[Frontiers in Bioscience 13, 3439-3447, May 1, 2008]

ADR1 interacts with a down-stream positive element to activate PS1 transcription

Hriday K. Das[1,2], Myrna L. Baez[1]

[1]Department of Pharmacology and Neuroscience, [2] Department of Molecular Biology and Immunology, and Institute of Cancer Research, University of North Texas Health Science Center at Fort Worth, 3500 Camp Bowie Boulevard, Fort Worth, Texas 76107

TABLE OF CONTENTS

1. ABSTRACT

We have identified downstream promoter sequence of the PS1 gene that may be regulated by novel transcription factors. 3' deletion from +178 to +165 had no effect on PS1 transcription. 3' deletion from +178 to +140 decreased promoter activity by 50%. Further 3' deletion from +178 to +114 decreased promoter activity by 80%. Therefore, a crucial element controlling over 80% of the promoter activity in SK-N-SH cell line is located between +114 and +165. Electrophoretic mobility shift assays suggested that zinc finger proteins Sp1 and ADR1 interacted with the PS1 promoter sequence (+114 to +140) and promoter region (+140 to +165) respectively. A three base pair substitution within the core sequence (GGCGGGGA to GGCGactA) of the ADR1 consensus in the element (+140 to +165) that abolished ADR1-DNA interaction, reduced PS1 transcription by 50%. The substitution mutation in the sequence (+114 to +140) that abolished Sp1-DNA interaction had no effect on PS1 expression. These data suggest that a novel mammalian trans-activator protein ADR1 binds to the downstream element (+140 to +165) to activate PS1 transcription.

2. INTRODUCTION

Presenilin genes (PS1 and PS2) encode highly homologous integral membrane proteins (1, 2). Mutations within the presenilin genes lead to the most aggressive form of early-onset familial Alzheimer's disease (FAD) and account for more than 75% of early onset cases (1, 3, 4). The pathogenesis of FAD is associated with the formation of amyloid plaques containing Aβ42/43 polypeptide (3, 5), a highly amyloidogenic variant of Aβ. Aβ42/43 polypeptide is produced by sequential proteolytic processing of APP (6). Presenilins are required for the final step of the intramembrane cleavage of APP by γ-secretase (7). γ-secretase activity has been shown to be carried out by a multimeric protein complex including PS1 (7). PS1 appears to be the γ-secretase catalytic subunit itself (8) and has been found tightly associated with a number of proteins such as nicastrin (9) which participates in the APP as well as in Notch processing pathways (10, 11). Notch receptor signaling is an evolutionarily conserved pathway which controls cell fate in *Caenorhabditis elegans* where PS1 homologue SEL-12 is required for Notch cleavage and Notch receptor signaling

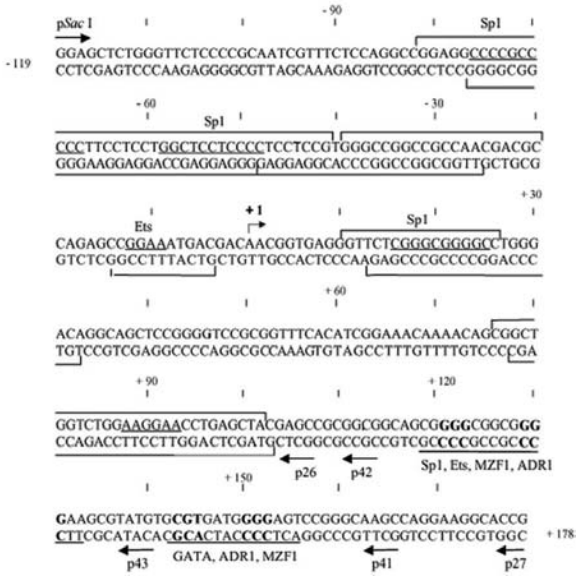

Figure 1. PS1 promoter sequence. PS1 promoter sequence from –118 to +178. The end point of the 3' deletions used in this study are indicated by arrows. The transcription initiation site is shown (+1). The position of Ets and Sp1 binding sites as identified by DNAseI footprinting are underlined. Putative binding sites for Sp1, Ets, GATA, MZF1, and ADR1 within the region (+114 to +165) are also underlined. Alteration of three nucleotides by mutations in the PS1 promoter sequence are bolded. The end points of the 5' and 3' deletions analyzed by transfection assays in this study are shown by *arrows.*

and cell fate determination (12) Studies of KO mice indicate that PS1 function is required for embryogenesis including central nervous system and skeletal development (13, 14). Furthermore, PS1 controls multiple aspects of γ-secretase processing, including the maturation, processing and intracellular translocation of component (s) of the γ-secretase complex (15). Some of the developmental function (s) of presenilins may also involve other proteins found in its association including β/γ-catenins (16). Thus regulation of PS1 level may play a critical role in mammalian development via Notch receptor signaling and in the pathogenesis of Alzheimer's disease.

We have analyzed the transcriptional control of the human PS1 gene, and we have previously identified a promoter fragment (-118/+178) that promotes efficient transcription in human neuroblastoma SK-N-SH cells (17). We have shown that a mutation altering two nucleotides of the consensus motif of an Ets transcription factor binding site present at – 10 reduces the activity of the PS1 gene promoter by 80% (17, 18). On the contrary mutations at consensus Ets binding sites at +65, +90, and +129 alone reduces PS1 transcription by 40% (18). We have now mapped two crucial downstream positive elements (+114 to +140) and (+140 to +165) that controls more than 80% promoter activity of the PS1 gene. In this report we have shown that transcription factor ADR1 or an ADR1 related protein interacts with the downstream positive element (+140 to +165) to activate PS1 transcription in SK-N-SH cell line.

