Molecular pathogenesis of spinocerebellar ataxias

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The autosomal dominant spinocerebellar ataxias (SCAs) are a group of neurodegenerative diseases, clinically and genetically heterogeneous, characterized by loss of balance and motor coordination due to dysfunction of the cerebellum and its afferent and efferent connections. Despite a well-described clinical and pathological phenotype, the molecular and cellular events that underlie neurodegeneration are still poorly understood. Compelling evidence points to major aetiological roles for interference with transcriptional regulation, protein aggregation and clearance, the ubiquitin-proteasome system and alterations of calcium homeostasis in the neuronal loss observed during the neurodegenerative process. But novel molecular routes that might be disrupted during disease progression are also being identified. These pathways could act independently or, more likely, interact and enhance each other, triggering the accumulation of cellular damage that eventually leads to dysfunction and, ultimately, the demise of neurons through a series of multiple events. This suggests that simultaneous targeting of several pathways might be therapeutically necessary to prevent neurodegeneration and preserve neuronal function. Understanding how dysregulation of these pathways mediates disease progression is leading to the establishment of effective therapeutic strategies in vivo, which may prove beneficial in the treatment of SCAs. Herein, we review the latest evidence for the proposed molecular processes to the pathogenesis of dominantly inherited spinocerebellar ataxias and the current therapeutic strategies.

Keywords: spinocerebellar ataxias; cerebellum; neurodegenerative disorders; neurodegenerative mechanisms; therapy

Abbreviations: ADCA = autosomal dominant spinocerebellar ataxia; CAG = DNA sequence coding for glutamine; DRPLA = dentatorubral pallidoluysian atrophy; ER = endoplasmic reticulum; FGF14 = fibroblast growth factor 14; HDACs = histone deacetylases; KCNC3 = potassium voltage-gated channel subfamily C member 3; PP2 = protein phosphatase 2 (formerly 2A); PRKCG = protein kinase C, gamma; Q = glutamine; SCA = spinocerebellar ataxia; SPTBN2 = beta-III spectrin; TBP = TATA box binding protein; UPS = ubiquitin-dependent proteasome system


Introduction

The autosomal dominant spinocerebellar ataxias (SCAs) are a complex group of neurodegenerative disorders characterized by progressive cerebellar ataxia of gait and limbs variably associated with ophthalmoplegia, pyramidal and extrapyramidal signs, dementia, pigmentary retinopathy and peripheral neuropathy (Zoghbi, 2000). Disease onset is usually between 30 and 50 years of age, although early onset in childhood and onset in later decades after 60 years have been reported. The prognosis is variable depending on the underlying cause of the spinocerebellar ataxia subtype. Epidemiological data indicate that SCAs might be more common than that previously estimated with prevalences of up to 5–7 in 100 000 in some populations (van de Warrenburg et al., 2002; Craig et al., 2004). This is similar to the prevalence of other uncommon motor neurodegenerative diseases, such as Huntington’s or motor neuron diseases. Founder mutation effects appear to contribute to the variable prevalence detected in specific SCA subtypes.

In the pre-genomic era, SCAs have been particularly controversial in terms of nomenclature and classification. Harding first proposed a classification of the autosomal dominant cerebellar ataxias (ADCAs) on the basis of the clinical symptoms, and differentiated this group of disorders...
into three main groups (Table 1) (Harding, 1993). It is important to note that Harding’s classification has not been overridden by the genetic classification and is still invaluable as a guideline in clinical practice and to prioritize genetic tests for diagnosis. ADCA type I is characterized by ataxia of the gait variably associated with ophthalmoplegia, pyramidal and extrapyramidal signs, cognitive impairment, optic atrophy or peripheral neuropathy. The clinical features in this group of ataxias are caused by a combination of degeneration of the cerebellum, basal ganglia, cerebral cortex, optic nerve, pontomedullary systems, spinal tracts or peripheral nerves. ADCA type II is distinct from ADCA type I by the presence of pigmented retinopathy. A third group, ADCA type III, includes relatively pure cerebellar ataxias where the degenerative process is limited to the cerebellum. ADCAs I and III are clearly genetically heterogeneous, whereas at least two different genes are associated with ADCA II (Table 1) (Giunti et al., 1999). Although the aetiology of SCAs is still poorly understood, genetic analyses, epidemiological data, neuropathological investigations and new experimental models are providing important new insights into the pathogenic mechanisms. At least 28 distinct loci are responsible for rare Mendelian forms of SCA (Table 2). Interestingly, a few SCA subtypes, including SCAs 1, 2, 3, 4, 8, 10, 12, 13, 17, 18, 19/22, 20, 21, 23, 24, 25, 27, 28, DRPLA, are caused by the expansion of a CAG (DNA sequence coding for glutamine) repeat sequence located within the coding region of specific genes, leading to an abnormally long polyglutamine (polyQ) tract in the encoded proteins named ataxins 1, 2 and 3, alpha 1A-voltage-dependent calcium channel, ataxin 7, TATA box binding protein (TBP) and atrophin 1, respectively. These SCAs show, as common features, the progressive neurodegeneration of neuronal subsets in distinct brain areas and the formation of polyQ-containing protein aggregates forming characteristic nuclear or cytoplasmic inclusions (Zoghbi and Orr, 2000). The age at onset and severity of disease symptoms inversely correlate with the length of the glutamine repeat. A second group of SCAs, including SCAs 8, 10 and 12, are caused by a repeat expansion located outside of the coding region of the disease genes leading to dysregulation of gene expression (Table 2) (Holmes et al., 1999; Koob et al., 1999; Matsuura et al., 2000). While the molecular mechanisms underlying SCAs 8 and 10 are unclear, SCA12 appears to be caused by dysregulation of the activity of the crucial enzyme protein phosphatase 2 (PP2, formerly named PP2A) in cerebellar Purkinje cells. Different mechanisms cause cerebellar ataxia and neurodegeneration in SCAs 5, 13, 14 and 27, where alterations in amino acid composition in beta-III spectrin (SPTBN2) (Ikedo et al., 2006), potassium channel KCNC3 (Waters et al., 2006), protein kinase C (PRKCG) (Chen et al., 2003a; Yabe et al., 2003) and fibroblast growth factor 14 (FGF14) (van Swieten et al., 2003), respectively, elicit disease symptoms in these four SCA subtypes. In the rest of SCAs, the genes and, therefore, the mutations remain to be identified and characterized. Understanding the pathogenetic mechanisms of neurodegeneration in spinocerebellar ataxias should lead to the identification of potential therapeutic targets and ultimately facilitate drug discovery.

This review highlights the scientific evidence for the major pathways leading to neurodegeneration in autosomal dominant SCAs, generated from genetic analysis, in vitro studies, experimental animal models and post-mortem brain studies (Fig. 1). We discuss the convergence of these pathways in the identification of potential molecular targets that could be used effectively to develop treatment (Table 3).

