Transcriptional Regulation of the Mouse Presenilin-1 Gene*

Noriaki Mitsuda‡, Allen D. Roses‡, and Michael P. Vitek‡§¶

From the ‡Division of Neurology, Duke University Medical Center, Durham, North Carolina 27710 and §Department of Molecular Pharmacology, Glaxo Wellcome, Research Triangle Park, North Carolina 27709

The presenilin-1 (PS-1) gene encodes at least three separate mRNA transcripts from its 12 exons, which are spread over 50 kilobase pairs of mouse DNA. The first transcript begins with exon 1A, whereas the other transcripts begin with exon 1B. Different portions of exon 1B are spliced to give long and short mRNAs. The expression of all of these transcripts depends on a single promoter located just upstream of exon 1A. Although this region lacks a TATA box and a number of common initiator sequences, it does contain a CAAT box, a heat-shock responsive element, a polyomavirus enhancer activator-3 site, an Ets 1–3 site, and multiple-Sp1 and multiple-Ap2 binding sites, which are typically found in eukaryotic promoters. We have combined a reporter gene with various portions of this putative PS-1 promoter and measured firefly luciferase activity relative to an internal renilla luciferase standard. We identified a 25-base pair fragment spanning the 5′-transcription start site of exon 1A as containing the core of the promoter activity. The sequences downstream of this region had undetectable promoter activity, suggesting that this core element is the gene’s only promoter, and it controls expression of all three transcripts. Although human PS-1 mRNA expression is clearly different from the mouse PS-1 mRNA pattern, the human and mouse core promoters do share limited homology.

Alzheimer’s disease is a devastating neurological disorder and the most common cause of dementia. The genetics of this disorder suggests that multiple genes are involved. To date, mutations in four genes have been found to be associated with Alzheimer’s disease phenotypes including the amyloid precursor protein gene on chromosome 21 (1, 2), the apolipoprotein E gene on chromosome 19 (3–5), the presenilin-1 (PS-1) gene on chromosome 14 (6), and the presenilin-2 (PS-2) gene on chromosome 1 (7). Although an unknown gene on chromosome 12 appears to associate with a large percentage of late-onset Alzheimer’s patients (8), the majority of familial Alzheimer’s disease cases are associated with mutations in the PS-1 gene. To date, over 30 independent mutations in PS-1 have been described in unrelated Alzheimer’s families displaying an early age-of-onset phenotype. Most of these mutations are missense mutations that result in single amino acid changes (6, 9–15). Deletions found in exon 4 and exon 9 cause additional mutations as do several truncations of the RNA transcripts arising through differential splicing (16). Although clustering of these mutations within the protein suggests the location of functionally important domains, the exact function of presenilin proteins is a matter of active investigation.

One approach to find gene function is to study the regulation of PS-1 gene expression. Using in situ hybridization, we and others demonstrate that PS-1 mRNA is most highly expressed in neurons of the brain (17). Immunohistochemistry revealed that the PS-1 protein was abundant in neurons, but was also associated with amyloid plaques and some glial cell types (18, 19). In contrast, Sherrington et al. (6) reported that PS-1 mRNA is widely expressed in a variety of organs throughout the body. This raises the question why mutations in the PS-1 gene product appear to confer a disease state in familial Alzheimer’s patients without apparent effect on their peripheral organs. The situation is further compounded because PS-1 mRNA and protein levels have not been reported thus leaving open the possibility that pathological regulation of PS-1 gene expression in familial Alzheimer’s disease patients, compared with age-matched healthy controls, contributes to the disease state.

Mutations in the PS-1 gene’s promoter and non-protein encoding regions are not known and reports on the gene’s wild-type sequence are lacking. Similarly, no functional analysis of the gene’s ability to promote transcription has been reported. Combined with recent reports that PS-1 knockout mice are embryonic lethal (20), knowledge of the PS-1 gene sequence and its transcriptional regulation may be important clues that help to identify PS-1 function in both normal and diseased states. To begin answering some of these questions, we now report a detailed sequence of the mouse PS-1 gene including its promoter, the entire protein encoding region, and ending in exon 12. In addition to the usefulness of this sequence in creating additional PS-1 knockout mice, we have defined the PS-1 promoter’s transcriptional regulatory elements. Our data on promoter activity suggests that the presenilin-1 gene is preferentially expressed and transcribed in neurons.

