Differential regulation of neuronal nicotinic acetylcholine receptor subunit gene promoters by Brn-3 POU family transcription factors

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The regulatory region of the neuronal nicotinic acetylcholine (nACh) receptor $\alpha 2$ subunit gene is activated by the Brn-3b POU family transcription factor but not by the closely related factors Brn-3a and Brn-3c. This pattern of regulation has not previously been observed for other neuronally expressed genes, several of which, such as those encoding α -internexin or SNAP-25, are activated by Brn-3a and Brn-3c but repressed by Brn-3b. The $\alpha 3$ nACh receptor subunit gene is also shown to be activated by Brn-3a but is repressed by Brn-3b and Brn-3c. In contrast, the

Brn-3 POU family transcription factors have no effects on either the $\alpha 7$ or $\beta 4$ nACh receptor subunit genes. The actions of Brn-3b on the $\alpha 2$ subunit are thus in contrast to the inhibitory actions of Brn-3b on several promoters that are activated by Brn-3a. The different actions of the Brn-3 POU factors on the range of nACh receptor genes tested suggests that the novel stimulation of the $\alpha 2$ subunit by Brn-3b is specific to this subunit and not a general feature of nACh receptor genes.

INTRODUCTION

Nicotinic acetylcholine (nACh) receptors play a crucial role in signal transduction within the nervous system. A family of nACh receptor α and β subunits have been identified by molecular cloning techniques in the chick and rat [1,2]. These various subunits show distinct patterns of expression within neuronal cells in developing and adult organisms [2–11]. Functional nACh receptors can be formed by complexes of α and β subunits or homomeric complexes of $\alpha 7$, $\alpha 8$ or $\alpha 9$ subunits [3,12,13]. The regulation of the expression of these subunits is controlled at the gene level by the actions of cellular transcription factors which modulate gene activity in both positive and negative directions.

The $\alpha 2$ nicotinic receptor subunit is one of the most restricted in its expression, being expressed in chickens for example only in the spiriformis lateralis nucleus of the diencephalon [14,15]. Its expression appears to be under negative regulation by an inhibitor element that reduces the activity of the promoter itself and also has this effect when linked to the heterologous promoter of simian virus 40 (SV40) [16]. The regulatory region of the $\alpha 2$ subunit contains six copies of the sequence CCCATGCAAT, which appears to be responsible for its inhibitory effect [16]. This sequence shows strong sequence similarity to the octamer consensus sequence ATGCAAAT which is bound by members of the POU family of transcription factors (for reviews see [17,18]). The three closely related members of the Brn-3 POU family Brn-3a (also known as Brn-3 or Brn-3.0; [19–21]), Brn-3b (also known as Brn-3.2; [20,22–24]) and Brn-3c (also known as Brn-3.1; [20,21,25]) are specifically expressed in neuronal cells of the peripheral and central nervous systems with each factor having distinct but overlapping expression patterns [19–21,23–25]. We have previously shown [26] that the Brn-3b factor activates a construct containing the $\alpha 2$ nACh receptor-regulating region linked to the SV40 promoter while Brn-3a and Brn-3c have no effect. The actions of the Brn-3 transcription factors on the $\alpha 2$

subunit regulatory region contrast with their actions on other neuronal promoters such as those of the genes encoding SNAP-25 or α -internexin which are activated by Brn-3a and Brn-3c but repressed by Brn-3b [27–30]. Thus the $\alpha 2$ nACh regulatory region is the first natural target for activation by Brn-3b to be described.

As the effects of the Brn-3 POU factor family on the $\alpha 2$ nACh receptor subunit regulatory region appear to be so unique, we wished to test the possibility that members of this family could regulate some of the other nACh receptor subunits to determine the nature of nACh receptor regulation by Brn-3. We have therefore investigated the effects of each of the forms of Brn-3 on the $\alpha 2$, $\alpha 3$, $\alpha 7$ and $\beta 4$ nACh receptor subunit gene promoters.

