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Rat B-50 gene transcription and translation

Bart J.L. Eggen [a,1], Dieta Brandsma [a], Marcellé Kasperaitis [c], Willem Hendrik Gispen [b], Loes H. Schrama [a,*]

[a] *Laboratory for Physiological Chemistry, Rudolf Magnus Institute for Neurosciences, Utrecht University, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands*

[b] *Department of Medical Pharmacology, Rudolf Magnus Institute for Neurosciences, Utrecht University, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands*

[c] *Department of Molecular Cell Biology, Padualaan 8, 3584 CH Utrecht, The Netherlands*

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Abstract

Previously we reported that the rat B-50/GAP-43 gene contains two promoters (P1 and P2). This study describes the contribution of these two promoters to the mRNA population in several paradigms leading to an altered B-50 mRNA expression. In 8-day-old rat brain we found that P1 transcripts (1676 ± 50 nt) account for 5% and P2 transcripts (1462 ± 46 nt) for 95% of the B-50 mRNAs. The expression of P1 and P2 derived transcripts is high at postnatal day 8 and the ratio between the amount of transcripts derived from P1 and P2 did not change during (embryonal and postnatal) development or aging. After peripheral nerve crush or transection B-50 mRNA expression is induced in the distal nerve stump. The amount of transcript in the nerve stump distal of the lesion derived from both P1 and P2 was increased and the ratio between P1 and P2 transcripts was not altered. To determine whether both P1 and P2 transcripts are translated, a polyribosomal profile from 8-day-old rat brain was generated. Northern analysis showed that both transcripts were associated with approximately four ribosomes. Since no change could be found in the activity in either of the two promoters under the different circumstances tested, we conclude that the activity of the two rat B-50 gene promoters is regulated by a similar mechanism.

Keywords: B-50/GAP-43; Promoter; Transcription; Translation; Polyribosome

1. Introduction

During development or regenerative outgrowth of the nervous system a specific set of growth associated genes is expressed [26]. The expression of these genes is both spatially and temporally regulated. One of the growth associated proteins involved in the development and regeneration of the nervous system is B-50, also known as GAP-43, pp46, neuromodulin and F1. B-50 is a 24 kDa membrane-associated calmodulin-binding phosphoprotein predominantly localized at the axonal growth cone [6,27]. A role for B-50 has been suggested in neuritic outgrowth and synapse formation, neurotransmitter release and in the process of regenerative neurite outgrowth [8,27,28]. The expression of B-50 is greatly enhanced during development and is mostly neuron-specific [1]. Transient expression is observed in non-neuronal cells of the developing chicken limb [29], glial cells [5] and in reactive Schwann cells during Wallerian degeneration following peripheral nerve lesion [21,31]. Vanselow et al. [33] showed that a crush lesion of the sciatic nerve in mice transgenic for the B-50 promoter fused to the bacterial *LacZ* gene increased the expression of β-galactosidase in the dorsal root ganglia and in a population of motor neurons in the ventral horn of the spinal cord at lumbar segments 4–5. Nuclear run-on studies showed that the rate of transcription was responsible for the abundance of B-50 mRNA in developing versus adult rat brain [1]. The rate of transcription was however unaffected during regenerative outgrowth of the goldfish optic nerve [20] or by NGF in PC12 cells [12,20]. Furthermore, it has been shown that rat B-50 promoter constructs could be used as a template for initiation of transcription in developing *Xenopus laevis* embryos [34] and zebrafish [22]. In PC12 cells it has been shown that the TPA-induced increase in B-50 mRNA was due to an increased stability

* Corresponding author. Fax: (31) (30) 539035. E-mail: schrama@med.ruu.nl

[1] Present address: Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, NY 11794-5230, USA.

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of the messenger [19]. Thus, regulation of B-50 gene expression occurs at the both the transcriptional and the post-transcriptional level.

In this study we confirmed that the B-50 mRNA population exists of two sizes transcripts, T1 and T2 (from promoters P1 and P2 respectively) and that T2 accounts for approximately 95% of the total population in 8-day-old rat brain [9]. Additionally we attempted to discern whether the relative abundance of the two transcripts is altered when a change in B-50 mRNA expression occurs. We investigated whether the activity of these two promoters is altered during (embryonal and postnatal) development or aging.

Lesioning of the sciatic or the facial nerve by crush or transection induces B-50 protein and mRNA expression in the distal nerve stump associated with Schwann cells as was determined by immunocytochemistry [32,35] and in situ hybridization [21]. Crush lesioning of the sciatic nerve results in re-innervation of the target cells in muscle or skin within 3 weeks following crush, whereas transection of the nerve results in permanent separation of the proximal and distal nerve portion. We determined whether the induced expression of the B-50 gene could be attributed to induction of one or two promoters.

In vitro translation studies of B-50 cRNAs mimicking the native B-50 transcripts suggested that only T2 transcripts were translated [10]. In this study we compared the in vivo translation efficiency of the two transcripts by generating a polyribosomal profile and identifying the polyribosomal fractions containing the respective transcripts.

