mutations in the human tau gene cause frontotemporal dementia and Parkinsonism associated with chromosome 17 (FTDP-17). One of the major disease mechanisms in FTDP-17 is the increased inclusion of tau exon 10 during pre-mRNA splicing. Here we show that modified oligonucleotides directed against the tau exon 10 splice junctions suppress inclusion of tau exon 10. The effect is mediated by the formation of a stable pre-mRNA-oligomer hybrid, which blocks access of the splicing machinery to the pre-mRNA. Correction of tau splicing occurs in a tau minigene system and in endogenous tau RNA in neuronal pheochromocytoma cells and is specific to exon 10 of the tau gene. Antisense oligonucleotide-mediated exclusion of exon 10 has a physiological effect by increasing the ratio of protein lacking the microtubule-binding domain encoded by exon 10. As a consequence, the microtubule cytoskeleton becomes destabilized and cell morphology is altered. Our results demonstrate that alternative splicing defects of tau as found in FTDP-17 patients can be corrected by application of antisense oligonucleotides. These findings provide a tool to study specific tau isoforms in vivo and might lead to a novel therapeutic strategy for FTDP-17.

Alternative splicing is a major regulatory mechanism in gene expression. An estimated 40% of human genes are alternatively spliced, and about 15% of the reported mutations in human genetic diseases affect pre-mRNA splicing (1–3). One of the diseases directly caused by mutations that result in pre-mRNA splicing defects is frontotemporal dementia and Parkinsonism associated with chromosome 17 (FTDP-17) (4–6). FTDP-17 is an autosomal-dominant disorder related to Alzheimer’s disease. Symptoms of FTDP-17 include personality changes, reduced speech, and dementia. In late stages, memory loss occurs, and the disease becomes virtually indistinguishable from Alzheimer’s disease (7). The disease gene for FTDP-17 has been identified by positional cloning as the tau gene, which encodes a microtubule-binding protein (8, 9). In FTDP-17, tau is incorporated into aggregates of fibrillar tangles and paired helical filaments, leading to neuronal cell death causing the neurological symptoms of the disease (4, 6, 10, 11).

Apart from FTDP-17, tau deposits are also the defining pathological feature of several neurological disorders including Alzheimer’s disease, progressive supranuclear palsy, and Niemann-Pick disease (4, 6).

Tau is a microtubule-binding protein that normally promotes microtubule assembly and stability (12–15). The microtubule-binding domains are encoded by exons 9–12 (16). In human brain, six tau isoforms are expressed as the result of alternative use of exon 2, exon 3, and exon 10, which encodes one of the microtubule-binding domains (17). Increased inclusion of exon 10 results in an elevated level of tau protein containing an additional fourth microtubule-binding domain (the 4R isoform) relative to tau protein containing only three microtubule-binding domains (the 3R isoform). Increased inclusion of tau exon 10 is tightly correlated with the disease phenotype, and overproduction of 4R tau is sufficient for disease (10, 18). The increase in exon 10 inclusion is due to mutations at the 3’-end of exon 10, which result in the destabilization of an RNA stem-loop (19). In healthy individuals, this loop prevents access of the splicing machinery to exon 10, thus blocking its inclusion into the mRNA. When mutated, the stem-loop is destabilized, and the splice junction in exon 10 can be used (4, 19–23). In addition, cis-acting elements within exon 10 also contribute to splice site choice (23, 24).

Here we report the application of antisense oligonucleotides to reverse the inclusion of tau exon 10 into mRNA as observed in FTDP-17 patients. Modified antisense oligonucleotides directed against either splice junction of exon 10 efficiently corrected its inclusion into tau mRNA. The oligonucleotides elicited a physiological effect by reducing the level of tau protein containing the microtubule-binding domain encoded by exon 10, and as a consequence the cytoskeleton morphology was altered. The use of antisense oligonucleotides will be useful to study the physiological role of specific tau isoforms in disease and cellular differentiation, and it might provide a novel therapeutic strategy to treat FTDP-17 and other tauopathies.

