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Interactions between regulatory proteins that bind to the nicotinic receptor β4 subunit gene promoter

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Abstract

The genes encoding the α3, α5 and β4 subunits of nicotinic acetylcholine receptors are tightly clustered within the genome. As these three subunits constitute the predominant acetylcholine receptor subtype expressed in the peripheral nervous system, their genomic proximity suggests a regulatory mechanism ensuring their coordinate expression. We previously identified two transcriptional regulatory elements within the β4 promoter. One of these elements, a CT box, interacts with the regulatory factors heterogeneous nuclear ribonucleoprotein K and Purα. Another element, a CA box, interacts with Sp1 and Sp3. The binding site for a fifth factor, Sox10, overlaps the CT and CA boxes. As the CT and CA boxes are adjacent, we postulated that the proteins that bind to the elements interact. Here we report that the CT box-binding factors interact with each other as do the CA box-binding factors. However, there are no direct associations between the two pairs of proteins. Interestingly though, Sox10 directly interacts with all four proteins, suggesting a central role in β4 gene expression for this member of the Sox family of regulatory factors.

Keywords: Acetylcholine receptor; Sox protein; Sp1; Transcriptional regulation; hnRNP K

1. Introduction

Neurotransmission within the nervous system is mediated in part by members of a superfamily of ligand-gated ion channels, those forming neuronal nicotinic acetylcholine receptors. Mature nicotinic receptors are pentameric structures with distinct pharmacological and physiological properties that result from incorporation of different receptor subunits into heteromeric and homomeric channels (Schoepfer et al., 1990; Elgoyhen et al., 1994; McGehee and Role, 1995; Role and Berg 1996; Gerzanich et al., 1997). Eleven genes encoding receptor subunits have been identified, and include α2–α9 and β2–β4 (Boyd, 1997). The functional diversity displayed by mature neuronal nicotinic acetylcholine receptors is thought to result primarily from the differential expression of the subunit genes and the subsequent incorporation of the subunits into functional receptors. While the consequences of the functional variability between neuronal nicotinic acetylcholine receptors are beginning to be appreciated, an understanding of the molecular basis governing expression of receptor subunits remains to be elucidated. However, it is clear that regulation of the receptor subunit genes at the level of transcription plays a critical role.

Several laboratories, including ours, are attempting to decipher the molecular mechanisms underlying the expression of three tightly clustered genes, those encoding the β4, α3 and α5 subunits. Interestingly, these subunits form the predominant receptor subtype expressed in the peripheral nervous system (Conroy and Berg, 1995; Conroy et al., 1993). We and others have identified several *cis*-acting elements (Boyd, 1994; Yang et al., 1994, 1997; Hu et al., 1995; Bigger et al., 1996; Fornasari et al., 1997; McDonough and Deneris, 1997) and *trans*-acting factors (Yang et al., 1995; Fyodorov and Deneris, 1996; Fyodorov et al., 1998; Milton et al., 1996; Bigger et al., 1997; Du et al., 1997, 1998; Campos-Caro et al., 1999, Liu et al., 1999) that are important for the transcriptional regulation of the β4, α3 and α5 subunit genes. Clustering of these

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genes and the fact that they represent the predominant neuronal nicotinic acetylcholine receptor subtype expressed within the peripheral nervous system raises the possibility that they are coordinately expressed via a common regulatory mechanism. Indeed, it has been demonstrated that transcription factor Sp1 is important for the regulation of expression of all three genes (Yang et al., 1995; Bigger et al., 1996, 1997; Campos-Caro et al., 1999). In addition, a POU-domain factor SCIP/Tst-1/Oct6 can transactivate both the α3 (Yang et al., 1994; Fyodorov and Deneris, 1996) and β4 (Liu et al., 1999) gene promoters. Moreover, we have recently demonstrated that the β4 and α3 promoters are transactivated in a neuron-specific manner by a member of the Sox family of transcription factors, Sox10 (Liu et al., 1999). It should be noted however, that although the β4, α3 and α5 genes are co-expressed within certain structures of the nervous system, their expression patterns are not completely identical (Wada et al., 1989, 1990; Dineley-Miller and Patrick, 1992; Patrick et al., 1993; Amador and Dani, 1995; McGehee and Role, 1995; Zoli et al., 1995), indicating that in addition to a possible common regulatory mechanism, each gene is most likely subject to independent regulation. Consistent with this idea is the demonstration that another family of POU domain factors, the Brn-3 family, can differentially regulate α3 subunit promoter activity in vitro, but has no effect on β4 subunit gene expression (Milton et al., 1996).