2. Materials and methods

2.1. Construction and purification of plasmid DNA

B-50 cDNA clones with an extended 5′ end were generated by insertion of a 0.8 kb genomic fragment in the *Acc*I site of pG3F1 (a generous gift from Dr. A. Routtenberg [23]). The resulting insert was transferred to a pGEM3Z plasmid (Promega) using *Bam*HI-*Sac*I generating pG3ZF1BS, a cDNA with a 5′ end at −1016. To generate a cDNA with a 5′ end at −481, pG3ZF1X, an *Xba*I fragment, was excised from pG3ZF1BS and religated. To create in vitro transcripts with a 5′ end at −40, a B-50 cDNA, isolated by Nielander et al. [17] was excised as an *Eco*RI fragment and blunt ligated in an *Sph*I linearized pGEM3Z generating pG3ZS. All plasmids used for in vitro transcription were isolated using alkaline lysis [24] and purified by two rounds of CsCl density centrifugation [24].

2.2. Animals, surgery and dissection

The surgery and dissection methods used are essentially as described in Plantinga et al. [21]. In short, male Wistar rats (120–140 g, TNO, Zeist) were anesthetized with Hypnorm (Duphar, Weesp, The Netherlands) at a dose of 0.8 ml/kg body weight. The left and right sciatic nerves were either crushed or transected as described in detail by de Koning et al. [7] (crush) and Plantinga et al. [21] (transection). At various time-points after nerve damage the rats were sacrificed by decapitation, the distal and proximal nerve segments were dissected and RNA was isolated. The experimental protocols used involving laboratory animals were reviewed and approved by the Animal Welfare Officer of the Utrecht University and are registered under number FDC 94004.

2.3. RNA isolation

Total RNA from rat brain and sciatic nerves was prepared using RNAzol (Cinna/Biotecx, Friendswood, USA), a isothiocyanate/phenol based single-step extraction method according to the manufacturer's protocol. Poly(A)$^+$ RNA was purified using the PolyATract mRNA isolation system (Promega) according to the supplier's protocol. The amount of total and poly(A)$^+$ RNA was determined spectrophotometrically by UV absorption at $A_{260\ nm}$.

2.4. Labeling of probes, Northern blotting and hybridization

DNA probes were prepared by random primed labeling of PCR products or inserts of plasmids according to the manufacturer's protocol (Boehringer Mannheim). RNA was separated on formaldehyde agarose gels and blotted on GeneScreen membranes (DuPont) using a posiblotter (Stratagene). The blots were hybridized overnight in 0.5 M Na_2HPO_4/NaH_2PO_4, pH 7.2, 7% SDS and 5 μg/ml denatured herring sperm DNA at 65°C, the final washing step was performed in 0.01 M Na_2HPO_4/NaH_2PO_4, pH 7.2, 1% SDS and 1 mM EDTA at 65°C.

2.5. In vitro transcription, dot blot and quantification

Uncapped RNA was synthesized on a linearized plasmid using the SP6/T7 transcription kit according the protocol supplied by the manufacturer (Boehringer Mannheim). RNA dot blot analysis was performed according to the Hybond N protocol of Amersham. Northern blots and dot blots were quantified using a BAS1000 Fuji Phosphoimager (Raytest, Germany).

2.6. Isolation of polyribosomes by iso-kinetic sucrose gradient centrifugation

Polyribosomes were isolated by homogenization of 8-day-old rat brains (n = 15) in 20 ml homogenization buffer (100 mM NH_4Cl, 5 mM $MgAc_2$, 0.2 M sucrose, 14 mM β-mercaptoethanol, 1 μg/ml cycloheximide and 20 mM Tris-HCl, pH 7.5) in the presence of the translation in-

hibitor cycloheximide. After centrifugation for 20 min at 20,000 × *g* at 4°C sodium desoxycholate was added to the supernatant to a final concentration of 1% (w/v). In a SW27 (Beckman) ultracentrifuge tube 22 ml of this supernatant was layered on a discontinuous gradient of 8 ml 1.8 M sucrose and 8 ml 1.0 M sucrose in homogenization buffer and centrifuged for 18 h at 26 krpm at 4°C. The polyribosomal pellet was resuspended in 1 ml 4 mM $MgAc_2$, 100 mM KAc, 0.25 M sucrose, 0.1 mM EDTA and 20 mM Tris-HCl, pH 7.6 using a type A dounce homogenizer. In a SW41 (Beckman) ultracentrifuge tube 20 $OD_{260\ nm}$ of the polyribosomes were layered on a

