The Human Synapsin II Gene Promoter

POSSIBLE ROLE FOR THE TRANSCRIPTION FACTORS ZIF268/EGR-1, POLYOMA ENHANCER ACTIVATOR 3, AND AP2*

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Synapsin II is a neuron-specific phosphoprotein that selectively binds to small synaptic vesicles in the presynaptic nerve terminal. Here we report the cloning and sequencing of the 5'-flanking region of the human synapsin II gene. This sequence is very GC-rich and lacks a TATA or CAAT box. Two major transcriptional start sites were mapped. A hybrid gene consisting of the Escherichia coli chloramphenicol acetyltransferase gene under the control of 837 base pairs of the synapsin II 5'-upstream region was transfected into neuronal and non-neuronal cells. While reporter gene expression was low in neuroblastoma and non-neuronal cells, high chloramphenicol acetyltransferase activities were monitored in PC12 pheochromocytoma cells. However, there was no correlation between reporter gene expression in the transfected cells and endogenous synapsin II immunoreactivity. Using DNA-protein binding assays we showed that the transcription factors zif268/egr-1, polyoma enhancer activator 3 (PEA3), and AP2 specifically contact the synapsin II promoter DNA in vitro. Moreover, the zif268/egr-1 protein as well as PEA3 were shown to stimulate transcription of a reporter gene containing synapsin II promoter sequences. In the nervous system, zif268/egr-1 functions as a "third messenger" with a potential role in synaptic plasticity. PEA3 is expressed in the brain and its activity is regulated by proteins encoded from non-nuclear oncogenes. We postulate that zif268/egr-1 and PEA3 couple extracellular signals to long-term responses by regulating synapsin II gene expression.

The synapsins are a family of neuronal phosphoproteins that are localized on the cytoplasmic surface of small synaptic vesicles (for review, see Greengard et al. (1993) and Thiel (1993)). This family consists of four proteins, synapsin Ia and synapsin Iib (collectively termed synapsin I) and synapsin IIa and synapsin IIb (collectively termed synapsin II). These isoforms are generated via alternative splicing from two different genes (Südhof et al., 1989). Molecular cloning of bovine, human, and rat synapsins revealed striking homologies in the amino-termini of all four synapsins. The major difference between synapsins I and II is the presence of a very proline-rich COOH-terminal domain in the synapsin I isoforms that contains clusters of basic amino acids as well as two recognition sites for Ca

Synapsin I has been postulated to link synaptic vesicles to the cytoskeleton, thus regulating the availability of synaptic vesicles for exocytosis. In addition, a role for synapsin I in the regulation of short-term plasticity has recently been suggested (Rosahl et al., 1993). The functional role of synapsin II is not yet clear. However, the fact that synapsin II interacts with both synaptic vesicles (Thiel et al., 1990; Siow et al., 1992) and actin filaments (Chilcote et al., 1994) in a similar fashion as synapsin I suggests that all synapsin isoforms share basic functional properties. This hypothesis was supported by results obtained with mice lacking synapsin I (Rosahl et al., 1993). These mice seem to have no serious impairment of brain function indicating that synapsin II might be compensating the loss of synapsin I.

Synapsins I and II have no non-neuronal counterpart in contrast to the synaptic vesicle proteins synaptobrevin and synaptophysin (Zhong et al., 1992; McMahon et al., 1993). The synapsin genes are therefore good candidates for an investigation of neuron-specific gene expression. Virtually all neurons express the synapsins, but not every isoform of synapsin is expressed in every nerve cell (Südhof et al., 1989). The human and rat synapsin I genes have been analyzed and it was shown that the 5'-flanking region contains neuron-specific enhancer elements (Sauerwald et al., 1990; Thiel et al., 1991). Here, we report the sequence of the human synapsin II promoter. Our studies have revealed similarities as well as distinct differences in comparison with the synapsin I promoter. Moreover, we present data indicating that the transcription factors zif268/egr-1, PEA3, and AP2 regulate synapsin II gene expression.

