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Review

Polyglutamine diseases: a transcription disorder?

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Abstract. Various molecular processes including unfolded protein response, protein transport, synaptic transmission and transcription are implicated in the pathology of polyglutamine diseases caused by the expanded polyglutamine-containing proteins. More than 20 transcription-related factors have been reported to interact with disease proteins, and the pathological interaction is known to repress gene expression. The whole shape of nuclear events evoked by disease proteins is now emerging with information on these transcription-related factors and with findings on the similarity between nuclear bodies and pathological inclusion bodies. This article reviews 'transcription theory', a rapidly growing hypothesis in polyglutamine diseases.

Key words. Polyglutamine disease; transcription; cofactor; splicing; RNA; nuclear body; aggregation; ubiquitin; SUMO; proteasome; neuronal death.

Introduction

Polyglutamine diseases are a specific group of hereditary neurodegeneration caused by expansion of CAG triplet repeats in an exon of disease genes which leads to production of a disease protein containing an expanded polyglutamine stretch (see review [1–4]). Among various triplet repeats, CAG expansion is the only one that causes neurondegeneration. Expansion of GCG repeats produces abnormal polyA-binding protein containing an elongated polyalanine tract, whereas the mutant protein causes cell death limited to skeletal muscles, known as oculopharyngeal muscular dystrophy [5]. The expanded polyglutamine tract is sufficient to cause neurodegeneration, because transgenic mice expressing the mutant polyglutamine exclusively show similar pathology [6, 7]. So far, nine neurodegenerative disorders, including Kennedy's disease, Huntington's disease, spinocerebellar atrophy-1, -2, -3, -6, 7, 17 and dentatorubral pallidoluysian atrophy (DRPLA) are known to belong to the polyglutamine diseases. In most polyglutamine diseases, expansion to over 40 repeats leads to onset (see review [4]). The threshold number is consistent with the length necessary for polar zipper formation [8, 9]. The only exception is SCA6, in which more than 21 repeats in the α1-voltage-dependent calcium channel subunit leads to onset. The discrepancy might be relevant to loss of channel function, but this speculation has not been proved. Most of the disease proteins are cleaved to peptides before they aggregate and form inclusion bodies. Mutant huntingtin, the disease protein of Huntington's disease, is the earliest example that is cleaved in culture cells and neurons [10–14]. Androgen receptor, ataxin-3 and atrophin-1/DRPLA (disease proteins of Kennedy's disease, SCA3 and DRPLA, respectively) are cleaved by caspases [15–18]. N-terminal cleavage of ataxin-7 by unknown proteases was also reported [19]. Li's group recently reported that the N-terminal fragment of huntingtin is selectively accumulated in the striatal neurons, and proposed that the brain region-specific cleavage leads to selective neuronal death [20]. However, this view was challenged by a re-

port showing that mutant huntingtin is more resistant to proteolysis, and N-terminal peptides arise from normal huntingtin in diseased brains [21]. Collectively, the polyglutamine diseases share a common molecular pathology and can be considered as a homogeneous disease entity.

At present, a critical question to be answered is how mutant proteins cause human pathology. It is generally accepted that aggregate or inclusion bodies are an important hallmark of polyglutamine diseases. However, it is not clear whether the aggregate directly induces neuronal dysfunction and death. It is also suggested that soluble proteins or cleaved peptides in monomer or oligomer states are toxic before aggregation. Soluble or oliogomer disease proteins may bind to cellular proteins and abrogate their functions during the process of aggregation. If we accept this hypothesis, it is essential to know which cellular proteins bind to the polyglutamine disease proteins. Actually, a large number of binding proteins have already been reported. They can be classified into nuclear transcription-related factors, ubiquitin-proteasome proteins and cytoskeleton-related proteins. The ubiquitin-proteasome group includes Hsp70, Hsp 72, Hsp90, Nedd8 and HDJ-2/HSDJ. These proteins regulate various cellular functions by changing the protein concentration in cells through degradation; thus, interaction with proteasome-related proteins could be a physiological reaction to degrade abnormal polyglutamine proteins. Alternatively, unfolded mutant protein in the endoplasmic reticulum (ER) may trigger the ER stress response, leading to proteasome activation and apoptosis cascade (see review [22]). As for cytoskeletal proteins, abnormal function of HIP [23, 24] may lead to vesicle transport dysfunction, and inhibition of HAP [25] may lead to dysfunction of microtubule-associated protein transport (see review [26]). Furthermore, results from many laboratories have shown that numerous transcription-related factors interact with polyglutamine disease. In contrast to other types of neurodegeneration, interaction with transcription-related factors is specifically reported in polyglutamine diseases. In this sense, these results may suggest that transcriptional dysfunction is a specific cue of polyglutamine disease pathology. In this review, I will focus on this new hypothesis of polyglutamine diseases and will ask whether the transcription theory has been established. Naturally, the aim is not to rule out the other pathological elements but to seek mutual links among the pathological elements.

Interaction between disease proteins and transcription-related factors

So far, more than 20 nuclear proteins relevant to transcription are suggested to interact with polyglutamine disease proteins (table 1). They include LANP, PQBP-1, N-CoR, ARA24, CBP, p53, mSin3A, $TAF_{II}130$, ETO/MTG8, p160/GRIP1, Sp1, C-terminal binding protein (CtBP), CA150, SC35, MLF1 and so on [27–47]. In some of them, interaction was verified by biochemical experiments and colocalization. In other proteins, interaction was suggested only with colocalization. Nuclear receptors such as glucocorticoid receptor are known to affect nuclear translocation of mutant andogen receptor [48, 49]. Promyelocytic leukemia protein (PML) is partially colocalized with ataxin-1 [50]. TATA-binding protein (TBP) and PML are colocalized with ataxin-3 in nuclear inclusion bodies of human brain [51, 52]. Rich et al. reported colocalization of ataxin-1 and RED [53]. Another TBP-associating factor, $TAF_{II}30$ (not $TAF_{II}130$), was reported to accumulate in nuclear inclusions of

Table 1. The polyglutamine disease protein-binding proteins.

Interacting protein, binding domain	Disease protein	Reference
Interaction was confirmed by biochemical and morphological experiments		
LANP:	ataxin-1	[27]
PQBP-1, polar amino acid rich domain	ataxin-1, poly-Q	[28, 29]
N-CoR, C-terminal domain	huntingtin	[30]
ARA24, N-terminal domain	androgen receptor, poly-Q	[31]
p53	huntingtin	[32]
mSin3A	huntingtin	[32]
ETO/MTG8, N-terminal domain, non poly-Q	atrophin-1	[35]
P160/GRIP1, C-terminal domain	androgen receptor	[36]
A2BP1	ataxin-2, C-terminal domain	[37]
CBP, CH3 domain/ a part of acetyl-transferase domain	huntingtin	[32, 34, 38, 39,]
$TAF_{II}130$	atrophin-1	[42]
(colocalization with ataxin-2, -3 and huntingtin was also reported)		
CBP	atrophin-1	[34]
CA150	huntingtin	[43]
CRX	ataxin-7	[44]
Sp1	huntingtin	[45]
C-terminal binding protein [CtBP]	huntingtin	[46]
Colocalization confirmed by morphological experiments		
PML	ataxin-1	[50]
TATA-binding protein [TBP]	ataxin-3	[51]
PML	ataxin-3	[51, 52]
RED	ataxin-1	[53]
$TAF_{II}30$	ataxin-7	[19]
SC35	huntingtin	[46]
Genetic relationship and colocalization reported		
MLF 1	polyglutamine tract	[47]

ataxin-7 [19]. A *Drosophila* homologue of MLF1 (myeloid leukemia factor 1) was shown to suppress polyglutamine toxicity [47]. Furthermore, although not included in the table, androgen receptor and TBP are the causative genes of polyglutamine diseases, and these transcription factors with abnormal polyglutamine tracts are known to self-aggregate [54–57]. Some factors such as polyglutamine binding protein-1 (PQBP-1) [28, 29] and ataxin-2 binding protein (A2BP1) [37] are also implicated in splicing. In addition, ataxin-1 itself possesses RNA-binding activity [58]. The recent notion that RNA modifications are tightly linked to transcription at the C-terminal domain of RNA polymerase II (Pol II) indicates that perturbation of these factors leads to transcriptional dysfunction in a large sense.

How, then, do polyglutamine disease proteins interact with transcription-related factors? As far as we know, binding domains to the polyglutamine disease protein are not identical. Moreover, the binding domain to the polyglutamine disease proteins has not been exactly determined in most cases. Unfortunately, we have only limited information. ETO/MTG binds to a domain other than the polyglutamine sequence [35]. LANP binds strongly to the C-terminal portion of ataxin-1, but interacts only weakly with the N-terminal part, including the polyglutamine tract [27]. Meanwhile, PQBP-1 binds to the polyglutamine tract via its polar amino acid-rich domain [29]. $TAF_{II}130$ also seems to interact with the polyglutamine region [42]. Conversely, the binding domains of these nuclear factors seem to be divergent (table 1). Therefore, in contrast to the established self-interaction mechanism among disease proteins (reviewed by [9]), motifs necessary for the binding between disease proteins and partner molecules cannot be extracted from these data.

