

## Review

# Mutant huntingtin can paradoxically protect neurons from death

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Huntington's disease (HD) is a progressive neurodegenerative disorder caused by a mutation in the gene *huntingtin* and characterized by motor, cognitive and psychiatric symptoms. Huntingtin contains a CAG repeat in exon 1. An expansion of this CAG repeat above 35 results in misfolding of Huntingtin, giving rise to protein aggregates and neuronal cell death. There are several transgenic HD mouse models that reproduce most of the features of the human disorder, for example protein inclusions, some neurodegeneration as well as motor and cognitive symptoms. At the same time, a subgroup of the HD transgenic mouse models exhibit dramatically reduced susceptibility to excitotoxicity. The mechanism behind this is unknown. Here, we review the literature regarding this phenomenon, attempt to explain what protein domains are crucial for this phenomenon and point toward a putative mechanism. We suggest, that the C-terminal domain of *exon 1* Huntingtin, namely the proline rich domain, is responsible for mediating a neuroprotective effect against excitotoxicity. Furthermore, we point out the possible importance of this mechanism for future therapies in neurological disorders that have been suggested to be associated with excitotoxicity, for example Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.

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Huntington's disease (HD) is a neurodegenerative disorder that affects approximately 1 in 10 000 individuals. Symptoms and signs include depression, personality changes, weight loss, dementia and motor disturbances. The latter are initially characterized by chorea and in the terminal stage they transform into akinesia.

The disease is inherited in an autosomal dominant fashion. The underlying gene mutation was identified in 1993 as a CAG-triplet repeat expansion in the gene *huntingtin*, which is located on chromosome 4.<sup>1</sup> The protein Huntingtin is 3144-amino-acid long and is encoded over 67 exons with the CAG-triplet repeat located within the first exon. The CAG sequence codes for glutamine and in HD, an expansion of the poly-glutamine (poly-Q) stretch, above 35 glutamines results in pathogenicity. The length of the poly-Q stretch inversely correlates with the age of onset of symptoms.<sup>2</sup> A repeat length of more than 70 glutamines leads to a juvenile form of the disease and the more commonly occurring poly-Q length of 40–55 typically leads to onset of symptoms at an age of 35–50 years.<sup>2</sup> Most commonly patients die 15–20 years after the onset of the first symptoms, due to complications of immobilization.

In the end stage of the disease, the total brain weight of HD patients is reduced by 10–20%. Mainly the caudate nucleus, putamen and neocortex exhibit cell loss and undergo atrophy. Due to the loss of neurons in these brain regions, the lateral ventricles enlarge. The medium-sized spiny neurons are the first to degenerate in the striatum, whereas large- and medium-sized aspiny striatal interneurons are less affected.<sup>3,4</sup>

Although several binding domains (see below for further details) have been identified in Huntingtin, the normal function of the protein is poorly understood. The protein has been suggested to play a role in microtubule-mediated transport and vesicle function.<sup>5</sup> The pathobiology of mutant Huntingtin is also not well understood. Huntingtin is cleaved and then, in its mutated variant, forms intracellular aggregates, particularly in cell nuclei, and, to a lesser extent, in the cytoplasm, neurites and terminals.<sup>6</sup> The aggregates are also composed of other proteins, such as transcription factors, chaperones, synaptic proteins and components of the ubiquitin–proteasome pathway.<sup>7,8</sup> The Huntingtin aggregates have both been suggested to be toxic to the cell as well as neuroprotective. In the latter case, it is argued that the neuroprotective effect of the

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**Abbreviations:** CBS, cystathionine beta-synthase; FIP2, Rab11 family-interacting protein 2; GRB2, growth factor receptor-bound protein 2; HD, Huntington's disease; HIP1, huntingtin interacting protein 1; HIP14, huntingtin interacting protein 14; N-CoR, nuclear receptor corepressor 1; NMDA, *N*-methyl-D-aspartic acid; PACSIN1, protein kinase C and casein kinase substrate in neurons protein 1; poly-Q, polyglutamine; PSD95, postsynaptic density protein 95; QA, quinolinic acid; RasGAP, RAS-GTPase-activating protein; SH3, Src-homology 3

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aggregates is due to conversion of a soluble toxic species into an insoluble form.<sup>9</sup>

### Huntingtin Mediates Reduced Susceptibility Against Excitotoxicity

A large variety of mouse models of HD exist with each expressing different variations of the Huntingtin protein. Several of these have been demonstrated to result in reduced susceptibility against excitotoxicity, and other forms of neural insults, in the mouse brain. Excitotoxicity entails overactivation of glutamate receptors, which leads to neuronal death.<sup>10</sup> Receptors that can mediate such effects include the *N*-methyl-D-aspartic acid (NMDA) and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors. Quinolinic acid (QA) is an agonist of NMDA receptors and is widely used in experiments to induce excitotoxicity.<sup>11</sup>

In the literature, we have found reports of reduced susceptibility to neural damage in six mouse HD models. We have also identified studies on an additional six models where susceptibility to excitotoxicity was not different from that seen in wild-type control mice. In this review, we describe the models that exhibit neuroprotection, briefly review the changes in the physiology of their brains and, finally, try to explain why some HD mouse models exhibit reduced susceptibility to excitotoxicity while others do not.