Experimental Procedures

Isolation and Characterization of Genomic Clones—Labeled oligonucleotides and polymerase chain reaction (PCR) products of the mouse PS-1 cDNA were used as probes to screen mouse libraries for genomic PS-1 clones. Based on the mouse PS-1 cDNA sequence (GenBank™ accession no. L42177), an upstream primer of sequence 5′-CGGAGAGAAGGAACACACAC-3′ and a downstream primer of sequence 5′-TCAGCTTTTCGTCCTTCCTCCTAC-3′ were used, with Quick Clone Mouse Brain cDNA (CLONTECH) as template, to amplify a portion of the mouse PS-1 cDNA by PCR. Amplification reactions were performed in a 100-μl volume containing 1 × PCR buffer II (Perkin Elmer), MgCl2 (1.5 mM), dATP, dGTP, dCTP, and dTTP (0.2 mM each, Perkin Elmer), DNA primers at 0.5 μM, 1 μl of cDNA template (0.1 ng),...
and Ampli-Taq DNA polymerase (5 units, Perkin-Elmer). The reaction
was 95 °C for 1 min, 50 °C for 1 min and 72 °C for 2 min for a total of
30 cycles. This PCR product was gel purified and labeled with [32P]ATP and T4-poly nuclide kinase and used to identify
plasmid subclones by hybridization. Based on the partial sequence of
plasmid clone Ph-2, the PCR primers 1C-US-for (GATCACAGTCTAGGTTGCT-
CAAGTC, 0.2 μM) and the CLONTECH AP1 adaptor primer (CCATCCTA-
CTTGGGACTATATAGGGC, 0.2 μM) were used further to screen a mouse ES-129/SVJ genomic library in a P1
vector (Genome Systems Inc.) by PCR. Of the three P1 clones identified,
P1–10809 was digested with EcoRI or HindIII, and these restriction
enzyme fragments were subcloned and sequenced as described above.

Rapid Amplification of cDNA Ends (RACE)—The 5′ end of PS-1
cDNA was identified using mouse cDNA. Marathon-Ready cDNA (mar-
BALB/c, 9–11 weeks of age, CLONTECH). Briefly, a 50-μL PCR reaction
containing a PS-1-specific reverse primer (TGTCGACTGGTTCAGGT-T-
CAAGTC, 0.2 μM), the CLONTECH API adaptor primer (CCATCCTA-
CTTGGGACTATATAGGGC, 0.2 μM), 2.5 ng of Marathon-Ready
cDNA, 1 × PCR buffer (Life Technologies, Inc.), MgCl2 (1.5 mM), di-

human PS-1 gene and exon 1C are 141, 371, and 139 bp, respectively.

results

Mouse Presenilin-1 Promoter Activity

Medium (Life Technologies, Inc.), 2.5% fetal calf serum, and 7.5% bo-
vine serum (HyClone). Differentiation of P19 cells to neuron-like cells
was treated with 0.5 μM all-trans-retinoic acid (22). Differenti-

ation of P19 cells to muscle-like cells followed treatment with 1% dimethyl sulfoxide (23). NIH/3T3 cells were routinely propagated in
Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) plus 10% fetal calf serum.

For transient transfection, Neuro2a, P19, retinoic acid-treated-P19,
dimethyl sulfoxide-treated P19, and NIH/3T3 cells were plated in six-
well tissue culture dishes at 9 × 10^4 cells/well and allowed to recover
for 1 day. Cells containing PS-1-promoter/reporter constructs were then
co-transfected with 0.3 pmol of one of the promoter firefly luciferase
plasmid constructs, pGL3 basic vector or pGL3 promoter plasmid (which
contains an SV40 promoter upstream of the firefly luciferase
Promega), and 0.3 pmol of pRL-TK plasmid (which contains a
herpes simplex virus thymidine kinase promoter upstream of the ren-
ila luciferase gene, Promega), using the Lipofectin procedure (Life
Technologies, Inc.) as described in the manufacture’s protocol.

Relative Luciferase Activity Measures—Transfected cells were cul-
tured for 24 h, washed twice with 2 ml of Ca2+- and Mg2+-free
phosphate-buffered saline, and lysed with Passive lysis buffer (Promega).
Firefly luciferase and renilla (sea pansy) luciferase activities were
measured sequentially using a Dual-Luciferase Reporter assay system
(Promega) and a model TD-20E Luminometer (Turner Design). After
measuring the firefly luciferase signal (Lafi) and the renilla (sea pansy)
luciferase signal (Lrni), the relative luciferase activity (RLA) was cal-
culated as: 

\[
\text{RLA} = \frac{\text{Lafi}}{\text{Lrni}}
\]

The ratio of firefly luciferase signal with an SV40 promoter in pGL3
divided by the renilla luciferase signal in pRL-TK.

results

RACE Detects Multiple Transcripts—As a prelude to cloning the
PS-1 promoter, the exact 5′-end of PS-1 mRNA from mouse
brain was identified by the RACE technique. Using the anti-
sense oligonucleotide “101–80-reverse” found in exon 2 of
mouse PS-1, 5'-RACE gives a major broad band of 210 bp and
a minor band of 430 bp from single-stranded cDNA templates
complementary to mouse brain mRNA (Marathon Ready
cDNA, CLONTECH, data not shown). Each of these bands was
isolated from agarose gels, subcloned into the pGEM-T vector (Promega) and sequenced. Sequencing revealed the presence of
three different PS-1 transcripts which appear to derive from
two unique transcription start sites marked by the vertical arrows.