**Experimental Procedures**

**Cell Lines—**COS-1 green monkey kidney cells (ATCC entry CRL-1650) were grown in Dulbecco's modified Eagle’s medium plus 10% fetal bovine serum at 37 °C. Rat PC12 cells (ATCC entry CRL-1721) were grown in RPMI 1640 medium, 10% fetal bovine serum, 5% horse serum, 2.5 g/liter glucose, 1 μM sodium pyruvate. Cells were split every other day at a ratio of about 2:3. A Pasteur pipette was used to degagregate cell clusters. Rat AR42J pancreatic acinar cells (ATCC entry CRL-1492) were grown in F-12/Ham medium, 10% fetal calf serum.

**Modified Antisense Oligonucleotides—**All oligonucleotides were 2’-O-methyl-oligoribonucleotides, contained a phosphorothioate backbone, and were purified by high pressure liquid chromatography. Identical results were obtained with oligonucleotides from either Operon Technologies or from Hybridon: E10a, 5’-UACUCGACCCUUUGGUGA-3’; E10b, 5’-UGAAGGUACUCACUGCGGC-3’; rE10β, 5’-CGACAG-UACUCACUGGCUCC-3’.

**Transfection—**Twenty hours prior to the experiment, 1.2 × 10⁶ cells were plated on a 60-mm Petri dish. For co-transfection of the tau...
minogene and oligonucleotides into COS-1 cells, GenePorter lipofection reagent was used according to the manufacturer’s protocol using a DNA/GenePorter ratio of 1:3. Typically, 21 μl of GenePorter reagent in 2 ml of serum-free Dulbecco’s modified Eagle’s medium were used per sample. 2 ml of 20% fetal bovine serum medium were added 5 h later. For electroporation, 2.5 x 10^6 PC12 cells, each well of a 24-well plate was used, were electroporated with 0.4 ml 1:500 of RNAWiz. Precipitated and washed RNA pellets were resuspended in diethyl pyrocarbonate-treated water, and concentrations were measured using a spectrophotometer.

PCR—cDNA was reverse transcribed via MultiScribe (Applied Biosystems) and cDNA was amplified with the Exon Trapping System (Life Technologies). The primers were SD2 (5’-TGAACGTACGATGGAACAGC-3’) and SA4 (5’-CACGCTACGGATGCCTGGC-3’), and the lengths of amplified products were 246 bp (10−) and 153 bp (10−). For amplification of endogenous tau from PC12 cells or AR42J cells, primers tauex9 (5’-TCAAGAAGAAGTGATGT-3’) and tauxe13 (5’-TGTTGCTGTGCTTTGCTTGGC-3’) located in exon 9 and 13, as described by Grover et al. (21), were used. The length of the amplified products was 367 bp (10−) and 297 bp (10−). PCR conditions were as follows: 10 min at 95°C; 42 cycles of 30 s at 95°C, 1 min at 72°C; 5 min at 72°C. For PCR of tau minigene, the conditions were: 10 min at 95°C; 40 s at 95°C; 40 s at 56°C; 1 min at 72°C; 5 min at 72°C. Conditions for PCR analysis of tau minigene were 10 min at 95°C; 40 s at 95°C; 40 s at 56°C; 1 min at 72°C; 5 min at 72°C. The length of the amplified products was 365 nucleotides for the isoform including exon 2 and 3, 268 nucleotides for the isoform including exons 2 and 1, and 180 nucleotides for the isoform without exons 2 or 3. The linear range for PCR conditions was tested in pilot experiments with wild type and −1 tau minigene in the range of 20–40 cycles and with endogenous tau from PC12 cells in the range of 25–45 cycles, and bands were visualized by ethidium bromide staining. The linear ranges were 28–38 cycles for tau minigenes and 32–42 cycles for rat tau. All subsequent reactions were carried out within the linear range. Samples were separated on 2% agarose gels containing 0.5% ethidium bromide. Data were recorded using a Geprint 2000i CCD imaging system and quantitated with NIH Image.

