Ataxin-10, the Spinocerebellar Ataxia Type 10 Neurodegenerative Disorder Protein, Is Essential for Survival of Cerebellar Neurons*

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A number of human genetic diseases are associated with the expansion of short tandem repeats in coding or noncoding gene regions (1, 2). Spinocerebellar ataxias and related genetic disorders are often caused by trinucleotide expansions of coding sequences conferring either loss-of-function as in Friedreich’s ataxia (3) or gain/change-of-function by the translation of CAG sequences conferring either loss-of-function as in Friedreich’s ataxia, is caused by a novel pentanucleotide (ATTCT) repeat expansion in the SCA10 gene. Although clinical features of the disease are well characterized, nothing is known so far about the affected SCA10 gene product, ataxin-10 (Axt-10). We have cloned the rat SCA10 gene and expressed the corresponding protein in HEK293 cells. Axt-10 has an apparent molecular mass of ~55 kDa and belongs to the family of armadillo repeat proteins. In solution, it tends to form homotrimeric complexes, which associate via a tip-to-tip contact with the concave sides of the molecules facing each other. Axt-10 immunostaining of mouse and human brain sections revealed a predominantly cytoplasmic and perinuclear localization with a clear restriction to olivocerebellar regions. Knock down of SCA10 in primary neuronal cells by small interfering RNAs resulted in an increased apoptosis of cerebellar neurons, arguing for a loss-of-function phenotype in SCA10 patients.

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The abbreviations used are: SCA, spinocerebellar ataxia; siRNA, small interfering RNA; bia-Tris, 2-[bis(2-hydroxyethyl)aminol]-2-(hydroxymethyl)propane-1,3-diol.

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amplified DNA into the corresponding sites of the eukaryotic expression vector pCEP-Pu. Primers used were 5′-TTT AAG CTT GCT AGC GAT GGC GGC GCC CAG GAT G-3′ and 5′-CTT GGA TCC TTA AGG CCG GGG GAT-3′. The resulting vector that introduces a polyhistidine tag, a Myc tag, and an enterokinase cleavage site at the N-terminal end of the protein sequence was used for transfecting HEK293 cells. For stable transfection, HEK293 cells were kept in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 1% Glu, and penicillin/streptomycin. Cells were grown to 80% confluence in 6-well plates and transfected overnight with 1 μg of vector DNA using 5 μl of LipofectAMINE reagent. The selection of positive clones was performed by culturing transfected cells with 2 μg/ml puromycin with frequent changes of medium until a resistant population appeared. All reagents were purchased from Invitrogen. HEK293 cells and ATX-10 were additionally transfected with SCA10 cDNA were grown to high density in 125-m1 cell culture flasks using complete medium. For expression of recombinant protein, cells were switched to serum-free expression medium. Cell supernatants were harvested frequently until the cells detached, pooled, and passed through a syringe filter. Conditioned medium was then purified using nickel-Sepharose chromatography according to the manufacturer’s instructions (Qiagen). Eluted fractions were analyzed by SDS-PAGE and dialyzed against 20 mM Tris-HCl, pH 7.4, and 150 mM NaCl.

The P4 fragment comprising amino acids 228–476 was expressed in E. coli BL21 (DE3) pLysS cells (Stratagene Europe). The corresponding cDNA fragment was produced by PCR with 5′-GCC GCC AGC GGA CTT GGC GCC CAG GAT G-3′ as forward and 5′-GCC CCA AGC TTA ACG GGG GAT GGC ATT-3′ as reverse primers and introduced into the pRSET 5d vector using NcoI and HindIII ends, respectively. Protein synthesis was induced at an A600 of 0.5 with 0.4 mM isopropyl-1-thio-β-D-galacto-pyranoside for 2 h. Cultures were centrifuged, and pellets were resuspended in lysis buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1% Tween 20, 1 mM dithiothreitol). P4 inclusion bodies were then prepared by several freeze-thaw and sonication cycles followed by extensive washing with lysis buffer. For refolding, inclusion bodies were solubilized in 6 M GuHCl, 100 mM dithiothreitol, 50 mM Tris/HCl buffer at pH 8.0 and then submitted to dialysis against refolding buffer containing 20 mM phosphate-buffered saline (PBS) and 0.5 mM dithiothreitol. Protein solution was purified on a Superdex 75 (26/60) column (Amersham Biosciences) using refolding buffer as eluent. Fractions were analyzed by SDS-PAGE, pooled, and concentrated.