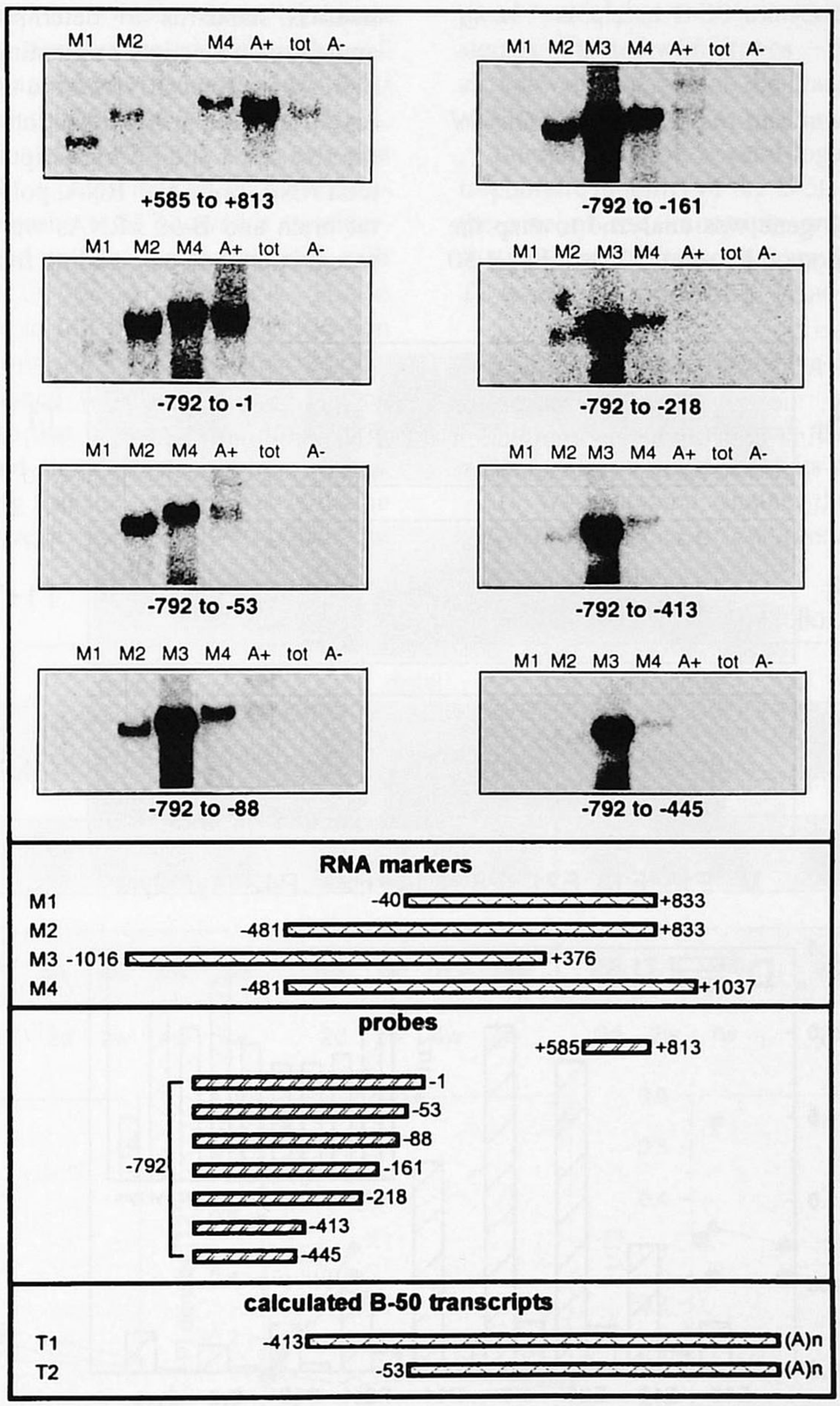

Fig. 1. Contribution of P1 and P2 transcripts to the B-50 mRNA population in 8-day-old rat brain and their respective length. Four B-50 cRNAs (see panel RNA markers) were generated by SP6 in vitro transcription of (1) *Dra*I linearized pG3ZS (M1, 873 nt), (2) *Dra*I linearized pG3ZF1X (M2, 1314 nt), (3) *Nae*I linearized pG3ZF1BS (M3, 1392 nt) and (4) *Ssp*I linearized pG3ZF1X (M4, 1518 nt). A dot blot containing 25, 50, 100 and 200 pg of each in vitro transcript was prepared and hybridized with probe −53, quantified by phosphoimaging and the amount of each marker resulting in equal hybridization signals was calculated using linear regression. Phosphoimages of Northern blots containing 4 μg poly(A)$^+$ RNA and 16 μg poly(A)$^-$ RNA (lanes A +), 20 μg total RNA (lanes tot), 20 μg poly(A)$^-$ RNA (lanes A −), 87.5 pg M1 (lanes M1), 100 pg M2 (lanes M2), 630 pg M3 (lanes M3) and 645 pg M4 (lanes M4) are shown. To the four in vitro transcripts (M1–M4) 20 μg poly(A)$^-$ RNA was added. At the bottom of each phosphoimage the 5′ and 3′ end of the used probe is indicated. The location B-50 in vitro transcripts (panel 'RNA markers'), probes (see panel 'probes') and the calculated T1 and T2 transcripts (see panel 'calculated B-50 transcripts') with their relative positions are shown, the numbers indicate the 5′ and 3′ ends, the adenosine of the translation start codon is numbered +1 and the poly(A) tail of the mRNA is indicated by (A)n.

continuous iso-kinetic gradient from 33.5% to 15% sucrose and centrifuged at 41 krpm for 90 min at 4°C. The gradient was fractionated and the $A_{260\ nm}$ absorption determined using an Uvicord (LKB). RNA was extracted using the method described by Chomczynski and Sacchi [4] and Northern blots were prepared.