EXPERIMENTAL PROCEDURES

DNA Cloning—A genomic clone termed pSyC58-10 that contained the synapsin II promoter DNA was kindly provided to us by Thomas Südhof, University of Texas, Dallas, TX. Mapping of the Start Site of Transcription—Human brain RNA (Clontech) was analyzed by S1 nuclease protection assay. To synthesize a single-strand DNA probe complementary to the synapsin II mRNA, a Pst I/Pvu II fragment of the genomic clone pSyC58-10 was inserted into M13 mp18, generating M13SyII-1. Using the oligonucleotide 5'-CCGCCCTTAGGAAATTTATCCTG-3', a primer extension reaction was performed in the presence of radiolabeled dCTP. The extension product was digested with EcoRI and purified on a denaturing polyacrylamide gel. The S1 digestion was done with 10 μg of human brain RNA as described (Wille et al., 1991).

Reporter Constructs—Plasmid pSyCAT-10 has been described previously (Jüngling et al., 1994). Plasmid pSyICAT contains the sequence

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) X89851.

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1 The abbreviations used are: PEA3, polyomavirus enhancer activator 3; CAT, chloramphenicol acetyltransferase; CHO, Chinese hamster ovary; GST, glutathione S-transferase; CMV, cytomegalovirus; LTP, long-term potentiation.
from ~837 to +110 of the human synapsin II gene inserted into the HindIII and XbaI sites of pCAT-Basic (Promega). Plasmids OVEC, JH514ref, and EBS-2/OVEC have been described (Thiel et al., 1994). To construct plasmid EBS-3/OVEC that contains 2 copies of the putative zif268/egr-1 binding site of the human synapsin II promoter, the oligonucleotides 5'TCGAGCTGCCTTCCTCCCTAG-3' and 5'TCGACTTGCAGGAGGGGCGACCCAGCTCGAGTCGCAGGAAGGAGGG-3' were annealed and ligated into the SstI and SalI sites of OVEC and the sites duplicates as described (Wosten et al., 1987). Reporter plasmids SyIPEA3CAT and p38PEA3CAT, containing 1993), and PC12/HD (Gerdes et al., 1991; Ju¨ngling et al., 1994). The synapsin II promoter driven CAT reporter gene was inserted into the minimal promoter consisting of (GCG) package, version 7, Genetics Computer Group.

RESULTS

Cloning of the 5’-Flanking Region of the Human Synapsin II Gene—The sequence of the 5’-flanking region of the human synapsin II gene is shown in Fig. 1A. The promoter does not contain a TATA or a CAAT box. The two major transcriptional initiation sites as well as consensus binding sites for the transcription factors zif268/egr-1, PE3, and AP2 are depicted. The NH2-terminal amino acid sequence of human synapsin II as deduced from the genomic clone revealed typical synapsin specific amino acid changes in comparison to synapsin I, i.e. Phε instead of Tyr, Ser13 instead of Asn, Ile15 instead of Met, and Glu30 instead of Gln. These changes are conserved for mouse, rat, and human synapsin II. The sequence surrounding the transcriptional start sites, as well as the proximal promoter region, is very GC-rich (Fig. 1B), suggesting the possibility that methylation might have an effect upon synapsin II gene regulation (Tate and Bird, 1993). A dot matrix analysis between the human synapsin I and II promoters revealed low homology in comparison to high conservation of the coding region (Fig. 2).

Mapping of Transcription Initiation Sites in the Human Synapsin II Gene—Identification of the synapsin II transcription initiation site was revealed by S1 nuclease mapping. A cDNA probe was synthesized by primer extension from a M13 template that contained synapsin II gene sequence from 301 to ~241 (Fig. 3A). Human brain total RNA was hybridized to this probe as well as total RNA from HeLa cells and yeast tRNA as negative controls. Incubation with S1 nuclease revealed two major protected fragments in the reaction containing human brain RNA (Fig. 3B) but not in the control reactions. Sequences are numbered with +1 being the first transcribed nucleotide corresponding to the start site most 3’ to the ATG codon.

