Spinocerebellar ataxias: an update

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Purpose of review

Here we discuss recent advances regarding the molecular genetic basis of dominantly inherited ataxias.

Recent findings

Important recent observations include insights into the mechanisms by which expanded polyglutamine causes cerebellar degeneration; new findings regarding how noncoding expansions may cause disease; the discovery that conventional (i.e. nonrepeat) mutations underlie recently identified ataxias; and growing recognition that multiple biological pathways, when perturbed, can cause cerebellar degeneration.

Summary

The dominant ataxias, also known as spinocerebellar ataxias, continue to grow in number. Here we review the major categories of spinocerebellar ataxias: expanded polyglutamine ataxias; noncoding repeat ataxias; and ataxias caused by conventional mutations. After discussing features shared by these disorders, we present recent evidence supporting a toxic protein mechanism for the polyglutamine spinocerebellar ataxias and the recognition that both protein misfolding and perturbations in nuclear events represent key events in pathogenesis. Less is known about pathogenic mechanisms in spinocerebellar ataxias due to noncoding repeats, though a toxic RNA effect remains possible. Newly discovered, conventional mutations in spinocerebellar ataxias suggest a wide range of biological pathways can be disrupted to cause progressive ataxia. Finally, we discuss how new mechanistic insights can drive the push toward preventive treatment.

Keywords

ataxia, cerebellum, neurogenetics, polyglutamine, repeat expansion

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Abbreviations

CHIP	carboxy-terminus of Hsc70 interacting protein
FXTAS	fragile X-associated tremor ataxia syndrome
SCA	spinocerebellar ataxia

1350-7540

Introduction

The dominantly inherited ataxias, or spinocerebellar ataxias (SCAs), are progressive disorders in which the cerebellum slowly degenerates, often accompanied by degenerative changes in the brainstem and elsewhere. SCAs remain a growth industry: another genetic locus for a new SCA is discovered nearly every year. At last count, at least 28 genetic loci for SCAs had been discovered (Table 1). The past year has witnessed new insights into the molecular mechanisms underlying various classes of SCAs. Here we focus on these newest findings and their implications for potential therapy in this growing family of progressive, often fatal disorders.

Categories of spinocerebellar ataxia

Breaking the SCAs down into subclasses provides the reader with a conceptual framework for this dauntingly large group. Currently, there are at least three major classes. The first includes SCAs caused by CAG repeat expansions that encode a pure repeat of the amino acid glutamine in the disease protein. These 'polyglutamine' diseases include SCA1, 2, 3, 6, 7 and 17, as well as at least three other diseases that are not primarily ataxic syndromes: Huntington disease, spinobulbar muscular atrophy, and dentatorubropallidoluysian atrophy. Together, the six known polyglutamine SCAs constitute the most common causes of dominant inherited ataxia, together accounting for well over 50% of affected families in nearly all regions of the world.

A second category comprises the SCAs that are due to repeat expansions falling outside of the protein-coding region of the respective disease genes. In other words, the pathogenic expansion does not encode glutamine or any other amino acid in the disease protein. Ataxic syndromes included in this category are SCA8, 10 and 12, although, as discussed later, there is some uncertainty about SCA8 as recent findings suggest a dual pathogenic mechanism. In all three diseases, it remains unclear how a noncoding repeat causes neurodegeneration. A dominant toxic mechanism occurring at the RNA level is the prevailing theory, reminiscent of myotonic dystrophy in which

Table 1 Classification of spinocerebellar ataxias (SCAs)