**Antibody Production**—An antisera against Atx-10 was obtained by immunization of rabbits with P4 inclusion bodies (BioScience, Göttingen, Germany) according to a standard protocol.

**SDS-PAGE and Western Blot Analysis**—For resolving Atx-10 oligomers by SDS-PAGE, gradient gels (3–10%) were applied with 2.5% albumin, 10% glycerol. For resolving Atx-10 oligomers on a 12% gel, 0.1% Triton X-100 for 30 min on ice. Reactions were stopped by the addition of Laemmli buffer and subsequent heat denaturation. Samples were analyzed by Western blotting using anti-Atx-10 antibody.

**Characterization of Rat Ataxin-10**

**Cell Fractionation**—The subcellular localization of Atx-10 in PC12 cells was analyzed after lysis of 3 × 10⁹ cells by passing them several times through a 0.6-mm syringe needle. Cytosolic and organelle fractions were separated by centrifugation in a table top centrifuge at 10,000 × g for 10 min. A further separation into microsomal and cytosolic fractions was obtained by ultracentrifugation of the soluble fraction at 100,000 × g for 1 h. Both fractions (200 μl) were then treated with 4 μl of protease K (1 mg/ml) in the presence or absence of 0.1% Triton X-100 for 30 min on ice. Reactions were stopped by the addition of Laemmli buffer and subsequent heat denaturation. Samples were analyzed by Western blotting using anti-Atx-10 antibody.

**Primary Neuronal and Glial Cultures**—Primary cerebellar and cortical neurons were prepared from postnatal Wistar rats (P1). Briefly, cerebellar and cortical cortices were prepared using 12% gels. Western blot analysis of Atx-10 was performed at 20,000 rpm for P4 at a filling height of 3 mm and 1400 rpm for Atx-10, respectively. Sedimentation equilibrium runs were performed at 20,000 rpm for 4 h. The CD spectrum of recombinant Atx-10 in phosphate-buffered saline (PBS) was acquired on a Jasco J720 spectropolarimeter (Japan Scientific Co.). The far-UV spectrum (200–400 transmission electron microscope.**

**CD Spectroscopy**—The CD spectrum of recombinant Atx-10 in phosphate-buffered saline (PBS) was acquired on a Jasco J720 spectropolarimeter (Japan Scientific Co.). The far-UV spectrum (200–400 nm) was measured in path length quartz cuvette and expressed as the mean residue ellipticity (°) at 20°C. The spectrum was normalized for concentration and path length to obtain the mean molar residue ellipticity after subtraction of the buffer contribution. Helix content was calculated using the k2d program (23).
with microwave heating at 90 °C for 30 min before incubation with Atx-10 antibody. All sections were counterstained with hematoxylin. For control stainings, the antibody solution was preincubated with recombinant Atx-10 at 1 mg/ml.

siRNA Transfections—21-nucleotide sense and 21-nucleotide antisense siRNA strands with symmetric 2-nucleotide 3′-overhangs consisting of 2′-deoxythymidines were designed and synthesized by Qiagen-Xeragon (Germantown, MD). The following rat SCA10 gene-specific target sequences were used: si-SCA10.3 (5′-AAGGGTCACACTGTTA-3′) and si-SCA10.4 (5′-AAGGATGAAGGACCTGGAAGA-3′).

siRNA duplexes were transfected into primary cerebellar and cortical neurons using LipofectAMINE 2000 (Invitrogen) in 24-well plates. Before transfection, the culture medium was replaced with 0.3 ml/well neurobasal medium without antibiotics. Briefly, 1 μl of LipofectAMINE 2000 and 4 μl of Opti-MEM I medium per well were preincubated for 5 min at room temperature. Meanwhile, 1 μl of si-SCA10.3RNA and 1 μl of si-SCA10.4RNA were mixed with 48 μl of Opti-MEM I medium. The two mixtures were combined and incubated for 20 min at room temperature for complex formation. The entire mixture (100 μl) was added to the cells in one well, resulting in a final concentration of 100 nM for the siRNAs. After 2 h of incubation, 0.2 ml of neurobasal medium containing 3% concentrated antibiotics, B27 supplement, and glutamate was added to the cells.