In our experience of screening polyglutamine-binding proteins by the yeast two-hybrid method, various types of proteins with no definite motif were isolated, and they share only polar amino acid sequences [59]. Meanwhile, an interaction between polyglutamine disease proteins and transcription-related proteins had been suspected before cloning of binding proteins because the polyglutamine tract is found in many nuclear transcription factors. They include Oct-2, Brn-2, CBP, TBP, androgen receptor, glucocorticoid receptor and so on. Cytoplasmic proteins containing a polyglutamine stretch are far fewer than nuclear proteins with polyglutamine repeats. Although the physiological functions of the polyglutamine sequence have not been clarified, it was reported that the polyglutamine stretch functions as a transcriptional activation domain in Oct-2 [60–62]. It suggests that the polyglutamine tract may function as a protein-binding motif and play a certain role in transcription.

Functions of interacting nuclear factors

Functions of the transcription factors that bind to polyglutamine disease proteins are essential to understand the overall effect of disease proteins on transcription (fig. 1). TBP binds to a TATA box located upstream of various genes. It is a main component of the TFIID fraction that associates with nearly 10 TBP-associating factors (TAFs). TBP recruits RNA polymerase II to the core promoter region of DNA (reviewed by [63]). LANP is a leucine-rich acidic nuclear protein with unknown function [64]. It was also reported as a human leucocyte-associated antigen (HLA) class II associate protein, an inhibitor of phosphatase 2A, an inhibitor of oncogenic transformation and a possible modulator of interactions between microtubule-associated proteins (MAPs) and microtubles [65–68]. PQBP-1 binds to the C-terminal domain of Pol II [29] and a splicing factor, U5-15kD [69, 70], and connects transcription and splicing machineries. It binds to the RNA polymerase II C-terminal domain of Pol II (Pol II-CTD) via its WW domain, and the interaction seems to be intensified by phosphorylation of the 2^{nd} serine residue in the repeat sequences (YSPTSPS) of Pol II-CTD [29]. CA150, possessing specific Ala-Gln (AQ) repeats and two WW domains, also binds to hyperphosphorylated Pol II-CTD more strongly [71], suggesting that these two polyglutamine binding factors seem to have a close relationship. It is of note that another hereditary neurodegeneration gene product 'survival of motor neurons' protein (SMN) also associates with Pol II [72]. N-CoR is a repressive transcription cofactor for nuclear steroid receptors. In addition to steroid receptors, it interacts with histone deacetylase and DNA methytransferase, and forms a repressor complex. RED is a specific protein containing acidic/basic repeats with unknown function [53]. Since proteins containing the acidic/basic repeats, such as RD, MURED and PQBP-1, are involved in splicing or transcription, RED might participate in such nuclear functions. ARA24 was isolated as a binding protein to the polyglutamine sequence of androgen receptor [31] and turned out to be identical to Ran. Ran is a critical guanosine triphosphatase (GTPase) that supplies energy for nuclear import and export of proteins [73]; thus, interaction between AR and Ran/ARA24 might be involved in nuclear transport. Genetic studies have actually shown that Ran/ARA24 affects protein/RNA nuclear transport, cell cycle regulation nuclear structure for mitotic regulation and RNA/DNA synthesis. Interestingly, the affinity of ARA24 to AR is decreased by expansion of poly-Q in AR [31].

CREB-binding protein (CBP) is a platform protein on which various kinds of transcription-related factors gather (see review [74, 75]). It contains multiple protein interaction domains, including three zinc fingers, CREB-binding domain, bromo-domain, and glutamine-rich domain, to

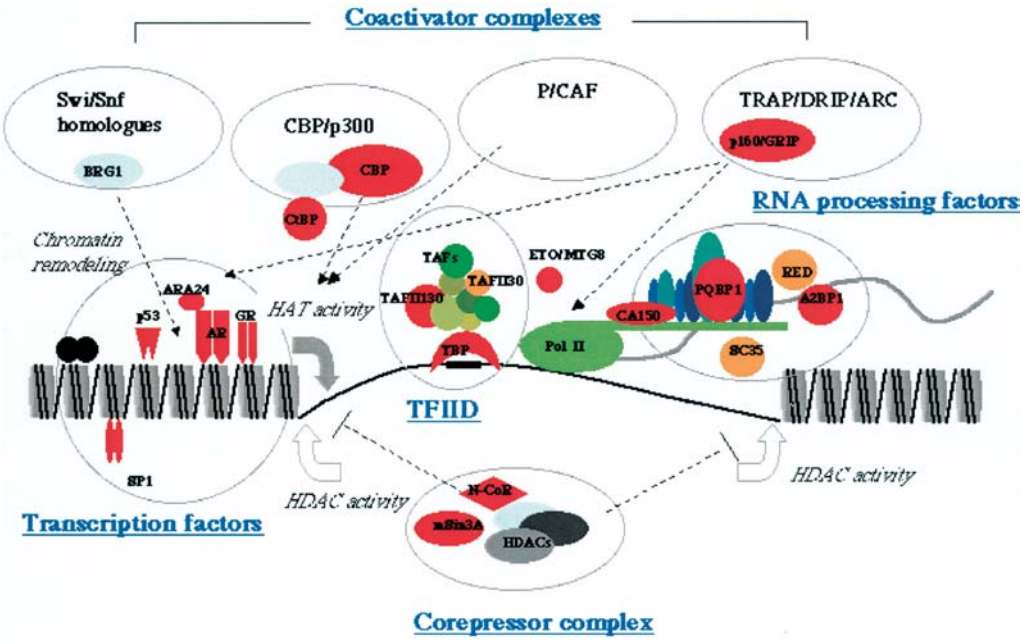

Figure 1. Functional distribution of binding partners of polyglutamine disease proteins. Proteins whose interaction was verified by biochemical and morphological analyses are indicated in red, while those suggested by morphological observation are shown in orange. See table 1 for which disease protein interacts with each protein. Proteins whose functions remain unclear are not shown in this figure. Functions of coactivator complexes are different. Swi/Snf complex possesses DNA remodeling activity. CBP/p300 and P/CAF groups possess HAT activity. TRAP/DRIP/ARC complex does not possess either activity. Corepressor complexes repress transcription basically via HDAC activity.

which more than 40 partners bind. Between the 2nd and 3rd zinc fingers, CBP has a domain carrying histone acetyltransferase (HAT) activity. However, as is well known, CBP is not the sole protein possessing HAT activities. In addition to MOZ and MLL, which belong to the CBP family, numerous proteins of the GNAT, MYST, SRC and $TAF_{II}250$ families possess HAT activity (see review [74, 75]). p53 is a well-known transcription factor that exerts various physiological effects, including cell cycle arrest and apoptosis. One of the famous targets of p53 is p21. After DNA damage by X-ray irradiation, activated ATM phosphorylates p53, increases the binding of p53 to regulatory cis elements and increases transcription from target genes, including p21 (reviewed by [76]). p21 suppresses G1/S and S cdk proteins. $TAF_{II}130$ is one of the numerous TBP-associating factors. ETO/MTG8 is a nuclear protein expressed in the brain, and it is known as a fusion partner of the AML1/ETO chimera oncoprotein [77–79]. ETO has a homologous domain to TAF110 and other motifs for protein interaction, suggesting that ETO is a TAF-like factor. N-CoR interacts with mSin3A and suppresses transcription [80]. p160 is another transcriptional cofactor that mediates interaction between a transcription factor and the core transcription machinery. Sp1 is a ubiquitous transcription factor involved in essential gene regulation. CtBP was isolated as a binding protein to adenovirus E1A [81], and shown to interact with mammalian polycomb proteins and delta-EF1, which function as their transcription corepressors [82]. CRX is a homeobox transcription factor that binds to the TAATCC/A sequence upstream of photoreceptor-specific genes [83–86]. Mutation of CRX leads to abnormal development of photoreceptors, the autosomal dominant form of cone-rod dystrophy and other retinal diseases. the *Drosophila* homologue of MLF-1, a fusion gene of the t(3;5)(q25.1;q34) of myelodysplastic syndrome and acute leukemia [87], was found to be a suppressor of polyglutamine toxicity in the poly-glutamine transgenic fly [47]. PML was isolated as a fusion gene at the t(15;17) translocation found in acute promyelocytia leukemia. It has a self-associating nature through the coiled-coil domain [88], and is now known to be a scaffold for nuclear bodies regulating the concentration, modification and/or compartmentalization of transcription factors in the nucleus [89].

Taken together, the proteins that interact with polyglutamine disease proteins are distributed around the core transcription machinery or Pol II (fig. 1), which is now known to exert DNA methylation, histone acetylation and RNA modification simultaneously (see review [90, 91]). It is of note that polyglutamine disease proteins bind to multiple components of coactivator complexes except Swi/Snf DNA remodeling factors. Disease proteins also bind to corepressor complex components such as N-CoR or CtBP. The distribution of nuclear proteins that interact

with polyglutamine disease proteins (fig. 1, indicated in red and orange) suggests that polyglutamine disease proteins would disturb a wide range of transcriptional functions. Furthermore, together with recent notions that transcription and RNA modifications are simultaneous processes conducted by multiple groups of proteins on the genomic DNA (see review [90, 92]), the map suggests that the target of polyglutamine disease proteins is extended to the functional link between transcription and RNA processing.

Nuclear localization of aggregates: where in the nucleus?