We have previously identified two adjacent regulatory elements, CT and CA boxes, in the promoter region of the β4 gene that are critical for its activity (Hu et al., 1995; Bigger et al., 1996). The CT box interacts with the transcriptional regulatory factors Purα (Du et al., 1997) and heterogeneous nuclear ribonucleoprotein K (hnRNP K) (Du et al., 1998), as well as with two as yet unidentified proteins (Du et al., 1997). The CA box serves as a binding site for the Sp1, Sp3 (Bigger et al., 1997) and Sox10 (Liu et al., 1999) transcription factors. We hypothesized that the proteins that bind to the CT and CA elements functionally interact to regulate β4 gene expression (Bigger et al., 1997). Indeed, Sp1 and Sp3 can directly interact and activate the β4 promoter in a synergistic manner (Bigger et al., 1997). Activation of the β4 promoter by Sp1 and Sp3 can be down-regulated by hnRNP K, which differentially affects the ability of these proteins to bind to DNA (Du et al., 1998). In an ongoing effort to test the hypothesis that the various transcription factors that interact with the CT and CA elements might physically associate to form a multi-protein complex that modulates β4 promoter activity, we performed in vitro-binding assays to assess protein–protein interactions between these factors. We demonstrate that the CT box-binding proteins Purα and hnRNP K directly interact, however, neither of the proteins is capable of associating with either Sp1 or Sp3. On the other hand, Sp1 and Sp3 directly bind to Sox10. Interestingly, Sox10 is also capable of directly interacting with Purα and hnRNP K. These results further underscore the complexity of transcriptional regulation of neuronal nicotinic acetylcholine receptor gene expression.

2. Materials and methods

2.1. Plasmids

Glutathione *S*-transferase (GST)-hnRNP K fusion constructs, except for GST-K3, were generously provided by Dr. David Levens (National Cancer Institute) and are described elsewhere (Tomonaga and Levens, 1995). GST-K3 was kindly provided by Dr. Karol Bomsztyk (University of Washington) (Denisenko et al., 1996). GST-Purα fusion constructs were a kind gift of Drs. Phang-Lang Chen and Wen-Hwa Lee (University of Texas Health Science Center at San Antonio) (Johnson et al., 1995). GST-Sp1 was a generous gift of Dr. Jonathan Horowitz (North Carolina State University) (Murata et al., 1994). GST-Sp3 was constructed by excising Sp3 cDNA from pPacUSp3, kindly provided by Dr. Guntram Suske (Philipps-Universität Marburg, Germany) (Hagen et al., 1994), with *Bam*HI and *Xho*I and ligating it into pGEX4T-1 (Pharmacia Biotech). pcDNA3/N-FLAG-hnRNP K was generously provided by Dr. Michael Lai (University of Southern California School of Medicine) (Hseih et al., 1998). pcDNA3.1-Purα was generated by subcloning an *Eco*RI fragment of Purα cDNA (from GST-Purα) into pcDNA3.1 vector. Construction of pBSKS plasmids, containing the Sox10 gene and its mutants, was performed as follows: Sox10 and Sox10ΔN, appropriate fragments were excised from pCMV5-Sox10 and pCMV5-Sox10ΔN, respectively (Kuhlbrodt et al., 1998a) with *Eco*RI and were cloned into the *Eco*RI site of pBSKS (Stratagene); Sox10MIC, Sox10WS029, Sox10059, fragments were excised from appropriate pCMV5-mutant Sox10 plasmids (Kuhlbrodt et al., 1998b) and subcloned into *Eco*RI/*Bam*HI-digested pBluescript KS; Sox10HMG, a *Bam*HI/*Xho*I fragment of Sox10HMG was cut out from pCMV5-Sox10HMG (Kuhlbrodt et al., 1998a) and ligated into *Bam*HI/*Sal*I-cut pBluescript KS.

2.2. Protein binding assays

Expression and purification of GST and GST-fusion proteins were carried out as described previously (Du et al., 1997, 1998). ^{35}S-labeled proteins were produced by transcribing and translating circular plasmid DNAs in the presence of ^{35}S-labeled methionine using the TNT coupled reticulocyte lysate system (Promega) as recommended by the manufacturer. ^{35}S-labeled proteins were incubated with GST-fusion proteins immobilized on glutathione–Sepharose 4B beads. In the assays detecting interactions between hnRNP K and Purα, the binding reactions were performed in 500 μl of standard lysis buffer containing

100 mM NaCl, 50 mM Tris–HCl (pH 7.4), 5 mM EDTA (pH 8.0), 0.5% Nonidet P-40 for 1 h at room temperature with continuous rocking. The rest of the assays were performed in 100 μl of BC100N buffer (20 mM HEPES (pH 7.9), 100 mM KCl, 20% glycerol, 0.02% Nonidet P-40, 1 mM dithiothreitol, 0.5 mM phenylsulfonyl fluoride, 0.5 mg/ml bovine serum albumin) for 3 h at 4°C. After incubation, the beads were washed five times with an appropriate buffer, resuspended in 20 μl of Laemmli sodium dodecyl sulfate (SDS) loading buffer (2% SDS, 100 mM dithiothreitol, 50 mM Tris–HCl, pH 6.8, 10% glycerol, 0.1% bromophenol blue). Samples were boiled for 5 min and analyzed by SDS–polyacrylamide gel electrophoresis (SDS-PAGE). The gels were then treated with Enhance (Du Pont) and dried. Proteins were detected by autoradiography.