FIG. 1. Sequence of the rat SCA10 cDNA. Composite nucleotide sequence and deduced amino acid sequence of the rat Atx-10 protein from PC12 cells. The numbers at the right indicate the positions of nucleotides and amino acid residues.
mine was added to each well to reconstitute the optimal medium. Specific silencing of the SCA10 gene product was confirmed by Western blot analysis. Cells were lysed in 2X Halt®/1003
Laemmli buffer 24, 48, and 72 h after transfection, and proteins were separated on 4–12% bis-Tris Nu-gels (Invitrogen, Basel, Switzerland) and blotted onto polyvinylidene difluoride membranes. Membranes were blocked for 1 h in 5% bovine serum albumin, PBS, 0.1% Tween 20 and incubated with primary rabbit anti-Atx-10 antibody (1:4000) overnight at 4 °C followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:4000; Sigma). The blots were developed using the ECL detection system (Amersham Biosciences). To confirm equal loading, the same membrane was incubated for 15 min at 50 °C with stripping buffer (62.5 mM Tris-HCl, pH 6.7, 2% SDS, 100 mM 2-mercaptoethanol), washed with PBS plus 0.1% Tween 20, and reprobed with an anti-β-actin monoclonal antibody (Sigma), followed by incubation with horse-radish peroxidase-conjugated goat anti-mouse antibody (1:4000; Sigma) and ECL detection.

Neuronal survival was assayed 48 h after transfection by measuring lactate dehydrogenase release into culture supernatant using standard procedures.

**Immunohistochemistry**—Primary cultures of cerebellar and cortical neurons were plated on poly-L-lysine-coated 8-well CultureSlides (BD...
Biosciences, Belgium); PC12 cells and primary cerebellar astrocytes were plated on poly-D-lysine-coated tissue culture dishes with four compartments. Cells were fixed in 2% p-formaldehyde, 0.1% glutaraldehyde, 0.1% tannic acid for 15 min at room temperature, washed in PBS, and permeabilized in PBS plus 0.2% Triton X-100 for 5 min at room temperature, followed by three washes with PBS. Primary polyclonal antibody (rabbit) against Atx-10 was applied at 1:1000 in PBS overnight at 4 °C. After washing with PBS, a 1:800 dilution of Alexa Fluor® 488 goat anti-rabbit IgG (Molecular Probes, Leiden, The Netherlands) was applied for 1 h at room temperature. Finally, cells were washed three times with PBS before being mounted with coverslips using Mowiol 4–88 (Sigma) containing 2.5% 1,4-diazabicyclo-(2.2.2) octane (Sigma) to reduce fading. Fluorescence microscopy was performed using a DP-50 digital camera coupled to an Olympus IX51 inverted microscope.

RESULTS

Cloning and Sequence Characterization of Rat SCA10—The rat SCA10 gene was found as a false positive clone during a cDNA library screen for differentially up-regulated transcripts during inflammatory response in PC12 cells. Initially, a full-length cDNA of 1992 bp was isolated containing an open reading frame from base pair positions 201–1626. Sequence comparison data base searches with the rat SCA10 cDNA resulted

| Protein          | Concentration | Sedimentation coefficient ($s_{20, w}$) | Molecular mass (gDa) | Molecular mass (kDa) |
|------------------|---------------|----------------------------------------|----------------------|----------------------|
| P4 low $M_r$     | 1.1           | 33                                     | 28                   |
| P4 high $M_r$    | 1.1           | 47                                     | 84                   |
| Ataxin-10 low $M_r$ | 0.25         | 65                                     | 59                   |
| Ataxin-10 high $M_r$ | 0.25        | 47                                     | 213                  | 177                  |