MATERIALS AND METHODS

Plasmid DNA

The $\alpha 2$ receptor constructs were as described by Bessis et al. [16]. The $\alpha 2.6$ -SV40-Sil construct contains the promoter region from –810 to +81 relative to the transcriptional start site upstream of the SV40 promoter and the luciferase gene. The $\alpha 2.6$ -Sil construct contains the same promoter region upstream of the luciferase gene without any SV40 sequences.

The $\alpha 3$ receptor constructs were as described by Yang et al. [31]. The $\alpha 3$ -238 construct contains the promoter region from –238 to +47 relative to the $\alpha 3$ transcriptional start site upstream of the luciferase gene. The $\alpha 3$ -1607 construct contains the promoter region from –1607 to +47 upstream of the luciferase gene.

The $\alpha 7$ receptor constructs were as described by Matter-Sadzinski et al. [32]. The NR, NP and NSa constructs contain respectively the promoter regions from –1383 to –188, –406 to –188 and –230 to –188 respectively, upstream of the $\alpha 7$ initiator ATG inserted immediately upstream of the chloramphenicol acetyltransferase (CAT) gene.

The $\beta 4$ receptor construct was as described by Hu et al. [33] and contains the region from -89 to $+137$ relative to rat $\beta 4$ transcription initiation site upstream of the luciferase gene.

The Brn-3a, Brn-3b and Brn-3c expression vectors contain full-length cDNA clones for each of these proteins [20] cloned under the control of the Moloney murine leukaemia virus promoter in the vector pLTRpoly, which has been modified by deletion of a cryptic splice site in the SV40 3' untranslated region [20,27,28]. In the case of Brn-3a an expression vector containing cDNA derived from the spliced form of the Brn-3a mRNA was used. The Brn-3b vector contains a full-length cDNA derived from the intronless RNA which encodes Brn-3b, while the Brn-3c vector similarly contains a full-length cDNA derived from the mRNA encoding Brn-3c which is produced from the primary transcript by the removal of a single intron [20]. Brn-3 chimeras contained PCR-generated sequences encoding the subdomains of Brn-3a and Brn-3b cloned into the same vector [20]. The subdomains of Brn-3a and Brn-3b were as follows, Brn-3a: subdomain I, amino acids (aa) 1 to 40; subdomain II, aa 41 to 108; subdomain III, aa 109 to 267; subdomain IV (POU domain), aa 268 to the end. Brn-3b: subdomain II, aa 1 to 92; subdomain III, aa 93 to 169; subdomain IV (POU domain), aa 170 to the end. Chimeric construct 1 contained subdomains I, II and III from Brn-3a plus subdomain IV from Brn-3b; chimeric construct 2 contained subdomains I, II and IV from Brn-3a plus subdomain III from Brn-3b; chimeric construct 3 contained subdomains I and II from Brn-3a plus subdomains III and IV from Brn-3b; chimeric construct 4 contained subdomain IV from Brn-3a plus subdomains II and III from Brn-3b.

Transfection

Transfection of DNA was carried out according to the method of Gorman [34]. Routinely 1×10^6 BHK-21 cells [35] were transfected with $10 \mu\text{g}$ of the reporter plasmid and $10 \mu\text{g}$ of the expression vector. In all cases cells were harvested 72 h later. The amount of DNA taken up by the cells in each case was measured by slot blotting the extract and hybridization with a probe derived from the ampicillin resistance gene in the plasmid vector [36]. This value was then used to normalize the values obtained in the luciferase or CAT assay as a control for differences in uptake of plasmid DNA in each sample.

Luciferase and CAT assays

Assays of luciferase activity were carried out using a commercially available kit (Promega) and a Turner luminometer with extracts which had been equalized for protein content according to the method of Smith et al. [37]. Assays of CAT activity were carried out according to the method of Gorman [34] using samples which had been equalized for protein content according to the method of Smith et al. [37].