In 1999, Hansson *et al.*<sup>12</sup> described reduced susceptibility against NMDA receptor-mediated excitotoxicity in the R6/1 mouse model of HD for the first time. The R6/1 mouse model expresses human *exon 1* mutant Huntingtin with a poly-Q stretch of around 115 glutamines. Hansson *et al.* injected 30 nmol QA into the striatum of 18-week-old presymptomatic R6/1 mice and into age-matched controls. The results revealed that cell death in the striatum of R6/1 animals was dramatically reduced compared to that seen in wild-type controls (Figure 1). Interestingly, the brains of R6/1 HD mice are not resistant to excitotoxic damage from birth, but the phenomenon develops with age. The temporal development of this resistance was evaluated in another study that examined both R6/1 and R6/2 mice.<sup>13</sup> R6/2 mice also express human mutant *exon 1* Huntingtin, but with an even longer poly-Q stretch of around 150 glutamines. In agreement with findings on earlier symptomatic onset in HD patients with longer poly-Q repeats,<sup>14</sup> the symptoms appear at a younger age in the R6/2 mouse model, with its even longer poly-Q repeat stretch, compared to R6/1 mice.<sup>13</sup>

Interestingly, reduced susceptibility to excitotoxicity develops gradually with age in both R6/1 and R6/2 mice. The time

courses resemble those for the development of motor symptoms in the two models.<sup>13</sup> For example, in the R6/1 mouse, there is no difference in susceptibility against toxin at 3 weeks of age, a partial reduction in susceptibility at 8–13 weeks and complete protection against QA-induced NMDA receptor-mediated excitotoxicity at 22 weeks of age. In R6/2 animals, the protection is complete already at 6 weeks of age.<sup>13</sup>

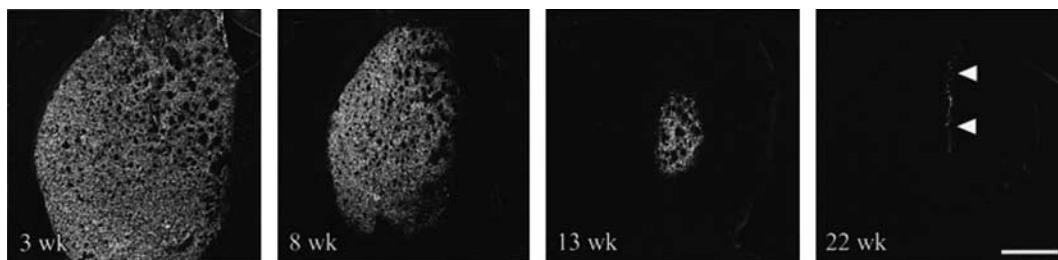
Eventually it became clear that the R6 mice are not the only transgenic HD mouse models that display reduced susceptibility to excitotoxicity. In 2004, Jarabek *et al.*<sup>15</sup> reported resistance against QA-induced damage in the N171-82Q mouse model of HD. This model overexpresses a construct consisting of human *exon 1* and parts of *exon 2* with an 82-glutamine-long stretch.

In 2005, Slow *et al.*<sup>16</sup> examined the 'shortstop' mouse model of HD. This mouse expresses human *exons 1* and *2* with a poly-Q stretch of approximately 120 glutamines. At 6 months of age, the striatum was resistant to damage following injections of QA. It is particularly interesting that this mouse model does not exhibit any evidence of neurological dysfunction or neuronal degeneration, although intraneuronal Huntingtin aggregates can be found throughout the brain.

The fourth mouse model found to display reduced susceptibility to excitotoxicity differs from the others in that it overexpresses full-length human Huntingtin with a short non-pathogenic poly-Q stretch (YAC 18).<sup>17</sup> Thus, this is not really a model of HD, in that the poly-Q stretch is of a normal length, but the cells overexpress Huntingtin. Furthermore, the level of protection in the YAC18 model is rather low compared to all other models that show reduced susceptibility: a much lower QA concentration than that used in several other models led only to approximately one-third of the protection observed in all other models. Whatever the mechanism behind the reduced susceptibility against excitotoxicity might be, it is clear that only the mutated form of Huntingtin has the full potential to mediate it.