SCA1	6p23; CAG expansion in *ATXN1* gene; polyQ dz	Ataxia with ophthalmoparesis, pyramidal and extrapyramidal findings
SCA2	12q24; CAG expansion in *ATXN2* gene; polyQ dz	Ataxia with slow saccades, peripheral neuropathy; less frequent extrapyramidal findings
Machado-Joseph disease/SCA3	14q32; CAG expansion in *ATXN3* gene; polyQ dz	Ataxia with ophthalmoparesis; pyramidal, extrapyramidal and amyotrophic signs
SCA4	16q22	Ataxia with sensory axonal neuropathy and pyramidal signs
SCA5	11p13; mutations in *SPTBN2* gene	Relative pure cerebellar ataxia with dysarthria; includes Lincoln descendants
SCA6	19p13; CAG expansion *CACNA1A* gene; polyQ dz	Pure cerebellar ataxia with dysarthria, nystagmus; occasional mild sensory loss
SCA7	3p14; CAG expansion in *ATXN7* gene; polyQ dz	Ataxia with ophthalmoparesis, retinal degeneration, dysarthria, variable pyramidal signs
SCA8	13q21; CTG/CAG expansion in *ATXN8* gene	Gait ataxia, dysarthria, nystagmus, spasticity, and reduced vibratory sensation
SCA10	22q13; ATTCT expansion in *ATXN10* gene	Gait ataxia, dysarthria, nystagmus; frequent seizures; neuropathy
SCA11	15q14–q21.3 by linkage	Slowly progressive, relatively mild gait and limb ataxia
SCA12	5q32; noncoding CAG expansion in *PPP2R2B*	Ataxia with tremor, dysarthria, increased reflexes; occasional dystonia, late onset dementia
SCA13	19q13.3–q14.4; mutations in *KCNC3* gene	Ataxia, varying onset including childhood; occasional delayed motor development and mental retardation
SCA14	19q13.4; mutations in *PRKCG* gene	Ataxia w/dysarthria; facial myokymia; occasional myoclonus, dystonia; vibratory loss; late onset can be pure ataxia
SCA15	3p24.2-3pter	Slowly progressive, relatively pure cerebellar ataxia
SCA16	3p26.2-pter	Ataxia with head tremor, dysarthria
SCA17	6q27; CAG expansion in *TBP* gene; polyQ disease	Ataxia with dementia, extrapyramidal signs; widespread cerebral and cerebellar atrophy
SCA18	7q22–q32	Ataxia with sensorimotor neuropathy
SCA19	1p21–q21	Slowly progressive ataxia, hyporeflexia, cognitive impairment; occasional tremor and myoclonus
SCA20	Chromosome 11	Ataxia with dysarthria; dentate calcification on CT
SCA21	7p21–p15	Ataxia with dysarthria, extrapyramidal features; cognitive defects; hyporeflexia
SCA22	1p21–q23	Pure cerebellar ataxia with dysarthria, nystagmus
SCA23	20p13–12.3	Slowly progressive ataxia with vibration loss
SCA25	2p21–p13	Ataxia with severe sensory neuropathy; gastrointestinal symptoms
SCA26	19p13.3	Pure cerebellar ataxia with dysarthria
SCA27	13q34; mutations in *FGF14* gene	Ataxia with tremor; orofacial dyskinesias; psychiatric symptoms and cognitive deficits
SCA28	18p11.22–q11.2	Ataxia with dysarthria, ophthalmoparesis, hyperreflexia
SCA29	3p26	Early onset, nonprogressive ataxia; vermian hypoplasia

expanded RNA repeats sequester RNA-binding proteins leading to aberrant RNA splicing [1,2]. This predicted mechanism, however, remains unproven for SCA8, 10 and 12. Fragile X-associated tremor ataxia syndrome (FXTAS) also belongs to this class of diseases, even though technically it is not an SCA (i.e. a dominantly inherited disorder). FXTAS is a progressive neurodegenerative ataxia that occurs in older men who carry a 'premutation' expansion in the *FMR1* gene [2]. Whereas in fragile X mental retardation the full-blown expansion results in loss of transcription of the disease protein FMRP, the premutation in FXTAS leads to accumulation of the expanded repeat-containing transcript, which likely acts in a dominant manner to perturb the regulation of gene expression in neurons.

A third category contains SCAs that are not due to dynamic repeat expansions. Instead, they are caused by conventional mutations in specific genes: deletion, missense, nonsense and splice site mutations. Just a few years ago, no SCAs were known to belong to this family. Now, however, at least four are known: SCA5, 13, 14 and 27. To date, the genes mutated in these SCA disorders are not obviously linked to a single biological pathway. This suggests that cerebellar and brainstem degeneration can be the biological consequence of perturbations in any one of many distinct cellular pathways. This category of SCAs will continue to grow in the coming years as the technology to pinpoint genetic defects in rare disorders gets easier and faster.