RESULTS

In previous studies we transfected BHK-21 fibroblasts cells [36] which lack any endogenous Brn-3 [22] with plasmids expressing each of the forms of Brn-3 [20] and a reporter plasmid containing the entire $\alpha 2$ gene inhibitory region ($\alpha 2.6$) upstream of the SV40 promoter driving the luciferase gene [26]. This construct was strongly activated by Brn-3b and unaffected by Brn-3a and Brn-3c. As the construct used in these studies contained the $\alpha 2$ regulatory region from -810 to $+81$ relative to the transcriptional start site cloned upstream of the SV40 promoter, we wished to test whether the effects we observed could also be obtained in the absence of any SV40 sequences. In the present

Table 1 Effect of Brn-3 forms on the $\alpha 2$ nACh receptor subunit promoter

Luciferase activity in BHK-21 cells transfected with intact $\alpha 2.6$ constructs with or without an SV40 promoter and either expression vector alone (vect) or the same vector expressing the indicated Brn-3 form. Values are expressed as mean $\pm$ S.E.M. in terms of ng of luciferase/ml of extract. * $P < 0.005$ compared with $\alpha 2.6$ -sil + vect by unpaired Student's *t*-test. † $P < 0.01$ compared with $\alpha 2.6$ -SV40-sil + vect by unpaired Student's *t*-test.

Construct	Luciferase activity (ng of luciferase/ml of extract)
$\alpha 2.6$ -sil + vect	0.67 ± 0.08
$\alpha 2.6$ -sil + Brn-3a	0.79 ± 0.10
$\alpha 2.6$ -sil + Brn-3b	$2.94 \pm 0.39^*$
$\alpha 2.6$ -sil + Brn-3c	0.79 ± 0.15
$\alpha 2.6$ -SV40-sil + vect	2.41 ± 0.78
$\alpha 2.6$ -SV40-sil + Brn-3a	3.07 ± 1.07
$\alpha 2.6$ -SV40-sil + Brn-3b	$37.80 \pm 8.82^\dagger$
$\alpha 2.6$ -SV40-sil + Brn-3c	1.81 ± 0.29

Table 2 Effect of Brn-3 forms on the $\alpha 3$ nACh receptor subunit promoter

Luciferase activity in BHK-21 cells transfected with the $\alpha 3$ (1607) or $\alpha 3$ (238) constructs and either expression vector alone (vect) or the same vector expressing the indicated Brn-3 form. Values are expressed as mean $\pm$ S.E.M. in terms of ng of luciferase/ml of extract. * $P < 0.005$ compared with $\alpha 3$ (1607) + vect by unpaired Student's *t*-test. † $P < 0.001$ compared with $\alpha 3$ (239) + vect by unpaired Student's *t*-test.

Construct	Luciferase activity (ng of luciferase/ml of extract)
$\alpha 3$ (1607) + vect	0.57 ± 0.02
$\alpha 3$ (1607) + Brn-3a	$2.35 \pm 0.32^*$
$\alpha 3$ (1607) + Brn-3b	$0.29 \pm 0.04^*$
$\alpha 3$ (1607) + Brn-3c	$0.24 \pm 0.06^*$
$\alpha 3$ (239) + vect	2.01 ± 0.15
$\alpha 3$ (239) + Brn-3a	$7.79 \pm 0.89^\dagger$
$\alpha 3$ (239) + Brn-3b	$0.72 \pm 0.11^\dagger$
$\alpha 3$ (239) + Brn-3c	$0.51 \pm 0.06^\dagger$

study we therefore compared the effects of Brn-3 forms on the $\alpha 2.6$ construct containing the -810 to $+81$ region with and without the SV40 promoter (Table 1). Both reporter gene constructs were strongly transactivated by co-transfection with the Brn-3b expression vector compared with the level of promoter activity when co-transfected with the expression vector lacking any insert. In contrast neither the Brn-3a or Brn-3c constructs had any effect on the activity of either $\alpha 2.6$ construct. Hence Brn-3b can activate the $\alpha 2$ nACh receptor promoter in the absence of any SV40 sequences.