3. MATERIALS AND METHODS

3.1. Construction of human PS1 promoter CAT reporters

A genomic fragment including 118 bp of upstream sequences, and 39 bp of the first intron was obtained as described before (17) by PCR amplification. Primers were designed on the basis of the published PS1 sequence (19) to incorporate restriction enzyme sites at the end of the amplified fragment. SacI and XbaI sites were introduced in forward and reverse primers, respectively. The PS1 promoter fragment (-118 to +178) obtained by PCR using pSacI and p27 primers, was then inserted into the promoterless pKT vector (20) upstream from the CAT gene. 3' deletion constructs including only sequences downstream from the SacI site were generated by amplification using the primer pairs pSacI-p41, pSacI-p42, pSacI-p43, and pSacI-p26 and inserted into pKT after digestion with SacI and XbaI (Figure 1). The (-118, +178) PS1CAT constructs containing point mutations were generated by PCR-based site directed mutagenesis using QuickChange kit from Stratagene and the complementary primer pairs with (-118, +178) PS1CAT plasmid as a template. Primer pairs p121mF-p121R, p129mF-p129mR, p143mF-p143mR, and p150mF-p150mR were used to generate point mutations (-118, +178) m121, (-118, +178) m129, (-118, +178) m143, and (-118, +178) m150 respectively.

3.2. Sequences of oligonucleotides used in this study

The sequences of the primers used in PCR are derived from the published sequence of the human PS1 gene (19). Forward (F) and reverse ® primers are shown in Table 1.

Double-stranded oligonucleotides containing the binding sites for Sp1, mutant Sp1, AP2, Ets, and GATA were purchased from Santa Cruz Biotechnology Inc. Santa Cruz, CA. All other oligonucleotides were synthesized by IDT (Corelville, IA).

3.3. Cell culture and transfection

Human neuroblastoma SK-N-SH cell line was obtained from ATCC and cultured as recommended. Cells were transfected using the calcium phosphate precipitation method (23). pSV-βGal plasmid (6 μg) was cotransfected with each PS1 construct (6 μg) as an internal control. A 12.5% glycerol shock was performed for 90 sec for SK-N-SH cells 5 h after adding the DNA to the cells. CAT and β-galactosidase activity were assayed as described previously (24).

3.4. Preparation of nuclear extracts

The preparation of small scale nuclear extracts is derived from a protocol described previously (25). Cell were lysed in 5 volumes of hypotonic buffer A (10 mM Hepes, pH 7.9, 10 mM KCl, 1.5 mM MgCl2, and0.5 mM DTT) by 20 passages through a 21-gauge needle. Nuclei were collected by centrifugation at 17,000 × g for 10 min in a Beckman microcentrifuge and homogenized with an added equal volume of buffer C (20 mM Hepes, pH 7.9, 600 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM

Table 1. Primer sequences

Primers used to generate 3' deletion mutants	
Gene	**5'–3' Sequence**
pSacI	5′-gatcGGAGCTCTGGGTTCTCCCCG-3′
p27	5'-gatctctagaCGGTGCCTTCCTGGCTTGC-3'.
p41	5′-gatctctagaGCTTGCCCGGACTCCCCATC-3′
p42	5′-gatctctagaCGCCGCGGCTCGTAGCTCAG-3′
p43	5′-gatctctagaCATACGCTTCCCCGCCGCCC-3′
p26	5'-gatctctagaGCTCGTAGCTCAGGTTC-3'
Forward primers used to generate point mutations	
p121mF	5′-CCGCGGCGGCAGCGactCGGCGGGGAAGCGTATG-3′
p129mF	5′-CGGCGGCAGCGGGGCGGCGactAAGCGTATGTGCGTGATG-3′
p143mF	5′-GGCGGGGAAGCGTATGTGataGATGGGGAGTCCGGGCAAGCCAGG-3′
p150mF	5′-GGGGCGGCGGGGAAGCGTATGTGCGTGATGtGtAGTCCGGGCAAGCCAGGAAGGCACCG-3′
Primers used to generate probes by PCR for electrophoretic mobility shift (EMSA) assay	
p100F	5′-CTACGAGCCGCGGCGGCAGC-3′
p106F	5′-GCCGCGGCGGCAGCGGGGCG-3′
p114F	5′-GGCAGCGGGGCGGCGGGGAA-3′
p175R	5′-TGCCTTCCTGGCTTGCCCGGACTCCCCATCACGCACATAC-3′
p165R	5′-GCTTGCCCCGGACTCCCCATC-3′
p140R	5′-CATACGCTTCCCCGCCGCCCCGCTGCC-3′
Double-stranded consensus sequence for the oligonucletides (coding strand) used in EMSA	
(+107 to +140)m121F	5′-CCGCGGCGGCAGCGactCGGCGGGGAAGCGTATG-3′
(+114 to 140)m129F	5′-GGCAGCGGGGCGGCGactAAGCGTATG-3′
(+136 to +175)m143F	5′-GTATGTGataGATGGGGAGTCCGGGCAAGCCAGGAAGGCA-3′
(+136 to +175)m150F	5′-GTATGTGCGTGATGtGtAGTCCGGGCAAGCCAGGAAGGCA-3′
MZF1AF	5′-GATCTAAAAGTGGGGAGAAAA-3′ (21)
MZF1BF	5′-GATCCGGCTGGTGAGGGGGAATCG-3′ (21)
ADR1F	5′-GATCGTTCTCCAACTTATAAGTTGGAGATGCCCGG-3′ (22)
ADR1mF	5′-GATCGTTCTCCAAC ccgcgg GTTGGAGATGCCCGG-3′ (22)
Sp1F	5′-ATTCGATCGGGGCGGGGCGAGC-3′
Sp1mF	5′-ATTCGATCGG tt CGGGGCGAGC-3′
EtsF	5′-GATCTGGAGCAGGAAGTTCGA-3′
AP1F	5′-CGCTTGATGACTCAGCCGGAA-3′
GATAF	5′-CACTTGATAACAGAAAGTGATAACTCT-3′

DTT, and 25% glycerol), in order to keep the ionic strength of the final extract between 280 and 320 mM NaCl. After 40 min at 2-4 °C, the homogenate was centrifuged for 10 min at 17,000 × g. The supernatants were frozen in dry ice-ethanol and stored at -80 °C.