### Polyglutamine neurotoxicity: protein aggregation, misfolding, stability and clearance

Seven spinocerebellar ataxia subtypes including SCAs 1, 2, 3/Machado-Joseph disease, 6, 7, 17 and DRPLA are caused by the expansion of a CAG-repeat sequence in specific genes, leading to abnormally long polyQ tracts in the encoded proteins (Zoghbi and Orr, 2000). Presumably, a similar pathogenic pathway is involved in these SCAs, since each of the expanded CAG repeats encodes polyglutamine and the pathogenic threshold for disease is roughly the same, at around 40 copies of the repeat in most of the different subtypes. It is assumed that the common toxic gain-of-function mechanisms for the polyglutamine-containing protein are aggregation and deposition of misfolded proteins leading to neuronal dysfunction and eventually cell death.

### Table 1 Modified Harding’s classification of ADCAs

<table>
<thead>
<tr>
<th>ADCA type</th>
<th>ADCA I</th>
<th>ADCA II</th>
<th>ADCA III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical presentation</td>
<td>Cerebellar syndrome with ophthalmoplegia/pyramidal/extrapyramidal signs/cognitive impairment/peripheral neuropathy</td>
<td>Cerebellar syndrome with pigmented retinopathy</td>
<td>Pure cerebellar syndrome</td>
</tr>
<tr>
<td>Neuropathology</td>
<td>Degeneration of the cerebellum, and of the basal ganglia/cerebral cortex/optic nerve/pontomedullary systems/spinal tracts/peripheral nerves</td>
<td>Cerebellar and pigmented retinal degeneration</td>
<td>Cerebellar degeneration</td>
</tr>
<tr>
<td>Genetic loci</td>
<td>SCAs 1, 2, 3, 4, 8, 10, 12, 13, 17, 18, 19/22, 20, 21, 23, 24, 25, 27, 28, DRPLA</td>
<td>SCA7**</td>
<td>SCAs 5, 6, 11, 14, 15, 16, 26</td>
</tr>
</tbody>
</table>

**A British ADCAII family negative for the SCA7 mutation has been reported (Giunti et al., 1999).
The gene encoding puratrophin 1 lies on the same chromosomal region where the SCA4 *SCAs 19 and 22 are likely allelic forms of the same gene.

<table>
<thead>
<tr>
<th>SCA subtype</th>
<th>Genomic location</th>
<th>Gene/locus</th>
<th>Protein</th>
<th>Mutation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCA1</td>
<td>6p22.3</td>
<td>ATXN1</td>
<td>Ataxin 1</td>
<td>CAG repeat</td>
<td>Orr et al. (1993)</td>
</tr>
<tr>
<td>SCA2</td>
<td>12q24.13</td>
<td>ATXN2</td>
<td>Ataxin 2</td>
<td>CAG repeat</td>
<td>Imbert et al. (1996)</td>
</tr>
<tr>
<td>SCA3</td>
<td>14q32.12</td>
<td>ATXN3</td>
<td>Ataxin 3</td>
<td>CAG repeat</td>
<td>Pulst et al. (1996)</td>
</tr>
<tr>
<td>SCA4</td>
<td>16q24-pter</td>
<td>ATXN4</td>
<td>U</td>
<td>U</td>
<td>Kawaguchi et al. (1994)</td>
</tr>
<tr>
<td>SCA5</td>
<td>11q13.2</td>
<td>SPTBN2</td>
<td>Beta-III spectrin</td>
<td>D, MM</td>
<td>Flanigan et al. (1996)</td>
</tr>
<tr>
<td>SCA6</td>
<td>19p13.13</td>
<td>CACNA1A</td>
<td>CACNA1A</td>
<td>CAG repeat</td>
<td>Ikeda et al. (2006)</td>
</tr>
<tr>
<td>SCA7</td>
<td>3p14.1</td>
<td>ATXN7</td>
<td>Ataxin 7</td>
<td>CAG repeat</td>
<td>Zhaochenko et al. (1997)</td>
</tr>
<tr>
<td>SCA8</td>
<td>13q21</td>
<td>KLHL1A5</td>
<td>Kelch-like 1</td>
<td>CTG repeat</td>
<td>David et al. (1997)</td>
</tr>
<tr>
<td>SCA9</td>
<td>Reserved</td>
<td></td>
<td>U</td>
<td>U</td>
<td>Koob et al. (1999)</td>
</tr>
<tr>
<td>SCA10</td>
<td>22q13.31</td>
<td>ATXN10</td>
<td>Ataxin 10</td>
<td>ATTCT repeat</td>
<td>Matsuura et al. (2000)</td>
</tr>
<tr>
<td>SCA11</td>
<td>15q14-q21.3</td>
<td>SCA11</td>
<td>U</td>
<td>U</td>
<td>Worth et al. (1999)</td>
</tr>
<tr>
<td>SCA12</td>
<td>5q32</td>
<td>PPP2R2B</td>
<td>PPP2R2B</td>
<td>CAG repeat</td>
<td>Holmes et al. (1999)</td>
</tr>
<tr>
<td>SCA13</td>
<td>19q13.33</td>
<td>KCNC3</td>
<td>KCNC3</td>
<td>MM</td>
<td>Waters et al. (2006)</td>
</tr>
<tr>
<td>SCA14</td>
<td>19q13.42</td>
<td>PRKCG</td>
<td>PRKCG</td>
<td>MM</td>
<td>Chen et al. (2003a)</td>
</tr>
<tr>
<td>SCA15</td>
<td>3p24.2-pter</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>Gardner et al. (2005)</td>
</tr>
<tr>
<td>SCA16</td>
<td>8q23-q24.1</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>Miyoshi et al. (2001)</td>
</tr>
<tr>
<td>SCA17</td>
<td>6q27</td>
<td>TBP</td>
<td>TBP</td>
<td>CAG repeat</td>
<td>Nakamura et al. (2001)</td>
</tr>
<tr>
<td>SCA18</td>
<td>7q31-q32</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>Devos et al. (2001)</td>
</tr>
<tr>
<td>SCA19*</td>
<td>1p21-q21</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>Verbeek et al. (2002)</td>
</tr>
<tr>
<td>SCA20</td>
<td>11</td>
<td></td>
<td>U</td>
<td>U</td>
<td>Knight et al. (2004)</td>
</tr>
<tr>
<td>SCA21</td>
<td>7p21.3-p15.1</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>Vuillaume et al. (2002)</td>
</tr>
<tr>
<td>SCA22*</td>
<td>1p21-q23</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>Chung et al. (2003)</td>
</tr>
<tr>
<td>SCA24</td>
<td>1p36</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>Swartz et al. (2002)</td>
</tr>
<tr>
<td>SCA25</td>
<td>2p21-p15</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>Stevanin et al. (2005)</td>
</tr>
<tr>
<td>SCA26</td>
<td>19p13.3</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>Yu et al. (2005)</td>
</tr>
<tr>
<td>SCA27</td>
<td>13q33.1</td>
<td>FGF14</td>
<td>FGF14</td>
<td>MM</td>
<td>van Swieten et al. (2003)</td>
</tr>
<tr>
<td>SCA28</td>
<td>18p11.22-q11.2</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>Cagnoli et al. (2006)</td>
</tr>
<tr>
<td>DRPLA</td>
<td>12p13.31</td>
<td>ATN1</td>
<td>Atrophin 1</td>
<td>CAG repeat</td>
<td>Koide et al. (1994)</td>
</tr>
<tr>
<td>Undefined***</td>
<td>16q22.1</td>
<td>PLEKKG4</td>
<td>Puratrophin 1</td>
<td>S’ SNS</td>
<td>Ishikawa et al. (2005)</td>
</tr>
</tbody>
</table>

*SCAs 19 and 22 are likely allelic forms of the same gene.

