Dynamics of ataxin-1 in spinocerebellar ataxia type 1
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CHAPTER 5

Spinocerebellar ataxia-1: insights in the function of ataxin-1 and the significance for other polyglutamine expansion disorders
PolyQ disorders and spinal cerebellar ataxia’s

Polyglutamine (polyQ) expansion disorders is a group of dominantly inherited neurodegenerative disorders that includes Huntington’s disease, dentatorubropallidoluysian atrophy, spinobulbar muscle atrophy and the spinocerebellar ataxias (SCAs) types 1, 2, 3, 6, 7 and 17. These polyQ disorders are caused by an expansion of the CAG-trinucleotide repeat that encodes for the amino acid glutamine. In the non-pathogenic form, these disease-related proteins contain a short polyQ-rich region, whereas the protein is converted into a disease-inducing form when the glutamine expansion exceeds the threshold of 36-40 amino acids (Orr and Zoghbi, 2007). SCA6 is an exception since a repeat of 20-33 amino acids is enough to trigger disease (Riess et al., 1997; Zhuchenko et al., 1997). The length of the polyQ stretch is inversely related to the age of onset. Although the disease-related proteins are widely expressed, specific cell types appear to be particular vulnerable. Each polyQ disorder is characterized by neurodegeneration in specific restricted regions of the brain. PolyQ disorders are generally characterized by the formation of intracellular aggregates in the patient’s brain, which can be mimicked in vitro. These aggregates were previously considered to be toxic but recent studies suggest that they have a protective role by sequestering smaller oligomeric aggregates. These small aggregates are composed of polyQ protein fragments and are now considered to represent the toxic species (Arrasate and Finkbeiner, 2005). In addition, the polyQ expansion alters the normal function of the protein. For example, polyQ expansion in the huntingtin protein (htt) affects its normal role in vesicle trafficking (Arrasate and Finkbeiner, 2005; Trushina et al., 2004).

SCA types 1, 2, 3, 6, 7 and 17 are autosomal dominant SCAs. At present, 29 SCAs have been identified that all cause cerebellar atrophy leading to ataxia, tremor and dystarthritis. There are three different types of mutational mechanisms that can cause SCA: polyQ expansions, non-coding repeat expansions and missense mutations. In all SCAs, the Purkinje cells in the cerebellum are affected. Since different mutations in seemingly unrelated proteins can cause a similar disease phenotype and neuropathological changes, one or multiple common pathways
likely underlie the pathogenesis of the SCAs.

SCA1 is by far the most extensively studied and described SCA disorder. However, the pathogenic mechanisms causing disease are still unclear. SCA1 is caused by a polyQ expansion mutation in the protein ataxin-1, and is characterized by a neurodegenerative process in the cerebellum which initially mainly affects the Purkinje cells. In time, however, degeneration of other brain regions such as specific brain stem neurons and loss of motoneurons in the spinal cord becomes apparent (Matilla-Duenas et al., 2008; Robitaille et al., 1995). This leads to clinical symptoms that become manifest around middle age and include slurred speech, problems with swallowing, cognitive impairments and spasticity.

**The function of ataxin-1**

Functions of ataxin-1 are still elusive and this complicates the understanding of the SCA1 disease process. Nonetheless, various studies have revealed important information about the functional domains of ataxin-1 as well as ataxin-1-interacting proteins. The majority of these interacting proteins play a role in transcriptional regulation or RNA synthesis, processing and degradation.