Most polyglutamine disease proteins form aggregates in the nucleus. Although nuclear aggregates are reported to be dispensable for cell death in SCA2 and other polyglutamine diseases [93], it is definitely true that aggregate formation is an important hallmark of polyglutamine diseases. Even if nuclear aggregates are dispensable for cell death, many reports suggest that nuclear localization of the disease protein is relevant to the pathological process of polyglutamine diseases. Therefore, it is essential to know exactly where in the nucleus the mutant protein accumulates, in order to understand complex events induced by the disease protein. Davies et al. and Waelter et al. reported beautiful pictures of nuclear aggregates in transgenic mouse and culture cells using electron microscopy [94, 95]. According to their data, huntingtin seems to aggregate in the nuclear matrix but spares peripheral chromatins and nucleorus. However, it should be mentioned that they did not focus exclusively on this question. We found that PQBP-1/ataxin-1 coinclusions are also distributed in the nuclear matrix outside of heterochromatin [29]. In addition, the Wanker group and the Pittman group reported that normal ataxin-3 distributes in the nuclear matrix [96, 97]. These data suggest that mutant protein does not accumulate in the heterochromatin where DNA is densely compacted; instead, they aggregate in the nuclear matrix, where proteins can move freely among nuclear bodies such as Cajal/coiled bodies and speckles/gems.

Several research efforts using marker proteins of various types of nuclear bodies are very helpful in investigating the accumulation site of abnormal proteins. The Orr group reported that mutant ataxin-1 is involved in the PML body, but does not colocalize with SC35, a marker protein of spliceosome. Meanwhile, Kegel et al. have shown colocalization of huntingtin and SC35 [46]. On the other hand, atrophin-1/DRPLA gene product does not colocalize with PML but with ETO, which is considered as a new nuclear body marker protein [35]. Similarly, PQBP-1 can be considered to be a new marker of the nuclear body that colocalizes with ataxin-1 [29]. These findings suggest that even in the nuclear matrix, different disease proteins accumulate at distinct sites. In the cases of ETO and PQBP-1, the nuclear bodies enlarge when the elongated polyglutamine protein is coexpressed. As for normal polyglutamine proteins, the Orr group reported that normal ataxin-1 distributes in the nuclear matrix, and some forms relatively small nuclear body-like structures [50]. Collectively, these results suggest that polyglutamine disease proteins are located in the nuclear matrix under physiological conditions and some of them form relatively small nuclear bodies.

Nuclear aggregates/inclusions and nuclear bodies

Despite the aforementioned similarities between nuclear bodies and nuclear aggregates, it is not clear whether nuclear aggregates originate from nuclear bodies. The concept of hard and insoluble inclusion bodies originates from pathological observations. The fibrous aggregate structure of polyglutamines is very similar to that of beta-amyloid, the main insoluble component of senile plaque in Alzheimer's disease [8]. Biochemical experiments further supported that at least some pathological inclusion bodies are detergent insoluble, leading to the concept of 'aggregates'. They were believed to be an isolated territory in the nucleus. On the other hand, nuclear body is an anatomical or cell-biological term. Cajal body and speckles were found by anatomical observations. Considering that these two terms possess different historical backgrounds, it is necessary to compare these two concepts with updated data to know the origin of polyglutamine disease inclusions. Many people believed that nuclear inclusions and nuclear bodies are totally different structures. The most significant difference between nuclear inclusion bodies and nuclear bodies was assumed to be protein transport. Transcription and splicing factors shuttle between the nuclear bodies and nuclear matrix. Depending demand, these factors are stored in the nuclear bodies or transported to appropriate sites in the nucleus (mainly, the margin between chromosome territory and the nuclear matrix). On the other hand, it was believed that transport does not occur in nuclear inclusions (= aggregates).

However, the concept of nuclear inclusion bodies began to change with recent observations. An initial observation would be disassembly of nuclear proteins in the dividing cell [53]. Despite the suspected nature of 'aggregates', fluorescent fusion proteins of mutant ataxin-1 disperse when cells divide. Furthermore, three reports directly showed dynamics of nuclear inclusions using new techniques. The first one came from the Paulson group [98]. They used fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP) on aggregates formed by green fluorescent protein-tagged polyglutamine disease proteins. In the case of

ataxin-3 and huntingtin fusion proteins, recovery after photobleaching at aggregates was very slow, and FLIP did not remarkably induce protein release from aggregates. On the other hand, ataxin-1 showed rapid recovery from photobleach and rapid release from aggregates. Their findings clearly indicated heterogeneity among so-called aggregates, and showed that ataxin-1 inclusion body has a homologous character to nuclear bodies. Similar approaches were reported from the other two groups. The Mancini group [99] partially reconfirmed the data of the Paulson group. However, they added the result that larger inclusions of mutant ataxin-1 showed slower exchange of the disease proteins. The Morimoto group compared the exchange of different components in the same aggregate [100]. According to their data, polyglutamine, especially elongated, proteins are tightly associated with the inclusion body structure, whereas other proteins involved in inclusion dynamically go into or out of the inclusion bodies.

These reports suggest similarities between inclusions and nuclear bodies. We can now imagine the following story (fig. 2). Mutant proteins accumulate in nuclear bodies to form insoluble fibrils based on tight interactions via the polar zipper. Some nuclear proteins with a high affinity for the disease protein are harbored in the nuclear bodies. Meanwhile, other components leave the pathological nuclear bodies just as they move away from physiological nuclear bodies. However, the release becomes difficult when the pathological nuclear body becomes the large nuclear aggregates composed of insoluble fibrils (fig. 2). It is of note that inclusions of mutant protein emerge exactly at the same site in the nucleus after photobleaching. This suggests that nuclear inclusion bodies have a certain anchor in the nuclear matrix. The character is again similar to that of nuclear bodies. PML bodies are known to locate almost at the same positions in the nucleus, except smaller nuclear bodies can move slightly in an energy-dependent manner [101]. Collectively, nuclear bodies and nuclear inclusions are closely related, and the nature of nuclear inclusion bodies is dependent on the type of disease protein, the length of polyglutamine stretch and the size of inclusions.

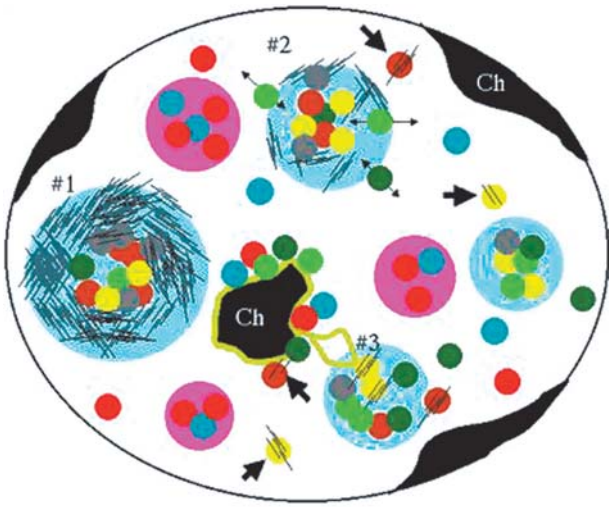

Figure 2. Schematic representation of the nucleus, indicating the hypotheses for transcriptional repression. Short black lines indicate fibrous aggregates of polyglutamine disease proteins. They aggregate in nuclear bodies (blue or pink circles). Fibrous aggregates develop the large nuclear body (#1) to a nuclear inclusion, from which various transcription factors (various colors of small circles) are not released (sequestration theory). Meanwhile, in a relatively smaller body (#2), transcription factors are still able to move in or away from the nuclear body (dynamic inclusion). The polyglutamine disease protein prefers blue bodies to pink bodies. Thus, factors involved (brown, yellow, gray) in blue bodies are more easily affected, but the influence is dependent on affinity for the disease protein and is variable among the factors. Transcription is performed at the boundary between chromatin territory (Ch) and nuclear matrix (light green belt). Red, blue and green factors are abundant, but gray, yellow and brown factors are decreased at the transcription site. Some of the brown and yellow factors interact with the soluble or preaggregate form of the disease protein (arrows), and recruitment to the transcription site or transcription activity is abrogated. It is also possible that the functions of transcription-related factors are disturbed in nuclear bodies (#3).

Transcriptional repression: general or specific?

A number of reports support transcriptional repression in polyglutamine diseases. As described, this idea originated from the observation that mutant proteins interact with various transcription-related proteins. However, it remains unclear whether polyglutamine disease proteins generally repress transcription or suppress transcription of a specific gene. Specific suppression might be possible if a disease protein interacts with a specific transcription factor that regulates the enhancer of a specific gene. It was actually reported that interaction of huntingtin and SP1 repressed transcription from the D1A dopamine receptor gene [45], although this finding did not rule out suppression of other neuronal genes. On the other hand, recent investigations have increased the number of candidate transcription factors that bind to mutant proteins. Among those transcription factors, CBP has attracted attention. Many groups have investigated the effect of CBP on transcription in the presence of disease protein because CBP is a representative coactivator that possesses HAT activity and interacts with numerous transcription factors (fig. 1). CBP has been suggested to interact and colocalize with androgen receptor, huntingtin and atrophin-1 [32–34]; thus it is plausible that mutant proteins affect coactivator function. Actually, it was reported that huntingtin binds to a domain of CBP (other than the HAT domain) and represses HAT activity [38, 39]. This story was supported by the observation that the inhibitor of histone deacetylase (HDAC), the counterpart of HAT, res-

cues neurodegeneration by mutant proteins (see review [40]) and by the results of the knockout mice of CREB, the partner of CBP, showing neurodegeneration [41]. Histone acetylation unwinds DNA from nucleosome and activates transcription. Since CBP interacts with many transcription factors, its binding to disease proteins should affect a wide range of gene expression rather than that of a specific gene. We also found interaction between PQBP-1 and Pol II and observed that interaction between PQBP-1 and ataxin-1 affects general transcription via Pol II dysfunction [29].