3. Results

3.1. hnRNP K and Purα can directly interact

We employed in vitro protein binding assays to further investigate the possibility that the CT- and CA-binding factors may physically interact to modulate the promoter activity of the β4 gene. We previously reported that CA-binding proteins, Sp1 and Sp3, directly interact and synergistically activate the β4 promoter (Bigger at al., 1997). The CT-binding protein, hnRNP K, can inhibit the transactivation potentials of Sp1 and Sp3, however, it does not physically associate with either of the Sp factors (Du et al., 1998). As described earlier, Purα and hnRNP K were isolated by virtue of their abilities to interact with the CT box of the β4 promoter. Interestingly, the two proteins have high affinities for opposite strands of the CT box; hnRNP K favors the upper strand (Du et al., 1998), while Purα favors the lower strand (Du et al., 1997). Exactly how these binding characteristics relate to regulation of the β4 gene remains to be elucidated. In this context, it seems highly probable that the two proteins may directly interact with each other in order to exert their regulatory functions. As an initial test of this possibility, GST-pull-down assays were performed. First, beads bearing GST or GST-hnRNP K fusion proteins were incubated with in vitro-translated Purα. In this and the following experiments, the beads contained equivalent amounts of the GST and the GST-fusion proteins of interest, as judged by the Coomassie blue staining of a separate gel (data not shown). After binding, the beads were washed, the bound proteins were eluted by boiling in SDS loading buffer, resolved by electrophoresis and detected by autoradiography. As shown in Fig. 1B, radioactively labeled Purα was retained on the beads containing hnRNP K, but not on the beads containing just GST. In a reciprocal experiment, beads bearing GST or

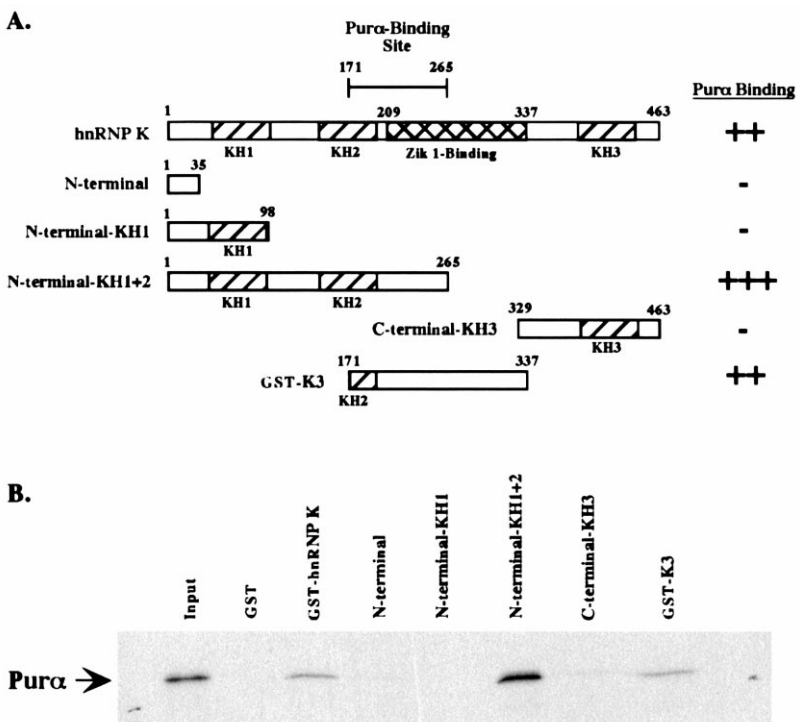

Fig. 1. Purα directly interacts with the region between amino acids 171 and 265 of hnRNP K in vitro. (A) Depiction of GST-hnRNP K deletion mutants. The hatched boxes represent conserved KH domains (Bomsztyk et al., 1997), while the cross-hatched box represents the Zik1-binding site. The N-terminal GST portion of each fusion protein is not shown. (B) In vitro-translated ^{35}S-labeled Purα was incubated with glutathione–Sepharose beads coupled to GST or the indicated fusion protein. The beads were washed, eluted with SDS buffer, and analyzed by SDS-12% PAGE. The autoradiograph of the region of the gel containing signal from in vitro-translated Purα is shown. The first lane (''Input'') shows the in vitro-translated ^{35}S-labeled Purα.