The same group of investigators then generated additional HD models in which they expressed full-length mutant Huntingtin with 133 glutamine residues and inserted a mutation in either a caspase 3 or a caspase 6 cleavage site. Both the models that lacked a mutation and those with a mutation in the caspase 3 cleavage site were normosensitive to QA, whereas a mutation in the caspase-6 cleavage site (the model named C6R) led to protection against QA-induced excitotoxicity.<sup>18</sup>

Six other transgenic mouse models of HD have been injected with QA into the striatum and exhibited either



**Figure 1** The vulnerability of R6/1 striatal neurons to QA decreases with age. Number of dying neurons was detected with fluorescent death marker Fluoro-Jade. Arrowheads indicate needle track, scale bar 700  $\mu\text{m}$  (from Hansson *et al.*<sup>13</sup>)

increased or normal sensitivity to the neurotoxin. Two models expressing the full-length huntingtin protein with either 72 or 128 glutamines (YAC72 and YAC128) showed slightly increased sensitivity to QA compared to matched wild-type control mice.<sup>17,19,20</sup> In another study, three models using a construct encoding for the first 22 exons of human Huntingtin (equivalent to around 30% of the protein) with 18, 46 or 100 glutamines (tgHD18, tgHD46 and tgHD100) were given intrastriatal injections of QA.<sup>21</sup> Mice aged up to about 8 months were studied and they all exhibited the same sensitivity to QA as wild-type mice. Finally, injections of malonate into the striatum of a mouse model overexpressing human exons 1–6 Huntingtin with 18 glutamines led to an extent of neuronal death similar to that seen in control mice.<sup>22</sup>

### Reduced Susceptibility to Excitotoxins is not Limited to the Striatum

Several studies have demonstrated that reduced susceptibility to excitotoxins is not limited to the striatum. Moreover, some of the mouse models have been shown to be insensitive to additional toxins. Both R6/1 and R6/2 mice are protected also against damage induced by intrastriatal injections of malonate,<sup>22</sup> dopamine and 6-hydroxydopamine.<sup>23</sup> Furthermore, R6/2 mice are protected against 3-nitropropionic acid,<sup>24</sup> kainic acid<sup>25</sup> and methamphetamine-induced reactive gliosis.<sup>26</sup> Interestingly, R6/1 mice also exhibit reduced brain damage compared to controls following global cerebral ischemia.<sup>27</sup> This wide range of reduced susceptibility to neuronal damage makes this phenomenon highly interesting for future research and applications. Moreover, the striatum is not the only brain region that has been shown to be less sensitive to damage in transgenic mouse models of HD. Hansson *et al.*<sup>13</sup> showed that the hippocampus of 16-week-old R6/1 mice is also protected against QA insults. Furthermore, a protection of the hippocampus in R6/1 animals toward global ischemia has been demonstrated.<sup>27</sup>

### What are Common Features between Transgenic Mouse HD Models that Display Reduced Sensitivity to Excitotoxins?

What conclusions can we draw from the studies described above, in an attempt to explain the underlying mechanism responsible for reduced susceptibility to neuronal insults?

One possible explanation for reduced sensitivity to excitotoxins in several HD mouse models could be that the expressed huntingtin fragments, which are expressed in those models throughout development, are highly toxic and may lead to cellular stress. The cellular adaptive response may also provide protection against excitotoxins. However, some observations clearly speak against this hypothesis: first of all, there are also six other transgenic HD models, which are not resistant but express toxic huntingtin constructs. If resistance would occur due to a general stress response, then one would expect also those HD models to display resistance. Furthermore, the shortstop model does not show any neurodegeneration but still shows a high level of resistance. This shows that resistance can also develop without toxic fragments. This argues for a more specific and less general mechanism

**Table 1** Transgenic models expressing Huntingtin

Mouse model	Exon	Poly-Q length	[QA] Used	% Protection
R6/1	1	115	30 nmol	~99
R6/2	1	150	30 nmol	~99
Shortstop	1+2	~120	6 nmol	~90
N171-82Q	1+part of 2	82	30 nmol	78
C6R	1–67	133	4 nmol	81–91*
YAC72	1–67	72	8 nmol	Not protected
YAC128	1–67	128	6 nmol	Not protected
tgHD18	1–22	18	30 nmol	Not protected
tgHD46	1–22	46	30 nmol	Not protected
tgHD100	1–22	100	30 nmol	Not protected
HDex6	1–6	18	1000 nmol (Malonate)	Not protected

Mouse models resistant to excitotoxicity are shown without background, non-resistant with gray background. Column 'Exon' indicates total length of Huntingtin construct expressed with 67 exons being full-length Huntingtin. \*: % protection depending on comparison to wild type (81%) or to HD55 (91%).