**The gene encoding puratrophin 1 lies on the same chromosomal region where the SCA4 gene localizes. Genes in genomic location are noted according to Ensembl. D, deletions; MM, missense mutations; SNS, single-nucleotide substitutions; U, unknown.

Proteins with expanded stretches of polyglutamine appear to take on an abnormal configuration resulting in the formation and deposition of polyglutamine aggregates in disease neurons forming characteristic nuclear or cytoplasmic inclusions, which are neuropathological hallmarks in these diseases (Ross and Poirier, 2004). These inclusions contain cellular components such as ubiquitin, the proteasome, HSP70 and transcription factors (Cummings et al., 1998; McCampbell et al., 2000; Schmidt et al., 2002). Whether the toxicity is a direct result of the aggregate or results from intermediary structures formed during the process of aggregation remains to be determined. However, blocking aggregation has been one approach attempted to minimize toxicity. Expanded polyglutamine proteins form fibrillar proteinaceous aggregates much more rapidly than normal proteins (Chow et al., 2002). Whether the toxicity is a direct result of the aggregate or results from intermediary structures formed during the process of aggregation remains to be determined. However, blocking aggregation has been one approach attempted to minimize toxicity. Expanded polyglutamine proteins form fibrillar proteinaceous aggregates much more rapidly than normal proteins (Chow et al., 2002).

The relationship between SCA neurodegeneration and the ubiquitin-dependent proteasome system (UPS), the main cellular machinery to degrade aberrantly folded proteins, is evidenced by the consistent findings of ubiquitin-positive protein aggregates in neuropathological studies (Ross and Poirier, 2004). In addition, there is evidence that a few ataxins, such as ataxins 1, 3 and 7, are susceptible to be ubiquitinated and targeted by the proteasome for degradation and clearance (Cummings et al., 1999; Matilla et al., 2001; Chai et al., 2004). Protein misfolding exerted by the expanded
Fig. 1 Molecular mechanisms of neurodegeneration in spinocerebellar ataxias. 1, aggregation; 2, apoptosis; 3, autophagy; 4, Ca\textsuperscript{2+} homeostasis alterations; 5, disruption of axonal transport and vesicle trafficking; 6, excitotoxicity; 7, interference with gene transcription; 8, mitochondrial impairment; 9, oxidative stress; 10, alterations of proteasome degradation; 11, synaptic dysfunction; 12, unfolded protein response (UPR); 13, potassium channel dysfunction; Ca\textsuperscript{2+}, calcium ions; ER, endoplasmic reticulum; Glu, glutamate; K\textsuperscript{+}, potassium ions; Na\textsuperscript{+}, sodium ions; Q, glutamine; Ub, ubiquitin.

Table 3 Potential molecular targets that could be used for treating spinocerebellar ataxias

<table>
<thead>
<tr>
<th>Molecular target</th>
<th>SCA subtype</th>
<th>Pathway</th>
<th>Potential drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akt</td>
<td>1</td>
<td>PI3K/Akt</td>
<td>Akt regulators, IGF-1</td>
</tr>
<tr>
<td>PP2</td>
<td>1, 12</td>
<td>PP2</td>
<td>PP2 regulators</td>
</tr>
<tr>
<td>PRKC</td>
<td>1, 14</td>
<td>PRKC</td>
<td>PRKC regulators</td>
</tr>
<tr>
<td>Gene transcription</td>
<td>1, 2, 3, 6, 7, 17, DRPLA</td>
<td>Several</td>
<td>HDACis: SAHA, Sirtuins, etc.</td>
</tr>
<tr>
<td>Aggregation</td>
<td>1, 2, 3, 6, 7, 17, DRPLA</td>
<td>Several</td>
<td>Suppressor polypeptides, chaperones, Congo Red, etc.</td>
</tr>
<tr>
<td>Aggregation</td>
<td>1, 2, 3, 6, 7, 17, DRPLA</td>
<td>Autophagy</td>
<td>Rapamycin</td>
</tr>
<tr>
<td>Aggregation</td>
<td>1, 2, 3, 6, 7, 17, DRPLA</td>
<td>SUMOylation</td>
<td>Regulators</td>
</tr>
<tr>
<td>Aggregation</td>
<td>1, 2, 3, 6, 7, 17, DRPLA</td>
<td>Transglutaminase</td>
<td>Transglutaminase inhibitors: cystamine</td>
</tr>
<tr>
<td>Molecular chaperones</td>
<td>1, 2, 3, 6, 7, 17, DRPLA</td>
<td>ER UPR, HSR</td>
<td>HSR induction: arimoclomol, geldanamycin, etc.</td>
</tr>
<tr>
<td>Ubiquitin/proteasome</td>
<td>1, 2, 3, 6, 7, 17, DRPLA</td>
<td>UPS</td>
<td>UPS enhancers</td>
</tr>
<tr>
<td>Mitochondrial function</td>
<td>All</td>
<td>Several</td>
<td>Coenzyme Q10, creatine, TUDCA</td>
</tr>
<tr>
<td>Calcium homeostasis</td>
<td>All: 1, 6</td>
<td>Calcium</td>
<td>Ca\textsuperscript{2+} signalling blockers</td>
</tr>
<tr>
<td>Dopaminergic signalling</td>
<td>1, 2, 3, 6, 17, 27</td>
<td>Dopamine</td>
<td>Amantadine, levodopa, dopaminergic and anticholinergic drugs</td>
</tr>
<tr>
<td>Neurotransmission</td>
<td>All</td>
<td>Glutamate, GABA</td>
<td>Inhibitors of mGlu and NMDA receptors, gabapentin</td>
</tr>
<tr>
<td>Caspases</td>
<td>All</td>
<td>Apoptosis/caspases</td>
<td>Caspase inhibitors: cystamine, CrmA, minocycline, FADD DN</td>
</tr>
<tr>
<td>Mutant ataxins</td>
<td>All</td>
<td>RNAi</td>
<td>RNAi</td>
</tr>
</tbody>
</table>