**Functional domains of ataxin-1**

Ataxin-1 contains three regions that regulate the function of ataxin-1 (Figure 1): the AXH-domain, the nuclear localization signal (NLS), and the phosphorylation site at Ser776. The AXH domain is a domain that is highly homologous to a portion of the HMG box transcription factor binding protein1 (HBP1) (Chen et al., 2004; de Chiara et al., 2005). In ataxin-1, the AXH domain is able to bind to RNA and a number of proteins including transcription regulators such as capicua, the silencing mediator of retinoic acid (SMRT) and thyroid hormone receptors (Tsai, 2004; Mizutani et al., 2005; Tsuda et al., 2005; (Lam et al., 2006; Mizutani et al., 2005; Serra et al., 2006; Tsai et al., 2004; Tsuda et al., 2005). In addition, the AXH domain acts as a dimerization domain and has a cluster of charged surface residues (Chen et al., 2004). This cluster is very well conserved among species and has been suggested to constitute a second binding surface for a yet unidentified partner (Carlson et al., 2008; Chen et al., 2004). The second region is the NLS which targets ataxin-1 to the nucleus. Lastly, the phosphorylation status of ataxin-1 at Ser776 has been described to be involved in nuclear body formation and interactions with several proteins such as RBM17 and 14-3-3 (Chen et al., 2003b; Emamian et al., 2003; Lim et al., 2008).

Purified ataxin-1 can bind to RNA (Yue et al., 2001). Ataxin-1 may therefore function as an RNA-binding and RNA-transporting protein, which is also suggested by its ability to shuttle between the nucleus and the cytoplasm (Irwin et al., 2005; Krol et al., 2008). A screen for modifiers of ataxin-1 function in Drosophila resulted in a nuclear pore protein and five proteins containing RNA-binding domains, one of which was the protein pumilio (Fernandez-Funez et al., 2000). In a yeast, two hybrid screens for protein interactors involved in SCAs and Purkinje cell degeneration identified, three proteins (RBM9, A2BP1 and RBPMS) which are involved in RNA binding and splicing. These proteins may link ataxia-causing proteins as most of the proteins causing SCA are not functionally related, but likely they interplay in common pathways. RNA binding and splicing could be one of these pathways. All these data suggest that ataxin-1 may indeed be involved in particular stages of RNA tran-
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scription or processing, such as transcription regulation, mRNA nuclear export, translation, mRNA stability and splicing.

**Cellular localization and nuclear body formation by ataxin-1**

Whereas ataxin-1 is a predominantly nuclear protein in most neurons, Purkinje cells show both nuclear and cytoplasmic staining (Koshy et al., 1998; Servadio et al., 1995; Skinner et al., 1997). This difference may contribute to the specific sensitivity of Purkinje cells for polyQ-expanded ataxin-1, as it may be the cytoplasmic function of ataxin-1 that is specifically affected by the polyQ expansion. Overexpression of wildtype or mutant ataxin-1 protein leads to the formation of nuclear structures *in vitro* (Krol et al., 2008; Stenoien et al., 2002) Fig. 2A,B). This is in accordance with transgenic mice studies that show similar nuclear bodies upon overexpression of wildtype or mutant ataxin-1 (Koshy et al., 1998, Fig. 2C,D). Initially, these structures were regarded as insoluble polyQ aggregates. However, more recent studies showed that these nuclear bodies do not represent sequestered aggregates but ataxin-1 accumulations with a high on/off rate of ataxin-1 and may therefore represent functional protein complexes.

**The role of ataxin-1 in RNA transport**

The ability of ataxin-1 to bind RNA, as well as the shuttling of ataxin-1 between nucleus and cytoplasm suggests that ataxin-1 may be involved in a particular stage of RNA transport (Cooper et al., 2009). Gene transcription takes place in the nucleus where several proteins and protein complexes interplay to transcribe DNA into pre-mRNA. This pre-mRNA often transiently accumulates at the sites of transcription to undergo a number of modifications that determines the protein encoded as well as the stability and translatability of the mRNA, such as capping of the 5’ end (Cooper et al., 2009; Neugebauer and Roth, 1997). Once the mRNA is transcribed, it is transported to the cytoplasm for translation into proteins. It is not known what mechanism is involved in this transport and whether this is an active or passive process (Cooper et al., 2009; Politz and Pederson, 2000). The mRNA is shuttled in so-called ribonucleotide protein (RNP) complexes. These complexes contain RNA
and trans-acting factors (TAFs) that support RNA transport and/or localization by binding to cis-acting elements of the mRNA (Kiebler and Bassell, 2006; Kindler et al., 2005). Once outside the nucleus, the RNP complexes travel along cytoskeletal filaments (actin filaments or microtubules) with the help of motor proteins until they reach their destination (Bassell and Kelic, 2004; Muslimov et al., 2002).

