Expression of the nerve growth factor-regulated NGFI-A and NGFI-B genes in the developing rat

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Summary
NGFI-A and NGFI-B are two genes that are induced in PC12 cells treated with nerve growth factor and encode zinc-finger proteins that may act as transcription factors. To study the function of these genes in vivo, their expression in rat embryos and postnatally developing tissues was examined. Both genes are expressed during embryogenesis from E12 to E18. In postnatally developing animals, these genes are induced in cortex, midbrain and cerebellum, suggesting that they may function in one or many of the changes that occur during postnatal maturation of the brain. Both genes are expressed at a low, constitutive level in a subset of other non-neuronal tissues, but also demonstrate developmental changes in expression in many other organs. To examine expression of these genes in ganglia of the peripheral nervous system, a sensitive and quantitative assay employing reverse transcription and polymerase chain reaction was developed. Results obtained with this assay demonstrated that expression of both NGFI-A and NGFI-B is modulated in the peripheral nervous system during postnatal maturation, but not in a pattern concordant with previously characterized NGF receptor expression. These studies suggest that NGFI-A and NGFI-B serve multiple, tissue-specific functions and are under complex regulation in vivo.

Key words: NGFI-A, NGFI-B, gene expression, rat, nerve growth factor.

Introduction
NGFI-A (Milbrandt, 1987) and NGFI-B (Milbrandt, 1988) are two genes originally isolated based on their induction by nerve growth factor (NGF) in the rat pheochromocytoma cell line, PC12. This neural-crest-derived cell line responds to NGF by differentiating into a post-mitotic cell type with many neuronal characteristics (Greene and Tischler, 1982). The murine homologs of both NGFI-A (Egr1 (Sukhatme et al. 1988), krox24 (Chavrier et al. 1988) and zif268 (Lau and Nathans, 1987)) and NGFI-B (nur77 (Hazel et al. 1988), N10 (Ryseck et al. 1989)) also have been isolated from 3T3 fibroblasts, where serum stimulation causes coincident cell proliferation and induction of both genes. NGFI-A and NGFI-B genes encode zinc-finger proteins that may act as transcription factors based upon their homologies to previously characterized proteins. NGFI-A is homologous to a family of zinc-finger proteins whose archetype is the Xenopus transcription factor, TFIIB (Ginsberg et al. 1984). NGFI-B is a member of the steroid hormone receptor gene superfamily (Evans, 1988), a group of ligand-dependent, zinc-finger-containing transcription factors. In addition to their expression in the PC12 and fibroblast cell lines from which they were initially characterized, both genes are expressed in a number of adult rat tissues including lung, brain and adrenal gland (Milbrandt, 1987; Milbrandt, 1988).

There is now extensive evidence that transcription factors, including zinc-finger proteins, play a crucial role in developmental processes. In Drosophila, mutations in the kruppel gene, which encodes a protein containing five zinc fingers, result in gross abnormalities in body morphology (Preiss et al. 1985). The mouse zinc-finger gene, Mkr2, is expressed in a number of specific neuronal cell types and is modulated during brain growth and development (Chowdhury et al. 1988). Similarly, the krox20 gene, whose primary structure is the most homologous to NGFI-A of all zinc-finger genes characterized thus far, is expressed in several specific hindbrain regions and peripheral nervous system cell types during embryogenesis (Wilkinson et al. 1989). Members of the steroid hormone receptor gene family are also regulated during growth and development. Many of the identified ligands for these receptors are important in a variety of developmental events. These include retinoic acid in the establishment of the limb anterior–posterior axis (Maden et al. 1989), thyroid hormone in the morphological changes accompanying Xenopus development (Allen, 1938), and estrogen and testosterone in sex-specific neuronal proliferation (Beyer and Feder, 1987). Although most studies have examined expression of
transcription factors during early embryogenesis, a number of development processes continue postnatally and into adulthood: myelination and dendritic elaboration of the nervous system (Gould et al. 1989; Schaffer and Friede, 1988), maturation of the immune system, and metabolic changes in the enteric system, among others. In the peripheral nervous system, NGF can affect the survival and phenotype of several different neuronal populations (Harper and Thoenen, 1981; Johnson et al. 1989), suggesting that these tissues also undergo postnatal developmental processes that might be modulated by NGF. In this study, we have examined the expression of NGFI-A and NGFI-B in a number of neonatal and postnatal rat tissues to determine whether these genes might function in any of these processes. Because many neural tissues of interest yielded small quantities of RNA, an assay employing reverse transcription and the polymerase chain reaction (RT/PCR) was developed to detect low abundance mRNAs from small amounts of tissue. This assay is quantitative and was used to detect gene expression in several different peripheral ganglia. This report demonstrates that both genes are expressed in a number of developing organs, but the differential patterns of expression observed suggest that NGFI-A and NGFI-B serve multiple and distinct, tissue-specific functions.