Isolation of the Mouse PS-1 Gene—A mouse genomic DNA
library in Lambda FIX-II was screened with the 5′-portion of
the mouse PS-1 cDNA (Fig. 2A, probe A). Of the positively

FIG. 1. Structure of three different presenilin-1 transcripts from mouse brain. DNA sequencing of the cloned products of 5′-RACE
of mouse brain cDNA revealed the presence of three independent tran-
scripts (A, B, and C), which appear to derive from two unique transcrip-
tion start sites marked by the vertical arrows. The distance between the
two transcription start sites is 410 bp. The sizes of exon 1A, exon 1B,
and exon 1C are 141, 371, and 139 bp, respectively.
Mouse Presenilin-1 Promoter Activity

Characterization of the PS-1 Gene’s Exon-Intron Structure

The sequence of almost 50 kbp of the P1–10809 clone was mapped and its entire sequence subcloned into multiple pBlueScript II KS(+) vector. Sequencing strategy: lambda phage clones and P1–10809 were restricted and subcloned into pBlueScript II KS(+) vector. Thick lines correspond to individual plasmid subclones from corresponding regions of PS-1 genomic DNA found in P1–10809. Double arrows represent PCR products from the P1–10809 template that were sequenced directly. Restriction endonucleases are abbreviated as: H, HindIII; E, EcoRI; N, NotI; X, XhoI. C, exon-intron structure of the mouse PS-1 gene. Exons are boxed and double lines represent introns. Filled boxes correspond to the protein coding and untranslated regions, respectively. The translation start codon ATG begins at position +11,420, the translation termination codon TAG is at +45,627, and the putative polyadenylation signal (AATTAA) is at position +46,612.

FIG. 2. Cloning and sequencing strategy elucidates the mouse presenilin-1 gene’s exon-intron structure. A, Screening strategy: screening A utilized a fragment of the mouse PS-1 cDNA as probe A (filled box) to identify lambda phage clones of the mouse PS-1 genomic DNA (represented as double lines). Screening B utilized PCR primers to identify a P1 clone of the mouse PS-1 gene, P1–10809, as represented by the hatched horizontal box. B, sequencing strategy: lambda phage clones and P1–10809 were restricted and subcloned into pBlueScript II KS(+) vector. Thick lines correspond to individual plasmid subclones from corresponding regions of PS-1 genomic DNA found in P1–10809. Double arrows represent PCR products from the P1–10809 template that were sequenced directly. Restriction endonucleases are abbreviated as: H, HindIII; E, EcoRI; N, NotI; X, XhoI. C, exon-intron structure of the mouse PS-1 gene. Exons are boxed and double lines represent introns. Filled boxes correspond to the protein coding and untranslated regions, respectively. The translation start codon ATG begins at position +11,420, the translation termination codon TAG is at +45,627, and the putative polyadenylation signal (AATTAA) is at position +46,612.
Characterization of the Mouse Presenilin-1 Promoter—The DNA located upstream and surrounding the transcription initiation sites typically confers the gene’s promoter activity. When the DNA surrounding the transcript A initiation of transcription site was compared with its human PS-1 genomic DNA counterpart (Fig. 3), the region of maximal similarity extends from positions 1–39 to +117 of the mouse PS-1 sequence. This region is rich in guanosine (G) and cytosine (C) residues containing the sequence motifs: GCCGGAAGT resembling an Ets region and GGGCGGG motif resembling an Sp-1 hexanucleotide binding element, which is commonly found in the promoters of other genes. The mouse sequences upstream of this region do not share similarity with the human sequence nor do they contain the most common eukaryotic promoter element, a TATA box (Fig. 4). Instead, this unique mouse sequence contains two CAAATA motifs, at positions 11210–11276 and 11367–11506 (Fig. 3, 4), which resemble CAAT boxes found in other eukaryotic promoters. This region unique to mouse also contains an Ap-2 binding site of transcription initiation with exon 1A and human PS-1 transcription begins with “A” (data not shown.). By DNA sequence similarity searching with BLAST network service available from National Center Biotechnology Information, regions of mouse/human homology are found around the transcription initiation sites for both genes. Consensus binding sites for the transcription factors ETS1 and SPI are underlined and are conserved in both mouse and human genes.

We employed a Dual-Luciferase reporter assay system (Promega) to test whether these elements function to promote transcription. In general, we assayed the promoter activity of DNAs flanking the transcription initiation site of PS-1 by inserting these DNA fragments in front of a basic, promoterless firefly luciferase reporter gene in plasmid pGL3. Constant amounts of pGL3 containing PS-1 promoter fragments and of pRL-TK plasmid containing a herpes simplex virus thymidine kinase promoter driving expression of sea pansy luciferase, were co-transfected into a constant number of cells. After 24 h, lysates of transfected cells were sequentially assayed for firefly luciferase (LAF) and sea pansy luciferase activity (LAR) so that a ratio of firefly to sea pansy activity (Fig. 5 and Table II), which we defined as 100% activity. Larger fragments in LUC 1 (−2322 to +1436), LUC 3 (−499 to +1171), and LUC 16 (−276 to +519) display only a small percentage of the LUC 29 activity, suggesting the presence of negative elements that apparently reduce their activities. Interestingly, the high activity of LUC 29 is not found in its flanking fragments such as LUC 2 (−2322 to −496) and LUC 23 (+188 to +519), which both lack significant promoter activity. The LUC 23 result is particularly interesting because its flanking fragments such as LUC 2 (−2322 to +496) and LUC 23 (+188 to +519), which both lack significant promoter activity. The LUC 23 result is particularly interesting because the alternative transcription start site begins at position +411 of exon 1B/exon 1C and apparently lacks meaningful promoter activity.

