Rat \( B_2 \) Sequences Are Induced in the Hippocampal CA1 Region After Transient Global Cerebral Ischemia*

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Global brain ischemia causes cell death in the CA1 region of the hippocampus 3–5 days after reperfusion. The biological pathway leading to such delayed neuronal damage has not been established. By using differential display analysis, we examined expression levels of poly(A) RNAs isolated from hippocampal extracts prepared from rats exposed to global ischemia and found an up-regulated transcript, clone 17a. Northern blot analysis of clone 17a showed an approximately 35-fold increase in the ischemic brain at 24 h after four-vessel occlusion. Rapid amplification of cDNA ends of clone 17a revealed a family of genes (160–540 base pairs) that had the characteristics of rodent \( B_2 \) sequences. In situ hybridization demonstrated that the elevated expression of this gene was localized predominantly in the CA1 pyramidal neurons. The level of expression in the CA1 region decreased dramatically between 24 and 72 h after ischemia. The elevated expression of clone 17a was not observed in four-vessel occlusion rats treated with the compound LY231617, an antioxidant known to exert neuroprotection in rats subjected to global ischemia. Since delayed neuronal death has the characteristics of apoptosis, we speculate that clone 17a may be involved in apoptosis. We examined the expression level of clone 17a in \textit{in vitro} models of apoptosis using cerebellar granule neurons that were subjected to potassium removal, glutamate toxicity, or 6-hydroxydopamine treatment and found that clone 17a transcripts were induced in cerebellar granule neurons by glutamate or 6-hydroxydopamine stimulation but not potassium withdrawal.

A short period of global cerebral ischemia in rodents causes neurons in the striatum, hippocampus, and lateral thalamus to die (1). Intriguingly, pyramidal neurons in the CA1 region of the hippocampus undergo delayed neuronal death 3–5 days after the insult (1, 2). A similar phenomenon occurs in human cerebral ischemia (3). This time lag provides a window of opportunity for therapeutic interventions after ischemic injury. However, the molecular mechanisms that trigger and lead to delayed neuronal death have not been well established, although many hypotheses have been proposed such as excitotoxicity of glutamate, disturbed calcium homeostasis, altered lipid metabolism, free radicals, and mitochondrial involvement (for reviews, see Refs. 4 and 5). There is a growing body of evidence suggesting that apoptotic events occur in both global and focal brain ischemia (6–8). It is possible that many of these hypotheses may in fact represent different aspects of a common mechanism. One of the approaches that can be used to further our understanding of the molecular mechanism of delayed neuronal death is to establish the gene expression profile of the process. Differential expression of many genes has been observed in ischemic brains, including some immediate early genes, heat shock proteins, and factors controlling apoptosis such as \( Bcl2 \), \( Bcl-x \), \( Bax \), and caspases (for reviews, see Refs. 9 and 10).

Differential display (DD)\(^1\) is a technique based on reverse transcription (RT) and PCR (11) and is widely used for identifying genes with altered expression in pathological and special physiological conditions. RT-PCR, open reading frame; PCR, polymerase chain reaction; polIII, RNA polymerase III; RACE, rapid amplification of cDNA ends; RT, reverse transcription; TF, transcription factor.

EXPERIMENTAL PROCEDURES

Animals—Transient forebrain ischemia was induced by the 4VO treatment as described by Pulsinelli and Brierley (17). Briefly, Wistar rats (Hilltop Labs, Scottsdale, PA) were prepared for forebrain ischemia by electrocauterizing the bilateral vertebral arteries and placing atrumatic clamps around the common carotid arteries without interrupting the arterial blood flow. Rats were anesthetized for the whole procedure using 2% halothane inhalation. On the following day, forebrain ischemia was induced by tightening the clamps for 30 min. In the case of sham-treated animals, the carotids were exposed but not occluded. Body temperature was maintained at 37 °C for 1 h during and after the 4VO treatment by means of heat lamps. Animals were sacrificed by decapitation at 24, 48, and 72 h after ischemia or sham operation. All

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank\(^*\)/EBI Data Bank with accession number(s) AF168585, AF168586, AF168587, AF168588, AF168589, AF168590, AF168591, AF168592, AF168593, AF168594, AF168595, and AF168596.

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\(^*\) The abbreviations used are: DD, differential display; 4VO, four-vessel occlusion; 6-OHDA, 6-hydroxydopamine; bp, base pair(s); CGN, cerebellar granule neurons; GSP, gene-specific primer; H&E, hematoxylin and eosin; kb, kilobase(s); ORF, open reading frame; PCR, polymerase chain reaction; polIII, RNA polymerase III; RACE, rapid amplification of cDNA ends; RT, reverse transcription; TF, transcription factor.