To investigate whether the effects were specific to the $\alpha 2$ nACh receptor promoter we used a construct containing the region from -1607 to $+47$ relative to the transcriptional start site of the $\alpha 3$ nACh receptor promoter linked to the luciferase gene. When BHK-21 fibroblasts were co-transfected with this construct and plasmids expressing each of the forms of Brn-3 the $\alpha 3$ (1607) construct was transactivated by Brn-3a and repressed by Brn-3b and Brn-3c (Table 2). In the case of the $\alpha 2$ promoter transactivation by Brn-3b is lost when the regulatory region containing six copies of the CCCATGCAAT motif is truncated [26]. In contrast, in the case of the $\alpha 3$, similar strong activation by Brn-3a and mild repression by Brn-3b and Brn-3c were also observed for a truncated $\alpha 3$ promoter construct containing the region of the promoter from -238 to $+47$ (Table 2). In other promoters activated by Brn-3a and repressed by Brn-3b, Brn-3b can also

Table 3 Effect of combinations of Brn-3 forms on the $\alpha 3$ nACh receptor subunit promoter

Luciferase activity in BHK-21 cells transfected with the $\alpha 3$ (1607) construct and combinations of either expression vector alone (vect) or the same vector expressing the indicated Brn-3 forms. Values are expressed as mean $\pm$ S.E.M. in terms of ng of luciferase/ml of extract. * $P < 0.01$ compared with $\alpha 3$ (1607) + vect + vect by unpaired Student's *t*-test.

Construct	Luciferase activity (ng of luciferase/ml of extract)
$\alpha 3$ (1607) + vect + vect	0.52 $\pm$ 0.02
$\alpha 3$ (1607) + Brn-3a + vect	2.12 $\pm$ 0.37*
$\alpha 3$ (1607) + Brn-3b + vect	0.15 $\pm$ 0.06*
$\alpha 3$ (1607) + Brn-3c + vect	0.14 $\pm$ 0.04*
$\alpha 3$ (1607) + Brn-3a + Brn-3b	1.97 $\pm$ 0.21*
$\alpha 3$ (1607) + Brn-3a + Brn-3c	3.20 $\pm$ 0.60*

interfere with activation by Brn-3a [27–29]. This was not so in the case of the $\alpha 3$ promoter, however, since transactivation of each construct by Brn-3a was unaffected by co-transfection with either Brn-3b or Brn-3c (Table 3). In previous experiments using chimeric constructs containing different regions of Brn-3a and Brn-3b we have shown that different regions are required for activation depending on the target promoter involved (Figure 1). In view of the apparent difference between Brn-3a activation of the $\alpha 3$ promoter compared with other neuronal promoters we examined the effects of these chimeric constructs and the isolated POU domains of Brn-3a and Brn-3b on the $\alpha 3$ promoter. In these experiments the $\alpha 3$ promoter was activated by Brn-3a and chimeric construct 4 (Table 4), suggesting that subregions II and III must come from the same Brn-3 form and be combined with a Brn-3a POU domain (subregion IV) for activation of this promoter.

To test whether Brn-3a or Brn-3b could activate other nACh receptor gene promoters we used constructs either containing the region of the $\alpha 7$ promoter from –1383 (NR), –406 (NP) or –230 (NSa) to –188 relative to the translational start site [32] or the region of $\beta 4$ from –89 to +137 relative to the transcriptional start site [33]. When BHK-21 fibroblasts were co-transfected with either the $\alpha 7$ (NR), $\alpha 7$ (NP) or $\alpha 7$ (NSa)

Table 4 Effect of Brn-3 chimeric and POU domain constructs on the $\alpha 3$ nACh receptor subunit promoter