Cloning and Sequence Characterization of Rat SCA10—The rat SCA10 gene was found as a false positive clone during a cDNA library screen for differentially up-regulated transcripts during inflammatory response in PC12 cells. Initially, a full-length cDNA of 1992 bp was isolated containing an open reading frame from base pair positions 201–1626. Sequence comparison data base searches with the rat SCA10 cDNA resulted
in several matches with the mouse SCA10 gene (originally named E46 mouse brain protein, accession number BC046802) showing the highest homology. The identity between the murine sequence and the rat SCA10 cDNA is 94%. The homologous sequence of human origin (accession number BC007508) shares 86% identity with the rat gene. Related sequences identified in the *Drosophila* and *Saccharomyces* genomes show identities ranging from 24 to 35%. We speculate that the translation starts at position 201, since in the mouse E46 sequence, the translation starts at the same position. The open reading frame thus codes for altogether 476 amino acids. The nucleotide and putative amino acid sequences are shown in Fig. 1. Atx-10 does not contain a signal sequence for secretion or any subcellular compartment arguing for a cytoplasmic localization of the protein.

**Oligomer Formation and Secondary Structure Analysis of Recombinant Atx-10**—We have expressed full-length Atx-10 in HEK293 cells together with a BM-40 signal sequence for secretion and an N-terminal polyhistidine tag allowing purification via affinity chromatography. For antibody production, a frag-
ment containing amino acids 228–476 (P4) was expressed in E. coli and refolded from purified inclusion bodies. Recombinant full-length Atx-10 showed an apparent molecular mass of ∼58 kDa in SDS-PAGE and Western blots using anti-Atx-10 antibody (Fig. 2A). The difference in the molecular mass of the recombinant protein from the calculated value of 53.7 kDa corresponds to the added tag sequences, which code for an additional 5.5 kDa. The endogenous Atx-10 protein in PC12 cell lysates showed the expected molecular mass (Fig. 2A), which was also observed for recombinant Atx-10 protein lacking a secretion signal sequence and tag sequences overexpressed in COS-7 cells (data not shown). The recombinant protein expressed in HEK293 cells exhibits a slight heterogeneity (triple band), probably due to a different behavior in SDS-PAGE caused by the added tag sequences. During purification of the P4 fragment via size exclusion chromatography, we have observed, beside the monomer peak, a pronounced second elution peak in a higher molecular mass region indicative of a distinct oligomer state (data not shown). Analytical ultracentrifugation experiments confirmed that both P4 and recombinant Atx-10 existed predominantly as oligomers with molecular masses suggesting a trimer formation (Table I). A similar result was obtained by gradient SDS-PAGE applying unheated Atx-10 samples in which the oligomeric band (∼150 kDa) was retained during electrophoresis (Fig. 2B). Electron micrographs of rotary-shadowed Atx-10 showed horseshoe-shaped complexes with sometimes three curved arms, suggesting a tip-to-tip association of the molecules with the concave sides facing each other (Fig. 2C). Since the P4 fragment has apparently retained the capacity for forming oligomers, we suppose that the homoa ssociation site is situated at the C terminus of the Atx-10 molecule.

Circular dichroism analysis resulted in a predominantly α-helical signal with characteristic minima at 208 and 222 nm (Fig. 2D). The calculated helix content is 36%. A secondary structure prediction for the Atx-10 protein sequence revealed a repetitive pattern of helical stretches (Fig. 2E) interspersed with turn structures. Fold recognition analysis using the 3D-PSSM program (16) clearly defined Atx-10 as a member of the armadillo repeat protein family with β-catenin and importin-β structures as best fitting models.