DRPLA, dentatorubral pallidolysian atrophy; ER, endoplasmic reticulum; HSR, heat-shock response; IGF-1, insulin growth factor 1; mGluR, metabotropic glutamate receptor; NMDAR, N-methyl-d-aspartate glutamate receptor; PP2, serine/threonine protein phosphatase 2; PRKC, protein kinase C; RNAi, RNA interference; SUMO, small ubiquitin-related modifier; UPR, unfolded protein response; UPS, ubiquitin/proteasome system; TUDCA, taurosodeoxycholic acid.
polyglutamine might lead to difficulties in the recognition and degradation process by the proteasome and, hence, in subsequent impaired clearance of mutant proteins. Further evidence indicates that polyglutamine expansions and aggregates may also derange UPS function. For instance, polyglutamine-expanded ataxin 1 appears to decrease the activity of the proteasome in cell culture (Park et al., 2005). In SCA3, ataxin 3 is an ubiquitin-specific cysteine protease that associates with the proteasome (Chai et al., 2004; Nicastro et al., 2005). It de-ubiquinates proteins by binding polyubiquitin chains through several ubiquitin interaction motifs (UIMs) within the Josephin domain, which is located near the polyQ tract. Elucidation of the molecular structure of the Josephin domain within ataxin 3 has led to the proposal of a model for recognition of interactions and formation of stable complexes with HHR23B located in the same surface involved in the interaction with UBA domains, the S5a polyUb-binding site and the proteasome (Nicastro et al., 2005). These observations provide a link between ataxin 3 and the UPS that is of particular relevance to neurodegenerative diseases. The expanded polyglutamine within ataxin 3 might alter its normal function and produce functional disruptions of the UPS pathway. Alternatively, reduction of proteasome activity may result from caspase-dependent cleavage of proteasome subunits owing to aggregated protein-induced apoptosis (Sun et al., 2004). Since the UPS plays a prominent role in the detoxification and targeting of damaged proteins for degradation, failure of this system might lead to an abnormal accumulation of a variety of toxic proteins, including those containing the polyglutamines, that would otherwise have been degraded, ultimately leading to neuronal dysfunction and/or death. Molecular chaperones, proteins that can repair or facilitate proteasome degradation of abnormally folded proteins, may play a role in SCAs as aggregates in human postmortem tissue often immunostain for chaperones (Ross and Poirier, 2004). This evidence indicates that the mechanisms of cell survival mediated by the endoplasmic reticulum (ER) chaperones and the unfolded protein response (UPR) are activated during neurodegeneration in spinocerebellar ataxias. The presence of unfolded proteins in the ER can cause ER stress or an imbalance between the load of unfolded proteins and the capacity of the ER protein-folding machinery. In order to restore ER homeostasis, neurons activate the ER stress response or UPR, eventually leading to transcriptional activation of genes encoding for chaperons. Consistent with this hypothesis, experimental overexpression of molecular chaperones modulates the formation of protein aggregates in cultured cells, transgenic mice and fruit flies, diminishing the toxicity of glutamine expansions (Cummings et al., 1998; Cummings et al., 2001; Bonini, 2002). How do chaperones modulate protein toxicity? A possible mechanism could be by stabilizing the misfolded monomeric conformation; chaperones might prevent the intramolecular transition that gives rise to spherical and annular oligomers and, simultaneously, stabilize a conformation that promotes inclusion body formation (Sakahira et al., 2002). Alternatively, by interacting with the disease protein, chaperones might prevent abnormal interactions with other proteins in the cell that are causal in toxicity.

Proteins that remain misfolded are degraded primarily by the ubiquitin-proteasome system, but also by the phagosome–lysosome system, a cellular process known as autophagy, which contributes to the routine turnover of cytoplasmic components (Shintani and Klionsky, 2004). Paradoxically, autophagy is used to protect cells, but also contributes to cell death. The accumulation of autophagic vesicles (bodies) has been observed in many neurodegenerative diseases where the associated proteins misfold and aggregate. Recent evidence has demonstrated that autophagy plays an essential role in the cellular clearance of toxic aggregated proteins in a cell culture model of polyglutamine-expanded ataxin 1-mediated neurodegeneration (Iwata et al., 2005). The precise mechanism by which aggregated mutant ataxin 1 is captured by autophagosomes is unclear, but it appears that inhibition of mTOR, a negative regulator of autophagic clearance, induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of neurodegeneration (Ravikumar et al., 2004). This study has established that autophagy efficiently degrades cytoplasmic insoluble aggregates and points to a possible therapeutic strategy by modulating the autophagic process to promote clearance of aggregated disease proteins. It is important to note that autophagy may also induce cell death in neurons that accumulate protein aggregates in a way that results in a pathological condition, although at present the mechanisms are unknown.

In 1993, a hypothesis was proposed where the neuronal protein inclusions induced by the expanded polyglutamines were stabilized by isopeptide cross-links introduced by transglutaminase (Green, 1993; reviewed in Hoffner and Djian, 2005). It was postulated that, as a result of excessive glutamine reiteration, the mutant proteins would become substrates of transglutaminase. Indeed, in vitro experiments demonstrated later that a glutamine repeat, if exposed on the surface of a protein, should form cross-linked aggregates in the presence of any transglutaminase activity activated by Ca\textsuperscript{2+}. While it has not been easy to examine for transglutaminase activity, the treatment of cultured cells bearing a truncated atrophin 1 in the presence of the transglutaminase inhibitor cystamine reduced aggregate formation and cell death (Igarashi et al., 1998). These observations provide arguments in favour of the transglutaminase hypothesis.

**Alterations in calcium homeostasis**

Alteration of calcium homeostasis plays a central role in apoptosis, and Ca\textsuperscript{2+} overload or perturbation of intracellular Ca\textsuperscript{2+} compartmentalization has long been recognized to be potentially cytotoxic (Orrenius et al., 2003). Calcium storage and signalling, as well as folding, modifying and sorting of newly synthesized proteins are among the main functions...
of the ER in mammalian cells. Disturbances in any of these functions can lead to the so-called ER stress. Both Ca\textsuperscript{2+} overload and depletion of the ER Ca\textsuperscript{2+} pool or alterations in the Ca\textsuperscript{2+} transport systems can result in changes in protein folding, in ER stress and activation of pro-apoptotic pathways. Mitochondria are known to participate actively in intracellular Ca\textsuperscript{2+} compartmentalization (Petersen, 2002). It has become apparent that mitochondrial calcium fluxes are integrated parts of cellular Ca\textsuperscript{2+} signalling. Calcium regulates outer mitochondrial membrane (OMM) permeabilization and, therefore, release of pro-apoptotic mitochondrial proteins into the cytoplasm, such as cytochrome c and apoptosis inducing factor (AIF), which is coupled to a stress response process known as inner-membrane permeability transition.

Strong evidence points to derangements of neuronal calcium signalling in neurodegeneration of spinocerebellar ataxias. Cerebellar Purkinje cells seem to be particularly sensitive to fluxes in intracellular calcium levels, which could result from different sources, such as the reduction of chaperone activity and ER stress. Several neuronal genes abundantly expressed in Purkinje cells that are involved in calcium signalling or homeostasis are downregulated in the cerebellum of SCA1 mutant mice before the occurrence of detectable motor impairment or pathology (Lin et al., 2000; Serra et al., 2004). This suggests a major role of disruption of calcium homeostasis in cerebellar Purkinje cells that could be importantly involved in the pathogenic process and/or the survival of these cells in SCA1.