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animal procedures were performed in accordance with institutional guidelines. Compound LY231617 (Lilly) was given orally 30 min before 4VO and again 4 h after 4VO at a dose of 50 mg/kg. Control rats received 2% acacia vehicle.

**Cardio-Rat Coronal Granule Neurosphere**—Primary cultures of CGNs were prepared from 8-day-old-Harlan Sprague-Dawley rat pups (Harlan Breeders, Indianapolis, IN) as described by Gallo et al. (18). Cells were dissociated from freshly dissected cerebella by mechanical disruption in the presence of trypsin and DNase (Sigma) and were resuspended in basal medium, Eagle's Life Technologies, Inc.), supplemented with 10% fetal bovine serum, 25 mM KCl, and 0.1 mg/ml gentamicin (Life Technologies, Inc.). Cells were seeded at a density of 1–2.5 × 10^5 cells/ml in poly-L-lysine-coated dishes. Cytosine arabinoside (10 μM; Sigma) was added to the culture medium 24 h after initial plating to arrest the growth of non-neuronal cells. Cultures were maintained at 37 °C in a humidified incubator with 95% air, 5% CO2 and were fed with glucose (10 mM) after 7 days in vitro and thereafter every 4th day. Cultures generated by this method have been shown to contain 95% granule neurons (19). In all experiments, neurons were used after being cultured 7–8 days in vitro.

For low K+ -induced neuronal apoptosis, the concentration of KCl in the conditioned medium was switched from 25 to 5 mM for an overnight treatment. For glutamate-induced apoptosis, the concentration of KCl in the conditioned medium was switched from 25 to 5 mM in a 12-well plate (3.8 cm²/well) and incubated overnight in a humidified incubator with 5% CO2.

**Amplification**—Total RNA was isolated using the RNAgents® total RNA isolation system (Promega Corp., Madison, WI) following the manufacturer's instructions.

**Differential Display**—To remove DNA contamination, 5 μg of total RNA from each animal was incubated at 37 °C for 30 min with 1 unit of amplification grade RNase-free DNase I (Life Technologies, Inc.) in 10 mM Tris-HCl, pH 7.5, and 10 mM MgCl2. The DNA digestion was terminated by adding 1 μl of 0.5 × EDTA and 3 μl of 2 M sodium acetate per 50-μl reaction. RNA was then phenol-extracted and precipitated.

For first strand cDNA synthesis, total RNA (2 μg) pooled from four animals was used for each reaction. The reaction contained 20 pmol of T12G, T12C, or T12A as primers. Other reagents were obtained from the SuperScript II RT kit (Life Technologies, Inc.).

The DD-PCR was performed by 20 ng of first strand cDNA, 20 pmol of each primer, 20 pmol of each dNTP, 1 μl of dNTPs (NEN Life Science Products), 10 units of AmpliTaq Gold® DNA polymerase (Perkin-Elmer) per 60-μl reaction. Thermocycles were performed in a Peltier Thermal Cycler, PTC-225 (MJ Research, Inc., Watertown, MA) as follows: 92 °C, 1 min; 40 times (92 °C, 10 s and 68 °C, 1 min); 68 °C, 2 min and kept at 4 °C thereafter until further processed.

The PCR products were incubated with 15 μl of loading buffer at 95 °C for 2 min. Samples, 3 μl each, were electrophoresed at 1700 V on a 6% polyacrylamide gel containing 8.3 M urea until the xylene cyanol stain was visible. The gel was stained with ethidium bromide to visualize the RNA ladder and to check RNA loading in each lane, some membranes were stripped and rehybridized with probes synthesized from a human actin cDNA (CLONTECH, Palo Alto, CA).

**Northern Blot Analysis**—Total RNA was isolated using the PicoPure RNA isolation kit (Arcturus) according to the manufacturer's instructions. RNA elution and sample loading were done as described previously by Alwine et al. (22). RNA samples were allowed to run off their own weight and were fixed on nylon membranes (Zeta-Probe GT, Bio-Rad) for Southern blot analysis. Biotinylated oligo(dT)17 was used as a probe for hybridization with RNA samples.

