Intracellular inclusions, pathological markers in diseases caused by expanded polyglutamine tracts?

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Abstract
The largest group of currently known trinucleotide repeat diseases is caused by (CAG)n repeat expansions. These (CAG)n repeats are translated into polyglutamine tracts from both mutant and wild type alleles. Genetic and transgenic mouse data suggest that the expanded polyglutamines cause disease by conferring a novel deleterious gain of function on the mutant protein. These mutations are associated with the formation of intracellular inclusions. This review will consider findings from necropsy studies of human patients and transgenic mouse models of these diseases, along with in vitro models, in order to try to synthesise the current understanding of these diseases and the evidence for and against inclusion formation as a primary mechanism leading to pathology.

Keywords: intracellular inclusions; polyglutamine; Huntington’s disease; spinocerebellar ataxia

The largest group of currently known trinucleotide repeat diseases are those caused by (CAG)n repeat expansion mutations and include spinobulbar muscular atrophy (SBMA), Huntington’s disease (HD), dentatorubral-pallidoluysian atrophy (DRPLA)/Haw River syndrome, and spinocerebellar ataxias (SCA) types 1, 2, 3 (or Machado-Joseph disease (MJD)), 6, and 7. All of these diseases follow autosomal dominant inheritance, except for SBMA which is X linked.

Each of these diseases is associated with distinct (but sometimes overlapping) patterns of neurodegeneration. However, they share a number of features, which suggests that they may involve similar pathological processes. First, the mutations are in the coding region of the genes and are translated into abnormally expanded polyglutamine tracts. The border between normal and disease alleles is at 36-40 repeats for most of the diseases, except for SCA6, where the mutation may act via a distinct pathological mechanism (table 1). These diseases tend to manifest the clinical phenomenon of anticipation, a tendency for the severity of symptoms to increase and/or the age at onset to decrease in successive generations. Disease chromosomes have high germ-line mutation rates, compared to normal chromosomes, which generally are stably transmitted. The disease chromosomes show a tendency to increase repeat numbers in successive generations, particularly when transmitted through the male line. This can account for anticipation, as all diseases show significant correlations of increasing repeat numbers on disease chromosomes and earlier age at onset of symptoms.

Polyglutamine expansions confer a deleterious gain of function on mutant proteins
Studies of HD and SBMA suggest that the (CAG)n repeat diseases are caused by gain of function mutations. In HD, a haplinsufficiency model is not supported by the following observations. Patients with Wolf-Hirschhorn syndrome have a terminal deletion of one chromosome 4p, including the HD gene, and do not show features of HD. Transgenic mice expressing only one HD allele do not show overt symptoms. The subtle brain abnormalities in the heterozygous knockout mice reported by the Vancouver group may have

Table 1 Trinucleotide repeat diseases that are caused by a CAG/polyglutamine repeat expansion in the relevant protein.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Normal range (CAG repeats)</th>
<th>Disease range (CAG repeats)</th>
<th>Presence of inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huntington’s disease (HD)</td>
<td>6–35</td>
<td>36–121</td>
<td>Yes</td>
</tr>
<tr>
<td>Dentatorubral-pallidoluysian atrophy (Haw River syndrome/DRPLA)</td>
<td>3–35</td>
<td>49–85</td>
<td>Yes</td>
</tr>
<tr>
<td>Spinobulbar muscular atrophy (Kennedy’s/SBMA)</td>
<td>11–33</td>
<td>38–62</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Spinocerebellar ataxia 1 (SCA1)</td>
<td>6–44</td>
<td>40–81</td>
<td>Yes</td>
</tr>
<tr>
<td>Spinocerebellar ataxia 2 (SCA2)</td>
<td>15–29</td>
<td>35–59</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Spinocerebellar ataxia 3 (Machado-Joseph disease/SCA3)</td>
<td>12–41</td>
<td>55–84</td>
<td>Yes</td>
</tr>
<tr>
<td>Spinocerebellar ataxia 6 (SCA6)</td>
<td>4–17</td>
<td>20–30</td>
<td>Not assessed</td>
</tr>
<tr>
<td>Spinocerebellar ataxia 7 (SCA7)</td>
<td>4–35</td>
<td>37–200</td>
<td>Yes</td>
</tr>
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resulted from the production of a short N-terminal fragment, as the model used a targeted disruption of exon 5 of the HD gene. These abnormalities were not reported by the two other groups whose gene targeting strategies were likely to have resulted in true null alleles. The absence of any abnormal phenotype in a human female carrying a balanced translocation disrupting the HD gene (breakpoint between exons 40 and 41) provides further support that a 50% reduction in HD gene activity is unlikely to be associated with deleterious consequences.