Materials and methods

RNA isolation and Northern blot analysis

For embryonic analysis, timed pregnant rats of indicated gestational age (±12h) were killed by decapitation and embryos dissected free from maternal tissue and immediately frozen. Appropriate tissues from 4 to 12 rat pups of indicated age were dissected and immediately frozen. Total RNA was prepared from tissues as previously described (Milbrandt, 1987). RNA concentrations were determined spectrophotometrically by measuring the A260. Twenty micrograms of each RNA sample was electrophoresed on 1.2% agarose formaldehyde gels as previously described (Milbrandt, 1987). Gels were stained with 0.5/µgml−1 ethidium bromide for 15min and destained in 10XSSC (Sambrook et al., 1989) for 30min. Ribosomal bands were visualized by U.V. transillumination to confirm that all samples were equally loaded. Gels were blotted to nitrocellulose (S&S) with 20XSSC as previously described (Milbrandt, 1987).

RNA probes were made using α-32P-UTP (New England Nuclear) and riboprobe transcription reagents (Promega) following the manufacturers’ protocol. The NGFI-A antisense RNA probe encompasses nucleotides 414 to 184 of the NGFI-A cDNA (Milbrandt, 1987). The NGFI-B probe corresponds to cDNA nucleotides 1537 to 1577 (Milbrandt, 1988). Hybridizations were carried out with 1X106 cts min−1 ml−1 probe at 65°C in 50% formamide, 5XSSC, 1% SDS, 5% dextran sulfate, 5XDenhardt’s, 1µg ml−1 PolyA, and 100µg ml−1 sonicated herring sperm DNA. Blots were washed in 0.2XSSC at room temperature followed by two washes at 65°C with 0.2XSSC and 0.5% SDS. Filters were exposed to Kodak XAR-5 film at −80°C using DuPont intensifying screens for 1 to 5 days.

RT/PCR analysis

Total RNA was isolated from 4 to 6 pairs of ganglia, nerves or adrenal glands using guanidine thiocyanate/acid phenol as previously described (Chomczynski and Sacchi, 1987). To synthesize control RNA, the plasmid vector Bluescript-KS (Stratagene) was linearized at an AvaI site within the polylinker sequence and a double-stranded oligo dT17 was ligated in place. This resulting construct was linearized with Sall and a double-stranded oligo 5′-TCGACGGCTTAGCTGGAAGGCCAGCCGC-3′ was ligated in place. This oligo contains two sequences in tandem to which the forward PCR primers for NGFI-A and NGFI-B will anneal. This construct was then linearized with SacI and a double-stranded oligo 5′-TCGACGGCTTAGCTGGAAGGCCAGCCGC-3′ was ligated in place. This oligo contains two sequences in tandem to which reverse PCR primers for NGFI-A and NGFI-B will anneal. This entire construct was sequenced and then used as a template for in vitro RNA transcription (Promega) following the manufacturer’s protocol.

For the reverse transcriptase (RT) reaction, 0.5 µg total target RNA was added to 200 ng of oligo dT17 and 2.5X105 copies of control RNA in a volume of 11 µl and heated to 68°C for 5min. After cooling to 27°C, RT reaction mix was added, containing a final concentration of 50 nm Tris−HCl (pH 8.3), 50 mm KCl, 10 mm MgCl2, 1 mm DTT, 10 µg ml−1 BSA, 1 mm dNTPs, 10 units RNAsin (Promega), and 10 units AMV reverse transcriptase (Molecular Genetic Resources) in a final volume of 20 µl. Reactions were carried out at 42°C for 2h. RT reaction was diluted five-fold with H2O and boiled for 5min. These first strand reactions were stored at −20°C for subsequent PCR analysis.