Three groups recently reported how gene expressions are affected in the transgenic mouse or striatal neuron models of Huntington's disease by microarray analyses [102–104]. Simultaneously, the Fischbeck group reported altered gene expression in cells expressing mutant androgen receptor [105]. According to their data, expression levels of many genes are changed. In addition, Luthi-Carter et al. pointed out that expression of the Pol II large subunit gene was changed significantly [102]. Interestingly, the message of Pol II was increased, while the protein was decreased. Their data show that multiple transcription factors but not a specific one are influenced in the transgenic mouse model. These results seem to support further the general transcription hypothesis. According to the data of Sipione et al., expression of the DNA remodeling complex, which includes no binding protein to the polyglutamine disease proteins so far, was also affected [104]. In addition, some RNA-processing factors were influenced. Accumulating results collectively suggest that transcription is affected generally but not specifically. However, the relative importance of each transcriptional dysfunction in the pathology must be evaluated with further data.

Why is transcription repressed by nuclear inclusions?

Why then is transcription repressed by nuclear inclusion bodies? The most prevailing idea came from findings that the polyglutamine-interacting protein, CBP, is sequestered into aggregates by disease proteins [32–34]. These reports have shown that sequestration of the essential nuclear protein into inclusion bodies dramatically disturbs transcription. The sequestration theory couples with the depletion of nuclear factors from the nuclear matrix. The total amount of CBP in the nuclear matrix is decreased by sequestration into the inclusion bodies, and the transcriptional activity is attenuated. The hypothesis is very attractive, and explains well the mechanism of transcriptional repression (fig. 2). Meanwhile, it was recently reported that the amount of CBP protein is not decreased in the nuclear matrix even after inclusion body formation [106]. It is also noteworthy that CBP is one of many coactivators possessing HAT activity; thus, HAT activity might be supplied from the other coactivators (fig. 1). In addition, recent findings on the nature of inclusion bodies described above have challenged the sequestration theory. It is possible that proteins in some kinds of inclusion bodies are completely sequestered from other nuclear portions. It is also possible that proteins could not leave the inclusions once they are included. However, on the other hand, some types of inclusion bodies, such as those of ataxin-1, actively import and export proteins. In addition, disease proteins do not exist in all the nuclear bodies, as we will see later in this section. Therefore, sequestration theory may require a kind of update together with the new data (fig. 2).

Alternatively, how can we explain transcriptional repression by polyglutamine disease protein? If sequestration alone cannot fully explain the repression mechanism, interaction with disease proteins in a nuclear compartment other than inclusion bodies must affect the functions of transcription factors. New findings in cell biology are useful for this consideration. Fine structures in the nucleus that were not known in classical morphology have been recently elucidated, and the view of the functions of nuclear compartments has been changing rapidly. Genomic DNA inactive for transcription is compacted in chromatin territory. Transcription and RNA modification factors are released from nuclear bodies, recruited to the relaxed genomic DNA at the transitional zone between chromatin territory and nuclear matrix, and conduct transcription of messenge RNA (mRNA) and transfer RNA (tRNA) by Pol II (see review [89, 107, 108]). Considering these findings, the first explanation will be that transcription is disturbed by the interaction between disease proteins and transcription factors that takes place at the critical zone in the nucleus (fig. 2). On the other hand, nuclear bodies have been considered as the storage compartment for nuclear factors not used currently. Recent data are also changing the view of nuclear bodies. HDAC1 and mSin3A which wind DNA around histones to repress transcription, are recruited to the PML body, and PML bodies are enlarged when the transcription is repressed [109], suggesting that the PML body is a functional platform for the nuclear factors to actively repress transcription, instead of being a simple storage place. The Cajal body is also reported to function as an essential place for assembling splicing factors, and this assembly is critical for transcription [110]. Nuclear bodies might be an active factory rather than a simple buffer compartment. Therefore, the second explanation will be that disease proteins disturb functions of nuclear bodies (fig. 2) in addition to simply shifting nuclear factors from the matrix to the nuclear body. According to this theory, disease proteins repress transcription in nuclear bodies before they grow into nuclear inclusions. These hypotheses should be examined once the detailed functions of nuclear bodies and the relationship between nuclear bodies and nuclear inclusions are clarified.

It is noteworthy that disease proteins are not present in all nuclear bodies that carry a nuclear protein marker. For example, huntingtin does not exist in all of the PML bodies [46]. A similar finding was also reported in the cases of huntingtin/CtBP [46] and ataxin-1/PML [50]. It may suggest that even high expression of the disease protein affects the function of a nuclear protein in the nuclear body only partially, because the proteins that do not colocalize with the disease protein should function normally. If not all the nuclear protein molecules are affected or sequestered, the result might suggest dominance of the nuclear matrix for transcriptional repression.

Other possible effects of interaction between transcription factors and disease proteins

Interaction between polyglutamine disease proteins and transcription factors also occurs outside the nucleus. The Manicini group reported that steroid receptor coactivator 1 (SRC1) is sequestered by androgen receptor into cytoplasmic inclusions, and the aggregates stained positively for Hsp 70, Hsp90, Nedd8 and HDJ-2/HSDJ [111]. It is also noteworthy that colocalization of disease proteins with $TAF_{II}130$ is mainly observed in the perinuclear cytoplasmic inclusions [42], corresponding to the aggresome, which contains many proteasome proteins and functions as the center for protein degradation [112]. These results suggest that transcription factors interacting with mutant disease proteins also are also sequestered into the protein degradation compartment in the cytoplasm and that the proteasome system is activated by these substrates. In addition to protein degradation, the proteasome activation may also trigger apoptosis cascades. Cosegregation of mitochondria and various cellular organelles with mutant proteins into the aggresome [95] have been well described, and recent investigations revealed that mutant disease proteins accumulate in the ER and trigger the ER stress response [113 and see review 22].

Is there any other effect of ubiquitination induced by the interaction with disease proteins? The physiological functions of ubiquitination may suggest the answer. For example, the effects of the proteasome system on nuclear transport of transcription factors have been reported. Poly-ubiquitinated p53 in the nucleus is exported to the cytoplasm for degradation by the proteasome system. Covalent binding of SUMO (small ubiquitin-related modifier) to Mdm2 represses the ubiquitination of p53 and activation of the proteasome system [114]. In the case of nuclear factor kappa B (NF-κB), nuclear import is activated by degradation of I-κB, an inhibitory factor of NF-κB in the cytoplasm (see review [115]), through ubiquitination. These findings indicate that ubiquitination critically regulates nuclear import and export of these transcription factors. Therefore, it is possible that disease proteins affect the nuclear transport of transcription factors through ubiquitination. These possible effects via ubiquitination (fig. 3) have not been examined extensively, but would be worthwhile to investigate.

It is also possible that the SUMOylation affects pathology by changing the metabolism of transcription factors. As mentioned above, SUMOylation antagonizes ubiquitination and degradation of Mdm2, an enhancer of p53 ubiquitination. Thus, SUMO accelerates p53 function indirectly [113]. SUMOylation of RanGAP (Ran-GTPase activating protein-1) is known to regulate association of RanGAP to the nuclear pore complex (NPC) and control nuclear transport (see review [116]). SUMOylation of PML leads to incorporation of nuclear proteins into the PML nuclear body (see review [89]). Considering these findings, we recently tested the hypothesis that SUMOylation might be activated in human brain affected by polyglutamine disease. We found that SUMO-1 protein is increased in the neurons of human polyglutamine diseased brains and of ataxin-1 transgenic mice [117]. Two subsequent papers supported the change of SUMOylation in polyglutamine diseases, whereas their conclusions on the effect of SUMOylation seem to be mutually contradictory. The Bonini group reported that a mutant form of the SUMO-1 activating enzyme Uba2 enhanced pathology in the *Drosophila* model [118]. This finding suggests that SUMO has a protective role against polyglutamine disease pathology. On the other hand, Terashima et al. reported that SUMOylation leads to inclusion body formation and accelerates cell death [119]. Taken together, the meaning of activated SUMOylation in affected neurons awaits further analysis.