Males with SBMA, who have CAG repeat expansions in the androgen receptor gene, develop progressive weakness and muscle atrophy owing to loss of their motor nerve supply and mild androgen insensitivity. This contrasts with the effects of point mutations elsewhere in the gene, which can result in severe androgen insensitivity but never cause a neuromuscular disease. Indeed, the neuromuscular features of SBMA are not seen even in a patient with a complete androgen receptor gene deletion. This supports the model that a gain of function is an important consequence of a CAG/polyglutamine repeat expansion. However, the loss of a component of the function of the androgen receptor in SBMA, which manifests as mild androgen insensitivity, should not be ignored. While it is likely that these polyglutamine gain of function mutations share similar pathological mechanisms, it is possible that they are also associated with loss of function of specific facets of the different proteins’ functions. This may contribute to the overall disease phenotype and could be one of the factors contributing to the different patterns of neurodegeneration.

A gain of function mutational mechanism is supported by transgenic mouse and Drosophila experiments, where fragments of the HD and SCA3 genes and the entire SCA1 gene with expanded repeats caused phenotypes consistent with these diseases, while transgenes containing normal repeat sizes were not associated with any phenotype. These data by themselves do not exclude the possibility of a dominant negative mutation, where the mutant allele reduces the activity of the wild type allele, so that the overall activity is less than 50%. This model was a possibility, since mice expressing no HD alleles showed early embryonic lethality and impaired neurogenesis. A dominant negative mechanism was formally excluded by a knock-in mouse expressing expanded repeats in the context of the endogenous mouse HD gene. Expression of a single knock-in mutant allele rescued the embryonic lethality associated with absent HD gene expression, suggesting that the expanded CAG repeat does not impair HD function, at least in the context of embryonic lethality and neurogenesis.

Intracellular inclusions. Although these inclusions were first noted by Roizin et al in HD brains in 1979, they were rediscovered and became a focus of research in 1997. In HD brains these inclusions comprise truncated derivatives of the mutant proteins, which only appear to be recognised by antibodies to epitopes close to the expanded polyglutamines. Inclusions were found in neuronal nuclei and were also associated with extracellular structures with a morphology consistent with dystrophic neurites. The intranuclear inclusions are positioned variably in the nucleus, tend to be significantly larger than the nucleolus, and are not separated from the rest of the nucleoplasm by a membrane (fig 1). The inclusions are spherical, ovoid, or elliptical in shape and are concentrated in neurons (and not glial cells) in areas of the brain which degenerate in HD and SCA3. A proportion of the inclusions are ubiquitinated. Electron microscopy showed that the inclusions were heterogeneous in composition and contained a mixture of granules, straight and tortuous filaments, and many parallel and randomly oriented fibrils. Generally only one inclusion is seen per cell in vivo. The proportion of neurons containing inclusions correlated with CAG repeat number and with the severity of neurodegeneration, suggesting that inclusions accumulate as the disease progresses.

While inclusions are seen in brains of patients with DRPLA, SCA1, SCA3, and SCA7, they have not been detected in controls. In SCA3 and HD, the nuclear localisation of the inclusions contrasts with the normally predominantly cytoplasmic localisation of the wild type protein.