The forward PCR primer was end labeled for 1h at 37°C in a reaction containing 500 ng oligonucleotide primer, 100µCi 32P-γ-ATP, (New England Nuclear 3000Ci mmol−1, 10mCi ml−1), T4 polynucleotide kinase buffer (70 mm Tris−HCl pH 7.6, 10 mm MgCl2, 5 mm DTT), and 10 units T4 polynucleotide kinase (New England Biolabs). Labeled oligonucleotide was separated from free label by passing the reaction over a NAP-5 Sephadex column (Pharmacia) and typically yielded a specific activity of 2X108 cts min−1 µg−1 oligonucleotide.

For PCR analysis, a reaction mix was made containing 50 µm KCl, 10 µm Tris−HCl (pH 9.0 at 25°C), 1.5 mm MgCl2, 0.01% gelatin, 0.1% Triton X-100, 0.4 mm dNTPs, 200 ng forward oligo, 200 ng reverse oligo, 10 ng (2X105 cts min−1) labeled forward oligo, and 2 units Taq DNA polymerase (Promega) in a total volume of 45 µl. To this mix, 5 µl of each diluted, first-strand RT reaction was added and then layered with 100 µl light mineral oil. All PCR reactions were carried out with an initial denaturation at 94°C for 2min, followed by 20−25 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min.

Products from the PCR reactions were run on 6% acrylamide gels in 1X TBE (0.045 M Tris–borate/0.001 M EDTA), dried and exposed for appropriate times on Kodak XAR-5 film. Results were quantitated by excising bands after autoradiography and performing scintillation counting on the dry gel slices. Copy number of target messages was calculated based on the ratio of target to control cts min−1 multiplied by the number of control RNA copies added to each reaction.

Results

NGFI-A and NGFI-B expression during rat embryogenesis

Many genes encoding putative transcription factors are
NGFI-A and NGFI-B gene expression

expressed at different times during embryogenesis. Therefore, we first determined whether NGFI-A and NGFI-B were expressed during similar periods of development. Northern blot analysis with NGFI-A probe detected a single transcript in embryonic RNAs that co-migrated with the transcript found in NGF-induced PC12 cell RNA (Fig. 1A). This transcript was detected as early as E12 and appeared throughout development to age E18. No change in expression level was detected during this time period. Furthermore, during later stages of embryogenesis (E16–E18), NGFI-A was detected at equal levels in both head and trunk sections. Similar analysis with NGFI-B probe demonstrated that this gene is also expressed from E12 to E18 with no significant change in message level (Fig. 1B). Although these results indicate that both NGFI-A and NGFI-B are expressed during embryonic development, they cannot differentiate whether mRNA levels of these two genes are constant throughout embryogenesis or whether these genes are induced in certain cell populations and attenuated in others.

**Tissue-specific expression during postnatal development**

Because NGFI-A and NGFI-B are activated not only by NGF, but also by serum stimulation, we were interested in determining whether these genes demonstrate a neuronal or other tissue-specific response during postnatal growth and differentiation in vivo. To answer this question, we surveyed a variety of tissues at several times during postnatal development. In many of the neonatal and postnatal tissues examined, NGFI-A and NGFI-B expression was minimal and constant. In lung, heart, liver and skin, mRNA from both genes was detected in approximately equal abundance and demonstrated few significant changes in level between E21 and adult ages (Fig. 2). The level of NGFI-A expression in adult lung is lower than that reported in an earlier study (Milbrandt, 1987). However, the present data were obtained from multiple RNA samples and Northern blot analyses and is representative of the message content found in the normal rat lung.