Luciferase activity in BHK-21 cells transfected with the $\alpha 3$ (1607) construct and vectors expressing each of the indicated forms of Brn-3, the isolated POU domains of Brn-3a or Brn-3b, the chimeric constructs coded as indicated in Figure 1. Values are expressed as mean $\pm$ S.E.M. in terms of ng of luciferase/ml of extract. * $P < 0.005$ compared with $\alpha 3$ (1607) + vect by unpaired Student's *t*-test. † $P < 0.05$ compared with $\alpha 3$ (1607) + vect by unpaired Student's *t*-test.

Construct	Luciferase activity (ng of luciferase/ml of extract)
$\alpha 3$ (1607) + vect	0.60 $\pm$ 0.04
$\alpha 3$ (1607) + Brn-3a	2.35 $\pm$ 0.32*
$\alpha 3$ (1607) + Brn-3b	0.29 $\pm$ 0.04*
$\alpha 3$ (1607) + POU-A	0.24 $\pm$ 0.10†
$\alpha 3$ (1607) + POU-B	0.10 $\pm$ 0.03*
$\alpha 3$ (1607) + 1	0.08 $\pm$ 0.01*
$\alpha 3$ (1607) + 2	0.08 $\pm$ 0.02*
$\alpha 3$ (1607) + 3	0.24 $\pm$ 0.11†
$\alpha 3$ (1607) + 4	2.44 $\pm$ 0.75†

construct and plasmids expressing each of the forms of Brn-3, all three $\alpha 7$ constructs were unaffected by Brn-3a, Brn-3b or Brn-3c (Table 5). Similarly, when BHK-21 fibroblasts were co-transfected with the $\beta 4$ construct and plasmids expressing each of the forms of Brn-3 all three Brn-3 forms (Brn-3a, Brn-3b and Brn-3c) failed to affect the basal expression of the $\beta 4$ construct (Table 6).

DISCUSSION

The activation of the neuronal nACh receptor $\alpha 2$ region by Brn-3b reported here demonstrates that our previous reports of such activation [26] are independent of the presence of an SV40 promoter within the construct and is due to a direct action of Brn-3b on the $\alpha 2$ promoter. Such activation of this nACh promoter by Brn-3b thus far appears to be unique since the promoters of several other neuronally expressed genes such as α -internexin [29] or SNAP-25 [30] have been shown to be activated by Brn-3a, but repressed by Brn-3b. The present studies also

	I	II	III	IV POU	tK-Oct	α -internexin	$\alpha 2$	$\alpha 3$
A	■	■	■	■	+	+	-	+
B		□	□	□	-	-	+	-
1	■	■	■	□	-	+	-	-
2	■	■	□	■	+	+	-	-
3	■	■	□	□	-	+	-	-
4		□	□	■	+	-	-	+

Figure 1 Summary of gene transactivation data with the Brn-3a (A) and Brn-3b (B) vectors and chimeric proteins containing different subregions of each factor

Data are summarized for constructs containing a consensus octamer motif cloned upstream of a test thymidine kinase promoter [27], the α -internexin promoter [29], the $\alpha 2$ promoter [26] and the $\alpha 3$ promoter (Table 4, this paper). The division of Brn-3a and Brn-3b subdomains I, II, III and IV is as follows, Brn-3a: subdomain I, amino acids (aa) 1 to 40; subdomain II, aa 41 to 108; subdomain III, aa 109 to 267; subdomain IV (POU domain), aa 268 to the end. Brn-3b: subdomain II, aa 1 to 92; subdomain III, aa 93 to 169; subdomain IV (POU domain), aa 170 to the end.