During transport, mRNAs in these RNP s are translationally arrested by the action of regulatory RNAs and RNA-binding proteins. Finally, translation can be initiated by several signaling pathways that target the translation initiation factor eIF4E, such as the mitogen-activated protein kinase (MAPK) signaling pathway. This mechanism enables local and controlled gene expression, which is particularly relevant in neurons such as Purkinje cells where distances between cell body and the endings of axon and dendrites can be significant and the localization of mRNA at the synapses has been proposed as a mechanism for synaptic plasticity and thus Purkinje cell function (Klann and Dever, 2004). This mRNA at the synapses has been transported from the nucleus in the cell body to the endings of the axon and dendrites. An example is mRNA of PKCγ, which has been reported to be present either in proximal dendrites or at low concentrations within the dendritic arbor (Moriya and Tanaka, 1994). PKCγ is highly expressed in Purkinje cells, is involved in synaptic plasticity and when mutated it causes SCA14. It is also a protein that interacts with ataxin-1 and promotes ataxin-1 dynamics, phosphorylation, and nuclear body formation (Verbeek et. al 2009, submitted). The targeting and local translation of mRNA has also been shown to have a role in axon guidance and neurodegeneration, which are important processes during brain development and plasticity (Willis et al., 2005). Several TAFs have indeed been shown to be nuclear-cytoplasmic shuttling proteins that first appear to associate with transcripts in the nucleus and subsequently direct mRNA transport to the cytoplasm (Farina and Singer, 2002).

Besides the presence of RNA in RNP-bodies during transport, cytoplasmic RNA can also be present in so-called P-bodies that appear to be related to neuronal RNP granules (Chen et al., 2003a). In P-bodies, mRNA accumulates and is stalled together with proteins involved in small RNA-mediated gene silencing, translational repression, mRNA surveillance and mRNA degradation (Eulalio et al., 2007; Parker and Sheth, 2007). The final destiny of mRNA in cells is determined in these P-bodies. RNP-and P-bodies share certain components and a common function is that of storing non-translated RNA. A difference between RNP-bodies and P-bodies is that P-bodies are not associated with translation-initiation factors (Eulalio et al., 2007). Other granules which contain RNA are stress granules, which are formed upon stress and keep mRNA in translation arrest. Recently, it has been reported that mRNAs can shuttle between stress granules and P-bodies (Spector, 2006). One of the P-body-marker proteins, GW182, contains a polyQ-rich domain that is essential for its P-body recruitment (Eulalio et al., 2007). It is confirmed in a recent study in yeast that proteins with Q/N rich prion-like domains contribute to the recruitment of RNP s to P-bodies (Savas et al., 2008). The RNA-binding protein pumilio and the stress granule protein TIAR both contain a glutamine rich region (Karlin and Burge, 1996; Vessey et al., 2006; Zhang et al., 2005), which is in agreement with the observation that Q stretches are needed for recruitment to P-bodies and stress granules. Thus, polyQ-
rich regions are present in RNA-binding proteins and can function as a domain that is essential in protein-protein interactions and protein complex formation in RNA granules, P-bodies and stress granules.

Thus, ataxin-1 may have functions in both the nucleus and the cytoplasm in Purkinje cells. The possible functions in each cellular compartment are summarized in Figure 3. In the nucleus, ataxin-1 forms nuclear bodies that are dynamic and disappear upon transcriptional inhibition. We suggest that ataxin-1 plays a role here in transcription, splicing and/or nuclear-cytoplasmic shuttling. No studies have been performed thus far to unravel ataxin-1 function in the cytoplasm. Here, ataxin-1 may play a role in the transport of mRNA to specific locations such as the post synapse in dendrites where it may function in repressing or activating the translation of mRNA that is present in the periphery of Purkinje cells.

