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Polyglutamine spinocerebellar ataxias — from genes to potential treatments

Henry L. Paulson¹, Vikram G. Shakkottai¹, H. Brent Clark², and Harry T. Orr^{2,3}

¹Department of Neurology, University of Michigan, Ann Arbor, Michigan, 48109, USA.

²Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, Minnesota, 55455, USA.

³Institute for Translational Neuroscience, University of Minnesota, Minneapolis, Minnesota, 55455, USA.

Abstract

The dominantly inherited spinocerebellar ataxias (SCAs) are a large and diverse group of neurodegenerative diseases. The most prevalent SCAs (SCA1, SCA2, SCA3, SCA6 and SCA7) are caused by expansion of a glutamine-encoding CAG repeat in the affected gene. These SCAs represent a substantial portion of the polyglutamine neurodegenerative disorders and provide insight into this class of diseases as a whole. Recent years have seen considerable progress in deciphering the clinical, pathological, physiological and molecular aspects of the polyglutamine SCAs, with these advances establishing a solid base from which to pursue potential therapeutic approaches.

The spinocerebellar ataxias (SCAs) are a large and diverse group of autosomal-dominant, progressive neurodegenerative diseases. They share the clinical feature of progressive ataxia, reflecting degeneration of the cerebellum and, often, other connected regions of the nervous system. The prevalence of the SCAs varies markedly depending on geography and ethnicity but is estimated to be 1–3 per 100,000 among Europeans¹. Although many of the SCAs result from point mutations, DNA rearrangements (SCA15, SCA16 and SCA20) or expansions of non-coding repeats (SCA8, SCA10, SCA31 and SCA36) (BOXES 1,2), the most common SCAs are caused by expansion of a CAG nucleotide repeat that encodes polyglutamine (polyQ) in the relevant disease proteins. These polyQ SCAs include SCA1–SCA3, SCA6, SCA7 and SCA17, which are caused by expanded polyQ sequences in ataxin 1 (ATXN1), ATXN2, ATXN3, subunit- α of the Cav2.1 voltage-gated calcium channel (CACNA1A), ATXN7 and TATA-box-binding protein (TBP), respectively. Disease severity

Correspondence to H.L.P. and H.T.O., henryp@med.umich.edu; orrx002@umn.edu.

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tends to be greater, and survival shorter, for patients with a polyQ SCA than for those with a non-polyQ SCA².

The genetic diversity of the SCAs, combined with the relative conservation of cerebellar structure among mammals, make these diseases a biologically robust experimental platform for discovering fundamental aspects of cerebellar function and dysfunction. In turn, these discoveries have implications for the development of potential therapeutic approaches and for the understanding of other neurodegenerative diseases — particularly other polyQ neurodegenerative diseases. In this article, we review the clinical, molecular and physiological aspects of the most common SCAs, the polyQ SCAs, and describe how molecular genetic approaches and model systems have advanced our understanding of essential features of their underlying pathogenesis. An emerging theme is that although the individual disease proteins that result in the polyQ SCAs are structurally and functionally different, the effects of the mutations on cellular processes and neuronal activity overlap considerably, leading to similar pathology and clinical features.

Clinical and pathological changes

The symptoms of polyQ SCAs typically start in adulthood and slowly progress over many years, culminating in death by brainstem failure, although this is not the case for SCA6, which typically spares cranial motor nerves³. Gait ataxia is most often the initial sign in patients; later signs include limb incoordination, speech disturbance and oculomotor abnormalities^{4, 5}. There is considerable variability in disease features, however, partly owing to differences in the size of the polyQ repeat expansion; longer expansions are associated with earlier symptom onset and a broader range of neurological symptoms. Symptoms include cerebellar ataxia, pyramidal symptoms and extrapyramidal symptoms, as well as impaired control of eye movements sometimes culminating in ophthalmoplegia and peripheral neuropathy. Individuals with SCA1, SCA2 or SCA3 usually show signs of clinical sensorimotor or sensory neuropathy, consistent with peripheral nerve involvement. Brainstem dysfunction in the SCAs leads to abnormal visual and brainstem auditory evoked potentials^{6, 7}.

The neuropathology of the polyQ ataxias is also complex and varied. In SCA1, SCA2 and SCA3, cerebellar and brainstem degeneration are nearly always prominent, whereas more anterior regions (such as the basal ganglia) and posterior regions (such as the spinal cord) exhibit a variable degree of damage^{8–10}.

Perturbations in neuronal circuitry and eventual neuronal loss affect many aspects of the cerebellar motor system in the SCAs (FIG. 1). The affected afferent components include the mossy fibres that arise in the spinocerebellar tracts, the vestibular nuclei and basal pontine nuclei, as well as climbing fibres from the olivary nuclei. Other proprioceptive sensory pathways, such as those ascending from the posterior columns of the spinal cord, can also be impaired. The cerebellar cortex itself is a frequent target of the SCAs, with Purkinje cells being the neuronal population that is most frequently affected by these diseases. The efferent components that are affected include the deep nuclei of the cerebellum, as well as their targets in the red nucleus and thalamus. The pathological variability within each polyQ SCA

makes it difficult to define a distinctive pattern for each type, but certain areas tend to be affected preferentially in each SCA.

SCA1.

The age of onset of SCA1 ranges from childhood to late adult life, with a mean onset in the fourth decade^{11, 12}. Earlier age at onset and larger expanded alleles are associated with faster symptom progression¹¹. Of the polyQ SCAs, SCA1 progresses the fastest (REF. ¹¹) and is associated with concomitant progression of cerebellar and non-cerebellar symptoms.

The typical pathological pattern in SCA1 is olivopontocerebellar atrophy¹³, which also occurs in SCA2 and several other SCAs. In addition to the profound loss of cerebellar Purkinje cells and the neurons of the dentate, basal pontine and olivary nuclei, other brainstem areas, including the red nuclei, vestibular nuclei and motor cranial nerve nuclei⁴, are often affected, whereas the pars compacta of the substantia nigra is usually spared. Involvement of the spinal cord varies greatly, and the basal ganglia, thalamus, cerebral cortex and hippocampus are typically spared^{9, 13}.

SCA2.

SCA2 typically presents with progressive ataxia, slowed eye movements and sensorimotor neuropathy. Extrapyramidal manifestations, including parkinsonism, motor weakness, ocular palsies and cognitive impairment, may also occur. The age of onset ranges from infancy to later adult life, with a mean onset in the mid-30s^{11, 12}. As in SCA1, an earlier age at onset and larger expanded alleles are associated with faster symptom progression¹¹.

As in SCA1 brains, most SCA2 brains at autopsy have olivopontocerebellar atrophy. Whereas the cerebellar cortical changes associated with SCA2 resemble those that occur in SCA1, the deep cerebellar nuclei are mostly spared in SCA2 (REFS ^{14–16}). The striatonigral pathways are more severely affected in SCA2 than in SCA1, as dopaminergic neurons in the substantia nigra and neurons in the neostriatum and pallidum are lost^{14, 15}. The red nucleus and thalamus are also usually affected in SCA2¹⁵. Motor cranial nerve nuclei are variably involved¹⁶, and the spinal cord often shows a loss of neurons in the anterior horn and the dorsal nucleus of Clarke (also known as the posterior thoracic nucleus), along with atrophy of the spinocerebellar tracts and posterior columns^{14, 15}.

Intermediate-range CAG repeat expansions (29–34 repeats) at the *ATXN2* locus are also a risk factor for amyotrophic lateral sclerosis (ALS)¹⁷. *ATXN2*-associated ALS is classic ALS: it is associated with a combination of upper and lower motor neuron signs; disease onset typically occurs in the 50s; and the survival time after onset is less than 3 years¹⁸. However, selective loss of Purkinje cells in the cerebellar vermis has been reported in *ATXN2*-associated ALS, unlike in other types of ALS¹⁹.

SCA3.

Also known as Machado–Joseph disease, SCA3 most commonly presents with progressive ataxia and spasticity⁸. The clinical phenotype, however, is so variable that some have classified SCA3 into the following subtypes: early-onset disease with extrapyramidal signs

and spasticity but minimal ataxia (Type 1); midlife progressive ataxia (Type 2); later-onset ataxia accompanied by neuropathy, amyotrophy and loss of reflexes (Type 3); and parkinsonism with or without ataxia (Type 4)^{8–10}.

SCA3 neuropathology differs from that of SCA1 and SCA2 in that the cerebellar cortex and olivary nuclei are relatively less affected in SCA3, whereas the deep cerebellar nuclei and basis pontis are usually more severely affected^{20–22}. Spinal, vestibular and pontine afferents to the cerebellum are also lost, along with their nuclei. The red nucleus is affected, but the thalamus is usually only minimally involved. Extrapyramidal pathology involves the substantia nigra, the subthalamic nuclei and the globus pallidus. There is often a loss of motor neurons in the cranial nerve nuclei and the anterior horns of the spinal cord.