Table 5 Effect of Brn-3 forms on the $\alpha 7$ nACh receptor subunit promoter

Luciferase activity in BHK-21 cells transfected with the $\alpha 7$ (NP), $\alpha 7$ (NR) or $\alpha 7$ (NSa) constructs and either expression vector alone (vect) or the same vector expressing the indicated Brn-3 form. Values are expressed as mean $\pm$ S.E.M. in terms of percentage of [14 C]chloramphenicol converted into monoacetate form. *Not significantly different from $\alpha 7$ (NP) + vect by unpaired Student's *t*-test. †Not significantly different from $\alpha 7$ (NR) + vect by unpaired Student's *t*-test. ‡Not significantly different from $\alpha 7$ (NSa) + vect by unpaired Student's *t*-test.

Construct	Chloramphenicol conversion (%)
$\alpha 7$ (NP) + vect	25.66 $\pm$ 7.56
$\alpha 7$ (NP) + Brn-3a	30.52 $\pm$ 3.51*
$\alpha 7$ (NP) + Brn-3b	31.87 $\pm$ 4.32*
$\alpha 7$ (NP) + Brn-3c	29.71 $\pm$ 5.67*
$\alpha 7$ (NR) + vect	10.95 $\pm$ 1.18
$\alpha 7$ (NR) + Brn-3a	11.13 $\pm$ 3.98†
$\alpha 7$ (NR) + Brn-3b	10.23 $\pm$ 1.45†
$\alpha 7$ (NR) + Brn-3c	11.86 $\pm$ 3.71†
$\alpha 7$ (NSa) + vect	4.47 $\pm$ 0.84
$\alpha 7$ (NSa) + Brn-3a	4.05 $\pm$ 0.37‡
$\alpha 7$ (NSa) + Brn-3b	4.89 $\pm$ 0.84‡
$\alpha 7$ (NSa) + Brn-3c	5.79 $\pm$ 0.63‡

Table 6 Effect of Brn-3 forms on the $\beta 4$ nACh receptor subunit promoter

Luciferase activity in BHK-21 cells transfected with the $\beta 4$ construct and either expression vector alone (vect) or the same vector expressing the indicated Brn-3 form. Values are expressed as mean $\pm$ S.E.M. in terms of ng of luciferase/ml of extract. *Not significantly different from $\beta 4$ + vect by unpaired Student's *t*-test.

Construct	Luciferase activity (ng of luciferase/ml of extract)
$\beta 4$ + vect	7.83 $\pm$ 1.08
$\beta 4$ + Brn-3a	8.82 $\pm$ 1.17*
$\beta 4$ + Brn-3b	6.48 $\pm$ 1.44*
$\beta 4$ + Brn-3c	9.09 $\pm$ 1.89*

demonstrate that such activation by Brn-3b is not a general feature of its actions on nACh receptor subunits. Thus $\alpha 3$ nACh receptor reporter constructs were activated by Brn-3a but not Brn-3b and Brn-3c, whereas the $\alpha 7$ and $\beta 4$ subunits were unaffected by any of the factors. These results demonstrate that different nACh receptor subunits show regulation by the Brn-3 family POU factors but suggest that these POU factors do not control all nACh receptor subunit expression.

The activation of the $\alpha 2$ nACh receptor promoter by Brn-3b depends on the intactness of the regulatory region from -144 to $+81$ containing six copies of the motif CCCATGCAAT. Truncation of this regulatory region so that one, two or four copies of the motif are present results in loss of Brn-3b activation, although these truncated constructs, unlike the full-length construct, are activated by Brn-3c [26]. In contrast similar activation by Brn-3a and repression by Brn-3b and Brn-3c was observed when the $\alpha 3$ construct was truncated from -1607 to -238 .