3.5. Electrophoretic mobility shift assay (EMSA)

Labeled probes were generated by kinasing double-stranded oligonucleotides and PCR products with γP32 ATP (New England Nuclear) and T4 polynucleotide kinase (New England Biolab). EMSAs were carried out by incubating 0.1 ng of probe with 5 µg of SK-N-SH cell nuclear extracts or approximately 1 ng of purified yeast mini Adr1 protein (26) in the presence of 1µg of poly (dI-dC)·poly (dI-dC) in 10 mM Hepes, pH 7.9, 50 mM NaCl, 0.75 mM MgCl2, 0.1 mM EDTA, 1 mM DTT, 0.5% NP40, and 10% glycerol for 20 min at 4 °C (17, 18). Protein-DNA complexes formed were then analyzed by electrophoresis on nondenaturing 4% polyacrylamide gels containing 0.1% NP40. The electrophoresis buffer was 0.25× TBE (89 mM Tris, 89 mM boric acid, and 1 mM EDTA). The gels were prerun for 30 min, and sample electrophoresis was for 90 min at 10 V/cm at 4 °C.

3.6. Preparation of yeast mini Adr1 protein

Yeast mini Adr1 protein (amino acids 17-160) contains a DNA binding domain (ADB) fused to a single activation domain TADII (26). Mini Adr1 protein was expressed in *E. coli* and purified in the laboratory of Dr. E.T. Young, University of Washington, Seattle (26).

4. RESULTS

4.1. Identification of a downstream positive cis-acting region (+114 to +165) of the PS1 promoter

When inserted into the pKT vector, PS1 sequences from –118 to +178 (Figure 1) promoted maximum level of CAT expression into neuroblastoma SK-N-SH cell line, since the CAT activity produced was about 100% of that observed by transfecting pSVCAT into SK-N-SH cells (Figure 2, ref. 17). We have shown previously by deletion analysis and *in vitro* mutagenesis that Ets binding site at -10 controls 80% of PS1 transcription (17,18). On the other hand mutations of Ets site at +65 or +90 or +129 control only 40% of PS1 transcription (18) In this study, deletion of 3' sequences between +178 and +165 produced no change in transcription of the PS1-CAT reporter gene into SK-N-SH cells (Figure 2). Deletion of 3' sequences between +165 and +140 decreased transcription by 50% to the level produced by the fragment from –118 to +178. Further 3' deletion of sequences between +165 and +114 reduced transcription by about 10-fold, to 8-10% of the maximum level observed. Therefore, it appears that a strong positive element controlling more than 80% promoter activity is present between +114 and +165. Since mutation of the +129 Ets site alone accounts for only 40% reduction of PS1 promoter activity (18), it appears that other factor binding sites present in the promoter region (+114 to +165) may be involved in the activation of the PS1 gene. Deletion of sequence from +114 to +107 did not produce any significant effect on PS1 transcription. The region (+114 to +140) of the promoter contains sequence motifs similar to

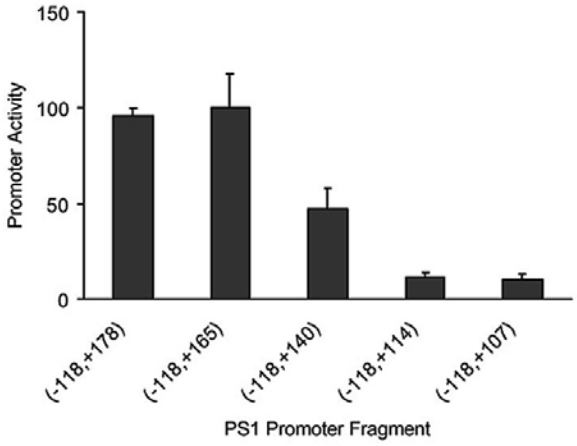

Figure 2. Mapping of 3' sequence determinants required for activity of the human PS1 promoter by deletion analysis. The positions of the 5'- and 3'-ends of each deletion fragment are indicated in the parenthesis. 6 μg of PS1-CAT reporter plasmid and 6 μg of pSV-β-gal plasmid were cotransfected in SK-N-SH cells. Promoter activity was expressed as the ratio of CAT to β-galactosidase activity for each transfected plate. The mean values for each construct (n = 3 or 4) are indicated. S.D. values were 10-20% in all cases. All constructs were tested in at least three different experiments. In all experiments, the relative activity of different constructs was consistent within statistical variations. The construct with the highest activity was expressed as 100%.

consensus for the binding sites of Sp1, Ets, ADR1, and MZF1 transcription factors (Figure 1). Likewise the region (+140 to +165) also contains sequence motifs similar to consensus for the binding sites of ADR1, GATA and MZF1 transcription factors (Figure 1). Hence, this region spanning between +114 and +165 is likely to be of crucial importance for the basal as well as the regulated expression of the PS1 gene.

4.2. Identification of nuclear factor binding sites on the PS1 promoter region (+114 to + 165) by electrophoretic mobility shift assay (EMSA)

We sought to visualize the interaction of nuclear factors with DNA sequences that affect transcription in transient transfection assays by EMSA. In these experiments radiolabeled PS1 DNA fragments extending from +100 to +175, and +100 to +165 generated by PCR were incubated with nuclear extracts prepared from human neuroblastoma SK-N-SH cell line (Figure 3A). There were five major bands, designated B1, B2, B3, B4 and B5 present in the cell extract with fragments (+100 to +175) and (+100 to +165). This data suggests that nuclear factor binding sites are located between +100 to +165. When the probes +106 to +165, and +114 to +165 were used for EMSA (Figure 3B, lanes 4 and 5), band B4 was abolished. This data suggests that the binding site for the nuclear factor corresponding to B4 is located between +100 and +106 and binding sites for nuclear factors corresponding to B1, B2, B3 and B5 are located between +114 and +165. Since binding site for B4 is located outside the positive element (+114 to +165), B4 may not be involved in the upregulation of the PS1 gene by the element (+114 to +165) . When (+100 to +140) fragment was used as a probe in EMSA (Figure 3B, lane 3) band B5 disappeared. This result suggests that binding sites for nuclear factors corresponding to B1, B2, and B3 are located between +114 and +140; and the binding site for nuclear factor corresponding to B5 is located between +140 and +165. This result also appears to suggest that the downstream positive element (+114 to +165) contains two *cis*-acting sequences (+114 to +140) and (+140 to +165). The sequences (+114 to +140) and (+140 to +165) appear to possess positive activity (Figure 2).