behind the observed reduced susceptibility to neuronal insults. In Table 1 and in the following section we summarize factors that may provide insights into underlying mechanisms. When examining the length of the protein that is necessary to induce resistance, it appears that the crucial part of the gene for mediating resistance must be *exon 1*. Every mouse model which is resistant expresses at least *exon 1*, some of them only *exon 1*. This suggests that only the protein fragment encoded by *exon 1* is necessary to mediate resistance. In the C6R model, which also exhibits resistance, a Caspase-6 cleavage site located in exon 13 is mutated.<sup>18</sup> This fact is, at first sight, difficult to reconcile with the concept that *exon 1* is crucial and will be discussed later on in this review.

We conclude that one commonality of all resistant mouse models is the expression of *exon 1* human huntingtin. At the same time, it is evident that expression of *exon 1* huntingtin, even with an expanded poly-Q stretch, does not always lead to resistance. This leads to the conclusion that the precise protein context in which the *exon 1* is expressed is vital in deciding over its neuroprotective properties. What other commonalities do the mouse models exhibiting reduced susceptibility have? Looking at the length of the poly-Q stretch, one common property can be found: the length of the poly-Q stretch of strongly protected mouse models is in the pathogenic range. Notably, the length of the poly-Q stretch does influence the development of the reduced susceptibility, as R6/2 mice develop the protective response at much lower ages than R6/1 mice.<sup>13</sup> The fact that R6 mice are not resistant to toxin-induced damage from birth also provides a clue to underlying mechanisms. From the R6 models it is clear that the process takes time and that it is not an all-or-nothing phenomenon, but develops gradually. Thus, it appears that either the transgene protein product has to accumulate to a minimal crucial level for it to be effective in this respect or that the protein undergoes conformational changes (e.g. formation of aggregates) once a crucial level is exceeded in the cells.

Possibly the altered conformation is associated with changes in intracellular location of the huntingtin fragment, another process that can require time to complete. There is a strong correlation between the appearance of intranuclear aggregates of huntingtin at the light microscopical level in the striatum and hippocampus of R6/1 mice and the onset of resistance in different neurons in these regions.<sup>13</sup> This suggests that this event is either important or that it correlates to another crucial change in the neurons that mediates the reduced susceptibility. In summary, we can conclude that resistance against NMDA receptor-mediated excitotoxicity seems to be related to the part of human Huntingtin encoded by *exon 1*.

What is the reason that wild-type Huntingtin normally does not mediate resistance? In an attempt to shed more light on this issue we will first shortly review what is already known about the structure of Huntingtin. As we hypothesize *exon 1* of the Huntingtin gene is crucial for the neuroprotection phenomenon, we will focus on this structure and its known properties and binding partners.

### Structural Aspects and Protein Interactors of Human Exon 1 Huntingtin

Huntingtin is 3144-amino-acid long and is a multi-domain protein that does not exhibit any significant sequence homology with other known proteins. The first 17 amino acids at the N terminus have been identified as a nuclear localization signal (NLS).<sup>28</sup> The poly-Q stretch, which in its mutated form causes HD, is located directly C terminus of the NLS and is followed by a proline-rich domain. *Exon 1* of huntingtin consists of the N-terminal NLS, the poly-Q stretch and an additional 50 amino acids of the protein.

Known interaction partners of *exon 1* Huntingtin are GRB2, p53, protein kinase C and casein kinase substrate in neurons protein 1 (PACSIN1), postsynaptic density protein 95 (PSD95), RAS-GTPase-activating protein (RasGAP) and SH3GL3.<sup>29–33</sup> Other candidates that possibly could interact with the domain of the protein encoded by *exon 1*, but have not been shown to interact only with this short sequence, include huntingtin interacting protein 1 (HIP1), Rab11 family-interacting protein 2 (FIP2), huntingtin interacting protein 14 (HIP14), nuclear receptor corepressor 1 (N-CoR) and cystathionine beta-synthase (CBS)<sup>34–38</sup> (for overview see Figure 2).

What is known about the six proteins that have been found to interact with *exon 1* of Huntingtin? First of all, all these proteins interact with the proline-rich domain of Huntingtin, which is localized directly at the C terminus of the poly-Q stretch. GRB2 interacts through its Src-homology 3 (SH3) domain with Huntingtin. It activates Ras by forming a complex with EGFR and SOS1.<sup>39</sup> GRB2 also is involved in the regulation of the ERK pathway. It is not known if the interaction between GRB2 and Huntingtin is dependent on the poly-Q length of Huntingtin.<sup>30</sup>

A GRB2-like protein, SH3GL3 (endophilin 3), also binds to *exon 1* Huntingtin. This interaction is discussed as a regulating factor for dynamin and synaptojanin function.<sup>29</sup> SH3GL3 only interacts with *exon 1* Huntingtin if it contains a pathogenic poly-Q stretch. As discussed above, resistance not only occurs in pathogenic Huntingtin, for which reason

SH3GL3 is unlikely to be critically involved in mediating this mechanism.