The full-length $\alpha 3$ nACh receptor has previously been shown to be strongly activated by the POU domain factor SCIP/Tst-1 [31]. The POU domain factors Oct-2 and Brn-4 were also shown to weakly activate this promoter although Oct-1 had no effect [31]. The minimal promoter region from -238 is also activated by SCIP/Tst-1 but lacks upstream SCIP/Tst-1-binding sites [31]. Moreover, mutants of SCIP/Tst-1 which cannot bind to DNA

still activate the $\alpha 3$ promoter [31] and it has therefore been suggested that SCIP/Tst-1 activates this promoter by protein-protein interactions with other transcription factors rather than by direct DNA binding. The lack of obvious octamer or octamer-related motifs in the -238 to $+47$ region of the $\alpha 3$ promoter might suggest that Brn-3a activates the $\alpha 3$ promoter by a similar mechanism not involving DNA binding. This would explain some of the differences in $\alpha 3$ promoter activation from that observed for example on a test promoter containing Brn-3a-binding sites [27,28] or on the α -internexin gene promoter [29] which contains Brn-3a-binding sites [38]. Thus unlike the $\alpha 3$ promoter these promoters are also activated by Brn-3c and activation by Brn-3a can be inhibited by Brn-3b, whereas this is not the case on the $\alpha 3$ promoter. The results obtained for activation of the $\alpha 3$ promoter by Brn-3a and the chimeric construct 4 might suggest that if activation is occurring via this mechanism the protein-protein interactions require subregions II and III from the same Brn-3 form combined with a POU domain (subregion IV) from Brn-3a. This contrasts with the thymidine kinase promoter with an added octamer motif (tK-oct) which was activated by all constructs containing a Brn-3a POU domain [27], and the α -internexin promoter which was activated by constructs containing a Brn-3a-derived N-terminus [29]. The strong activation of the $\alpha 3$ promoter by SCIP/Tst-1 was cell-type-dependent [31] and much reduced in non-neuronal cells. In our study we have used the BHK-21 cell line and the relatively small activation by Brn-3a may thus be a consequence of the use of a non-neuronal cell type.

Alternatively it is possible that the $\alpha 3$ promoter from -238 to $+47$ does contain binding sites for Brn-3a. Thus several different consensus binding sites for the factor have been reported by different groups [39,40]. Moreover, in the α -internexin promoter removal of all the octamer-like motifs which can bind Brn-3a to yield a promoter containing only the region downstream of -77 did not abolish the ability of the promoter to respond to Brn-3a [29]. However, further truncation to -46 rendered the promoter non-responsive to Brn-3a and a novel binding site for Brn-3a was subsequently detected in the region from -77 to -46 [38].

Whatever the precise mechanism by which Brn-3a activates the $\alpha 3$ promoter, it is clear that this promoter is one of several neuronal promoters activated by this factor. In contrast, the activation of the $\alpha 2$ promoter by Brn-3b is thus far unique. The $\alpha 2$ nACh receptor subunit is the most restricted in its distribution and clearly the ability of Brn-3b to activate its promoter may play a role in its limited expression within the nervous system. The $\alpha 3$ subunit is more widely distributed and activation by Brn-3a, together with suppression by Brn-3b and Brn-3c, could play a role in its expression within the nervous system. Thus *in situ* hybridization studies comparing the distribution of the various forms of Brn-3 with that of the $\alpha 2$ and $\alpha 3$ subunits will be necessary to investigate this further. The $\alpha 7$ promoter is activated during development [32]; however, the Brn-3 POU factors do not appear to affect its expression. Similarly the Brn-3 factors do not modulate the activity of the $\beta 4$ promoter even though the $\alpha 3$ and $\beta 4$ subunit genes are on the same chromosome and are closely linked [31], suggesting that they could be subject to similar regulation.

In conclusion the Brn-3 POU family transcription factors are able to regulate the $\alpha 2$ and $\alpha 3$ nACh receptor subunit gene expression but not the $\alpha 7$ and $\beta 4$ subunits. The $\alpha 2$ subunit is activated by Brn-3b while the $\alpha 3$ subunit is repressed by this Brn-3 form but activated by Brn-3a. This indicates that the action of Brn-3 transcription factors on nACh receptor gene expression is dependent on the individual characteristics of each subunit promoter.

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