Short Isoform of POU Factor Brn-3b Can Form a Heterodimer with Brn-3a That Is Inactive for Octamer Motif Binding*

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The POU proteins Brn-3a and Brn-3b belong to a family of DNA binding transcription factors that share stretches of extensive homology. Both Brn-3a and Brn-3b are expressed as shorter and longer isoforms. The long form of Brn-3a is able to oncogenically transform primary fibroblasts. By contrast, the short form of Brn-3b (Brn-3b(s)) cannot transform fibroblasts but is able to specifically inhibit the transforming activity of Brn-3a(l). Moreover, Brn-3a(l) can act as a transcriptional transactivator, while Brn-3b(s) is not only unable to do so but in addition specifically inhibits the transactivating activity of Brn-3a(l). Here, we show that the opposite and antagonistic activities of Brn-3a(l) and Brn-3b(s) proteins are due to their different DNA binding properties; Brn-3a(l) but not Brn-3b(s) can form stable complexes with several octamer-related target DNA sequences. The presence of Brn-3b(s) completely inhibits the binding of Brn-3a(l) to DNA by preventing the formation of Brn-3a(l)-DNA complexes as well as by disrupting preformed complexes. Experiments with GST fusion proteins and in vitro binding studies suggest that the inhibition of Brn-3a(l) activity by Brn-3b(s) occurs via direct interaction of the two transcription factors in solution. Therefore, we hypothesize that Brn-3b(s) can act as a direct antagonist of Brn-3a(l) by inhibiting its DNA binding through the formation of an inactive hetero-oligomeric complex.

The POU domain represents a structural entity that was first recognized as a conserved motif in the transcription factors Pit-1, Oct-1, Oct-2, and Unc-86. All four proteins act as transcriptional regulators and play an important role in tissuespecific gene regulation, assuring the proper development and differentiation of target cells (for reviews, see Refs. 1 and 2). The POU domain is subdivided into a POU-specific domain and a POU homodomain; both subdomains are separated by a short linker sequence and are responsible for the DNA binding activity of POU transcription factors. In contrast to classical homodomain transcription factors, POU proteins seem to recognize longer DNA motifs, which represent variants of the octamer consensus sequence ATGCAAAT (3–6).

The family of transcription factors has grown substantially after a successful search for related genes based on PCR experiments with degenerated oligonucleotides (7) and has been subdivided into six classes named POU I–VI (1, 2). Among others, the rat homologue of the Caenorhabditis elegans gene encoding Unc-86 was isolated and termed brn-3a (7) due to its predominant expression in rat brain. Independently, a human homologue of unc-86, then called RDC-1, was isolated from a neuroepitheloma cDNA library and found to be identical to brn-3a (8). The analysis of the newly isolated brn-3a gene revealed also the existence of two other related genes, brn-3b and brn-3c (9–16). Brn-3a, brn-3b, and brn-3c have also been termed brn-3.0, brn-3.2, and brn-3.1, respectively (2). All three brn-3 genes are differentially expressed during the development of the nervous system, but expression of brn-3a has also been detected in lymphoid cells (9). Brn-3 proteins constitute together with Unc-86 and I-POU the class IV of POU domain transcription factors. Indeed, all proteins of the class IV share a stretch of homology at their amino-terminal ends that led to the identification of a new domain of 37 amino acids called the POU IV box (9, 13, 16). Both brn-3a and brn-3b genes give rise to two mRNA molecules that differ in their 5′-ends. Thus, both genes code for two distinct proteins, a longer and a shorter isoform, that differ in their amino-terminal regions (see Fig. 1A and Refs. 13 and 14). Brn-3a(s), the short isoform of Brn-3a, lacks in comparison to the longer isoform, Brn-3a(l), about 80 amino-terminal residues including the entire POU IV box and represents a truncated version of Brn-3a(l). In contrast, Brn-3b(s) lacks only part of this homology region. Due to the usage of an alternative translational start site, Brn-3b(s) has acquired nine amino-terminal residues that it does not share with the longer Brn-3b(l) protein. The POU domains of Brn-3a, -b, and -c are located at the carboxyl-terminal end of the proteins and have almost identical sequences, only 12 amino acids of 160 being exchanged (13). When individually expressed as GST fusion proteins, all three POU domains show very similar DNA binding characteristics with different octamer-related target sequences (13).