SCA6.

SCA6 begins later than other polyQ ataxias, with the median age of onset in the early 50s²³. Clinically, SCA6 is considered a relatively pure cerebellar syndrome with few extracerebellar signs¹, and seems not to reduce lifespan²³. Symptoms may be episodic in individuals with the shortest disease-causing repeat sequences⁴. Clinical dysfunction in SCA6 correlates best with the degree of cerebellar atrophy²⁴. Macroscopically, atrophy is confined to the cerebellum. Microscopically, however, the neuropathology in SCA6 shares features with that of other polyQ SCAs, although involvement beyond the cerebellar Purkinje neurons is much less prominent²². Loss of the giant pyramidal Betz neurons of the cerebral cortex has been described in SCA6, and there is variable involvement of the brainstem and cerebellar nuclei²⁵.

Functions and pathogenic mechanisms

Whereas it is widely recognized that expansion of the polyQ tract in each of the proteins associated with neurodegenerative diseases alters protein conformation and leads to protein aggregation²⁶, there remains debate about the mechanisms underlying the neuronal vulnerability in each of the polyQ SCAs. As reviewed below, data suggest that protein context matters greatly and that for any given polyQ SCA, the perturbations of the native functions of the disease protein and the mechanisms responsible for toxic gain of function are probably interrelated (FIG. 2). A common refrain is that the disease proteins affected in polyQ SCAs participate in the molecular regulation of gene expression at the transcriptional and/or post-transcriptional level and that polyQ expansion perturbs this regulation. An actively pursued hypothesis is that the misfolding, accumulation and/or aggregation of the expanded polyQ protein disrupts the delicate balance of RNA and protein homeostasis in the nervous system and does so in a disease-specific manner that is dictated in part by the structure (FIG. 3) and function of the protein. In this section, we describe the normal functions of the proteins affected by polyQ expansion in the polyQ SCAs and examine how a loss of these functions and/or a gain of toxic functions may be induced by expanded polyQ tracts.

ATXN1 and SCA1.

Wild-type ATXN1 includes a polyQ tract that normally contains 6–34 glutamines, with repeats longer than 20 glutamines containing 1–3 histidine interruptions. In addition, several other functional motifs and domains have been identified in ATXN1 (FIG. 3). A monopartite nuclear-localization signal (NLS) motif near the carboxyl terminus directs the localization of the protein to the nucleus²⁷. ATXN1 containing a wild-type polyQ tract shuttles between the cytoplasm and nucleus. By contrast, ATXN1 containing an expanded polyQ tract is transported to the nucleus but cannot be exported from the nucleus²⁸. A single amino-acid substitution within its NLS prevents expanded ATXN1 from entering the nuclei of Purkinje cells and eliminates its toxicity²⁷, indicating that at least in these cells, disease pathogenesis may be linked to a function of ATXN1 in the nucleus.

Wild-type and expanded ATXN1 interact with various nuclear components, including RNA^{28,29}, several regulators of transcription, SMRT³⁰ (silencing mediator of retinoic acid and thyroid hormone receptor; also known as NCOR2), capicua (CIC)³¹, the zinc finger protein GFI1 (homologue of senseless in *Drosophila*)³² and the nuclear receptor- α (ROR α)-60 kDa Tat-interactive protein (TIP60; also known as KAT5) complex^{33,34}. The interaction with the transcriptional repressor CIC is subserved by the 130-amino-acid region of ATXN1 known as the ATXN1 HBP1 (AXH) domain, which independently forms an oligonucleotide/oligosaccharide-binding (OB) fold^{35,36}. The importance of ATXN1–CIC complexes in SCA1 pathogenesis is suggested by the observation that reducing CIC levels — either genetically or through exercise — dampens the deficit in motor performance and the premature lethality of the SCA1-like phenotypes seen in *Atxn1*^{154Q/2Q}-knock-in mice³⁷ (see BOX 3 on modelling SCAs in mice).

Near the C terminus of ATXN1, just downstream of the NLS, is Ser776, one of seven phosphorylation sites in ATXN1 (REFS^{38,39}). Replacement of S776 with an alanine, which cannot be phosphorylated, mitigates the ability of ATXN1 [82Q] to induce Purkinje cell disease in transgenic mice³⁸. Substituting an aspartic acid at position 776, to mimic phosphorylation, enhances the ability of ATXN1[82Q] to induce Purkinje cell disease and converts ATXN1[30Q] into a protein that can induce ataxia in mice when expressed in Purkinje cells⁴⁰. Ser776 phosphorylation leads to a reduction in the proteolytic clearance of ATXN1 (REF. 41), activation of a binding motif in ATXN1 for the chaperone 14-3-3 (REE⁴²), an increase in ATXN1 binding to the splicing factor RBM17 (REF. 43) and a decrease in ATXN1 interactions with the splicing factor U2AF65 (also known as U2AF2)⁴⁴.

FIGURE 2 presents a model of SCA1 pathogenesis that incorporates the crucial nuclear interactions and functions of ATXN1. An important aspect underlying this model is the concept that transcription and RNA processing, including splicing, are coupled spatially and kinetically⁴⁵. On entering the nuclei of Purkinje cells, ATXN1 readily binds to CIC. Although ATXN1 alone does not bind to DNA²⁹, CIC can bind DNA; thus, the ATXN1–CIC complex is targeted to chromatin and transcription sites. Notably, as transcription proceeds, ATXN1 is hypothesized to shuttle between complexes with CIC and those with RBM17. This idea is supported by analyses of cerebellar extracts revealing that ATXN1 can be found in two high-molecular-weight soluble complexes: one that includes CIC and another, distinct complex that includes RBM17 (REF. 43). These two ATXN1 complexes are envisioned to be

in a dynamic equilibrium — perhaps determined by the length of the polyQ tract and the phosphorylation of Ser776 — and the dynamics of the complexes affect gene expression. Importantly, expansion of the polyQ tract in ATXN1 favours ATXN1-RBM17 complex formation⁴³, implicating this complex in SCA1 pathogenesis.

Cerebellar RNA-sequencing studies using transgenic mice expressing ATXN1 [82Q] identified a gene network whose expression correlated with disease progression⁴⁶. Pathway analysis indicated that genes involved in synaptic long-term depression (LTD) and glutamate receptor signalling were particularly affected in transgenic mice compared with controls; both of these pathways have been linked to the pathogenesis of SCA1 and other ataxias^{47–49}. Proteomic approaches indicate that alterations in mechanistic target of rapamycin (mTOR) signalling contribute to SCA1 cerebellar pathogenesis^{50,51}. In the cerebellar proteomes of transgenic mice with *ATXN1* [82Q]-expressing Purkinje cells, mTOR levels were decreased before the onset of symptoms⁵¹. Proteomic analysis of synaptic proteins in disease-susceptible Purkinje cells in early-stage *Scal*^{154Q/2Q}-knock-in mice showed that the levels of HOMER3, a synaptic scaffold protein enriched in Purkinje cells, were dramatically reduced⁵⁰. Importantly, either pharmacological or genetic reductions in Purkinje cell expression of mTOR complex 1 (mTORC1) worsened cerebellar-linked behaviour and pathology in the *Scal*^{154Q/2Q} mouse model, whereas increased Purkinje cell expression of HOMER3 attenuated these disease features.

Interestingly, mTORC1 signalling is also altered in Huntington disease (HD) and in mouse HD models⁵². Restoring mTORC1 activity in the N171-82Q mouse model of HD by expressing a constitutively active form of the GTP-binding proteins RHEB (an mTORC1 regulator) or RHES (an mTORC1 activator enriched in the striatum) alleviated HD-like motor deficits and pathology. Thus, a decrease in mTOR signalling — particularly mTORC1 signalling — is observed in SCA1 and HD and probably in several other neurodegenerative disorders as well.

Changes in DNA-damage and DNA-repair pathways have also been implicated in the pathogenesis of neurodegenerative disorders⁵³, including many polyQ diseases⁵⁴. In SCA1, enhanced expression of replication factor A1 (RPA1), a protein that functions in several DNA-repair pathways, was shown to improve motor performance and Purkinje cell morphology, as well as molecular functions, in *Scal*^{154Q/2Q} mice⁵⁵. In addition, restoration of mitochondrial DNA repair by expressing the DNA-repair protein high mobility group protein B1 (HMGB1) attenuated SCA1-like phenotypes in *Scal*^{154Q/2Q} mice⁵⁶.

Thus, several cellular pathways are implicated in SCA1 pathogenesis. The multi-pathway picture of SCA1 pathogenesis creates challenges for the development of new therapies, as discussed later in this Review.

ATXN2 and SCA2.

