Functional Architecture of Atrophins*

Received for publication, November 3, 2006  Published, JBC Papers in Press, December 6, 2006, DOI 10.1074/jbc.M610274200

Yiguo Shen, Gena Lee, Youngshik Choe, J. Susie Zoltewicz, and Andrew S. Peterson 1

From the Gallo Center and the Department of Neurology, University of California at San Francisco, Emeryville, California 94608

Vertebrate genomes harbor two Atrophin genes, Atrophin-1 (Atn1) and Atrophin-2 (Atn2). The Atn1 locus produces a single polypeptide, whereas two different protein products are expressed from the Atn2 (also known as Rere) locus. A long, or full-length, form contains an amino-terminal MTA-2-homologous domain followed by an Atrophin-1-related domain. A short form, expressed via an internal promoter, consists solely of the Atrophin domain. Atrophin-1 can be co-immunoprecipitated along with Atrophin-2, suggesting that the Atrophins ordinarily function together. Mutations that disrupt the expression of the long form of Atrophin-2 disrupt early embryonic development. To determine the requirement for Atrophin-1 during development we generated a null allele. Somewhat surprisingly we found that Atrophin-1 function is dispensable. To gain a better understanding of the requirement for Atrophin function during development, an analysis of the functional domains of the three different gene products was carried out. Taken together, these data suggest that Atrophins function as bifunctional transcriptional regulators. The long form of Atrophin-2 has a transcriptional repression activity that is not found in the other Atrophin polypeptides and that is required for normal embryogenesis. Atrophin-1 and the short form of Atrophin-2, on the other hand, can act as potent and evolutionarily conserved transcriptional activators.

The mouse and human genomes contain two Atrophin genes, the normal functions of which are not clear. Gain-of-function polyglutamine extension mutations in the Atrophin-1 gene (Atn1) cause dentato-rubro-pallidoluysial atrophy, a dominant neurodegenerative disease (1). The Atrophin-2 (Atn2) locus, also known as Arg-Glu repeat-encoding (Rere), was first described as encoding an Atrophin-1-related protein that could heterodimerize with Atn1 in vitro (2, 3). The Atn2 locus is more complex than the Atn1 locus, encoding two polypeptides from a larger palette of domains. In addition to the Atrophin domain, the Atn2 locus encodes the BAH, ELM2, SANT, and GATA domains, which are arranged in a configuration similar to that found in MTA-2, a protein that scaffolds the formation of a repressive chromatin remodeling complex, NuRD (4). Atn2 and Atn1 gene products can be co-immunoprecipitated from embryo extracts, indicating that Atrophins probably carry out at least some of their cellular functions by acting together in a molecular complex (5).

The single Atrophin gene products that are produced by the Drosophila melanogaster (Atro or grunge) and Caenorhabditis elegans (EGL-27) genomes have domain structures that are reminiscent of the long form of the vertebrate Atrophin-2 (6, 7), suggesting that this may be the ancestral function with Atn1 and the short form of Atn2 being a more recent evolutionary specialization. Genetic analysis of Atro in Drosophila indicates that it functions as a transcriptional co-repressor during development with important roles in segmentation and planar polarity (8). Overexpression of a polyglutamine-expanded version of Atn1 in flies indicates that it too can function as a transcriptional repressor in this context (7). These studies are consistent with the general idea that Atrophins have an evolutionarily conserved function as transcriptional co-repressors. This is an attractive conclusion, but it cannot be used to explain why evolution has elaborated distinct gene products from an apparently single ancestral form. This evolutionary innovation appears to have been an early and functionally important one, because the teleost, avian, and mammalian lineages all carry two types of Atrophin genes.

We have begun to explore the question of distinct functions of the two types of Atrophin gene products using a combination of genetic and biochemical analysis. We previously reported the phenotype of a mutation in Atn2 that selectively disrupts the function of the Atrophin-2 long-form gene product (5). Loss of Atr2L causes a diverse set of developmental defects, many of which are associated with a failure in the function of important signaling centers. For example, Shh is not expressed from the anterior notochord, Fgf8 expression in the anterior neural ridge is defective, and the Fgf8-expressing apical ectodermal ridge does not form properly in embryos unable to express Atr2L. Somitogenesis and heart development, processes that are not related in any obvious way to the signaling centers that are defective, also fail to proceed normally indicating that there are additional and diverse roles for Atr2L. To carry the analysis of Atrophin function further, we generated a null allele of Atn1 and carried out in vitro analyses of the Atrophin-2 and Atrophin-1 domains. These data support the idea that the Atrophin domain on its own has a distinct function that is supplied redundantly by the short form of Atrophin-2 (Atr2S) and Atrophin-1.