To more accurately define the minimal or core regions conferring promoter activity, we studied the −327 to +206 region of the PS-1 gene in greater detail. Sequence comparison showed this region to contain a CAAT box (−281), a heat shock element (−220), an AP2 site (−80), a PEA-3 site (−53), an Ets 1–3 site (−7), and Sp1 sites (+119, +161). To find which of these elements and/or new elements were functionally active, we resected this region and tested smaller fragments for promoter activity. Since LUC 24 and LUC 23 lacked significant activity, we initially focused on the fragments from −440 to +91 as shown in Fig. 5. The CAAT box at −281 plays an active role in the PS-1 promoter because LUC 8 (−261 to +91) has

Table I
Numbering scheme for the mouse PS-1 gene’s exon-intron structure
The positions of the 5′-end and the 3′-end of each exon were counted from the transcription start site of exon 1A being defined as position +1.

| Exon | Position |
|------|----------|
| 1A   | 1–141    |
| 1B   | 411–781  |
| 1C   | 411–549  |
| 2    | 11210–11276|
| 3    | 11367–11506|
| 4    | 39773–39871|
| 5    | 40245–40331|
| 6    | 42082–42255|
| 7    | 43217–43335|
| 8    | 45459–45545|
| 9    | 111420   |
| 10   | 45627    |
| 12   | 46612    |

3 N. Mitsuda, A. D. Roses, and M. P. Vitek, unpublished results.
less activity than LUC 6 (−327 to +91) which contains this CAAT box. A negative element must reside upstream of this CAAT box because the activity of LUC 4 (−244 to +91) is about half that of LUC 6 (−327 to +91). The heat shock element at −220 may not play a role in PS-1 promoter activity as fragments containing (LUC 8, −226 to +91) and lacking (LUC 10, −219 to +91) this element have similar activities. The AP2 site at −180 and/or the PEA-3 site at −153 appear to play positive roles in PS-1 promoter function as LUC 12 (−287 to +91) has about 4-fold more activity than LUC 13 (−232 to +91) which lacks these sites. Similarly, the Ets 1–3 site at position −278 plays a positive role as judged by the RLA activity of LUC 14 (−294 to +91) at 7.9% and LUC 29 (−218 to +91) at 0.7%.

Based on these experiments, we tested whether the region from −87 to +41 could contain the core promoter activity in two ways. First, LUC 25 (−87 to +41) had an RLA promoter activity of 28%. Second, the deletion of this region to give LUC 30 (delete −87 to +41 from −327 to +206) decreased activity from 100% (LUC 29) to 0.2% (LUC 30). Taken together, these results strongly suggest that the Ap2, PEA-3, Ets 1–3, and Sp1 elements comprise the major functional elements of the PS-1 promoter in the region −87 to +41.

**Cell-specific Transcription**—Using in situ hybridization to human brain slices, we found that PS-1 RNA was most abundant in neurons and below the limits of detection in other brain cells. This result suggested that the PS-1 promoter may preferentially function in neurons. To test this idea further, we compared the activity of the promoter-fragment/reporter plasmids LUC 1, LUC 3, LUC 4, LUC 27, and LUC 29 in different cell types. As reported above, the mouse Neuro-2A cell line of neuroectodermal lineage supports more RLA promoter activity from LUC 29 and LUC 4 than from LUC 27, LUC 3, and LUC 1 (Fig. 6 and Table II). In contrast, the mouse NIH/3T3 fibroblast cell line supports only minimal promoter activity with each of these promoter/reporter constructs (LUC 29, LUC 27, LUC 4, LUC 3, or LUC 1). To further test the idea that the PS-1 promoter activity is great in neurons, we transfected the mouse embryonal carcinoma cell line P19 with these reporter constructs. P19 cells are uniquely differentiated by all-trans-retinoic acid treatment into a neuron-like phenotype (22) or by dimethyl sulfoxide treatment into a muscle-like phenotype (23). Retinoic acid-treated P19 cells support as much as 2.5-fold more relative luciferase activity from plasmid LUC 29 compared with untreated P19 cells. Untreated P19 cells support as much as 1.3-fold more relative luciferase activity compared

---

**Fig. 4. Nucleotide sequence of the mouse PS-1 promoter region.** The sequence of mouse PS-1 gene flanking the two transcription initiation sites, marked with vertical arrows, is displayed. Some restriction endonuclease sites are underlined, and various promoter elements are boxed and labeled. Exon 1A and exon 1B are double-underlined.
FIG. 5. Mouse presenilin-1 promoter-reporter constructs and their relative luciferase activity (%RLA). A, structural organization of PS-1 promoter. Top line represents the region of the PS-1 gene which was analyzed for promoter activity where boxes for exon 1A and exon 1B are
with dimethyl sulfoxide-treated P19 cells. These results are consistent with the hypothesis in which PS-1 transcription is preferred in neuron-like cells.