ATXN2 is a 145 kDa protein that is highly conserved across species⁵⁷. Considerable evidence suggests that ATXN2 functions in cytoplasmic aspects of RNA metabolism. Initial evidence for this came from the finding that ATXN2 bears like-Sm (LSm) motifs, which are associated with mRNA decay in the cytoplasm⁵⁸. Many identified ATXN2-interacting

proteins were subsequently shown to function in RNA metabolism. For example, the polyadenylate-binding protein (PABP)-interacting motif of ATXN2, PAM2 (REF. 59), enables the protein to interact with PABP and a putative RNA-binding protein called ATXN2-binding protein⁶⁰. One way in which ATXN2 functions in RNA metabolism is in the assembly of stress granules and processing bodies (P-bodies): both ATXN2 and PABP are components of stress granules and P-bodies⁶¹. Moreover, the *Drosophila* homologue of ATXN2, *Atx2*, is needed for microRNA (miRNA)-mediated repression of the translation of several mRNAs, raising the possibility that ATXN2 might also act in miRNA pathways in mammals⁶².

Further studies of ATXN2 homologues in different model organisms provide evidence linking ATXN2 to the regulation of translation. Yeast Pab1 (a homologue of PABP1), which promotes translation, genetically interacts with the ATXN2 homologue, Pbp1 (REF. 63). Furthermore, overexpression of Pbp1 in yeast causes effects similar to those observed when translation is inhibited by cycloheximide. Direct evidence linking ATXN2 to translation comes from studies showing that ATXN2 or its homologue forms complexes with polyribosomes. *Atx2* in *Drosophila* (and ATXN2 in transfected human cells) co-sediments with polyribosomes, and this association is mediated by the LSm and PAM2 motifs⁶⁴. The association of ATXN2 with components of translationally active polyribosomes and translationally silent RNA granules suggests that it may regulate the trafficking of mRNA between these structures, allowing a cell to tailor translation to its needs in response to changes in the environment. For example, ATXN2 regulates the cellular growth response to nutrient signals: studies in yeast⁶⁵, nematode⁶⁶ and mammalian cells⁶⁷ show that ATXN2 inhibits the mTOR pathway in part by sequestering mTORC in stress granules.

Another intriguing functional link between ATXN2 and RNA metabolism relates to the observation that intermediate-length CAG-repeat tracts (encoding 27–33 glutamines) in *ATXN2* are an important risk factor for sporadic ALS. This finding fits with the emerging view that many ALS risk genes, including the gene encoding TAR DNA-binding protein 43 (TDP43; also known as TARDBP), regulate RNA metabolism⁶⁸; moreover, several findings functionally link TDP43 and ATXN2. TDP43 is a heterogeneous nuclear ribonucleoprotein that, similar to ATXN2, is involved in several aspects of RNA metabolism, including transcription, alternative splicing and RNA stability. In a yeast screen for modifiers of TDP43 toxicity, the yeast ATXN2 homologue, Pbp1, increased TDP43-mediated toxicity. Moreover, *Atx2* also increases TDP43 toxicity in *Drosophila*. In addition, in yeast and mammalian cells, the interaction between TDP43 and ATXN2 is RNA-dependent¹⁷. Perhaps most intriguing is that, similar to TDP43, ATXN2 mislocalizes to cytoplasmic puncta in ALS motor neurons. Intermediate-length CAG-repeat expansions in *ATXN2* (27–33 repeats) are considerably more prevalent in individuals with ALS than in unaffected individuals^{17,18}, suggesting that variation in polyQ tract length — even in the wild-type range — directly affects function, almost certainly through an aspect of RNA metabolism involving ATXN2 and TDP43.

How polyQ expansion in ATXN2 causes SCA2 remains unknown. The role of ATXN2 in stress granule formation and translational regulation, however, raises the intriguing possibility that polyQ expansion in ATXN2 alters its interactions with key proteins and

complexes (such as PABP, TDP43 and mTORC), thereby perturbing neuronal RNA and protein homeostasis. Evidence from a mouse model expressing ATXN2 [Q42] supports this view; in this model, the mutant ATXN2 was observed to sequester, in insoluble form, its known interactor PABPC1 (REF. 69). Recent expression profiling in a different SCA2 mouse model expressing ATXN2[Q127] revealed dysregulated expression of cerebellar gene clusters in several pathways, including GTPase signalling pathways, calcium signalling pathways and cell death pathways⁷⁰. These changes were largely absent in *Atxn2*^{-/-} mice, suggesting that toxic gain-of-function mutations rather than loss-of-function mutations are a key driver of disease⁷⁰.

ATXN3 and SCA3.

The SCA3 disease protein ATXN3 is a specialized deubiquitinase (DUB) involved in ubiquitin-dependent protein quality control (reviewed in REFS 71,72). This 42 kDa protein has an amino-terminal catalytic domain (termed the Josephin domain) and a flexible C-terminal tail that includes three ubiquitin-interacting motifs (UIMs) that flank the polyQ tract. In addition to the UIMs, which bind ubiquitin chains with high affinity (FIG. 3), the Josephin domain of ATXN3 contains other ubiquitin-binding sites that regulate catalytic activity and protein stability^{73,74}. As a DUB, ATXN3 preferentially acts on longer chains of at least four ubiquitins in length and may cleave K63-linked chains and mixed-linkage chains more readily than K48-linked chains. The UIMs probably specify the types of ubiquitin chains (in terms of both length and linkage) that are bound and cleaved by ATXN3, and mono-ubiquitylation of ATXN3 itself on a specific lysine residue, K117, enhances its activity⁷⁵. This post-translational modification may accelerate ATXN3 function under conditions of ubiquitin stress.

Evidence suggests that ATXN3 participates in various ubiquitin-dependent pathways to maintain protein homeostasis. A primary function of ATXN3 may be to partner with ubiquitin ligases to regulate, or edit, the lengths and linkage types of ubiquitin chains on substrates. Two important E3 ligase partners are parkin and CHIP (also known as STUB1), both of which have crucial roles in ubiquitin-dependent protein homeostasis^{76,77}. ATXN3 has also been implicated in endoplasmic reticulum-associated degradation⁷⁸, aggresome production⁷⁵ and DNA repair⁸⁰⁻⁸³, among other pathways. In all these processes, ATXN3 acts through ubiquitin-dependent functions. ATXN3 has been reported to interact with more than 100 proteins, many of which are directly linked to ubiquitin-dependent protein quality control. These include valosin-containing protein (VCP), UV excision repair protein RAD23 and many molecular chaperones. Strikingly, when overexpressed, non-expanded ATXN3 actually suppresses expanded polyQ-mediated neurotoxicity in *Drosophila* models⁸⁴, supporting the idea that ATXN3 has a role in maintaining ubiquitin-dependent protein homeostasis.

It is intriguing that a protein whose very function counters perturbations in protein homeostasis in neurodegeneration also causes neurodegeneration when harbouring a polyQ expansion. Although data indicate that polyQ expansion confers a toxic gain-of-function to the disease proteins, is it possible that, in the case of ATXN3 a loss of this vital quality-control protein contributes in some way to disease development? *In vitro*, at least, expanded

ATXN3 still binds and cleaves polyubiquitin chains, suggesting that it retains activity. However, polyQ expansion could conceivably alter the ability of ATXN3 to bind to or cleave ubiquitin chains in the cellular milieu. For example, the ability of expanded ATXN3 to interact with parkin or CHIP is indeed altered; expanded ATXN3 binds CHIP more tightly, reducing the levels of this important quality-control ubiquitin ligase⁷⁷. Further investigation of the DUB function of ATXN3 in neurons is needed, however, to support the notion that a partial loss of function contributes to disease. Nevertheless, it seems clear that a profound loss of ATXN3 function is not the primary cause of SCA3: mice lacking ATXN3 are essentially normal except for increased levels of polyubiquitin in the brain, and neurodegeneration in a knock-in mouse model of HD is not accelerated when ATXN3 is eliminated⁸⁵.

One attractive hypothesis for the pathogenesis of SCA3 is that conformational changes induced by polyQ expansion could alter ATXN3 DUB function in multiple ubiquitin-dependent pathways. The roles of ubiquitin in the nervous system extend far beyond proteasomal degradation, and mutant ATXN3 could precipitate myriad subtle changes in ubiquitin-dependent cellular processes that collectively prove deleterious ('death by a thousand cuts'). A normal-sized polyQ domain in ATXN3 may permit conformational flexibility in the C-terminal domain that facilitates binding to diverse polyubiquitin chains and ubiquitylated substrates, whereas expansion would favour a kinetically 'trapped' conformation that perturbs ATXN3 interactions with its normal binding partners, with many deleterious consequences for the cell.