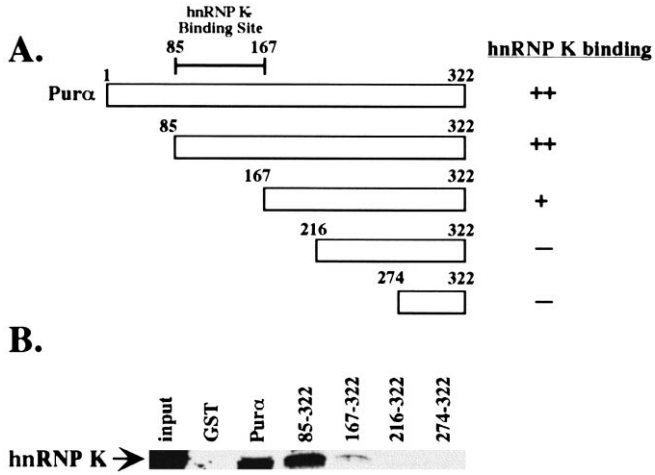

Fig. 2. hnRNP K directly interacts with the region between amino acids 85 and 167 of Purα in vitro. (A) Schematic diagram of amino-terminal deletions of Purα protein. The N-terminal GST portion of each fusion protein is not shown. (B) In vitro-translated ^{35}S-labeled hnRNP K was incubated with glutathione–Sepharose beads coupled to GST or the indicated fusion protein. The beads were washed, eluted with SDS buffer, and analyzed by SDS-12% PAGE. The autoradiograph of the region of the gel containing signal from in vitro-translated hnRNP K is shown. The first lane (''Input'') shows the in vitro-translated ^{35}S-labeled hnRNP K.

GST-Purα were incubated with in vitro-translated hnRNP K. Consistent with the above results, hnRNP K interacted with GST-Purα, but not with GST alone (Fig. 2B). These data indicate that in vitro, the binding of Purα and hnRNP K is direct and specific.

To identify the regions of hnRNP K involved in these interactions, various deletion mutants of hnRNP K (Fig. 1A) were produced as GST-fusion proteins and incubated with in vitro-translated Purα. As shown in Fig. 1B, Purα binds the amino-terminal half of hnRNP K, N-terminal KH1 + 2 (amino acids 1–265), as well as the middle portion of the protein, K3 (amino acids 171–337), but does not interact with the extreme amino or carboxy termini of hnRNP K, N-terminal (amino acids 1–35), N-terminal-KH1 (amino acids 1–98), C-terminal-KH3 (amino acids 329–463). Therefore, Purα-binding sites appear to be located between amino acids 171 and 265 of the hnRNP K protein. Next, we attempted to map the regions of Purα that are important for its interactions with hnRNP K. Radioactively labeled in vitro-translated hnRNP K was used in the GST-binding assays with GST-Purα deletion mutants encompassing various regions of Purα (Fig. 2A). As shown in Fig. 2B, binding of hnRNP K to Purα was gradually reduced upon removal of the first 167 amino acids of the latter protein, and was completely abolished when the amino-terminal half of Purα was deleted. These results suggest that the region between amino acids 85 and 167 are required for Purα interactions with hnRNP K.

3.2. The CT-binding proteins, Purα and hnRNP K, directly interact with Sox10

As mentioned above, we failed to detect direct interactions between hnRNP K and the CA-box binding proteins, Sp1 and Sp3. Interestingly, similar to hnRNP K, at least in vitro, Purα is also unable to bind to the Sp factors (data not shown). We next tested the ability of hnRNP K and Purα to interact with another CA-binding protein, Sox10. GST pull-down assays were performed as described above. As shown in Figs. 3B and 4B, in vitro-translated, radioactively labeled Sox10 sedimented with the beads containing both the GST-Purα and GST-hnRNP K fusion proteins, but not with the beads bound by GST alone. These results suggest that Sox10 can participate in direct protein–protein interactions with both hnRNP K and Purα in vitro.

