmic reticulum; HD, Huntington's disease; Hdj, human DNAJ; HHR23A, human homologs of yeast DNA repair protein Rad23; Hip-1, huntingtin-interacting protein 1; HSP, heat-shock protein; htt, huntingtin; IP3R1, inositol (1,4,5)-trisphosphate receptor type 1; mGluR5, metabotropic glutamate receptor 5; MPT, mitochondrial permeability transition; NF-Y, nuclear factor Y; PGC-1, PPARγ coactivator-1; polyQ, polyglutamine; ROS, reactive oxygen species; SAHA, suberoylanilide hydroxamic acid; SB, sodium butyrate; SBMA, spinal bulbar muscular atrophy; SCA, spinocerebellar ataxia; siRNA, small-interfering RNA; SP1, specificity protein 1; SUMO, small ubiquitin-like modifier; TAF, TBP-associated factor; TBP, TATA-binding protein; TFTC/STAGA, TBP-free TBP-associated factor-containing/suppressor of Ty 3 homolog (SPT3)/RNA polymerase II, TBP-associated factor (TAF9)/general control of amino-acid synthesis 5 (GCN5) acetyltransferase complex; TG, transglutaminase; TLS, translocated in liposarcoma; UPS, ubiquitin-proteasome system; YAC, yeast artificial chromosome.

Table 1 List of polyQ diseases with respective genomic loci, protein names, and the ranges of normal and pathological expansion

			PolyQ expansion	
Disease	Locus	Protein	Normal	Pathological
SBMA	Xq11-q12	Androgen receptor	6–36	38–62
DRPLA	12p13	Atrophin-1	3–38	49–88
HD	4p16.3	Huntingtin	6–35	36–121
SCA1	6p23	Ataxin-1	6–39	41–83
SCA2	12q24	Ataxin-2	14–32	34–77
SCA3	14q24-q31	Ataxin-3	12–40	62–86
SCA6	19p13	CACNA1A	4–18	21–30
SCA7	3p21–p12	Ataxin-7	7–18	38–200
SCA17	6q27	TATA-binding protein	25–43	45–63

SBMA, spinal bulbar muscular atrophy; DRPLA, dentatorubropallidoluysian atrophy; HD, Huntington's disease; spinocerebellar ataxia; polyQ, polyglutamine.

when the number of glutamines exceeds ~35–45. However, in the case of SCA6, the pathological threshold is ~20 repeats, and in SCA3 it is closer to 60 repeats (Shao and Diamond 2007). The expanded CAG repeat is unstable and tends to expand further leading to earlier age of onset and a more severe disease course in successive generations, a phenomenon called anticipation (Schols *et al.* 2004). Anticipation was also observed without an increase in CAG number indicating a complicated pathomechanism of polyQ diseases (Bauer *et al.* 2005a). In some diseases, such as SCA2 and SCA7, the CAG triplets can be extremely unstable. This instability results in either a very long repeat with juvenile age of onset or a *de novo* pathological expansion from the normal length allele in the absence of an intermediary length repeat (Mao *et al.* 2002; Bauer *et al.* 2004a).

No cases of HD, SBMA, DRPLA, or SCA1–3 and 7 with deletions or point mutations in their genes were reported, suggesting that these disorders do not result from a loss of gene function but rather from a gain of toxic function. The situation in SCA6 is less clear. Point mutations in the P/Q calcium channel gene cause the channel disorders episodic ataxia type 2, and familial hemiplegic migraine. There is significant overlap between these disorders and SCA6 in their clinical presentations (Jen *et al.* 1998; Alonso *et al.* 2003). Recently, polyQ-containing intranuclear inclusions in Purkinje cells and brainstem neurons were found in the brains of SCA8 patients and in a bacterial artificial chromosome (BAC)-transgenic mouse model of SCA8 (Moseley *et al.* 2006). SCA8 is believed to be caused by the expansion of a CTG repeat sequence, which resides in an untranslated endogenous antisense RNA that overlaps the Kelch-like 1 gene (Chen *et al.* 2008a). The newly discovered gene, ataxin-8, spans the repeat antiparallely to Kelch-like 1 and encodes a polyQ expansion in the CAG direction (Moseley *et al.* 2006).

Several shared features of polyQ diseases indicate a common toxic effect related to the polyQ expansion. They are all unremitting and progressive diseases. Both normal and abnormal proteins are usually expressed at the same level in the tissues. Also, there is no clear relationship between expression pattern and site of pathology, except for SCA6, where the gene product is expressed predominantly in Purkinje cells (Ishikawa *et al.* 1999). Although most of the proteins associated with polyQ diseases are expressed systemically, the cytotoxicity appears restricted to certain neuronal subtypes in the CNS. This suggests that probably certain specific cellular conditions exist in vulnerable neurons that may cause the selective cytotoxicity by their gene products.

In first part of this review, we discuss several points of the polyQ-related pathogenesis. The second part addresses different treatment strategies for polyQ diseases (Fig. 1) while providing an update of recent knowledge in this field.

Pathomechanism of polyglutamine diseases

Misfolding and aggregation of mutant polyglutamine proteins

It is hypothesized that polyQ diseases are the result of a toxic gain of function that occurs at the protein level. A prominent pathological feature in most of these diseases is the intranuclear and cytoplasmic accumulation of aggregated polyQ proteins inside neurons (Davies *et al.* 1997; DiFiglia *et al.* 1997). The role of the aggregation in disease pathogenesis is controversial. It is not completely clear whether the toxicity of the expanded polyQ proteins results from the presence of visible aggregates or from smaller intermediary species generated during the aggregation process. Aggregates may merely represent end products of the upstream toxic event. Some studies have suggested that the inclusions serve a protective role (Saudou *et al.* 1998). Several cellular models show the discrepancy between inclusion formation and cell death. For example, in rat primary striatal neurons, the inclusions were not a prerequisite for cell death but mutant huntingtin (htt) had to be present in the nucleus to induce apoptosis (Saudou *et al.* 1998). Apoptosis in neuroblastoma cell lines was increased in the presence of mutant htt however it did not correlate with aggregate formation (Lunkes and Mandel 1998; Lunkes *et al.* 1999). The first transgenic mouse modeling HD had a phenotype reminiscent of HD and extensive striatal intranuclear inclusions; however, there was minimal cell death (Mangiarini *et al.* 1996). Other mice had striatal loss but fewer or no inclusions (Reddy *et al.* 1998; Aronin *et al.* 1999; Hodgson *et al.* 1999). On the other hand, aggregates are usually found in affected areas of the patients' brains rather than in unaffected areas and the late onset and progressive nature of polyQ diseases can only be best explained by the slow process of protein aggregation. In SBMA, the inclusions are clearly associated with neuronal

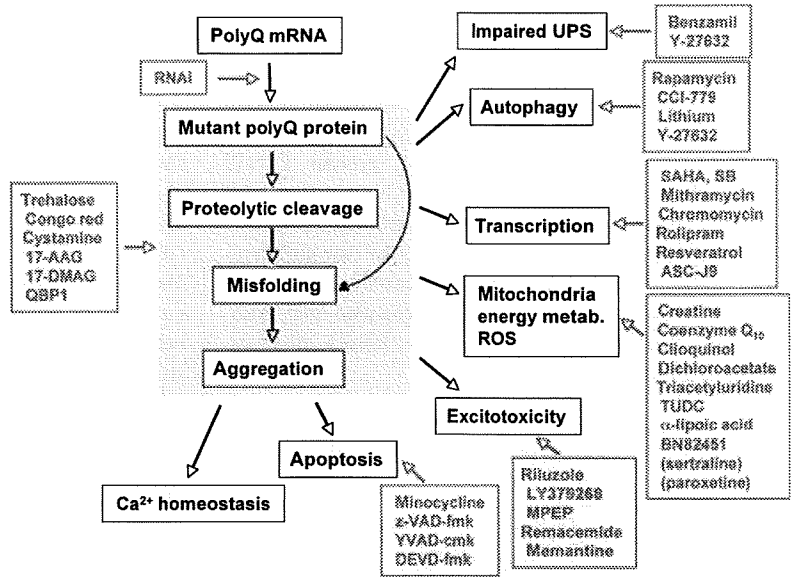

Fig. 1 Summary of therapeutic strategies in polyQ diseases. Molecules targeting different events of polyQ diseases pathogenesis (black boxes) are displayed in red boxes. Blue background highlights processing of the polyQ protein.

death. Despite the widely distributed androgen receptor (AR) throughout the CNS (Li *et al.* 1998), nuclear aggregates were observed only in affected motor neurons in the spinal cord and brainstem (Apostolinas *et al.* 1999). This may not be true in all polyQ diseases because the aggregates are also found in the dentate nucleus of HD cerebellum, a region of the brain unaffected by neurodegeneration in HD (Becher *et al.* 1998). Another exemption is, based on the observation in human necropsied cases of SCA1, SCA2, SCA3, and DRPLA, the absence of intranuclear inclusions in cerebellar Purkinje cells, which are targets of neurodegeneration in these polyQ diseases (Koyano *et al.* 2002).