**RNA Isolation**—Total RNA was isolated from the hippocampus of 8-day-old CGNs using RNAeasy® kit (Qiagen). The isolated RNA samples were treated with RNAse-free DNAse and were subjected to DNA-free RNA isolation for hybridization with probes synthesized from a human actin cDNA (CLONTECH, Palo Alto, CA).

**Rapid Amplification of cDNA Ends (RACE)**—For full-length cDNA clones, RNA samples were subjected to RACE using the random primed cloning kit (Roche Molecular Biochemicals). Hybridizations were performed in 50% formamide, 0.12 M Na2HPO4, 0.25 M NaCl, and 7% (w/v) sodium dodecyl sulfate (SDS) at 43 °C overnight. The blots were washed twice, each for 10 min, at room temperature in 30 mM sodium citrate buffer, pH 7.0, containing 30 mM NaCl and 0.1% SDS. A third wash was conducted at 65 °C, for 10 min, in 3 mM sodium citrate buffer, pH 7.0, containing 0.3 M NaCl and 0.1% SDS. DNA in RNA loading in each lane, some membranes were stripped and rehybridized with probes synthesized from a human actin cDNA (CLONTECH, Palo Alto, CA).
inserted into the plasmid vector pT7/T3-18 (Ambion, Austin, TX) with T4 DNA ligase (Life Technologies, Inc.). Vectors containing the insert were linearized with EcoRI or BamHI separately at 37 °C, purified with the QiAquick PCR purification kit (Qiagen), and used as the templates to synthesize the probes. Single-stranded RNA probes were synthesized in the presence of 35S-UTP using the riboprobe in vitro transcription system (T3/T7; Promega). Antisense and sense RNA probes were transcribed with either T3 or T7 RNA polymerase corresponding to the promoter region flanking the insert sequence in the T3/T7-18 vector. After in vitro transcription, RNA probes were treated with RNase-free DNase I to remove the template DNA.

The hybridization was conducted with the SureSite hybridization reagents kit (Novagen, Inc. Madison, WI) following the manufacturer’s instructions. Briefly, frozen tissue sections were fixed with 4% paraformaldehyde for 20 min and permeabilized with 1 μg/ml protease K for 10 min at room temperature. Each slide was covered with 80 μl of hybridization buffer containing 106 cpm of RNA probes. Hybridization was performed for 18 h at 50 °C in a humidified chamber. Background controls were performed on 4VO sections using the sense RNA as a probe. For experimental controls, sections of sham-operated animals were hybridized with the antisense probe. After hybridization, unbound probes were digested with RNase A (20 μg/ml) for 30 min at 37 °C. The post-hybridization washes were all performed at 50 °C. Air-dried slides were exposed to Hyperfilm-βMAX x-ray film (American Shamiah Biotech) for 1 h and developed manually in Kodak developer t-19. For higher resolution, sections were covered with 50% Kodak NTA-2 emulsion and exposed for 2.5 h in the dark. The slides were developed, counter-stained with hematoxylin and eosin (H&E), mounted with paramount, and photographed using both light- and dark-field microscopy.

RESULTS

Identification of Clone 17a—To identify differentially expressed genes in response to transient cerebral ischemia, poly(A) RNAs prepared from the dorsal hippocampus of 4VO- and sham-operated rats were compared by differential display analysis. We detected both up- and down-regulated transcripts. However, we focused on an ischemia-induced transcript, clone 17a. Fig. 1 displays the result of DD-PCR that contained clone 17a. The products from each DD-PCR reaction were loaded in two lanes. Each lane contained 70–150 bands. Interestingly, the arbitrary primer sequence was found at both ends of the amplified fragment of clone 17a (Fig. 2). Apparently, the same random primer was used as both 5' and 3' primers in the DD-PCR, and the anchored oligo(dT) primer was left unused. Conceivably, amplification of clone 17a fragments occurred in all DD-PCR reactions containing the random primer 5'-AATCGGGCTG-3', no matter which oligo(dT) primer was used.

Northern Blot Analysis—To confirm the gene expression patterns observed in differential display analysis, the clone 17a fragment was used as the template for probe synthesis in Northern blot analysis. Clone 17a transcripts showed an approximately 35-fold increase in 4VO rats versus sham (Fig. 3). The size range of the transcripts was 160–350 bases. Data base search of clone 17a in GenBankTM and GenEMBLTM using the BLAST and FastA programs revealed regions of homology to rodent B2 sequences and a rat somatotropin intron. To check whether somatotropin mRNA is elevated in the 4VO rat, the fifth exon (1909–2109) of rat presomatotropin gene (GenBankTM accession number J00740 or V01238) was used as a probe for Northern blot analysis. Hippocampal total RNA was loaded, 10 μg per lane, and probed with 32P-labeled fifth exon fragments generated by PCR. A higher level of somatotropin expression was not observed in 4VO rats (data not shown). On the other hand, B2 sequences are believed to be spread throughout the genome by retrotransposition and are found in both introns and exons of other genes (23).