Promoter Activity of the 5’-Flanking Region—Plasmid pSyI-CAT contains 837 nucleotides of 5’-flanking sequence, the transcription initiation site as well as 110 nucleotides of 5’-untranslated region of the human synapsin II gene fused to a promoterless CAT gene. This plasmid was transiently transfected into neuronal and non-neuronal cell lines together with plasmid pCMVβ to correct for variations in transfection efficiencies. Fig. 4A shows that the synapsin II promoter is weak in the neuronal cell lines N205Y, N262, and NG108-15 as well as in the fibroblasts CHO and HeLa. There is virtually no difference in CAT activity between these cell lines. In addition, we analyzed three different clones of the pheochromocytoma cell line PC12. CAT activity was 4–12 times higher in these cells than in the neuroblastoma cells or fibroblasts. As a control we introduced plasmid pSyCAT-10 into these cells (Fig. 4B). The

2 Thiel, G., Petersohn, D., and Schoch, S., (1995) Gene (Amst.), in press.
The synapsin I promoter-CAT gene was highly expressed in both neuroblastoma and PC12 cells, and poorly expressed in non-neuronal cells. Immunoblot analysis of the endogenous synapsins in neuronal cells used here revealed that both synapsins I and II are present in NS20Y, NS26, and NG108–15 cells (Fig. 4C). The expression of synapsins I and II in PC12 cells is in general much lower. A quantitative estimation of the bound iodinated protein A by PhosphorImager analysis revealed that PC12/HD and PC12/SF cells contain only 20 or 13% of the synapsin IIb expressed in NG108–15 cells. There was no correlation between reporter gene expression and endogenous synapsin II immunoreactivity. The synapsin II promoter-CAT gene was transcribed in PC12/NY cells 10 times more efficiently than in NS26 or NG108–15 cells although the endogenous synapsin II levels are comparable. Furthermore, transfection experiments of PC12/HD cells revealed 8 times higher CAT activities than in NS26 or NG108–15 cells despite the fact that endogenous synapsin II levels are much lower in the PC12 clone. Based on the low CAT activities in neuroblastoma cells as well as the
The Zinc Finger Transcription Factor Zif268/egr-1 Binds to the Proximal Region of the Synapsin II Promoter and Functions as a Transcriptional Activator—We recently demonstrated that the synapsin I gene is regulated by zif268/egr-1 (Thiel et al., 1994). The synapsin II 5′-flanking region contains a sequence 5′-CGCCCCCCTC-3′ that is a 8/9 match to the zif268/egr-1 consensus site 5′-CGCCCCCCTC-3′ (Fig. 5A). The zif268/egr-1 binding site located in the synapsin I promoter has been termed EBS-2 (Thiel et al., 1994). By analogy, we refer to the putative zif268/egr-1 binding site in the synapsin II promoter as EBS-3 (Fig. 5A).

To test whether zif268/egr-1 binds to this motif we performed electrophoretic mobility shift assays. As a source for zif268/egr-1, 293 human embryonic kidney cells were transfected with an expression vector encoding zif268/egr-1 and nuclear extracts were prepared. As a control, 293 cells were transfected with an expression vector for PEA3. Fig. 5B shows in the left panel the results obtained with the zif268/egr-1 site derived from the synapsin I promoter. Using nuclear extract prepared from 293 cells containing zif268/egr-1, we observed a specific DNA-protein complex that was absent in the reaction with nuclear extracts from 293 cells transfected with an expression vector for PEA3 instead of zif268/egr-1 (compare lanes 2 and 12, marked by an arrowhead). When unlabelled EBS-2 DNA was added to the reaction, this complex disappeared (lanes 3–5). Unlabelled oligonucleotides encompassing the EBS-3 site from the synapsin II promoter also competed efficiently (lanes 6–8), whereas an unrelated oligonucleotide containing the PEA3 site from the synapsin II promoter did not reduce the intensity of the observed DNA-protein complex (lanes 9–11). On the right panel of Fig. 5B, the results obtained with the probe containing the EBS-3 site are depicted. Nuclear extracts of 293 cells containing zif268/egr-1 revealed a similar DNA-protein complex (lane 14, marked by an arrowhead) that was competed efficiently by an excess of EBS-3 (lanes 15–17) or EBS-2 DNA (lanes 18–20). The control probe containing the PEA3 binding site failed to compete (lanes 21–23), suggesting that zif268/egr-1 recognizes the EBS-3 site in a sequence-specific manner. Moreover, the EBS-3–zif268/egr-1 complex was not detectable in nuclear extracts from 293 cells transfected with a PEA3 expression vector (lane 24). The binding experiments depicted in Fig. 5B show, in addition, unspecific complexes that are present in 293 extracts transfected with either zif268/egr-1 or PEA3 expression vectors. However, the retarded band originating from the presence of zif268/egr-1 is clearly defined. We conclude from these results that zif268/egr-1 binds specifically to the promoter region of the synapsin II gene.