In recent years, it has been suggested that oligomeric species such as protofibrils and microaggregates are the direct source of polyQ toxicity and that large aggregates are cytoprotective (Arrasate *et al.* 2004). It was proposed that above a certain length threshold, polyQ sequences form oligomers stabilized by hydrogen-bonded polar zippers and associate via formation of hydrogen bonds (Perutz *et al.* 1994). Non-covalent interactions with other proteins and effect of transglutaminase (TG) were also considered to play roles in polyQ proteins oligomerization (Kahlem *et al.* 1996; Green 1993). Expanded polyQ tracts are good substrates for TGs *in vitro* (Kahlem *et al.* 1998), and the presence of TG inhibitors prevented aggregate formation in COS-7 cells (these cells obtained by immortalizing a cell line derived from kidney cells of the African green monkey with a version of the SV40) over-expressing truncated forms of mutant atrophin-1 or htt (Saudou *et al.* 1998; Igarashi *et al.* 1998). Formation of a β-sheet structure may also cause generation of fibrillar and non-fibrillar aggregates (Tanaka *et al.* 2001; Perutz *et al.* 2002). *In vitro*, fibrillary appearances of inclusions under electron microscopy and double refraction after Congo red staining are reminiscent of amyloid and are consistent with the polar zipper hypothesis (Wanker 2000; Hollenbach *et al.* 1999). Tanaka *et al.* (2003) reported another form of aggregate named quasi-aggregate, which exposed polyQ tracts on the surface of non-fibrillar aggregates. Recently, it was reported a toxicity of even a monomeric β-sheet conformer of polyQ proteins (Nagai *et al.* 2007). Analysis of the effect of polyQ protein conformation on cytotoxicity revealed that mutant polyQ β-strand/β-turn structure results in aggregation and toxicity in neuronal cells while polyQ protein analogs unable to assume β-strand/β-turn structure do not aggregate and are not toxic (Poirier *et al.* 2005).

Modulation of polyQ aggregation by sequences flanking polyQ stretch has been reported in several studies showing this phenomenon in ataxin-1, ataxin-3, and htt (Ellisdon *et al.* 2006; Masino *et al.* 2004; Bhattacharyya *et al.* 2006; de Chiara *et al.* 2005; Thakur *et al.* 2009). In case of mutant htt, flanking sequences on both C- and N-terminal sides of the polyQ in exon 1 influence the aggregation. Presence of the C-terminal proline-rich domain reduces the htt exon 1 aggregation kinetics without changing the aggregation mechanism by favoring more aggregation-resistant conformations (Bhattacharyya *et al.* 2006). The N-terminal domain consisting of first 17 amino acids of the htt exon 1, on the other hand, determines the aggregation mechanism. This domain unfolds in a polyQ length-dependent manner and self-aggregates to form oligomers with cores composed of the N-terminal domains. PolyQ sequences stretch out during this process and during potential further nucleation. Monomers of expanded htt exon 1 can be then added to these prefibrillar aggregates forming amyloid-like structures (Thakur *et al.* 2009). The ability of the N-terminal sequence of htt

exon 1 to remodel the polyQ aggregation mechanism indicates that polyQ proteins may, depending on flanking sequences, aggregate by distinct mechanisms resulting in variable aggregate morphologies and having different cellular effects.

Recently, Furukawa *et al.* reported cross-seeding mechanism between polyQ- and RNA-binding protein T cell intracellular antigen-1 (TIA-1), in which the seed of polyQ aggregates induces T cell intracellular antigen-1 (TIA-1) aggregates. The result suggested that the different protein aggregates are induced through cross-seeding from the primary polyQ aggregates. This mechanism may also explain the diversity of pathology of polyQ diseases (Furukawa *et al.* 2009).

Post-translational modifications of mutant polyglutamine proteins

Proteolytic cleavage of polyglutamine proteins

Pathogenesis of several polyQ disorders including HD, SBMA, and SCA3, appears linked to proteolytic cleavage resulting in production of toxic polyQ-containing fragments. In other diseases, such as SCA1, the proteolysis of the mutant protein has been not confirmed. The conformational changes in the proteins with expanded polyQ lead to misfolding and proteolytic cleavage into smaller toxic fragments (Paulson 1999, 2000; Lunkes *et al.* 2002; Gardian *et al.* 2005). The use of several antibodies directed against N- and C-terminal parts of the protein showed that only a truncated version, including the polyQ expansion, aggregates in the vast majority of the polyQ diseases (Wellington and Hayden 2000). Caspase-mediated cleavage sites were identified or predicted in htt, atrophin-1, ataxin-3, and AR (Wellington *et al.* 1998). As a consequence of the cleavage, the resulting fragment could have an enhanced toxic effect (Ikeda *et al.* 1996; Ellerby *et al.* 1999) and/or could more easily enter the nucleus (Igarashi *et al.* 1998; Hackam *et al.* 1999) and/or more rapidly aggregate (Igarashi *et al.* 1998; Cooper *et al.* 1998). In several studies, truncated proteins with polyQ expansions appeared more prone than full-length proteins to form inclusions or cause cell death by apoptosis (Paulson *et al.* 1997; Ikeda *et al.* 1996; Martindale *et al.* 1998; Merry *et al.* 1998). Inclusions found in the brains of HD patients were stained by antibodies against sequences located close to polyQ stretch but not by those against more C-terminal parts of htt (Sieradzan *et al.* 1999; DiFiglia *et al.* 1997). The N-terminal fragments in the patients' brains have been identified as generated by caspases (Wellington *et al.* 2002). On the other hand, the presence of full-length mutant htt in the sodium dodecyl sulfate and urea insoluble material from HD patients' brains was reported (Dyer and McMurray 2001). A more recent study has shown that the inclusions isolated from HD brains contain broad ranges of N-terminal fragments of expanded htt with sizes of 50–150 kDa rather than intact mutant protein (Hoffner *et al.* 2005). These fragments may result from multiple proteolytic activities with little specificity. It is however possible, that the smaller products of specific cleavage seed the inclusions followed by recruitment of larger non-specific fragments. This is supported by the fact that the cleavage sites for caspases 2, 3, and 6 have been identified in human htt at amino acids 552, 513 (and 552), and 586, respectively, while the cleavage at amino acid 552 is an early event in the course of the disease (Wellington *et al.* 2002). The cleavage of mutant htt at the 586 amino acid by caspase 6 has been shown to be crucial in the HD pathogenesis as it was essential for the HD-related behavioral phenotype and selective neuropathology in yeast artificial chromosome HD mouse model expressing the whole human htt with 128Q (YAC128) (Graham *et al.* 2006). Modulating the mutant htt cleavage by caspase 3 at the amino acids 513 and 552, on the other hand, had no effect on disease progression and neurodegeneration in these mice. Caspase 6- but not caspase 3-resistant mutant htt protected neurons from excitotoxic stress suggesting that not all htt fragments contribute to excitotoxic neuronal death equally. Moreover, nuclear translocation of expanded htt was delayed in mice expressing mutant htt resistant to caspase 6 cleavage (Graham *et al.* 2006). This study strongly supports the hypothesis that generation of a specific htt fragment may represent an initial event in HD pathogenesis.