**Western Blotting**—Proteins were extracted essentially as described by Janke et al. (25). PC-12 cells (1.2 x 10⁶ per sample) were used, and the homogenization step was omitted. Dephosphorylation was performed for 4 h at 67°C using 18 units/ml alkaline phosphatase (Roche Molecular Biochemicals). Equal amounts of protein were separated on SDS-PAGE gels and transferred to membranes using a 10:1 buffer, then blocked with 5% milk in PBS. Membranes were probed overnight at 4°C with anti-tau-1 (Roche Molecular Biochemicals; 1:500 in PBS, 2% milk, 0.1% Tween) followed by goat anti-mouse horseradish peroxidase (Amersham Pharmacia Biotech; 1:1000 in PBS, 2% milk, 0.1% Tween) for 60 min at room temperature. The signal was visualized using the Amersham ECL or ECL plus Western blotting detection kits and Hyperfilm ECL (Amersham Pharmacia Biotech). Bands were quantitated on a FluorChem system (Alpha Innotech).

**Fluorescence Microscopy**—Cells were grown on coverslips and transfected as described for COS-1 cells. 48 h after transfection, cells were fixed for 12 min in fresh 3.7% paraformaldehyde in CSK buffer (10 mM Pipes, pH 6.8, 100 mM NaCl, 3 mM MgCl₂, 300 mM sucrose, 1 mM phenylmethylsulfonyl fluoride, 0.1% Triton X-100). Cells were rinsed in PBS and incubated for 60 min with anti-tubulin antibody (Sigma), 1:500 in PBS. Coverslips were then rinsed three times for 8 min each in PBS. Cells were incubated for 60 min with rabbit anti-mouse fluorescent isothiocyanate (Vector Laboratories), 1:250 in PBS. Cells were rinsed three times for 8 min each and mounted in Vectashield (Vector Laboratories). Fluorescence microscopy was performed on a Leica TCS-SF confocal microscope using a x 100, 1.4 NA objective. Excitation was at 488 nm, and emission was detected between 525 and 575 nm. Single optical sections of 500-nm nominal thickness were collected. Phase images were acquired on a Nikon Eclipse 500 microscope equipped with a MicroMax cooled CCD camera and Metamorph Imaging software. Raw images are shown.

**RESULTS**

We sought to correct inclusion of exon 10 into tau mRNA by use of antisense oligonucleotides. Our approach was to introduce into cells 2'-O-methyl-oligoribonucleotides containing sequence complementary to the 3’ or 5’ splice site of exon 10 (Fig. 1, a and c). In contrast to unmodified phosphorothioate oligodeoxynucleotides, 2'-O-methyl-modified antisense oligonucleotides do not promote RNase H-mediated RNA degradation but rather form stable RNA-DNA hybrids (26). Our rationale was that the oligonucleotides prevent access of the splicing machinery to the splice junction and in this way may block the inclusion of the protected tau exon 10 into the mRNA (Fig. 1, a and c).

**Exclusion of Tau Exon 10 in a Minigene System**—To test whether antisense oligonucleotides targeted to tau exon 10 sequences were capable of altering the splicing behavior of tau exon 10, we used a previously characterized minigene system that recapitulates to a large extent the behavior of exon 10 in the context of the full-length tau gene (21). The splicing pattern of exon 10 in the minigene was analyzed by RT-PCR on total RNA isolated 24 h after transfection of the minigene into COS-1 cells (Fig. 1b). As expected, expression of the wild-type exon 10 minigene in COS-1 cells resulted in predominant exclusion of exon 10 (Fig. 1b) (21). The fraction of the 10+ tau isoform was 0.27 ± 0.08 (Fig. 1b). In contrast, similar to the situation in FTDP-17 patients, expression of exon 10 containing a single point mutation at position −1 resulted in predominant inclusion of exon 10 (21). The fraction of the 10+ tau isoform was now increased to 0.87 ± 0.06 (Fig. 1b). Similar results were obtained with mutants at positions +3 and +14 (data not shown; Ref. 21). The identity of the PCR products was confirmed by sequencing (data not shown).