What causes disease in SCA1 patients?

Purkinje cells provide the sole output from the cerebellum and each Purkinje cell receives two types of excitatory inputs namely from climbing fibers and parallel fibers. Climbing fibers are axons from cells in the inferior olive and parallel fibers are axons from granular cells in the cerebellar cortex. Both form synaptic contacts all over the dendrites of Purkinje cells. Each Purkinje cell receives input from only one climbing fiber and from hundred thousands of parallel fiber axons (Ogasawara et al., 2008). Purkinje cells rely on two types of glutamate receptors: AMPA receptors and the metabotropic glutamate receptor, mGluR1, which is abundantly expressed in Purkinje cells. When climbing fibers and parallel fibers release pre-synapse glutamate, glutamate binds to the AMPA and mGluR1 receptors at the Purkinje cell post-synapse membrane thereby producing a complex post-synaptic response involving several proteins and consisting of a calcium-release signal from intracellular stores (Hartmann and Konnerth, 2008; Serra et al., 2004). In the SCA1 mutant mouse, some neuronal genes that are involved in glutamate signaling and calcium homeostasis are downregulated in Purkinje cells, already before the onset of motor impairment and neuropathology (Lin et al., 2000; Serra et al., 2004). These genes (Homer-3, G-substrate, EAAT4, IP3I and CARP) are normally highly expressed in Purkinje cells (Gold et al., 2003) where the proteins are localized in the dendritic tree of Purkinje cells. As a result of the downregulation in SCA1, intracellular calcium levels and calcium signaling may be altered, which is likely to be detrimental for the Purkinje neurons in particular. These cells are very sensitive to fluxes in intracellular calcium levels (Duenas et al., 2006) and disruption of calcium homeostasis in SCA1 may contribute or may even initiate the pathogenic process. The lurcher mouse demonstrates that indeed altered glutamate signaling can cause Purkinje cell degeneration. In this mouse, a gain of function mutation in the delta2 glutamate receptor (GluRdelta2) alters glutamate signaling that results in Purkinje cell degeneration (Yue et al., 2002). By taking the downregulation of the expression of these genes as a major pathological hallmark of SCA1, we will follow step-by-step how polyQ expansion can affect ataxin-1 function at different stages of SCA1 and how these alterations may contribute to the pathology of the disease.

I. Ataxin-1 sequestration into polyQ aggregates

A general neuropathological hallmark of polyQ disorders is the presence of intra-
cellular aggregates in the affected neurons of patients, and this has been assumed to be the common toxic gain-of-function leading to neuronal dysfunction and eventually cell death. Although aggregates are widespread in polyQ disorders, this phenomenon cannot explain the vulnerability of specific types of neurons in each polyQ disorder (Table 1). Nuclear bodies formed by ataxin-1 can easily be misinterpreted for aggregates but we and others have shown that ataxin-1 aggregates are highly dynamic structures (Krol et al., 2008; Stenoien et al., 2002). In addition, mutant ataxin-1 has a higher on/off rate and a faster diffusion speed between nuclear bodies. This may result in the higher percentage of larger nuclear bodies (Krol et al., 2008). Nuclear body formation can be affected by the phosphorylation status of ataxin-1 at Ser776 because substitution of Ser776 by alanine to mimic a non-phosphorylation status disrupts the formation of nuclear bodies (Emamian et al., 2003). Furthermore, when ataxin-1 and the kinase PKCγ are both over-expressed in cell culture there is an increase in ataxin-1 phosphorylation and nuclear body formation (Verbeek et al. 2009, submitted). This implicates that phosphorylation modifies ataxin-1 in such a way that the ability to form nuclear bodies is increased.