In contrast, changes in gene expression were observed in kidney, gut, spleen, thymus and muscle (Fig. 3). In the kidney, mRNA from both NGFI-A and NGFI-B increases approximately ten-fold between E21 and P1, followed by a rapid decline and return to basal levels by P3. This burst of expression at the time of birth is similar to that seen for the rat c-fos message (Kasik et al. 1987). However, while c-fos demonstrates this expression pattern in several tissues, the kidney was the only tissue for which this phenomenon was consistently observed for NGFI-A and NGFI-B. As shown in Fig. 2, this transient increase is seen to a lesser extent in heart as well, but is not observed in liver, lung and skin from the same group of animals. In the gut, both genes show a gradual decrease in expression from E21 to P21, and are not expressed in adulthood. This lack of expression in adult gut was confirmed by a segmental analysis of expression in adult tissue obtained from proximal small intestine, distal small intestine, large intestine and colon (data not shown). In contrast, NGFI-A and NGFI-B are gradually induced in both spleen and thymus. At age E21 and P1, neither gene exhibits detectable mRNA levels in the spleen. However, by two weeks after birth both genes are expressed at a level that is maintained into adulthood. In the thymus,
expression of both genes is easily detected at age E21, but also increases into adulthood. Finally, in muscle, levels of NGFI-B mRNA increase from E21 to adulthood, while those of NGFI-A mRNA are equally abundant, but relatively constant. Unlike other tissues, expression of these genes does not appear to be co-regulated in muscle. This analysis does not distinguish the cell types in which these genes are expressed. Therefore, whether expression in muscle originates from myocytes, supporting connective tissue, or associated nerve bundles must be clarified by in situ hybridization and immunohistochemical analysis.

Our analysis of these tissues involved many different Northern blot experiments. Therefore, we were able to compare levels of expression at different times for a given tissue, but not levels between different tissues. Fig. 4 compares all tissues surveyed at a single age midway through the times selected for our analysis, P8. In any one tissue, both genes are expressed at similar levels.

Expression during postnatal brain development
Because both NGFI-A and NGFI-B were isolated from a cell line undergoing neuronal differentiation, we were interested in examining the expression of these genes
during neuronal development and growth in vivo. In the first series of experiments, the expression of these genes was examined in postnatal brain that was partitioned into cerebellum/brainstem, midbrain and cortex. As shown in Fig. 5, both genes are expressed at low levels in neonatal and early postnatal brain. At later times and into adulthood, both genes show a dramatic increase in expression in all brain sections. Expression is most abundant and shows the greatest increase in cortex as compared to midbrain and cerebellum. As with most other tissues, NGFI-A and NGFI-B show similar patterns of expression. These results suggest that both genes may be important in a number of the developmental changes that occur in the brain subsequent to neuronal proliferation.

Development of an RT/PCR assay for gene expression

Many tissues of the peripheral nervous system in which we desire to study NGFI-A and NGFI-B gene expression are often too small to permit the use of standard techniques of RNA isolation and Northern blot analysis. This is especially true of embryonic and neonatal ganglia such as the dorsal root sensory ganglia (DRG) and the superior cervical sympathetic ganglia (SCG), two neuronal populations that are developmentally modulated by NGF (Harper and Thoenen, 1981). To expedite our analysis, an assay was developed using reverse transcription (RT) and polymerase chain reaction (PCR) to quantitate gene expression from small amounts of tissue.

Fig. 6A outlines the RT/PCR assay. In the first step, RNA is reverse transcribed with a poly-dT primer along with a control, synthetic cRNA that contains a poly(A) tail and sequences from NGFI-A and NGFI-B to which the PCR primers will anneal (see methods). A portion of this RT reaction containing the first strand cDNA is then added to a PCR reaction containing two radio-labeled oligonucleotide primers corresponding to the gene of interest. The resulting PCR products are then electrophoresed on an acrylamide gel and subjected to autoradiography.