To test whether antisense oligonucleotides could reverse the inclusion of the mutant exon 10, COS-1 cells were co-transfected with exon 10 minigene containing the −1 mutation and increasing concentrations of antisense oligonucleotide E10α or E10β, targeted to the 5’ or 3’ splice junction of exon 10, respectively (Fig. 1c). Both oligonucleotides prevented inclusion of exon 10 efficiently (Fig. 1, d and f). A half-maximal effect was achieved at 5–10 nM for either oligonucleotide (Fig. 1, d and f). A previously observed PCR band representing 10− exon 10 and 10− exon 10 was observed in all samples not included in the quantitation (21). The exclusion of exon 10 was oligonucleotide sequence-specific, since oligonucleotides containing the same nucleotide composition but in random order had no effect on exon 10 inclusion at concentrations up to 200 nM (Fig. 1, e and g). The fact that the RNA levels in control cells not treated with oligonucleotide and cells treated with concentrations of up to...
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a

b

 WT - 1

0.27 0.87

E10\alpha

E10\beta

\[ \text{E10} = \text{3'-gaugg uuuccuACGUC UAU-5'} \]

c\[ \text{cauuccuuc uuuggcucu gcag UGCG AUAAUAAUA AGAAGCUGGA...} \]

\[ \text{E10}\beta = \text{C GCGUGAcac ucauggaagu-5'} \]

\[ \text{AUCAACACAG UCCGGGGAGG GCAGUUug uauacucucu cacgucccc} \]

stem loop

d

E10\alpha

E10\beta

E10\alpha

E10\beta

0 2 5 10 20 50 no RT nM

0.83 0.69 0.61 0.43 0.42 0.24
s0.06 s0.11 s0.10 s0.10 s0.04 s0.04

0.89 0.65 0.50 0.36 0.31 0.18
s0.08 s0.20 s0.15 s0.14 s0.13 s0.11

0 2 20 200 no RT nM

0.68 0.61 0.69 0.69 0.66
s0.05 s0.06 s0.06 s0.06 s0.07

0.89 0.91 0.85 0.85 0.91
s0.04 s0.00 s0.00 s0.08 s0.04

f

E10\alpha

E10\beta

Exon 10 inclusion

Concentration (nM)

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9

0.01 1 10 100

Exon 10 inclusion

Concentration (nM)

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9

0.01 1 10 100

Exon 10 inclusion

Concentration (nM)

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9

0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9

0.01 1 10 100
Correction of Tau Splicing

200 nM oligonucleotides were identical confirms that the oligonucleotides did not act through an RNA degradation mechanism (Fig. 1, d and e).

E10β contains a single mismatch to −1 tau at position 7. To assess whether this mismatch reduced the effectiveness of the oligonucleotide, we tested E10β on tau minigenes containing point mutation in position +3, which, similar to the −1 mutation, is also located toward the center of E10β or, alternatively, a tau minigene with a point mutation in position +14, which is just outside of the E10β target region (21). E10β was equally efficient in preventing the inclusion of exon 10 into either one of these mutated tau minigenes (Fig. 1h). We conclude that a single mismatch in E10β to −1 tau does not significantly affect its inhibitory efficiency.

To ensure that this effect was specific to the tau minigene and was not due to changes in the global splicing pattern in COS-1 cells caused by the presence of the oligonucleotides, the effect of E10α and E10β on the alternative splicing pattern of a minigene containing the adenovirus 2 E1A transcript was analyzed (Fig. 2). E1A contains a single 3′ acceptor splice site but multiple upstream donor sites, which give raise to 9, 10, 12, and 13 S mRNAs (Fig. 2a) (27, 28). When COS-1 cells were co-transfected with E1A and either one of the antisense oligonucleotides, neither oligonucleotide had any effect on E1A splicing products (Fig. 2, b and c). Furthermore, neither oligonucleotide had any effect on a minigene containing the alternatively spliced fibronectin-EDI exon (29) (data not shown). Taken together, the results demonstrate that antisense oligonucleotides complementary to tau exon 10 splice junctions effectively prevent inclusion of exon 10 during the splicing reaction, that this effects occurs in a oligonucleotide sequence-specific manner, and that the effect on tau exon 10 splicing is gene-specific.