We have previously shown that Brn-3a(l) but not Brn-3b(s) is able to oncogenically transform primary rat embryo fibroblasts in cooperation with activated Ha-Ras according to an assay that was originally developed to demonstrate the oncogenic cooperation between c-Myc and Ha-Ras (17). The transformed cells resulting from Brn-3a(l) rat embryo fibroblast transfections are able to form tumors in nude mice and show anchorage-independent growth, unequivocally establishing a bona fide oncogenic potential for Brn-3a(l) (13). The presence of Brn-3b(s) in a rat embryo fibroblast transformation assay with Brn-3a(l) completely inhibited the oncogenic activity of Brn-3a(l) (13). Similarly, transactivation studies have shown that

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The abbreviations used are: PCR, polymerase chain reaction; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis; bp, base pair(s); CRH, corticotropin-releasing hormone.
Brn-3a(l) is able to stimulate transcription and expression of reporter constructs containing a minimal promoter and octamer-related upstream elements (9, 18, 19). By contrast, Brn-3b(s) cannot transactivate and even represses the transcription of similar reporter gene constructs (18, 19). Moreover, expression of Brn-3b(s) specifically inhibits Brn-3a(l)-mediated transactivation of reporter constructs in cotransfection experiments (18, 19). In addition, the expression of brn-3a and brn-3b is regulated in opposite directions in neuronal cells in response to cAMP or growth factor treatment, enhancing the level of brn-3a-specific transcripts while reducing the level of brn-3b mRNA molecules (18).

In the present study, we attempted to investigate how the inhibitory and antagonistic effects of Brn-3b(s) on the activity of Brn-3a(l) are mediated. We show here that Brn-3b(s) in contrast to Brn-3a(l) and Brn-3b(l) (15) does not bind to octamer or octamer-related DNA target sequences and that the inhibitory effect of Brn-3b(s) is due to its direct interaction with Brn-3a(l), leading to the formation of inactive hetero-dimeric complexes that are no longer able to bind these DNA elements.

**EXPERIMENTAL PROCEDURES**

**Generation and Manipulation of Proteins**—In vitro translation was performed in a single step transcription/translation system using the TNT-coupled reticulocyte lysate (Promega) according to the supplier’s information. The reaction contained 1 μg of plasmid DNA and 40 μCi of [35S]methionine in a total volume of 50 μl.

To generate GST fusion proteins, the appropriate DNA fragments were subcloned into the bacterial expression vector pGEX-2T and expressed as a fusion protein with GST according to the supplier’s instructions (Pharmacia Biotech Inc.). The eluates from the affinity columns showed one major band on 12% SDS-PAGE analysis of Coomassie-stained protein, indicating successful purification. For the analysis of protein-protein interactions, 0.5 μg of GST fusion protein or as a control 3 μg of GST protein was incubated with 2.5 μl of an in vitro translation reaction in 200 μl of binding buffer (50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 1 mM dithiothreitol, 0.5% (v/v) Nonidet P-40) for 1 h at 4 °C. Glutathione-Sepharose beads were washed several times in binding buffer and were added to the binding reaction that was then further incubated for 30 min at 4 °C. The beads were spun down and washed five times in binding buffer containing 0.5 × NaCl. Bound proteins were eluted from the beads by incubation with SDS sample buffer and were analyzed via SDS-PAGE.

**DNA Binding Studies**—Electrophoretic mobility shift assays were performed as described (13) using in vitro translated proteins. Briefly, 5'-end-labeled oligonucleotides were mixed with 2.5 μl of an in vitro translation reaction (see above) in electrophoretic mobility shift assay buffer (10 mM Hepes, pH 7.9, 60 mM KCl, 4% (w/v) Ficoll, 1 mM EDTA, 1 mM dithiothreitol) in a total volume of 20 μl. To avoid nonspecific interactions, 1 μg of poly(dI:dC) was added to the reaction. Binding was assessed by incubation of the mixture for 1 h at 4 °C. Free DNA and protein-DNA complexes were resolved on a native 6% polyacrylamide gel run in 0.5× TBE. After electrophoresis, the gel was dried and autoradiography was performed.