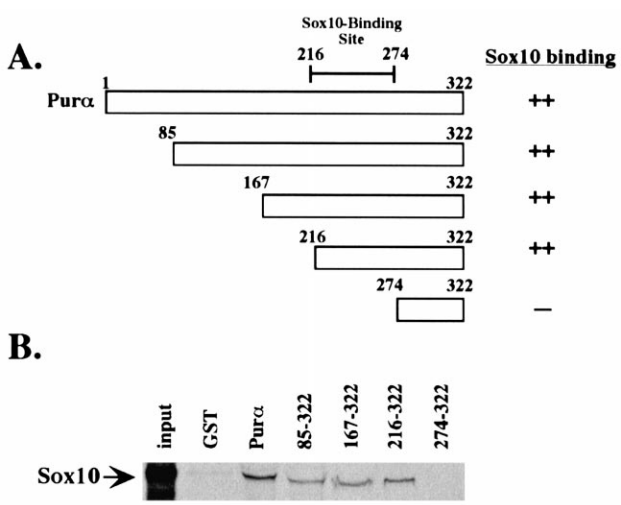

Fig. 3. Sox10 directly interacts with the region between amino acids 216 and 274 of Purα in vitro. (A) Depiction of deletion mutants of Purα protein. The N-terminal GST portion of each fusion protein is not shown. (B) In vitro-translated ^{35}S-labeled Sox10 was incubated with glutathione–Sepharose beads coupled to GST or the indicated fusion protein. The beads were washed, eluted with SDS buffer, and analyzed by SDS-12% PAGE. The autoradiograph of the region of the gel containing signal from in vitro-translated Sox10 is shown. The first lane (''Input'') shows the in vitro-translated ^{35}S-labeled Sox10.

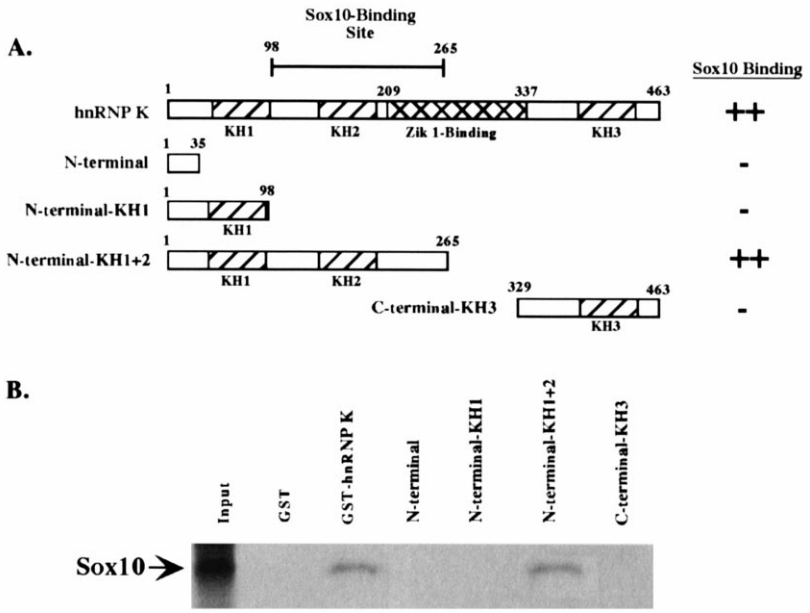

Fig. 4. Sox10 directly interacts with the region between amino acids 98 and 265 of hnRNP K in vitro. (A) Depiction of GST-hnRNP K deletion mutants. The hatched boxes represent conserved KH domains, while the cross-hatched box represents the Zik1-binding site. The N-terminal GST portion of each fusion protein is not shown. (B) In vitro-translated ^{35}S-labeled Sox10 was incubated with glutathione–Sepharose beads coupled to GST or the indicated fusion protein. The beads were washed, eluted with SDS buffer, and analyzed by SDS-12% PAGE. The autoradiograph of the region of the gel containing signal from in vitro-translated Sox10 is shown. The first lane (''Input'') shows the in vitro-translated ^{35}S-labeled Sox10.

To determine the region within Purα that mediate interactions with Sox10, in vitro-translated, radioactively labeled Sox10 was incubated with the GST-Purα variants containing different deletions of the protein (Fig. 3). Deletion of the C-terminal 48 amino acids of Purα completely abolished binding of Sox10 (Fig. 3B). However, deletion of the N-terminal 215 amino acids had little effect on its ability to interact with Sox10 (Fig. 3B). Together, these data indicate that direct protein–protein interactions between Sox10 and Purα are mediated by the region of Purα lying between amino acids 216 and 274.

Next, we tested which domains of hnRNP K are involved in its direct association with Sox10. As shown in Fig. 4B, similar to the binding of hnRNP K to Purα, the N-terminal half of the protein is involved in its binding to Sox10 (N-terminal KH1 + 2). However, the extreme N-terminus, KH1 domain and the C-terminal part of hnRNP K, that includes the KH3 domain, do not contribute to its interactions with Sox10 (Fig. 4). These results therefore suggest that the Sox10-binding site is located between amino acids 98 and 265 of the hnRNP K protein.