4.3. Transcription factor Sp1 interacts with the sequences (+114 to +140) and transcription factor ADR1 interacts with the sequence (+140 to +165)

To further delineate the region, double stranded oligonucleotides containing the binding sites of different transcription factors were used as competitors for EMSA. Radiolabeled DNA fragment from +100 to +165 was used as a template in this experiment. As shown in Figure 4A, Sp1 and (+114 to +140) specific unlabeled probes blocked the formation B1 and B4 complexes (lanes 3, 4 and 17, 18). Ets-specific unlabeled probe (Figure 4A, lanes 7 and 8) did not compete with any of these B1-B5 bands. These data suggest that interaction of Ets with +129 Ets element may be very weak. Similarly ADR1 and (+136 to +175) specific unlabeled probes efficiently competed for the formation of B5 complex (Figure 4A, lanes 15, 16 and 19, 20). These results suggest that B1 and B4 complexes are generated by Sp1 or Sp1 family of transcription factor (s) and B5 complex is formed by zinc finger containing transcription factor ADR1 or an ADR1 related protein. Competition with double-stranded oligonucleotides suggests that complexes B2 and B3 are generated by non-specific protein-DNA interaction. To confirm that B1 and B4 complexes are generated by Sp1 and B5 complex is formed by ADR1, competition experiments were carried out with Sp1 and ADR1 mutant oligonucleotides as well as PS1mutant (+114 to +140) and (+136 to +175) oligonucleotides (Figure 4B). Sp1 mutant and mutant oligonucleotide containing mutation in the Sp1 binding site (+107 to +140)m121 failed to abolish the formation of B1 or B4 complex only (Figure 4B, lanes 4, 5 and 12, 13). On the contrary, a mutant oligonucleotide (+114 to +140)m129 containing mutation in the putative Ets binding site in the sequence (+114 to +140) (Figure 1) competed efficiently for B1 or B4 complex (Figure 4B, lanes 10 and 11). These results confirm that B1 or B4 complex is generated by Sp1 or Sp1 related proteins. Similarly ADR1 mutant, and mutants (+136 to +175)m150 and (+136 to +175)m143 containing mutations in ADR1 binding site failed to abolish B5 band (Figure 4B, lanes 7, 8 and 15, 16, 17, 18). These results suggest that zinc finger protein ADR1 or an ADR1 related protein binds to the sequence (+140 to +165). Therefore, Sp1 and ADR1 appear to interact with the PS1 sequences (+114 to +140) and (+140 to +160) respectively.

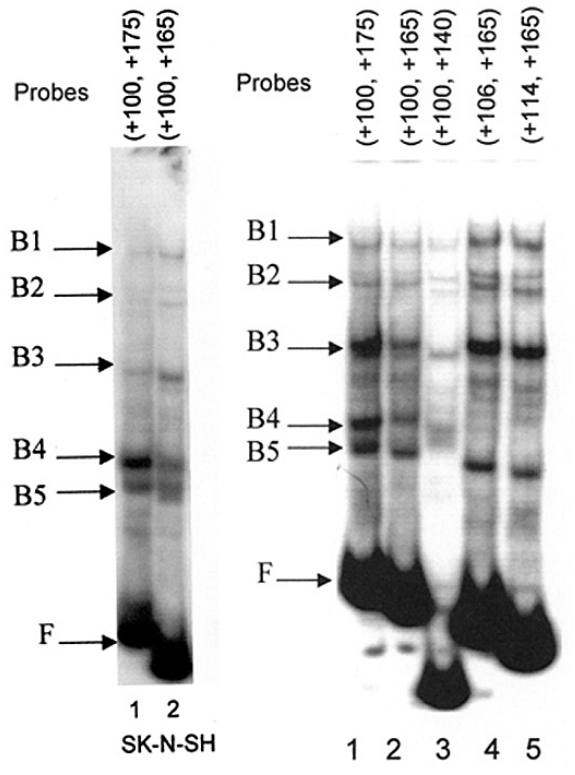

Figure 3. Protein factors bind to PS1 downstream positive *cis*-acting element. A, radiolabeled DNA fragments containing PS1 sequences from +100 to +175 and +100 to +165 (0.1ng of DNA= 20,000 cpm) were incubated with nuclear extracts prepared from SK-N-SH cells and analyzed by EMSA. *Lanes 1 and 2* contain 5 μg of SK-N-SH cell nuclear protein. F denotes the free (unbound) fragment and B1-B5 indicate the position of bands representing protein-DNA complexes. Probes are indicated at the top of each lane. B, Radiolabeled DNA fragments containing PS1 sequences from +100 to +175, +100 to +165, +100 to +140, +106 to +165, and +114 to +165 were used in EMSA using SK-N-SH cell nuclear extracts. All others are same as in A.

4.4. Transcription factors Sp1 and ADR1 bind to the sequences (+114 to +140) and (+140 to +165) respectively but only ADR1-DNA interaction activates PS1 gene transcription

To confirm that Sp1 and ADR1 binding sites are involved in the transcription of the PS1 gene, three nucleotides at the Sp1 (m121), the putative Ets, ADR1 (m129) sites in the sequence (+114 to +140), as well as the ADR1 (m143, m150) consensus in the sequence (+140 to +165) were altered in the (-118, +178) PS1-CAT reporter gene by PCR. These mutant PS1-CAT reporters were cotransfected along with β-gal plasmid into SK-N-SH cells and their respective CAT activities were determined. As shown in Figure 5, mutations in the ADR1 binding sites (-118, +178)m150, (-118, +178)m143 reduced CAT activity by 50% virtually at the same level produced by the deletion mutant (-118 , +140). On the contrary, mutations in the Sp1 binding site (-118, +178)m121 had no effect on PS1 transcription. Mutation on the putative Ets binding site (-118, +178)m129 at +129 reduced PS1 transcription by 40% which is consistent with our previously published data (18). These results appear to suggest that ADR1 or an ADR1 related protein binds to the downstream element (+140 to +165) and activates transcription of the PS1 gene whereas binding of Sp1 transcription factor to the downstream promoter element (+114 to +140) has no significant role in PS1 expression. It also confirms our previously published data that interaction of Ets factor with the +129Ets binding site within the (-114 to +140) element is responsible for transcriptional activity of the positive *cis*-acting element (+114 to +140) (18).