### Table 1. Polyglutamine disorders

<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene</th>
<th>Normal repeat length</th>
<th>Expanded repeat length</th>
<th>Primary affected brain area</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD</td>
<td>Huntingtin</td>
<td>6-34</td>
<td>36-121</td>
<td>Striatum and cortex</td>
<td>Cummings and Zoghbi, 2000;</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vonsattel et al., 1985</td>
</tr>
<tr>
<td>SCA1</td>
<td>Ataxin-1</td>
<td>6-44</td>
<td>39-82</td>
<td>Cerebellum, brain stem and spinal cord</td>
<td>Matilla-Duenas et al., 2007;</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Robitaille et al., 1995</td>
</tr>
<tr>
<td>SCA2</td>
<td>Ataxin-2</td>
<td>15-24</td>
<td>32-200</td>
<td>Cerebellum and brain stem</td>
<td>Durr et al., 1995; Orozco et al., 1989</td>
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<tr>
<td>SCA3</td>
<td>Ataxin-3</td>
<td>13-36</td>
<td>16-84</td>
<td>Cerebellum, basal ganglia, brain stem, and spinal cord</td>
<td>Durr, 1996;Takiyama, 1994;</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Woods, 1972</td>
</tr>
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<td>SCA6</td>
<td>CAGNA1a</td>
<td>4-19</td>
<td>10-33</td>
<td>Cerebellum</td>
<td>Ikeuchi et al., 1997; Zhuchenko et al., 1997</td>
</tr>
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<td>SCA7</td>
<td>Ataxin-7</td>
<td>4-35</td>
<td>37-306</td>
<td>Cerebellum and inferior olive</td>
<td>David et al., 1998; Martin et al., 1994</td>
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<tr>
<td>SCA17</td>
<td>TBP</td>
<td>25-42</td>
<td>47-63</td>
<td>Cerebellum, brain stem and cerebrum</td>
<td>Koeppen et al., 1981; Rolfs et al., 2003</td>
</tr>
<tr>
<td>SBMA</td>
<td>Androgen receptor</td>
<td>9-36</td>
<td>38-62</td>
<td>Anterior horn, bulb region and dorsal root ganglion</td>
<td>Sobue et al., 1989</td>
</tr>
<tr>
<td>DRPLA</td>
<td>Atrophin</td>
<td>7-34</td>
<td>49-88</td>
<td>Cerebellum, globus pallidus, striatum, and the dentate,</td>
<td>Burke et al., 1994; Takahashi et al., 1988</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>subthalamic and red nuclei</td>
<td></td>
</tr>
</tbody>
</table>

Disease Gene Normal repeat length Expanded repeat length Primary affected brain area References
HD Huntingtin 6-34 36-121 Striatum and cortex Cummings and Zoghbi, 2000; Vonsattel et al., 1985
SCA1 Ataxin-1 6-44 39-82 Cerebellum, brain stem and spinal cord Matilla-Duenas et al., 2007; Robitaille et al., 1995
SCA2 Ataxin-2 15-24 32-200 Cerebellum and brain stem Durr et al., 1995; Orozco et al., 1989
SCA3 Ataxin-3 13-36 16-84 Cerebellum, basal ganglia, brain stem, and spinal cord Durr, 1996;Takiyama, 1994; Woods, 1972
SCA6 CAGNA1a 4-19 10-33 Cerebellum Ikeuchi et al., 1997; Zhuchenko et al., 1997
SCA7 Ataxin-7 4-35 37-306 Cerebellum and inferior olive David et al., 1998; Martin et al., 1994
SCA17 TBP 25-42 47-63 Cerebellum, brain stem and cerebrum Koeppen et al., 1981; Rolfs et al., 2003
SBMA Androgen receptor 9-36 38-62 Anterior horn, bulb region and dorsal root ganglion Sobue et al., 1989
DRPLA Atrophin 7-34 49-88 Cerebellum, globus pallidus, striatum, and the dentate, subthalamic and red nuclei Burke et al., 1994; Takahashi et al., 1988

References
Krol et al., 2008; Stenoien et al., 2002.
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Severe Purkinje cell pathology (Cummings et al., 1999). Surprisingly, a mouse model expressing ataxin-1 with a deletion of the self-association AXH region developed a similar pathology as SCA1-mutant mice but without nuclear ataxin-1 bodies (Klement et al., 1998). These findings suggest that the nuclear bodies represent functional complexes involving ataxin-1 protein and inhibition of their formation does not prevent SCA1 pathology.