Exclusion of Tau Exon 10 from the Endogenous mRNA—We next sought to test whether antisense oligonucleotides can also force exclusion of tau exon 10 in the context of the endogenous transcript. To this end, we used rat neuronal pheochromocytoma PC12 cells. PCs express predominantly the 10+ isoform and were thus useful to test whether modified oligonucleotides could shift the predominant form to the 10− isoform (30) (Fig. 3). The predominant inclusion of exon 10 in rat tau is most likely due to three nucleotide changes near the 5′ splice site, which resemble stem-loop-disrupting mutations in human tau (21). PCs were transfected with increasing amounts of E10α or rat E10β. Rat E10β is identical to E10β except that it contains three nucleotide changes to make it fully complementary to the rat sequence. The alternative splicing pattern of rat tau mRNA was analyzed 24 h after transfection by RT-PCR using primers in exons 9 and 11 (Fig. 3a). As in the minigene system, both antisense oligonucleotides effectively excluded exon 10 from the tau mRNA (Fig. 3, b and d). A half-maximal effect was achieved at −1.5 μM for both E10α and rat E10β.

FIG. 2. Antisense oligonucleotides against tau have no effect on alternative splicing of adenovirus E1A. a, schematic representation of the E1A splicing reporter minigene containing multiple donor sites and a common acceptor site. The observed splicing isoforms are indicated as 9, 10, 12, and 13 S. b and c, titration of antisense oligonucleotides E10α or E10β into COS-1 cells co-transfected with the E1A minigene construct 24 h after transfection. The oligonucleotides have no effect on E1A splicing. An additional band representing a PCR artifact commonly seen with E1A is indicated by an asterisk. c, quantitation of the relative amounts of E1A isoforms. Values are averages from three experiments.

Compared with the minigene system, the required concentrations were significantly higher due to lower transfection efficiency of PC12 cells with oligonucleotides (data not shown).
Control antisense oligonucleotides of the same base composition as E10α or rat E10β, but in random order, had no effect on tau splicing at concentrations up to 10 μM (Fig. 3, c and e). E10β targeted against the human tau gene had a 2–3-fold lower inhibitory activity than rE10β (data not shown).

To test whether the effect of the antisense oligonucleotides was specific to tau exon 10, we probed the splicing pattern of the alternatively spliced exons 2 and 3 in tau using primers located in exons 1 and 4 (Fig. 4a). Neither E10α nor rat E10β had any effect on the splicing pattern of exons 2 and 3 in endogenous tau (Fig. 4, b and c). Similarly, neither oligonucleotide had any effect on alternative splicing of the ARP3β gene, encoding an actin-related protein (31) (data not shown). These results show that inclusion of tau exon 10 in the context of its endogenous pre-mRNA is effectively prevented by the presence of antisense oligonucleotides, that this effects occurs in an
oligonucleotide sequence-specific manner, and that the effect on tau exon 10 splicing is specific and has no effect on other alternative splicing events in the same gene.

Reduction of 4R Tau Protein—To probe whether the effect of antisense oligonucleotides was restricted to the RNA or was translated into a change in the relative levels of 3R and 4R protein isoforms, we performed Western blotting. PC12 cells were transfected with either a control oligonucleotide of scrambled sequence or either E10/H9251 or rat E10/H9252. Cells were grown for 48 h and tau proteins isolated using several precipitation steps and dephosphorylated as previously described (25). All six tau protein isoforms were detected using the phosphorylation-independent anti-tau-1 antibody. As expected, three pairs of bands were detected in PC12 cells (Fig. 5a). In each pair, the upper band represents the 4R isoform, and the lower band represents the 3R isoform (4, 25). The three pairs correspond to the isoforms containing exons 2 and 3 together, exon 2 only, or neither exon 2 nor exon 3. Exon 3 alone is never included (4). In cells treated with control oligonucleotides, the 4R isoform was predominant in each pair (Fig. 5a). In contrast, treatment of PC12 cells with E10α or rat E10β resulted in the increased accumulation of the 3R isoform in all pairs as indicated by the appearance of the lower bands (Fig. 5a). Quantitative analysis of Western blots demonstrated that treatment with antisense oligonucleotides increased the relative amount of 3R tau isoforms. Values are averages from three experiments.