EXPERIMENTAL PROCEDURES

Atrophin-1 Knock-out Mice—The targeting construct was produced by PCR amplification of fragments arms from...
ES² cell DNA. The fragments were assembled together with Lox sites made from synthetic oligonucleotides and a PKG-Neo cassette. The construct was sequence verified before being electroporated into the A14 ES cell lines, which were a kind gift from Dr. Bill Skarnes. Correct targeting events (INeo allele) were identified using a PCR strategy and confirmed by Southern blotting. A correctly targeted ES cell line was electroporated with a Cre expression plasmid, and Cre-mediated deletion events that produced the Attn1Δ allele were identified using PCR. Both Attn1-INeo and the Attn1Δ ES cell lines were used to generate chimeras and germ-line transmission.

**mRNA Expression**—Radioactively labeled probes were synthesized from cDNA template using a Random Primed DNA labeling kit (Roche Applied Science) and [³²P]dCTP (Amersham Biosciences), mouse multiple tissue Northern blots (Clontech) were hybridized and stripped according to manufacturer's guidelines. For detection of Atrophin-2 transcript isoforms, two Atrophin-2 cDNA fragments (residues 1–1242 and 3162–4680) were used as template for 5' and 3' probe synthesis, respectively. To detect Atrophin-1, full-length cDNA was used as template. Human α-actin probe was synthesized from template supplied by Clontech.

**Constructs**—Full-length Atrophin-1 and Atrophin-2 cDNAs were described in Zoltewicz et al. (5). Truncated versions of Atrophin-2 were constructed from the full-length clones by restriction fragment ligation or PCR amplification from the cloned cDNA using Expanded High Fidelity PCR (Roche Applied Science). All constructs were sequence verified. For transcription activity assays, full-length or fragments of Atrophin cDNAs were cloned into pFA-CMV vector (Stratagene) using appropriate restriction enzyme sites to produce the amino-terminal Gal4 fusion protein. Constructs E through O of Fig. 3 encode the following Atrophin-2 amino acid residues: E, 1–616; F, 1–343; G, 344–570; H, 481–1559; I, 571–1140; J, 1055–1559; K, 1011–1360; L, 1011–1360 without “RE” repeat domain; M, 1148–1360; N, 1323–1559; O, 1055–1148.

For protein localization studies, full-length or fragments of Atrophin-2 were cloned into pEGFP-N3 vector (Clontech) to generate carboxyl-terminal GFP fusion proteins. These truncated Atrophin-2-GFP constructs encode the following Atrophin-2 amino acid residues, two Atrophin-2 cDNA fragments (residues 1–1219 and 1323–1559). Atrophin-2 fragments were constructed on pFLAG7 vector (Sigma) to produce amino-terminal FLAG-tagged fusion proteins.

**RESULTS**

**A Null Allele of Attn1**—To assess the requirement for Attn1 during development we generated an allele in which six of ten exons are Floxed (Flanked with Lox) sites (Fig. 1A). We then generated mice carrying a germ-line allele in which Cre-mediated recombination deleted the Floxed region, which includes the initiator codon and ~90% of the coding region. Both PCR and Southern blot analysis were used to confirm the structure of the deletion (data not shown). Intercrosses between heterozygotes produced viable and fertile homozygotes at Mendelian ratios, indicating, somewhat surprisingly, that Attn1 is dispensable under ordinary circumstances. To confirm that the desired mutation had been produced, we also carried out Northern blot analysis using total brain RNA. The predicted truncated message was produced by the deleted allele (Fig. 1B). Given the existence of two Attn genes, a likely explanation is that the Attn2 gene provides a redundant source of Atrophin function.