The well-described aggregation of mutant ATXN3 in SCA3 occurs both in the form of large intranuclear inclusions in neurons and as smaller cytoplasmic and axonal puncta. In SCA3, or when cells are stressed, ATXN3 also moves from the cytoplasm, its primary site of residence, into the nuclei of neurons. Thus, its nuclear relocation in disease may reflect an indirect response to stress or may directly result from changes to the mutant protein itself. Its presence in the nucleus contributes to its toxicity⁸⁶, but precisely how its nuclear localization contributes to such toxicity remains unclear.

Although the downstream consequences of polyQ expansion in ATXN3 that are principally responsible for its toxicity are currently unknown, the mutant gene product is clearly somehow neurotoxic. As *ATXN3* is a nonessential gene, reducing levels of the mutant protein — that is, to intervene proximally in the neurotoxic cascade — is an attractive therapeutic option. Nucleotidebased gene-silencing approaches, including RNA interference and antisense oligonucleotides, have established that suppressing expression of the mutant protein in mouse models does not have deleterious consequences for the nervous system^{87–90}. Moreover, small-molecule screens have begun to identify existing US-approved drugs that can reduce the toxicity, or steady-state levels, of mutant ATXN3. For example, citalopram and other selective serotonin-reuptake inhibitors reduce ATXN3 toxicity in nematode and mouse models of SCA3 through a serotonin receptor-dependent pathway⁹¹, and aripiprazole, an atypical antipsychotic with complex pharmacology, recently surfaced from a cell-based drug screen as a compound that reduces the levels of mutant ATXN3 (REF. 92). Studies of these and other compounds will be required to determine their potential as disease-modifying agents and their mechanisms of action. One of many

potential mechanisms of action is through the boosting of one or more protein quality-control pathways, such as molecular chaperone-mediated clearance or autophagy^{93,94}, the efficacy of which has been shown in animal models. In addition to strategies intended to lower mutant ATXN3 levels, strategies intended to alter its nuclear relocation, phosphorylation and proteolysis — all of which can modulate toxicity — are under investigation^{95–97}.

CACNA1A and SCA6.

With the discovery of CACNA1A as the disease gene affected in SCA6 (REFS 98,99), the basis of the disease was attributed to an expanded C-terminal polyQ tract in the gene product, the α 1A transmembrane subunit of the Cav2.1 calcium channel. The Cav2.1 channel is abundantly expressed by cerebellar Purkinje cells, and CACNA1A is mutated in several other inherited neurological disorders, including episodic ataxia type 2 and familial hemiplegic migraine type 1 (REF. 100). The possibility that the function of the channel is altered, however, continues to be debated, and recent data suggest that the pathogenesis of the disease is driven by an alternative mechanism.

In wild-type mice and in individuals without SCA6, the polyQ-containing cytoplasmic C terminus of the α 1A subunit of the Cav2.1 channel is cleaved, generating a stable C-terminal peptide that transits to the nuclei of Purkinje cells. In culture, the nuclear localization of the expanded polyQ-containing C-terminal peptide is toxic to cells¹⁰¹. In addition, CACNA1A encodes a bicistronic mRNA (with an internal ribosomal entry site (IRES)) that can be translated into two proteins¹⁰²(FIG. 2): the α 1A subunit of the Cav2.1 channel and the transcription factor α 1ACT, which enhances the expression of numerous genes implicated in neuronal differentiation and Purkinje cell development, including GRN (which encodes granulin), PMCA2 (which encodes a calcium ATPase) and BTG1 (which encodes B cell translocation gene 1 protein). α 1ACT containing an expanded polyQ is less able to enhance the expression of these genes, and blocking the IRES-driven translation of polyQ-expanded α 1ACT rescues SCA6-like phenotypes in a mouse model of SCA6. These results suggest that an expanded polyQ-containing C-terminal domain of the gene product — whether by cleavage of the α 1A subunit of the Cav2.1 channel or by IRES-mediated translation of α 1ACT — and its subsequent translocation to the nucleus contribute to disease.

Compared with other polyQ SCAs, SCA6 contains several unique features, including a much lower repeat-length threshold for disease (disease-causing tracts are only 20–33 repeats long) and the fact that the Cav2.1 protein is embedded in the plasma membrane (all other polyQ disease proteins are soluble). Perhaps linked to these distinctive features, there is much less evidence to suggest that disease-protein accumulation and aggregation occur in SCA6 compared with what is known about the other polyQ SCAs. Arguably, the best evidence is found in mouse models expressing hyper-expanded forms of CACNA1A. For example, knock-in mice expressing the major Cav2.1 subunit splice isoform with a 118Q expansion show selective degeneration of Purkinje cells and accumulation of the disease protein in what seem to be lysosomes¹⁰³. Moreover, inclusion formation and Purkinje cell degeneration in this mouse model were accelerated by the absence of the key lysosomal protease cathepsin B. Although these findings suggest that the endolysosomal pathway has a

role in SCA6, further studies exploring the behaviour of the Cav2.1 channel subunit and α 1ACT with polyQ-repeat lengths in the actual disease range are needed.

Other polyQ proteins implicated in cerebellar ataxia.

Three other polyQ diseases can present primarily with ataxia: SCA7, SCA17 (REF. 104] and dentatorubral-pallidoluysian atrophy (DRPLA)¹⁰⁵. SCA7 resembles SCA1, SCA2 and SCA3 clinically and pathologically but is unique among the SCAs in that it is typically accompanied by profound retinal degeneration. SCA17 and DRPLA are rather rare, show more widespread degeneration than most SCAs and are markedly heterogeneous in clinical features; sometimes, they resemble HD more than an SCA. The disease proteins in all three diseases are closely linked to transcription, supporting that alterations in transcription-associated complexes are a common feature of polyQ disease pathogenesis, as already illustrated in SCA1.

The SCA7 protein ATXN7 is a component of the SPT-ADA-GCN5 acetyltransferase complex (SAGA complex)¹⁰⁶. SAGA broadly regulates transcription through its dual histone-modifying enzymes, the histone acetyltransferase GCN5 (also known as KAT2A) and the DUB ubiquitin C-terminal hydrolase 22 (USP22). Although ATXN7 itself has no enzymatic activity, it anchors USP22 within the DUB module of the SAGA complex, enhancing the DUB activity of SAGA. *In vitro*, normal and polyQ-expanded ATXN7 enhance DUB activity equally; however, *in vivo*, expanded ATXN7 forms insoluble complexes that sequester other components of the DUB module such that the SAGA complex can no longer remove ubiquitin from its substrates, including histone H2B¹⁰⁷. This dominant-negative effect of expanded ATXN7 probably disrupts the careful balance between the acetylation and de-ubiquitylation mediated by SAGA, leading to deleterious changes in gene expression that help to precipitate retinal and neurodegeneration in SCA7 (REFS 107–109.).

Studies of mice in which the expression of mutant, expanded *ATXN7* could be conditionally inactivated in Bergmann glia or in Purkinje cells and inferior olivary neurons revealed that cerebellar disease involves the action of mutant ATXN7 in both neurons and glia¹¹⁰ and implicated the dysfunction of inferior olivary neurons, whose climbing fibre projections are one of the two major excitatory Purkinje cell inputs, in disease pathogenesis. These findings nicely illustrate the complexity of SCA7 — and, presumably, other SCAs — and remind us that much more must be discovered about the contributions of cell types besides neurons to SCA disease pathogenesis.

Among the rarest polyQ SCAs is SCA17, which is caused by a repeat expansion in TBP¹⁰⁴. TBP is an essential general transcription factor that forms dimers and binds the TATAAA sequence just upstream of the transcription start site in most genes; here, it recruits the many other factors needed to initiate transcription. Expansion of the polyQ tract in TBP reduces dimer formation and DNA binding by TBP; alters TBP interactions with various transcription factors, including the general transcription initiation factor IIB; and promotes aggregation of TBP in neurons.

Given that TBP is such an important transcription factor throughout the body, its involvement in selective neurodegeneration in SCA17 is particularly intriguing. It has been speculated, but not yet experimentally proven, that polyQ-expanded TBP may disrupt neuron-specific transcriptional events. Similar to TBP, the DRPLA protein atrophin is a transcription co-regulator that may, when harbouring a polyQ expansion, elicit cell-specific transcriptional changes that are deleterious in the nervous system¹⁰⁵. Although less common than the other polyQ SCAs, both SCA17 and DRPLA warrant further investigation, as such investigation may reveal that these diseases share pathways of relevance to the broader field of polyQ diseases.

Changes in physiology

In the course of a neurodegenerative disorder, alterations in neuronal structure and function are believed to result in part from perturbations to intracellular homeostatic processes. These alterations may represent relatively nonspecific changes in the ability of neurons to maintain the normal trafficking, assembly and energetics of membrane proteins required for regulating excitability (FIG. 4). Nevertheless, some of these changes may contribute to behavioural dysfunction in ataxia and may thus represent potential targets for symptom improvement in SCA. Recent work suggests that early changes in the expression of specific receptors and ion channels that are important for regulating membrane excitability contribute not only to motor dysfunction but also to structural changes in neurons that consistently precede cell death. Thus, targeting these changes might not only improve symptoms but also protect neurons from degeneration.