In SCA6, Purkinje cell degeneration is associated with polyglutamine expansions within the CACNA1A gene (Zhuchenko et al., 1997). The CACNA1A gene encodes an alpha (2.1) subunit, formerly named [alpha]1A, which is the major pore-forming subunit of the Ca\textsubscript{v}2.1 voltage-dependent P/Q-type calcium channel (Pietrobon, 2002). Voltage-dependent calcium channels are made up of beta and gamma-s accessory subunits. Alpha subunits are membrane glycoproteins of ~2400 amino acids in length in which primary structure predicts the presence of four homologous domains, each consisting of six transmembrane domains and a pore-forming P loop. P/Q-type calcium channels are high-voltage-activated calcium channels found primarily in neurons and expressed at high levels in granule cells and Purkinje cells of the cerebellar cortex. Their principal role is believed to be in synaptic transmission. It remains to be demonstrated whether the action of the mutant gene product is to perturb calcium channel function, to serve as a target for transaminases or to bind to nuclear-binding proteins. Interestingly, three allelic diseases are caused by different types of mutations in the CACNA1A gene including SCA6, episodic ataxia type 2 and familial hemiplegic migraine (Mantuano et al., 2003). One form of cerebellar ataxia associated with a G293R mutation in the P loop of the first domain of the Ca\textsubscript{v}2.1 channel has a very similar phenotype to that of SCA6 associated with CAG-repeat expansions. As point mutations within the CACNA1A gene are not likely to act via the hypothetical nuclear-binding mechanisms or have any effects on transamination, an alteration of calcium channel function might be a possible scenario for the pathogenic alleles. Polyglutamine expansions in SCA6 might interfere with the Ca\textsuperscript{2+} channel to reduce Ca\textsuperscript{2+} influx and impaired function of the mutant Ca\textsuperscript{2+} channels, rendering them incapable of preventing cell death (Matsuyama et al., 2004). Still more electrophysiological studies are needed to clarify the molecular mechanisms underlying disease pathogenesis in SCA6 and other related ataxias.

**Mitochondrial stress and apoptosis**

Multiple lines of evidence suggest that neuronal death in spinocerebellar ataxias might result from the direct activation of apoptotic pathways (Lipinski and Yuan, 2004). In addition to the role of mitochondria in intracellular calcium homeostasis as discussed earlier, polyglutamine-expanded cellular death of cerebellar neurons by ataxins 3 and 7 in SCA3 and SCA7, respectively, is preceded by recruitment of caspases into polyQ aggregates. This is followed by activation of caspases 3 and 9, and of mitochondrial apoptotic pathways mediated by members of the Bcl-2 family, such as Bax and Bcl-x(L) (Chou et al., 2006; Wang et al., 2006). Both factors are known key components of neuronal apoptosis by regulating mitochondrial release of cytochrome c and Smac/DIABLO. Consistent with these observations, mutant ataxin 7 in SCA7 induces translocation of cytochrome c and Smac/DIABLO to the cytosol preceded by activation of caspases 9 and 3. This suggests that mutant ataxin 7 causes apoptotic death of cerebellar neurons by promoting mitochondrial release of cytochrome c and Smac/DIABLO. Alternatively, pro-apoptotic pathways could be activated by displacement of harmful factors sequestered by expanded polyglutamines, or through non-canonical mechanisms of caspase activation. In any case, the toxic proteins may promote mitochondrial dysfunction and increased free radical production associated with oxidative damage, and abnormal energy metabolite concentration and utilization. Further evidence of mitochondrial dysfunction resulting or contributing to ataxia is exemplified by diseases caused by mutations in polymerase gamma resulting in predominant cerebellar dysfunction/atrophy. While relevant, these mechanisms of toxicity do not explain for the time being the selective vulnerability of distinct cell types and brain regions in spinocerebellar neurodegenerations.

**Emerging pathways**

Most of the pathways leading to toxicity by disease proteins in spinocerebellar ataxias remain to be discovered. Increasing evidence reveals that nuclear expression of polyglutamine-expanded proteins is an essential step in polyglutamine pathogenesis, and that the resulting transcriptional dysregulation caused by ataxins containing expanded polyglutamine results in neuronal dysfunction and death. Interference
with gene expression would occur by the effects of the mutation exerted on the interaction with transcriptional regulatory proteins. For instance, ataxin 1 interacts with the transcriptional co-repressor SMRT (silencing mediator of retinoid and thyroid hormone receptors) through the AXH domain within Atxn1 (Tsai et al., 2004). Since SMRT regulates the expression of the retinoid and thyroid hormone receptors, it is feasible that the mutant form of ataxin 1 might exert deleterious effects on SMRT-dependent transcriptional pathways. Similarly, ataxins 3 and 7, and the SCA17 and DRPLA gene products, TBP and atrophin 1, respectively, are directly involved in transcription as components of transcriptional regulatory complexes (Shimohata et al., 2000; Li et al., 2002; Zhang et al., 2002; Helmlinger et al., 2004). But the genes and pathways regulated by these factors remain unknown. Transcriptional dysregulation might account for the cell-type specificity degeneration observed in spinocerebellar ataxias, through the sequestration of factors, by long glutamine stretches within the mutant proteins, whose expression is confined to cells that degenerate in these conditions. Therefore, they would then become unavailable to perform their normal cellular duties with potentially lethal consequences. In SCA1, sequestration of the acidic nuclear protein 32 (Anp32a/Lanp) (Matilla et al., 1997), PQBP-1 (Okazawa et al., 2002), Boat (Mizutani et al., 2005) and Gfi-1/Senseless Senseless (Tsuda et al., 2005) might lead to selective cerebellar neurodegeneration by impairing transcription and dysregulating gene expression in specific cells. Remarkably, Anp32a/Lanp is a potent specific regulator of PP2 activity and its expression is predominantly confined to cerebellar Purkinje cells (Matilla and Radrizzani, 2005). Therefore, dysregulation of PP2 activity is a likely scenario underlying cerebellar neurodegeneration in SCA1. Accordingly, in both SCA1 and SCA12, cell death might be provoked by similar pathogenic mechanisms. Similarly, inactivation of the tissue-specific transcription factor CRX in SCA7 by the polyglutamine-based association with mutant ataxin 7 could contribute to the cone-rod dystrophy observed in SCA7 mice and patients (La Spada et al., 2001). One group of nuclear proteins that bind to and whose activity may be altered by mutant ataxins is histone acetyltransferases (HATs). HATs are responsible for the acetylation of histones and several other important proteins, and this modification results in altered function of the target protein (Kouzarides, 2000). HATs also regulate cellular processes at levels as different as modifying transcriptional competence of chromosomes, temporal regulation of promoter activity and protein activation/inactivation. The altered balance between protein acetylation and deacetylation may be a key process contributing to pathogenesis induced by mutant proteins containing expanded polyglutamines (Steffan et al., 2001). Importantly, restoration of this balance is possible by genetic or pharmacological reduction of the opposing enzyme group, that is, the histone deacetylases (HDACs). This is leading to new therapeutic strategies with histone deacetylase inhibitors (HDACis) to treat neurodegeneration (McCampbell et al., 2001; Steffan et al., 2001; Hockly et al., 2003).