We sought additional information with which to evaluate possible redundancy between Attn1 and Attn2. Previous analysis had indicated widespread expression of Attn1 and Attn2 but had

---

² The abbreviations used are: ES cell, embryonic stem cell; RERE, Arg-Glu repeat-encoding; CMV, cytomegalovirus; GFP, green fluorescent protein; CBP, CREB-binding protein; CREB, cAMP-response element-binding protein; PML, promyelocytic leukemia; POD, PML oncopgenic domain; Atr2L, Atrophin-2 long form; Atr2S, Atrophin-2 short form; NLS, nuclear localization signal; HDAC, histone deacetylase; EGFR, epidermal growth factor receptor; E, embryonic day (e.g. E17).
not distinguished between the expression of the two Atn2 gene products (2, 3, 5). To see whether the expression of the Atrophins overlapped during embryogenesis, we probed Northern blots with probes recognizing the Atr2L and Atr2S messages. All three of the gene products are expressed throughout post-implantation embryogenesis at varying levels (Fig. 2B and data not shown). The Atr2L message has fairly uniform expression through most of gestation but is up-regulated as embryos approach term, whereas the Atr2S message has two discrete peaks, early and late in embryogenesis. From this expression pattern, it seems plausible that one or both of the Atrophin-2 gene products could compensate for the loss of Atrophin-1.

To determine whether up-regulation of Atn2 expression might contribute to the ability of Atn2 to compensate for loss of Atn1, we measured the protein level of Atn2 in both Atn1 knock-out and wild-type littermates. As shown in Fig. 1C, the expression of Atr2S protein is unaffected by loss of Atn1.

Mutations that disrupt Atr2S expression were not available, but previously we had described two different alleles that block the expression of Atr2L (5). To see whether the loss of Atr2L could uncover a requirement for Atrophin-1, we intercrossed the two mutations. Compound heterozygotes were obtained at Mendelian ratios and were viable and fertile. We were also able to obtain Atn1-/-Atr2L+/+ mice that were viable and fertile without difficulty. Animals that are homozygous for Atr2L mutations are not viable past mid-gestation, which complicated our ability to assess a role for Atr2L in the background of an Atn1-null embryo. Thus, although this analysis had some limitations, it is consistent with the idea that either Atn1 is completely unnecessary or, alternatively, that Atr2S is capable of supplying a similar function that compensates for the loss of Atn1. A number of protein-protein interactions and functional domains have been described for Atrophin-1 and, to a lesser degree, Atrophin-2 (9–11). To see whether we could identify functions that were shared between Atrophin-1 and Atr2S we carried out an in vitro analysis of their functional domains. Because Atr2L has unique functions that cannot be provided by Atrophin-1 or Atr2S, identification of its functional domains was carried out as well.
Functional Domains of Atrophin-2

Identification of a Conserved Domain for Transcriptional Activation—Prior studies have shown that the disease-causing, poly(Q)-extended version of human Atrophin-1 can function as a transcriptional co-repressor when expressed in mammalian cells. The Drosophila Atrophin gene can also function as a co-repressor in this assay system, supporting the idea that this is an inherent property of Atrophins rather than a neomorphic function produced by the dominant poly(Q) mutation. The domain structure of the Drosophila gene product is most similar to that of Atr2L, however, not that of Atrophin-1; and Atrophins can heterodimerize, making it difficult to conclude that co-repression is the only or even the predominant function of Atrophin-1. To address this question more directly, we carried out a comparison of Atrophin-1 and Atrophin-2 functions in a transcriptional reporter assay. A dual luciferase reporter system in mammalian cells was used in which Atrophin-1, Atr2L, and Atr2S were independently fused with the Gal4 DNA-binding domain and co-transfected with a luciferase reporter gene carrying five copies of Gal4 binding sites upstream of a basal promoter. Surprisingly, we found that unlike the human poly(Q)-extended Atrophin-1, the wild-type mouse Atrophin-1 has potent transcriptional activation ability, producing \( \sim 14 \)-fold activation (Fig. 3). Atr2S can also function as an effective transcriptional activator in this assay system, driving transcription \( \sim 8 \) -fold. When Atr2L was tested in this assay system, it exhibited only weak transcriptional activation, suggesting that the Atrophin-2 amino-terminal domain may have co-repressor ability as implied by its MTA-2 homolog domain and physical association with HDAC1 (5).

To explore this further we made truncated versions of Atr2L and Atr2S. As shown in Fig. 3, this analysis showed that the MTA-2 homologous amino-terminal region has the ability to repress transcription. Although we did not examine this in detail, repression is most potent with constructs containing the full, Atr2L-specific domain (compare for example constructs E versus F and B versus H).