Common features of spinocerebellar ataxias

Before discussing mechanistic insights, we should review shared features among the SCAs. Perhaps the most important unifying feature is the pattern of neurodegeneration. Neurologists naturally associate these disorders with the typical clinical features reflecting damage to the cerebellum. Indeed, many SCAs have extensive cerebellar atrophy involving molecular, Purkinje cell and granule cell layers as well as the deep cerebellar nuclei. It is important to remember, however, that many SCAs are characterized almost as much by their extracerebellar

brain involvement. For example, all but one of the six polyglutamine SCAs display significant brainstem involvement. The exception, SCA6, is typically a 'pure' cerebellar ataxia in which Purkinje cells degenerate but very little else does. Basal ganglionic involvement is also common in many SCAs and cerebral cortical involvement contributes significantly to clinical features in a few SCAs, most notably SCA17. Spinal cord and peripheral nerve involvement are also fairly common. In contrast, some features are relatively unique to specific SCAs, such as retinal degeneration in SCA7 and epilepsy in SCA10.

A second feature is the remarkably wide range in phenotype. This heterogeneity stems primarily from the fact that expansions, which cause most SCAs, can vary greatly in size. Indeed the tendency for expansions to change size is why these mutations are also described as 'dynamic'. Larger expansions typically cause more severe, earlier onset disease. Normal repeats are also polymorphic, varying in size among individuals. For most expanded repeat diseases, we know very little about how variation in normal repeat length affects the expression or function of the gene product or influences the onset of disease caused by pathogenic expansions.

The dynamic nature of expanded repeats is illustrated by the fact that they often expand further when transmitted from one generation to the next. The intriguing clinical phenomenon of 'anticipation' is coupled to this molecular observation. Anticipation is the tendency for disease to worsen from generation to generation within a family. Two facts explain anticipation: expansions frequently enlarge upon transmission and larger expansions typically cause earlier onset disease. Of course, anticipation does not occur in every SCA, only in those due to expanded repeats. Even among the SCAs due to repeat expansions, anticipation occurs more robustly in some disorders than in others. Anticipation is particularly severe in SCA7, when it can result in extremely large expansions causing disease in newborns.

The dynamic nature of repeat expansions also explains the continuing appearance of new disease-causing expansions in the population. New cases arise from intermediate size repeats that, while not large enough to cause disease, are large enough to be prone to further expansion in the next generation. Thus, in individuals with progressive adult onset ataxia without a family history of similar disease, the clinician should consider the possibility of a new onset expansion in one of the known SCAs. The SCA most commonly found in this scenario is SCA6.

A final shared feature of SCAs is, unfortunately, their relentless progression. In most SCAs an inexorably progressive degenerative process leads to death over a 15–30-year period. Exceptions include the later onset, pure cerebellar forms of SCA such as SCA6. Currently we have no preventive treatment for any SCA or, for that matter, any polyglutamine disorder. While there may not yet be cures for these disorders, affected individuals and their treating physicians need to be aware that symptomatic therapies do exist for many symptoms occurring in SCA. For example, many individuals who manifest parkinsonism as part of their ataxia syndrome will respond to dopaminergic therapy; this is particularly true for SCA2 and SCA3/MJD.

Insights into pathogenesis of polyglutamine spinocerebellar ataxias

Since the discovery that many neurodegenerative diseases are caused by expansion of polyglutamine-encoding CAG repeats, the prevailing view has been that the toxic action of the mutations occurs primarily at the protein level [3]. Most evidence from in-vitro studies, cellular and animal models and human disease tissue supports this view. The discovery that human polyQ disease brain contains intracellular inclusions of the disease protein suggested that the expansion promotes misfolding of the disease protein, resulting in aggregation. In-vitro tests with recombinant polyQ disease proteins have supported this model. Expanded polyQ proteins are intrinsically prone to aggregate, forming amyloid-like aggregates *in vitro* (see [4] for recent illustration). Importantly, the repeat length threshold for aggregation *in vitro* closely mirrors the repeat length known to cause disease. Further supporting a toxic protein model is the fact that expanded CAG repeats engineered to be expressed at the mRNA level, but not at the protein level, do not display toxicity when introduced into cells or animals. Thus, polyglutamine protein aggregation, or at least a biochemical process associated with aggregation, is integral to the disease process.

What is less clear is the precise relationship between protein misfolding, the process of aggregation, and the formation of macroscopic inclusions observed in disease brain tissue. While inclusions can be viewed as a biomarker of a problem in protein misfolding and accumulation, they are not always found in affected brain cells. In several studies, inclusions have correlated with neuronal survival rather than neuronal cell death, and in some mouse models of disease there is a divergence between the degenerating brain areas and the areas with inclusions. Thus, inclusions may reflect a protective response to the presence of accumulated, abnormal protein – essentially a pathway by which neurons wall off abnormal polyglutamine protein into a relatively inert structure.