Subcellular Localization and Detection of Atx-10 in Primary Neuronal Cells—To assess the subcellular localization of Atx-10, we fractionated PC12 cell lysates by centrifugation at 10,000 × g and looked for Atx-10 in the pellet and supernatant fractions by Western blotting. As shown in Fig. 3A, Atx-10 was exclusively found in the soluble cell fraction, indicating that it did not associate with organelles or the cell membrane. In a second experiment, the soluble fraction of the PC12 cell lysate obtained in the first experiment (Fig. 3A) was pelleted by ultracentrifugation at 100,000 × g to separate cytosolic (supernatant) and microsomal (pellet) fractions. Both samples contained comparable amounts of Atx-10 in this experiment, indicating that the protein was not largely attached to membranes. The detection of Atx-10 in the pellet fraction in this experiment can be explained by residual cytoplasmic solution, which cannot be removed completely from the sample without loss of microsomes. Both fractions were treated with proteinase K in the absence or presence of detergent to exclude the possibility that Atx-10 was localized on the lumen side of vesicles that is protected from protease cleavage. Proteinase K treatment removed the Atx-10 signal both in the intact microsomal fraction and in the detergent-treated microsomal fraction, confirming its cytoplasmic localization (Fig. 3B).

Detection of Atx-10 in PC12 cells by fluorescence microscopy using Atx-10 antibody revealed a cytoplasmic pattern confirm-

### Table II

| Human brain | Mouse brain |
|-------------|-------------|
| Offactory bulb (mitral cells) | ND | ++ |
| Cerebral cortex | 0/+ | 0/+ |
| Striatum | 0 | 0/− |
| Locus coeruleus | + | + |
| Substantia nigra | 0 | 0 |
| Midbrain raphe | ++/− | ++ |
| Nuclei pontis | ++/− | ++/− |
| Nuclei cuneatus | +++ | ND |
| Nuclei vestibularis | ++ | ++ |
| Nuclei olivaris | ++ | ++ |
| Nuclei hypoglossus | ++/− | ND |
| Purkinje cells | ++/− | ++/− |
| Deep cerebellar nuclei | ND | ++ |
| Spinal motor neurons | + | + |

* 0, no observable staining reaction; ++, very strong staining reaction; /, "from . . . to"; ND, not determined.

SCA10 Expression Pattern in Rat Tissues and Atx-10 Immunostaining in Human and Mouse Brain Sections—The expression pattern of the rat SCA10 gene was analyzed by Northern blotting using tissues of various rat organs (Fig. 4). As shown in Fig. 4A, the SCA10 transcript showed a ubiquitous distribution. SCA10 gene expression was, however, markedly increased in tissues of testis, adrenals, and brain. For a more detailed neuronal expression pattern at the protein level, we performed Atx-10 immunostainings using mouse and human brain sections. The intensity of Atx-10 reactivity differed significantly between the different neuronal populations and between the nerve cell bodies of individual nuclei (Fig. 5 and Table II). Atx-10 immunostaining was diffusely distributed in the cell bodies of neurons and was also present in the proximal parts of dendritic processes. Only in the human cerebellum was a perinuclear ring of increased reactivity observed in some Purkinje cells (Fig. 5F). A strong Atx-10 reactivity in the cell nucleus was only observed in the spinal ganglia neurons. Strong axonal Atx-10 reactivity was observed in long tracts of the human and murine spinal cord. However, the main fiber tracts (corpus callosum and fornix) of the cerebral hemispheres remained unstained. The most intense reactivities were observed in the olivary nuclei (Fig. 5, A and B), the nuclei pontis (Fig. 5, C and D), the deep cerebellar nuclei, the Purkinje cells (Fig. 5, E and F), the vestibular and cuneate nuclei, and the sensory ganglion cells. The mouse neuronal populations showing lesser Atx-10 reactivities were the mitral cells of the olfactory bulb, pyramidal cells of the upper cortical layers of the cerebral cortex, neurons of the midbrain tectum, the raphe nuclei, the locus coeruleus, and the motor nuclei in brain stem and spinal cord. Variable intensity of staining was observed in pyramidal cells of the human cerebral cortex, neurons of the midbrain tectum, the raphe nuclei, and the motor nuclei in brain stem and spinal cord. Very weak staining of nerve cells was seen in the thalamus. The dentate gyrus and the pyramidal cell layer of the hippocampus remained unstained (Fig. 5, G and H). Interestingly, strong Atx-10 staining was observed in clustered small neuropil granules in the hippocampus, cerebellum, and brain stem of the mouse brain, very similar to the granules reported by Jucker et al. (17) in aging mice. Some Atx-10-positive cells were also found in the Purkinje cell layer of the human cerebellum (Fig. 5F).
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Functional Analysis of Atx-10 Using in Vitro siRNA Knock Down Technology—Our immunohistochemical data revealed a predominant expression of Atx-10 in neuronal subpopulations of the central nervous system. Therefore, to assess the functional role of Atx-10, we decided to use primary neurons as potential targets to interfere with SCA10 gene expression. Primary cerebellar and cortical neurons were transfected with a combination of two cognate siRNA duplexes 7 days after plating or with transfection reagent as control. To evaluate the ability of siRNA transfection to abolish target gene expression, cell lysates were analyzed 24, 48, and 72 h after transfection by immunoblotting. As shown in Fig. 6, A and B, specific silencing of the SCA10 gene product was confirmed in siRNA-transfected neurons, whereas in control transfected neurons, Atx-10 expression remained unchanged. Atx-10 protein levels were significantly reduced already after 48 h, being hardly detectable after 72 h. Equal loading was confirmed by the presence of consistent amounts of β-actin protein.