Clone 17a Matches Characteristics of B2 Sequences—To get full-length cDNA, the sequence of clone 17a obtained from DD-PCR was used to design primers for 5' and 3'-RACE (Fig. 2). The 5'-RACE was conducted using GSP1. Heterogeneity at

The sequence of the eighth exon (2219–2419) of rat presomatotropin gene (GenBankTM accession number J00740 or V01238) was used as a probe for Northern blot analysis. Hippocampal total RNA was loaded, 10 μg per lane, and probed with 32P-labeled eighth exon fragments generated by PCR. A higher level of somatotropin expression was not observed in 4VO rats (data not shown). On the other hand, B2 sequences are believed to be spread throughout the genome by retrotransposition and are found in both introns and exons of other genes (23).

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the 5' end was observed in both length and base composition (data not shown); however, the consensus sequences started at GAGATG (Fig. 2). By using GSP2, the 3'-RACE produced multiple sequences as well (Fig. 4). Sequence alignment using GCG software showed a consensus sequence of about 160 bases. Most of the RACE sequences contained open reading frames
(ORF) of either 29- or 40-amino acid peptides. Further analysis of these transcripts revealed promoter regions, box A and B (24), of RNA polymerase III (polIII) within the transcripts. The poly(A) addition signal, transcription stop sequence, and poly(A) tail was found at the ends of most 3' sequences. The sequence results indicate that the transcripts are products of polIII and match the characteristics of rodent B2 sequences.

**Hypothetical Peptide**—The ORFs seen in clone 17a gene family suggest that peptides could be generated by these sequences. Proteins coded by small open reading frames (<100 amino acids) belong to a number of important categories, such as ATPase modulators, stress proteins, transcriptional regulators, and antioxidants (for review, see Ref. 25). To see whether the clone 17a gene is translated, a rabbit polyclonal antibody was generated against the synthetic peptide CSS-RGHEFNSQQPHGGSQPSVKRSD deduced from the clone F3 sequence (Fig. 4). The full-length peptide is predicted to have a molecular mass of 4.4 kDa and an isoelectric point of 8.7. To search for the hypothetical peptide, proteins were extracted from the hippocampus of 4VO and sham rats and analyzed, 80 ug per lane, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. The peptide was not detected by the antibody raised against the synthetic peptide. Transfection of 293 cells with the clone F3 sequence in a pcDNA3.1 vector yielded no detectable peptide expression. To date, translation of B2 sequences has not been reported.

**Clone 17a Transcripts Are Localized in Pyramidal Neurons**—To examine the tissue-specific expression of clone 17a in rat brain, an antisense riboprobe was transcribed from clone 17a and hybridized with rat brain sections. After x-ray film exposure, strong expression was detected mainly in the CA1 region of 4VO hippocampi (Fig. 5A). Some expression was observable in the cortex of 4VO brains as well. Brain sections of sham-treated rats showed a background level expression with the antisense probe (Fig. 5B). The specificity of the hybridization reaction was confirmed by comparison of signals generated from the antisense probe (Fig. 5A) and the sense probe (Fig. 5C) on 4VO brain sections. By using emulsion, higher resolution (1000 x) was achieved (Fig. 6). In the CA1 region of a 4VO hippocampus, silver grains were found in the cytoplasm of pyramidal neurons. Since sections were counter-stained with H&E, nuclei are shown as dark gray areas in the bright-field image. In dark-field microscopy, silver grains reflect light and are shown as bright particles in a dark background. In the same brain region of sham animals, silver grains were also observed around nuclei with a much less density. The same observation was made in brain sections derived from five individual animals. This result confirms the differential expression of clone 17a in 4VO rats discovered by RT-PCR and Northern blot analysis and is consistent with the fact that B2 poly(A) RNAs are mainly distributed in the cytoplasm (26).