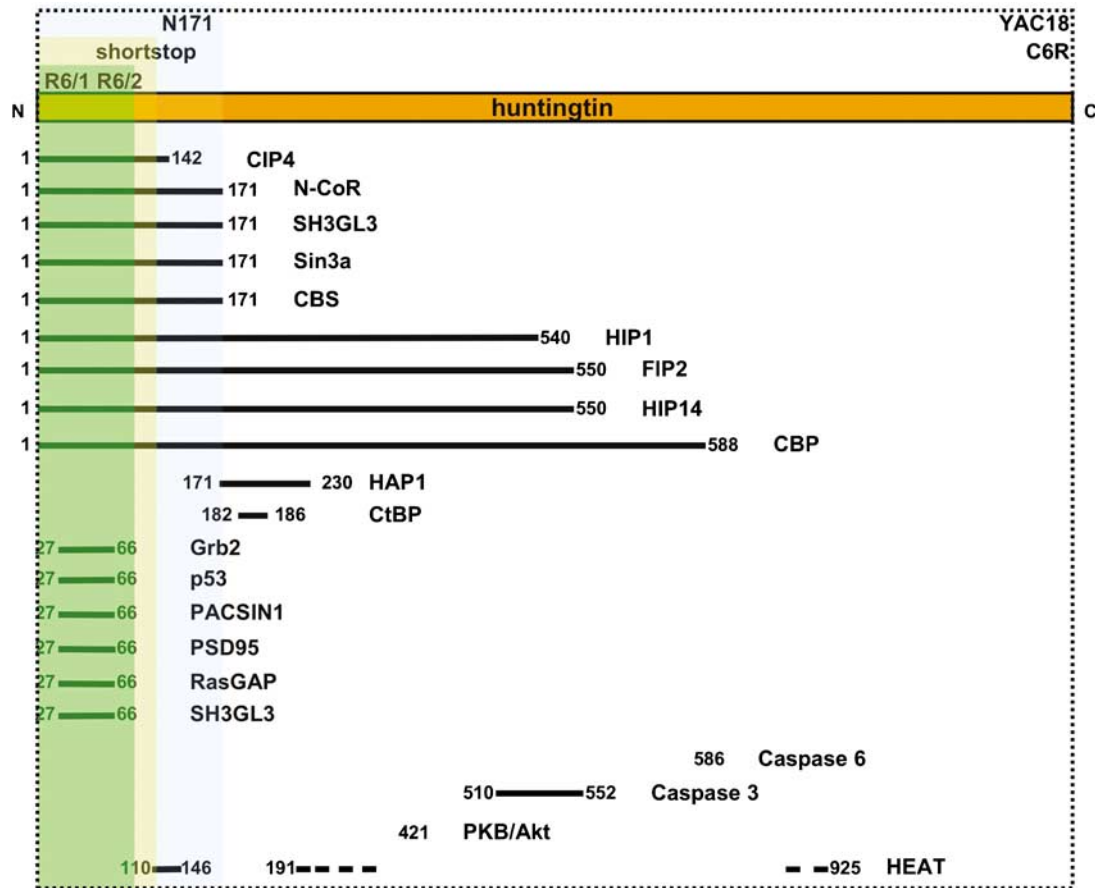
Another protein that interacts with Huntingtin by a SH3 domain is PACSIN1. This interaction is poly-Q repeat length dependent and is enhanced in the mutated form with 44 glutamines.<sup>32</sup> PACSIN1 is believed to play a role in synaptic vesicle recycling.

The tumor suppressor protein p53 has also been found to be one of the interaction partners of *exon 1* Huntingtin.<sup>31</sup> As p53 is critically involved in regulating cell death, it may be involved in mediating reduced susceptibility to excitotoxicity. However, it was also shown<sup>31</sup> that the length of the poly-Q stretch does not directly influence p53 binding. Indeed, p53 binds to both *exon 1* Huntingtin protein constructs either with 20 or 51 glutamines. Interestingly, *exon 1* Huntingtin with a 103-long glutamine stretch lacking the proline-rich domain displayed dramatically reduced affinity to p53.<sup>31</sup>