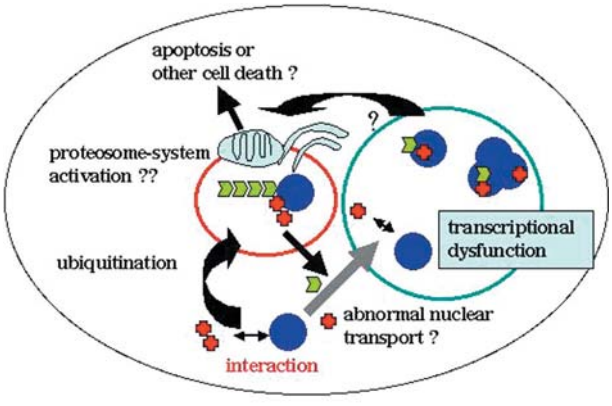

Figure 3. Possible pathological processes surrounding transcriptional repression. Cell, nucleus and aggresome are indicated with black, light blue and red circles, respectively. Transcription-related factors (blue) interact with polyglutamine disease proteins (red cross) either in the cytoplasm or the nucleus. Interaction induces ubiquitination (green arrow) of the factors, and the resultant increase of the substrate possibly leads to proteasome system activation. Ubiquitination may also affect nuclear transport. Apoptosis or the other cell death cascade may be activated by transcriptional dysfunction and/or the proteasome system.

Transcriptional repression causes neuronal dysfunction and death

In this review, I have shown the possible involvement of transcription-related factors in pathology. However, at present, it is not clear whether transcriptional dysfunction results in neuronal death. It is plausible that transcriptional repression, either specific or general, leads to downregulation of genes essential for neuronal function. Several reports show this line of evidence. Dunah et al. reported that huntingtin represses transcription of D1A dopamine receptor via its effect on the transcription factor SP1 [45]. The Sobue group reported that androgen deprivation suppresses progression of phenotype in the transgenic mouse model of Kennedy's disease [120]. The report suggested that transcriptional disturbance of a specific gene might be able to affect neuronal death. Similar cases suggesting dysfunction of specific neuronal gene transcription will probably be reported in cellular and animal models. However, we should not neglect a wide range of transcriptional effects induced by disease proteins that occur simultaneously, as we have already seen. So far, we have only a little evidence that transcriptional repression leads to neuronal death. Nor do we know what kind of cell death is induced by transcriptional repression in neurons. More than 20 years ago, it was reported that suppression of transcription by a specific inhibitor of Pol II, α-amanitin, leads to necrosis in culture cells [121]. Meanwhile, it was also reported that the drug induces upregulation of tumor necrosis factor (TNF), which leads to apoptosis [122, 123]. The mechanism inducing cell death after transcriptional repression was not clarified, and we do not know exactly what kinds of effects are induced by transcriptional repression. Furthermore, the extent of transcriptional repression necessary for cell death is not known. The answer is critical because transcription does not completely cease in neurons expressing mutant polyglutamine disease proteins. These points must be settled in order to confirm transcriptional repression as a cause of neuronal death in polyglutamine diseases.

Slow death or fast death?

The mechanisms of neuronal death in polyglutamine diseases have been investigated with cellular models and transgenic animal models, including mice and flies. Although these models help in understanding the pathology, we cannot rule out the possibility that the types of cell death observed might be different in different models. Mutant polyglutamine proteins induce apoptosis in cellular models and *Drosophila* models (see review [124]), while mouse models have not shown typical apoptosis of affected neurons [125, 126]. Furthermore, there is no definite proof that disease proteins induce apoptosis in human disease brains. The Bates group reported that transgenic mice expressing mutant huntingtin showed volume reduction of brain structures, while neuronal density did not change microscopically [6]. They reported later that neuronal death in transgenic mice is distinct from apoptosis [125]. The Tsuji group recently reported a similar case in the transgenic mouse of atrophin-1 [127 and oral presentation by Tsuji 2003]. They found neither decrease in neuronal density nor morphological change indicating neuronal death, but did find reduction of brain volume with aging. These reports collectively suggest that non-apoptotic neuronal death progresses gradually in their mouse models. It is possible that a similar type of non-apoptotic cell death occurs in human disease brains. Another important claim of the Tsuji group is that neurological symptoms could occur before neuronal death. Their data suggest that the stage of functional disturbance precedes the stage of neuronal death in their mouse model. The suggestion is very attractive and agrees well with recent reports that transcriptional repression occurs in important functional genes for neurons. However, since the pathology of most symptomatic patients clearly shows neuronal loss, further investigations are necessary to explain the entire pathological cascade.

Numerous reports suggest apoptosis in cellular models of neurodegenerative disorders (see review [22]). Cell-biological experiments have also supported the pathway from aggregation to apoptosis. Meanwhile, most data suggesting apoptosis in human disease brains are based on TdT-mediated dUTP-biotin nick end labeling (TUNEL) staining. As well known, TUNEL staining is not specific for apoptosis. It only detects cleaved ends of DNA that are produced both in apoptosis and in other types of cell death. Furthermore, peri- or postmortem events could lead to apoptosis of neurons in human pathology. Thus, these data have not proved apoptosis definitely, which is one reason views of apoptosis remain skeptical. A second reason for skepticism stems from the fact that apoptosis induces only rapidly progressive cell death. Even if we assume a kind of two-hit theory that different processes affect neurons at random, like cancer cells, the progress of neurodegeneration over decades in polyglutamine diseases is too slow for the apoptosis theory. Meanwhile, only a few results support transcriptional repression as the cause of slowly progressive neuronal death. One example is CREB knockout mice [41] and the other is our PQBP-1 transgenic mouse, which shows a late onset motor neuron disease-like phenotype [128]. However, it is clear that this hypothesis is worthwhile testing, since the molecular process of transcriptional death has not been clarified sufficiently.

Conclusion

This review has focused on one of the growing theories for the pathology of polyglutamine diseases. At present, despite of a number of experimental data the transcription theory remains inconclusive and further investigations is essential to establish it. Also, I would like to stress that we should not isolate this pathological mechanism from other possible mechanisms, but should seek mutual links among different pathological mechanisms to understand the whole shape of pathogenesis. Pathological and physiological analyses of the similarity between nuclear bodies and nuclear inclusions based on progress in the basic molecular biology of nuclear functions might open a window on the complex pathology of polyglutamine diseases.

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Note added in Proof. After acceptance, I noticed two important papers are lacked. The first paper describes interaction between NF-kB and huntingtin: Takano H. and Gusella J. F. (2002) The predomantly HEAT-like motif structure of huntingtin and its association and coincident muclear entry with dorsal, and NF-kB/Rel/dorsal family transcription factor. BMC Neurosci. **3:** 15.
The second one is on a possible transcriptional function of LANP: Seo S. B., McNamara P., Heo S., Turner A., Lane W. S. and Chakravarti C. (2001) Regulation of histone acetylation and transcription by INHAT, a human cellular complex containing the set oncoprotein. Cell **104:** 119–130.

1 Cummings C. J. and Zoghbi H. Y. (2000) Fourteen and counting: unraveling trinucleotide repeat diseases. Hum. Mol. Genet. **9:** 909–916
2 Gusella J. F. and MacDonald M. E. (2000) Molecular genetics: unmasking polyglutamine triggers in neurodegenerative disease. Nat. Rev. Neurosci. **1:** 109–115
3 Orr H. T. (2001) Beyond the Qs in the polyglutamine diseases. Genes Dev. **15:** 925–932
4 Fischbeck K. H. (2001) Polyglutamine expansion neurodegenerative disease. Brain Res. Bull. **56:** 161–163
5 Brais B., Bouchard J. P., Xie Y. G., Rochefort D. L., Chretien N., Tome F. M. et al. (1998) Short GCG expansion in the PABP2 gene cause occulopharyngeal muscular dystrophy. Nat. Genet. 18, 164–167
6 Mangiarini L., Sathasivam K., Seller M., Cozens B., Harper A., Hetherington C. et al. (1996) Exon 1of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell **87:** 493–506
7 Ikeda H., Yamaguchi M., Sugai S., Aze Y., Narumiya S. and Kakizuka A. (1996) Expanded polyglutamine in the Machado-Joseph disease protein induces cell death in vitro and in vivo. Nat. Genet. **13:** 196–202
8 Scherzinger E., Sittler A., Schweiger K. Heiser K., Lurz R., Hasenbank R. et al. (1999) Self-assembly of polyglutamine-containing huntingtin fragments into amyloid-like fibrils: implication for Huntington's disease pathology. Proc. Natl. Acad. Sci. USA **96:** 4604–4609
9 Perutz M. F. (1999) Glutamine repeats and neurodegenerative diseases: molecular aspects. TIBS **24:** 58–63
10 Difiglia M., Sapp E., Chase K. O., Davies S. W., Bates G. P., Vonsattel J. P. et al. (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science **277:** 1990–1993
11 Hackam A. S., Singaraja R., Wellington C. L., Metzler M., McCutcheon K., Zhang T. et al. (1998) The influence of huntingtin protein size on nuclear localization and cellular toxicity. J. Cell Biol. **141:** 1097–1105
12 Li S.-H. and Li X.-J. (1998) Aggregation of N-terminal huntngtin is dependent on the length of its glutamine repeats. Hum. Mol. Genet. **7:** 777–782.
13 Miyashita T., Matsui J., Ohtsuka Y., Mami U., Fujishima S., Okamura-Oho Y. et al. (1999) Expression of extended polyglutamine sequentially activates initiator and effector caspases. Biochem. Biophys. Res. Commun. **257:** 724–730
14 Martindale D., Hackam A., Wieczorek A., Ellerby l., Wellington C., McCutcheon K. et al. (1998) Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. Nat. Genet. **18:** 150–154
15 Wellington C. L., Ellerby L. M., Hackam A. S., Margolis R. L., Trifiro M. A., Singaraja R. et al. (1998) Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. J. Biol. Chem. **273:** 9158–9167
16 Butler R., Leigh P. N., McPhaul M. J. and Gallo J.-M. (1998) Truncated forms of the androgen receptor are associated with polyglutamine expansion in X-linked spinal and bulbar muscular atrophy. Hum. Mol. Genet. **7:** 121–127
17 Merry D., Kobayashi Y., Bailey C. K., Taye A. A. and Fischbeck K. H. (1998) Cleavage, aggregation and toxicity of the expanded androgen receptor in spinal and bulbar muscular atrophy. Hum. Mol. Genet. **7:** 693–701
18 Kobayashi Y., Miwa S., Merry D. E., Kume A., Mei L., Doyu M. et al. (1998) Caspase-3 cleaves the expanded androgen receptor protein of spinal and bulbar muscular atrophy in a polyglutamine-length dependent manner. Biochem. Biophys. Res. Commun. **252:** 145–150
19 Yvert G., Lindenberg K. S., Devys D., Helmlinger D., Landwehrmeyer G. B. and Mandel J.-L. (2001) SCA7 mouse models show selective stabilization of mutant ataxin-7 and similar cellular reponses in different cell types. Hum. Mol. Genet. **10:** 1679–1692
20 Li H., Li S.-H., Johnston H., Shelbourne P. F. and Li X.-J. (2000) Amino-terminal fragments of mutant huntingtin show selective accumulation in striatal neurons and synapti toxicity. Nat. Genet. **25:** 385–389
21 Dyer R. B. and McMurray C. T. (2001) Mutant protein in huntingtin disease is resistant to proteolysis in affected brain. Nat. Genet. **29:** 270–278
22 Soto C. (2003) Unfolding the role of protein misfolding in neurodegenerative diseases. Nat. Rev. Neurosci. **4:** 49–60
23 Kalchman M. A., Koide H. B., McCutcheon K., Graham R. K., Nichol K., Nishiyama K. et al. (1997) HIP1, a human homologue of *S. cervisiae* Sla2p, interacts with membrane-associated huntingtin in the brain. Nat. Genet. **16:** 44–53
24 Wanker E. E., Rovira C., Scherzinger E., Hasenbank R., Walter S., Tait D. et al. (1997) HIP-1: a huntingtin interacting protein isolated by the yeast two-hybrid system. Hum. Mol. Genet. **6:** 487–495
25 Li X. J., Li S. H., Sharp A. H., Nucifora F. C. Jr, Schilling G., Lanahan A. et al. (1995) A huntingtin-associated protein enriched in brain with implications for pathology. Nature **378:** 398–402
26 Wanker E. E. (2002) Hip1 and Hippi participate in a novel cell death-signaling pathway. Dev. Cell **2:** 126–128
27 Matilla A., Koshy B. T., Cummings C. J., Isobe T., Orr H. T. and Zoghbi H. Y. (1997) The cerebellar leucine-rich acidic nuclear protein interacts with ataxin-1. Nature **389:** 974–978