**In Vitro Binding Assay and Co-immunoprecipitation**—The coding sequences of brn-3a(l) and brn-3b(s) were transferred into Bluescript vectors that permit transcription with T7 or SP6 polymerase, respectively. [35S]Methionine-labeled proteins were produced by the coupled TNT transcription/translation system (Promega) using linearized plasmid DNA. Both brn-3a(l) and brn-3b(s) were translated separately or cotranslated in one reaction. 10 μl of the reaction was mixed with 1 ml of cold 250 mM NaCl, 0.5% Nonidet P-40, 3% bovine serum albumin and spun at 14 000 rpm for 10 min at 4 °C. Brn-3a-Brn-3b complexes were precipitated from the supernatant with specific anti-Brn-3a antiserum and collected with protein-A Sepharose. The precipitated complexes were then analyzed by SDS-PAGE (12%) and visualized by autoradiography.

**RESULTS**

**Coexpression of brn-3 Genes**—The mRNA molecules coding for the Brn-3 proteins Brn-3a, -3b, and -3c that are schematically depicted in Fig. 1A had previously been shown to be coexpressed in murine embryonic spinal cord (13) with the exception of the long isoform of brn-3b (brn-3b(l)) that was reported in retina and F9 teratocarcinoma cells (15, 16). To clarify if brn-3b(l) is coexpressed with brn-3b(s) and both isoforms of brn-3a, we performed an reverse transcription-PCR reaction with RNA from E 13.5 spinal cord and specific primer pairs that distinguish between mRNA molecules coding for Brn-3b(l) and Brn-3b(s) (Fig. 1B). The combination of primers b and c and primers b and d yielded DNA fragments of the expected lengths of 511 and 489 bp representing the mRNA molecules of Brn-3b(s) and Brn-3b(l), respectively (Fig. 1B, lanes 6 and 8) for 511 and 930 bp for the controls with genomic DNA (Fig. 1B, lanes 4 and 5). All PCR products were recognized when hybridized to an internal oligonucleotide probe (primer i, Fig. 1B). This clearly indicates that both brn-3b isoforms are coexpressed with Brn-3a(l) in embryonal spinal cord.

**DNA Binding of Brn-3 Proteins**—To investigate the specific inhibition of Brn-3a(l) activity by Brn-3b(s), we performed DNA binding studies with in vitro translated proteins. First, we tested in vitro translated Brn-3a(l) and Brn-3b(s) proteins that are schematically represented in Fig. 1A with a series of different octamer-related elements in gel mobility shift experiments (Fig. 2). The same amount of protein was incubated with oligonucleotides containing an octamer consensus sequence (HSV-Oct and wt-Oct), an octamer-related sequence (Ota), or a sequence from the CRH gene promoter (23). Brn-3a(l) bound very efficiently to the CRH element and with lower affinity to the octamer elements and the related sequence Ota (Fig. 2; see also Ref. 9). Whereas the long isoform of Brn-3b (Brn-3b(l)) had previously been shown to also bind to the CRH element (15), we show here that Brn-3b(s) did not seem to bind with appreciable affinity to the CRH oligonucleotide or the octamer-related elements (Fig. 2), although a correct in vitro translated Brn-3b(s) protein was obtained (see below). As a control, an aliquot of the