Emerging lines of evidence highlight the importance of understanding the normal function of the disease proteins associated with spinocerebellar ataxias to unravel the molecular mechanisms underlying the detrimental effects of the mutations. In SCA1, expansion of the polyglutamine tract or nuclear expression alone is not sufficient to cause disease. It appears that phosphorylation of a specific serine residue in ataxin 1 by protein kinase B/Akt plays a crucial role in modulating the ability of the mutant form of ataxin 1 to induce neurodegeneration by influencing its biological interactions with other proteins and the formation of nuclear inclusions (Chen et al., 2003b). Therefore, the protein context and the cellular proteins with which ataxin 1 normally interacts are likely to be important in the disease process, indicating that, for instance, blocking the phosphorylation events could be a viable treatment. In support of this, the protein context appears to be a modifying factor of the age of onset of spinocerebellar degeneration (van de Warrenburg et al., 2005). In SCA2, ataxin 2 contains an RNA-binding Lsm domain characterized by a conserved sequence motif consisting of two short segments known as Sm1 and Sm2, which are separated by a variable linker (Albrecht et al., 2004). Lsm domain proteins are involved in a variety of essential RNA-processing events including RNA modification, pre-mRNA splicing and mRNA decapping and degragation, and some of them are also important components of spliceosomal small nuclear ribonucleoprotein (snRNPs) complexes (He and Parker, 2000). Interestingly, ataxin 2 interacts with A2BP1 (ataxin 2 binding protein 1) (Shibata et al., 2000), whose RNA-binding Caenorhabditis elegans homologue, fox-1, regulates tissue-specific alternative splicing (Jin et al., 2003). Deciphering the mechanisms by which ataxin 2 regulates alternative splicing should provide insights into the pathways dysregulated during disease progression leading to the identification of potential therapeutic targets.

RNA-mediated neurotoxicity has been implicated in SCA8 pathogenesis. It seems that the SCA8 gene is transcribed as a part of an untranslated RNA that overlaps with the transcription and translation start sites and the first splice junction of KLHL1, a gene that encodes a protein with structural similarities to a family of factors involved in the organization of the cytoskeleton (Koob et al., 1999). Since the SCA8 gene functions as a gene regulator, it has been proposed that a RNA gain-of-function mechanism might underlie neurodegeneration in SCA8 as is the case for myotonic dystrophy (DM) (Philips et al., 1998; Liquori et al., 2001). Genetic screens in flies have revealed that mutations in staufen, muscle-blind, split ends and CG3249 modulate neurodegeneration (Mutsuddi et al., 2004). These observations suggest that CUG expansions in SCA8 might alter associations with specific RNA-binding proteins. It is important to note that the molecular defect in SCA8 is subject of
controversy, since expansions in the SCA8 gene are not exclusively detected in patients with spinocerebellar ataxia (Worth et al., 2000; Corral et al., 2005).

SCA10 is uniquely caused by an intronic pentanucleotide ATTCT repeat within the E46L gene, now designated ATXN10 (Matsura et al., 2000). The E46L protein is widely expressed throughout the brain, contains two armadillo (arm) repeat domains and interacts with the heterotrimeric GTP-binding protein (G-protein) (Lin and Ashizawa, 2005). It has been proposed that E46L enhances heterotrimeric G-protein signalling to mediate neurite formation. E46L belongs to the armadillo repeat family of proteins where the arm repeats mediate protein–protein interactions to modulate a myriad of cellular processes, including intracellular signalling, cytoskeletal regulation, nuclear transport and regulation of gene expression both during development and throughout adult life (Coates, 2003). Therefore, E46L might be regulating important cellular processes through the mediation of G-protein intracellular signalling.

An expanded CAG trinucleotide repeat located within the promoter region of the gene encoding a brain-specific regulatory subunit of the PP2 (formerly 2A) (PPP2R2B) has been associated with SCA12 (Homes et al., 1999). Neurodegeneration in SCA12 is confined to the cerebellum. The serine/threonine PP2 regulates a wide array of cellular processes including cell growth and differentiation, DNA replication, cellular morphogenesis, long-term depression and apoptosis (Sonntag, 2001). It has been proposed that the SCA12 repeat expansion may alter the levels of expression of one splice variant (termed B beta1) of PPP2R2B by altering the efficiency of the promoter driving expression, influencing the efficiency of the promoter driving expression, with potentially lethal consequences. It remains possible that these alterations in amino acid composition within the gamma arm repeats mediate protein–protein interactions to modulate a myriad of cellular processes, including intracellular signalling, cytoskeletal regulation, nuclear transport and regulation of gene expression both during development and throughout adult life (Coates, 2003). Therefore, E46L might be regulating important cellular processes through the mediation of G-protein intracellular signalling.

The disease symptoms in SCAs 14 and 27 are elicited by alterations in amino acid composition within the gamma subunit of protein kinase C (PRKCG) (Chen et al., 2003; Yabe et al., 2003) and FGFI4 (van Swieten et al., 2003), respectively. These alterations appear to dysregulate protein function and specific cellular pathways. Calcium-phospholipid-dependent protein kinase C comprises a family of enzymes that transduce the plethora of signals promoting lipid hydrolysis, thus regulating a variety of cellular processes, such as membrane-receptor signal transduction, control of gene expression and synaptic plasticity (Newton, 2001). PRKCG is highly expressed in brain and spinal cord, with particularly high expression in Purkinje cells of the cerebellar cortex during dendritic development, where it seems to act as a negative regulator of dendritic growth and branching (Schrenk et al., 2002). Mutant PRKCG gene products are less stable than the normal protein leading to abnormal activation patterns, altered membrane targeting and enhanced activity. It is speculated that the SCA14 phenotype results from gain-of-function mechanisms rather than haploinsufficiency, because no chain-terminating mutations have been found and heterozygous PKC-null animals are neurologically normal (Abeliovich et al., 1993). However, a dominant-negative mechanism cannot be ruled out at this time. Alterations in protein stability of FGFI4 underlie neurodegeneration of the cerebellum and basal ganglia in SCA27 (van Swieten et al., 2003). FGFI4 is a member of a subclass of fibroblast growth factors that is expressed in the developing and adult central nervous system, and has been implicated in neuronal signalling, axonal trafficking and synaptosomal function (Wang et al., 2002). Neuropharmacological studies in mice showed that FGFI4-deficient mice have reduced responses to dopamine agonists indicating that FGFI4 is required for striatopallidal-mediated dopaminergic signalling. Therefore, dysfunction of this pathway could account for the cortical hyperexcitability and the parkinsonism-associated symptoms in SCA27.