4.5. Yeast Adr1 protein and an ADR1 related protein present in SK-N-SH neuroblastoma cells interact with the (+140 to +165) promoter element

To demonstrate that ADR1 protein is involved in the activation of the PS1 promoter, EMSAs were performed with purified yeast Adr1 miniprotein expressed in *E. coli* (26). As shown in Figure 6A, Adr1 miniprotein interacted strongly with both the wild type ADR1 (lane 1) and mutated ADR1 probes (lane 2). This result is in agreement with the previous observation that mutation (ADR1m) in the central 6 bp (-TTATTA-) sequence flanking the two inverted repeats has no effect on Adr1 binding (22). Figure 6A also shows that Adr1 miniprotein bound very strongly to the wild type promoter fragment (+136, +175) (lane 3). However, in contrast to ADR1m, mutations at the +150 element (+136, +175)m150 (lane 4) abolished the binding of Adr1 drastically. These results suggest that yeast Adr1 protein interacts specifically with the ADR1 consensus sequence present within the element (+140 to + 165). These results also suggest that yeast Adr1 miniprotein behaves differently in recognizing the ADR1 consensus binding sites present in the yeast ADH2 and the human PS1 promoters. To identify mammalian ADR1 protein, EMSAs were performed with SK-N-SH cell nuclear extract (Figure 6B). Several protein-DNA complexes were formed both with wild type and mutated probes. However, a specific protein-DNA complex was formed with both the wild type ADR1 (Figure 6B, lane 1) and the PS1 promoter fragment (Figure 6B, lane 3) and this complex was absent with mutated probes (Figure 6B, lanes 2 and 4, indicated by an arrow). Another faster moving band present with the wild type probe (lane 3) was found to be absent with mutated probe (lane 4). This faster moving band may represent monomer of ADR1 or an ADR1 related protein. Since the mutation at the +150 site (+136, +175)m150 reduced PS1 transcription by 50%, the protein-DNA complex which is abolished by the mutated probe (+136, +175)m150 must be important for PS1 activation. Therefore, these results suggest that an ADR1 related protein present in SK-N-SH cell nuclear extracts interacts specifically with the +150 element and its interaction with the ADR1 binding site requires the presence of the central 6bp sequence (-TTATAA-) (22). Therefore, the ADR1 protein present in SK-N-SH cells appears to be different from the yeast Adr1 protein.

5. DISCUSSION

We have examined the transcriptional regulation of human PS1 expression by linking the promoter

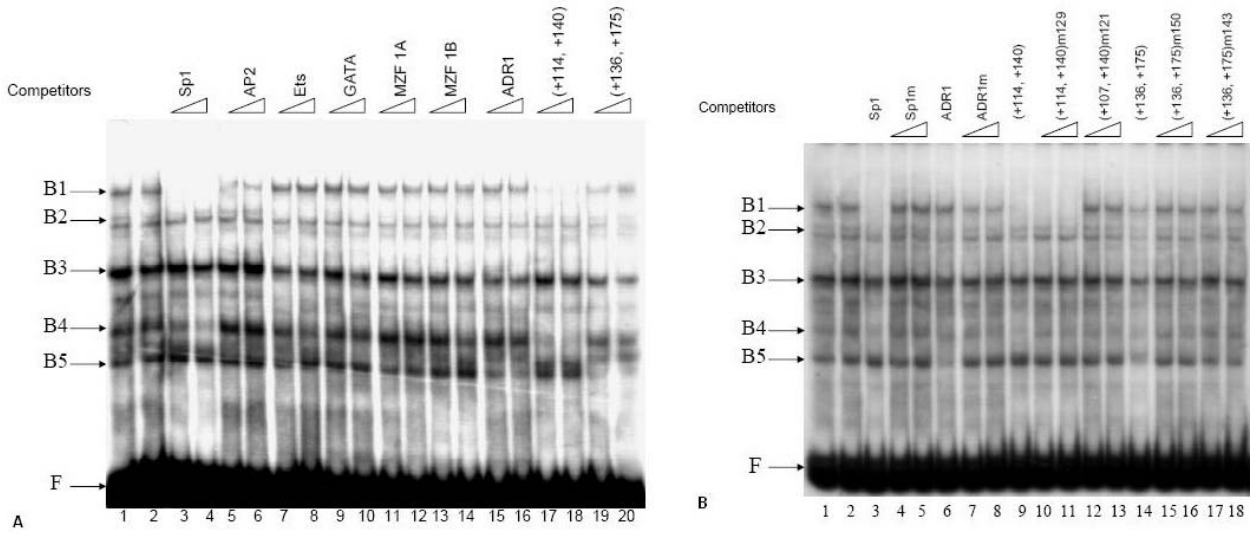

Figure 4. Competition of specific DNA-protein complexes by various DNA fragments of the PS1 promoter and other DNA binding site oligonucleotides. A, a radiolabeled DNA fragment containing the PS1 promoter sequence from +100 to +165 (0.1ng of DNA= 20,000 cpm) was incubated with 5 μg of SK-N-SH cell nuclear extracts and protein-DNA complexes were analyzed by EMSA. Different double stranded oligonucleotides containing binding sites for different transcription factors as well as PS1 promoter sequences as indicated above each lane was used as specific competitors (see "Experimental Procedures"). *Odd-numbered lanes* contain a 100-fold molar excess of unlabeled competitors, while *even-numbered lanes* contain 200 fold molar excess of specific competitors as indicated. *Lanes 1 and 2* contain only 5 μg of SK-N-SH cell nuclear extracts. F denotes the free (unbound) fragment and B1-B5 indicate the position of bands representing protein-DNA complexes. B, same as in A. *Lanes 3, 6, 9 and 14* contain 100 fold molar excess of wild type double stranded oligonucleotide competitors indicated on the top of each lane. *Lanes 4, 7, 10, 12, 15, and 17* contain 100 fold molar excess of mutant double stranded oligonucleotide competitors indicated on the top of each lane. *Lanes 5, 8, 11, 13, 16, and 18* contain 200 fold molar excess of mutant double stranded oligonucleotide competitors indicated on the top of each lane. *Lanes 1 and 2* contain only 5 μg of SK-N-SH cell nuclear extracts. F denotes the free (unbound) fragment and B1-B5 indicate the position of bands representing protein-DNA complexes.