It has been reported that ataxin-3 is the target of caspase 1 at 241, 244, and 248 amino acids, and that the brains from SCA3 transgenic mice contained a C-terminal fragment (Goti *et al.* 2004; Berke *et al.* 2004; Colomer Gould *et al.* 2007). It has also been shown that normal ataxin-3 was extensively proteolysed in COS-7 cells with cleavage occurring at six other sites throughout the protein. Interestingly, mutant ataxin-3 underwent proteolysis to a much lesser extent with expanded polyQ masking the C-terminal cleavage sites (Pozzi *et al.* 2008) suggesting that SCA3 pathology can arise also through the accumulation of uncleaved protein. On the other hand, toxic fragment hypothesis has been introduced by Haacke *et al.* (2006), stating that expanded polyQ fragment must be released from the protective sequence context of full-length protein to be able to trigger the aggregation process. It is therefore possible that a toxic fragment initiates the aggregation process and later, as the intracellular levels of uncleaved expanded ataxin-3 increase, this process becomes independent of the presence of toxic fragments (Pozzi *et al.* 2008).

Mutant htt alters calcium signaling and increases Ca^{2+} levels in different cells (Bezprozvanny and Hayden 2004). Consequently, a calcium-dependent protease, calpain is activated in the brains of HD patients, where calpain co-localizes with htt aggregates and calpain cleavage products are present (Gafni and Ellerby 2002). Another cleavage of mutant htt is performed by an aspartyl protease between amino acids 104 and 114 (Lunkes *et al.* 2002). It is not known yet whether this cleavage is specific to mutant htt.

Proteolytic cleavage may be not an essential step in pathogenesis of all polyQ diseases. For polyQ protein normally localized in the cytoplasm, such as htt, ataxin-3, or atrophin-1, proteolysis appears to facilitate their translocation into nucleus enhancing their toxicities. For proteins already localized in the nucleus, for example, TATA-binding protein (TBP) or ataxin-7, proteolytic cleavage may not have so important role.

Phosphorylation of polyglutamine proteins

Phosphorylation may affect proteolytic cleavage, the initial conversion of mutant polyQ proteins to pathogenic conformations and nuclear transport (Warby *et al.* 2009). The impact of htt phosphorylation on HD pathogenesis has been demonstrated in several studies. Phosphorylation of htt at S421 by protein kinase Akt or serum and glucocorticoid-induced kinase SGK is neuroprotective against HD cellular toxicity (Humbert *et al.* 2002; Rangone *et al.* 2004). Recently, it has been reported that S421 phosphorylation of htt decreased the accumulation of both full-length htt and htt fragments (including those generated by caspase 6) in the nucleus (Warby *et al.* 2009). This observation is consistent with a previous study showing the effect phosphorylation status of S421 on vesicular transport in neurons. When phosphorylated, htt recruited kinesin-1 to the dynactin complex on vesicles and microtubules and promoted the anterograde transport of vesicles away from nucleus. Conversely, when htt was not phosphorylated, kinesin-1 was released and vesicles were more likely to undergo retrograde transport back to nucleus (Colin *et al.* 2008). Calcineurin (calcium/calmodulin-regulated serine/threonine protein phosphatase) dephosphorylates S421 *in vitro* and in cells. Using a rat model of HD with lentiviral-mediated expression of a polyQ htt fragment in the striatum, it has been shown that inhibition of calcineurin activity either by over-expression of a dominant-interfering form, by RNA interference, or by the specific inhibitor FK506, leads to an increased phosphorylation of S421 and prevents mutant htt-mediated death of striatal neurons (Pardo *et al.* 2006).

Phosphorylation of mutant htt at S434 by serine-threonine kinase Cdk5 reduced htt cleavage at 513 amino acid by caspase 3 and inhibited polyQ aggregation and cytotoxicity (Luo *et al.* 2005). The aggregation and cellular toxicity of mutant htt has also been shown to decrease upon phosphorylation at S536, which inhibits the cleavage of htt by calpain at this amino acid (Gafni *et al.* 2004; Schilling *et al.* 2006). Many phopshorylation sites were identified throughout whole htt sequence and several kinases have been proposed to be involved including MAPK/ERK kinase 1/2 or extracellular signal regulated kinase 1 (Schilling *et al.* 2006). Further studies are needed to understand how all the phosphorylation events affect the function and regulation of htt and their role in HD pathogenesis.

As mentioned in the section 'Phosphorylation of polyglutamine proteins', htt phosphorylation by Akt has been shown neuroprotective. In contrary, phosphorylation of mutant ataxin-1 by Akt at S776 promotes its binding to 14-3-3, which in turn leads to ataxin-1 accumulation and neurodegeneration (Chen *et al.* 2003). Abnormal nuclear stabilization of mutant ataxin-1 by Akt was shown to have critical role in SCA1 pathogenesis in ataxin-1[82Q]-A776 transgenic mice (Emamian *et al.* 2003). In a recent study, however, inhibition of Akt either *in vivo* or in cerebellar extract did not decrease the phosphorylation of ataxin-1 at S776 arguing against involvement of Akt as the key kinase in SCA1 pathogenesis. The same study suggested that cAMP-dependent protein kinase is responsible for the S776 phosphorylation in the cerebellum (Jorgensen *et al.* 2009). Other phosphorylation sites that may play a role in ataxin-1 localization and interactions in SCA1 are S239 and probably T236. S239 is within the consensus sequences targeted by casein kinase 1 (CK1), Cdc2/Cdk5, and extracellular signal regulated kinase (Vierra-Green *et al.* 2005).

Ataxin-3 has been reported to undergo phosphorylation at S256 by glycogen synthase kinase 3β and this phosphorylation resulted in reduced mutant ataxin-3 aggregation *in vitro* (Fei *et al.* 2007). Another kinase, CK2 has been shown to be a regulator of nuclear localization of ataxin-3. CK2-dependent phosphorylation of ataxin-3 at S236 and S340/S352 decreased the appearance of nuclear inclusions and controlled the nuclear translocation of ataxin-3 providing a reasonable therapeutic approach for SCA3 (Mueller *et al.* 2009).

The polyQ expansion in AR activates major mitogen-activated protein kinase pathways, from which the p44/42 pathway in turn phosphorylates at S514. This phosphorylation has been shown to promote the mutant AR cytotoxicity (LaFevre-Bernt and Ellerby 2003). In atrophin-1, S734 has been identified as a phosphoacceptor in cell cultures and rat brain. Both normal and mutant protein forms are phosphorylated by c-Jun N-terminal kinase (JNK), but precise analyses revealed a reduced affinity of c-Jun N-terminal kinase (JNK) to expanded AR (Okamura-Oho *et al.* 2003). Biological significance of atrophin-1 phosphorylation needs to be evaluated.

With the development of specific inhibitors of kinases and proteases, modulating phosphorylation and proteolytic processing of mutant polyQ proteins could be a promising treatment strategy.

Sumoylation of polyglutamine proteins

Small ubiquitin-like modifier (SUMO) proteins bind covalently to specific lysine residues in target proteins regulating their cellular localization, protein–protein interactions, and transcription factors transactivation (Sarge and Park-Sarge 2009). Antagonistic relationship between sumoylation and ubiquitination has been proposed as they share common

consensus sequence (Muller *et al.* 2001). Enhanced immunoreactivity of SUMO1 has been observed in affected neurons of HD, SCA1, SCA3, and DRPLA patients (Ueda *et al.* 2002).

Sumoylation of N-terminal fragment htt at K6, K9, and K15 was suggested to enhance the stability and reduce the aggregation of mutant htt, which however seemed to result in toxic intermediate polyQ oligomers accumulation and transcriptional repression (Steffan *et al.* 2004). Importantly, the same lysines are also ubiquitinated, however generally, sumoylation made pathology substantially worse while ubiquitination made it modestly better in *Drosophila* model of HD suggesting that (Steffan *et al.* 2004).