**DISCUSSION**

From promoter to polyadenylation signal, the full sequence of the mouse presenilin-1 gene and its exon-intron structure set the stage to describe some of its unique functions. In contrast to the reported PS-1 cDNA sequence, 5'-RACE surprised us by amplifying three different mRNA transcripts which share two unique transcription start sites. Sequence analysis showed that transcript A begins with exon 1A while transcript B and transcript C begin with exon 1B. Exon 1C is a fragment of exon 1B sharing its 5'-end at position +411, but only extending to position +549. This example of alternative splicing in exon 1B versus exon 1C yields multiple RNA transcripts and has also been described for exon 9 in the human PS-1 gene (16). Two distinct transcription start sites, however, have been reported for only a few genes including human catechol-O-methyl transferase (24), mouse neurotrophin-3 (25), and rat aromatic L-amino acid decarboxylase (26). In these cases, each transcriptional start site was associated with a distinct promoter so that a stoichiometry of one promoter per transcription start site was observed.

Our characterization of promoter activities for the PS-1 gene, however, revealed a much different picture. Using a promoter-fragment coupled to the firefly luciferase reporter with sea pansy Renilla luciferase as an internal standard, we found that the -327 to +206 fragment (LUC 29) contains most of the PS-1 promoter activity. The known sequence motifs which apparently contain this activity are a CAAT box (-281), an AP2 site (-80), a PEA-3 site (-53), an Ets 1-3 site (-7), and an Sp1 site (+25). While this region overlaps some of exon 1A, deletion of the -87 to +41 region in LUC 30 reduces promoter activity by 50-fold. To measure promoter activity around the alternative transcription start site, we tested LUC 23 (+118 to +519) containing Sp1, Ap2, and Ets 1-3 sites and found these exon 1B/exon 1C sequences to confer about 1% of the activity surrounding the exon 1A promoter. These results suggest to us that the region surrounding the +1 position of exon 1A may promote the expression of transcript A, transcript B, and transcript C. Alternatively, a weak promoter controlling transcription initiation at position +410 in exon 1B/exon 1C may amount to only 1% of the transcription initiation at position +1. By cloning all of the products of the 5'-RACE into plasmid vectors and counting each clone carrying exon 1C, we estimate the abundance of transcript C to approach 30% of all of the PS-1 transcripts (data not shown), further supporting the idea that the major promoter at +1 functions to control transcription initiation from both the +1 and the +410 sites. Quantitative measurement of transcript A, transcript B, and transcript C levels will help to further resolve this issue. The high homology between human and mouse promoters combined with our description of multiple start sites and alternative splicing for the mouse PS-1 gene reasonably suggests how the human PS-1 promoter may function.

Recently, PS-1 was reported to be expressed predominantly in neurons of the central nervous system (17). This result matches our own data that PS-1 RNA, by in situ hybridization, is strongly expressed in neurons and at undetectable levels in other cell types. Similarly, several immunohistochemical studies report primarily neuronal localization of PS-1 protein with weak staining of amyloid plaques and some glia surrounding those plaques. On the other hand, Sherrington et al. (6) showed that Northern blots of RNA from different organs all hybridized to a PS-1 cDNA probe, suggesting that PS-1 RNA is ubiquitously expressed. At present, these results can not be easily reconciled.

While not rigorous proof, our data clearly shows preferential promoter activity in neuron-like cells supporting a cell-type-specific pattern of PS-1 expression. We find the greatest amount of PS-1 promoter activity in the mouse Neuro2a neuroblastoma cell line, followed by the P19 embryonal carcinoma cell line and almost no activity in the mouse NIH/3T3 fibroblast cell line (Table II). To further confirm this finding, we employed the P19 mouse embryonal carcinoma cell line because of its unique ability to be differentiated into a muscle-like phenotype following dimethyl sulfoxide treatment or into a neuron-like phenotype (P19-RA-neuron) following all-trans-retinoic acid treatment (22, 23). If our hypothesis that PS-1 promoter activity is preferred in neuron-like cells, then we would predict that P19 cells differentiated with retinoic acid into neuron-like cells would display more PS-1 promoter activity than P19 cells differentiated with dimethyl sulfoxide into muscle-like cells. As clearly shown in Fig. 6 and Table II, P19-RA-neuron cells display the most PS-1 promoter activity followed by untreated P19 cells and the least activity in P19 dimethyl sulfoxide-treated muscle cells. These results, combined with the Neuro2a and NIH/3T3 results, indicate a clear pattern of PS-1 promoter activity which is preferred in neurons.