To analyze whether zif268/egr-1 functions as a transcriptional activator for synapsin II gene expression, two copies of the EBS-3 sequence were cloned immediately upstream of the TATA box of the OVEC plasmid that contains the rabbit β-globin gene as a reporter (Fig. 5C). As a positive control, a plasmid containing two copies of the zif268/egr-1 binding site derived from the synapsin I promoter was used. These plasmids were transfected into CHO-K1 cells together with pCMV5 DNA or an expression vector for zif268/egr-1 (Fig. 5C). Plasmid J H514ref containing a mutated β-globin gene under the control of the SV40 promoter/enhancer was included in the transfection to control for variability in transfection efficiency (Thiel et al., 1994). 48 h post-transfection, cytoplasmic RNA of the transfected cells was isolated, hybridized to a β-globin derived cRNA probe, and analyzed by RNase protection mapping (Fig. 5D). When zif268/egr-1 was overexpressed in cells transfected with EBS-2/OVEC and EBS-3/OVEC plasmids, transactivation was observed. A quantitative estimation of the transactivation properties of zif268/egr-1 by PhosphorImager analysis revealed
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Zif268/egr-1 binds to the synapsin II promoter in vitro and transactivates a reporter gene containing zif268/egr-1 binding sites derived from the synapsin II promoter in its upstream regulatory region. A, sequence of zif268/egr-1 consensus sites in the upstream regions of the synapsin I and synapsin II gene; B, electrophoretic mobility shift assay using nuclear extract derived from human 293 cells transfected with expression vectors for zif268/egr-1 (lanes 1–12) or pCMVzif (lanes 13–24). The radiolabeled probes were the zif268/egr-1 site of the synapsin I promoter, termed EBS-2 (left panel, lanes 1–12), and the putative zif268/egr-1 site of the synapsin II promoter (~174 to ~153), termed EBS-3 (right panel, lanes 13–24). Binding experiments without extract are depicted in lanes 1 and 13. Competitor DNA was added to the reaction at 10-fold (lanes 3, 6, 9, 15, 18, and 21), 100-fold (lanes 4, 7, 10, 16, 19, and 22), and 500-fold (lanes 5, 8, 11, 17, 20, and 23) molar excess to the probe. Synthetic oligonucleotides containing the PEA3 binding motif (denoted “PEA3”) were used as an unrelated competitor (lanes 9–11 and 21–23). The arrowheads indicate the protein-DNA complexes originating from zif268/egr-1 binding to its cognate binding site. C, reporter plasmids containing rabbit β-globin as reporter gene, a TATA box (plasmid OVEC), and 2 copies of the zif268/egr-1 binding site of the synapsin I promoter (EBS-2/OVEC) and synapsin II promoter (EBS-3/OVEC). The zif268/egr-1 cDNA is expressed under control of the cytomegalovirus IE gene promoter (pCMVzif). D, reporter plasmids, expression plasmids, and the JHS14ref internal standard plasmid were introduced into CHO-K1 cells. Cytoplasmic RNA was analyzed for β-globin mRNA by RNase protection mapping. The bands labeled test indicate correctly initiated β-globin transcripts, and the bands labeled ref were generated by the internal standard plasmid JHS14ref. RT indicates incorrectly initiated read-through transcripts of the test templates. Also shown is an aliquot of undigested cRNA (riboprobe). Size markers, BstEII cut λ DNA (left) and HaeIII-digested pBR322, are shown in lanes M.

a 5.8- and 3.6-fold increase in transcription for EBS-2 and EBS-3 binding sites, respectively. These results indicate that zif268/egr-1 functions as a transcriptional activator after binding to the zif268/egr-1 motif of the synapsin II promoter.