Some components of cerebellar circuitry seem to be preferentially vulnerable early in the disease. Climbing fibre, but not parallel fibre, innervation of Purkinje cells undergoes structural and functional alterations in a transgenic mouse model of SCA1, and these alterations are concurrent with the onset of motor symptoms. A failure of climbing fibres to extend fully onto Purkinje cell dendrites is associated with impaired Purkinje neuron responsiveness to climbing fibre activation¹¹¹. Although the degeneration of Purkinje cell dendrites that is associated with SCA1 would inevitably lead to synaptic changes, recent work suggests that early involvement of specific synaptic signalling pathways contributes to motor dysfunction. Specifically, before substantial dendritic degeneration has occurred and while fast synaptic glutamatergic transmission is still intact, metabotropic glutamate receptor (mGluR) signalling and the amplitude of mGluR-mediated excitatory postsynaptic currents (EPSCs) in Purkinje cells are reduced¹¹². This impairment in mGluR-mediated signalling also impairs LTD of Purkinje cell-parallel fibre synapses in transgenic and lentivirus-mediated models of mutant *ATXN1* expression. In a lentivirus-mediated model of SCA1, baclofen, a GABA type B receptor agonist that also potentiates mGluR1 responses in Purkinje neurons, partially rescued impairment in Purkinje neuron-parallel fibre LTD and motor dysfunction. Surprisingly, despite a reduction in the maximum amplitude of mGluR1-mediated responses observed in this study, mGluR1-mediated slow EPSCs were observed to be prolonged in a different study employing *ATXN1* transgenic mice, possibly secondary to loss of glutamate transporter activity¹¹². Systemic administration of a negative allosteric modulator of mGluR1, JNJ 16259685, improved the motor phenotype in mutant *ATXN1*-

expressing mice. Hence, modulating mGluR signalling in Purkinje neurons may be an attractive therapeutic strategy for SCA1.

Purkinje neurons exhibit remarkably regular autonomous pacemaker spiking with little variability in interspike intervals, even in the absence of synaptic input. Altered Purkinje cell spiking occurs in many mouse models of ataxia^{113,114}, including models of SCA1 (REFS 115,116), SCA2 (REFS 117–119), SCA3 (REF. 120.) and SCA6 (REF. 121). In mouse models of SCA1 and SCA2, a progressive reduction in firing frequency precedes cell loss^{115–117}. In SCA1 Purkinje cells, this reduction in firing frequency may reflect increased A-type voltage-gated potassium channel currents¹¹⁵. In one mouse model of SCA1, the pacemaker firing of Purkinje cells was found to become disrupted with the onset of motor dysfunction, and these cells showed abnormal depolarization¹¹⁶. The inability of SCA1 Purkinje cells to support repetitive spiking reflected reduced levels of large-conductance calcium-activated potassium (BK) channels and subthreshold-activated potassium channels. Virus-mediated upregulation of BK channel expression in SCA1 Purkinje cells improved motor function and partly restored Purkinje cell morphology¹¹⁶. In a transgenic model of SCA2, Purkinje cell spiking in cerebellar slices and *in vivo* is irregular, with considerable variability among interspike intervals^{98,117}. Although the basis for these alterations in spiking is still unclear, a positive modulator of small-conductance calcium-activated potassium channels improved the regularity of spiking to control levels, preventing motor dysfunction and reducing the number of darkly stained Purkinje cells on electron microscopy (so-called dark cell degeneration)¹¹⁷. In a different transgenic model of SCA2, reduced BK channel transcripts, and the associated impaired function of BK channels and other potassium channels, are responsible for impaired Purkinje neuron spiking¹⁶⁴.

These studies suggest that changes in the intrinsic excitability of Purkinje neurons occur early in both SCA1 and SCA2 and are due in part to dysregulation of calcium-activated potassium channel physiology. These channels may be important targets for the treatment of motor dysfunction and neurodegeneration.

Therapeutic perspectives

Emerging insights into the mechanisms underlying polyQ SCAs suggest that these diseases may be treated through at least two principal therapeutic routes. The first is to pharmacologically modulate the perturbed cerebellar circuitry, as a symptomatic or possibly diseasemodifying therapy. The second is to reduce levels of toxic disease-gene products.

Given that mutant polyQ disease proteins can perturb gene expression, RNA homeostasis and protein homeostasis through diverse pathways with myriad downstream consequences, acting proximally in the disease cascade to lower levels of the toxic protein is currently the most compelling strategy towards developing a disease-modifying therapy. To date, efforts to target the expression of polyQ SCA proteins have used antisense oligonucleotides (ASOs) or virus-mediated delivery of short hairpin RNAs or artificial miRNAs to interfere with translation. Preclinical tests of ASOs or miRNA-based drugs for SCA1, SCA2, SCA3 and SCA6 (reviewed else-where¹²²; also see REFS 90,123) show considerable promise, making clinical trials in the near future very likely. Notably, ATXN2-targeting ASOs ameliorated

disease not only in SCA2 model mice¹²⁴ but also in a TDP43 transgenic mouse model of ALS¹²⁵. Certainly, the recent clinical trial successes with an ASO-based drug for spinal muscular atrophy^{126,127}, as well as a phase I clinical trial for ASOs in superoxide dismutase 1 (SOD1)-variant-mediated ALS¹²⁸, demonstrate that CNS delivery of ASOs by lumbar puncture is a viable therapeutic approach. The future looks promising for gene suppression as disease-altering therapy for the polyQ SCAs.

Well-designed interventional studies of SCA are crucial for a better understanding of the natural history of disease progression and to identify biomarkers for assessing disease progression and therapeutic target engagement. The rates of progression in SCA1, SCA2, SCA3 and SCA6 have been assessed by the European and US consortia of ataxia investigators (the European Integrated Project on SCAs (EUROSCA) and the Clinical Research Consortium for SCAs, respectively), permitting calculation of the sample sizes needed in SCA clinical trials^{129,130}. Moreover, structural MRI and proton magnetic resonance spectroscopy detect disease-relevant changes with higher sensitivity than do clinical scales and thus have potential as noninvasive surrogate markers in clinical trials^{130,131}. Transcranial magnetic stimulation is showing promise for detecting alterations in corticospinal and corticomuscular coherence in presymptomatic and symptomatic individuals harbouring the *ATXN2* mutation^{132,133}. As recently shown for another polyQ protein, huntingtin¹³⁴, detection of the SCA polyQ proteins in the cerebrospinal fluid of patients may also prove to be an effective biomarker of target engagement for the polyQ SCAs.

Conclusions

The polyQ SCAs are a prime example of the power of genetics to advance the understanding of the cellular pathways crucial for neuronal function and dysfunction. One example highlighting this point is the recent elucidation of the role of *CACNA1A* bicistronic mRNA and the effects of a cleaved C-terminal peptide on nuclear transcription in SCA6¹⁰²; this study identified an exciting new signal transduction pathway in neurons. As a group, the polyQ SCA proteins function in diverse fundamental processes affecting neuronal function and survival. With this in mind, the relationship between *ATXN2* polyQ tract length and the risk of developing ALS^{17,18} probably portends the existence of similar effects of polyQ tract length in other SCA proteins.

As in other dominant neurodegenerative disorders, several modes and pathways have been identified as potential contributors to disease pathogenesis in the polyQ SCAs^{46,70,135}. Understanding the relative contribution of each to pathogenesis in a given disease, however, remains a work in progress. Regardless of the precise molecular underpinnings of the toxic gain of function in polyQ SCAs, targeting the expression of the mutant disease proteins is a particularly compelling therapeutic strategy. Two therapeutically relevant observations from SCA mouse models are that neurological dysfunction precedes neuronal loss and that mouse models in which the mutant gene can be conditionally inactivated demonstrate an age-dependent recovery, with earlier silencing of mutant SCA gene expression conferring more complete recovery. Importantly, even at a relatively late stage in disease progression, but before neuronal loss, neurons may retain some ability to recover¹³⁶, a finding that

emphasizes the importance of intervening early in the disease. It remains unclear, however, how early in polyQ SCA a therapeutic intervention must be administered to have a disease-modifying effect.

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Pyramidal symptoms

Spasticity, increased reflexes and weakness caused by pathology in cerebral cortical primary motor neurons or their axonal projections.

Extrapyramidal symptoms

Rigidity, tremor, difficulty initiating movement and gait disturbances typical of parkinsonism or, occasionally, increased involuntary movement, such as chorea. These symptoms are secondary to pathology in pathways involving the basal ganglia and substantia nigra.

Ophthalmoplegia

Inability to move the eyes, secondary to pathology in the oculomotor cranial nerve nuclei (cranial nerves III, IV and VI) or the nerves themselves. In some cases, the pathology may be above the cranial nerve nuclei (supranuclear), in the voluntary gaze centres.