RasGAP downregulates the level of Ras-GTP and opposes the effect of GRB2, which was discussed above.<sup>40</sup> The interaction between RasGAP and Huntingtin might therefore play a role in regulating Ras-GTP levels. Furthermore, it was shown that mice with a null mutation of either the RasGAP or huntingtin gene exhibit increased neuronal apoptosis.<sup>41</sup>

Finally, it is well established that PSD95 (postsynaptic density protein 95) interacts with Huntingtin.<sup>33</sup> PSD95 binds to certain glutamate receptors, that is NMDA- or kainate receptors, and to cytoplasmic signaling proteins such as one member of the above-mentioned RasGAP, SynGAP. As mentioned earlier, the NMDA receptor is a primary mediator of excitotoxicity, especially in response to the NMDA-receptor ligand QA. Huntingtin, through its interaction partners PSD95, RasGAP and GRB2, might be involved in organizing the postsynaptic density and thereby could affect signaling downstream from the synaptic receptors. The N171-82Q transgenic HD mouse, which exhibits reduced sensitivity to excitotoxin, has reduced levels of PSD95.<sup>15</sup> Sun *et al.*<sup>33</sup> reported that a pathogenic poly-Q stretch (48 Q) impairs PSD95 binding to Huntingtin. In the same study, a Huntingtin construct with a non-pathogenic, 16 glutamine long poly-Q stretch protected cells *in vitro* from glutamate-mediated neuronal death by 82%. In contrast, the same concentration of glutamate led to cell death when cells were transfected with a Huntingtin construct with an expanded (48 Q) poly-Q stretch. Thus, in this case, the mutant huntingtin construct did not mediate neuroprotection, but, on the contrary, it exacerbated excitotoxic death. The Huntingtin constructs used in this particular study spanned the first three exons of the huntingtin gene. This supports the idea that the length of the fragment of huntingtin protein is crucial regarding whether it will be protective or promote cell damage, regardless of the length of the poly-Q stretch. Recall that transgenic mice expressing *exon 1* or *exons 1* and *2* exhibit reduced susceptibility to excitotoxic damage. In contrast, transgenic mice expressing 22 exons of the huntingtin gene or the full-length protein exhibit normal or increased damage, respectively, following intra-striatal injection of QA. Taken together, PSD95 remains an interesting candidate that could be directly involved in the resistance phenomenon.

Interestingly, all six interacting proteins bind via a SH3 domain to the proline-rich domain of Huntingtin. For some of



**Figure 2** Comparison of Htt domain structure of six HD mouse models that show resistance against excitotoxicity. Different models cover different parts of Htt (full-length Htt shown in orange, covered parts shown in green, yellow, blue and white) (not in scale). Numbering is based on poly-Q length of 9. CIP4, cdc42-interacting protein 4; N-CoR, nuclear receptor corepressor 1; CBS, cystathionine beta-synthase; HIP1, huntingtin interacting protein 1; FIP2, Rab11 family-interacting protein 2; HIP14, huntingtin interacting protein 14; CBP, CREB binding protein; HAP1, huntingtin-associated protein 1; CtBP, C-terminal-binding protein 1; GRB2, growth factor receptor-bound protein 2; PACSIN1, protein kinase C and casein kinase substrate in neurons protein 1; PSD95, postsynaptic density protein 95; RasGAP, RAS-GTPase-activating protein; PKB/Akt, protein kinase B; HEAT, for Huntingtin, Elongation factor 3, Protein phosphatase 2A, TOR1

them the binding is affected by the length of the poly-Q stretch, whereas for others it is not. PSD95 together with GRB2 and RasGAP are an interesting group of huntingtin interactors, because their functions are related to NMDA receptors. It should be remembered, however, that mouse models expressing Huntingtin fragments do not only display reduced susceptibility to NMDA receptor-mediated excitotoxicity, but are also partially resistant to other insults such as those induced by malonate,<sup>22</sup> dopamine and 6-hydroxydopamine.<sup>23</sup> This could imply that the effects of toxic levels of malonate, dopamine and 6-hydroxydopamine are partially mediated via the NMDA receptor, which has been suggested to be the case to varying degrees.<sup>22,23</sup> Alternatively, the neuroprotective effects elicited by truncated Huntingtin target a cellular pathway that is not specific for NMDA receptor-mediated death.