The mechanisms controlling neuron-specific promoter activity are poorly understood. The most direct mechanism would be for a positive regulator, that is only present in neuronal cells, to singularly activate the neuron-specific promoter. Alternatively, a negative regulator, that is only present in non-neuronal cells, could globally repress the neuron-specific promoter in all but

| Promoter | Cell line<sup>a</sup> | N2a | P19N | P19 | P19M | NIH/3T3 |
|----------|----------------------|-----|------|-----|------|---------|
| RLA      | SY40 (control)       | 2.1 | 10.6 | 5.0 | 35.9 | 1.1     |
| Core promoter | Total promoter     | 36.8| 114.1| 21.2| 114.9| 0.3     |
| IRLA     | Total promoter       | 17.8| 10.8 | 4.3 | 3.2  | 0.3     |
| Core promoter | Total/Core         | 1.7 | 1.1  | 0.7 | 0.5  | 0.1     |

<sup>a</sup> Cell lines are defined in the legend to Fig. 6.
neuronal cells. Depending upon the exact DNA elements within the promoter, some combination of positive and negative controls of transcriptional activity might also yield neuron-preferred promoter function. Going beyond our characterization of the regions conferring PS-1 promoter activity in Neuro2a cells, we may now look at the data to suggest which of the DNA elements might confer neuron-preferred promoter function. The region showing the highest activity in Neuro2a neuron-like cells extends from $-2329$ to $-1206$ (LUC 29) and contains a CAAT box at $-2281$, a heat-shock inducible element at $-2218$, an Ap2 site at $-280$, a PEA-3 site at $-253$, an Ets 1–3 site at $-7$, and an Sp1 site at $+25$. The CAAT box could be the source of about a third of the positive control of neuron-specific activity as its deletion reduces promoter activity by about a third when comparing LUC 6 ($-2327$ to $-1201$) with LUC 8 ($-2261$ to $-1201$, Fig. 4). Based on the 4-fold greater activity of LUC 12 ($-87$ to $+91$) compared with LUC 13 ($-32$ to $+91$), it appears that both the Ap2 site and the PEA-3 site are good candidates for the positive control of neuron-specific promoter function. Ap2 sites are reported to be most frequently found in promoters active in cells of neural crest lineage, and several examples exist of their involvement with neuron-specific activity (27–29). In contrast, the 5-fold less activity of LUC 26 ($-9$ to $+41$) compared with LUC 27 ($-9$ to $+16$) implicates the Sp1 site at $+25$ as a negative regulator of neuron-specific promoter function. These same data could also be interpreted as the Ets 1–3 site having a positive function, possibly as part of a core promoter element from $-9$ to $+16$. Direct measurement of LUC 27 ($-9$ to $+16$) shows that Neuro2a and P19-RA-neuron cells have more activity than do P19 dimethyl sulfoxide-treated muscle or NIH/3T3 non-neuronal cells supporting the idea that this 25-bp region contributes to neuron-preferred promoter activity. The major transcription start site at position $+1$ is

| Cell Line | Clone# | 0% | 10% | 20% | 30% | 40% | 50% | 60% | 70% | 80% | 90% | 100% |
|-----------|-------|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|
| LUC29     |       |    |     |     |     |     |     |     |     |     |     | 100% |
| N2a       | LUC27 | 9.8%|     |     |     |     |     |     |     |     |     | 37.9%|
|           | LUC4  | 3.8%|     |     |     |     |     |     |     |     |     | 1.0% |
|           | LUC3  |     |     |     |     |     |     |     |     |     |     | 1.0% |
|           | LUC1  |     |     |     |     |     |     |     |     |     |     | 1.0% |
| LUC29     |       |    |     |     |     |     |     |     |     |     |     | 60.7%|
| P19N      | LUC27 | 6.2%|     |     |     |     |     |     |     |     |     | 24.2%|
|           | LUC4  | 11.2%|    |     |     |     |     |     |     |     |     | 10.7%|
|           | LUC3  | 1.2% |    |     |     |     |     |     |     |     |     | 0.5% |
|           | LUC1  | 0.3% |    |     |     |     |     |     |     |     |     | 0.1% |
| LUC29     |       |    |     |     |     |     |     |     |     |     |     | 18.0%|
| P19       | LUC27 | 3.9%|     |     |     |     |     |     |     |     |     | 2.8% |
|           | LUC4  | 10.7%|    |     |     |     |     |     |     |     |     | 3.9% |
|           | LUC3  | 0.5% |    |     |     |     |     |     |     |     |     | 0.3% |
|           | LUC1  | 0.1% |    |     |     |     |     |     |     |     |     | 0.1% |
| LUC29     |       |    |     |     |     |     |     |     |     |     |     | 18.0%|
| P19M      | LUC27 | 2.8%|     |     |     |     |     |     |     |     |     | 0.6% |
|           | LUC4  | 3.9% |    |     |     |     |     |     |     |     |     | 0.2% |
|           | LUC3  | 0.1% |    |     |     |     |     |     |     |     |     | 0.1% |
|           | LUC1  | 0.1% |    |     |     |     |     |     |     |     |     | 0.1% |
| LUC29     |       |    |     |     |     |     |     |     |     |     |     | 1.7% |
| P19       | LUC27 | 0.6%|     |     |     |     |     |     |     |     |     | 0.2% |
|           | LUC4  | 0.2% |    |     |     |     |     |     |     |     |     | 0.1% |
|           | LUC3  | 0.1% |    |     |     |     |     |     |     |     |     | 0.1% |
|           | LUC1  | 0.1% |    |     |     |     |     |     |     |     |     | 0.1% |