Since the RT/PCR assay employs an enzymatic amplification step, it was important to demonstrate that quantitation of the final product is a linear extrapolation of the actual target mRNA level. Fig. 6B shows the results of this assay when two-fold serial dilutions (4 ng to 125 ng) of total RNA from P22 cortex was analyzed for NGFI-A expression. When ten percent of each RT reaction was used in the following PCR reaction (A lanes), each reaction yielded a signal that was approximately two-fold lower than the previous sample. This relationship was confirmed by excising the bands and subjecting them to scintillation counting. When five percent of each RT reaction was used in the subsequent PCR reaction (B lanes), the signal was two-fold lower in each case, as expected. Furthermore, the signal obtained from using five percent of the RT reaction for a given PCR reaction was identical to that seen using ten percent of the RT reaction primed with half as much RNA (e.g. compare lane B at 1 µg vs. lane A at 0.5 µg). These results indicate that the amount of radioactive PCR product produced is directly related to the amount of first-strand cDNA synthesized in the RT reaction and therefore, a direct measure of mRNA abundance. This RT/PCR assay is linear over two orders of magnitude and is sensitive enough to detect two-fold differences in message levels with as little as 6 ng of total RNA. Because only a small amount of RNA is needed for the initial RT reaction, and because only a small fraction of the RT reaction is used for each PCR reaction, multiple reactions may be performed with the same RNA sample to analyze the expression of many different gene products.

Finally, to demonstrate that the results obtained with
Fig. 5. Expression of NGFI-A and NGFI-B in postnatally developing rat brain. Total RNA (20 μg) was isolated from designated brain regions at indicated postnatal ages and subjected to RNA transfer analysis. Filters were hybridized with NGFI-A probe (A) or NGFI-B probe (B) and exposed for 24 h.

Expression in the PNS during postnatal development

Using the RT/PCR assay described above, the expression of NGFI-A (Fig. 7A) and NGFI-B (Fig. 7B) was analyzed in SCG, DRG, spinal cord, sciatic nerve, and adrenal gland. The left panels of Fig. 7 show an example of the results obtained using this assay. After appropriate exposures, the bands from each gel were excised and counted. The right panels of Fig. 7 show the graphical representation of these results, calculated from three independent trials.

NGFI-A is most abundant in the adrenal gland and sciatic nerve. In the adrenal gland, there is a two-fold increase in expression between E21 and P8 which then appears to decrease to prenatal levels in adulthood. Message levels in neonatal sciatic nerve are very high, but rapidly decrease over the first postnatal week of development. Subsequently, levels continue to decrease and reach their lowest level in adults. Surprisingly, we were unable to detect significant expression of NGFI-A in SCG and DRG, two tissues known to be subject to developmental effects of NGF (Harper and Thoenen, 1981). From E21 to adult, there is neither a significant increase nor decrease in expression. Likewise, in the spinal cord, no significant change in NGFI-A expression is seen. This is in marked contrast to the dramatic increase in expression seen in many different regions of the brain (Figs 5A and 6C).

When an aliquot of the same first-strand reaction was subjected to PCR analysis using oligonucleotides directed to NGFI-B mRNA, a very different pattern of expression was observed. In the adrenal gland, prenatal NGFI-B mRNA is in low abundance. However, after birth these levels increase twenty-fold within the first three weeks of postnatal life and then continue to increase into adulthood. In contrast, expression of NGFI-B in the sciatic nerve is high in the neonatal animal and remains so into adulthood. We noted a transient, but consistent attenuation of NGFI-B mRNA levels at P8, although the significance of this change is not known. In DRG and SCG, NGFI-B is induced approximately three-fold from birth to adulthood, and in the spinal cord, an eight-fold induction is seen starting at P8, similar to that seen for certain regions of the brain. The distinct expression patterns observed for
NGFI-A and NGFI-B suggest that these two genes serve different biological functions in the developing peripheral nervous system.

Discussion

NGFI-A and NGFI-B are two genes whose products were isolated from a rat pheochromocytoma cell line undergoing neuronal differentiation in response to NGF (Milbrandt, 1987; Milbrandt, 1988), and from serum-stimulated fibroblast cell lines (Sukhatme et al. 1988; Chavrier et al. 1988; Lau and Nathans, 1987; Hazel et al. 1988; Ryseck et al. 1989). These observations, in conjunction with studies identifying both transcripts in neuronal and non-neuronal adult tissues, suggested that these genes may be important for NGF-modulated neuronal maturation, but may also respond to different growth stimuli in other developing organs.