PEA3 Interacts in a Sequence-specific Manner with the Synapsin II Promoter and Functions as a Transcriptional Activator—The PEA3 motif 5’-AGGAAG-3’ was first recognized in the polya enhancer (Martin et al., 1988). The PEA3 cDNA was recently cloned and the PEA3 mRNA was found to be expressed in brain (Xin et al., 1992). We recognized a sequence identical to the PEA3 motif in the human synapsin I (~638 to ~633) as well as in the human synaptophysin promoter (~657/~652) (Fig. 7A). The synaptophysin promoter is numbered relative to the A of the start codon as +1. To test whether PEA3 binds to the synapsin II and synaptophysin promoters, the PEA3 DNA-binding domain was expressed in E. coli as a fusion protein with Schistosoma japonicum GST (Fig. 6A) and purified by glutathione affinity chromatography (Fig. 6B). The recombinant GST-PEA3 fusion protein was subsequently used in DNA-protein binding assays. Using the PEA3 binding site derived from the synapsin II promoter as a probe, a DNA-protein complex was observed in the absence of competitors (Fig. 7B, middle panel; lane 12). When unlabeled DNA encompassing the synapsin II promoter PEA3 binding site was added to the reaction, the complex disappeared (lanes 13 and 14). Unlabeled oligonucleotides encompassing the PEA3 consensus site as well as the PEA3 site from the synaptophysin promoter also competed with the complex in proportion to the amount of competitor (lanes 15–18), whereas the unrelated oligonucleotide containing the zif268/egr-1-like binding site from the synapsin I promoter (Thiel et al., 1994) interfered only slightly with binding when used at a 100-fold molar excess (lane 20). Incubation of the PEA3 probe derived from the synaptophysin promoter with the GST-PEA3 fusion protein revealed a DNA-protein complex (lane 22) that was competed efficiently by an excess of cold probe or by probes containing the consensus or the synapsin II promoter PEA3 binding site (lanes 23–28). The control oligonucleotide containing a zif268/egr-1 binding site failed to compete (lanes 29 and 30). The PEA3 consensus site (Xin et al., 1993) was tested as a positive control. The GST-PEA3 protein formed a complex with this DNA probe (Fig. 7B, lane 2) that could be competed by an excess of unlabeled probe (lanes 3 and 4) as well as by probes containing the synapsin II and synaptophysin PEA3 binding sites (lanes 5–8). An unrelated DNA probe did not compete with this complex. In the negative con-
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**Fig. 7.** PEA3 binds to the synapsin II and synaptophysin promoters in vitro. A, sequence of the PEA3 consensus site (PEA3 cons.) (Xin et al., 1993). PEA3 (SyII) and PEA3 (p38) denotes sequences of the human synapsin II and synaptophysin promoter that contain the PEA3 binding core motif. B, electrophoretic mobility shift assay using recombinant GST-PEA3 (lanes 2–10, 12–20, and 22–30). The assay was performed with radiolabeled double-stranded, synthetic oligonucleotides listed under A. Binding experiments without fusion protein are depicted in lanes 1, 11, and 21. Competitor DNA was added to the reaction at 10-fold (lanes 3, 5, 7, 9, 13, 15, 17, 19, 23, 25, 27, and 29) or 100-fold (lanes 4, 6, 8, 10, 14, 16, 18, 20, 24, 26, 28, and 30) molar excess to the probe. Synthetic oligonucleotides containing the zif268/egr-1-like motif of the synapsin I promoter (denoted zif268/egr-1) were used as an unrelated competitor (lanes 9, 10, 19, 20, 29, and 30). The arrowheads indicate the protein-DNA complexes originating from GST-PEA3 binding to its cognate binding site. C, DNase I protection analysis was performed with a radiolabeled DNA probe from the human synapsin II promoter (sequence -657 to -302). The lower strand was radiolabeled with the Klenow fragment of E. coli polymerase I including radioactive dCTP. The lane marked with control are the result of a DNase I reaction without adding protein. The other reactions contained either 25 μg of GST or GST-PEA3 fusion protein. The region protected by GST-PEA3 is indicated on the right-hand side.

control experiments no protein was added to the probe. No retarded DNA-protein complex was observed (Fig. 7B, lanes 1, 11, and 21). We conclude from these data that PEA3 interacts specifically with both synapsin II and synaptophysin promoters.