**Fig. 6. Cell type-specific PS-1 promoter activity.** PS-1 promoter-reporter constructs LUC 29, LUC 27, LUC 4, LUC 3, and LUC 1 were transiently transfected into Neuro2a neuroblastoma (N2a), undifferentiated P19 (P19), all-trans-retinoic acid differentiated neuron-like P19 (P19N), dimethyl sulfoxide-differentiated muscle-like P19 (P19M), and NIH/3T3 fibroblast cells. An SV40 promoter driving firefly luciferase in pGL3-basic plasmid (Promega) and pRL-TK (thymidine kinase promoter driving renilla luciferase gene) was also transfected into each cell line as external and internal controls, respectively. After measuring luciferase activity from all combinations of plasmids, the IRLA was calculated as RLA/RLASV40, where RLA is the ratio of firefly luciferase signal in the external control divided by the renilla luciferase signal in the internal control, to compare the activity of different promoter fragments in different cell lines. Plasmid LUC 29 transfected into N2a cells showed the greatest IRLA value which we defined as 100% activity.
located in this proposed core promoter element. The ETS-1 transcription factor prefers binding to the Ets 1–3 binding site found in this core by a ratio of five to one over the PEA-3 binding site (30). This finding is particularly interesting as the ETS-1 transcription factor is thought to be specific for B cells and resting T cells of the immune system and not been previously described for neuronal cells. Sp1 binding sites appear to be ubiquitously distributed in all promoters of all cell types and their ability to function as negative elements appears to be novel.

In summary, we described a complete sequence of the mouse presenilin-1 gene from its tip to its tail. This sequence has shown us that there are two independent transcription start sites. Functional testing of the DNA regions surrounding these start sites showed that they both were apparently controlled by a single, major promoter that includes the +1 position of exon 1A. This promoter was also quite interesting because it is mostly active in neuron-like cells. Further characterization can now progress to a complete description of those positive and negative DNA elements and transcription factors which function to control presenilin-1 gene expression.

Acknowledgments—We thank John Gilbert, Kalina Boteva, Marilyn Jansen, and Osamu Onodera for many helpful discussions.