3. Results

3.1. Mapping of the amount and the size of promoter P1 and P2 transcripts

The 5′ end of the B-50 gene was analyzed to map the contribution of the promoters P1 and P2 to the B-50 mRNA population and to measure their respective transcripts sizes. B-50 cDNA clones were extended at their 5′ end and used as templates for in vitro transcription. These in vitro transcripts (M1 to M4) differed in both their size (873, 1314, 1392 and 1518 nt) and their 5′ ends (−40, −481, −1016 and −481 as 5′ nucleotide (nt) respectively, see Fig. 1, panel 'RNA markers') and were used as internal standards to determine the ratio between the amount of transcripts originating from P1 and P2. Furthermore, these four cRNAs were used as a standard to correct for differences in hybridization conditions and to estimate the size of P1 and P2 transcripts. Northern blots containing total RNA, poly(A)$^+$ RNA, poly(A)$^-$ RNA from 8-day-old rat brain and B-50 cRNAs were hybridized with a probe recognizing the open reading frame (ORF, +585 to +813)

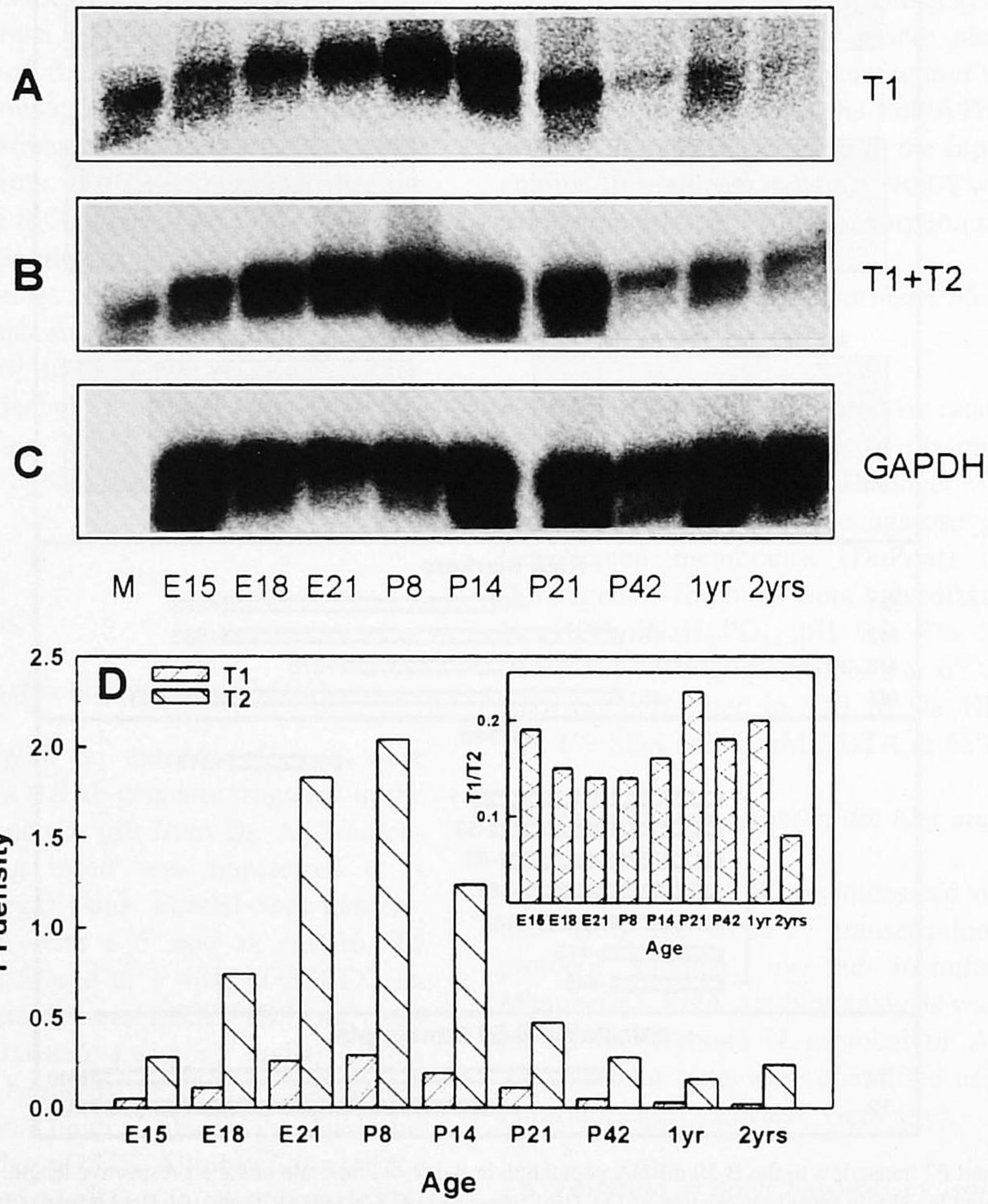

Fig. 2. Amount of T1 and T2 transcripts during (embryonal) development or aging. Phosphoimages of a Northern blot hybridized with B-50 probes T1 (−465 to −218, panel A) and T1 + T2 (+585 to +813, panel B) and an autoradiograph of an hybridization with a GAPDH probe (panel C) are shown. Each lane contained 2.5 μg poly(A)$^+$ RNA isolated from E15, E18, E21, P8, P14, P21, P42, 1 year and 2 years old rat brain. Lanes M contain 200 pg of B-50 in vitro transcript M2 (1349 nt, see Fig. 1); to each sample tRNA was added to a final quantity of 10 μg RNA per lane. The relative amounts of T1 and T2 transcripts expressed during different developmental stages were quantified using a BAS1000 Phosphoimager and are expressed as phosphoimage density (PI density, panel D). After quantification and correction for differences in hybridization using the B-50 cRNA marker the signal of T2 was obtained by subtraction of the corrected T1 signal from the corrected ORF signal. The inset of panel D shows the ratio between the amount of T1 and T2 transcripts at these time-points obtained by dividing the PI density of T1 transcripts by the PI density of T2 transcripts (T1/T2).