**Interaction between POU Factors Brn-3a and Brn-3b**

The murine Brn-3a genomic sequence encoding the amino-terminal region of the protein from amino acid position 1–109 was subcloned into pGEX-2 and expressed as a fusion protein with GST according to the supplier’s instructions. The region of the protein from amino acid position 1–109 was subcloned into the murine Brn-3a genomic sequence encoding the amino-terminal region of the protein from amino acid position 1–109 was subcloned into pGEX-2 and expressed as a fusion protein with GST according to the supplier’s instructions. The region of the protein from amino acid position 1–109 was subcloned into the murine Brn-3a genomic sequence encoding the amino-terminal region of the protein from amino acid position 1–109 was subcloned into pGEX-2 and expressed as a fusion protein with GST according to the supplier’s instructions.
A schematic representation of the different Brn-3 proteins and their homology domains. Regions with high homology are depicted by the same shading. Numbers represent the positions of amino acids in each individual Brn-3 protein delineating the borders of the individual homology regions. The amino-terminal region in Brn-3b(l) is interrupted by a nonconserved stretch of amino acids (checkered box) ranging from amino acid position 33 to 87. The POU IV box is located between amino acid positions 28–66 with regard to Brn-3a(l). B, coexpression of brn-3b(l) and brn-3b(s) in embryonal spinal cord of the mouse at developmental stage E 13.5 analyzed by reverse transcription-PCR. Upper panel, PCR products separated on an agarose gel and hybridization of the products after transfer to nitrocellulose to an internal oligonucleotide (primer“i”) to demonstrate that the amplified DNA fragments represent brn-3b-specific sequences. Lanes 1 and 2, marker; lane 3, no template and primers b, c, and d (water control); lane 4, PCR products with genomic phage clone λ47 (13), which contains brn-3b genomic sequences as template and primers b and c; lane
unprogrammed transcription/translation lysate (Promega) was also used in these gel shift experiments (Fig. 2). Binding reactions with oligonucleotides and unprogrammed lysate resulted in the formation of complexes also present in binding reactions with Brn-3 proteins (marked with asterisks, Fig. 2). Therefore, they account for unspecific interactions of substances in the lysate with the labeled oligonucleotide and do not represent complexes between Brn-3 proteins and DNA.

To assess specificity of the binding of Brn-3a(l) and the absence of any interaction of Brn-3b(s) with specific octamer-related recognition sequences or the CRH element, competition experiments were performed (Fig. 3). Binding of in vitro translated Brn-3a(l) to the CRH, wt-Oct, and Ota elements was clearly inhibited in a binding reaction by a 100-fold excess of the respective unlabeled oligonucleotide but not with an unlabeled competitor containing a random DNA sequence (RCO) also given at 100-fold excess (Fig. 3A). This further establishes the ability of Brn-3a(l) to recognize and to specifically bind not only to the CRH element but also to several octamer consensus sequences (HSV-Oct, wt-Oct) and octamer-related sequences (Ota, CRH). The arrow marks specific protein-DNA complexes; asterisks indicate unspecific complexes between substances in the lysate and the oligonucleotides.

Interaction between POU Factors Brn-3a and Brn-3b

Brn-3b(s) Inhibits DNA Binding of Brn-3a(l)—Although Brn-3b(s) is unable to bind to various DNA recognition sites (Fig. 2), it specifically inhibits the binding of Brn-3a(l) to the CRH element when it is present in the binding reaction (Fig. 3C). Incubation of both in vitro translated Brn-3a(l) and Brn-3b(s) proteins with the CRH element does not result in the formation of any protein-DNA complex in a standard gel shift assay (Fig. 3C). This strongly suggests that Brn-3b(s) is not only unable to bind to this DNA target sequence but can act as a specific inhibitor of the interaction of Brn-3a(l) with the CRH binding site. Moreover, this is also true for other DNA target sequences. Mixing of both Brn-3a(l) and Brn-3b(s) together in binding reactions with the HSV-Oct, wt-Oct, and Ota elements did not result in the formation of any Brn-3a(l)-DNA complexes (Fig. 4A). This inhibition is dependent on the concentration of Brn-3b(s) protein in the binding reaction (Fig. 4B). Increasing amounts of Brn-3b(s) in a binding reaction consisting of Brn-3a(l) and the CRH element gradually inhibit the formation of the proper Brn-3a(l)-CRH complex (Fig. 4B, arrow). That this inhibition is specific for Brn-3b(s) and is not due to components in the in vitro transcription/translation lysate is shown by incubation of Brn-3a(l) with the CRH element and an appropriate amount of unprogrammed lysate (Fig. 4B, lysate). In addition, Brn-3b(s) can not only inhibit the binding of Brn-3a(l) to the CRH element but is also able to disrupt the preformed complex (Fig. 4B, last lane). Brn-3a(l) protein and the CRH oligonucleotide were allowed to react for 1 h and a protein-DNA complex before the same amount of Brn-3b(s) protein was added. The electrophoretic analysis of the resulting complexes showed that Brn-3b(s) can disrupt the preformed complex (Fig. 4B, last lane), strongly suggesting a direct protein-protein interaction between Brn-3a(l) and Brn-3b(s). So far, we have not obtained any evidence that Brn-3b(s) has a similar inhibitory activity on DNA binding of Brn-3a(l) (data not shown).