Inclusions in transgenic models of polyglutamine diseases

Inclusions were first observed in a transgenic mouse model expressing exon 1 of the HD...
gene with expanded repeats.\textsuperscript{18} The morphology of these inclusions is very similar to that seen in patients and they are also ubiquitinated. In longitudinal studies, the appearance of the inclusions appears to predate the phenotypic abnormalities. Such inclusions are also seen in mouse models expressing the full length SCA1 and HD genes containing expanded repeats.\textsuperscript{19} The appearance of intranuclear inclusions and a progressive neurological phenotype in mice expressing expanded CAG repeats placed ectopically in the \textit{hprt} gene argues that the expanded polyglutamines are the cause of the pathology in all of these disorders and that the expanded repeats can cause disease even in unnatural contexts.\textsuperscript{21}

Intranuclear inclusions and late onset cell degeneration are also seen in the \textit{Drosophila} models expressing portions of the SCA3 and HD genes, containing expanded repeats.\textsuperscript{11}\textsuperscript{12} Besides confirming that the pathophysiological processes leading to cellular dysfunction/death owing to polyglutamine expansions are conserved in invertebrates, these \textit{Drosophila} experiments confirmed data from mouse models of HD that suggested that the phenotypic severity correlated with the level of transgene expression.\textsuperscript{10}\textsuperscript{15} In the fruit fly model of SCA3, the expression of the anti-apoptotic gene \textit{p35} blocked the cell death resulting from the polyglutamine expansion containing transgene.\textsuperscript{11} However, \textit{p35} overexpression did not rescue cell death in the \textit{Drosophila} model of HD.\textsuperscript{12}

**Intracellular inclusions in tissue culture models of polyglutamine diseases**

Intranuclear and extranuclear inclusion formation in tissue culture models of polyglutamine diseases may provide important insights into the pathogenic mechanisms in vivo, as these models seem to show many similarities to the in vivo situation, including ubiquitination of inclusions. These models have shown inclusion formation in both neuronal and non-neuronal cell lines (fig 2). As one would expect, the inclusion number and cellular toxicity in cell lines in vitro correlate with CAG repeat number. Models of HD, SCA3, and DRPLA expressing different sized fragments of normal or mutant cDNAs in cell lines suggest that fragments of polyglutamine disease proteins with expanded repeats are more toxic than the full length proteins with similar expansions.\textsuperscript{16}\textsuperscript{22}\textsuperscript{26} The generation of truncated proteins may be an important step in the pathogenetic pathway in some polyglutamine diseases, since in HD brains and in vitro models where cells were transfected with full length mutant genes the inclusions contain short derivatives of huntingtin, which are only recognised by antibodies to epitopes close to the polyglutamine stretch.\textsuperscript{15} The HD, SCA1, SCA3, and androgen receptor proteins are cleaved by caspses,\textsuperscript{27} a class of proteases which are activated in apoptosis. It has been proposed that the production of toxic fragments from proteins containing expanded polyglutamines may lead to caspase activation and further toxic fragment formation in a positive feedback cycle, ultimately leading to cell death.\textsuperscript{25} In HD, it is likely that other proteases are responsible for a further reduction in the size of the protein, as the inclusions are not recognised by an antibody to an epitope between the caspase cleavage site and the polyglutamine repeats.\textsuperscript{28}

While inclusions appear to be frequently found in the nucleus in postmortem brains of patients with polyglutamine diseases, they also are found in extranuclear structures like neurites.\textsuperscript{27} In transient transfection of cell lines with HD gene fragments, longer cDNA constructs were associated with cytoplasmic inclusions, which were less toxic than the intranuclear inclusions characteristic of the shorter cDNA constructs containing equivalent numbers of expanded repeats.\textsuperscript{22}\textsuperscript{27} The inducible in vitro model system of Lunken and Mandel,\textsuperscript{16} which has allowed a study of the time course of inclusion formation, suggested that the cytoplasmic inclusions may be precursors of intranuclear inclusions. It is not clear whether aggregation occurs in the cytoplasm before cleavage in vivo. Cleavage would facilitate nuclear entry, since protein entry to the nucleus requires specific nuclear localisation signals for molecules larger than about 46 kDa, while smaller molecules can enter the nucleus by passive diffusion. However, the presence of full length mutant huntingtin in the nucleus in the inducible cell lines of Lunken and Mandel\textsuperscript{16} suggest that the full length protein can be transported into the nucleus and is compatible with previous reports suggesting that huntingtin can be found in the nucleus.