II. Altered transcriptional complex formation

The presence of mutant ataxin-1 in the nucleus is crucial for the induction of SCA1 pathogenesis. Disruption of the NLS in ataxin-1 inhibits SCA1 pathogenesis despite of the polyQ expansion mutation (Klement et al., 1998). Ataxin-1 may become incorporated into a nuclear body when transcription is initiated and stay in this complex until the process of splicing starts.

The fact that ataxin-1 is involved in transcription is also implied by the disappearance of ataxin-1 nuclear bodies when a transcription inhibitor is added to cells that express GFP-ataxin-1[Q2/A85] (unpublished data; (Irwin et al., 2005). Ataxin-1 has been described to be present in at least two different protein complexes. The first complex is involved in transcription and in this complex ataxin-1 interacts with capicua, Rora and tip 60 (Lam et al., 2006; Zoghbi and Orr, 2009). In the second protein complex, ataxin-1 interacts with RBM17 and phosphorylation of ataxin-1 at Ser776 strengthens this interaction (Lim et al., 2008; Zoghbi and Orr, 2009). RBM17 is an RNA-binding protein that is involved in splicing. Recently, Lim et al. (2008) reported that the polyQ expansion in ataxin-1 increases the number of ataxin-1/RBM17 complexes, whereas the amount of mutant ataxin-1 is decreased in complex with capicua. Thus, polyQ expanded ataxin-1 may cause a loss of ataxin-1/capicua complexes and an increase in ataxin-1/RBM17 complexes. As a result, mutant ataxin-1 may mediate toxicity by affecting the function of protein partners that are specific for vulnerable types of neurons such as the Purkinje cells, especially since Purkinje cells are one of the few cells that co-express ataxin-1, capicua and Rora. Here, Rora is an orphan nuclear receptor that mediates the expression of a group of genes known to have a role in Purkinje cell development and function (Gold et al., 2003).

Twelve genes that have a Purkinje cell-specific function are downregulated in the SCA1 mouse model and also a decrease of the Rora protein itself has been reported. In addition, partial loss of Rora enhanced the pathogenicity of mutant ataxin-1 (Serra
et al., 2004). This could be a direct result of reduced transcription triggered by the reduced interaction between capicua, Rora and mutant ataxin-1.

RBM17 has a relatively high expression level in Purkinje cells compared to other neurons (Zoghbi and Orr, 2009). Mutant ataxin-1 can possibly interact with splicing through the direct interaction with RBM17 resulting in either impaired splicing or incorrect splicing. Incorrectly spliced mRNAs is degraded by cellular mechanisms, such as nonsense-mediated RNA decay (Chang et al., 2007; Culbertson, 1999). As a result, these specific mRNAs and their subsequent proteins are downregulated.