To further assess interactions between Sox10 and the CT box-binding proteins, GST-pull-down assays were performed using in vitro-translated, radioactively labeled variants of Sox10 and GST-fusion Purα and hnRNP K. Mutant Sox10ΔN lacks the amino terminal 89 amino acids, while Sox10HMG contains only the DNA-binding HMG domain of the protein (Fig. 5A). Three of the Sox10 mutations, WS029, MIC and 059 (Fig. 5A) were originally identified in patients with Waardenburg–Hirschsprung syndrome, a disease characterized by defects in tissues of neural crest origin (Pingault et al., 1998). Mutant WS029 is a nonsense mutation that converts tyrosine 83 to a stop codon; MIC is also a nonsense mutation leading to a truncated protein of only 188 amino acids; mutant 059 lacks the last 106 amino acids as a consequence of a deletion of two nucleotides at position 1076 resulting in a frameshift. As shown in Fig. 5B and C, the HMG domain of Sox10 appears to be sufficient to mediate its interactions with both Purα and hnRNP K. From all the mutants tested, only WS029 containing the N-terminal 83 amino acids of Sox10 was unable to bind to either Purα or hnRNP K (Fig. 5B and C). These results indicate that the HMG domain of Sox 10 is critical for its interactions with the CT-binding proteins.

3.3. Sox10 directly interacts with Sp1 and Sp3

Finally, we tested whether Sox10 can directly interact with Sp1 and Sp3. In vitro-translated, radioactively labeled wild type and mutant Sox10 proteins, described above, were used in the GST-binding assays with GST, GST-Sp1 and GST-Sp3. As shown in Fig. 6, GST protein by itself did not show any significant binding to Sox10. On the other hand, GST-Sp1 (Fig. 6B) and GST-Sp3 (Fig. 6C) strongly interacted with the full-length Sox10 protein,

suggesting that indeed Sox10 can directly bind to both Sp1 and Sp3. Removing the N- or C-terminal part of Sox10 was not detrimental to Sp factor recognition (Fig. 6B and C). In fact, both regions of the molecule could be deleted with the resulting HMG domain being sufficient to observe specific binding between Sox10 and Sp1 and Sp3. Similar, to interactions of Sox10 with the CT-binding proteins, the extreme N-terminus of the molecule (mutant WS029) did not support its association with either Sp1 or Sp3 (Fig. 6B and C). Thus, the HMG domain of Sox10 appears to be sufficient to bind to the Sp factors.

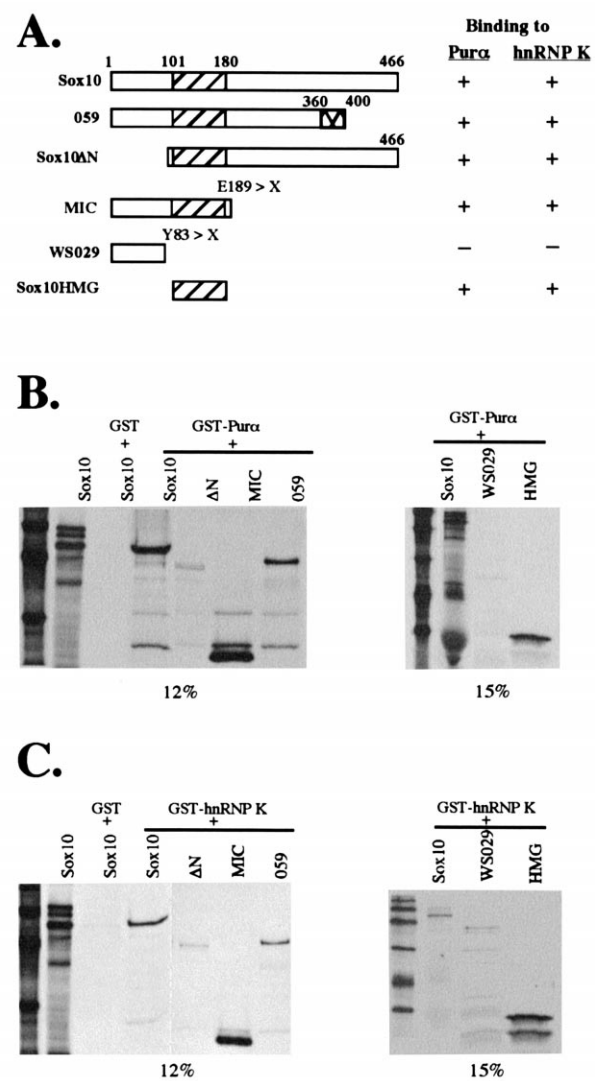

Fig. 5. Sox10 directly interacts with the CT-element binding-proteins Purα and hnRNP K in vitro. (A) Depiction of Sox 10 protein and its mutants. The hatched box represents the HMG domain. The cross-hatched box in mutant 059 represents 40 unrelated amino acids created by a frameshift (see text). (B) In vitro-translated ^{35}S-labeled Sox10 or one of its derivatives was incubated with glutathione–Sepharose beads coupled to GST or GST-Purα as labeled. The beads were washed, eluted with SDS buffer, and analyzed by SDS-PAGE. The percent of acrylamide in the gel is indicated under the autoradiograph. (C) Same as in (B), except that in vitro-translated ^{35}S-labeled Sox protein and its mutants were incubated with either GST or GST-hnRNP K.