Appropriately, then, the focus of research has recently shifted to earlier steps in the aggregation pathway [4,5,6•]. Small oligomers of mutant protein may prove to be the toxic species, engaging in deleterious interactions with additional polyQ proteins and other cellular proteins.

In contrast, larger fibrillar complexes formed further downstream in the aggregation pathway may contain mutant protein 'packaged' into relatively neutral structures. Studying the toxicity of abnormal polyglutamine conformers in animals and cells is not a simple matter because many different species may exist simultaneously: native and misfolded monomer, oligomeric complex, partially aggregated intermediates and mature aggregate. Sorting out which species are neutral and which are toxic is not easy. While the precise nature of the toxic species remains uncertain, a recent study showed that the cellular chaperonin protein, TRiC, suppressed polyglutamine toxicity while promoting its assembly into larger, nontoxic 500 kDa oligomers [6•]. These larger oligomers proved to be conformationally distinct from smaller 200 kD complexes that are associated with toxicity.

Perturbations in protein quality control

Precisely why polyQ disease proteins are toxic to neurons remains unclear. One possibility is that the production of misfolded polyQ protein places a continual burden on quality control pathways in cells. Neurons possess hundreds of proteins that facilitate the correct folding of proteins, the efficient refolding, disaggregation and degradation of abnormally folded or aggregated proteins. If this elaborate machinery for maintaining protein homeostasis is impaired in the presence of mutant polyQ protein, then other proteins that otherwise would fold correctly may also begin to accumulate as misfolded proteins. Using the nematode as a model system, Gidalevitz and colleagues [7•] recently showed that this is indeed the case. When mutant polyQ proteins were expressed in the worm, other metastable (i.e. temperature-sensitive) cellular proteins were driven to misfold. Thus, expression of mutant polyQ protein can induce global misfolding in cells. Chronic perturbation of global protein homeostasis in this manner would be expected to have deleterious effects in neurons.

The cellular pathways to handle abnormal polyglutamine proteins include three major pathways: molecular chaperones, the ubiquitin–proteasome degradation and autophagy. All three have been implicated in the polyglutamine disorders, and now there is growing consensus that they are functionally linked. For example, a quality control protein recently shown to modulate polyglutamine toxicity is carboxy-terminus of Hsc70 interacting protein (CHIP). CHIP functions both as a co-chaperone and as a ubiquitin ligase, thereby linking the chaperone and proteasome pathways [8•]. Indeed, CHIP reduces levels of aggregated polyQ protein in various model systems, and may do so both by favoring the formation of soluble, nontoxic conformers of the protein and by facilitating the degradation of aberrant polyglutamine protein [9,10].

Numerous molecular chaperones, including heat shock protein (Hsp) 70 and Hsp40, have been shown to suppress polyQ toxicity in numerous model systems. Three groups recently added an important new wrinkle to the chaperone story: the cytosolic chaperonin, TRiC, which participates in nascent protein synthesis, prevents aggregation and toxicity of polyglutamine proteins in cell-based and in-vitro assays [6•,11,12]. It will be interesting to see whether this effect translates *in vivo* to mouse models of disease.

While the notion that chaperones modulate disease is increasingly compelling, evidence directly implicating the proteasome pathway in disease is less clear cut (reviewed in [13]). Proteasome function declines with age, paralleling the age-related symptoms in SCAs, and overexpressed polyQ proteins impair proteasome function in transfected cells. Nevertheless, there is no compelling in-vivo evidence to date that mutant polyQ proteins directly inhibit the proteasome in brain. Indeed, some carefully controlled studies in mice have failed to show proteasomal compromise in affected tissues.

Recently, autophagy [14] has been implicated in a variety of neurodegenerative diseases. The importance of autophagy in neurons was recently confirmed when knockout of the autophagy gene *Atg7* in mice led to age-related neurodegeneration with accumulation of ubiquitinated inclusions [15•]. At this point, no published studies have confirmed a significant role for autophagy in the polyglutamine SCAs, but researchers are actively pursuing this possibility.