28 Waragai M., Lammers C.-H., Takeuchi S., Imafuku I., Udagawa Y., Kanazawa I. et al. (1999) PQBP-1, a novel polyglutamine tract-binding protein, inhibits transcription activation by Brn-2 and affects cell death. Hum. Mol. Genet. **8:** 977–987

29 Okazawa H., Rich T., Chang A., Lin X., Waragai M., Kajikawa M. et al. (2002) Interaction between mutant ataxin-1 and PQBP-1 affects transcription and cell death. Neuron **34:** 701–713

30 Boutell J. M., Thomas P., Neal J. W., Weston V. J., Duce J., Harper P. S. et al. (1999) Aberrant interaction of transcriptional repressor proteins with Huntington's disease gene product, huntingtin. Hum. Mol. Genet. **8:** 1647–1655

31 Hsiao P.-W., Lin D.-L., Nakao R. and Chang C. (1999) The linkage of Kennedy's neuron disease to ARA24, the first identified androgen receptor polyglutramine region-associated coactivator. J. Biol. Chem. **274:** 20229–20234

32 Steffan J. S., Kazantsev A., Spasic-Boskovic O., Greenwald M., Zhu Y. Z., Gohler H. et al. (2000) The Huntington's disease protein interacts with p53 and CREB-binidng protein and represses transcription. Proc. Natl. Acad. Sci. USA **97:** 6763–6768

33 McCampbell A., Taylor J. P., Taye A. A., Robitschek J., Li M., Walcott J. et al. (2000) CREB-binding protein sequestration by expanded polyglutamine. Hum. Mol. Genet. **9:** 2197–2202

34 Nucifora F. C. Jr, Sasaki M., Peters M. F., Huang H., Cooper J. K., Yamada M. et al. (2001) Interference by huntingtin and atrophin-1 with CBP-mediated transcription leading to cellular toxicity. Science **291:** 2423–2428

35 Wood J. D., Nucifora F. C. Jr, Duan K., Zhang C., Wang J., Kim Y. et al. (2000) Atrophin-1, the dentate-rubral and pallido-luysian atrophy gene product, interacts with ETO/MTG8 in the nuclear matrix and represses transcription. J. Cell Biol. **150:** 939–948

36 Irvine R. A., Ma H., Yu M. C., Ross R. K., Stallcup M. R. and Coetzee G. A. (2000) Inhibition of p160-mediated coactivation with increasing androgen receptor polyglutamine length. Hum. Mol. Genet. **9:** 267–274

37 Shibata H., Huynh D. P. and Pulst S.-M. (2000) A novel protein with RNA-binding motifs interacts with ataxin-2. Hum. Mol. Genet. **9:** 1303–1313

38 McCampbell A., Taye A. A., Whitty L., Penney E., Steffan J. S. and Fischbeck K. H. (2001) Histone deacetylase inhibitors reduce polyglutamine toxicity. Proc. Natl. Acad. Sci. USA. **98:** 15179–15784

39 Steffan J. S., Bodal L., Pallos J., Poelman M., McCampbell A., Apostol B. et al. (2001) Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. Nature **413:** 739–743

40 Taylor P. and Fischbeck K. H. (2002) Altered acetylation in polyglutamine disease: an opportunity for therapeutic intervention? TIMM **8:** 195–197

41 Mantamadiotis T., Lemberger T., Bleckmann S. C., Kern H., Kretz O., Villalba A. M. et al. (2002) Disruption of CREB function in brain leads to neruodegeneration. Nat. Genet. **31:** 47–54

42 Shimohata T., Nakajima T., Yamada M., Uchida C., Onodera O., Naruse S. et al. (2000) Expanded polyglutamine streches interact with TAFII130, interfering with CREB-dependent transcription. Nat. Genet. **26:** 29–36

43 Holbert S., Denghien I., Kiechle T., Rosenblatt A., Wellington C., Hayden M. R. et al. (2001) The Gln-Ala repeat transcriptional activator CA150 interacts with huntingtin: neuropathologic and genetic evidence for a role in Huntington's disease pathogenesis. Proc. Natl. Acad. Sci. USA **98:** 1811–1816

44 La Spada A. R., Fu Y. H., Sopher B. L., Libby R. T., Wang X., Li L. Y. et al. (2001) Polyglutamine-expanded ataxin-7 antagonizes CRX function and induces cone-rod dystrophy in a mouse model of SCA7. Neuron **31:** 913–927

45 Dunah A. W., Jeong H., Griffin A., Kim Y.-M., Standaert D. G., Hersch S. M. et al. (2002) Sp1 and TAFII130 transcriptional activity disrupted in early Huntington's disease. Science **296:** 2238–2243

46 Kegel K. B., Meloni A. R., Yi Y., Kim Y. J., Doyle E., Cuiffo B. G. et al. (2002) Huntingtin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein and represses transcription. J. Biol. Chem. **277:** 7466–7476

47 Kazemi-Esfarjani P. and Benzer S. (2002) Suppression of polyglutamine toxicity by a *Drosophila* homolog of myeloid leukemia factor 1. Hum. Mol. Genet. **11:** 2657–2672

48 Diamond M. I., Robinson M. R. and Yamamoto K. R. (2000) Regulation of expanded polyglutamine protein aggregation and nuclear localization by the glucocorticoid receptor. Proc. Natl. Acad. Sci. USA **97:** 657–661

49 Welch W. J. and Diamond M. I. (2001) Glucocorticoid modulation of androgen receptor nuclear aggregation and cellular toxicity is associated with distinct forms of soluble expanded polyglutamine protein. Hum. Mol. Genet. **10:** 3063–3074

50 Skinner P .J., Koshy B. T., Cummings C. J., Klement I. A., Hellin K., Servadio A. et al. (1997) Ataxin-1 with expanded glutamine tract alters nuclear matrix-associated structure. Nature **389:** 971–974

51 Perez M. K., Paulson H. L., Pendse S. J., Saionz S. J., Bonini N. M. and Pittman R. N. (1998) Recruitment and the role of nuclear localization in polyglutamine-mediated aggregation. J. Cell Biol. **143:** 1457–1470

52 Yamada M., Sato T., Shimohata T., Hayashi S., Igarashi S., Tsuji S. et al. (2001) Interaction between neuronal intranuclear inclusions and promyelocytic leukemia protein nuclear and coiled bodies in CAG repeat diseases. Am. J. Pathol. **159:** 1785–1795