**Animals Treated with LY231617**—The antioxidant LY231617 has been shown to reduce delayed neuronal death in the CA1 region (27). Although LY231617 possesses antioxidant activity as one of its properties, the precise mechanism through which LY231617 prevents neuronal injury after global ischemia is not clear. To establish that clone 17a gene is closely related to delayed neuronal death, rats were treated with the compound as described above. At 24 h post-ischemia, com-
pound-treated animals had a background level expression of clone 17a much less than those of untreated 4VO rats (Fig. 7). This result suggests that clone 17a expression was closely associated with neuronal degeneration. The same blot also demonstrates that a higher level expression of clone 17a occurred around 24 h post-ischemia and was not observed at 72 h post-ischemia (Fig. 7). Decreased expression of clone 17a beyond 24 h post-ischemia was also observed in the CA1 region probed with the antisense RNA (Fig. 8), which suggests that induction of clone 17a transcripts was an early event in delayed neuronal death. Interestingly, an increasing amount of B2 transcripts in the dentate gyrus was detected over the same period (Fig. 8).

**Clone 17a Was Induced by Glutamate and 6-OHDA in CGNs**—Delayed neuronal death has the morphological and biochemical characteristics of apoptosis (6, 8, 28). We postulated that clone 17a may be involved in apoptosis. B2 induction has been observed in the apoptotic cell death of PC-12 cells upon nerve growth factor deprivation (29). To investigate the possible involvement of clone 17a in apoptosis, in vitro models of neuronal apoptosis in cultured CGNs were examined for the expression of clone 17a. CGNs from early postnatal rats can be maintained in the culture medium containing serum and high potassium (25 mM) (30). The apoptotic cell death of cultured CGNs can be induced by switching potassium to a lower but more physiological concentration (5 mM) (31). Relatively low concentrations of glutamate or 6-OHDA, as applied in this...
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study, are also causative factors of CGN apoptosis (32, 33). Glutamate is a known potent excitotoxin critical for the neuronal damage in ischemic stroke (34). 6-OHDA is proposed to be the endogenous toxin involved in the neuronal damage of Parkinson’s disease (35). In contrast to low K⁺ model, glutamate-induced apoptosis does not require RNA or protein synthesis but does require post-translational activation (32).

In Northern blot analysis, total RNA isolated from apoptotic and control CGNs was loaded 10 μg per lane. Induction of clone 17a transcripts was found most significant in the glutamate- and 6-OHDA-induced cell death (Fig. 9, lanes 3 and 4). However, a higher expression level of clone 17a was not detected in the low K⁺-induced cell death (Fig. 9, lane 2). It is known that apoptosis of CGNs can be induced by different stimuli and environmental conditions and that different intracellular mechanisms are likely to be involved (36). Results in this study indicate that B₂ expression was associated with certain types of apoptosis. Since it is easy to detect, B₂ sequences could be used as a marker to detect or distinguish different types of cell death. Further experiments are needed to establish its specific expression in apoptosis and whether it is apoptotic or protective in delayed neuronal death.

**DISCUSSION**

The primary goal of this study is to identify genes involved in ischemic cell death in the rat 4VO model. We are interested, specifically, in early gene products that mediate or control delayed neuronal death. The rats that we used in this study were sacrificed 24 h after the 4VO treatment. At this time point, there is no histological evidence of CA1 neuronal damage (16). Extensive neuronal damage occurs at 72 h after ischemia.

In mammalian genomes, there are families of highly repeated DNA sequences, including long and short interspersed elements (37). B₂ sequences are a family of short interspersed elements consisting of about 10⁵ related sequences dispersed throughout the genome (23, 38). The consensus region of B₂ sequences is 180 bp long and contains the RNA polymerase III promoter, poly(A) addition signal, and transcription stop signal (24). A member of the B₂ family deviates 3–5% in the consensus region (38). Sequences at the 3′ end following the consensus sequence are most variable. On Northern blots, B₂ sequences are generally displayed as a smear in the range of 200–600 bp (39). The function of B₂ sequences has not been established, although a general regulatory role in gene expression and RNA processing has been suggested (38). The RACE products of clone 17a contained multiple sequences with variable 5′- and 3′-flanking regions and possessed all the structural characteristics of B₂ sequences. Northern blot analysis showed that the size range of clone 17a expressed in 4VO rats was between 160 and 540 bp. Sequence analysis revealed that the RACE products contained the internal promoter regions of polIII and a consensus region of approximately 160 bp with less than 4% deviation. The length of the poly(A) tail obtained by RACE is not the authentic length of poly(A) tail but the length of the oligo(dT) primer used for first strand cDNA synthesis. Although these transcripts contained ORFs, the translated product was not detected. B₂ poly(A) RNAs are believed to be the final functional products involved in regulation of mRNA processing, transport, stability, and translation (26, 40, 41). In situ hybridization demonstrates that these transcripts were predominantly located in the neurons within the hippocampal CA1 region, indicating that B₂ sequences may be closely related to the distinct effect of 4VO on pyramidal neurons. The specific association of B₂ sequences in delayed neuronal death was further manifested in LY231617-treated 4VO animals, where the compound-protected neurons in the CA1 region showed background levels of clone 17a transcripts.