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Oligonucleotide/oligosaccharide-binding fold

(OB fold). A structural feature found in many oligonucleotide-binding proteins.

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Stress granules

Subcellular organelles that are major sites for the regulation of mRNA translation. These are non-membrane-bound aggregates composed of proteins and mRNA molecules that form in a reversible manner during cellular stress.

Processing bodies

(P-bodies). Cytoplasmic domains that contain collections of proteins involved in diverse processes, such as mRNA degradation, nonsense-mediated mRNA decay, translational repression and inhibitory-RNA-mediated gene silencing.

Cycloheximide

An antibiotic that interferes with protein synthesis by blocking translational elongation. The drug is used experimentally as a rapidly reversible means to block protein synthesis *in vitro*.

Heterogeneous nuclear ribonucleoprotein

Protein that forms nuclear complexes with RNA during gene transcription and post-translational modification of pre-mRNA.

Aggresome

A cytoplasmic collection of misfolded proteins that forms when the normal protein degradation system is overtaxed. This possibly protective mechanism may result in formation of the intracellular inclusions observed in neurodegenerative diseases.

Bicistronic mRNA

mRNA that has two open-reading frames, both of which are translated into a polypeptide. These polypeptides often are functionally related and may be regulated together.

Internal ribosomal entry site

(IRES). RNA structures that allow initiation of translation independent of the usual 5'-cap mechanism, thus enabling translation of protein in the middle of an mRNA.

SPT-ADA-GCN5 acetyltransferase complex

(SAGA complex). A histone acetyltransferase complex that regulates gene transcription by altering chromatin structure via histone modification. The complex is also involved in the biogenesis and nuclear export of mRNA.

Bergmann glia

A type of astrocyte found only in the cerebellar cortex. These cells have intimate contacts with Purkinje cell dendrites in the superficial layer of the Cortex and are involved in the re-uptake of glutamate.

Negative allosteric modulator

A molecule that decreases the function of a receptor or an enzyme by binding to a locus that is not the active enzymatic site, typically by effecting a conformational change.

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Box 1 |**Mutational basis for some non-polyglutamine spinocerebellar ataxias**

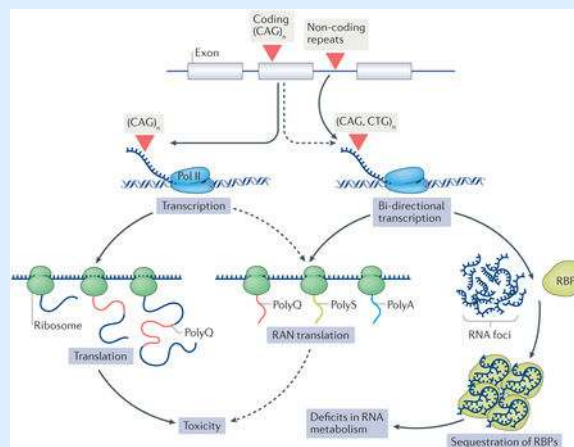
Genetics has played a seminal role in revealing the varied biology of the spinocerebellar ataxias (SCAs). In addition to the polyglutamine (polyQ) SCAs, which are the most common SCAs and the focus of this Review, many other less common forms of SCA are caused by other repeat expansions, DNA deletions or classical point mutations. Together, they demonstrate the many cellular and molecular pathways that, if disrupted, can cause spinocerebellar degeneration. The table lists non-polyQ SCAs that are associated with point mutations or DNA deletions, as well as the proteins affected in the diseases and their physiological functions.

Disorder	Mutant protein (mutated gene)	Function of protein	Refs
SCA5	β 2-spectrin (<i>SPTBN2</i>)	Helps to form neuronal membrane skeleton	137
SCA11	Tau tubulin kinase 2 (<i>TTBK2</i>)	Tau phosphorylation	138
SCA13	Voltage-gated potassium channel subunit Kv3.3 (<i>KCNC3</i>)	Regulates membrane potential	139
SCA14	Protein kinase C γ (<i>PRKCG</i>)	Protein phosphorylation	140
SCA15/16	Inositol 1,4,5-triphosphate receptor type 1 (<i>ITPR1</i>)	Ca ²⁺ signalling	141–143
SCA23	Prodynorphin (<i>PDYN</i>)	Synaptic transmission	144
SCA26	Eukaryotic translation elongation factor 2 (<i>EEF2</i>)	Translation	145
SCA27	Fibroblast growth factor 14 (<i>FGF14</i>)	Cell growth and survival	146
SCA28	AFG3-like protein 2 (<i>AFG3L2</i>)	ATP-dependent protease	147
SCA34	Elongation of very long chain fatty acids 4 (<i>ELOVL4</i>)	Lipid metabolism	148
SCA35	Transglutaminase 6 (<i>TGM6</i>)	Protein crosslinking	149
SCA38	Elongation of very long chain fatty acids 5 (<i>ELOVL5</i>)	Lipid metabolism	150
SCA40	Coiled-coil domain-containing protein 88C (<i>CCDC88C</i>)	WNT signalling	151
SCA41	Transient receptor potential 3 (<i>TRPC3</i>)	Regulates membrane potential, Ca ²⁺ signalling	152
SCA42	Voltage-gated calcium channel subunit- α 1G Cav3.1 (<i>CACNA1G</i>)	Ca ²⁺ signalling	153
SCA43	Nephrilysin (<i>MME</i>)	Zinc-dependent metalloprotease	154

Box 2 |**Toxic RNA and RAN peptides in spinocerebellar ataxias**

Considerable evidence suggests that the toxicity of RNA that contains non-coding expanded repeat sequences can underlie the pathogenesis in spinocerebellar ataxias (SCAs). This notion is underlined by the toxicity of RNA containing the large non-coding ATTCT repeat expansion in SCA10. RNA molecules that contain the expanded repeat form foci that bind and sequester RNA-binding proteins (RBPs)¹⁵⁵, resulting in a loss of RBP function and thus impairing aspects of RNA metabolism such as splicing and translation, depending on the normal role of the sequestered RBP (see the figure).

In addition, expanded repeats can cause protein-mediated effects through repeat-associated non-ATG initiated (RAN) translation, as first demonstrated in SCA8 (REF. 156). RAN translation, which may contribute to disease pathogenesis in many repeat expansion diseases¹⁵⁷, including polyglutamine (polyQ) diseases, can occur across the expanded repeat in all three frames and possibly in both the sense and antisense directions, producing putatively toxic RAN peptides. Experimental evidence supports the presence of RAN-translated peptides in Huntington disease and their toxicity when overexpressed in tissue culture cells¹⁵⁸. Although it remains unclear whether RAN-translated peptides are generated in the polyQ SCAs and contribute to toxicity *in vivo* (depicted as dashed arrows in the figure), the role of such peptides is an active topic of investigation in the polyQ SCAs. Pol II, polymerase II; polyA, polyalanine; polyS, polyserine.



Box 3 |**Mouse models of polyglutamine spinocerebellar ataxias**

An important approach to understanding spinocerebellar ataxia (SCA) pathogenesis has been to model disease features in mice. An issue in using mice to model SCAs is the extent to which slowly progressive, mid-life diseases, such as the SCAs, can be recapitulated in the short lifespan of a mouse, which is typically 2 years. Two general approaches to creating a discernible clinical phenotype in mice are to overexpress an SCA-associated transgene or to express a disease-associated transgene with a CAG-repeat expansion that is longer than the maximum repeat length seen in humans. These manipulations create the potential to produce results unrelated to the human disease and thus require careful comparison to controls, such as transgenic mouse lines that overexpress normal alleles to the same degree.

Another strategic issue is whether to target the SCA-associated transgene such that it is selectively expressed in particularly relevant populations, such as Purkinje cells^{118, 159, 160}, or such that it is expressed in all neurons or cells of the CNS that normally express the gene. The former approach can be advantageous when applied to Purkinje cells, for example, as these cells are crucial for cerebellar function and are affected in nearly all forms of SCA, and when these cells are damaged, a common result in mouse models is an easily discernible motor deficit. The latter approach uses either knock-in strategies to insert an expanded polyglutamine-encoding region into the mouse disease gene with its endogenous transcriptional regulator elements^{161, 162} or transgenic strategies using vectors capable of carrying an entire human gene, including its regulatory elements¹³⁵. One advantage of such a strategy is that the resulting model is arguably more genetically precise and more fully captures the molecular genetic features of the human disease.

