, the expression levels of various forms of *TAF1* in the XDP caudate nucleus, using quantitative RT-PCR by gning specific probes for the TaqMan assay (fig. 5*a*). One of these probes, TA14-391—with an alternative exon of 6 additional bp, named "exon 34"—showed a highly significant decrease in its expression level in the caudate nucleus of the patient with XDP (fig. 5*a*), which was less than ~1/40 of that in the normal control, and its expression was virtually limited to brain and neurons (table 7). TA14-391 was the second-most abundant among all *TAF1* species (fig. 5*a*), and its expression level in the control caudate nucleus was 1/4–1/5 of that of the major form of *TAF1* (table 7). TA14-391 also showed a significantly decreased level of expression in the cortex and the nucleus accumbens of the patient with XDP (fig. 5*b*), although these regions had no neuronal loss. These findings suggest that the decreased level of expression was the cause rather than the result of neuronal loss in the caudate nucleus of the patient with XDP. In addition to the TaqMan assay, in situ hybridization was performed in the caudate by use of a riboprobe common to TAF1 isoforms that are located in exon 38 (probe 3 in fig. 2*a*). The riboprobe showed decreased expression in the caudate neurons of the patient, although the weak expression was still present in glial cells (fig. 5*e*). Immunohistochemical examination by use of a polyclonal antibody against the common epitopes of *TAF1* also showed decreased immunoreactivity in the XDP neurons in the caudate nucleus from other patients with XDP (fig. 5*f*). These findings suggest that the deficiency of the neuron-specific isoform of TAF1, TA14-391, reflects these histologically verified neuron-specific decreases of *TAF1* expression and that TA14-391 is one of the neuron-specific isoforms, whereas apparently similar levels of mRNA expression for TAF1 or its isoforms as a whole (figs. 2*b*, 2*c*, and 5*a*) can be accounted for by increased glial expression of *TAF1* due to intensive astrogliosis[4,5] (fig. 5*d*–5*f*), obscuring the decreased expression in neurons. The TA14-391 isoform may represent the decreased expression of many TAF1 isoforms in the XDP neurons, because of its original neuron specificity of expression. Finally, to determine the complete structure of the isoform containing the 6 bp of exon 34′, full-length cloning from libraries consisting of cDNAs enriched in their 5′ ends[10] was performed. A single cDNA containing exon 34′ was successfully cloned (fig. 6), and this had the complete translation frame of the major form of *TAF1* with an insertion of two amino acid residues, alanine and lysine, in the carboxyl terminal kinase domain.

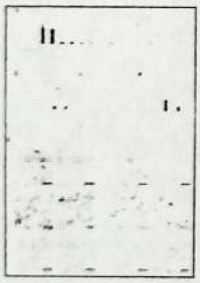

Figure 5.

Expression of the TAF1 isoforms in the caudate. *a,* Expressions are shown relative to the expression of 18S rRNA (as an internal control). The label "relative mRNA expression" means relative mRNA expression level to 1/20 × 18S rRNA. ...

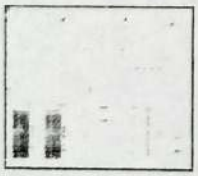

Figure 6.

a, Full-length cloning of a TAF1 isoform sequence containing alternative exon 34′. The 5′ end was obtained from CapSite cDNA from brain. The 3′ end was obtained from Marathon-Ready cDNA from brain. The complete DNA sequences of...

Table 7.

Abundance of the Probe TA14-391 in Various Tissues and Cell Lines[Note]

Discussion

Go to:

SVA retrotransposon insertions are thought to be active in the human genome and to alter the expression level of adjacent genes that cause diseases, such as autosomal recessive hypercholesterolemia (ARH [MIM 605747])[14] and Fukuyama-type congenital muscular dystrophy (FCMD [MIM 607440]).[15] SVA insertion was found in the 3′ UTR region of the FCMD gene and in an intronic region of the ARH gene, which showed association with the reduced