and with seven different 5′ UTR derived probes (for location of probes, see Fig. 1, panel 'probes'). The 5′ end of all 5′ derived probes was −792; the 3′ nt of these probes was at −1, −53, −88, −161, −218, −413 and −445 respectively; the 5′ UTR probes are referred to regarding their 3′ nt (for phosphoimages see Fig. 1, top panel). Comparison of the hybridization signal obtained with the ORF probe (+585 to +813), which hybridizes with all messengers, to the signal obtained with probe −1 showed a decrease indicating that the majority of the B-50 messengers contains a relatively short 5′ UTR (Fig. 1, panels (+585 to +813) and (−792 to −1), compare lanes A+ to M2 and M4). Comparison of the hybridization signal obtained with probe −53 showed a considerable decrease as compared to the signal obtained with probe −1 (Fig. 1, panels (−792 to −1) and (−792 to −53), compare lanes A+ to M2 and M4). Quantification of the phosphoimages shown in Fig. 1 revealed that the hybridization signals obtained with probes −88, −161 and −218 was identical to that obtained with probe −53 and reduced to background signal when probes −413 or −445 were used indicating that the transcription initiation site of P1 is located between −213 and −413 and no transcription initiation site is present 5′ of −413. To determine the relative amount of T1 and T2 transcripts, a quantitative analysis of the Northern blots was performed using a BAS1000 Phosphoimager. Both B-50 mRNA and cRNA signals were corrected for the number of bases binding to the probe. Quantification revealed that T1 is located at ±−413 and T2 at ±−53 (see Fig. 1, see panel 'calculated B-50 transcripts') and the hybridization signals obtained with probes −53, −88, −161 and −218 all showed that 5% of the transcripts originates from P1 and 95% from P2.

Interpolation of a semi-logarithmic plot of the electrophoretic mobility of the B-50 cRNA markers to estimate the size of the transcripts originating from P1 and P2 showed that T1 transcripts are 1676 ± 50 nt in length and T2 transcripts are 1461 ± 46 nt in length respectively.

3.2. P1 and P2 activity during development

Previous studies showed that the expression of B-50 mRNA in rat brain reaches its peak around post-natal day 7 [1]. We examined whether the activity of promoters P1 and P2 alters during (embryonal and postnatal) develop-