53 Rich T., Assier E., Skepper J., Segard H. B., Allen R. L., Charron D. et al. (1999) Disassembly of nuclear inclusions in the dividing cell-a novel insight into neurodegeneration. Hum. Mol. Genet. **8:** 2451–2459

54 La Spada A. R., Wilson E. M. Lubahn D. B., Harding A. E. and Fischbeck K. H. (1991) Androgen receptor gene mutations in X-linked spinobulbar muscular atrophy. Nature **352:** 77–79

55 Koide R., Kobayashi S., Shimohata T., Ikeuchi T., Maruyama M., Saito M. et al. (1999) A neurological disease caused by an expanded CAG trinucleotide repeat in the TATA-binding protein gene: a new polyglutamine disease? Hum. Mol. Genet. **8:** 2047–2053

56 Zuhlke C., Hellenroich Y., Dalski A., Kononowa N., Hagenah J., Vieregge P. et al. (2001) Different types of repeat expansion in the TATA-binding protein gene are associated with a new form of inherited ataxia. Eur. J. Hum. Genet. **9:** 160–164

57 Nakamura K., Jeong S. Y., Uchihara T., Anno M., Nagashima T., Ikeda S. et al. (2001) SCA17, novel autosomal dominant cerebellar ataxia caused by an expanded polyglutamine in TATA-binding protein. Hum. Mol. Genet. **10:** 1441–1448

58 Yue S., Serra H. G., Zoghbi H. Y. and Orr H. T. (2001) The spinocerebellar ataxia type 1 protein, ataxin-1, has RNA-binding activity that is inversely affected by the length of its polyglutamine tract. Hum. Mol. Genet. **10:** 25–30

59 Imafuku I.,Waragai M., Takeuchi S., Kanazawa I., Kawabata M., Mouradian M. M. et al. (1998) Polar amino acid-rich sequences bind to polyglutamine tracts. Biochem. Biophys. Res. Commun. **253:** 16–20

60 Muller-Immergluck M. M., Schaffner W. and Matthias P. (1990) Transcription factor Oct-2A contains functionally redundant activating domains and works selectively from a promoter but not from a remote enhancer position in non-lymphoid (Hela) cells. EMBO J. **9:** 1625–1634

61 Gerster T., Balamaceda C. G. and Roeder R. G. (1990) The cell type-specific octamer transcription factor OTF-2 has two domains required fro the activation of transcription EMBO J. **9:** 1635–1643

62 Tanaka M., Clouston M. W. and Herr W. (1994) The Oct-2-glutamine-rich and proline-rich activation domains can synergize with each other or duplicates of themselves to activate transcription. Mol. Cell Biol. **14:** 6046–6055

63 Woychik N. A. and Hampsey M. (2002) The RNA polymerase II machinery; structure illuminates function. Cell **108:** 453–463

64 Matsuoka K., Taoka M., Satozawa N., Nakayama H., Ichimura T., Takahashi N. et al. (1994) A nuclear factor containing the leucine-rich repeats expressed in murine cerebellar neurons. Proc. Natl. Acad. Sci. USA. **91:** 9670–9674

65 Vaesen M., Barnikol-Watanabe S., Gotz H., Awni L. A., Cole T., Zimmerman B. et al. (1994) Purification of two putative HLA class II associated proteins. PHAPI and PHAPII. Biol. Chem. Hoppe-Seyler **375:** 113–126

66 Li M., Makkinje A. and Damuni Z. (1996) Molecular identification of I1PP2A, a novel potent heat-stable inhibitor protein of protein phosphatase 2A. Biochemistry **35:** 6998–7002

67 Chen T. H., Brody J. R., Romantsev F. E., Yu J. G., Kayler A. E., Voneiff E. et al. (1996) Structure of pp32, an acidic nuclear protein which inhibits oncogene-induced formation of transformed foci. Mol. Biol. Cell **7:** 2045–2056

68 Ulitzur N., Humbert M. and Pfeffer S. R. (19979 Mapmodulin: a possible modulator of the interaction of microtuble-associated proteins with microtubles Proc. Natl. Acad. Sci. USA **94:** 5084–5089

69 Zhang Y.-Z., Lindblom T., Chang A., Sudol M., Ann E., Sluder A. E. et al. (2000) Evidence that dim1 associates with a network of proteins involved in pre-mRNA splicing, and delineation of residues for dim1 interactions with hnRNP F and Npw38/PQBP-1. Gene **257:** 33–43

70 Waragai M., Junn E., Kajikawa M., Takeuchi S., Kanazawa I., Shibata M. et al. (2000) PQBP-1/Npw38, a nuclear protein binding to the polyglutamine tract, interacts with U5-15kD/dim1p via the carboxyl-terminal domain. Biochem. Biophys. Res. Commun. **273:** 592–595

71 Sune C., Hayashi T., Liu Y., Lane W. S., Young R. A. and Garcia-Blanco M. A. (1997) CA150, a nuclear protein associated with the RNA polymerase II holoenzyme, is involved in Tat-activated human immunodeficiency virus type 1 transcription. Mol. Cell. Biol. **17:** 6029–6039

72 Pellizzoni L., Charroux B., Rappsilber J., Mann M. and Dreyfuss G. (2001) A functional interaction between the survival motor neuron complex and RNA polymerase II. J. Cell Biol. **152:** 75–85

73 Alberts B., Johnson A., Lewis J., Raff M., Roberts K. and Walter P. (2002) Molecular Biology of the Cell, 4th ed., 673–674, Garland Science, New York

74 Goodman R. H. and Smolik S. (2000) CBP/p300 in cell growth, transformation, and development. Genes Dev. **14:** 1553–1577

75 Chan H. M. and La Thangue N. B. (2001) p300/CBP proteins: HATs for transcriptional bridges and scaffolds. J. Cell Sci. **114:** 2363–2373

76 Nakamura Y. (1998) ATM: the p53 booster. Nat. Med. **4:** 1231–1232

77 Erickson P., Gao J., Chang K. S., Look T., Whisenant E., Raimondi S. et al. (1992) Identification of breakpoints in t(8;21) acute myelogenous leukemia and isolation of a fusion transcript, AML1/ETO, with similarity to *Drosophila* segmentation gene, runt. Blood **80:** 1825–1831

78 Nisson P. E., Watkins P. C. and Sacchi N. (1992) Transcriptionally active chimeric geen derived from the fusion of the AML1 gene and a novel geen on chromosome 8 in t(8:21) leukemic cells. Cancer Genet. Cytogenet. **63:** 81–88

79 Miyoshi H., Kozu T., Shimizu K., Enomoto K., Maseki N., Kaneko Y. et al. (1993) The t(8:21) chromosomal translocation in acute myeloid leukemia results in production of an AML1-MTG8 fusion transcript. EMBO J. **12:** 2715–2721

80 Hildebrand D., Tiefenbach J., Heinzel T., Grez M. and Mauer A. B. (2001) Multiple regions of ETO cooperate in transcriptional repression. J. Biol. Chem. **276:** 9889–9895

81 Schaeper U., Boyd J. M., Verma S., Uhlmann E., Subramanian T. and Chinnadurai G. (1995) Molecular cloning and characterization of a cellular phosphoprotein that interacts with a conserved C-terminal domain of adenovirus E1A involved in negative modulation of oncogenic transformation. Proc. Natl. Acad. Sci. USA **92:** 10467–10471

82 Sewalt R. G. A. B., Gunster M. J., van der Vlag J., Satijn D. P. E. and Otte A. P. (1999) C-terminal binding protein is a transcriptional repressor that interacts with a specific class of vertebrate polycomb proteins. Mol. Cell. Biol. **19:** 777–787

83 Freund C. L., Gregory-Evans C. Y., Furukawa T., Papaioannou M., Looser J., Ploder L. et al. (1997) Cone-rod dystrophy due to mutations in a novel photoreceptor-specific homeobox gene (CRX) essential for maintenance of the photoreceptor. Cell **91:** 543–553

84 Swain P. K., Chen S., Wang Q.-L., Affatigato L. M., Coats C. L., Brady K. D. et al. (1997) Mutations in the cone-rod homeobox gene are associated with the cone-rod dystrophy photoreceptor degeneration. Neuron **19:** 1329–1336

85 Sohocki M. M., Sullivan L. S., Mintz-Hittner H. A., Birch D., Heckenlively J. R., Freund C. L., et al. (1998) A range of clinical phenotypes associated with mutations in CRX, a photoreceptor transcription-factor gene. Am. J. Hum. Genet. **63:** 1307–1315

86 Swaroop A., Wang Q. L., Wu W., Cook J., Coats C., Xu S. et al. (1999) Leber congenital amaurosis caused by a homozygous mutation (R90W) in the homeodomain of the retinal transcription factor CRX: direct evidence for the involvement of CRX in the development of photoreceptor function. Hum. Mol. Genet. **8:** 299–305

87 Yoneda-Kato N., Look A. T., Kirstein M. N., Valentine M. B., Raimondi S. C., Cohen K. J. et al. (1996) The t(3;5)(q25.1;q34) of myelodysplastic and acute myeloid leukemia produces a novel fusion gene, NPM-MLF1. Oncogene **12:** 265–275