Last, there are transgenic approaches to generating conditional mouse models that allow the disease phenotype to be measured after stopping the expression of a mutant SCA protein^{136, 163}. For example, a model of SCA1 pathogenesis in which mutant ataxin 1 (ATXN1) was conditionally expressed in Purkinje cells revealed that the disease is dependent on the continuous expression of mutant ATXN1. The extent of recovery when mutant ATXN1 was no longer expressed was directly related to how long the *ATXN1* transgene had already been expressed. When mutant ATXN1 expression was discontinued at an early stage, the disease was completely reversed; by contrast, discontinuation of ATXN1 expression at a later stage led to only partial recovery. Notably, Purkinje cells were at least partly able to recover from mutant ATXN1 expression even at a late stage of the disease.

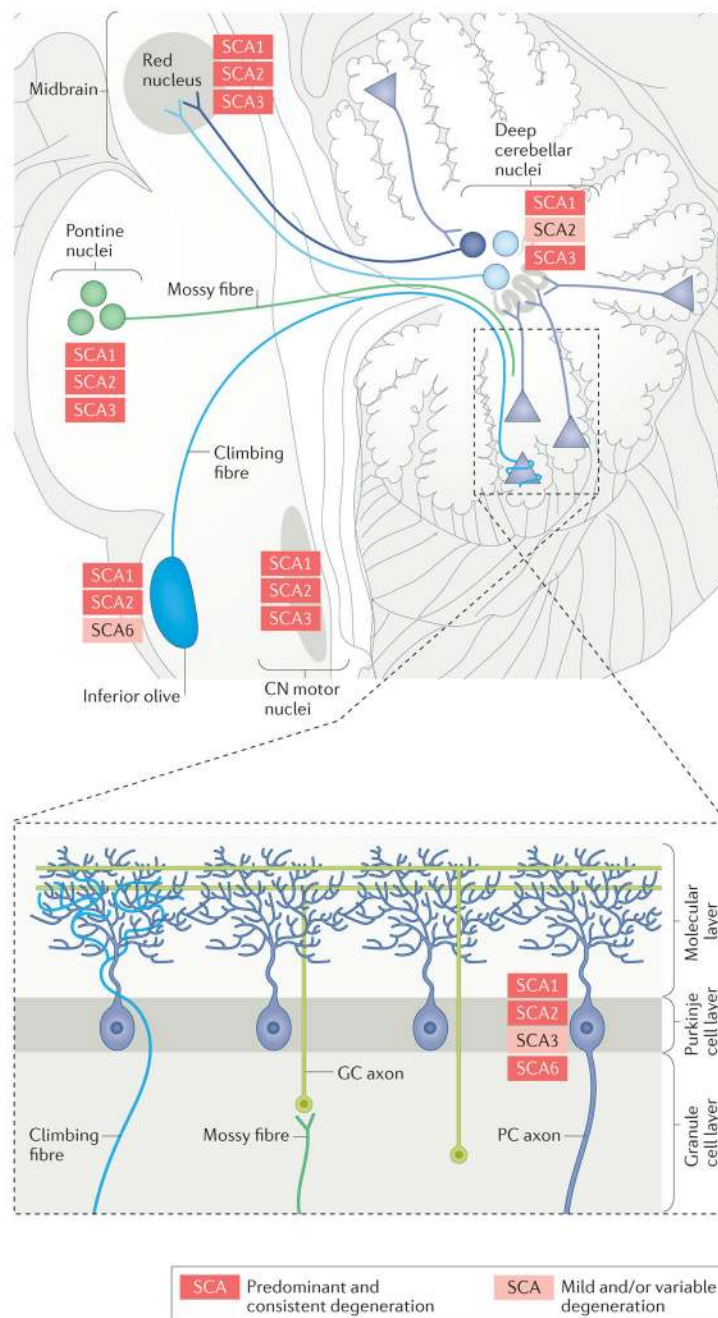


Figure 1 | Components of the cerebellar circuitry showing degeneration in the polyglutamine spinocerebellar ataxias.

The different spinocerebellar ataxias (SCAs) have similar but not identical patterns of pathological involvement, shown here for SCA1, SCA2, SCA3 and SCA6. The figure shows the areas that are consistently affected in the different SCAs (red), as well as those that are mildly or variably affected in the different SCAs (pink). The areas and cell types in the cerebellar circuitry that are affected include the cerebellar cortex (particularly Purkinje cells (PCs), the cell bodies of which are depicted by triangles here), the inferior olive and its

climbing fibres, the pontine nuclei and their mossy fibres, the deep cerebellar nuclei, the red nucleus and the cranial nerve (CN) motor nuclei. GC, cerebellar granule cell.

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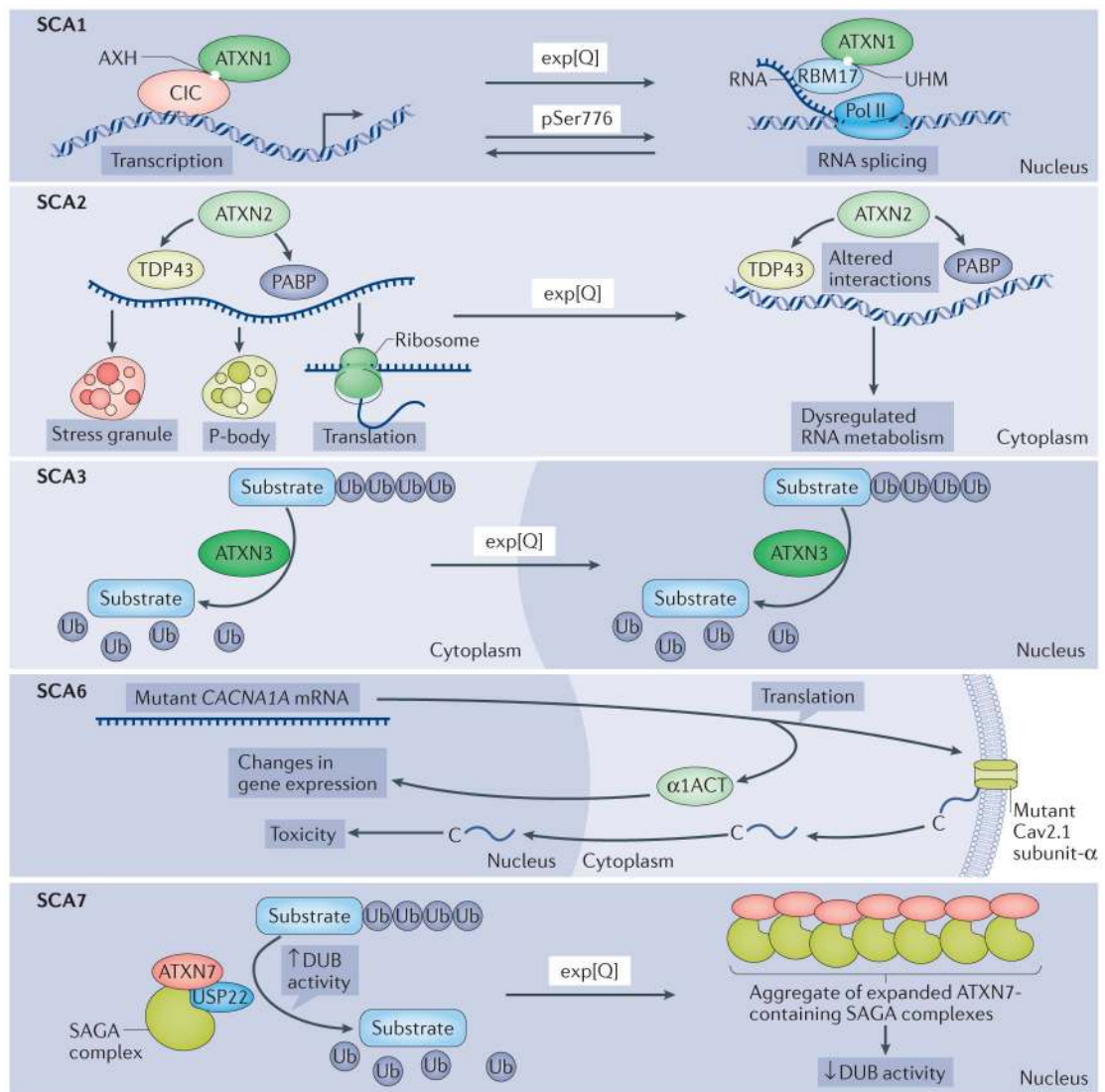


Figure 2 | Cellular processes affected by mutant polyglutamine proteins in spinocerebellar ataxias.