In ataxin-1, five major sumoylation sites have been identified (K16, K194, K610, K697, and K746). Sumoylation of ataxin-1 was dependent on its nuclear localization, phosphorylation at S776, self-association domain and polyQ length, all having role in the subcellular distribution of ataxin-1 in COS-1 cells. Nuclear localization, on the other hand, did not appear dependent on sumoylation, although it could have role in the efficiency of nucleocytoplasmic shuttling of ataxin-1. The lack of nucleus-to-cytoplasmic traffic of mutant ataxin-1 with disrupted interactions of ataxin-1 with other proteins leading to transcription dysregulation could be attributed to the decreased sumoylation (Riley *et al.* 2005).

Neuronal intranuclear inclusions in brains of DRPLA patients and mutant atrophin-1 aggregates in DRPLA cellular model were highly sumoylated. The results of this study showed active role of sumoylation in the pathogenesis of DRPLA accelerating aggregate formation and cell death (Terashima *et al.* 2002).

Androgen receptor is another target of sumoylation and this modification inhibits AR activity while significantly reducing mutant AR aggregation without affecting the levels of the receptor. Interestingly, SUMO inhibited AR aggregation through a unique mechanism that does not depend on critical features essential for its interaction with canonical SUMO-binding motifs (Mukherjee *et al.* 2009). Precise role of sumoylation in the polyQ-related neurodegenration is still not completely understood. Interestingly, while sumoylation appears neuroprotective in SBMA and SCA1, in case of HD and DRPLA it seems to accentuate the pathological process.

Role of nuclear localization of mutant polyglutamine proteins

Nuclear accumulation of expanded polyQ proteins has been shown to contribute to pathogenesis of several polyQ diseases (Saudou *et al.* 1998; Peters *et al.* 1999; Ross *et al.* 1999; Orr 2001; Klement *et al.* 1998) by affecting gene expression (Zoghbi and Orr 2000) or by disrupting nuclear organization and function (Sun *et al.* 2007). Yang *et al.* (2002) showed that polyQ aggregates were toxic only when localized in the nucleus but not in the cytoplasm. Proteolytic cleavage of mutant polyQ proteins with predominantly cytoplasmic localization of the normal copies may enhance the nuclear access as the upper limit for passive nuclear translocation has been proposed at about 46 kDa (Rubinsztein *et al.* 1999). Putative nuclear localization signal sequences have been found in some polyQ proteins including ataxin-1, ataxin-7, and atrophin-1 (Schilling *et al.* 1999b; Kaytor *et al.* 1999; Klement *et al.* 1998). Similarly, nuclear export signal sequences have been identified in htt, ataxin-7, and atrophin-1 with expanded polyQ tracts reducing nuclear export of these proteins (Cornett *et al.* 2005; Nucifora *et al.* 2003; Xia *et al.* 2003; Taylor *et al.* 2006). Nuclear accumulation of mutant proteins and inclusions have been identified as predominant in HD, SCA1, SCA3, SCA7, SCA17, DRPLA, and SBMA patients (Schols *et al.* 2004); however, cytoplasmic inclusions have also been found in affected brain regions of HD and SCA2 patients (Huynh *et al.* 2000; DiFiglia 2002).

Vast majority of htt is normally localized to cytoplasm (Gafni *et al.* 2004) but in HD, htt migrates to nucleus (DiFiglia *et al.* 1997) where it disrupts the activities of transcription factors and alters the normal transcriptional profile of neurons (Chan *et al.* 2002; Panov *et al.* 2002). Nuclear translocation of mutant htt is required to induce neurodegeneration. Inhibiting this translocation by blocking htt cleavage reduces the toxicity and retards the disease progression (Saudou *et al.* 1998; Wellington *et al.* 2000; Gafni *et al.* 2004). On the other hand, cytoplasmic mutant htt can inhibit the axonal transport and disrupt synaptic function and glutamate release (Gunawardena *et al.* 2003; Li *et al.* 2000a, 2001, 2003; Szebenyi *et al.* 2003) suggesting that both nuclear and cytoplasmic expanded htt polyQ is able to trigger certain pathological events. Htt contains two nuclear export signal sequences, one near the C-terminal and the other one being the N-terminal 17 amino acids. PolyQ expansion together with htt cleavage (e.g. by caspase 6) impedes their function resulting in accumulation of mutant htt in the nucleus (Cornett *et al.* 2005; Xia *et al.* 2003). The first 17 amino acids have also been identified as directing htt to various subcellular compartments including plasma membrane, autophagic vesicles, endoplasmic reticulum (ER) and Golgi apparatus (Kegel *et al.* 2005; Atwal *et al.* 2007; Rockabrand *et al.* 2007).

Ataxin-1 contains a C-terminal nuclear localization signal and is therefore localized to the nucleus (Skinner *et al.* 1997). The nuclear localization of ataxin-1 is essential for neurotoxicity and SCA1 disease as the disruption of nuclear localization signal inhibited the formation of intranuclear inclusions and reduced the Purkinje cells pathology and SCA1 phenotype in a transgenic mouse model (Klement *et al.* 1998).

Ataxin-2 is a cytoplasmic protein with highest expression in Purkinje cells, the most affected neurons in SCA2 (Huynh *et al.* 1999). Ubiquitinated intranuclear inclusions have been

found only in few pontine neurons of SCA2 patients, but not in Purkinje cells (Koyano *et al.* 1999). Cytoplasmic localization with microaggregation or accumulation of ataxin-2 has been shown to be sufficient to cause SCA2 pathology in humans and mice. The 42 kDa, N-terminal fragments produced by proteolytic cleavage of ataxin-2 are believed to act as seeds of cytoplasmic aggregation in the Purkinje cells of SCA2 patients (Huynh *et al.* 1999, 2000).

Ataxin-3 is mostly cytoplasmic protein, but, depending on cell type, it displays subcellular distribution involving both the cytoplasm and the nucleus (Wang *et al.* 1997; Paulson *et al.* 1997; Tait *et al.* 1998; Trottier *et al.* 1998). Intranuclear inclusions have been observed in human SCA3 brains (Paulson *et al.* 1997; Schmidt *et al.* 1998). Recently, it has been found that full-length ataxin-3, regardless of polyQ tract length, is also localized to mitochondrial matrix and membrane and that the polyQ expansion promotes binding to the mitochondrial membrane. Although lacking predicted nuclear or mitochondrial localization signal, first 27 amino acids of ataxin-3 might play role in cell sorting as cleavage at this site caused absence of ataxin-3 in the nucleus or mitochondria (Pozzi *et al.* 2008). It is apparent that the disease results from the presence of mutant ataxin-3 in several cellular compartments such as nucleus, cytosol and mitochondria although it remains to clarify which localization causes primary abnormalities in SCA3 pathogenesis.

Ataxin-7 contains a functional nuclear localization signal and is primarily localized in the nucleus (Kaytor *et al.* 1999) where it is a subunit TFTC/STAGA transcriptional complex (Helmlinger *et al.* 2004, 2006). Nuclear localization of mutant ataxin-7 appears to be essential for toxicity with profound effect on transcriptional dysregulation (Helmlinger *et al.* 2004; La Spada *et al.* 2001). Similarly in SCA17, the localization of TBP as a transcription factor is nuclear, where the polyQ expansion results in transcriptional dysregulation (Friedman *et al.* 2007).

Atrophin-1 contains both nuclear localization (at N-terminal) and export (at C-terminal) signal sequences resulting in ubiquitous cellular localization (Schilling *et al.* 1999b; Nucifora *et al.* 2003). Neuronal inclusions in DRPLA are predominantly nuclear, but they do not seem to correlate with the sites of neurodegeneration (Sato *et al.* 1999; Koyano *et al.* 2002). On the other hand, an N-terminal fragment of mutant atrophin-1 was found to accumulate in the neuronal nuclei of DRPLA patients and model mice while lacking its C-terminal with nuclear export signal. This accumulation occurred before the first symptoms of the disease (Schilling *et al.* 1999b; Nucifora *et al.* 2003). In addition, mutation within the nuclear localization signal resulted in retention of the protein in the cytoplasm and reduced the toxicity of atrophin-1 (Nucifora *et al.* 2003).