88 Contegno F., Cioce M., Pelicci P. G., and Minucci S. (2002) Targeting protein inactivation through an oligomerization chain reaction. Proc. Natl. Acad. Sci. USA **99:** 1865–1869

89 Zhong S., Salomoni P. and Pandolfi P. P. (2000) The transcriptional role of PML and the nuclear body. Nat. Cell Biol. **2:** E85–E90

90 Hirose Y., and Manley J. L. (2000) RNA polymerase II and the integration of nuclear events. Genes Dev. **14:** 1415–1429

91 Orphandis G. and Reinberg D. (2002) A unified theory of gen expression. Cell **108:** 438–451

92 Glass C. K. and Rosenfeld M. G. (2000) The coregulator exchange in transcriptional functions of nuclear receptors. Genes Develop. **14:** 121–141

93 Huynh D. P., Figueroa K., Hoang N. and Pulst S.-M. (2000) Nuclear localization or inclusion body formation of ataxin-1 are not necessary for SCA2 pathogenesis in mouse and human. Nat. Genet. **26:** 44–50

94 Davies S. W., Turmaine M., Cozens B. A., Difiglia M., Sharp A. H., Ross C. A. et al. (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell **90:** 537–548

95 Waelter S., Boeddrich A., Lurz R., Scherzinger E., Lueder G., Lehrach H. et al. (2001) Accumulation of mutant huntingtin fragments in aggresome-like inclusion as a result of insufficient protein degradation. Mol. Biol. Cell **12:** 1397–1407

96 Tait D., Riccio M., Sittler A., Scherzinger E., Santi S., Ognibene A. et al. (1998) Ataxin-3 is transported into the nucleus and associates with the nuclear matrix. Hum. Mol. Genet. **7:** 991–997

97 Perez M. K., Paulson H. L., and Pittman R. N. (1999) Ataxin-3 with an altered conformation that exposes the polyglutamine domain is associated with the nuclear matrix. Hum. Mol. Genet. **8:** 2377–2385

98 Chai Y., Shao J., Miller V. M., Williams A. and Paulson H. L. (2002) Live-cell imaging reveals divergent intracellular dynamics of polyglutamine disease proteins and supports a sequestration model of pathogenesis. Proc. Natl. Acad. Sci. USA **99:** 9310–9315

99 Stenoien D. L., Mielke M. and Mancini M. A. (2002) Intranuclear ataxin1 inclusions contain both fast- and slow-exchanging components. Nat. Cell Biol. **4:** 806–810

100 Kim S., Nollen E. A. A., Kitagawa K., Bindokas V. P. and Morimoto R. I. (2002) Polyglutamine protein aggregates are dynamic. Nat. Cell Biol. **4:** 826–831.

101 Muratani M., Gerlich D., Janicki S. M., Gebhard M., Elis R. and Spector D. L. (2002) Metabolic-energy-dependent movement of PML bodies within the mammalian cell nucleus. Nat. Cell Biol. **4:** 106–110

102 Luthi-Carter R., Hanson S. A., Strand A. D., Bergstrom D. A., Chun W., Peters N. L. et al. (2002) Dysregulation of gene expression in the R6/2 model of polyglutamine disease: parallel changes in muscle and brain. Hum. Mol. Genet. **11:** 1911–1926

103 Chan E. Y. W., Luthi-Carter R., Strand A., Solano S. M., Hanson S. A., DeJohn M. M. et al. (2002) Increased huntingtin-protein length reduces the number of polyglutamine-induced gene expression changes in mouse models of Huntington's disease. Hum. Mol. Genet. **11:** 1939–1951

104 Sipione S., Rigamonti D., Valenza M., Zuccato C., Conti L., Pritchard J. et al. (2002) Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses. Hum. Mol. Genet. **11:** 1953–1965

105 Lieberman A. P., Harmison G., Strand A. D., Olson J. M. and Fischbeck K. H. (2002) Altered transcriptional regulation in cells expressing the expanded polyglutamine androgen receptor. Hum. Mol. Genet. **11:** 1967–1976

106 Yu Z.-X., Li S.-H., Nguyen H.-P. and Li X.-J. (2002) Huntingtin inclusions do not deplete polyglutamine-containing transcription factors in HD mice. Hum. Mol. Genet. **11:** 905–914

107 Lewis J. D. and Tollervey D. (2000) Like attracts like: getting RNA processing together in the nucleus. Science, **288:** 1385–1389

108 Gall J. G., Bellini M., Wu Z. and Murphy C. (1999) Assembly of the nuclear transcription and processing machinery: Cajal bodies (coiled bodies) and transcriptosomes. Mol. Biol. Cell **10:** 4385–4402

109 Croce L. D., Raker V. A., Corsaro M., Fazi F., Fanelli M., Faretta M. et al. (2002) Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. Science **295:** 1079–1082.

110 Stanek D., Rader S. D., Kingauf M. and Neugebauer K. M. (2003) Targeting of U4/U6 small nuclear RNP assembly factor SART3/p110 to Cajal bodies. J. Cell Biol. **160:** on line

111 Stenoien D .L., Cummings C. J., Adams H. P., Mancini M. G., Patel K., DeMartino G. N. et al. (1999) Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by HDJ-2 chaperone. Hum. Mol. Genet. **8:** 731–741

112 Jonhston J. A., Ward C. L. and Kopito R. R. (1998) Aggresomes: a cellular response to misfolded proteins. J. Cell Biol. **143:** 1883–1898

113 Nishitoh H., Matsuzawa A., Tobiume K., Saegusa K., Takeda K., Inoue K. et al. (2002) ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. Genes Dev. **16:** 1345–1355

114 Buschmann T., Lerner D., Lee C. G. and Ronai Z. (2001) The Mdm-2 amino terminus is required for Mdm2 binding and SUMO-1 conjugation by the E2 SUMO-1 conjugating enzyme Ubc9. J. Biol. Chem. **276:** 40389–40395

115 Silverman N. and Maniatis T. (2001) NF-kappaB signaling pathways in mammalian and insect innate immunity. Genes Dev. **15:** 2321–2342

116 Muller S., Hoege C., Pyrowolakis G. and Jentsch S. (2001) SUMO, ubiquitin's mysterious cousin. Nat. Rev. Mol. Cell Biol. **2:** 202–210

117 Ueda H., Goto J., Hashida H., Lin X., Oyanagi K., Kawano H. et al. (2002) Enhanced SUMOylation in polyglutamine diseases. Biochem. Biophys. Res. Commun. **293:** 307–313

118 Chan H. Y. E. Warrick J. M., Andriola I., Merry D. and Bonini N. (2002) Genetic modulation of polyglutamine toxicity by protein conjugation pathways in *Drosophila*. Hum. Mol. Genet. **11:** 2895–2904

119 Terashima T., Kawai H., Fujitani M., Maeda K. and Yasuda H. (2002) SUMO-1 co-localization with mutant atrophin-1 with expanded polyglutamines accelerates intranuclear aggregation and cell death. Neuroreport **13:** 2359–2364

120 Katsuno M., Adachi H., Kume A., Li M., Nakagomi Y., Niwa H. et al. (2002) Testosterone reduction prevents phenotypic expression in a transgenic mouse model of spinal and bulbar muscular atrophy. Neuron **35:** 843–54

121 Stirpe F. and Fiume L. (1967) Studies on the pathogenesis of liver necrosis by alpha-amanitin. Effect of alpha-amanitin on ribonucleic acid synthesis and on ribonucleic acid polymerase in mouse liver nuclei. Biochem. J. **105:** 779–782

122 Voitenok N. N., Misuno N. I., Panyutich A. V. and Kolesnikova T. S. (1989) Induction of tumor necrosis factor synthesis in human monocytes treated by transcriptional inhibitors. Immunol. Lett. **20:** 77–82

123 Leist M., Gantner F., Naumann H., Bluethmann H., Vogt K., Brigelius-Flohe R. et al. (1997) Tumor necrosis factor-induced apoptosis during the poisoning of mice with hepatotoxins. Gastroenterology **112:** 923–34

124 Ross C. A. (2002) Polyglutamine pathogenesis: emergence of unifying mechanisms for Huntington's disease and related disorders. Neuron **35:** 819–822

125 Turmaine M., Raza A., Mahal A., Mangiarini L., Bates G. P. and Davies S. W. (2000) Nonapoptotic neurodegeneration in atransgenic mouse model of Huntington's disease. Proc. Natl. Acad. Sci. USA **97:** 8093–8097

126 Garden G. A., Libby R. T., Fu Y. H., Kinoshita Y., Huang J., Possin D. E. et al. (2002) Polyglutamine-expanded ataxin-7 promotes non-cell-autonomous purkinje cell degeneration and displays proteolytic cleavage in ataxic transgenic mice. J. Neurosci. **22:** 4897–4905

127 Sato T., Oyake M., Nakamura K., Nakao K., Fukusima Y., Onodera O. et al. (1999) Transgenic mice harboring a full-length human mutant DRPLA gene exhibit age-dependent intergenerational and somatic instabilities of CAG repeats comparable with those in DRPLA patients. Hum. Mol. Genet. **8:** 99–106

128 Okuda T., Hattori H., Takeuchi S., Shimizu J., Ueda H., Palvimo J. J. et al. (2003) PQBP-1 transgenic mice show a late–onset motor neuron disease-like phenotype. Hum. Mol. Genet. **12:** 711–725