fragment extending from -118 to +178 (with respect to the PS1 start site of transcription) to the CAT reporter gene. When this construct was transfected into human neuroblastoma SK-N-SH cells, maximal level of expression was observed (17). We have previously identified a positive *cis*-acting element at (-22 to +6). The region (-22 to +6) contains an Ets transcription factor binding motif, and a 2-base pair alteration within the core sequence (GGAA to TTAA) of the Ets consensus reduced PS1 transcription by more than 80% (17, 18). We have also shown that Ets1/2 factors bind specifically to the –10 Ets element and activate PS1 transcription which is also modulated by p300 and p53 (27). In the present study, we have mapped a downstream positive *cis*-acting element located in the region (+114 to +165) and have identified at least one transcription factor ADR1 that interacts with the sequence (+140 to +165) within the element (+114 to +165).

3' deletion analysis has suggested that the positive effect of the downstream element is mediated by two distinct sequences located between +114 and +140; as well as between +140 and +165. Computer search for the binding sites of known transcription factors reveals that the sequence (+114 to +140) has the putative binding sites for transcription factors Sp1, Ets, ADR1, and MZF1 whereas the sequence (+ 140 to +165) contains the consensus binding sites for ADR1, MZF1, and GATA (Figure 1). *DNaseI* footprining analysis of the region (+100 to +178) with SK-N-SH cell nuclear extracts failed to identify any protected region. This indicates that protein factors interacting with this region may be present in very small amount in the nuclear extracts or protein-DNA interaction is very weak. Therefore, EMSAs were carried out to identify nuclear factors that interacted with the above two sequences. Competition with wild type Ets binding site oligo (Figure 4A, lanes 7 and 8) and with PS1 promoter specific oligo containing mutation at +129 Ets site (Figure 4B, lanes 10 and 11) indicated that none of the protein-DNA complexes (B1-B4) was generated by Ets transcription factor. However, transcription factor Sp1 binds to the sequence (+114 to +140) but fails to activate PS1 expression (Figure 5). We have previously shown that mutations of the Ets site at +129 alone reduces PS1 transcription by 40% (18) which is consistent with our 3' deletion analysis (Figure 2) suggesting that deletion of sequences from +140 to +114 reduces PS1 transcription by more than 50%. We have also demonstrated that transcription factor ADR1 binds to the sequence (+140 to +165) and activates PS1 transcription.

The yeast transcriptional activator protein Adr1 controls the expression of genes required for ethanol, glycerol, and fatty acid utilization (26). Adr1

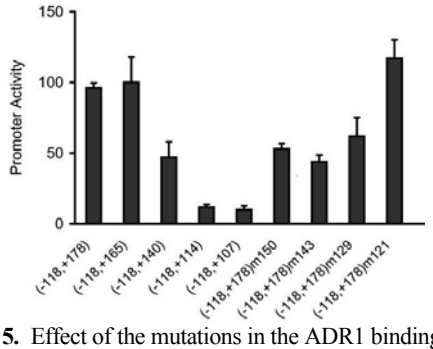

Figure 5. Effect of the mutations in the ADR1 binding site on the transcriptional activity of the PS1 promoter. PS1-CAT fusion genes containing various fragments of the PS1 promoter linked to the CAT reporter gene were transfected into SK-N-SH cells. The end-points of the promoter fragments used in each of the constructs are indicated below the graph. Mutations (m) of three consecutive nucleotides starting at positions +150, +143, +129, and +121 are also indicated below the graph (see Figure 1 and "Experimental Procedures"). Promoter activity was expressed as the ratio of CAT to β-galactosidase activity for each transfected plate. The mean values for each construct (n = 3 or 4) are indicated. S.D. values were approximately 10% in all cases. All constructs were tested in at least three different experiments. In all experiments, the relative activity of different constructs was consistent within statistical variations. The construct with the highest activity was expressed as 100%.

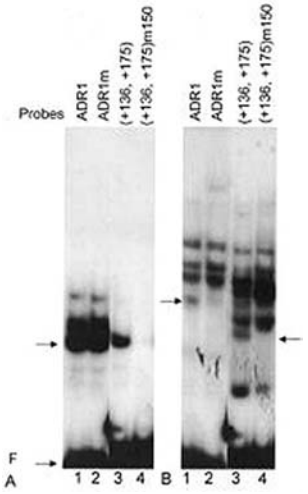

Figure 6. Yeast Adr1 protein and ADR1 or an ADR1 related protein present in SK-N-SH cells bind specifically to the PS1 promoter sequence (+140 to +175). A, radiolabeled double-stranded oligonucleotides containing the binding sites for yeast Adr1 and the human PS1 sequence (+140 to +175) (see "Materials and Methods") (0.1ng of DNA= 20,000 cpm) as indicated above each lane, were incubated with approximately 1 ng of pure yeast mini Adr1 protein. *Lanes 1 and 2* contain wild type ADR1 and mutated ADR1m probes, *lanes 3 and 4* contain wild type (+140 to +175) and mutated (+140 to +175)m150 PS1 probes. *Arrows* indicate the specific protein-DNA complexes. F denotes the free (unbound) probes. B, same as in A. 5 μg of nuclear extracts prepared from SK-N-SH cells were analyzed by EMSAs.