From molecular point of view, SBMA is a unique type of polyQ disease, because a specific ligand can alter the subcellular localization of AR. The unliganded AR is localized to the cytoplasm while binding of an androgen ligand, such as testosterone, translocates AR to the nucleus and triggers the expression of androgen-responsive genes (Stenoien *et al.* 1999; Katsuno *et al.* 2002). Intranuclear inclusions have been observed in SBMA patients in motor neurons in brainstem and spinal cord (Li *et al.* 1998). SBMA affects only males, with female carriers remaining largely asymptomatic as binding of testosterone accelerates AR transportation to the nucleus (Brooks and Fischbeck 1995). Nuclear localization of AR seems to be the main site of SBMA-related pathogenesis (Brooks and Fischbeck 1995; Katsuno *et al.* 2002) further supporting the concept that toxicity of polyQ proteins depends on their localization.

Role of heat-shock proteins in preventing aggregation

The presence of expanded polyQ proteins causes a cellular stress response, which may include the up-regulation of heat-shock proteins (HSPs). HSPs levels have been shown to have a biphasic response to expanded polyQ expression where the initial phase of increased expression of HSPs is induced by cellular stress response, followed by progressive reduction of HSPs levels during later stages of neurodegeneration (Huen and Chan 2005; Hay *et al.* 2004). HSPs recognize misfolded proteins, stabilize them in monomeric conformation and suppress their aggregation (Muchowski *et al.* 2000). By interacting with the mutant proteins, chaperones may prevent abnormal interactions with other cellular proteins. The critical role of chaperones in polyQ diseases has been evidenced in many studies (Muchowski 2002). The most investigated chaperones have been Hsp70 and heat-shock cognate 70. A co-chaperone class known as Hsp40, which includes human DNAJ1 (Hdj1) and Hdj2, facilitates the action of Hsp70. Both Hsp40 and Hsp70 are involved in the clearance of misfolded proteins via the ubiquitin-proteasome system (UPS) pathway (Bercovich *et al.* 1997).

The members of Hsp40 and/or Hsp70 families co-localize with nuclear aggregates in human brain tissue of SCA1 (Cummings *et al.* 1998), SCA3 (Schmidt *et al.* 2002), and SCA7 (Zander *et al.* 2001) patients along with SCA1 (Cummings *et al.* 1998) and SCA7 (Yvert *et al.* 2000) transgenic mouse brains. Hdj2 and heat-shock cognate 70 were associated with nuclear aggregates in HD cell culture as well as in transgenic mice models and their over-expression reduced the aggregate formation (Jana *et al.* 2000). An Hsp90 family member, Hsp84, was found to co-localize with nuclear aggregates in HD transgenic mouse brains, and its over-expression reduced both polyQ aggregation and toxicity (Mitsui *et al.* 2002). Hsp105α in SBMA patients' and transgenic mouse brains suppresses polyQ toxicity (Ishihara *et al.* 2003).

The over-expression of Hsp40 and/or Hsp70 chaperones in cell culture, or in *in vivo* polyQ diseases models reduced the inclusion formation of mutant htt, AR, ataxin-3, or polyQ-green fluorescent fusion proteins and/or suppressed cell death

(Kobayashi *et al.* 2000; Chai *et al.* 1999a; Kazemi-Esfarjani and Benzer 2000; Stenoien *et al.* 1999; Warrick *et al.* 1999; Jana *et al.* 2000; Muchowski *et al.* 2000; Cummings *et al.* 1998). Over-expression of Hsp70 in SCA1 transgenic mice also suppressed neurodegeneration and improved motor function (Cummings *et al.* 2001). The aggregation reduction by HSPs probably results from increased solubility of polyQ proteins and improved access to the degradation machineries. On the other hand, it has been shown that the suppression of the polyQ cytotoxicity did not depend on the aggregation reduction by Hsp40 and Hsp70 (Zhou *et al.* 2001). Moreover, a small chaperone Hsp27 could reduce the mutant htt toxicity by inhibition of the accumulation of the reactive oxygen species (ROS) without affecting the aggregation (Wyttenbach *et al.* 2002). Thus, whether chaperones are directly involved in aggregate formation itself remains to be determined.

The C-terminal Hsp70-interacting protein (CHIP) links the molecular chaperones with UPS. CHIP interacts with Hsp70 and Hsp90 through its tetratricopeptide repeat domains and with proteasome through an E4/U-box domain (Jiang *et al.* 2001; Ballinger *et al.* 1999). CHIP has been demonstrated to reduce the expanded polyQ aggregation, to enhance the clearance of mutant polyQ proteins and to reduce their cytotoxicity in different polyQ diseases *in vitro* and *in vivo* models (Jana *et al.* 2005; Al-Ramahi *et al.* 2006; Miller *et al.* 2005; Williams *et al.* 2009). These studies provided further evidence for the protein misfolding and aggregation model for polyQ toxicity.

Apoptosis and polyglutamine diseases

The mechanism of cell death in polyQ diseases is very complex as many processes triggered by the presence of expanded polyQ proteins can lead to the cell death, such as direct activation of cell death pathways, mitochondrial abnormalities, transcriptional dysregulation, proteasome impairment, defects in axonal transport, or unfolded protein response. Other pathophysiological events such as excitotoxicity, metabolic stress, or accumulation of free radicals may promote the cell fate toward death by further enhancing the mitochondrial dysfunction. Many proteins usually involved in apoptosis are sequestered, redistributed or activated by expanded polyQ proteins. Caspases are activated (Wang *et al.* 1999; Li *et al.* 2000b), and can be recruited in inclusions (Sanchez *et al.* 1999). Cytochrome *c* release was observed in cells transfected with mutant htt (Li *et al.* 2000b). Cytochrome *c* released from mitochondria interacts with apoptotic peptidase activating factor 1 (Apaf-1) and procaspase 9, which in turn activate caspase 3 and the caspase cascade (Li *et al.* 1997). On the other hand, certain apoptotic events may be inhibited by the presence of mutant htt. For example, htt aggregates were shown to sequester the proapoptotic protein kinase Cδ, and therefore block the protein kinase Cδ-dependent DNA fragmentation (Zemskov *et al.* 2003).

The expression of expanded polyQ protein in rat neurons resulted in the activation and recruitment of caspase 8 to the aggregates (Sanchez *et al.* 1999). In this apoptotic pathway, the caspase cascade is initiated and caspase 8 deficiency or expression of a dominant-negative mutant suppressed neurodegeneration. The sequestration of caspase 8 to polyQ aggregates required Fas-associated protein with death domain, because both caspase 8 binding and neuronal death were blocked by dominant-negative Fas-associated protein with death domain (Sanchez *et al.* 1999).

Other caspases may also have a role in polyQ toxicity. Activation of caspases 1 and 3 in a HD transgenic mouse model has been demonstrated. Inhibition of these two caspases by minocycline, had neuroprotective effect, delayed the symptoms and extended the lifespan of these mice (Chen *et al.* 2000).

Calcium increases outer mitochondrial membrane permeability resulting in release of proapoptotic proteins into the cytosol including cytochrome *c* and apoptosis inducing factors. Caspases 1 and 9 were also activated through disruption of mitochondrial membrane potential and released cytochrome *c* into the cytosol in mouse neuroblastoma cells expressing N-terminal htt with expanded polyQ (Jana *et al.* 2001). This process was associated with impaired proteasomal function. It has been also shown that the altered Ca^{2+} signaling is strongly involved in the mitochondria-mediated activation of the apoptotic caspases and calpain in HD and SCA3 models (Tang *et al.* 2003; Chen *et al.* 2008b). Expanded polyQ protein causes increased mitochondrial apoptosis activation by at least three synergistic mechanisms. Mutant htt elevated cytosolic Ca^{2+} levels by enhancement of NMDA receptor function and by strong binding to inositol (1,4,5)-trisphosphate receptor type 1 (IP3R1). IP3R1 is then sensitized to activation by IP3 in medium spiny neurons resulting in abnormal Ca^{2+} influx via NMDA receptor and Ca^{2+} release from ER through IP3R1. The presence of expanded htt may also directly affect the permeability of the mitochondrial membrane (Bezprozvanny and Hayden 2004).