Phenotypic effects of Atx-10 knock down on cultured neurons were first observed by phase microscopy, indicating that neuronal survival was impaired as compared with control-transfected cells (not shown). Therefore, neuronal cell loss was evaluated measuring lactate dehydrogenase release into the culture medium of siRNA-transfected and control-transfected central nervous system neurons. To compare different neuronal target populations, lactate dehydrogenase release was analyzed 48 h after siRNA transfection in primary cortical and cerebellar neurons. Silencing of SCA10 gene expression resulted in highly significant increase in cytotoxicity in both cerebellar and cortical neurons as compared with controls (Fig. 6C). However, cerebellar neurons appeared to be significantly more sensitive to reduced Atx-10 levels (38.3 ± 7.9%; mean ± S.D.) than cortical neurons (9.8 ± 1.9%; mean ± S.D.), although Atx-10 expression was knocked down equally. These results indicate that the presence of Atx-10 is essential for the survival of this subpopulation of neurons.

Discussion

In this study, we report the cloning of the rat SCA10 gene, which codes for an intracellular protein of ~55 kDa with clear sequence homologies to related proteins in mice and humans. Our attempts to express the full-length protein in E. coli were unsuccessful, although fragments of Atx-10 were well expressed in a bacterial system, and a large C-terminal fragment (P4) could be refolded from inclusion bodies, allowing the production of a polyclonal antibody. Full-length Atx-10 was expressed in HEK293 cells as a secreted fusion protein showing a slightly higher molecular mass in SDS-PAGE than the endogenous protein of PC12 cells due to the added tag and protease cleavage sequences (~4 kDa). Homology searches and fold prediction programs classify Atx-10 as member of the armadillo repeat protein family with an all-a-helical fold. This was confirmed by CD-spectroscopic analysis of both Atx-10 and the P4 fragment (not shown) and electron micrographs of recombinant Atx-10 showing molecules with an elongated curved shape characteristic for this protein family (Fig. 3C).

The armadillo repeat motif usually confers protein-protein interactions with diverse cellular binding partners and elicits functions in many different biological contexts (18). A tendency for homo-oligomerization as in the case of Atx-10 represents a hitherto novel feature of this domain. We have observed apparent oligomer formation both for the recombinant proteins and for endogenous Atx-10 as deduced from gel filtration analysis of PC12 cell lysates (not shown). As pointed out above, we have indications that the homoassociation site is located near the protein’s C terminus. Crystal structures of complexes with armadillo repeat proteins have identified binding regions exclusively in grooves on the concave surfaces of the superhelix (19, 20). As our electron micrographs of recombinant Atx-10 suggest a tip-to-tip association in a horseshoe-shaped fashion, it can be concluded that oligomerization might either open a more extended polypeptide binding region or render the binding site inaccessible, thereby inactivating the protein function. Interestingly, alignments of the SCA10 gene with homologous sequences from phylogenetically distant organisms as Drosophila (accession number AAF57806), Saccharomyces (accession number Q09888), and Arabidopsis (accession number NM116241) display a clear gradient of conservation toward the C terminus of the protein (not shown), indicating that the proposed homoassociation site is essential for its cellular function.