More recently, the genetic defects have been identified in three spinocerebellar ataxias. In-frame deletions in the beta-III spectrin gene SPTBN2 co-segregate with SCA5 in American and French families (Ikeda et al., 2006). It appears that these spectrin mutations alter the levels, distribution and stability of the beta-III spectrin-associating protein and Purkinje cell-specific glutamate transporter EAAT4. In a German family, missense mutations within the SCA5 gene co-segregating with the disease in affected individuals may disrupt the ability of spectrin to bind to the actin cytoskeleton. These observations suggest that disruption of glutamate signalling and vesicle trafficking may play a role in the pathogenic mechanisms in SCA5, which are pathways previously implicated in neurodegeneration in SCA1, Alzheimer’s and Huntington’s diseases and amyotrophic lateral sclerosis. In SCA13, missense mutations in exon 2 of the potassium channel KCNC3 gene have been found associated with neurodevelopmental and neurodegenerative phenotypes (Waters et al., 2006). KCNC3 (also known as Kv3.3) is a fast-rectifying voltage-gated Shaw subtype potassium channel abundantly expressed in the cerebellum, and the mutations appear to impair channel activity, being consistent with a dominant-negative effect of the mutant allele. It appears that the channel mutations shift the activation curve in the negative direction and slowed channel closing and, therefore, might change the output characteristics of fast-spiking cerebellar neurons, in which KCNC channels confer capacity for high-frequency firing. A single-nucleotide substitution
in the 5′ untranslated region of the gene encoding the Purkinje cell atrophy associated protein puratrophin 1, also known as pleckstrin homology domain, containing family G (with RhoGef domain) protein 4 (PLEKHG4), a protein implicated in intracellular signalling and actin dynamics at the Golgi apparatus, is associated with a spinocerebellar ataxia subtype characterized by pure cerebellar atrophy and sensorineural hearing impairment (Ishikawa et al., 2005). Interestingly, the puratrophin 1 gene is located on the same chromosomal region where the SCA4 gene localizes (Flanagan et al., 1996). Although the heterozygous C/T single-nucleotide substitution within the puratrophin 1 gene detected in the chromosome 16q22.1-linked ADCA Japanese families are not present in the German SCA4 family (Zuehlke and Hellenbroich, personal communication), the puratrophin 1 gene cannot be excluded from being associated with disease symptoms in SCA4 at this time.

Although ataxia is the prominent symptom, few mutations cause an almost pure cerebellar syndrome and isolated degeneration of the cerebellar cortex. On the contrary, most SCAs are multisystemic disorders showing neurodegeneration not only in the spinocerebellar tracts but also in the basal ganglia and midbrain (Schols et al., 2000). Parkinsonism symptoms in SCA1 (Gilman et al., 1996), SCA2 (Infante et al., 2004; Simon-Sanchez et al., 2005), SCA3/MJD (Gwinn-Hardy et al., 2001), SCA6 (Khan et al., 2005), SCA17 (Gunther et al., 2004) and SCA27 (van Swieten et al., 2003) are indicative of possible impairment of striatongrinal and/or striatopallidal projections in these spinocerebellar ataxia subtypes.

Alterations in glutamate synaptic neurotransmission have been proposed as a possible mechanism mediating neurodegeneration. In SCA1 transgenic mice, motor dysfunction precedes neuronal death, demonstrating that mutant ataxin 1 produces disruption of motor function in SCA1 not by killing cells but by affecting some Purkinje cellular functions including alterations in synaptic plasticity (Clark et al., 1997). Elevated intracellular calcium levels by calcium-permeable glutamate receptors are suggested as a possible mechanism (Serra et al., 2004). The importance of maintaining proper glutamate transmission in the Purkinje cell/parallel fibre synapse for proper motor function is supported by studies with ataxia mouse mutants. For instance, mice lacking mGluR1 display significant motor deficits, and introduction of mGluR1 expression into Purkinje cells of mGluR1−/− mice restores normal motor function (Ichise et al., 2000).

**Therapeutic strategies**

There are currently no known effective treatments to modify disease progression in any of the SCAs or related neurodegenerative disorders, although some benefits on ataxic symptoms have been reported in a few therapeutic trials. A preliminary open trial with acetazolamide temporally reduced the severity of symptoms in SCA6 (Yabe et al., 2001). Dopaminergic and anticholinergic drugs have been used to alleviate tremor, bradykinesia or dystonia in SCA2 and SCA3 (Buhmann et al., 2003; Nandagopal and Moorthy, 2004). Spasticity in SCAs could be effectively treated with baclofen, tizanidine or mimentine. In selected cases where other treatments have failed, botulinum toxin has been successfully used to treat dystonia and spasticity. Intention tremor has been ameliorated with benzodiazepines, β-blockers or chronic thalamic stimulation (Pirker et al., 2003). Muscle cramps, which are often present at the onset of the condition in SCAs 2, 3, 7 and DRPLA are alleviated with magnesium, chinine or mexiletine. Preliminary data from a recent open trial with gabapentin have shown improvement of cerebellar symptoms in ataxia patients by increasing GABA concentration in the central nervous system and, thus, by stimulating GABAergic neurotransmission (Gazulla et al., 2004).