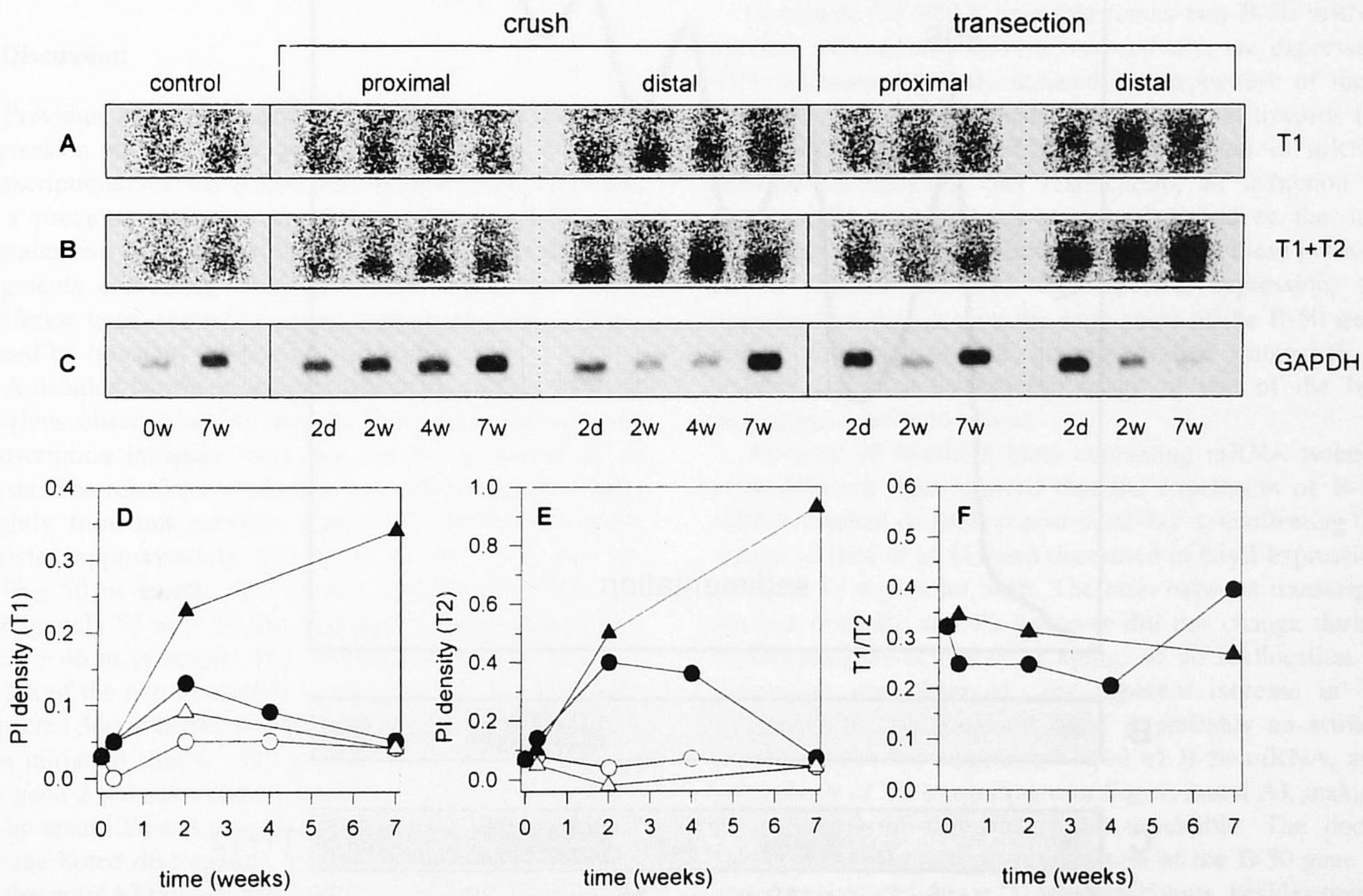

Fig. 3. Amount of T1 and T2 transcripts in proximal and distal nerve portions determined at different time-points after sciatic nerve crush or transection. Panel A shows the phosphoimage obtained by hybridization with probe T1 (−465 to −218) and panel B the signal obtained with probe T1 + T2 (+585 to +813). In panel C an autoradiograph obtained by hybridization with an GAPDH probe is shown. Each lane contains 20 μg total RNA. The relative amounts of T1 (panel D) and T2 (panel E) transcripts at different time-points after sciatic nerve lesion were quantified using a BAS1000 Phosphoimager and are expressed as PI density. Panel F shows the ratio between T1 and T2 transcripts (PI density of T1 divided by PI density of T2, T1/T2). (○) crush, proximal nerve portion; (●) crush, distal nerve portion; (△) transection, proximal nerve portion; (▲) transection, distal nerve portion.

ment or aging. Northern blots containing poly(A)$^+$ RNA isolated from E15, E18, P1, P8, P14, P21, P42, 1 year and 2 years old rat brain (Fig. 2, lanes E15 through 2yrs) and B-50 in vitro transcript M2 (−481 to +833, lanes M) were prepared. GAPDH was used to correct for the amount of RNA in each lane (Fig. 2, panel C). The Northern blots were hybridized with a probe specific for T1 transcripts (−242 to −55, Fig. 2, panel A) and with a probe hybridizing within the ORF (+585 to +813, see Fig. 2, panel B). After quantification and correction for differences in hybridization using the B-50 cRNA marker the signal of T2 was obtained by subtraction of the corrected T1 signal from the corrected ORF signal. The expression of both transcripts reached its peak at post-natal day 8 with regard to the other time points investigated (see Fig. 2, panel D). Comparing the amount of T1 and T2 transcripts (T1/T2) showed that the relative contribution of P1 and P2 to the B-50 mRNA population remains unchanged during (embryonal) development or aging (see Fig. 2, inset of panel D).

3.3. P1 and P2 activity in regenerating and transected sciatic nerve

The activity of promoters P1 and P2 was examined in the peritraumatic region at various time points following sciatic nerve crush or transection. Northern blots containing total RNA isolated from the proximal and distal portion of the nerve stump following crush or transection were prepared. The hybridization signals obtained with a probe only hybridizing with P1 transcripts (−242 to −55, Fig. 3, panel A) and a probe binding within the ORF (+585 to +813, Fig. 3, panel B) were compared. Northern blots were hybridized with a GAPDH probe (Fig. 3, panel C) to correct for the amount of RNA in each lane. Quantification showed that the amount of transcripts derived from both P1 and P2 increased dramatically in the distal stump as compared to the normal situation. After sciatic nerve crush, the increase in expression was at its peak at 2 weeks post-lesion and declined to control levels after 7 weeks, whereas after transection expression re-