Nucleus as site of toxicity

Perturbation in protein homeostasis is not incompatible with an alternative hypothesis: that the mutant polyQ protein interacts aberrantly with various cellular proteins, including both normal protein partners and novel interactors [16]. While some overlap would be expected across diseases, the set of interacting proteins for each disease protein would be unique. Such a scenario could lead to disease-specific, deleterious consequences of mutant polyQ proteins. Lim *et al.* [17] recently reported a systematic analysis of SCA protein–protein interactions that is consistent with this model. Recent findings further supporting this model include aberrant interactions between polyQ proteins and transcription factors and nonhistone chromatin proteins in the nucleus [18•,19•, 20,21]. Alterations in protein quality control pathways could promote such aberrant interactions and thus impair the exquisite regulation of neuronal gene expression.

As most polyQ proteins normally reside in the nucleus or become concentrated in the nucleus during disease, the hypothesis that they trigger disease by perturbing gene expression is compelling [22]. Expanded polyglutamine proteins or polyQ-containing proteolytic fragments engage in aberrant protein–protein interactions in the

nucleus, including with important transcriptional components and chromatin proteins. Interactions with polyQ oligomeric complexes may functionally deplete certain transcription factors and other important nuclear proteins in affected neurons, resulting in altered activity at specific promoters and perturbed chromatin modification by histone acetyltransferases. Moreover, several polyQ-containing proteins are directly involved in transcription: among the SCAs, the SCA7 protein ataxin-7 is now known to be part of the STAGA transcriptional complex, and the SCA17 protein is the basal transcription factor, TATA-binding protein. Other SCA proteins, such as ataxin-1, interact with and regulate specific transcriptional complexes. Moreover, many transcription factors contain polyQ or glutamine-rich domains, suggesting that polyQ motifs directly modulate transcription.

Consistent with transcriptional dysregulation as a pathogenic mechanism, microarray analyses of tissue from many mouse and cell models of SCAs have demonstrated transcriptional alterations. The challenge now is to define which gene expression changes directly relate to pathogenesis and which are merely compensatory responses. It will also be important to determine how well the observed changes in transcripts correlate with altered protein levels. Advances in proteomics now make it possible to explore changes in protein profiles systematically, but discerning which changes are proximal, pathogenic events and which are epiphenomena remains a challenge.

Transcriptional dysregulation caused by mutant polyQ proteins reflects, in part, changes in histone acetylation. Certain transcription factors known to interact with polyQ proteins, for example cAMP response element binding protein (CBP), possess histone acetyltransferase (HAT) activity. Since polyQ proteins can inhibit HAT activity and thereby repress transcription, this suggests a potential form of therapy: administration of histone deacetylase (HDAC) inhibitors. Indeed, HDAC inhibitors have rescued polyQ toxicity in cells and flies and may even reduce further repeat instability upon transmission, based on recent studies in transgenic flies. The HDAC inhibitor, phenylbutyrate, has already been tested in Huntington disease patients, with the results of this trial still pending. If this compound or another HDAC inhibitor proves effective in such patients, it should also be tested promptly in the polyglutamine SCAs.

The reader should keep in mind that changes in gene expression need not occur only at the transcriptional level or only involve protein-coding genes. For example, the SCA2 disease protein ataxin-2 may modulate protein translation by assembling with ribosomes [23]. Moreover, recent findings suggest that noncoding microRNAs, a hot topic in biology, are altered in disease [24,25]. Since microRNAs regulate the expression of protein-coding genes, their dysregulation could have profound effects on protein expression profiles.

Influence of protein context: specific proteins for specific diseases

The marked clinical differences among the polyQ SCAs despite their shared mutational mechanism illustrate the importance of disease protein context in pathogenesis. Here we will discuss recent findings in SCA1, SCA3 and SCA6 to illustrate this point.

It has been known for some time that the toxicity of the SCA1 disease protein, ataxin-1, depends on protein elements far removed from the actual polyglutamine expansion. For example, a carboxy-terminal nuclear localization signal and a distal serine residue known to be phosphorylated (serine at position 776) are both required for mutant ataxin-1 to mediate toxicity. New evidence now links ataxin-1 toxicity to its ability to associate with the transcriptional repressor, capicua [18•]. The serine-mutated form of ataxin-1, which is nontoxic even with a polylglutamine expansion, fails to enter this repressor complex. Moreover, when the ataxin-1 paralog, ataxin-1 like protein, is overexpressed in mice, it displaces mutant ataxin-1 from the repressor complex and thereby suppresses toxicity [26]. Ataxin-1 also forms a complex with retinoid-related orphan receptor α (RORα), a transcription factor important for cerebellar development. Expression of mutant ataxin-1 leads to depletion of this critical transcription factor, which likely contributes to pathogenesis [19•]. Mutant ataxin-1 interacts deleteriously with RORα during cerebellar development. If mutant ataxin-1 is not expressed until after this developmental window has passed, cerebellar degeneration is substantially reduced, suggesting that early developmental events influence later neurodegeneration in the adult. Taken together, the above findings strongly support a model of toxicity in which specific protein interactions made by ataxin-1, interactions which require the surrounding protein context of ataxin-1, are essential to pathogenesis.