An alternative pathway by which ataxin-1 could affect splicing is through its interaction with polyQ-binding protein-1 (PQBP-1). PolyQ expansion in ataxin-1 increases its affinity for PQBP-1, resulting in reduction of transcription through the interaction with RNA polymerase II (Okazawa et al., 2002). PQBP-1 is abundant in the central nervous system, and is particularly highly expressed in the cerebellum (Okazawa et al., 2002). PQBP-1 contains a domain rich in polar amino acids which is a known binding site for polyQ repeats in proteins like htt, the androgen receptor and ataxin-1, and this binding affinity is increased when the polyQ tract is expanded (Okazawa et al., 2002; Waragai et al., 1999). Overexpression of PQBP-1 in transgenic mice results in neuronal dysfunction with loss of Purkinje cells and granular cells in the cerebellum as well as loss of spinal motor neurons (Okuda et al., 2003). Overexpression of PQBP-1 in cultured cells results in the formation of nuclear bodies, similar to bodies formed by ataxin-1. PQBP-1 has been functionally linked to pre-mRNA splicing, as it has been identified as a component of spli-
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cosomal complexes and has been described to interact with the pre-mRNA splicing factor U5-15kDA (Waragai et al., 2000; Zhang et al., 2000) and the activator of pre-mRNA splicing SIPP1 (Nicolaescu et al., 2008). PQBP-1 interacts also with RNA polymerase II. Therefore, it may be a link between transcription and splicing (Okazawa et al., 2002). When PQBP-1 and SIPP1 are co-expressed in cell culture, nuclear bodies are formed (Nicolaescu et al., 2008) that resemble ataxin-1 nuclear bodies that we have observed (Krol et al., 2008). These PQBP-1/SIPP1 nuclear bodies are highly dynamic (Nicolaescu et al., 2008) like ataxin-1 bodies. Moreover, SIPP1 is a nuclear-cytoplasmic shuttling protein and binds to RNA (Craggs et al., 2001; Llorian et al., 2005).

Colocalisation of PQBP-1 and ataxin-1 has not been demonstrated yet in nuclear bodies but the interaction of ataxin-1 and PQBP-1 in the nucleus points at a role in transcription and/or splicing. Possibly, ataxin-1 alters the normal interaction between proteins that are present in these transcription and/or splicing complexes causing the downregulation of gene transcripts. However, we did not find any significant differences between the dynamics of LANP, RBM17 and PQBP-1 which all interact with ataxin-1 in the presence of wildtype and mutant ataxin-1 (Krol et al., unpublished data).

III. Impaired nuclear-cytoplasmic shuttling

Since ataxin-1 can shuttle between the nucleus and the cytoplasm, it has been suggested that ataxin-1 transports mRNA to the periphery of cells (Irwin et al., 2005; Krol et al., 2008). Earlier reports suggested that polyQ expansion of ataxin-1 impaired nuclear shuttling (Irwin et al., 2005), but our data show that polyQ-expanded ataxin-1 is still capable of shuttling between nucleus and cytoplasm with similar kinetics (Krol et al., 2008). Therefore, it is unlikely that impaired nuclear-cytoplasmic shuttling of polyQ-expanded ataxin-1 is underlying SCA1 disease.

One of the proteins that interacts with ataxin-1 in the nucleus, leucine-rich acidic nuclear protein (LANP), is a nuclear-cytoplasmic shuttling protein and this protein is predominantly present in Purkinje cells (Matilla et al., 1997). The polyQ expansion in ataxin-1 enhances the interaction between ataxin-1 and LANP. LANP is involved in processes such as regulation of gene expression, RNA transport, apoptosis, intracellular signaling and cytoskeletal dynamics (Matilla and Radrizzani, 2005). When co-transfected with ataxin-1, LANP is recruited into ataxin-1 nuclear bodies (Matilla et al., 1997). LANP is homologous to the U2A small nuclear ribonucleoprotein particle, snRNP U2A, that is involved in alternative splicing of RNA. In addition, LANP has been shown to bind and shuttle the RNA-binding protein HUR, which is involved in RNA stability and transport (Opal et al., 2003). The dynamics of LANP are not or only marginally affected by polyQ-expanded ataxin-1.

IV. Altered posttranscriptional regulation: RNA bodies and local translation

Ataxin-1 can be a component of RNP complexes or P-bodies where it contributes to the transport and/or local translation of specific mRNAs. When ataxin-1 binds directly to RNA, it can be a TAF that binds to specific cis-elements in mRNAs. Here, the polyQ expansion mutation may disrupt this interaction. Indeed, it has been described that the binding to RNA is diminished when the length of the polyQ tract increases (Yue et al., 2001). This specific element could be present in mRNAs of proteins downregulated in glutamate signaling. However, no known RNA-binding element in ataxin-1 has been
discovered yet. Other possibilities are that mutant ataxin-1 disrupts transport towards the periphery of a neuron so that mRNAs do not arrive at the destination or that mutant ataxin-1 affects local translation of specific mRNAs, for example mRNAs involved in glutamate signaling. It may keep mRNA in translational arrest or interfere with signaling pathways like that of MAPK that is required for local translation. This all can result in downregulation of the expression of glutamate signaling proteins leading to disturbed signaling and calcium homeostasis. Thus, mutant ataxin-1 may perturb mRNA processing and trafficking or local translation in critical neurons leading to the unique pathology of SCA1.