There is increasing evidence that transcription factors play a central role in embryonic development of the nervous system. NGFI-A is a member of the ‘zinc finger’ multigene family. A number of these genes are developmentally expressed in the nervous system. Mkr2 is a zinc-finger gene isolated from mouse and is specifically expressed in the central nervous system and peripheral neural ganglia of the early embryo (Chowdhury et al. 1988). Krox20, the member of this family most homologous to NGFI-A, is expressed in specific
Fig. 7. RT/PCR analysis of peripheral neural tissues. (A) Left panel shows representative autoradiographic results of NGFI-A expression in adrenal gland, sciatic nerve and DRG at indicated ages. Right panel shows the numerical results of three independent trials examining NGFI-A expression in sciatic nerve (SN), adrenal gland (ADR), superior cervical ganglia (SCG), dorsal root sensory ganglia (DRG) and spinal cord (SC) at indicated ages. Results are expressed in mRNA copy number (see methods). (B) As in panel A, results obtained using NGFI-B specific PCR oligonucleotides.

regions of the embryonic mouse hindbrain and in early neural crest cells (Wilkinson et al. 1989).

NGFI-B is a member of the steroid hormone receptor gene family which includes nuclear receptors for retinoic acid, thyroid hormone and sex steroids (Evans, 1988) as well as a number of Drosophila genes, which may play a role in early developmental processes (Rothe et al. 1989; Mlodzik et al. 1990; Segraves and Hogness, 1990). Although the putative ligand for NGFI-B is not known, its homology with these other receptor proteins suggests that it may function in early developmental processes as well. In fact, our initial analysis demonstrated that both NGFI-A and NGFI-B are expressed in rat embryos from E12 to E18. In the case of NGFI-A, this result has been confirmed by in situ studies which demonstrated expression of this gene in cartilage and bones of E14 and E17 mouse embryos (McMahon et al. 1990).

While most studies have been carried out at embryonic ages, a number of developmental processes occur postnatally as well. Maturation of the immune system and metabolic changes in the enteric system are two examples. In the rat nervous system, thyroid hormone can affect postnatal dendritic elaboration and glial proliferation in the rat hippocampus and cerebellum (Dussault and Ruel, 1987). Similarly, sex steroids modulate synaptic arrangements in many CNS regions during postnatal brain maturation (Beyer and Feder, 1987). Myelination, dendritic elaboration, synapse elimination and rearrangement, changes in electrical activity, and alteration in neurotransmitter complement, learning and memory are all developmental processes that occur postnatally. For this reason, our analysis was designed to study the role of NGFI-A and NGFI-B in postnatally developing tissues and to determine whether expression of these genes could be correlated with specific neuronal or non-neuronal developmental events.

We first examined expression of these genes in several non-neuronal tissues. In liver, skin, lung and heart, both genes are expressed at varying levels that do not change significantly between birth and adulthood.
All of these tissues are undergoing somatic growth; the fact that neither NGFI-A nor NGFI-B is modulated specifically in these cell populations suggests that these genes are regulated by more complex biological signals than those that lead to an 'early growth response'. In the kidney, both genes show a burst of expression at birth, followed quickly by attenuation to undetectable levels. A similar response is seen for the c-fos gene product (Kasik et al. 1987). However, unlike NGFI-A and NGFI-B, this burst of c-fos activity at birth is seen in many different tissues.

In a third group of tissues including gut, muscle, spleen and thymus, both genes show gradual changes in expression during postnatal growth. In the gut, transcripts of both genes are present at relatively high levels in the neonate, but gradually decrease and are absent in the adult. Although anatomic localization of these gene products will provide more insight as to their function in the gut, it is possible that NGFI-A and/or NGFI-B may regulate metabolic changes that occur as the gut matures. Alternatively, these gene products may play a role in development of the enteric nervous system, its glial components, or companion neuroendocrine cells, all derivatives of the neural crest (Le Douarin, 1986).