Despite being the smallest polyglutamine disease protein, the SCA3 protein ataxin-3 functions in diverse cellular pathways. It is a ubiquitin chain-binding protein and deubiquitinating enzyme that participates in the 'handling' of abnormal proteins from the cytoplasm and endoplasmic reticulum [27,28]. Ataxin-3 seems prone to aggregate *in vitro* even in the absence of polyglutamine expansion, although in the presence of expansion an irreversible form of aggregation takes place [29]. The relationship of normal ataxin-3 function to disease was suggested by studies in *Drosophila* showing that normal ataxin-3 suppresses toxicity induced by various expanded polyglutamine proteins. This suppressor activity was linked to its ubiquitin-associated functions. When the enzymatic activity of expanded ataxin-3 was made catalytically inactive by

mutating a single amino acid, the protein became much more highly toxic. The evidence supports a model in which mutant ataxin-3 possesses an intrinsic activity to suppress its own polyglutamine toxicity. This may explain why full-length, mutant ataxin-3 does not cause human disease until the repeat is typically at least 55 residues in length, much larger than the repeats in other polyglutamine diseases [30].

SCA6 is a special case precisely because of its unique protein context: it is the only polyQ disease caused by a mutation in a membrane protein, the voltage-gated calcium channel α-1A subunit. Expansion may lead directly to channel dysfunction in SCA6, as expression of the mutant channel subunit shifts the voltage dependence of channel inactivation. This is attractive as a disease mechanism because many other conventional mutations in this same channel subunit are known to cause episodic ataxia type 2. This fact notwithstanding, abnormal accumulations of the disease protein have been observed in SCA6 disease brain, reminiscent of other polyQ SCAs. It is even possible that the disease protein is proteolytically cleaved, releasing a polyglutamine-containing fragment that acts in the nucleus, as proposed for other polyQ diseases [31].

Thinking beyond the neuron

Understandably, research has focused on neurons because they are the cell type most prone to degenerate. Disease, however, reflects pathology not of individual neurons; rather, it is networks of cells, some of which are not neurons, that constitute the in-vivo environment in which degeneration takes place. Custer and colleagues [32•] have now shown that selective expression of a polyQ disease protein, ataxin-7, in Bergmann glia of the cerebellum leads to degeneration of neighboring Purkinje cells. Expression of expanded ataxin-7 in glial cells may cause neurodegeneration by inhibiting glutamate uptake, leading to excessive, toxic glutamate exposure to nearby Purkinje cells. Similar nonneuronal involvement in pathogenesis is actively being sought for other SCAs.

Leads to potential therapy for polyglutamine spinocerebellar ataxias

Very few preventive clinical trials have been performed in the SCAs. Most clinical trials in polyglutamine diseases have focused on Huntington disease and likely will continue to for the near future given the well developed clinical trial structure in the Huntington disease community. One hopes that clues to preventive therapy in the polyglutamine SCAs will come from these studies. Any trial drug that benefits such patients immediately becomes a candidate for testing in any of the polyglutamine SCAs. This is especially true if the compound's mechanism of action counteracts a shared feature of expanded polyglutamine proteins rather than a unique property of the Huntington disease protein, huntingtin.

Among candidate therapies, the electron transport molecule coenzyme Q10 slowed disease progression in Huntington disease mouse models and led to a statistically insignificant benefit in a carefully controlled trial in Huntington disease patients (the HD-CARE study) [33]. One hopes that higher doses of coenzyme Q10, soon to be tested in a similar Huntington disease clinical trial, will show real benefit. Oral administration of another compound to counteract cellular energy depletion, creatine, also proved beneficial in Huntington disease mouse models and reduced a marker of DNA damage, 8-hydroxydeoxyguanosine, in a short-term human trial [34]. As mentioned earlier, HDAC inhibitors may hold promise [35•] based on successful treatment trials in mouse and fly models of disease. If such treatment also reduced repeat instability, as suggested by recent findings in flies [36], this would be an unexpected bonus. Given the central role of protein aggregation in disease, the search for antiaggregation compounds [37] may yield a compound that acts on all members of the polyQ class. Finally, RNA interference or antisense approaches to decrease expression of the mutant gene product have been successful in mouse models [38], though the preclinical studies required to bring this to the clinic will take some time.