Innovative approaches, such as RNA interference (RNAi) with the aim of inhibiting polyglutamine-induced neurodegeneration, prevention of protein misfolding and aggregation by overexpression of chaperones, and regulation of gene expression by application of HDACis, are proving effective in pre-clinical trials. In SCA1, intracerebellar injection of vectors expressing short hairpin RNAs profoundly improves motor coordination, restores cerebellar morphology and resolves characteristic ataxin 1 inclusions in Purkinje cells of transgenic mice (Xia et al., 2004). While these results prove that RNAi therapy improves cellular and behavioural characteristics in pre-clinical trials, its application in patients to protect or even reverse disease phenotypes shall be delayed until proper toxicity tests are assessed. Pointing to a different target, molecular chaperones provide a first line of defence against misfolded, aggregation-prone proteins. Many studies have analysed the effects that chaperone overexpression has on inclusion body formation and toxicity of pathogenic polyQ fragments in cell culture, and it is clear that overexpression of molecular chaperones might prove beneficial for the treatment of neurodegenerative diseases (Muchowski and Wacker, 2005). They prevent inappropriate interactions within and between non-native polypeptides, enhance the efficiency of de novo protein folding and promote the refolding of proteins that have become misfolded as a result of the mutations and cellular stress (Chan et al., 2000). Chemical and molecular chaperones might also prevent toxicity by blocking inappropriate protein interactions, by facilitating disease protein degradation or sequestration or by blocking downstream signalling events leading to neuronal dysfunction and apoptosis. Congo Red, thioflavine S, chrysamine G and Direct Fast have proved effective in suppressing aggregation in vitro and in vivo (Heiser et al., 2000; Sanchez et al., 2003). Several low molecular mass compounds, such as the organic solvent dimethylsulphoxide (DMSO) and the cellular osmolytes glycerol, trimethylamine N-oxide and trehalose, increase the stability of proteins in their native conformation; hence they are called chemical chaperones on the basis of their influence on protein folding. They are effective in preventing cell death triggered by mutant ataxin 3.
(Yoshida et al., 2002). Trehalose was identified in an in vitro screen for inhibitors of polyQ aggregation, and its administration reduces brain atrophy, improves motor dysfunction and extends the lifespan of mice resembling the polyglutamine disorder Huntington’s disease (Tanaka et al., 2004). In vitro experiments suggest that the beneficial effects of trehalose result from its ability to bind and stabilize polyglutamine-containing proteins. More recently, a new generation of small chemical compounds that directly target polyQ aggregation without significant cytotoxicity have been identified in high-throughput screens using cell-free assays or by targeting cellular pathways (Heiser et al., 2002; Zhang et al., 2005). These compounds inhibit polyQ aggregation in cultured cells and intact neurons and can rescue polyQ-mediated neurodegeneration in vivo. By a different mechanism, a small molecule that acts as a co-inducer of the heat-shock response by prolonging the activity of heat-shock transcription factor HSF1, arimoclomol, significantly improves behavioural phenotypes, prevents neuronal loss, extends survival rates and delays disease progression in a mouse model of neurodegeneration (Kieran et al., 2004). Similarly, activation of heat-shock responses with geldanamycin inhibits aggregation and prevents cell death (Rimoldi et al., 2001). This suggests that pharmacological activation of the heat-shock response may, therefore, be an effective therapeutic approach to treating neurodegenerative diseases. However, excessive upregulation of chaperones might lead to undesirable side-effects, such as alterations in cell cycle regulation and cancer (Mosser and Morimoto, 2004). Therefore, a delicate balance of chaperones is likely to be required for a beneficial neuroprotective effect. For instance, chemical or molecular chaperones, used in combination with a pharmacological agent that upregulates the synthesis of molecular chaperones, might be a valid therapeutic approach for treating spinocerebellar ataxias caused by polyglutamine expansions. Aggregate formation has also been successfully targeted with inhibitors of transglutaminase, such as cystamine, which reduces apoptotic cell death and alleviates disease symptoms by the expanded polyglutamine (Dedeoglu et al., 2002; Karpuj et al., 2002).

Compounds targeting mitochondrial function such as coenzyme Q10 (Shults, 2003), creatine (Ryu et al., 2005) and tauroursodeoxycholic acid (TUDCA) (Keene et al., 2002), or autophagy, such as the mTor inhibitor rapamycin and its various analogous (Ravikumar et al., 2004), have proved effective at reducing cellular toxicity in animal models, and are currently being tested in clinical trials. Caspase activation, which usually precedes neuronal cell death, can be targeted with caspase inhibitors such as zVAD-fmk, CrmA, cystamine, FADD DN and minocycline. These have proved efficient to prevent disease progression, delay onset of symptoms and extend survival rates in several mouse models of neurodegeneration (Ona et al., 1999; Chen et al., 2000; Lesort et al., 2003). Other agents promoting the clearance of mutant proteins in the CNS or Ca\(^{2+}\) signalling blockers, such as specific inhibitors of the NR2B-subunit of N-methyl-D-aspartate receptors and blockers of metabotropic glutamate receptor mGluR5 and inositol 1,4,5-trisphosphate receptor InsP3R1, may be beneficial for the treatment of some spinocerebellar ataxia subtypes. Delaying protein accumulation and the associated deleterious effects by enhancing degradation of toxic proteins by the ubiquitin-proteasome pathway could also be employed as therapeutic approaches.

The role that some ataxins play in transcription and, more importantly, the suppression of cytotoxic effects mediated by some of their co-transcriptional regulators can be used to modulate the pathological effects of mutant ataxins, opening the path for new therapeutic strategies for treating some of the SCAs. Recent progress in HDAC research has made possible the development of inhibitors of specific HDAC family proteins and these compounds could be effective candidates for treatment of spinocerebellar ataxias (Dokmanovic and Marks, 2005). Neuroprotective strategies addressed at specific bioenergetic defects might hold particular promise in the treatment of spinocerebellar conditions. In vivo neuroprotection exerted by the insulin growth factor IGF-1 through the PP2-regulated PI3K/Akt signalling pathway raises its possible potential to halt cerebellar neurodegeneration (Leinninger and Feldman, 2005).

Gene therapy and stem cell approaches are being considered for treatment of spinocerebellar neurodegenerations. Delivery of proteins or compounds by viral vectors represents one such gene therapeutic approach. Neural cell replacement therapies are based on the idea that neurological function lost during neurodegeneration could be improved by introducing new cells that can form appropriate connections and replace the function of lost neurons. This strategy has proved to be effective in treating neurodegeneration in Parkinson’s disease and amyotrophy lateral sclerosis (Svendsen and Langston, 2004). Both mouse and human embryonic stem cells, when cultured in vitro to produce embryoid bodies, are able to differentiate towards a motor neuron lineage (Wichterle et al., 2002). Remarkably, when neurons created in vitro are introduced into an injured chick spinal cord, some stem cell-derived motor neuron extend axons and innervate appropriate targets. Since neurogenesis does occur in the adult nervous system, another approach could be to stimulate endogenous stem cells in the brain or spinal cord to generate new neurons. Studies to understand the molecular determinants and cues to stimulate endogenous stem cells are under way (Gage, 2002). Although promising, we are only starting to learn the potentials and challenges of stem cells, especially for use in neurodegenerative diseases.

**Concluding remarks**

Here we review common themes occurring in spinocerebellar neurodegenerative conditions. Progressive neurodegeneration in spinocerebellar ataxias is mediated by mutant proteins capable of inducing neuronal damage by interfering with several molecular pathways including protein aggregation.
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and clearance, transcriptional regulation, the ubiquitin-proteasome system, alterations of calcium homeostasis and activation of pro-apoptotic routes among others. These pathways could act independently or, more likely, interact and enhance each other, triggering the accumulation of cellular damage that eventually leads to dysfunction and, ultimately, the demise of neurons through a series of multiple events. This suggests that simultaneous targeting of several pathways might be therapeutically necessary for preventing neurodegeneration and preserving neuronal function. Understanding how dysregulation of these pathways mediates disease progression is leading to the establishment of effective therapeutic strategies in vivo, which may prove beneficial in the treatment of spinocerebellar ataxias.

Acknowledgements

We are indebted to Nick Wood (Institute of Neurology, Queen Square, London, UK) for his support, helpful comments and suggestions. This work was supported by the Child Health Research Appeal Trust (CHRAT) and the National Health System Executive. Research at the Institute of Child Health (ICH) benefits from Research and Development funding from the UK National Health System Executive.

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