contains two zinc fingers and a region amino terminal to the fingers, which together are essential for DNA binding (26, 28) and functions through UAS1, a perfect 22-bp repeat in the ADH2 promoter (29, 30]. The Adr1 binding site has been identified to be present either 5' or 3' to the start of transcription for several other genes (22, 31). The consensus binding site within each half of the inverted repeat which are essential, comprises of four essential base pairs GG (G/A)G (22). The preferred sequence, TTGG (G/A)GA, is found in both halves of the inverted repeat (22). Each half of the inverted repeat is an independent, functional binding site for one monomer of Adr1. However, both halves of UAS1 must be present in inverted orientation for full transcriptional activation (22). When the central 6 bp of the Adr1 binding consensus (22) were deleted, only a single monomer of yeast Adr1 was able to bind and activation *in vivo* was severely reduced (22). Therefore, it appears that yeast Adr1 does not care about the central 6 bp AT rich sequence. In this report we have shown that the mammalian homologue of the yeast adr1 protein requires the presence of the central 6 bp AT rich sequence for its function (Figure 6B). Therefore, the ADR1 protein identified in this report is distinct from the yeast Adr1 protein. It has been suggested that Adr1 plays an important role in mediating chromatin remodeling (26). It has also been shown that regulated Adr1 binding to the ADH2 promoter requires Snf1 kinase, a yeast homologue of AMP-activated protein kinase and is inhibited by Reg1-Glc7 protein phosphatase (26). It will be interesting to see if the function of the mammalian ADR1 protein identified in this report could also be modulated by protein kinases and phosphatases, and chromatin remodeling is involved in PS1gene activation by ADR1 .

Purified yeast Adr1 protein recognizes the +150 element (+140 to +165) in the PS1 gene and the mutation in the core sequence of ADR1 consensus present in the +150 element (+140 to +165) abolished Adr1-DNA interaction (Figure 6A). We have also shown that a nuclear protein present in the SK-N-SH cell nuclear extract interacts with the ADR1 and the PS1 sequence (+140 to +175) probes (Figure 6B). This protein-DNA interaction has been found to be abolished with the ADR1m and (+140, +175)m150 probes suggesting that mammalian homologue of the yeast Adr1 is present in SK-N-SH cells. Identification and characterization of this novel mammalian ADR1 transcription factor will be useful to understand its role in the transactivation of the PS1 and other mammalian genes.

6. ACKNOWLEDGEMENT

We wish to thank Dr. Elton T. Young (University of Washington, Seattle) for his very generous gift of purified yeast Adr1 miniprotein produced in bacteria, and Dr. Martine Pastorcic for transfection experiments. This work was supported by a grant from the National Institute of Aging, NIH (R01AG18452) to H.K.D. M.L.B was supported by undergraduate fellowships from SMART and McNair summer programs of The University of North Texas Health Science Center at Fort Worth.

7. REFERENCES

1. Rogaev E.I, R. Sherrington, G. Levesque, M. Ikeda, Y. Liang, H. Chi, C. Lin, K. Holman, T. Tsuda, L. Mar, S. Sorbal, B. Nacmias, S. Placentini, L. Amadicci, I. Chumakov, D. Cohen, L. Lennfelt, P. E. Fraser, J. M. Rommens and P.H. St George-Hyslop: Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* 376, 775-778 (1995)

2. Li X and I. Greenwald: Additional evidence for an eight-transmembrane-domain topology for Caenorhabditis elegans and human presenilins. *Proc. Natl. Acad. Sci. U. S. A*. 95, 7109-7114 (1998)

3. Tanzi R. E, D.M. Kowacs, T-W. Kim, R. D. Moir, R. D, S. Y. Guenette and W. Wasco: The gene defects responsible for familial Alzheimer's disease. *Neurobiol. Dis*. 3, 159-168 (1996)

4. Sherrington R, E. I. Rogaev, Y. Liang, E. A. Rogaeva, G. Levesque, M. Ikeda., H. Chi, C. Lin, G. Li, K. Holman, T. Tsuda, L. Mar, J-F. Foncin, A.C. Bruni, M. P. Montesi, S. Sorbi, I. Rainero, L. Pinessi, L. Nee, I. Chumacov, D. Pollen, A. Brookes, P. Sanseau, R .J. Polinsky, W. Wasco, H. A. R. Da Silva, J. L. Haines, M. A. Patricak-Vance, R. E. Tanzi, A. D. Roses, P. E. Fraser, J. M. Rommens and P. H. St George-Hyslop: Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* 375, 754-760 (1995)

5. Selkoe D. J: Translating cell biology into therapeutic advances in Alzheimer's disease. *Nature* 399, 23-31 (1999)

6. Duff K, C. Eckman, C. Zehr, X. Yu, C-M. Prada, J. Perez-tur, M. Hutton, L. Buee, Y. Harigaya, D. Yager, D. Morgan, M. N. Gordon, L. Holcomb, L. Refolo, B. Zenk, J. Hardy and S. Younkin: Increased amyloid-β42 (43) in brains of mice expressing presenilin 1. *Nature* 383, 710-712 (1996)

7. Li Y-M, M. T. Lai, M. Xu, Q. Huang, J. DiMuzio-Mower, M. K. Sardana, X.-P. Shi, K.-C. Yin, J. A. Shafer and S. J. Gardell: Presenilin 1 is linked with gamma-secretase activity in detergent solubilized state. *Proc. Natl.. Acad. Sci. U. S. A*. 97, 6138-6143 (2000).

8. Wolfe M. S, W. Xia, B. L. Ostaszewski, T. S. Diehl, W. T. Kimberly and D. J Selkoe: Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature* 398, 513-522 (1999)

9. De Strooper B. Aph-1, Pen-2, and nicastrin with presenilin generate an active γ-secretase complex. Neuron 38: 9-12 (2003).