Deletion analysis of Atr2S localized its ability to act as a transcriptional activator to the 94-amino acid stretch between amino acids 1055 and 1148 (Fig. 3). Truncated proteins in which this domain is removed are unable to activate transcription, whereas those constructs containing this domain, even in the absence of other Atrophin-2 sequences, efficiently activate transcription, demonstrating that it is both necessary and sufficient for the transcriptional activation ability of Attn2. This domain is highly conserved between Atrophin-1 and -2 with 71\% identity and 78\% similarity. The amino acid sequence is rich in serine and proline residues, as has been seen in other transcriptional activation domains.

The activation domain that we identified is adjacent to a stretch of repeating arginine and glutamic acid residues (RERE motif). Previous studies pointed toward the involvement of this motif in the interaction of Atrophins with each other. To examine the effect of this motif on transcriptional activation abilities, we deleted these RERE motifs using site-directed mutagenesis and found that they were not required for transcriptional activation (Fig. 3L).

Atrophin-2 Is Localized to Subnuclear Domain—Atr2L has three consensus nuclear localization signal (NLS) sequences, two in the MTA-2 homolog domain and one in the Atrophin homolog domain. Atr2S has one NLS sequence at its amino-terminal tip. A Previous report indicated that Atrophin-2 is localized to a subnuclear domain, the PML oncogenic domain (POD), although at the time of that study only the Atr2L isoform was known (2). To examine the relationship between the functional domains of Atrophin-2 and its localization, we fused GFP at the carboxyl termini of Atr2L and Atr2S and examined...
its localization in mammalian cells. As shown in Fig. 4, both Atr2L and Atr2S are nuclear proteins with a punctate distribution. The cellular localization patterns of the two isoforms are indistinguishable, suggesting that the sequences determining this subnuclear localization only require sequences within Atr2S. This would also imply that the NLS in the Atr2L amino-terminal domain is redundant or inactive. To further pursue the identity of the signal domain, we made a series of constructs with amino- or carboxyl-terminal truncations of Atr2S and Atr2L. Amino-terminal deletions that removed all three NLSs failed to localize to the nucleus (data not shown). Carboxyl-terminal deletions, on the other hand, revealed a domain required for subnuclear localization. Removal of the 150 carboxyl-terminal amino acids of either Atr2S or Atr2L produced a protein with nuclear localization but without the punctate, subnuclear localization characteristic of Atr2S and Atr2L. This indicates that the 150-amino acid carboxyl-terminal motif contains a novel localization signal. A previous report indicated that Atr2L co-localizes with the PML protein in a subnuclear domain. As shown in Fig. 5B, the punctuate nuclear distribution of Atr2L co-localized with PML. This suggests that the 150-amino acid motif at the carboxyl-terminal tip of Atr2S and Atr2L is a required element for the POD-directing signal.

Transcriptional Activity Correlates with HDAC1 and P300 Association—The analysis of Atr2L functional domains suggests that Atr2L can either activate or repress transcription, presumably depending upon regulatory factors. Transcriptional activators can act through recruitment of histone acetyltransferases such as P300 or CBP. Repression, on the other hand, is often the result of histone deacetylase recruitment. The 10–30 PODs found in each nucleus are enriched in histone acetylase P300 and in histone deacetylase HDAC1 (12). This suggests that Atrophin-2 may affect transcription by assembling transcriptional regulatory complexes in PODs. To test this idea, we chose HDAC1, P300, and CBP as candidate interactors and carried out co-immunoprecipitation experiments to
identify interacting proteins. As shown in Fig. 5A, when epitope-tagged Atn2 amino- or carboxyl-terminal fragments were immunoprecipitated with M2 antibody, HDAC1 was found to be associated with the amino-terminal fragment, consistent with the involvement of this domain in transcriptional repression. The carboxyl-terminal domain, on the other hand, is associated with P300. CBP was not detected in either of the immunoprecipitation experiments.

To test whether this association affects the localization of the endogenous proteins, cells that transfected with the Atr2L-GFP construct were stained with HDAC1 or P300 antibodies. As shown in Fig. 5B, HDAC1 was recruited into the Atrophin-2 subnuclear domain. In untransfected cells, HDAC1 was distributed equally throughout the nucleus. P300 had a more irregular nuclear distribution in untransfected cells; however, it also appears to have been recruited into the Atrophin-2 punctuate subnuclear domain.