Effect of Reduced 4R Tau Levels on the Cytoskeleton—Tau is a microtubule-associated protein, and functional differences between the 3R and the 4R isoform have been reported, although the molecular basis for these differences is not entirely clear (11, 32, 33). Due to the functional differences between the 3R and the 4R isoform, altering the ratio between the two isoforms might affect cytoskeletal architecture. We were not able to reliably analyze microtubule morphology in PC12 cells due to their small size and spherical shape. As an alternative, we analyzed the cytoskeletal morphology in rat AR42J pancreatic acinar cells, which have previously been demonstrated to strongly express 4R tau isoforms (34). AR42J cells were either mock-transfected or transfected with a control oligonucleotide of scrambled sequence or either E10α or rat E10β, and the cells were processed for microscopy (Fig. 6). As expected, in mock-transfected cells or cells treated with the scrambled control oligonucleotide, predominantly the 10+/H11001 tau isoform was expressed (Fig. 6a). In contrast, either one of the oligonucleotides targeted to the splice junction strongly promoted exon 10 exclusion and led to the predominant formation of the 10− tau isoform (Fig. 6a). By phase-contrast microscopy, cells treated with control oligonucleotide were normal in appearance and were indistinguishable from mock-transfected control cells, demonstrating that the administration of oligonucleotide alone
has no effect on cell morphology (Fig. 6b). The cells showed typical fibroblast-like cytoplasmic extensions, and in some cells characteristic leading edges could be observed (Fig. 6b). In contrast, cells treated with either E10α or rat E10β were round in shape, although they were still attached to the substratum (Fig. 6b). To directly visualize the microtubule cytoskeleton, cells were stained with an anti-tubulin antibody. In mock-transfected cells and in cells treated with scrambled control oligonucleotide, a typical microtubule stain was observed. Microtubule cables of several micrometers in length were seen throughout the whole cell, and parallel bundles were detected at the periphery of cells (Fig. 6, c and d). Note that the images shown are confocal sections through equatorial planes of the cell and thus do not show the cables above and below the nucleus. In contrast, in cells treated with oligonucleotides E10α or rat E10β, no distinct microtubule cables were observed (Fig. 6, c and d). Tubulin staining was confined to the very periphery of the cell, and no peripheral bundles could be detected. More than 90% of the cell population treated with oligonucleotide exhibited this phenotype. These results show that a reduction in 4R tau protein has a significant physiological effect by destabilizing the microtubule cytoskeleton and altering cell morphology.

**DISCUSSION**

We show here that modified antisense oligonucleotides complementary to the 5′- and 3′-splice sites of exon 10 of tau pre-mRNA effectively prevent inclusion of this exon into the mature mRNA during the splicing reaction. The effect is genespecific, extends to the protein level, and induces a physiological effect manifested by a change in the microtubule cytoskeleton.

The rationale of our approach was to block access of the splicing machinery to either splice junction, thus preventing inclusion of the exon. This strategy mimics the regulation of exon 10 splicing in vivo. In human brain, excessive inclusion of exon 10 is prevented by a stem-loop at the 3′-end of exon 10 formed by the terminal two bases of exon 10 and 16 bases in the adjacent intron (4, 19–23). The stem-loop generates an unfavorable binding site for the U1 small nuclear RNA and thus reduces the splicing efficiency of exon 10 (4, 19, 24). Loss of negative splicing regulation by the stem-loop is critical for the disease process. Patient mutations in the 3′-end of human tau exon 10 and the adjacent intron region contribute in two ways to exon 10 inclusion. First, mutations destabilize the stem-loop, resulting in increased access of the splicing machinery to the splice junction. Second, all reported mutations also increase the complementarity of the 5′ splice site sequence to the 5′-end of the U1 small nuclear RNA. The introduced antisense oligonucleotides therefore mimic the function of the stem-loop by impeding the access of U1 small nuclear ribonucleoprotein to the 3′-end of exon 10.