An effect of expanded ataxin-3 on the expression of Bcl-2 family proteins has been reported (Chou *et al.* 2006; Tsai *et al.* 2004). The expression of mutant ataxin-3 in cerebellar, striatal, or substantia nigra neurons specifically up-regulated proapoptotic Bax and down-regulated Bcl-xL proteins which may lead to mitochondrial release of apoptogenic proteins and apoptotic cell death (Chou *et al.* 2006). Similar effect on Bax and Bcl-xL had the expression of expanded ataxin-7 in primary neuronal culture from cerebellum or neocortex (Wang *et al.* 2006). These studies further underlined the complex effect of expanded polyQ on apoptotic cell death.

A unique aspect of the expression of mutant ataxin-2 was observed in PC12 (cells derived from pheochromocytoma of rat adrenal medulla) and COS-7 cells, where the apoptotic cell death through caspase 3 activation was correlated with

disruption of the Golgi complex. This is probably because of the primary localization of normal but not expanded ataxin-2 in Golgi apparatus (Huynh *et al.* 2003). These data supported previous observations that ataxin-2 co-localized with its binder, the ataxin-2-binding protein 1, which was also co-localized with Golgi proteins (Shibata *et al.* 2000). When compared with most of the other polyQ proteins, the ataxin-2-induced cell death does not require nuclear localization (Huynh *et al.* 2000).

Polyglutamine aggregation can also contribute to apoptosis activation by reduced binding to proapoptotic factors, which are normally sequestered by wild type polyQ proteins. Huntingtin-interacting protein 1 (Hip-1) is a protein with a pseudo death effector domain (Hackam *et al.* 2000) and was originally identified as an Hip. The affinity of Hip-1 to htt is reduced by the presence of expanded polyQ, resulting in elevated free Hip-1, which forms heterodimers with a Hip-1 interactor, Hippi, and they are able to activate apoptosis through binding and activating caspase 8 (Gervais *et al.* 2002).

Cell death in SCA6 has been attributed to $Ca_V2.1$ Ca^{2+} channel dysfunction, which normally prevents cell death by regulating Ca^{2+} influx, suggesting that the polyQ expansion in α1A subunit of voltage-dependent P/Q type calcium channel (CACNA1A) results in a channelopathy (Matsuyama *et al.* 2004).

In SCA17, the polyQ expansion in the TBP affects the interaction of TBP with its binding partners impairing the transcription of the target genes (Friedman *et al.* 2007). It was demonstrated, that the mutant TBP displays reduced binding to TATA box DNA *in vitro*. Interestingly, the same study also showed the inhibition of the TATA-dependent transcriptional activity by a mutant TBP fragment lacking the C-terminal DNA-binding domain. This fragment formed intranuclear aggregates and caused severe neurological phenotype in the SCA17 TBP-105Q-T transgenic mice indicating that the mutant TBP can induce neurotoxicity independent of its association with DNA (Friedman *et al.* 2008).

A recent study defined the pathway by which the AR with expanded polyQ activates neuronal apoptosis in SBMA. The expression of mutant N-terminal AR induced intrinsic pathway-mediated apoptosis. It is initiated by activation of c-JUN by Jun N-terminal kinase resulting in death protein 5/hara-kiri [member of the proapoptotic Bcl-2 homology domain 3 (BH3)-only family] up-regulation leading to Bax activation (Young *et al.* 2009).

Another polyQ-related insult leading to cell death is ER stress with consequent activation of apoptosis signal-regulating kinase 1 (Nishitoh *et al.* 2002). This hypothesis was supported by a study using a peptide inhibitor of apoptosis signal-regulating kinase 1, as it was able to reduce the apoptosis in a cellular model of DRPLA (Kariya *et al.* 2005).

Mitochondrial involvement in polyglutamine diseases

Impairment of mitochondrial functions is one of the key events in polyQ diseases leading to cell death via activation of apoptotic cascades. The process of mitochondrial dysfunction is accompanied by impaired respiration, stress-induced mitochondrial depolarization, increased free radical production with oxidative damage, and abnormal energy metabolism in polyQ diseases (Grunewald and Beal 1999; Panov *et al.* 2002; Browne and Beal 2006).

The importance of mitochondria in polyQ diseases pathogenesis and related cell death had been suggested by mimicking the HD phenotype using the complex II respiratory chain inhibitors 3-nitropropionic acid or malonate (Brouillet *et al.* 1995; Beal *et al.* 1993). In toxin models of HD, the activities of complex II and III were reduced and energy metabolism was impaired as observed in human HD brains and in cells derived from HD model mice, respectively (Gu *et al.* 1996; Milakovic and Johnson 2005). Another study supporting the direct effect of expanded polyQ proteins on mitochondria showed that polyQ proteins increased ROS in isolated mitochondria (Puranam *et al.* 2006). Mutant htt was also observed on neuronal mitochondrial membranes by electron microscopy and the incubation of normal mitochondria with fusion protein with expanded polyQ reduced their Ca^{2+} retention capacity (Panov *et al.* 2002). It has been demonstrated that recombinant mutant htt directly induced mitochondrial permeability transition (MPT) pore opening in isolated mouse liver mitochondria. Mutant htt decreased the Ca^{2+} threshold necessary to trigger MPT pore opening accompanied by a significant release of cytochrome *c* (Choo *et al.* 2004).

One study showed that the Ca^{2+} loading capacity of isolated mitochondria from YAC128 and R6/2 HD mouse models was increased compared with control animals, however this was not true in the mitochondria from an *Hdh*150 knock-in mouse model (Oliveira *et al.* 2007). In the knock-in mouse models, mitochondria were likely not to function at maximal capacity under resting conditions suggesting that only under conditions of neuronal stress does the mitochondrial impairment contribute to HD pathogenesis (Milakovic and Johnson 2005; Oliveira *et al.* 2007). No significant defects in basal respiratory capacity, basal ATP synthesis, or uncoupling were observed in *Hdh*150 striatal neurons, suggesting that there was no major bioenergetic defect in these neurons. Under neuronal stress though, some neurons may be unable to meet the increased ATP demand because of the defects in ATP synthesis and/or export and they become more vulnerable to Ca^{2+} deregulation (Oliveira *et al.* 2007). Altered Ca^{2+} buffering capacity of mitochondria is therefore probably a cause of enhanced NMDA-induced apoptosis occurring in HD brains (Fei *et al.* 2007). In a cellular and a *Caenorhabditis elegans* HD model, it has been shown that the over-expression of mutant htt diminished the normal dynamics of mitochondria fusion and fission inter-

fering with mitochondrial ATP generation providing another potential therapeutic target (Wang *et al.* 2009).

The expression of mutant AR caused mitochondrial abnormalities leading to caspase activation. Surprisingly, a polyQ-dependent decrease in mitochondrial number and impaired mitochondrial membrane potential was observed with increased ROS. Mutant AR also altered the expression of genes important for mitochondrial function including one of the key regulators of mitochondrial biogenesis and function, peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1β), and its target genes peroxisome proliferator-activated receptor γ, and mitochondrial transcription factor A. The mitochondrial genes regulated by mitochondrial transcription factor A and several cellular antioxidant genes were also down-regulated suggesting a profound role of mitochondrial dysfunction in SBMA pathogenesis (Ranganathan *et al.* 2009).