In vitro models have also helped to clarify possible processes leading to aggregate formation. Igarashi \textit{et al.}\textsuperscript{29} have shown that aggregate formation is partially inhibited by agents which inhibit transglutaminases. These findings complement those of Kahlem \textit{et al.}\textsuperscript{28} who have shown that this enzyme can catalyse aggregate formation by huntingtin with expanded repeats.
and that this process correlates with expansion size. In vitro studies of purified fragments of the HD gene with expanded polyglutamines showed that purified recombinant proteins with expansions formed stable aggregates which show polarisation microscopic properties with Congo Red, which are similar to those described for amyloids. Such staining is generally seen with proteins which consist of β pleated sheets and are consistent with the predictions made by Perutz that such structures were likely to arise through polar zipper formation by polyglutamine stretches.

The formation of inclusions in cell models is sometimes correlated with apoptosis or an increased susceptibility to agents which stimulate apoptosis. Thus, the cell models may provide a further link between inclusion formation and cell death or cellular dysfunction or both.22 24 26 30

Do inclusions cause cell death/dysfunction?

The data from in vitro and in vivo experiments have not formally shown that inclusions cause cell death but suggest a number of appealing possibilities.31 These inclusions are ubiquitinated and in SCA1, SBMA, and SC3A3 they appear to sequester the Hsp40 homologue, HDJ2/HSDJ, and the 20S proteasome,50–32 which are, along with the ubiquitin pathway, components of the cellular machinery responsible for the degradation of short lived proteins.53 The concentrations of short lived proteins are used to regulate diverse cellular functions. Inclusions may thus disrupt the control of degradation of such proteins. This could lead to an altered homeostasis of these molecules causing cellular dysfunction and ultimately apoptosis (fig 3A).

However, inclusions may not directly cause disease. Inclusion formation may be an epiphenomenon, possibly a side product of another cellular process culminating in pathology (fig 3B). Alternatively, some have even suggested that inclusions may represent a protective response (fig 3B), and that disruption of inclusions may be harmful.35 36 Sadou et al51 queried the role of inclusions in cell death using in vitro studies of primary striatal cultures. A number of their experiments suggested a dissociation between inclusion formation and cell death. First, they showed that constructs comprising the first 171 amino acids of huntingtin with 68 repeats formed greater numbers of inclusions between inclusion formation and cell death.

Figure 3 (A) Likely scenario leading to neuronal cell death/dysfunction in CAG/polyglutamine repeat diseases. Proteins containing an expanded polyglutamine tract initiate aggregation at an intracellular site which depends on the length of the entire protein. The process leading to aggregation or the aggregates themselves compromise cell viability. Proteolytic cleavage of the protein by caspases might generate a fragment which is more prone to aggregation leading to more toxicity, enhanced expression of caspases, and further protein cleavage, by a positive feedback loop. Heat shock proteins are expected to play an important role as molecular chaperones in (re)folding pathways. The ubiquitin-proteasome machinery is likely to be involved and may be sequestered by the protein aggregates leading to an altered homeostasis of short lived proteins. Each step in this model may be influenced by interacting proteins which themselves play a key role in the biology of specific types of neurones in each disease. Based on experiments performed in vitro and in vivo models, it seems likely that the “trigger” for cell death (apoptosis) is initiated by transport of the mutant protein to the nucleus. (B) Three possible scenarios for the role of protein aggregates in CAG/polyglutamine repeat diseases. (i) Aggregates are “toxic” and initiate pathology. (ii) Aggregates protect the cell from a “toxic” protein. (iii) Aggregates are a mere epiphenomenon (no function).

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pressed the first 80 amino acids of huntingtin with 120 repeats and observed a high accumulation of inclusions, but no increase in cell death, thus also showing a dissociation between inclusions and cell death.