Although expression of many genes is found to be altered during ischemic injury (10), the mechanism of delayed neuronal death is still vague. Many histological and biochemical observations in delayed neuronal death match features of apoptosis (6, 8, 28). Induction of several regulatory genes of apoptosis is associated with delayed neuronal death. For example, both mRNA and protein of Bax are expressed prior to delayed neuronal death in the CA1 region (42, 43). The anti-apoptotic gene bcl-2 is expressed in neurons that have survived delayed neuronal death (44). Increased levels of nuclear factor κB in the nuclei of pyramidal neurons are observed after ischemic injury (45). Nuclear factor κB has been shown to both promote (46) and suppress (47) apoptosis depending on conditions and cell types. Finally, caspase-3 and other caspase activities are also observed in delayed neuronal death (8). The finding that clone 17a was expressed in the glutamate- and 6-OHDA-induced apoptosis of CGNs is a suggestion of B₂ involvement in apoptosis and further indicates that delayed neuronal death was mediated by apoptosis. However, whether B₂ sequences promote neuronal apoptosis or protect neurons from delayed neuronal death needs further investigation. Since the amount of clone 17a transcripts decreased at 48 and 72 h in situ (Fig. 8) while cell death increases in the same time frame (16), high expression of the transcripts at 24 h post-ischemia is unlikely the consequence of cell death. It is known that DNA fragmentation caused by methyl methanesulfonate does not induce B₂ expression (48). The differences in clone 17a expression among the low K⁺-, glutamate-, and 6-OHDA-induced cell death in CGNs may be due to the severity of apoptosis or may reflect the differences in their apoptotic pathways. It is known that glutamate- or 6-OHDA-induced apoptosis in CGNs is p53-dependent (49, 50) and involves loss of mitochondrial function (51, 52), whereas low K⁺-induced apoptosis is p53-independent (53) and does not depend on a loss of mitochondrial function (54). It would be of interest to know whether B₂ sequences play a specific role in the apoptotic cascade and whether altered B₂ expression can affect apoptosis.

B₂ expression can be stimulated under various conditions. The level of B₂ transcripts is high in early embryos (40, 55) and virally transformed cells (56) and is low or absent in differentiated cell types (57). Transcription of B₂ sequences can be induced in somatic cells by heat shock (58, 59) and serum stimulation (60). Interestingly, apoptosis has been observed under similar circumstances (61, 62). B₂ sequences are transcription products of polIII. The possible involvement of B₂ sequences in apoptosis can be unveiled further by examining induction of polIII activity in various viral transformations.
Adenovirus E1A (63), simian virus 40 T antigens (64), X-protein of the human hepatitis B virus (65), and Tax protein of the human T-cell leukemia virus type 1 (66) are known to trigger apoptosis in mammalian cells. On the other hand, cells transformed with these agents exhibit an increased level of polIII transcripts (67–70).

During transcription of B2 sequences, polIII activity is modulated by transcription factors TFIIIB and TFIIIC (71). The rapid increase in B2 RNA levels is most likely mediated by these factors. SV40 T antigen is a transactivator of polIII (72), which increases activity of TFIIIB by changing its abundance or phosphorylation state (73). TFIIIC has been shown to be the rate-limiting factor in the formation of polIII initiation complexes (71) and probably the target of trans-activators (74). The ability of X-protein to activate polIII is due to its interaction with TFIIIB rather than TFIIIC (69). During X-protein activation, polII catalytic activity remains unchanged. TFIIIB is also associated with TFIIIC rather than TFIIIC (69). During X-protein activation, polII catalytic activity remains unchanged. TFIIIC has been shown to be the rate-limiting factor in the formation of polIII initiation complexes (71) and probably the target of trans-activators (74). The rate-limiting factor in the formation of polIII initiation complexes (71) and probably the target of trans-activators (74).

The action of B2 sequences can be predicted from their ability to serve as retroposons (75) and to interact with mRNAs (41). All these could serve as important mechanisms for gene regulation in delayed neuronal death.
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