The members of the family of CTS transcription factors bind to the core sequence 5'-A(C/G)GAAAG-3'. The flanking sequences are variable and there is evidence that these sequences are necessary to determine which CTS protein will bind (Wasfy et al., 1992, 1993). We performed DNase I footprinting analysis to identify the sequence of the synapsin II promoter that is protected by PEA3 from DNase I digestion. Fig. 7C shows that the GST-PEA3 protein protects the synapsin II promoter sequence -628 to -647 from DNase I digestion, including the core sequence 5'-AGGAAG-3'. No nuclease protection was observed by adding no protein (control) or GST protein. These data confirm the electrophoretic mobility shift analysis that PEA3 binds specifically to the synapsin II promoter through sequences containing the PEA3 binding motif.

We next asked if PEA3 functions as a transcriptional activator following binding to the synapsin II or the synaptophysin promoter. Using the CAT gene as a reporter, four binding sites for PEA3 from the synapsin II promoter or the synaptophysin promoter were cloned immediately upstream of the minimal promoter, thus generating plasmids SyIIPEA3CAT and p38PEA3CAT, respectively (Fig. 8A). As a negative control, four copies of the zif268/egr-1 site from the synapsin I promoter were inserted into the same vector (plasmid EBS-2CAT). These plasmids were transfected in CHO-KI cells together with the expression vector pCMV5 or an expression vector encoding PEA3. Also transfected in all experiments was a plasmid containing the β-galactosidase gene under control of the CMV promoter/enhancer to correct for differences in transfection efficiencies. The results of this experiment are depicted in Fig. 8B. Overexpression of PEA3 increased transcription of the CAT gene 7.5- and 15-fold, respectively, when PEA3 binding sites derived from the synapsin II or the synaptophysin gene promoter were present in the regulatory region. There was no increase in transcription when zif268/egr-1 binding sites were inserted upstream of the CAT gene. These data indicate that PEA3 functions as a transcriptional activator after binding to the synapsin II or synaptophysin promoter.

The Distal but Not the Proximal AP2 Consensus Site Functions as a Binding Site for Recombinant AP2—AP2 is a sequence-specific DNA-binding protein that interacts with enhancer elements of selected genes to stimulate transcription (Williams et al., 1988). The sequence 5'-CCC(C/A)N(C/G)(C/G)(C/G)-3' has been proposed as the AP2 consensus site (Faist and Meyer, 1992). In the human synapsin II promoter we recognized two sites that matched this consensus sequence (Fig. 9A). We there-
We have analyzed a genomic clone containing the human synapsin II 5'-flanking region. A comparison with the genes encoding the synaptic vesicle proteins synapsin I (Südhof et al., 1990), synaptophysin (Ozcelik et al., 1990), and synaptobrevin II (Archer et al., 1990) revealed that the promoter regions of these genes are very GC-rich and lack TATA as well as CAAT boxes. We also observed that there is no extensive promoter sequence homology between the human genes encoding synapsin I and synapsin II. This lack of homology has also been reported for the mouse synapsin promoters (Chin et al., 1994). Transcription factors interact in general with short consensus sequences, thus a lack in overall homology between the regulatory region of the synapsin genes does not necessarily imply different regulatory mechanisms.