Blocking of splice sites appears to be an efficient way to modulate splice site choice in vivo. Antisense oligonucleotides have been used in several other systems including β-globin, cystic fibrosis transmembrane conductance receptor, bcl-X, and dystrophin to block access of the splicing machinery to an undesirable splice site (35–41). Whereas all reported cases of antisense-mediated correction of splicing defects used oligonu-
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3. Cooper, T. A., and Mattox, W. (1997) *Am. J. Hum. Genet.* 61, 259–266
4. Spillantini, M. G., and Goedert, M. (1998) *Trends Neurosci.* 21, 428–433
5. Fischer, N. L., Wilhelmsen, K., LaFerla, F. M., Jones, M. Z., D'Amato, C. J., and Gilman, S. (1997) *Ann. Neurol.* 41, 706–715
6. Goedert, M., Spillantini, M. G., and Davies, S. W. (1998) *Curr. Opin. Neurobiol.* 8, 619–632
7. Wilhelmsen, K. C. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 7120–7121
8. Hong, M., Zhukareva, V., W sostek, Z., Reed, L., Miller, B. I., Geschwind, D. H., Bird, T. D., McKeel, D., Goate, A., Morris, J. C., Wilhelmsen, K. C., Schellenberg, G. D., To anxious, J. Q., and Lee, V. M.-Y. (1998) *Science* 282, 1914–1917
9. Hutton, M., Lendon, C. L., Rizzu, P., Baker, M., Frolich, S., Houlden, H., PICKERING-BROWN, S., and CHAKRAVARTY, S. (1998) *Nature* 393, 702–705
10. Clark, L. N., Porquier, J., W sostek, Z., Geschwind, D. H., Nasreddine, Z. S., Miller, B., Li, D., Payami, H., Awert, F., Markopoulou, K., Andreas, A., D’Souza, I., Lee, V. M., Reed, L., Trojanowski, J. Q., Zhukareva, V., Bird, T., Schellenberg, G., and Wilhelmsen, K. C. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 13103–13107
11. Barghorn, S., Zheng-Fischhofer, Q., Ackmann, M., Biernat, J., von Bergen, M., Mandelkow, E. M., and Mandelkow, E. (2000) *Biochemistry* 39, 11714–11721
12. Kase, Y., Chen, J., and Hirokawa, N. (1992) *EMBO J.* 11, 3853–3861
13. Goode, B. L., Chau, M., Denis, P. E., and Feinstein, S. C. (2000) *J. Biol. Chem.* 275, 38182–38189
14. Bue, T., Bue-Thierry, T., Bue-Scherrer, V., Delacourte, A., and Hof, P. P. (2001) *Brain Res.* 93, 95–130
15. Friedhoff, P., von Bergen, M., Mandelkow, E. M., and Mandelkow, E. (2000) *Biochem. Biophys. Acts* 1501, 123–132
16. Lee, G., Neve, R. L., and Kosik, K. S. (1989) *Neuron* 2, 1615–1624
17. Goedert, M., Spillantini, M. G., Jakes, R., Rutherford, D., and Crowther, R. A. (1989) *Neuron* 3, 519–526
18. Goedert, M., Crowther, R. A., and Spillantini, M. G. (1998) *Neuron* 21, 955–958
19. Varani, L., Hasegawa, M., Spillantini, M. G., Smith, M. J., Murrel, J. R., Ghatti, B., Klug, A., Goedert, M., and Varani, G. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 8229–8234
20. Hasegawa, M., Smith, M. J., Iijima, M., Tabira, T., and Goedert, M. (1999) *FEBS Lett.* 443, 93–96
21. Grover, A., Houlden, H., Baker, M., Adamson, J., Lewis, J., Prihar, G., Pickering-Brown, S., Duff, K., and Hutton, M. (1999) *J. Biol. Chem.* 274, 15134–15143