Similarly to previously cited studies, PGC-1α, another member of PGC-1 complex, is a mitochondrial biogenesis and function co-activator which regulates mitochondria response to oxidative stress (St-Pierre *et al.* 2006). Mutant htt was reported to directly bind to PGC-1α and interfere with its function (Weydt *et al.* 2006), supporting the connection between oxidative stress and mitochondrial dysfunction in HD. Moreover, PGC-1α is regulated by cAMP response element (CRE)-binding protein (CREB), a transcription factor down-regulated by expanded polyQ htt (Steffan *et al.* 2000) and mutant htt was shown to interfere with transcription of PGC-1α (Cui *et al.* 2006).

Expanded polyQ proteins were reported to impair axonal trafficking, which may lead to defective mitochondrial distribution and function providing another causal link between mitochondria and polyQ diseases (Szebenyi *et al.* 2003; Chang *et al.* 2006). This interaction correlates with decreased distribution and transport rate of mitochondria in the processes of cultured neuronal cells and reduced level of ATP in synaptosomal fractions (Orr *et al.* 2008). Mutant ataxin-3 has been recently reported to decrease the activities of antioxidant enzymes levels causing increased damage of mitochondrial DNA (Yu *et al.* 2009). Based on number of studies, it can be stated for almost a certainty that mitochondrial impairment is a common feature in the pathogenesis of polyQ diseases.

Involvement of proteasome in polyglutamine diseases

The UPS is a major cellular protein degradation pathway clearing short-lived and damaged proteins (Goldberg 2003; Chou *et al.* 2006). UPS degrades many regulatory proteins having roles in distinct cell signaling pathways and synaptic function and plasticity (Bingol and Schuman 2006). Impairment of the UPS, as a major regulator of normal cellular functioning and as a detoxification machinery targeting damaged proteins for degradation, may have therefore lethal consequences on the affected cells.

The impairment of UPS by mutant htt and other expanded polyQ proteins has been proposed and shown in several studies (Bence *et al.* 2001; Jana *et al.* 2001; Zemskov and Nukina 2003; Khan *et al.* 2006; Bennett *et al.* 2007) although it is still considered controversial. The relationship between UPS- and polyQ-related diseases is evidenced by the ubiquitination of several aggregated polyQ proteins (Ross and Poirier 2004). Indeed, expanded ataxin-1, -3, and -7, as well as atrophin-1, AR, and htt colocalize with the proteasome and several studies have shown a redistribution of the proteasome complex to the inclusions (Paulson *et al.* 1997; Chai *et al.* 1999b; Stenoien *et al.* 1999; Yvert *et al.* 2000; Cummings *et al.* 1998). Ubiquitinated ataxin-3 inclusions were observed in brains of SCA3 patients (Paulson *et al.* 1997). Ubiquitinated inclusions have been also reported in SCA2 and SCA7 brains (Holmberg *et al.* 1998; Koyano *et al.* 1999), although the exact distribution and relationship to disease remains unclear (Huynh *et al.* 2000). SCA7 intranuclear inclusions are ubiquitinated in severely degenerated areas such as the inferior olive, yet also in the cerebral cortex, which is unaffected (Holmberg *et al.* 1998; Yvert *et al.* 2001). In SCA17, ubiquitin- and TBP-positive inclusions were found in the putamen, which shows pattern of neurodegeneration in affected patients (Nakamura *et al.* 2001). The ubiquitination of the polyQ inclusions suggests that the UPS is attempting to clear the mutant proteins. Consistent with this, the 20S proteasome was shown to colocalize with ataxin-1 (Cummings *et al.* 1998) and ataxin-3 aggregates (Chai *et al.* 1999b).

Preventing the ubiquitination of mutant ataxin-1 resulted in increased cellular toxicity indicating that the ubiquitination of expanded polyQ may play an important detoxifying role (Cummings *et al.* 1999). Ataxin-3 was shown to interact with HHR23A and HHR23B, the human homologs of yeast DNA repair protein Rad23 (Wang *et al.* 2000). The recruitment of HHR23A to the intranuclear inclusions by mutant ataxin-3 probably impairs its normal interaction with UPS as Rad23 was shown to recruit polyubiquitinated proteins to the proteasome and facilitate their degradation in yeast (Verma *et al.* 2004; Elsasser *et al.* 2004). Proteasome subunits were also found colocalized with mutant ataxin-7 intranuclear inclusions in SCA7 transgenic mice (Yvert *et al.* 2001), transfected cells, and human brain (Zander *et al.* 2001). Moreover, S4 subunit of the 19S proteasome is specifically depleted in the regions of the brain affected in SCA7. Furthermore, a yeast two-hybrid assay has demonstrated interaction between ataxin-7 and this subunit (Matilla *et al.* 2001).

Two basic mechanisms are likely to account for the UPS impairment by expanded polyQ proteins. First, sequestration into polyQ aggregates and/or altered subcellular localization of the proteasome components may be responsible for the UPS dysfunction. This hypothesis is supported by seques-

tration of ubiquilin 1 and 2 as well as Tollip, which are thought to modulate UPS, in polyQ aggregates (Doi *et al.* 2004). Second, proteins with expanded polyQ may block the proteasome preventing other substrates to enter. Number of studies have been published supporting or disputing these theories and are discussed elsewhere (Davies *et al.* 2007). It has been also proposed that the reduction of UPS activity may result from caspase-dependent cleavage of proteasome components while the aggregated proteins induce apoptosis (Sun *et al.* 2004).

Although the precise role of UPS in polyQ diseases pathogenesis remains to be clarified, it is evident that the alteration of its activity is one of the common features in this group of disorders.

Transcriptional dysregulation in polyglutamine diseases

The mutant polyQ proteins interact with many other cellular proteins and may sequester them into cytoplasmic or nuclear aggregates (Mitsui *et al.* 2002; Doi *et al.* 2008). Htt, ataxins, atrophin-1, and AR have several interactions in common supporting the recent knowledge of pathogenic effect related to expanded polyQ stretch. As many of the aberrantly bound proteins are transcription-related factors, their dysbalance may have profound effects on gene expression with potentially toxic effects. Analyses of postmortem tissues of HD patients and HD or DRPLA models have revealed altered expression of many genes (Kotliarova *et al.* 2005; Chan *et al.* 2002; Kuhn *et al.* 2007; Luthi-Carter *et al.* 2002). Oyama *et al.* found that expression of sodium channel subunit β4 is severely reduced in the brains at an early stage in HD model mice, which might be involved in dendritic abnormalities of neurons observed in HD (Oyama *et al.* 2006; Miyazaki *et al.* 2007).

A variety of nuclear proteins relevant to transcription have been suggested to interact with different mutant polyQ proteins (Okazawa 2003). Some of them bind to more polyQ disease proteins while some are associating specifically, what could be one of the underlying mechanisms for the selective neurodegeneration in polyQ diseases. For example, the interaction of expanded ataxin-1 with leucine-rich repeat acidic nuclear protein 32 (Matilla *et al.* 1997), PolyQ-binding peptide-1 (Okazawa *et al.* 2002), Gfi-1/Senseless (Tsuda *et al.* 2005), and Boat (Mizutani *et al.* 2005) might lead to selective degeneration in cerebellum. Protein phosphatase 2 activity is regulated by leucine-rich repeat acidic nuclear protein 32, the factor predominantly expressed in Purkinje cells and therefore the dysregulation of protein phosphatase 2 is a possible mechanism of neurodegeneration in SCA1 (Matilla and Radrizzani 2005). Ataxin-7 is a subunit of TFTC/STAGA transcriptional complex, which interacts with the photoreceptor-specific transcriptional activator cone–rod homeobox protein (Helmlinger *et al.* 2004; Palhan *et al.* 2005; La Spada *et al.* 2001). Mutant ataxin-7 sequesters cone–rod homeobox protein and suppresses the acetyltransferase activity of the TFTC/STAGA complex, which results in down-regulation of genes vital for retinal function and accounts for the retinal degeneration seen in SCA7 (Palhan *et al.* 2005; La Spada *et al.* 2001).