Advances in noncoding repeat spinocerebellar ataxias

The pathogenic mechanisms in the known noncoding repeat SCAs remain uncertain. The past year has witnessed new advances in SCA8 and SCA10.

SCA8

SCA8 is associated with a large CTG repeat expansion that is not fully penetrant (i.e. not all individuals with the expansion develop disease). The CTG expansion resides in an antisense RNA for the Kelch-like 1 gene, *KLHL1* [39]. In the limited neuropathological analyses to date, degeneration of Purkinje cell, inferior olivary and nigral neurons has been documented, together with periaqueductal gliosis. The particular pattern of neurodegeneration and Purkinje cell cytopathology in SCA8 may be distinct from that of other SCAs [40].

Recent studies in mouse [41•] and *Drosophila* [42] models provide insight into the cellular role of the SCA8 locus and the role of the repeat expansion in disease. At this point the jury is still out on the pathogenic mechanism in SCA8. In fact, possibly more than one pathogenic mechanism is at play: the CTG expansion could alter *KLHL1* expression [43•], act through a dominant RNA-based mechanism as in myotonic dystrophy [44], or be transcribed in the opposite (CAG) direction and thus encode a toxic polyglutamine protein fragment [41•,44].

In a recent mouse model expressing the human SCA8 locus, the expansion caused a progressive neurological

phenotype accompanied by reduced cerebellar-cortical inhibition [41•]. Intriguingly, both in these mice and in human SCA8 autopsy tissue, intranuclear inclusions in Purkinje and brainstem neurons could be immunolabeled by an antibody specific for expanded polyglutamine. This suggested that the CTG repeat is also transcribed in the opposite (CGA) direction, producing an expanded polyglutamine protein. If this is the case, however, the protein would differ from other polyQ disease proteins in that it would be an isolated stretch of polyQ with no flanking protein sequence. Such a stripped-down polyQ fragment has been shown to be toxic and aggregation prone in some cell models.

It is also possible that the expansion alters antisense-mediated regulation of the *KLHL1* gene product, KLHL1. A brain-specific, cytosolic protein, KLHL1 likely functions as an actin-organizing protein that modulates neurite outgrowth, the dynamic properties of dendritic spines, and neuronal proteins essential for postsynaptic function. In mice, targeted deletion of the *Klhl1* gene in Purkinje neurons results in dendritic deficits, abnormal gait, and progressive motor incoordination [43•]. Importantly, mice heterozygous for the *Klhl1* deletion also have significant gait abnormalities and incoordination, suggesting that KLHL1 expression changes induced by the expansion could account for some of the pathology seen in this disorder. KLHL1 also has been shown to interact with and modulate voltage-gated calcium channels, in particular the principal subunit of P/Q-type channels, α (1A) subunit [45]. In the presence of KLHL1, P/Q-type current properties show a significant increase in mean current density and a shift in steady state activation.

SCA10

A distinctive ataxia because it so often is accompanied by seizures, SCA10 is caused by a huge ATTCT expansion in an intron of the *ATXN10* gene. This gene encodes a novel protein, ataxin-10, of unknown function but highly expressed in brain [46]. Thus far, the SCA10 mutation has been restricted to those of Amerindian ancestry [47]. In 2006, studies were conducted in two SCA10 families manifesting with distinct seizure frequencies [48]. One family displayed uninterrupted ATTCT expansions, while in the second family the expansion was interrupted by nonconsensus repeat units differing in length and sequence. This report challenged the convention that disease-causing microsatellite expansions consist only of uninterrupted pure repeats and suggested that the purity of the expanded repeat element may be a disease modifier in SCA10.