22. D’Souza, I., Porquier, J., Hong, M., Nochlin, D., Lee, V. M., Bird, T. D., and Schellenberg, G. D. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 5588–5593
23. Gao, Q.-S., Memmot, J., Lafayatis, R., Stamm, S., Serreton, G., and Andreas, A. (2000) *J. Neurochem.* 74, 490–500
24. D’Souza, I., and Schellenberg, G. D. (2000) *J. Biol. Chem.* 275, 17700–17709
25. Ji, N., Beck, M., Holzer, M., Bigl, V., and Arendt, T. (2000) *Brain Res.* 859, 231–242
26. Lamond, A. I., Sprott, B., Ryder, U., and Hamm, J. (1989) *Cell* 58, 383–390
27. Caceres, J. F., Stamm, S., Heftman, D. M., and Krainer, A. R. (1994) *Science* 265, 1706–1709
28. Bourgeois, C. F., Pupierla, M., Hildwein, G., and Stevenin, J. (1999) *Mol. Cell.* 39, 7347–7358
29. Kornblihtt, R. A., Pese, C. G., Alonso, C. R., Cramer, P., Serbrou, A., Werbajh, S., and Muro, A. F. (1996) *FASEB J.* 10, 248–257
30. Smith, C. J., Andvert, B. H., Davis, B. R., and Gallo, J. M. (1995) *FEBS Lett.* 375, 243–248
31. Jay, P., BERGE-LAFRANC, J. L., Massacrier, A., Roessler, E., Wallis, D., Muenke, M., GASTALDI, M., TAVIANO, S., Cau, P., and Berta, P. (2000) *J. Eur. Biochem.* 270, 2921–2928
32. Goedert, M., and Jakes, R. (1999) *EMBO J.* 9, 4225–4230
33. Goode, B. L., and Feinstein, S. (1994) *J. Cell Biol.* 124, 769–782
34. Vanier, M.-T., Nevir, P., Mihalk, L., and Laurain, J.-F. (1998) *J. Cell Sci.* 111, 1419–1432
35. Sierackowska, H., Sambade, M. J., Agrawal, S., and Kole, R. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 12840–12844
36. Lucarelli, A., Sierackowska, H., Caretta, C., Fuchareon, S., Summerton, J., Weller, D., and Kole, R. (2000) *Proc. Natl. Acad. Sci. U. S. A.* 97, 9591–9596
37. Dunckley, M. G., Manoharan, M., Villiet, P., Eperon, I. C., and Dickson, G. (1998) *Hum. Mol. Genet.* 7, 1083–1096
38. Mann, C. J., honeyman, K., Ceng, A. J., Lloyd, L., Fletcher, S., Morgan, J. E., Partridge, A. A., and Wilks, S. (2001) *Proc. Natl. Acad. Sci. U. S. A.* 98, 42–47
39. Wilts, S. D., Lloyd, L., Carville, K., Fletcher, S., honeyman, K., Agrawal, S., and Kole, R. (1999) *Neuromuscular Disorders* 9, 330–338
40. Friedman, K. J., Kole, R., Cohn, J. A., Knowles, M. R., Silverman, L. M., and Friedman, M. (1998) *Acta Neuropathol.* 81, 591–596
41. Ishikawa, K., Ishikawa, K., Davies, P., Delacourte, A., Tiseo, P., Yen, S. H., and Dickson, D. W. (2000) *Acta Neuropathol.* 100, 235–244
42. Conrad, C., Arendt, A., Trojanowski, J. Q., Dickson, D. W., Kang, D., Chen, X., Wiedervant, W., Hansem, L., Masliah, E., Thal, L. J., Karmazin, R., Xia, Y., and Saitoh, T. (1997) *Ann. Neurol.* 41, 277–281
43. Higgins, J. J., Litvan, I., Pho, T. L., Hill, W., and Nei, L. (1998) *Neurology* 50, 270–273

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REFERENCES

1. Hanke, J., Brett, D., Zastrow, J., Aydin, A., Delbruck, S., Lehmann, G., Luft, P., Reich, J., and Bork, P. (1999) *Trends Genet.* 15, 389–390
2. Phillips, A. V., and Cooper, T. A. (2000) *Cell Mol. Life Sci.* 57, 235–249

* Correction of Tau Splicing 42993

2. B. Kalbfuss and T. Misteli, unpublished result.