Evidence That Brn-3a(l) and Brn-3b(s) Can Interact with Each Other—As all protein-DNA binding reactions were performed in vast excess of radioactively labeled oligonucleotides, the lack of any DNA binding of Brn-3a(l) in the presence of Brn-3b(s) can only be explained by a direct interaction of both proteins and the formation of hetero-oligomers. To address this question, experiments with GST fusion proteins were performed. To this end, the full-length Brn-3a(l) cDNA clone was inserted into the bacterial expression vector pGEX-2T and expressed as a fusion protein in bacteria on induction with isopropyl-β-D-galactopyranoside. In addition, Brn-3b(s) was in vitro translated in the presence of [35S]methionine to obtain a labeled reaction partner for Brn-3a(l). In vitro translation of the full-length cDNA for Brn-3b(s) yielded two proteins of 35 and 29 kDa (Fig. 5A, first lane). The 35-kDa protein represents the complete Brn-3b(s) protein that consists of 322 amino acids (13, 14). The shorter 29-kDa protein probably represents a truncated version that is initiated from an internal AUG codon 171 bp downstream, yielding a protein of 265 amino acids (29 kDa). The observed additional bands (Fig. 5A, first lane) are also present in the unprogrammed lysate (not shown). To show that the two proteins of 35 and 29 kDa are the bona fide in vitro translation products of Brn-3b(s), the Brn-3b(s) cDNA was tagged at the carboxyl terminus with a 5, genomic phage clone λ47 as template and primers b and d; lane 6, first strand DNA generated with primer a and RNA from spinal cord as template and primers c and b; lane 7, generation of template from RNA without reverse transcriptase; lane 8, first strand DNA and primers b and d; lane 9, generation of template from RNA without reverse transcriptase. Lower panel, schematic representation of the two Brn-3b mRNA molecules, given is the location of the primers for first strand synthesis for the PCR reactions and for the subsequent hybridization as well as the size of the expected PCR products in base pairs.
quences encoding a hemagglutinin epitope adding 30 amino acids (4.3 kDa) to the Brn-3b(s) protein. Immunoprecipitation of in vitro translated hemagglutinin epitope-tagged Brn-3b(s) with anti-hemagglutinin epitope antibodies yielded only two proteins with a molecular mass of about 39.5 and 33.5 kDa (data not shown), clearly demonstrating that the two proteins of 35 and 29 kDa are translation products of the brn-3b(s) cDNA.

Affinity-purified GST-fusion protein was mixed with the radioactively labeled in vitro translated Brn-3b(s) proteins and incubated with glutathione-Sepharose beads. The mixture was washed several times to get rid of unspecific binding, incubated
with SDS-PAGE sample buffer, and analyzed on denaturing polyacrylamide gels. The GST/Brn-3a(l) fusion protein but not the GST protein alone was able to bind in vitro translated Brn-3b(s) proteins and retain them through the washing reactions (Fig. 5A), clearly demonstrating a direct interaction of Brn-3b(s) proteins and retain them through the washing reaction. (Fig. 5A) and the GST domain (lane 6). B, binding of in vitro translated intact Brn-3b(s) (lane 4) to GST-coupled Brn-3a(l) in the presence of two different concentrations of ethidium bromide (EtBr) (lanes 2 and 3) and GST alone (lane 1) (conditions as in A).