#### Possible Mechanisms Involved in Huntingtin-Mediated Resistance

The reduced neuronal damage seen following injection of toxins into the brains of transgenic HD mice could be classified

into a number of fundamentally different mechanisms. First, the neurons might express reduced numbers or impaired variants of glutamate receptors, leading to less calcium entry even when extracellular glutamate levels are high. Second, it is possible that the downstream events that cause cell death following increased levels of cytosolic calcium are defective. Third, the cells may have developed improved defenses against toxin-induced damage, for example higher levels of calcium buffering protein, antioxidant or anti-apoptotic molecules. Fourth, it is even conceivable that the reduced neuronal damage following injections of toxins is related to changes in surrounding glia that more effectively reduce the concentrations of the toxins or produce neuroprotective growth factors. Indeed, glial cells also express the huntingtin transgene in the mouse models that exhibit reduced sensitivity to toxins, so their functions may well be altered. It has been demonstrated in a *Drosophila* model of HD that selective overexpression of Huntingtin in glia can have a remarkable influence on the whole brain and on disease progression.<sup>42</sup>

Concerning the possible direct involvement of glutamate receptors in the phenomenon, it has been demonstrated that this neuroprotection is not due to a reduced number of

functional NMDA receptors in R6/1 or R6/2 animals.<sup>12</sup> In another study, no change in NMDA receptor subunit composition was shown (NR1, NR2A, NR2B).<sup>15</sup> Nevertheless, the authors were able to observe a decrease in phosphorylation of NR1 at Ser897, previously reported to decrease NMDA receptor current. However, this cannot sufficiently explain the phenomenon observed.

Some earlier studies have examined possible changes in candidate molecules that could explain the neuroprotective effect seen in transgenic mouse models of HD. None of these molecules that have been examined so far are altered in a way that would explain the effect. Thus, there are no major changes in the density and composition of NMDA receptors (as mentioned above) or the levels of brain-derived neurotrophic factor, ciliary neurotrophic factor,<sup>43,44</sup> Calbindin,<sup>12</sup> superoxide dismutase, Bcl-XL, X-linked inhibitor of apoptosis, ascorbate and glutathione<sup>23</sup> in the striatum of R6 mice at ages when they exhibit reduced susceptibility to excitotoxin. Interestingly, calcium levels are reported to recover more rapidly in striatal neurons from 8-week-old R6/2 mice than in wild-type control cells when exposed to QA in an acute *in vitro* model system.<sup>13</sup> Also, basal calcium levels were increased 5- to 6-fold in striatal neurons from R6/2 mice compared to wild-type striatal neurons. The cellular mechanism underlying this difference in calcium handling and its potential importance for the resistance phenomenon remains unclear.

Jarabek *et al.*<sup>15</sup> have shown that in N171-82Q HD mice, which display reduced sensitivity to excitotoxin, the levels of PSD95, SAP-102, nNOS, citron and PI-3 kinase are decreased at an age when resistance is developed. These proteins are involved in, for example, synaptic transmission and energy metabolism. The investigators speculated that those decreases could mitigate the detrimental effects of increases in cytosolic calcium. Furthermore, they suggested that observed decreases of dopamine receptor 1 and NR1 phosphorylation could be part of an adaptive response to handle chronically increased calcium levels. They, like others, conclude their findings with the comment that the resistance phenomenon is likely due to a similar mechanism found in ischemic preconditioning. The concept of ischemic preconditioning shows indeed many parallels to the reduced susceptibility in many HD models: after a relatively mild brain ischemia, neurons are less susceptible to severe brain ischemia.<sup>45</sup> It has been suggested that mild activation of NMDA receptors is a central mechanism of developing ischemic tolerance.<sup>46-48</sup> Although several other interesting studies have demonstrated potential neuroprotective properties of different factors investigated in the context of HD, none of these factors have afforded the same dramatic protection against excitotoxicity as is in the transgenic HD mouse models we have described in this review article (see e.g. Saydoff *et al.*,<sup>49</sup> Dedeoglu *et al.*,<sup>50</sup> McBride *et al.*,<sup>51</sup> Jin *et al.*<sup>52</sup> and Ferrante *et al.*<sup>53</sup>).

### Does the Proline-Rich Domain of Huntingtin Cause Resistance?

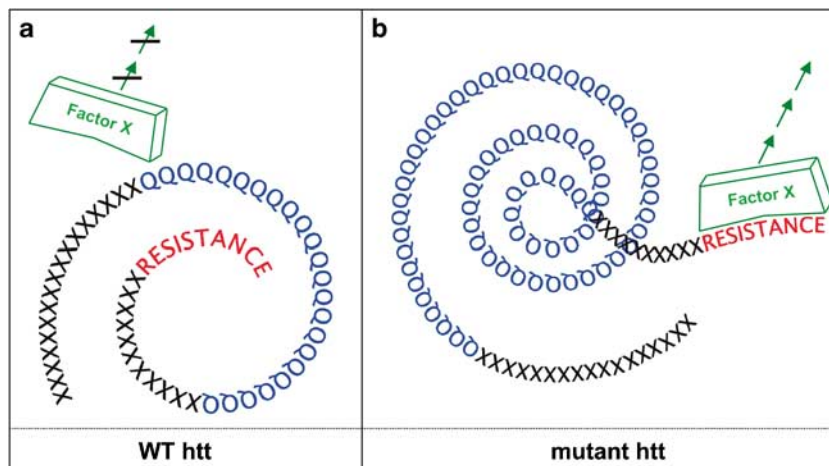
As mentioned earlier, there are several interactors for *exon 1* huntingtin. It is possible that one or more of them are involved in mediating the resistance of the cells to toxin-induced