Second, Sadou et al. showed that while both striatal and hippocampal neurons developed inclusions when transfected with N-terminal fragments of huntingtin containing expanded repeats, only the striatal neurons showed cell death. The dissociation between cell types showing inclusions and those which degenerate is also seen in SCA7, where inclusions are also found in regions of the brain which do not show severe neurodegeneration. Similarly, in the SCA3 Drosophila model, the formation of the inclusions was not sufficient to cause cell death, since inclusions were found in many cell types which did not degenerate but also in cells which were particularly prone to death, like neurones.

Third, the effect of inhibiting ubiquitination on inclusion formation and cell viability was tested. The inclusions seen in patients' brains and in in vivo and in vitro models of polyglutamine diseases are ubiquitinated. This process is used by cells to tag misfolded proteins and target them for degradation. Sadou et al. showed that expression of a dominant negative ubiquitin conjugating enzyme mutation, which inhibits ubiquitination, resulted in a dramatic reduction of inclusion formation but increased cell death by huntingtin constructs containing expanded repeats. However, inhibition of ubiquitination also resulted in increased cell death in cells expressing huntingtin constructs with 68 repeats, it is difficult to dissociate the cell lethality owing to the ubiquitin deficiency from any effects of the expanded glutamine repeats in these experiments.

Klement et al. suggested that aggregate formation may not be a prerequisite for pathology, since similar Purkinje cell pathology and ataxic phenotypes were observed in mice expressing the SCA1 gene with 77 repeats, with or without a deletion of the self-association domain. The mice expressing transgenes without the self-association domain had no intranuclear inclusions in their Purkinje cells, in contrast to the mice expressing the entire mutant gene. This conclusion may be simplistic, since later reports suggest that the phenotype in the mice with the deleted self-association domain may be non-progressive, in contrast to the mice with the full length gene. In addition, no data were presented for mice with normal repeat lengths containing deletions of the self-association domain in SCA1 and it is conceivable that deletion of the self-association domain is itself deleterious.

The above data suggest that inclusion formation may not be simply linked to cell death and further work needs to be done in order to clarify this controversy. This may have a much wider relevance, since the phenomenon of ubiquitinated inclusion bodies is not confined to polyglutamine diseases. Indeed, it seems to be an emerging theme in many other late onset neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and prion diseases.

Other questions

Despite the similarities between the different polyglutamine diseases and the possibility that the inclusions represent a common pathogenic pathway, it is still unclear why certain cells are particularly prone to inclusion formation and cell death in vivo, and why the regions of cell death differ in the different CAG repeat diseases. The cell specificity may be a function of a number of non-mutually exclusive factors. First, the cells which degenerate may show different expression patterns of the relevant disease gene. While there are no gross correlations between susceptible cell populations in HD and expression levels, variation in expression does occur in the adult striatum, and the cell types showing increased expression are those which are vulnerable to the mutation. Since the formation of inclusions may occur over a long period, possibly starting in embryogenesis, it is possible that vulnerability is related to expression patterns at particular times of development, or the overall cumulative expression over a lifetime. The specificity of neuronal vulnerability may be related to expression of proteins which interact with the polyglutamine disease proteins. For example, this model is supported in SCA1 by the interaction of ataxin-1 (the relevant protein) with the cerebellar leucine rich acidic nuclear protein, which is expressed mainly in the vulnerable cerebellar Purkinje cells. In addition, the strength of this interaction correlates with the number of repeats in the mutant ataxin and both proteins colocalise in matrix associated subnuclear structures. Another factor which may be relevant is the availability and expression level of different proteases in different cell types which can form toxic fragments from the full length proteins. Furthermore, one should not ignore the possibility of differential cellular susceptibility to death caused by intranuclear versus extranuclear inclusions.
Rubinsztein, Wytttenbach, Rankin

9 Ikeda H, Yamaguchi M, Sugai S, Aze Y, Narumiya S, Kaki-
zuuka A. Expanded P5 glutamine in the Machado-Joseph
disease protein induces cell death in vitro and in vivo. Nat