Altered posttranscriptional control of gene expression through the interaction of disease-related proteins with P-bodies is also observed in other polyQ disorders. The SCA-2-causing protein, ataxin-2, colocalizes and affects the assembly of P-bodies and stress granules through an interaction with the P-body component DDX6 (Nonhoff et al., 2007). Furthermore, genetic screens in Drosophila have revealed that mutations in the neuronally-expressed RNA-binding proteins staufen, muscle-blind, split ends and CG3249 modulate neurodegeneration in SCA8 (Mutsuddi et al., 2004). SCA8 is caused by a non-coding repeat in the SCA8 gene and is a controversial disease, since not all patients that have the mutation develop the disease. The SCA8 transcript functions as a gene regulator. Therefore, it has been proposed that an RNA gain-of-function mechanism underlies neurodegeneration. The CUG expansion in the SCA8 gene may alter the association of SCA8 protein with specific RNA-binding proteins. In addition, in the opposite direction, 3’ to 5’, the SCA8 gene contains a CAG expansion encoding a polyQ expansion protein (Ikeda et al., 2008). Thus, SCA8 could be both a non-coding expansion repeat and a polyQ expansion disease. Possibly, more than one pathogenic mechanism is involved. SCA8 mice and human patients have intranuclear inclusions in Purkinje cells and brainstem neurons that are positive for expanded polyQ (Soong and Paulson, 2007). Furthermore, Savas et al. (2008) showed that the Huntington’s disease causing protein htt plays a role in gene silencing by means of colocalization with P-bodies and the interaction with Argonaute-2 (Ago2). Overexpression of mutant htt reduced the number of P-bodies and reporter gene silencing activity (Savas et al., 2008). Both the polyQ and the nearby proline-rich regions of htt are involved in the interaction with Ago2 (Savas et al., 2008). Thus, two polyQ disease causing proteins (htt and ataxin-2) and one gene involved in SCA8 are linked to posttranscriptional control of gene expression through the interference with processes of RNA binding or metabolism. Altered posttranscriptional regulation may be a common factor in some if not all polyQ expansion disorders.

**Summary**

Ataxin-1 has a number of functions but it is unknown at what level mutant ataxin-1 induces toxicity. Ataxin-1 may initially be involved in transcription in the nucleus, then remains in a complex in the nucleus during splicing (together with RBM17), acts as an RNA-binding protein or TAF for mRNA nuclear-cytoplasmic shuttling, and finally is instrumental in targeting mRNAs to distants parts of cells (dendrites, axons) for local protein production. Perhaps if ataxin-1 is involved in the recruitment of several proteins and RNAs into nuclear bodies such as Cic, RBM17, RNA, a change in its interaction...
with one component may affect its interactions with the other component resulting in a gain of function alteration. Mutant ataxin-1 can induce toxicity in the nucleus by altering transcription and splicing of specific genes. It can also induce toxicity in the cell body as it may alter the transport of RNA in the cytoplasm or interfere with local translation of the mRNA.

So far, the cytoplasmic functions of ataxin-1 have hardly been investigated, which may be due to the most visible hallmark of ataxin-1, the nuclear bodies. While the nuclear function is indeed affected by polyQ expansion, understanding the role of ataxin-1 in the periphery of Purkinje cells may bring us closer to the understanding why these neurons are so vulnerable to mutant ataxin-1 expression, and hopefully lead to a better understanding of the dysfunction and atrophy of these cells.

References


Spinocerebellar ataxia-1: insights in the function of ataxin-1


Chapter 5


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