Each panel illustrates the cellular processes affected by the polyglutamine (polyQ) proteins in spinocerebellar ataxia 1 (SCA1), SCA2, SCA3, SCA6 and SCA7. In all of the SCAs depicted, except SCA3, the cerebellar Purkinje cells are prominently affected, whereas in SCA3, the Purkinje cells are more mildly affected. Top panel (SCA1): in the ataxin 1 (ATXN1) protein, it is believed that the AXTN1 HBP1 (AXH) domain and the U2AF homology (UHM) domain are interaction motifs for the transcriptional regulator capicua (CIC) and the RNA splicing factor RBM17, respectively, and a shift in these interactions is critical for mutant, polyglutamine-expanded ATXN1 to cause disease in Purkinje cells. PolyQ expansion is depicted by ‘exp[Q]’ in all panels. Phosphorylation of Ser776 in ATXN1 (pSer 776) also shifts the balance between these interactions. Second panel (SCA2): ATXN2 is known to interact directly or indirectly with numerous proteins implicated in RNA metabolism, as well as RNA itself, to regulate translation, stress granule formation and P-body formation. Of particular interest are the interactions of ATXN2 with polyadenylate-

binding protein (PABP) and TAR DNA-binding protein 43 (TDP43), each of which also binds directly to RNA. One hypothesis is that the polyQ tract length in ATXN2 impairs interactions with PABP and TDP43 and thereby contributes to SCA2 pathogenesis, as well as the risk for ALS (not shown). Third panel (SCA3): as a deubiquitinase (DUB), ATXN3 binds and cleaves polyubiquitin chains and has been implicated in a variety of ubiquitin (Ub)-dependent protein quality control pathways. Although expanded ATXN3 retains DUB activity *in vitro*, changes in polyQ-repeat length may alter its function in the complex cellular environment, with deleterious consequences. As with many polyQ disease proteins, mutant ATXN3 becomes concentrated in the nucleus. Fourth panel (SCA6): the *CACNA1A* gene encodes a bicistronic mRNA that, on translation, yields the following two proteins: the membrane-localized $\alpha 1A$ subunit of the Cav2.1 channel and the transcription factor $\alpha 1ACT$. Expansion of the polyQ-encoding repeat in *CACNA1A* leads to toxicity through altered $\alpha 1ACT$ -mediated regulation of transcription, as well as through nuclear translocation of a peptide cleaved from the carboxyl terminus of the mutant Cav2.1 channel subunit. Fifth panel (SCA7): ATXN7 is a component of the SPT-ADA-GCN5 acetyltransferase (SAGA) complex. SAGA regulates transcription through its dual histone-modifying enzymes, the histone acetyltransferase GCN5 and the DUB ubiquitin C-terminal hydrolase 22 (USP22). PolyQ-expanded ATXN7 forms insoluble complexes that are thought to sequester other components of the DUB module such that the SAGA complex can no longer remove ubiquitin from its substrates. Pol II, polymerase II.

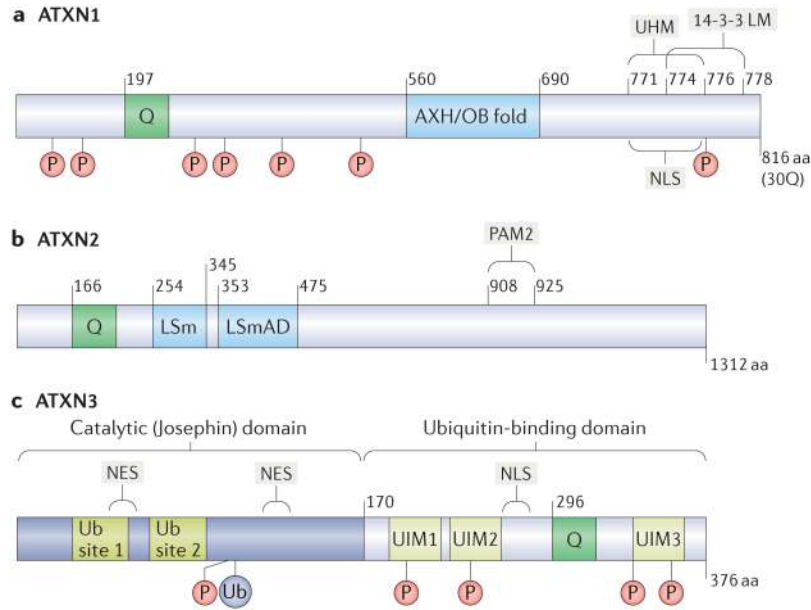


Figure 3 | Functional motifs in ATXN1, ATXN2 and ATXN3.

Functional motifs are diagrammed for each polyglutamine (polyQ) spinocerebellar ataxia (SCA)-associated ataxin (ATXN) protein. The diagrams also show the polyQ regions (denoted as ‘Q’), as well as the phosphorylation sites (denoted as ‘P’) and ubiquitylation sites (denoted as ‘Ub’ to represent ubiquitin). **a** | ATXN1 with a 30Q polyQ region is shown. The ATXN1 HBP1 (AXH) domain and the U2AF homology motif (UHM) of ATXN1 are interaction motifs for capicua (CIC) and RBM17, respectively; the AXH domain itself forms an oligonucleotide/oligosaccharide-binding (OB) fold. ATXN1 also features a nuclear-localization signal (NLS) near the carboxyl terminus of the protein that facilitates its localization to the nucleus and a phosphorylation-dependent binding motif for the chaperone 14-3-3 (14-3-3 LM). **b** | ATXN2 interacts directly or indirectly with numerous proteins implicated in RNA metabolism. Its poly(A)-binding protein (PABP)-interacting motif PAM2 enables ATXN2 to interact with PABP and TAR DNA-binding protein 43 (TDP43). ATXN2 also features a like-Sm(LSm) motif and an LSm-associated domain (LSmAD). **c** | The deubiquitinase (DUB) ATXN3 has an N-terminal catalytic (Josephin) domain, which contains two Ub-binding sites and two nuclear export sites (NES), and a C-terminal Ub-binding domain bearing three Ub-interacting motifs (UIMs) and an NLS.

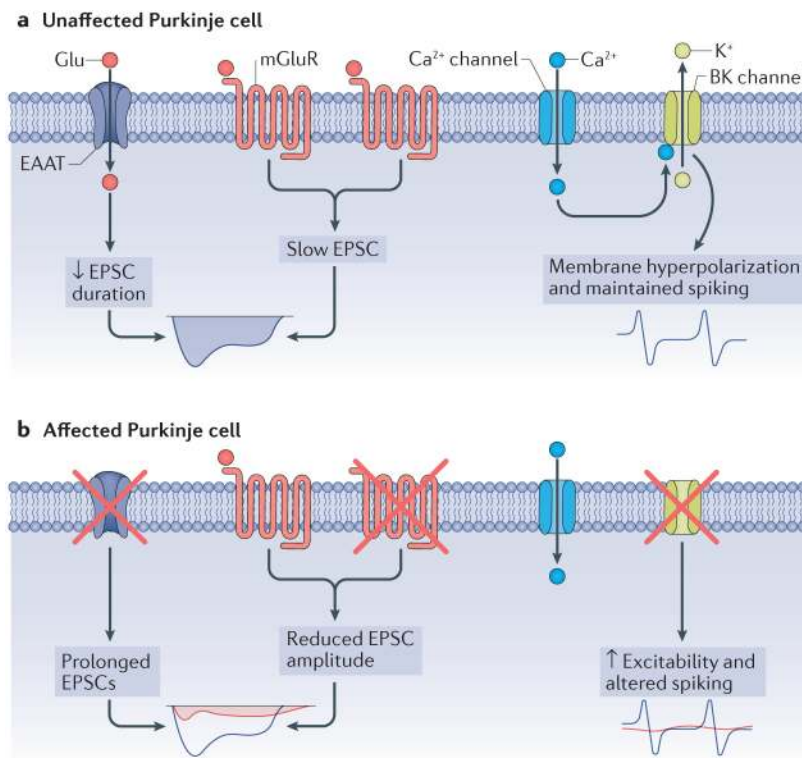


Figure 4 | Alterations in Purkinje cell electrophysiology in spinocerebellar ataxias.

Concurrent with the motor dysfunction that occurs in the spinocerebellar ataxias (SCAs), and before the onset of substantial cellular morphological alterations, the expression levels and functions of ion channels and receptors are altered in SCA. **a** | Expression and function of ion channels in an unaffected Purkinje cell. The function of excitatory amino acid transporters (EAATs), which carry glutamate, and metabotropic glutamate receptors (mGluRs) yield slow excitatory postsynaptic currents (EPSCs), whereas normal large-conductance calcium-activated potassium (BK) channel function keeps the cell membrane hyperpolarized, maintaining spiking, **b** | The reduction in mGluRs and EAATs results in reductions in the amplitude of slow EPSCs (normal currents are shown in blue, and the currents in SCA are shown in red). The reduction in glutamate transporters prolongs the effect of glutamate at the synapse and also prolongs the mGluR-mediated slow EPSCs (red). In addition to alterations in synaptic signalling, the intrinsic excitability of the neuron is altered secondary to a loss of potassium channels. A reduction in BK channel expression and function results in unopposed calcium entry through voltage-gated calcium channels, with impairments in Purkinje neuron spiking (normal spiking indicated in blue; altered spiking indicated in red).