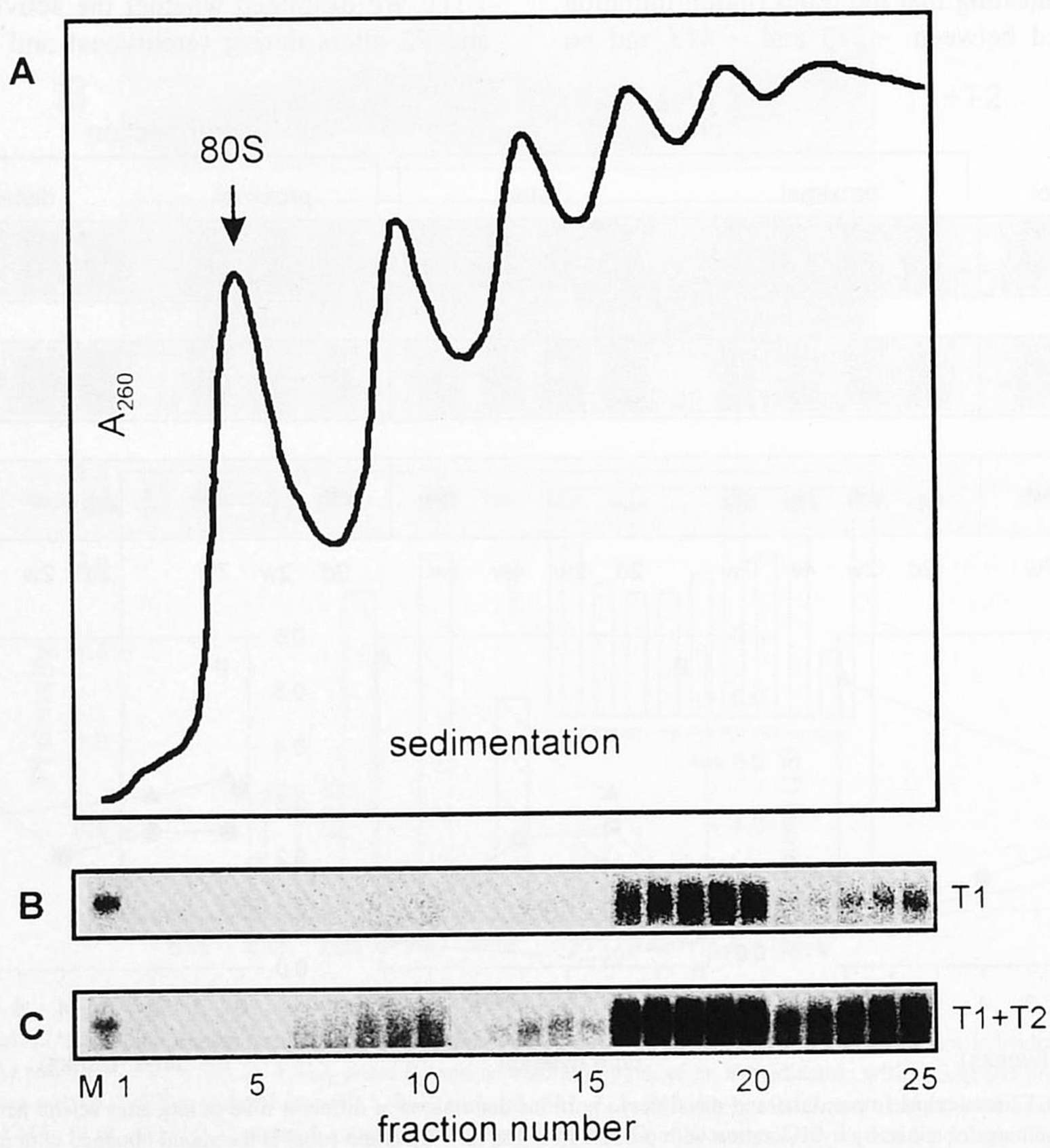

Fig. 4. The distribution of T1 and T2 transcripts in a polyribosomal profile of 8-day-old rat brains. Panel A shows the $A_{260\ nm}$ profile and sedimentation of polyribosomes in an iso-kinetic sucrose gradient. In panel B a phosphoimage of a Northern blot, containing the 25 fractions of the gradient, hybridized with probe T1 (−465 to −218) is shown. In panel C the result of hybridization with probe T1 + T2 (+585 to +813) is shown. Lanes M contain 200 pg of B-50 in vitro transcript M2 (1349 nt, see Fig. 1) to which 20 μg tRNA was added.

mained elevated up to 7 weeks post-lesion (Fig. 3, panels D and E). The expression level of B-50 mRNA transcripts was not altered in the proximal nerve stump after transection or crush with either probe. Comparing the amount of T1 and T2 transcripts (T1/T2) at these time points (Fig. 3, panel F) did not show an reallocation of transcription in favor of one of the two transcription initiation sites.

3.4. Polyribosomal distribution of P1 and P2 transcripts

To determine the in vivo translation efficiency of the two B-50 messengers, a polyribosomal profile was generated. Polyribosomes were isolated from 8-day-old rat brains and separated on an iso-kinetic sucrose gradient. Panel A of Fig. 4 shows the $A_{260\ nm}$ scan of the gradient. The gradient was fractionated in 25 samples from which RNA was extracted. Northern blots containing these 25 samples (lanes 1 through 25) and a B-50 in vitro transcript (−481 to +833, lanes M) were prepared and hybridized with a T1 probe (−242 to −55, Fig. 4, panel B) and an ORF probe (+585 to +813, Fig. 4, panel C). The majority of the T1 and T2 transcripts were located in the tetrasomal fraction (lanes 19–20), some T1 and T2 transcripts were found in higher polyribosomal fractions (lanes 23–25).

4. Discussion

Previous work from several laboratories reported that expression of the B-50 gene is regulated at both the transcriptional and the post-transcriptional level [12,19,20]. In a previous study we showed that the rat B-50 gene contains two transcription initiation sites and that the DNA fragments containing these start sites, when fused to a luciferase gene, served as a transcription template as determined by transient transfection assays [9].