damage. All of them interact via their SH3 domain with the proline-rich domain of Huntingtin. According to the database available at <http://smart.embl-heidelberg.de>, there are currently 413 identified proteins with at least one SH3 domain in *Homo sapiens* (336 for *Mus musculus*), most of them are possible interactors with the proline-rich domain of Huntingtin.

One possible explanation why so many different Huntingtin constructs with different properties lead to reduced susceptibility to excitotoxicity is that the poly-Q region does not directly mediate resistance. Instead a domain in the 50-amino-acid-long sequence (still encoded within *exon 1* of the gene) located after the poly-Q stretch could be crucial. The poly-Q stretch might influence the secondary and/or tertiary structure of these 50 amino acids so that an amino-acid sequence responsible for mediating resistance is exposed on the surface of the protein. It is also possible that the folding of, for example, full-length huntingtin exposes parts of *exon 1* in a different manner than it is in a shorter Huntingtin construct (Figure 3). This could explain why the length of the huntingtin fragment that is expressed can affect whether the resistance phenomenon develops or not. The same concept could explain why the C6R model is resistant whereas the C3R model is not, considering the possibility that a protein that is 512-amino-acid long will fold differently than one that is 585-amino-acid long. As a result, different parts of *exon 1* could be exposed in the two proteins and thereby lead to an altered interaction with proteins involved in mediating resistance.

There are several experiments that could help determine the mechanism underlying the resistance phenomenon. First of all, the number of possible proline-rich domain interactors is limited, and only a fraction of those are expressed in the brain of HD models that exhibit the resistance to toxins. Moreover, proline-rich domain interactors can readily be identified using, for example, protein-protein chips or yeast two hybrid screens. Viral vectors encoding the identified factors could then be used to examine the effects of overexpressing them in response to toxic insults *in vitro*. In addition, siRNA or specific small molecule inhibitors could be used to further elucidate the signaling pathways involved in mediating resistance. Finally, it should be possible to create viral vectors that either overexpress the protective protein or otherwise modify the signaling pathway, and could be tested for their neuroprotective effects in animal models of for example Parkinson's disease *in vivo*. An alternative approach would use the same viral vectors to transduce cells in wild-type animals, examine if they develop the resistance phenomenon, and then identify gene and protein expression changes that could be involved in the mechanism.

In summary, we hypothesize that resistance to NMDA receptor-mediated excitotoxicity is due to factors that bind to the proline-rich domain of huntingtin. Further, we suggest that the accessibility of this region changes due to conformational changes in the parts of the protein encoded by *exon 1*, and that these are caused by either changes in the poly-Q stretch or in the folding of Huntingtin in general. This could explain the reduced susceptibility found in different HD models with one underlying common mechanism. Clearly, it is still possible that cellular mechanisms underlying resistance in the various HD models differ, but on the whole this seems unlikely. A deeper understanding of the mechanisms mediating reduced



**Figure 3** Possible explanation how a prolonged poly-Q stretch could lead to resistance to excitotoxicity. (a) A non-pathogenic poly-Q stretch does not expose a resistance responsible sequence (shown in red) to the protein surface. A factor X, responsible for mediating resistance, cannot bind to Huntingtin and cannot signal. (b) The prolonged poly-Q stretch leads to a conformational change of Huntingtin, which exposes the resistance responsible sequence on the protein surface. Factor X, responsible for mediating resistance can bind and mediate its effect

susceptibility may mean that we can utilize it in attempts to devise novel neuroprotective strategies against neurodegenerative diseases such as Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease and other poly-Q disorders like SCA1 and SCA3. Of course, it will be essential to separate the beneficial effects of the mutant huntingtin fragment from its possible inherent capacity to cause neurodegeneration. The fact that the Shortstop transgenic mouse model of HD exhibits reduced susceptibility to excitotoxin but no neurological symptoms due to the transgene provides some hope in this regard. Thus, even overexpression of a short huntingtin fragment using a gene therapy approach could prove useful in attempts to stop neurodegenerative disease. It is envisaged that a pharmacological approach could eventually be developed to target the intracellular signaling that is downstream from the primary effect of the mutant huntingtin fragment and thereby trigger a neuroprotective response.

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