In a mouse model the mutant *ATXN10* allele was transcribed at normal levels, and in patient-derived cells the premRNA was processed correctly. While SCA10-null mice exhibit embryonic lethality, heterozygous mutants are overtly normal and do not develop an ataxic phenotype [49]. This suggests that a partial loss of *ATXN10* function is unlikely to be the major pathogenic mechanism. Using yeast-two-hybrid screening followed by confirmatory mammalian cell studies, Waragai *et al.* [50] identified a G-protein β subunit (Gβ2) as an ataxin-10 binding partner. Overexpression of ataxin 10 in PC12 cells induced neurite extension and enhanced neuronal differentiation induced by nerve growth factor. The ataxin-10/Gβ2 interaction may represent a novel mechanism for inducing neuritogenesis in neural cells by activating the Ras–mitogen-activated protein kinase–Elk-1 cascade.

Spinocerebellar ataxias caused by conventional mutations

Only in the past few years have SCAs been discovered to be caused by conventional mutations. To date, four have been identified: SCA5, 13, 14 and 27. The diverse functions of the defective genes suggest that a wide range of biological pathways can be disrupted to cause cerebellar degeneration.

SCA5

With the identification of mutations in the *SPTBN2* gene in SCA5 families, βIII spectrin became recognized as an ataxia disease gene [51•]. The involvement of this cytoskeletal component directs attention towards the possible role of organelle stability and altered membrane protein dynamics in neuronal health and function. The findings suggest that mechanical properties of neurons and their dynamics may be as important as altered Ca^{2+} homeostasis, transcriptional dysregulation, and impaired protein degradation in causing neurodegeneration.

SCA13

SCA13 was recently shown to be caused by mutations in the *KCNC3* gene, which encodes a voltage-gated K^+ channel (Kv3.3) highly enriched in cerebellum [52•]. Mutations in this gene have a dominant effect on electrophysiological properties of the multisubunit K^+ channel, and lead to disease phenotypes with neurodevelopmental and neurodegenerative features. Two missense mutations have been studied in *Xenopus laevis* expression systems. $KCNC3^{R420H}$, located in the voltage-sensing domain, possessed no channel activity when expressed alone and had a dominant-negative effect when coexpressed with the wild-type channel subunit. $KCNC3^{F448L}$ shifted the activation curve in the negative direction and slowed channel closing. Thus, these mutations are expected to change the output characteristics of fast-spiking cerebellar neurons, in which KCNC channels confer the capacity for high-frequency firing.

SCA14

SCA14 is caused by various missense, deletion and splice site mutations in the *PRKCG* gene encoding protein

kinase Cγ [53,54]. This member of the family of serine/threonine kinases is highly expressed in Purkinje cells. To date, at least 18 mutations have been reported, 12 of them located in exon 4 encoding the important Cys2 subdomain of protein kinase Cγ [55]. The clinical features induce by *PRKCG* mutations can be quite heterogeneous even in the same ethnic background [56]. While most affected patients display a late-onset uncomplicated form of spinocerebellar ataxia with occasional mild extrapyramidal features, one individual presented with a mild, childhood onset, nonprogressive ataxia accompanied by multifocal myoclonus [57].

SCA27

Characterized by impaired cognitive abilities and slowly progressive ataxia, SCA27 is caused by mutations in the fibroblast growth factor 14 (*FGF14*) gene [58]. The discovery of the SCA27 mutation was precipitated by the finding that *Fgf14* knockout mice have ataxia, paroxysmal dyskinesias and impaired sensorimotor function. Xiao and colleagues [59] further showed that synaptic transmission at hippocampal Schaffer collateral-CA1 synapses and short and long-term potentiation were impaired in Fgf14(−/−) mice. Findings suggest a novel role for FGF14 in regulating synaptic plasticity by controlling the mobilization, trafficking or docking of synaptic vesicles to presynaptic active zones. The similarities between Fgf14(−/−) mice and humans with SCA27 was extended by Wozniak *et al.* [60] in showing that Fgf14(−/−) mice exhibit spatial memory deficits in the Morris water maze. These results suggest a role for FGF14 in spatial learning and synaptic plasticity. What remains unclear is whether the dominant inheritance of SCA27 reflects haplo-insufficiency for this important brain gene.

Conclusion

As the number of SCAs continues to grow, so does the range of diverse molecular mechanisms causing cerebellar degeneration. While repeat expansions have been known to cause SCAs for over a decade, the emerging recognition of conventional gene mutations in various SCAs lends support to the view that many different biological pathways, when disrupted, can manifest primarily with cerebellar degeneration. Although more still needs to be learned about the molecular basis of SCAs, one especially promising advance is that new insights into the toxic protein mechanism in the polyglutamine SCAs have begun to suggest routes to preventive therapy.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 513).

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