A detailed Northern analysis was in agreement with our previous observation [9] that the B-50 gene contains two transcription initiation sites that are not separated by an intron. The relative contribution and calculated sizes differ slightly from our previous report; 5% of the transcripts initiates approximately 413 nt 5′ of the ATG and are 1676 ± 50 nt length; 95% of the transcripts initiates approximately 53 nt 5′ of the translation start codon and are 1462 ± 46 nt in length. The observed 200 nt difference in length of the two transcripts is not sufficient to explain the expected 360 nt difference in length based on the transcription initiation sites (−413 versus −53). In the 3′ end of the gene 2 polyadenylation signals are observed which are 65 bp apart [23] and may be alternatively used and account for the noted discrepancy in size. Furthermore the length of the poly(A) tail is unknown. The B-50 cRNA markers were used to quantify the obtained hybridization signals; the hybridization efficiency of the used probes to the B-50 cRNA markers and the native transcripts was equal. Moreover the size of the respective transcripts was estimated using the B-50 cRNAs, differences in secondary structure, interfering with the migratory behavior on agarose gels were absent. The results obtained are in agreement with data published by Grabczyk et al. [13] who showed that, using RNase protection and primer extension, the majority of the transcripts initiates at −51/−52. Our data do not support the location of the 'core promoter' as postulated by Nedivi et al. [16], with start sites from −482 to −456, since only 5% of the transcripts initiates at −413.

Several laboratories have documented genes containing multiple promoters controlling cell-specific and/or developmental stage-dependent expression. The human IGF-II gene contains four promoters, separated by introns, that exhibit a tissue-specific and developmental stage-dependent expression pattern [30]. In the rat IGF-I gene a tissue-specific start site usage is observed [25]. In several genes multiple promoters were characterized that regulate cell-specific expression. In the tropomyosin gene, alternative promoter usage and splicing leads to cell-specific expression [14]. In the dystrophin gene, cell-specific expression is obtained by a brain and muscle specific promoter, separated by an intron [3]. In the brain-derived neurotrophic factor gene contains four promoters, separated by introns, are alternatively used resulting in tissue-specificity [15].

In human SH-SY5Y neuroblastomas two B-50 mRNA species, 1400 nt and 1600 nt respectively, are expressed. TPA treatment not only induced the expression of these two transcripts but reallocated the expression towards the 1600 nt transcript [18]. Unless an alteration in mRNA stability accounts for this reallocation, an induction of promoter P1 expression is suggested. Based on the data that genes containing multiple promoters use these promoters to regulate time-and tissue-specific expression, we hypothesized that in vivo the expression of the B-50 gene during development and/or regenerative outgrowth is (partly) regulated by specific usage of one of the two transcription initiation sites.

Analysis of Northern blots containing mRNA isolated from different ages showed that the expression of B-50 mRNA reached its peak at post-natal day 8, confirming the results of Basi et al. [1], and decreased to basal expression levels at 42 days after birth. The ratio between transcripts derived from P1 and P2 however did not change during (embryonal) development or aging, so no reallocation of expression was observed. The apparent increase in T2 transcripts in 2-year-old rat brain is probably an artifact caused by the low expression level of B-50 mRNA, and particularly of T1 transcripts (see Fig. 2, panel A), making quantification of this time point unreliable. The documented increased rate of transcription of the B-50 gene in young versus adult brain [1] which accounts, besides possible differences in messenger stability, for an increased B-50 mRNA expression is not achieved by induction of one of the two promoters. The amount of both T1 and T2 transcripts increases and decreases concomitantly during (embryonal and postnatal) development or aging.

In the distal nerve stump, expression of B-50 mRNA is increased following sciatic nerve crush or transection [21]. Hybridization with probes discriminating between T1 and T2 transcripts revealed that both transcripts were expressed by reactive Schwann cells following peripheral nerve lesion (Fig. 3, panels A and B). After nerve lesion the ratio between the amount of T1 and T2 transcripts compared to the control situation was not altered in the distal nerve stump (see Fig. 3, panel F) indicating that the increased expression of the B-50 gene in reactive Schwann cells is not accounted for by a specific induction of one of the promoters. The ratio between T1 and T2 transcripts in the proximal nerve stump was not determined, low expression levels of the T1 transcripts made reliable quantification impossible. The ratio between T1 and T2 transcripts in the sciatic nerve is approximately 0.3 and in 8-day-old brain approximately 0.05, suggesting that in the peripheral nervous system a larger portion of the B-50 mRNA population originates from P1.

To determine whether the two transcripts differed in their translation efficiency in vivo, as was suggested by in vitro translation studies [10], the position of the two transcripts in a polyribosomal profile was determined. The amount of ribosomes associated with a messenger is a measure of the efficiency with which the ribosomes translate the messenger [2,11]. Northern blot analysis of the polyribosomal distribution of T1 and T2 transcripts revealed that both messengers were in the same polyribosomal fraction and associated with approximately four ribosomes. Thus the contribution of the two B-50 promoters to the B-50 protein pool is not regulated via differences in translation efficiency of their respective transcripts.

Taken together, these data show that the ratio between the amount of transcripts derived from P1 and P2 is neither altered during (embryonal) development nor after peripheral nerve lesion. The translation efficiency of the respective transcripts, as determined by ribosome association, was equal. The rat B-50 gene contains two distinct promoters and we hypothesize that the activity of these two promoters, under the conditions examined, is controlled by a similar mechanism.

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