REFERENCES
1. Citron, M., Oltersdorff, T., Haas, C., McConlogue, L., Hung, A. Y., Seubert, P., Vigo-Pelfrey, C., Lieberburg, I., and Selkoe, D. J. (1992) Nature 360, 672–674
2. Suzuki, N., Cheung, T. T, Cai, X.-D., Odaka, A., Otvos, L., Jr., Eckman, C., Geld, T. E., and Younkin, S. G. (1994) Science 264, 1336–1340
3. Corder, E. H., Saunders, A. M., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Small, G. W., Roses, A. D., Haines, J. L., and Pericak-Vance, M. A. (1993) Science 261, 921–923
4. Corder, E. H., Saunders, A. M., Rissh, N. J., Strittmatter, W. J., Schmechel, D. E., Gaskell, P. C., Rimmer, J. B. Locke, P. A., Connelly, P. M., Schmad, K. E., Small, G. W., Roses, A. D., Haines, J. L., and Pericak-Vance, M. A. (1994) Nat. Genet. 7, 180–184
5. Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M., Englund, J., Salvesen, G. S., and Roses, A. D. (1995) Proc. Natl. Acad. Sci. U. S. A. 90, 1977–81
6. Sherrington, R., Roganew, E. I., Liang, Y., Rogaczew, E. A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., Tsuda, T., Mar, L., Foncin, j.-F., Brun, A. C., Montesi, M. P., Sorhi, S., Rainero, I., Pines, L., Nee, L., Chumakov, I., Pollen, D., Brooks, A., Sunanpe, P., Poinlains, R. J., Wascow, W., Da Silva, H. A. R., Haines, J. L., Pericak-Vance, M. A., Tanzi, R. E., Roses, A. D., Frazer, P. E., Rommens, J. M., and St. George-Hyslop, P. H. (1995) Nature 375, 754–760
7. Levy-Lahad, E., Wasse, W., Poorkaj, P., Romano, D. M., Oshima, J., Pettingill, W. H., Yu, C. K., Jondro, P. D., Schmidt, S. D., Wang, K., Crowley, A. C., Fu, Y. H., Guenette, S. Y., Galas, D., Nemens, E., Wijisman, E. M., Bird, T. D., Schellenberg, G. D., and Tanzi, R. E. (1995) Science 269, 973–977
8. Stephenson, J. (1997) J. Am. Med. Ass. 277, 775
9. Wasse, W., Pettingill, W. P., Jondro, P. D., Schmidt, S. D., Guruhanagavatula, S., Rodes, L., Dibiase, T., Romano, D. M., Guenette, S. Y., Kovacs, D. M., Grown, J. H., and Tanzi, R. E. (1995) Nat. Med. 1, 848
10. Alzheimer’s Disease Collaborative Group (1995) Nat. Genet. 11, 219–222
11. Campion, D., Flamand, J. M., Brice, A., Hannequin, D., Dubois, B., Martin, C., Moreau, V., Charbonnier, F., Didierjean, O., Tardieu, S., Penet, C., Puel, M., Pasquier, F., Driant, F. L., Bellis, G., Calenda, A., Heilig, R., Martinz, M., Mallet, J., Bellis, M., Clerget-Darpoux, F., Agid, Y., and Freiburg, T. (1995) Hum. Mol. Genet. 4, 2373–2377
12. Cruts, M., Backhoven, H., Wang, S. Y., Gassen, G. V., Theuns, J., De Jonghe, C. D., Wohiert, A., De Vooch, J., De Winter, G., Cras, P., Brudyard, L., Datson, N., Weissenbach, J., Dunnen, J. T., Martin, J., Hendriks, L., and Broeckhoven, C. V. (1995) Hum. Mol. Genet. 4, 2363–2371
13. Boteva, K., Vitek, M., Mitsuha, H., de Silva, H., Xu, P. T., Small, G., and Gilbert, J. R. (1996) Lancet 347, 130–131
14. Rosso, M. N., Fox, N. C., Beck, J., Campbell, T. C., and Collinge, J. (1996) Lancet 347, 1560
15. Kamin, K., Sato, S., Sakaki, Y., Yoshihama, A., Nishikawa, Y., Takeda, M., Tanabe, H., Nishimura, T., Ik, K., St. George-Hyslop, P. H., Miki, T., and Ogihara, T. (1996) Neurosci. Lett. 208, 115–198
16. Perez-Tur, J., Froelich, S., Prihar, G., Crook, B., Muff, D., Kunn, M., Busfield, F., Lundon, C., Clark, R. F., Roques, P., Kullberg, R. A., Johnston, C., Mammam, F., Lassen, K., Louden, H., Karran, E., Roberts, G. W., Rasser, M., Adams, M. D., Hardy, J., Goate, A., Lanfert, L., and Hutton, M. (1995) Neuropept 7, 297–301
17. Kovacs, D. M., Faurett, H. J., Page, K. J., Kim, T., Mor, R. D., Merriam, D. E., Hollister, B. D., Halka, O. G., Mancini, R., Felsenstein, K. M., Hyman, B. T., Tanzi, R. E., and Wasse, W. (1996) Neurosci. Lett. 224–229
18. Scheuer, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T. D., Hardy, J., Hutton, M., Kukuki, W., Larson, E., Levy-Lahad, E., Viitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R. W., Wasse, W., Lannfett, L., Selkoe, D., and Younkin, S. (1996) Nat. Med. 2, 864–870
19. Lab, J. J., Hellman, C. J., Nash, N. R., Rees, H. D., Yi, H., Counts, S. E., and Levy, A. L. (1997) J. Neurosci. 17, 1971–1980
20. Shon, J., Bronson, R. T., Chen, D. F., Xia, W., Selkoe, D. J., and Tognawa, S. (1997) Cell 89, 629–639
21. Sambrook, J. F., Fison, E. F., and Maniatis, T. (1989) Mol. Cell. Biol. 23, 1971–1980
22. Petersohn, D., Schoch, S., Brinkmann, D. R., and Thiel, G. (1995) Neuropept 7, 297–301
23. Edwards, M. K. S., Harris, J. F., and McBurney, M. W. (1983) Mol. Cell. Biol. 3, 2271–2279
24. Tenhunen, J., Salminen, M., Lundstrom, K., Vihlkuoto, T., Savolainen, E., and Ullman, O. (1994) Eur. J. Biochem. 233, 1049–1059
25. Leinhardt, A., and Lindholm, D. (1994) Eur. J. Neurosci. 6, 1149–1159
26. Albert, R. V., Lee, M. R., Bolden, A. H., Wurzbinger, R. J., and Aunanato, A. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 12053–12057
27. Sato, T., Xiao, D. M., Li, H., Huang, F. L., and Huang, K.-P. (1995) J. Biol. Chem. 270, 10314–10322
28. Petersohn, D., Schoch, S., Brinkmann, D. R., and Thiel, G. (1995) J. Biol. Chem. 270, 24361–24369
29. Chino, L. S., Li, J., and Greenberg, P. (1994) J. Biol. Chem. 269, 18507–18513
30. Fisher, R. J., Mavrothalassitis, G., Kondoh, A., and Papas, T. S. (1991) Oncogene 6, 2249–2254
Transcriptional Regulation of the Mouse Presenilin-1 Gene
Noriaki Mitsuda, Allen D. Roses and Michael P. Vitek

J. Biol. Chem. 1997, 272:23489-23497.
doi: 10.1074/jbc.272.38.23489

Access the most updated version of this article at http://www.jbc.org/content/272/38/23489

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 29 references, 11 of which can be accessed free at http://www.jbc.org/content/272/38/23489.full.html#ref-list-1