DISCUSSION

In Drosophila, where there is only one Atrophin gene, loss-of-function mutations produce segmentation effects, cellular polarity defects, and embryonic lethality. The Drosophila Atrophin gene (DAtro) can function as a transcriptional co-repressor by co-operating with the even-skipped and tailless tran-
scription factors to regulate segmentation (7, 8). During development of the fly eye, DAstro physically interacts with the cytoplasmic domain of a cell surface molecule, Fat, to regulate planar polarity (13). In this context it is not clear whether it is also acting as a transcriptional co-repressor in the nucleus. During wing development, genetic studies support the idea that DAstro acts as repressor of genes downstream of the EGFR (14). In the absence of DAstro function, EGFR target genes are derepressed, effectively producing ectopic EGFR signaling.

Mammals have two Atn genes encoding three different iso-
forms. In a previous report, we described an Atn2 mutation that selectively disrupted expression of one of the two isoforms, Atr2L, expressed from this locus (5). Interestingly, the structure of the Atr2L isoform is the one that is most similar to that of DAstro. Atr2L mutant mice are embryonic lethal, dying around E9.5 with defects in heart looping, telencephalon and somite development, and, loss of Shh and Fgf8 expression from anterior signaling centers.

Zebrafish, like mammals, have two Atn genes, one Atn1 homologue and two Atn2 homologues. Although the protein isoforms expressed by the zebrafish Atn2 homologues have not been described, the genomic structure is consistent with one or both of the loci being capable of expressing both
tagged fragments of Atrophin-2 were expressed in HEK293 cells. The PML, HDAC1, and P300 antibodies detect co-localization with endogenous protein, the cells were stained with proteins co-localize with Atrophin-2 and the PML protein. Cells were trans-

Atrophin-2 with HDAC1 and P300 can be seen associated with either region. HEK293 cells were transfected with either empty vector (ci-

controls antibody (IgG), or anti-FLAG immunoprecipitates (IP). Association of endogenous HDAC1 with the amino-terminal transcriptional repressor region was detected by Western blotting. The association of the histone acetylase P300 with the carboxyl-terminal transcriptional activator region of Atrophin-2 was also detectable. CBP, a related histone acetylase, is not asso-

sociated with either region. HEK293 cells were transfected with either empty vector (first three lanes) or FLAG-tagged fusion constructs. B, the association of Atrophin-2 with HDAC1 and P300 can be seen in vivo where both of these proteins co-localize with Atrophin-2 and the PML protein. Cells were trans-

fected with an Atrophin-2-GFP construct, and GFP was visualized directly. To detect co-localization with endogenous protein, the cells were stained with PML, HDAC1, and P300 antibodies.

Atrophin-2 localization to PODs is regulated by a targeting signal, a requisite part of which at least is at its carboxyl-terminal tip. PODs are a subnuclear domain involved in regulating apoptosis, proliferation, and other important events in cells. More than 30 proteins, such as p53, CBP, and Rb, have been

FIGURE 5. Atrophin-2 co-localizes with HDAC1 and P300 in PODs. A, FLAG-tagged fragments of Atrophin-2 were expressed in HEK293 cells. The top panel shows Western blot detection of FLAG-tagged proteins in extracts (E), control antibody (IgG), or anti-FLAG immunoprecipitates (IP). Association of endogenous HDAC1 with the amino-terminal transcriptional repressor region was detected by Western blotting. The association of the histone acetylase P300 with the carboxyl-terminal transcriptional activator region of Atrophin-2 was also detectable. CBP, a related histone acetylase, is not asso-

associated with either region. HEK293 cells were transfected with either empty vector (first three lanes) or FLAG-tagged fusion constructs. B, the association of Atrophin-2 with HDAC1 and P300 can be seen in vivo where both of these proteins co-localize with Atrophin-2 and the PML protein. Cells were trans-

fected with an Atrophin-2-GFP construct, and GFP was visualized directly. To detect co-localization with endogenous protein, the cells were stained with PML, HDAC1, and P300 antibodies.