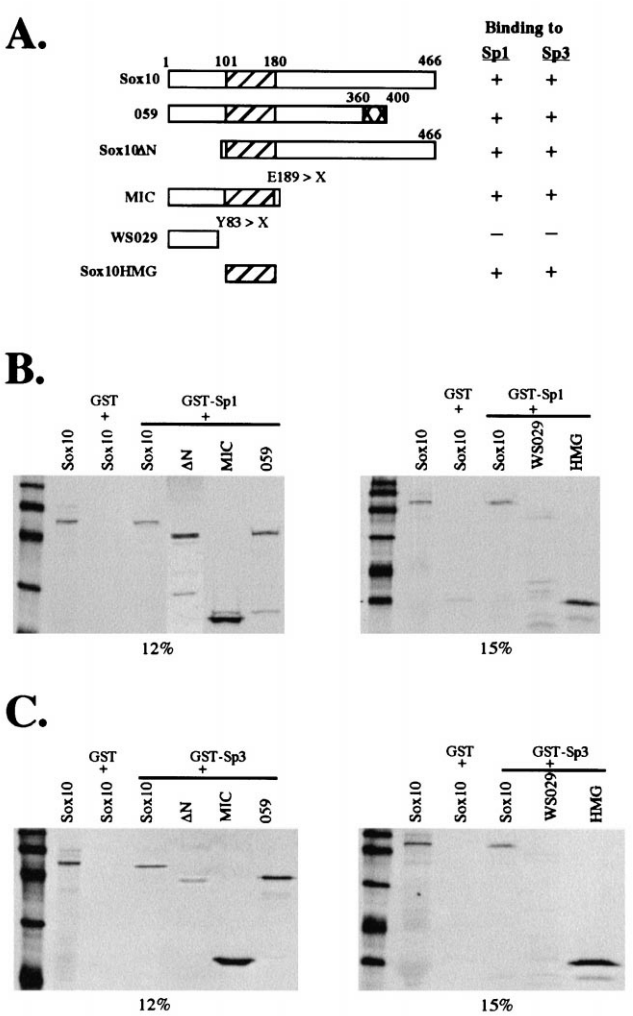

Fig. 6. Sox10 directly interacts with the CA-element binding-proteins Sp1 and Sp3 in vitro. (A) Depiction of Sox 10 protein and its mutants. (B) In vitro-translated ^{35}S-labeled Sox10 or one of its derivatives was incubated with glutathione–Sepharose beads coupled to GST or GST-Sp1 as labeled. The beads were washed, eluted with SDS buffer, and analyzed by SDS–PAGE. The percent of acrylamide in the gel is indicated under the autoradiograph. (C) Same as in (B), except that in vitro-translated ^{35}S-labeled Sox protein and its mutants were incubated with either GST or GST-Sp3.

4. Discussion

Development of a eukaryotic organism from a fertilized egg to an adult follows a predetermined pathway during which a great number of phenotypically different cells is generated. It is believed that the primary control for the generation of different cell types from an identical genetic background within an organism lies at the level of differential gene expression (Carey, 1998). How can the precise

spatial and temporal patterns of expression be achieved for over 50,000 genes in an organism? A widely held hypothesis is that cooperativity and synergy between a limited number of transcription factors, some of which are widely expressed and others that are tissue specific, are used to generate the desired complexity (Carey, 1998).

Our long-term goal is to understand the mechanisms that govern developmentally regulated and cell-type-specific expression of neuronal nicotinic acetylcholine receptors in the nervous system. Understanding how the expression of these synaptically located proteins is regulated will provide insight into the mechanisms underlying synaptogenesis as a whole. To date, we have identified several regulatory factors that appear to be involved in transcriptional regulation of the neuronal nicotinic acetylcholine receptor β4 subunit gene (Bigger et al., 1996, 1997; Du et al., 1997, 1998; Liu et al., 1999). Consistent with the hypothesis presented above, some of the identified factors are widely expressed, for example, Sp1 and Sp3 (Saffer et al., 1991; Hagen et al., 1992, 1994), while others have restricted patterns of expression, for example, Sox10 (Kuhlbrodt et al., 1998a). We have also demonstrated that, in the context of the β4 promoter, Sp1 and Sp3 can synergistically activate transcription (Bigger et al., 1997), while hnRNP K can differentially affect the transactivation potentials of these factors (Du et al., 1998).