To confirm that Brn-3a(l) and Brn-3b(s) proteins can interact and form complexes, we programmed an in vitro transcription/translation system with Brn-3a(l) and Brn-3b(s) plasmids together and used anti-Brn-3a antibodies to collect complexes. The antisera was raised against the amino terminus of Brn-3a(l) and was able to specifically precipitate Brn-3a(l) but not Brn-3b(s) (Fig. 6). Confirming the findings with GST fusion proteins, Brn-3b(s) specifically bound to Brn-3a(l) in this system, and both proteins were coprecipitated from the lysate (Fig. 6). In the GST pull down experiment performed in the presence of ethidium bromide, only the 35-kDa form of Brn-3b(s) appeared to bind efficiently to Brn-3a(l). Similarly, in the immunoprecipitation experiment, only the full-length 35-kDa form was found to be associated with Brn-3a(l) (Fig. 6, last lane). This demonstrates that the 35-kDa form and not the 29-kDa form of Brn-3b(s) is the bona fide reaction partner of Brn-3a(l). Moreover, when Brn-3b(s) was expressed in eukaryotic cells, the truncated 29-kDa form was not detected and therefore represents a peculiarity of the in vitro translation reaction (data not shown).

**DISCUSSION**

The regulation of the activity of upstream transcription factors can be realized through a number of mechanisms, one of which is the expression of a specific inhibitor or repressor. Studies presented here reveal that within the Brn-3 family of POU transcription factors, one isoform of the family member Brn-3b can act as a specific repressor of the activity of another family member, namely Brn-3a. While the isolated POU domain of Brn-3b as well as the long isoform of the protein, Brn-3a(l), is able to bind to specific octamer-related sequences (13, 15), we show here that the short isoform Brn-3b(s) is not only incapable to bind these target sequences but in addition can disrupt complexes between Brn-3a(l) and DNA. There are two possibilities to explain the lack of DNA binding of Brn-3b(s): the molecular structure of Brn-3b(s) in contrast to Brn-3a(l) could inhibit access of the POU domain to DNA by steric hindrance or, alternatively, Brn-3b(s) may contain an inhibitory domain that interacts with its own POU domain in a way that interferes with DNA binding. Regions that mediate inhibitory functions for DNA binding but are located outside the DNA binding domain itself have already been described in other DNA binding proteins, for example in the POU transcription factor Skn-1 (25). Similar to the situation found with the two Brn-3 POU factors, expression of the skn-1 gene yields two proteins Skn-1a and Skn-1i that differ in their amino-terminal ends due to a differential splicing process of the Skn-1 mRNA (25). Skn-1i contains an amino-terminal domain that renders the protein unable to bind DNA and to transactivate transcription from a reporter gene construct containing upstream octamer sequences (25). It is unclear at present if such a specific inhibitory region exists in the Brn-3b(s) protein and requires further investigation.

Previous experiments already suggested antagonistic activities of Brn-3b(s) and Brn-3a(l). Brn-3b(s) was found to inhibit the transforming activity of Brn-3a(l) assessed by focus formation after cotransfection of primary fibroblasts with activated Ha-Ras (13). Moreover, Brn-3b(s) has been shown to inhibit transcriptional transactivation of octamer-dependent reporter genes by Brn-3a(l) (19). Given the similarity of the POU domains of both proteins, the most obvious explanation for this inhibitory activity of Brn-3b(s) was that it competes with Brn-

**Fig. 5.** A, SDS-PAGE analysis of Brn-3b(s) in vitro translation products directly from the transcription/translation reaction (lane 1), after incubation with glutathione-coupled Sepharose beads (lane 2), after incubation with a GST/Brn-3a(l) fusion protein (lane 3), after incubation with fusion proteins between GST and the amino-terminal part of Brn-3a(l) from amino acid position 1–108 (lane 4), the middle part from amino acid positions 109–267 (lane 5), and the POU domain (lane 6). B, binding of in vitro translated intact Brn-3b(s) (lane 4) to GST-coupled Brn-3a(l) in the presence of two different concentrations of ethidium bromide (EtBr) (lanes 2 and 3) and GST alone (lane 1) (conditions as in A).