10. Yu G., M. Nishimura, S. Arawaka, D. Levitan, L. Zhang, A. Tandon, Y.-Q. Song, E. Rogaeva, F. Chen, T. Kawarai, A. Supala, L. Levesque, H. Yu, D.-S. Yang, E. Holmes, P. Milman, Y. Liang, D. M. Zhang, D. H. Xu, C. Sato, E. Rogaev, E. M. Smith, C. Janus, Y. Zhang, R. Aebersold, L. Farrer, S. Sorbi, A. Bruni, P. E. Fraser and P. H. St George-Hyslop: Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and betaAPP processing. *Nature* 407, 48-54 (2000)

11. Chen F., G. Yu, G. S. Arawaka, M. Nishimura, T. Kawarai,., H. Yu, A. Tandon, A. Supala, Y.-Q. Song, E. Rogaeva, P. Milman, C. Sato, L. Ahang, P. E. Fraser and P. St George-Hyslop: Nicastrin binds to membrane-tethered Notch. *Nat. Cell Biol.* 3, 751-754 (2001)

12. Li X, and I. Greenwald: Membrane topology of the C. elegans SEL-12 presenilin. *Neuron,* 17, 1015-1021 (1996)

13. Wong P. C, H. Zeng, H. Chen, M. W. Becher, D. J. S. Sirinathsinghji, M. E. Trumbauer, H. Y. Chen, D. L. Price, L. H. T. Van der Ploeg and S. S. Sisodia: Presenilin 1 is required for Notch 1 and DIIl expression in the paraxial mesoderm. *Nature* 387, 288-292 (1997)

14. Shen J, R. T. Bronson, D. F. Chen, W. Xia, D. J. Selkoe and S. Tonegawa,: Skeletal and CNS defects in presenilin-1-deficient mice. *Cell* 89, 629-639 (1997)

15. Leem J. Y, S. Vijayan , P. Han, D. Cai, M. Machura, K. O. Lopes, M .L. Veselits, H. Xu and G. Thinakaran: Presenilin 1 is required for maturation and cell surface accumulation of nicastrin. *J. Biol. Chem*. 277, 19236-19240 (2002)

16. Zhou J., U. Liyanage, M. Medina, C. Ho, A. D. Simmons, M. Lovett and K.S. Kosik: Presenilin 1 interaction in the brain with a novel member of the Armadillo family. *Neuroreport,* 8, 2085-2090 (1997)

17. Pastrocic M and H. K. Das: An upstream element containing an ETS binding site is crucial for transcription of the human presenilin-1 gene. *J. Biol. Chem* 274, 24297-24307 (1999)

18. Pastorcic M and H. K. Das: Alternative initiation of transcription of the human presenilin 1 gene in SH-SY5Y and SK-N-SH cells: the role of Ets factors in the regulation of presenilin 1. *Eur. J. Biochem* 271, 4485-4494 (2004)

19. Rogaev E. I., R. Sherrington, C. Wu, G. Levesque, Y. Liang, E. A. Rogaeva, M. Ikeda, K. Holman, C. Lin, W. J. Lukiw, P. J. De Jong, P. E. Fraser, J. M. Rommens and P. St. George-Hyslop: Analysis of the 5' sequence, genomic structure, and alternative splicing of the presenilin-1 gene (PSEN1) associated with early ons*et al*zheimer's disease. *Genomics* 40, 415-424 (1997)

20. Das H. K., T. Leff and J. L. Breslow: Cell type-specific expression of the human apoB gene is controlled by two cis-acting regulatory regions. *J. Biol. Chem*. 263, 11452-11458 (1988)

21. Hullinger T. G, R. L. Panek, X. Xu and S. K. Karathanasis: p21-activated kinase-1 (PAK1) inhibition of human scavenger receptor class B, type I promoter in macrophages is dependent of PAK1 kinase activity, but requires GTPase-binding domain *J. Biol. Chem.* 276, 46807-46814 (2001)

22. Cheng C, N. Kacherovsky, K. M. Dombek, S. Camier, E. Rhim and E. T. Young: Identification of potential target genes for Adr 1p through characterization of essential nucleotides in UAS1. *Mol. Cell. Biol.* 14, 3842-3852 (1994)

23. Gorman C. M., L. F. Moffat, and B. H. Howard: Recombinant genomes which express chloramphenicol acetyl transferase in mammalian cells. *Mol. Cell. Biol*. 2, 1044-1051 (1982)

24. Chuang S. S, H. Zhuang, S. R. Reisher, S. I. Feinstein and H. K. Das: Transcriptional regulation of the apolipoprotein B-100 gene: identification of cis-acting elements in the first nontranslated exon of the human apolipoprotein B-100 gene. *Biochem. Biophys. Res. Commun*. 215, 394-404 (1995)

25. Dignam J. D., R. M. Lebowitz and R. G. Roeder: Accurate transcription by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.*, 11, 1475-1489 (1983)
26. Young E. T, N. Kacherovsky and K. Van Riper: Snf1 protein kinase regulates Adr1 binding to chromatin but not transcription activation. *J. Biol. Chem.* 277, 38095-38103 (2002)
27. Pastrocic M and H. K. Das: Regulation of transcription of the human presenilin-1 gene by Ets transcription factors and the p53 protooncogene. *J. Biol. Chem* 275, 34938-34945 (2000)
28. Hartshorne T. A, H. Blumberg and E. T. Young: Sequence homology of the yeast regulatory protein ADR1 with xenopus transcription factor TFIIIA. *Nature* 320, 283-287 (1986)
29. Eisen A, W. E. Taylor, H. Blumberg and E. T. Young: The yeast regulatory protein ADR1 binds in a zinc-dependent manner to the upstream activating sequence of ADH2. *Mol. Cell. Biol*. 8, 4552-4556 (1988).
30. Shuster J., J. Yu, D. Cox, R. V. L. Chan, M. Smith and E. T. Young: ADR1-mediated regulation of ADH2 requires an inverted repid sequence. *Mol. Cell. Biol*. 6, 1894-1902 (1986)
31. Sleep D, J. E. Ogden, N. A. Robertsand A. R. Goodey: Cloning and characterization of the *Saccharomyces cerevisiae* glycerol-3-phosphate dehydrogenase (GUT2) promoter. *Gene* 101, 89-96 (1991)

Abbreviations: PS, PS1, and PS2, presenilin, presenilin type 1, and presenilin type 2, respectively; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase; bp, base pair (s); DTT, dithiothreitol; EMSA, electrophoretic mobility shift assay. Adr1or ADR1, Alcohol dehydrogenase gene regulator-1

Key Words: Presenilin 1, Transcription, Gene Regulation, Transcription Factors, Zinc Finger Protein

Send correspondence to: Dr Hriday K. Das, Department of Pharmacology and Neuroscience, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107, Tel: 817-735-5448, Fax: 817-735-2091, E-mail: hdas@hsc.unt.edu

http://www.bioscience.org/current/vol13.htm