10 Burright EN, Clark HB, Servadio A, et al. SCA1 transgenic
mice: a model for neurodegeneration caused by an

polyglutamine protein forms intranuclear inclusions and causes

12 Jackson GR, Salecker I, Dong X, et al. Polyglutamine-
expanded human huntingtin transgenes induce degenera-
tion and frequency of intracellular aggregates. J Neurobiol

13 White JK, Auerbach W, Duyao MP, et al. Immunohisto-
chemical localization and aggregation of polyglutamine in the

14 Roizin L, Stellar S, Liu JC. Neuronal nuclear-cytoplasmic
aggregate formation and apoptosis by transglutaminase
expression of mutated full-length HD cDNA. FEBS Lett

15 Skinner PJ, Koshy BT, Cummings CJ, et al. Suppression of
intracellular aggregation and apoptosis by transglutaminase
inhibitors in cells expressing truncated DRPLA protein
with an expanded polyglutamine stretch. Nat Genet

21 Reddy PH, Williams M, Charles V, et al. Polyglutamine-
explained human huntingtin transgenic mice: a model for
disease protein induces cell death in vitro and in vivo. Nat

disorders generates truncated fragments containing the

23 Lunkes A, Mandel JL. A cellular model that recapitulates
major pathogenic steps of Huntington’s disease. Hum Mol

influence of huntingtin protein size on nuclear localisation

25 Kahlem P, Green H, Dian F. Transglutaminase action imi-
itates Huntington’s disease: selective polymerization of
huntingtin containing expanded polyglutamine. Mol Cell

26 Lunkes A, Mandel JL. A cellular model that recapitulates

27 Klement IA, Skinner PA, Kaytor MD, et al. Formation of
neuronal intranuclear inclusions underlies the neurological
dysfunction in transgenic mice for the HD mutation. Cell

28 Kahlem P, Green H, Dian F. Transglutaminase action imi-
itates Huntington’s disease: selective polymerization of
huntingtin containing expanded polyglutamine. Mol Cell

29 Perutz MF. Glutamine repeats and inherited neurodegen-
erative diseases: molecular aspects. Curr Opin Struct Biol

N-terminal fragments of huntingtin with expanded
polyglutamine repeats form nuclear and cytoplasmic aggregates

31 Cummings CJ, Mancini MA, Antalffy B, DeFranco DB, Orr
HT, Zoghbi HY. Chaperone suppression of aggregation and
altered subcellular proteasome localization imply pro-

32 Paulson HL, Chai Y, Gray-Board GL, Bonini NM. Misfold-
ing and aggregation in spinocerebellar ataxia type 3: a role
for cellular chaperones in glutamine-repeat disease. Am J

33 Hochstasser M. Ubiquitin-dependent protein degradation.

34 Nudov D, Finheden S, Derry D, Greenberg ME. Hunting-
tin acts in the nucleus to induce apoptosis but death does
not correlate with the formation of intranuclear inclusions.

35 Sisodia S. Nuclear inclusions in glutamine repeat disorders:
are they pernicious, coincidental, or beneficial? Cell 1998;
95:1-4.

36 Holmberg M, Davyaerts M, Durr A, et al. Spinocerebellar
ataxia type 7 (SCA7): a neurodegenerative disorder with
neuronal intranuclear inclusions. Hum Mol Genet 1998;7:
913-18.

37 Klement IA, Skinner PA, Kaytor MD, et al. Ataxin-1 nuclear
localisation and aggregation: role in polyglutamine-

38 Orr HT, Skinner PJ, Klement CJ, Cummings CJ, Zoghbi
HY. The role of ataxin-1 nuclear expression and aggregates

39 Ferrante RL, Konrad NW, Beal MF, Martin JB, Bird ED,
Richardson EP. Morphologic and histochemical character-
istics of a spared subset of striatal neurons in Huntington’s

40 Matilla A, Koshy BT, Cummings CJ, Isobe T, Orr HT,
Zoghbi HY. The cerebellar leucine-rich acidic nuclear pro-