A common transcriptional activator, CREB, along with its co-activator CREB-binding protein (CBP), has been strongly implicated in expanded polyQ-induced gene repression (Steffan *et al.* 2000). CBP is an important mediator of survival signals in neurons. It has histone acetyltransferase activity, which is important for allowing transcription factors access to DNA. In the presence of mutant htt or atrophin-1, CBP is sequestered into aggregates (Nucifora *et al.* 2001). CBP recruitment to intranuclear inclusions or the interaction with mutant polyQ proteins also occurs in SBMA, SCA3, and SCA7 (McCampbell *et al.* 2000; Li *et al.* 2002a; Strom *et al.* 2005). In SCA1, CBP was not irreversibly trapped into nuclear aggregates but it was rapidly exchanged. Even short residence time of CBP within inclusions, however, may be sufficient to disrupt its normal function in maintaining cellular homeostasis (Stenoien *et al.* 2002). It appears that the disruption of CREB/CBP-mediated gene expression may be a common mechanism of neurodegeneration in polyQ repeat diseases. On the contrary, it was reported that in R6/2 HD mouse model, the CREB-mediated transcription was increased (Obrietan and Hoyt 2004) and CBP was not depleted in HD model mice brains (Yu *et al.* 2002).

TATA-binding protein was found to colocalize with htt, ataxin-2, ataxin-3, and atrophin-1 inclusions in human brains (Perez *et al.* 1998; Uchihara *et al.* 2001; van Roon-Mom *et al.* 2002), but similarly to CBP, the levels of TBP were not reduced in HD model mice (Yu *et al.* 2002). Specificity protein 1 (SP1), contains a glutamine-rich activation domain, which is responsible for regulation of the transcriptional machinery of transcription factor II D (TFIID), a protein complex composed of TBP and multiple TBP-associated factors (TAF_{II}s), including $TAF_{II}130$ (Tanese and Tjian 1993). The interaction of SP1 with mutant htt leads to suppression of SP1 transcriptional activity resulting in down-regulation of dopamine D_2 or nerve growth factor receptors (Li *et al.* 2002b; Dunah *et al.* 2002). The over-expression of SP1 and $TAF_{II}130$ was able to ameliorate the mutant htt toxicity and recover the dopamine D_2 receptor activity (Dunah *et al.* 2002) but it was also demonstrated in HD models that SP1 suppression can be neuroprotective (Qiu *et al.* 2006). The role of SP1 in polyQ toxicity is thus not clear. $TAF_{II}130$ itself also interacts with mutant htt and it was shown to colocalize with atrophin-1 and ataxin-3 (Shimohata *et al.* 2000; Dunah *et al.* 2002). Another TAF, $TAF_{II}30$, was reported to be sequestered in nuclear inclusions formed by mutant ataxin-7 (Yvert *et al.* 2001).

Expanded polyQ reduces the cytoplasmic interaction of htt with the repressor element-1 transcription factor/neuron restrictive silencer factor leading to nuclear enrichment of this factor and resulting in transcriptional repression of the

gene encoding brain-derived neurotrophic factor (BDNF) (Zuccato *et al.* 2003).

Sequestration of p53 into mutant htt aggregates was originally reported to repress the p53-mediated transcription (Steffan *et al.* 2000). It was later shown that the binding of mutant htt to p53 increased intranuclear levels of p53 and enhanced its transcriptional activity in HD cellular and in *in vivo* models as well as brain autopsies of HD patients. Genetic deletion of p53 then suppressed mutant htt-induced neurodegeneration in *Drosophila* (Bae *et al.* 2005).

Recently, Yamanaka *et al.* identified nuclear factor Y (NF-Y) to be associated with htt aggregates in cellular and mouse models of HD. This interaction led to sequestration of NF-Y components resulting in its decreased activity (Yamanaka *et al.* 2008). NF-Y has been reported to regulate Hsp70 gene transcription (Imbriano *et al.* 2001). Presence of mutant htt reduced the NF-Y-mediated expression of Hsp70 revealing the mechanism of progressive decrease of Hsp70 in model mice reported previously (Hay *et al.* 2004; Merienne *et al.* 2003).

Furthermore, Doi *et al.* (2008) found that TLS (translocated in liposarcoma) is a major polyQ aggregate-interacting protein. Mutations in fused in sarcoma/TLS were recently found to cause a familial form of amyotrophic lateral sclerosis (Kwiatkowski *et al.* 2009), thus TLS-related neuronal degeneration might play a significant role in polyQ diseases.

In addition to modulating the activities of transcription factors, a direct interaction of mutant htt with DNA was recently shown to perturb gene expression *in vitro* and *in vivo*. The enhanced binding of mutant htt to genomic DNA alters the DNA conformation and together with consequent disrupted binding of transcription factors alters the normal mRNA expression (Benn *et al.* 2008). The relationship between polyQ pathogenesis and transcription factors is however not clear yet and needs further investigation to resolve the above-mentioned conflicting observations.

In vivo models of polyglutamine diseases

Appropriate disease model system is an essential tool in understanding the disease pathomechanism and in the development of therapeutic strategies. For example, the formation of neuronal intranuclear aggregates was first observed in a HD transgenic mouse model (Davies *et al.* 1997) and this discovery led to identification of similar inclusions in brains of patients with polyQ diseases. Gain of function of the polyQ disease proteins enables relatively simple establishments of cellular model systems for these diseases requiring simply (over)expression of the mutant protein. Cellular models are especially useful in high throughput screenings. These include either random chemical libraries or different gene-silencing libraries with small-interfering RNAs (siRNA) or short-hairpin RNAs. Modern imaging equipments, together with different biochemical methods, enable to analyze many altering effects of chemical compounds or gene modifications, on polyQ proteins accumulation and aggregation, cytotoxicity, etc. Also, it is very important to determine the mechanism of action of the potential drugs and the cellular models offer an ideal starting point in this effort.

After the identification of the molecular genetic background, the modeling of neurodegenerative diseases in transgenic or mutant animal models has provided a great instrument for investigating the disease pathogenesis and testing treatment strategies. The well-characterized genetics, development and anatomy together with short lifespans of invertebrate organisms make them excellent model systems of polyQ diseases. The development and advantages of nematode *C. elegans* and the fruitfly *Drosophila melanogaster* models have been reviewed previously (Parker *et al.* 2004; Cauchi and van den Heuvel 2006; Driscoll and Gerstbrein 2003). The invertebrate models are also suitable for a large-scale screening studies because of relatively low cost, modest time requirements or absence of the complications of the blood–brain barrier (Bates and Hockly 2003; Hughes and Olson 2001). Invertebrate models are especially useful in modifying genetic screens and have revealed several candidate genes having role in protein folding and clearance and affecting the course of SCA1 and HD (Kazemi-Esfarjani and Benzer 2000; Fernandez-Funez *et al.* 2000).

Mouse models

The generation of mammalian models of polyQ diseases is crucial for providing researchers with an alternative approach to study molecular mechanism of respective disorders although some of these models do not completely simulate the neuropathological changes in humans. Generally, the CAG repeat expansions responsible for the diseases in humans are not sufficient to trigger disease in mice. Therefore, very large expansion or multiple gene copies have been used in mice to mimic the humans' conditions but there is still no model available which would fully reproduce the symptoms seen in patients. Nevertheless, some of the mouse models have been very useful in therapeutic testing. Number of various mouse models for SCA1, 2, 3, 7, DRPLA, SBMA, and HD have been generated and were, together with their potential utilization in therapeutic trials, reviewed recently (Ferrante 2009; Bates and Gonitel 2006; Heng *et al.* 2008; Yamada *et al.* 2008; Marsh *et al.* 2009).

The transgenic mouse models can be generally divided to fragment and full-length genetic models. Knock-in mice represent the most precise genetic models for polyQ diseases so far. An expanded CAG triplet repeat is inserted into the endogenous mouse gene and the expression is driven by the respective endogenous promotor. Alternatively, a whole mouse exon can be replaced by a human exon carrying expanded polyQ.