**Fig. 6.** In vitro binding and coimmunoprecipitation of Brn-3a(l) and Brn-3b(s). Brn-3a(l) and Brn-3b(s) were radiolabeled by in vivo translation either separately (lanes 3 and 4) or together (lane 5). The anti-Brn-3a antiserum recognizes specifically in vitro translated Brn-3a(l) (lane 6) but not Brn-3b(s) (lane 7) and was able to precipitate a complex between Brn-3a(l) and Brn-3b(s) (lane 8). Preimmune serum did not react with Brn-3a(l) (lane 1), and the anti-Brn-3a antibody did not react with components in the unprogrammed lysate (lane 2). Arrowheads indicate the Brn-3a(l) and Brn-3b(s) in vitro translation products (lanes 3 and 4). Under the conditions employed in this assay, Brn-3a(l) binds to the intact Brn-3b(s) protein (lane 8) but not to the truncated form, which initiates from an internal downstream AUG.
3a(l) for binding sites but is itself inactive. However, the data presented here now indicate that the inhibitory effect of Brn-3b(s) on Brn-3a(l) activity could be mediated by the formation of a complex between both POU factors that no longer binds DNA. It appears that the complex formation occurs in a stoichiometric fashion and that the amount of free and active or complexed and inactive Brn-3a(l) depends on the concentration of Brn-3b(s). Antagonistic effects between closely related transcription factors are not unprecedented, as the example of the POU factors Skn-1a and Skn-1i shows (25). Both are closely related to the POU protein Oct-1, and cotransfection experiments have shown that Skn-1i, which cannot bind DNA, inhibits the transactivating properties of Oct-1 (25). By contrast, Skn-1a that contains an alternative amino terminus and is able to bind DNA can indeed transactivate transcription from octamer consensus sequence containing reporter gene constructs (25). This behavior of Skn-1 proteins is very reminiscent to the situation described here where the POU factor Brn-3b(s) is able to bind DNA and to mediate a biological function through this interaction (15) but not the shorter isoform of this protein (Brn-3b(s)), which contains an alternative amino terminus. Furthermore, Skn-1i acts as an inhibitor of Oct-1 very much like Brn-3b(s), which inhibits the function of Brn-3a(l). However, while it is unclear to date whether the inhibitory function of Skn-1i is mediated through a direct interaction with Oct-1, data presented here argue for a direct interaction and heterooligomerization of Brn-3a(l) and Brn-3b(s).

Brn-3a(l) shows a high degree of sequence similarity to the POU factor Unc-86 (8), and it has been speculated that Brn-3a(l) is the vertebrate homologue of Unc-86 that has been isolated from C. elegans. Unc-86 interacts with the LIM homeodomain protein Mec-3 and forms heterodimers that can transactivate the Mec-3 promoter (26). In this system, the POU domain of Unc-86 is sufficient for heterodimer formation with Mec-3. However, the Mec-3 protein requires the homeodomain and additional carboxyl-terminal sequences to form complexes with Unc-86 (26). However, in contrast to the Brn-3a(l)-Brn-3b(s) interaction, the formation of heterodimers between Unc-86 and Mec-3 does not abrogate DNA binding of the complex but stabilizes DNA binding (26).

The expression patterns of brn-3a and brn-3b overlap during embryonal development of the peripheral nervous system, the retina, the spinal cord, the precerebellar anlage and the mesencephalic tectum (9, 11, 15, 16). We have shown that Brn-3a(l) and the two isoforms of Brn-3b are coexpressed in the murine spinal cord. It remains to be shown if indeed Brn-3a(l) and Brn-3b(s) are not only coexpressed within the same tissue but also in the same cells. This could be resolved by in situ hybridization of spinal cord tissue with probes specific for the different Brn-3 isoforms. However, given the evidence presented in this paper describing the in vitro behavior of Brn-3b(s) and Brn-3a(l), it is very attractive to speculate that the short isoform of Brn-3b acts as a cell lineage-specific repressor of the function of Brn-3a and that the relative expression levels and the ratio of Brn-3a(l) to Brn-3b(s) could be incremental for the decision of cells in the developing peripheral nervous system to proliferate or to differentiate. Also, further experimentation has to show whether the inhibitory activity of Brn-3b(s) extends to the other Brn-3 proteins besides Brn-3a(l) or even to other POU transcription factors.

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