Unlike most other tissues, NGFI-A and NGFI-B are not co-regulated in muscle. Expression levels of NGFI-A are relatively high and constant throughout postnatal development while NGFI-B shows a gradual increase in expression from birth to adulthood. Although immunohistochemical analysis will be required to identify whether expression is co-localized to the same cell type, this is one of the few examples where expression patterns of these two genes vary in vivo.

Finally, in the spleen, NGFI-A and NGFI-B are not expressed until several weeks after birth and attain their highest level of expression in the adult. In the thymus, both genes are abundantly expressed as early as E21, but also reach their highest expression levels in the adult. Although immunocytes are not commonly thought of as targets of NGF, recent evidence suggest that NGF receptors are present on rat spleen mononuclear cells (Thorpe et al. 1987). Furthermore, many immune cells respond to a variety of growth factors and immunogens with coincident induction of several different 'early response genes' (Harrigan et al. 1989). NGFI-A or NGFI-B may play a role in these responses, an idea substantiated by the fact that NGFI-A is induced in antigen-stimulated murine B lymphocytes in culture (Seyfert et al. 1989).

Because NGFI-A and NGFI-B are associated with NGF-induced neuronal differentiation of PC12 cells, we were interested in determining whether these genes are responsible for neural growth and development in vivo. As a first step toward this goal, we examined expression of both genes at different ages in postnatal rat brain. Brain regions were divided into cortex, midbrain and cerebellum/brainstem. Both NGFI-A and NGFI-B show a dramatic increase in expression in all three brain regions from birth to adulthood, a pattern similar to that seen for the c-fos gene (Gubits et al. 1988). NGFI-A and NGFI-B appear to be co-regulated, albeit differently in each area of the CNS examined. For example, induction and expression of each gene is greatest in cortex. In contrast, the induction and adult level of expression in the cerebellum and midbrain is much lower and occurs with slower kinetics. In fact, changes in electrical activity, myelination and dendritic elaboration occur at different times in the brain regions examined in our study. Furthermore, NGF receptor expression is differentially modulated in the cholinergic neurons of the basal forebrain (Buck et al. 1987) and caudate/putamen (Mobley et al. 1989), in cerebellar Purkinje cells, and in several brainstem nuclei (Yan and Johnson, 1988). This coincident induction of both NGF receptor and the NGFI-A and NGFI-B genes suggests that they may regulate some NGF-modulated responses in these specific populations of neurons.

Many tissues that are responsive to NGF, and therefore are potential targets for NGFI-A and NGFI-B gene action, are too small to generate sufficient material for RNA isolation and conventional Northern blot analysis. To circumvent this problem, we have developed an mRNA detection assay employing a combination of reverse transcription (RT) and polymerase chain reaction (PCR) to detect low abundance mRNA using small amounts of tissue. A similar technique has been developed to examine growth factor expression in small populations of cells (Wang et al. 1989). This technique has advantages over Northern blotting because less than one microgram of total RNA is needed and enrichment of poly(A) RNA is not required. Unlike in situ hybridization or immunohistochemical techniques, this assay is quantitative and can be done quickly and repetitively without the need for antibodies and multiple tissue sectioning. As demonstrated in the results, this assay is linear over at least two orders of magnitude and sensitive enough to detect two-fold changes in mRNA levels. In these experiments, RNA from four to six pair of ganglia or nerve trunks was sufficient to conduct an analysis of many different genes. The RT/PCR assay may be performed without a cloned, cDNA probe; for any gene whose sequence is known, two oligonucleotides may be designed to assess the level of its expression using this assay. We expect that this will be extremely useful in quantitatively analyzing the coordinate expression of a panel of gene products from small amounts of neural tissue. This will be important for future studies involving pharmacological manipulation of gene expression, where amounts of the administered agent are limiting. Finally, since both RT and PCR reactions can be performed directly from cell or tissue lysates without the need for prior RNA purification (Van Gelder et al. 1990; Higuchi, 1989), this assay will allow the examination of gene expression from small numbers of primary cultured neurons and tissue sections that have undergone previous pharmacologic or electrophysiologic manipulation.

In this study, we have used the RT/PCR assay to analyze NGFI-A and NGFI-B gene expression in the
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References


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