Neuron-specific control elements have been identified in the proximal region of the human, and rat synapsin I promoter (Sauerwald et al., 1990; Thiel et al., 1991). Recently, an analysis of the mouse synapsin II promoter showed that a reporter gene under the control of 5 kilobase pairs of the 5'-upstream region is highly transcribed in the neuronal cell lines PC12, NS20Y, NS26, and NG108–15, but not in the non-neuronal cells HeLa, L6, 3T3, or HepG2 (Chin et al., 1994). In addition, transfection experiments of deletion constructs into HeLa and PC12 cells implicated a neuron-specific core promoter between -79 and +153 of the mouse synapsin II gene (Chin et al., 1994). Our own transfection experiment in PC12 and HeLa cells using the 5'-proximal region of the human synapsin II promoter yielded results similar to those of Chin et al. (1994). There was, however, a striking lack of correlation between the endogenous synapsin II gene expression in PC12 cells and the high transcriptional activity of a transfected synapsin II promoter-CAT construct. We had noted this phenomenon before as problematic in respect to synapsin I gene expression as measured in transfected PC12 cells (Thiel et al., 1991). We therefore found it necessary to test other neuronal cell lines as model systems for measuring synapsin I gene expression. The data we obtained from these neuronal cell lines, along with the non-neuronal cell lines tested, indicated that the 5'-proximal region of the synapsin II promoter does not confer tissue specificity of transcription. Thus, we believe that, as was the case with synapsin I promoter transfection experiments (Thiel et al., 1991), the use of PC12 cells as the sole neuronal model system to define the neuron-specificity of a promoter sequence is ill advised since the high level of transcriptional activity from the transfected synapsins I and II promoter-CAT constructs in these cells, 1) show no correlation with endogenous synapsin levels in these cells and 2) is at variance with the other neuronal cell lines tested.

The synapsin II gene promoter contains binding sites for the transcription factors zif268/egr-1, PEA3, and AP2, transcription factors that have been suggested to be involved in connecting extracellular signals to gene expression. A cartoon depicting the binding sites of these proteins in the synapsin II promoter is shown in Fig. 10.

The zinc finger transcription factor zif268/egr-1 (Christy et al., 1988; Sukhatme et al., 1988), also known as NGFI-A (Millbrandt, 1987) and Krox24 (Lemaire et al., 1988) belongs to a group of genes termed the cellular immediate early genes (Sheng and Greenberg, 1990). Zif268/egr-1 gene expression is highly responsive to neuronal stimulation. In the hippocampal model of long-term potentiation (LTP), the zif268/egr-1 gene is
induced by high-frequency stimulation and this induction is inhibited by N-methyl-D-aspartic acid-receptor antagonists (Cole et al., 1989; Wisden et al., 1990). These findings suggest a potential role for zif268/egr-1 in synaptic plasticity. Recently we presented data indicating that the synapsin I gene is a target for zif268/egr-1 (Thiel et al., 1994). Here, our results suggest that zif268/egr-1 might also regulate synapsin II gene expression. LTP is known to cause an increase in synaptic contact area as revealed by morphological studies (Desmond and Levy, 1988; Schuster et al., 1990; Geinisman et al., 1991).

In addition, LTP-dependent changes in the number of vesicles in the synapse have been reported (Applegate et al., 1987; Meshul and Hopkins, 1990). It seems therefore likely that the concentrations of synaptic vesicle proteins are subject to regulation depending on the actual "need" of a particular neuron. In support of this view, Lynch et al. (1994) showed that LTP induced an increase of synapsin I, synaptotagmin, and synapto
tophysin immunoreactivity in the dentate gyrus. Thus, we propose a signaling cascade where induction of LTP is accomplished by an increase in zif268/egr-1 mRNA and protein (Abraham et al., 1993). The zif268/egr-1 protein translocates to the nucleus and activates synapsin I gene expression that can be monitored 3 h later by immunoblotting with specific antibodies to synapsin I. This cascade can be blocked at early stage by administration of an N-methyl-D-aspartic acid-receptor antagonist prior to induction of LTP. It will be of interest to determine whether synapsin II immunoreactivity also increases following LTP.

PEA3 was recently cloned and analyzed for tissue-specific expression. Detectable amounts of PEA3 mRNA were found only in brain and epididymides (Xin et al., 1992). Here, using DNA-protein binding experiments and transfection analysis, it was demonstrated that PEA3 binds to the synapsin II promoter and transactivates a reporter gene that has synapsin II promoter sequences in its regulatory region. Similar results were obtained for a PEA3-binding site in the synaptophysin promoter, which has been proposed to mediate the effect of protein kinase C and cAMP upon transcription (Imagawa et al., 1992; Berg et al., 1993).

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