To further investigate the potential functional interactions between the proteins that bind to the adjacent CT and CA regulatory elements within the β4 promoter, we used in vitro binding assays to detect direct protein–protein interactions among the various factors. We were able to detect direct and specific association between Purα and hnRNP K (Figs. 1 and 2), Purα and Sox10 (Figs. 3 and 5), hnRNP K and Sox10 (Figs. 4 and 5), Sox10 and the Sp factors, Sp1 and Sp3 (Fig. 6), but not between Purα/hnRNP K and Sp1/Sp3 (summarized in Fig. 7). Domains important for the interactions were mapped. Purα has distinct binding domains for interactions with hnRNP K (amino acids 85–167) and Sox10 (amino acids 216–274). Interestingly, the region between amino acids 85 and 215 of Purα is important for its interactions not only with hnRNP K, but also with another single-stranded DNA-binding protein, YB-1 (Safak et al., 1999). Interaction between Purα and YB-1 was shown to be cooperative and to result in synergistic activation of the human polyomavirus JCV promoter by these proteins (Safak et al., 1999). Therefore, it would be interesting to investigate whether the direct association between Purα and hnRNP K modulates their ability to bind to the CT-box of the β4 promoter and, importantly, whether it affects the transcriptional activity of this promoter.

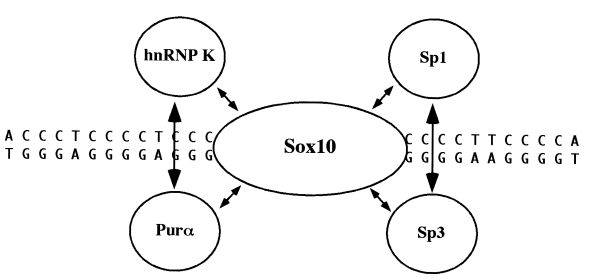

Fig. 7. A schematic diagram showing direct protein–protein interactions (arrows) among the CT- and CA-binding proteins at the relevant region of the β4 subunit gene promoter. The interactions between Sp1 and Sp3 were previously reported (Bigger et al., 1997).

It should be noted that hnRNP K is a highly interactive molecule that has been referred to as a ''nucleic acid-regulated docking platform'' (Bomsztyk et al., 1997). hnRNP K has been shown to bind to the transcription factor TBP (Michelotti et al., 1996), a transcriptional repressor Zik1 (Denisenko et al., 1996), the protein-tyrosine kinases Src, Fyn and Lyn, as well as other cellular factors (reviewed in Bomsztyk et al., 1997), suggesting potential roles of hnRNP K in gene regulation and signal transduction. In the case of interactions with Purα, amino acids 171 to 265 of hnRNP K are important. As the same region of hnRNP K is involved in its association with other proteins [e.g., Zik1 (Denisenko et al., 1996), protein-tyrosine kinases (Van Seuningen et al., 1995), Sox10 (this study)], these factors may disrupt the binding of hnRNP K with other partners and therefore affect its function. On the other hand, the dimerization domains present in hnRNP K may allow it to form higher order structures and thus allow it to be involved in the formation of complex multiprotein regulatory complexes (Schullery et al., 1999).

Of the proteins involved in the regulation of β4 promoter activity, hnRNP K interacts not only with Purα, but also with Sox10. The HMG domain of Sox10 is sufficient to mediate the binding. Sox10 belongs to the HMG family of transcription factors that has been shown to play important roles during a number of developmental processes such as sex determination, haemopoiesis, chondrogenesis, neurogenesis and lens formation (reviewed in Wegner, 1999). Members of this family are known for their ability to function cooperatively with other transcriptional regulators (Wegner, 1999). Interestingly, in numerous cases, the HMG domain of these factors seems to mediate the direct protein–protein interactions, for example such as those between Sox2 and Oct3 (Ambrosetti et al., 1997), and HMG-2 and Oct2 (Zwilling et al., 1995). Thus, the HMG domains of the Sox family members may contain common structural features for interactions with other transcription factors.

As shown in this study the HMG domain of Sox10 is also involved in binding to the general transcription factors Sp1 and Sp3. Sp1 and Sp3 have been demonstrated to regulate transcription of a wide variety of tissue-specific and ubiquitous genes (over 1500 citations). Sp1 has been found to directly interact and synergistically cooperate with other transcription factors, such as Ets, NF-κB, E2F and AP1 (Marin et al., 1997 and references therein). In addition, both Sp1 and Sp3 have been postulated to acti-

vate transcription of TATA-less promoters via protein–protein interactions tethering them to the RNA polymerase II complex (Pugh and Tjian, 1991; Dennig et al., 1996). This is of interest as the β4 gene promoter does not contain a TATA sequence (Hu et al., 1994) and, therefore, the coupling of protein interactions at the CT/CA elements with the basal transcriptional machinery must occur independently of TBP binding to DNA. One way by which this may be achieved is through protein–protein interactions (e.g., between Sp1 and TAF110). As mentioned above, hnRNP K directly interacts with TBP (Michelotti et al., 1996), therefore, the three proteins we have shown to bind to regulatory regions of the TATA-less β4 promoter each have the potential to interact with the general transcriptional apparatus. The functional interplay between these various regulatory proteins as it pertains to expression of the β4 subunit gene is under investigation.

Acknowledgements

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