The subcellular localization of Atx-10 was predominantly cytoplasmic ruling out a similar function as for β-catenin, which is associated with the intracellular domain of E-cadherin.
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and hence attached to the cell membrane. This was additionally confirmed by co-precipitation experiments using a fusion protein in which the intracellular portion of murine E-cadherin was fused to glutathione S-transferase (not shown). We can also exclude an association of Atx-10 with major components of the cytoskeleton, since Atx-10 was exclusively found in the cytosolic fraction of cell lysates, and double stainings of PC12 cells using phalloidin or anti-tubulin antibody did not result in overlapping patterns (not shown). We are currently working on a proteomics approach to identify binding partners of Atx-10 in PC12 cells, which might point to parallels in its cellular function in cerebellar neurons. Our Northern blot experiments have shown that SCA10 expression is up-regulated to a similar degree in brain and adrenal tissues (Fig. 4A).

Spinocerebellar ataxias constitute a fast growing group of hereditary diseases caused by different genetic defects that result in a similar neurological disorder (6). The generally unspecific clinical phenotype results from tissue degeneration affecting the cerebellum itself or its afferent and efferent pathways. No autopsy studies of SCA10 patients have been published to date. Thus, we are left with conjectures concerning the distribution of histopathological changes in this particular form of SCA. However, magnetic resonance imaging data in SCA10 patients have shown severe cerebellar atrophy (21), accounting for the cardinal clinical features of the disease. The observed distribution of Atx-10 reactivity together with the high protein expression in spinal ganglia neurons and areas known to project to the cerebellum, including the nuclei pontis and the olivary nuclei, is consistent with spinocerebellar involvement and with the reported clinical symptomatology in affected families (21). In addition, the high expression of Atx-10 in spinal ganglia neurons might be related to the peripheral neuropathy known to occur in a large proportion of SCA10 patients.

The SCA10 mutation, which has been localized to 22q13.3, represents a novel class of microsatellite expansion formed by a pentanucleotide repeat within a noncoding gene region. Microsatellite expansions in noncoding regions have been described for several other dominantly inherited genetic disorders, including myotonic dystrophies 1 and 2 and SCAS, which are caused by triplet or, as in the case of myotonic dystrophy 1, tetranucleotide expansions (2). For these, different mechanisms of pathogenesis are discussed: 1) haploinsufficiency on the protein level, 2) disregulated expression of neighboring genes, and 3) toxic effects by gain-of-function at the RNA level. Although several reports have determined monocausal mechanisms responsible for the clinical phenotypes in noncoding repeat expansion disorders, there is also growing evidence for additive disease models (22).

Our siRNA data demonstrate that loss of SCA10 expression is sufficient for cell degeneration (Fig. 6C). This effect was highly specific and restricted to cerebellar neurons, excluding a general toxicity induced by the applied siRNAs (Fig. 6A and B). This observation would argue for a loss-of-function phenotype in SCA10 patients, although definite results will only be obtained when patient material from affected tissues is available. Matsuura et al. (15) have reported that SCA10 mRNA levels in lymphoblastoid cell lines were unchanged in SCA10 patients. However, since elevated expression levels seem to be restricted to neuronal cell lines, we suggest that a reduced expression from haploinsufficiency alone, which might not add to a basal expression, would only affect tissues in which higher concentrations of Atx-10 are essential.

Our data present the first characterization of Atx-10 and suggest a possible pathogenic mechanism involving reduced protein expression levels in affected tissues, predominantly in sensitive neurons and tracts of the cerebellum. Further studies are needed to elucidate the functional role of Atx-10 in neuronal target cells.

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