A

B

VECTOR

N-616

C-990

E

IgG

IP

IP

E

IP

E

PODS

Atrophin-2 localization to PODs is regulated by a targeting signal, a requisite part of which at least is at its carboxyl-terminal tip. PODs are a subnuclear domain involved in regulating apoptosis, proliferation, and other important events in cells. More than 30 proteins, such as p53, CBP, and Rb, have been...
found co-localized in PODs (12). When overexpressed, Atrophin-2 localizes to PODs and recruits HDAC1 and P300. This indicates that Atn2 may interact not only with HDAC1 and P300 but perhaps also with other proteins existing in PODs. Interestingly, poly(Q) extension mutants have been shown to accumulate in subnuclear domains reminiscent of PODs under pathogenic conditions (17, 18), suggesting that this localization may play a role in neurodegeneration. We found that localization to PODs is not required for the transcriptional regulatory ability of Atrophin-2 in our experimental context, although it remains possible that it is required in vivo.

Acknowledgments—We are thankful to members of the Peterson laboratory for helpful discussions and criticism, to Dr. Scott May for providing reagents, and to Dr. Woody Hopf for help with microscopy.

REFERENCES
1. Nagafuchi, S., Yanagisawa, H., Sato, K., Shirayama, T., Ohsaki, E., Bundo, M., Takeda, T., Tadokoro, K., Kondo, I., Murayama, N., et al. (1994) Nat. Genet. 6, 14–18
2. Waerner, T., Gardellin, P., Pfizenmaier, K., Weith, A., and Kraut, N. (2001) Cell Growth & Differ. 12, 201–210
3. Yanagisawa, H., Bundo, M., Miyashita, T., Okamura-Ohno, Y., Tadokoro, K., Tokunaga, K., and Yamada, M. (2000) Hum. Mol. Genet. 9, 1433–1442
4. Bowen, N. J., Fujita, N., Kajita, M., and Wade, P. A. (2004) Biochim. Biophys. Acta 1677, 52–57
5. Zoltewicz, J. S., Stewart, N. J., Leung, R., and Peterson, A. S. (2004) Development (Camb.) 131, 3–14
6. Herman, M. A., Ch'ng, Q., Hettenbach, S. M., Ratliff, T. M., Kenyon, C., and Herman, R. K. (1999) Development (Camb.) 126, 1055–1064
7. Zhang, S., Xu, L., Lee, I., and Xu, T. (2002) Cell 108, 45–56
8. Wang, L., Rajan, H., Pitman, J. L., McKeown, M., and Tsai, C. C. (2006) Genes Dev. 20, 525–530
9. Okamura-Ohno, Y., Miyashita, T., Ohmi, K., and Yamada, M. (1999) Hum. Mol. Genet. 8, 947–957
10. Wood, J. D., Nucifora, F. C., Jr., Duan, K., Zhang, C., Wang, J., Kim, Y., Schilling, G., Sacchi, N., Liu, J. M., and Ross, C. A. (2000) J. Cell Biol. 150, 939–948
11. Wood, J. D., Yuan, J., Margolis, R. L., Colomer, V., Duan, K., Kushi, J., Kaminsky, Z., Kleiderlein, J. J., Sharp, A. H., and Ross, C. A. (1998) Mol. Cell. Neurosci. 11, 149–160
12. Salomoni, P., and Pandolfi, P. P. (2002) Cell 108, 165–170
13. Fanto, M., Clayton, L., Meredith, J., Hardiman, K., Charroux, B., Kerridge, S., and McNeill, H. (2003) Development (Camb.) 130, 763–774
14. Charroux, B., Freeman, M., Kerridge, S., and Baonza, A. (2006) Dev. Biol. 291, 278–290
15. Asai, Y., Chan, D. K., Starr, C. J., Kappler, J. A., Kollmar, R., and Hudspeth, A. J. (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 9069–9074
16. Nucifora, F. C., Jr., Sasaki, M., Peters, M. F., Huang, H., Cooper, J. K., Yamada, M., Takahashi, H., Tsuji, S., Troncoso, J., Dawson, V. L., Dawson, T. M., and Ross, C. A. (2001) Science 291, 2423–2428
17. Takahashi, J., Fujigasaki, H., Iwabuchi, K., Bruni, A., Uchihara, T., El Hachimi, K. H., Stevanin, G., Durr, A., Lebre, A. S., Trottier, Y., de The, H., Tanaka, J., Hauw, J. J., Duyckaerts, C., and Brice, A. (2003) Neurobiol. Dis. 13, 230–237
18. Yamada, M., Wood, J. D., Shihomata, T., Hayashi, S., Tsuji, S., Ross, C. A., and Takahashi, H. (2001) Ann. Neurol. 49, 14–23