ELSEVIER

European Journal of Pharmacology 393 (2000) 69–74

ejp

www.elsevier.nl/locate/ejphar

Transcriptional control of the neuronal nicotinic acetylcholine receptor gene cluster by the β43′ enhancer, Sp1, SCIP and ETS transcription factors

Evan S. Deneris *, Nicole Francis, Jenny McDonough, Dmitry Fyodorov, Tim Miller, Xiangdong Yang

Case Western Reserve University, School of Medicine, Department of Neurosciences, 2109 Adelbert Rd. Cleveland OH, 44106-4975, USA

Accepted 21 January 2000

Abstract

Receptors assembled from the products of a neuronal β4α3α5 NAChR gene cluster depend on these genes being coordinately regulated in particular populations of neurons. Little is known, however, about the transcriptional mechanisms that are likely to underlie their co-expression in correct neuronal cell types. We have identified several regulatory elements and transcription factors that influence transcription of the α3 and β4 genes. The promoters of these genes appear to contain a common *cis* element that binds Sp1 transcription factors. They can be activated by the POU-domain factor SCIP and activation does not require SCIP binding sites. Between these two promoters is a cell type specific enhancer called β43′. This enhancer has little activity in non-neuronal cells and is preferentially active in particular populations of central neurons. The clustered genes are potential targets of ETS factors as the ETS domain factor, Pet-1 can activate β43′-dependent transcription. The neuron-selective properties of β43′ and its location suggest that it is a component of the *cis* regulatory information required to control expression of the β4 and α3 genes in specific populations of neurons.

Keywords: Nicotinic receptor; Transcription; Enhancer; Promoter; Neuron

1. Introduction

The vertebrate genome provides a tremendous potential for generating nicotinic acetylcholine receptor diversity by encoding at least sixteen different subunits that can be used to assemble receptors. An important question currently under investigation is what are the transcriptional mechanisms that control the distribution of subunit mRNA within various populations of vertebrate neurons? As most nicotinic receptor subtypes expressed in neurons are heteromeric assemblies of two, three, or even four different subunits, the genes encoding them must be coordinately regulated to allow the co-expression of appropriate subunit mRNA in the correct neuronal cell types. Thus, as the protein-coding regions of the neuronal nicotinic receptor subunit genes diversified over time, the genetic regulatory information required to restrict and coordinate neuronal co-expression of individual subunit mRNAs must have evolved in parallel.

Three of the neuronal nicotinic receptor subunit genes are clustered in the order β4, α3, and α5 over about 50 kb in the vertebrate genome (Boulter et al., 1990; Couturier et al., 1990; Raimondi et al., 1992). Clustering of the β4, α3, and α5 genes suggests that this organization has been evolutionarily conserved in order to preserve regulatory information needed to control cell-type specific transcription of these genes. It is straightforward to imagine what might be the functional significance of this organization as the β4, α3, and α5 subunits are assembled together into at least one major ganglionic nicotinic receptor subtype (Conroy and Berg, 1995; Vernallis et al., 1993). β4 and α3 but not α5 are also likely to be assembled together into at least one retinal subtype well as other brain subtypes (Vailati et al., 1999; Zoli et al., 1998). It seems

* Corresponding author. Tel.: +1-216-368-8725; fax: +1-216-368-4650.

E-mail address: esd@po.cwru.edu (E.S. Deneris).

0014-2999/00/$ - see front matter
PII: S0014-2999(99)00883-3

reasonable to expect that the clustered organization of their respective genes is a particularly efficient solution to coordinating peripheral and central neuronal co-expression of one or more of them. Experimentally, the cluster offers a relatively compact genetic system with which to identify transcriptional mechanisms that coordinate expression of subunits that are to be assembled together into receptors in particular neuronal populations.

One way in which coordinate expression might be achieved is through the action of neuron selective enhancers that activate transcription of the clustered genes in specific populations of neurons. Perhaps clustering permits the sharing of these cell-type specific *cis* elements in order to coordinate subunit expression. We have searched for neuron-selective *cis* elements upstream of the rat β4 and α3 genes using a combination of reporter assays in cell lines, central and peripheral primary neurons, and transgenic animals. This investigation has resulted in the identification of a non-cell type-specific α3 and β4 promoters and neuron selective enhancer within the β4 3′-untranslated region.

2. Methods and materials

Reporter plasmids used for transfection assays were made with pGL2 or pGL3 based luciferase vectors (Promega). Transfections were performed using either electroporation or calcium phosphate precipitation. Luciferase enzyme assays were performed using standard methods as described previously (Yang et al., 1994). PC12 cells were grown using conditions previously described (McDonough and Deneris, 1997). Transfections in dissociated retinal cultures will be described elsewhere.

To prepare nuclear extracts, PC12 cells were grown to near confluence in 150 mm dishes, washed with phosphate-buffered saline, and collected by centrifugation. The cells were resuspended in 10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 4 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin and incubated on ice for 15 min. Then 10% NP40 was added to solubilize the cellular membrane and the solution was centrifuged for 30 s at 14,000 rpm. The pellet was resuspended in 20 mM HEPES (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 4 μg/ml leupeptin, 1 μg.ml aprotinin, 1 μg/ml pepstatin and centrifuged for 5 min at 14,000 rpm. The crude nuclear extract was then aliquoted and stored at −80°C. Mobility shift assays (EMSA) were performed essentially as described (McDonough and Deneris, 1997). [^{32}P]-end-labeled double-stranded oligonucleotides were incubated with PC12 extract in EMSA binding buffer (15 mM HEPES pH 7.9, 60 mM KCl, 1.5 mM DTT, 7.5 mM $MgCl_2$, 0.5 mM EDTA, 10% glycerol, 2 μg poly dI · dC) for 30 min at 4°C. Oligonucleotide competition experiments were performed with a 10-min preincubation of excess unlabeled, double-stranded competitor oligonucleotides added to nuclear extracts prior to the addition of labeled oligonucleotide. Anti-Sp1 or anti-Sp4 antibodies were obtained from Santa Cruz Biotechnologies, Santa Cruz, CA. and oligonucleotides from Life Technologies, Gaithersburg, MD. DNA–protein complexes were loaded on 4% polyacryamide gels in 1× Tris–glycine buffer (50 mM Tris base, 380 mM glycine, and 2 mM EDTA) and visualized by autoradiography using Kodak XAR-5 film.

3. Results and discussion

3.1. Rat α3 and β4 promoters

In neurons and neural cell lines the rat α3 gene initiates transcription at multiple sites within a G + C-rich region. A TATA box is not recognizable in this region (Yang et al., 1994). Based on the extensive co-expression of the β4 and α3 genes, their promoters might be expected to share a significant degree of sequence identity. Sequence comparisons between the two rat promoters, however, reveals no sequence similarity other than scattered 6–10 bp matches. As is expected for TATA-less G + C-rich promoters there are several Sp1 consensus binding sites in or near the α3 start site region. Indeed one of these sites (α3 GA), which is positioned immediately upstream of the start site region, has been shown to be a major *cis*-acting element for rat α3 basal promoter activity in PC12 cells (Yang et al., 1995). This site binds recombinant Sp1 and Sp1-immunoreactive material in present in PC12 cells and can mediate Sp1 transcriptional activation in co-transfection assays. The β4 promoter has a similar element, which is also able to bind Sp1 present in PC12 nuclear extracts (Fig. 1A, B). These results indicate that the α3 and β4 GA motifs appear to be functionally equivalent *cis* elements. Therefore, it is likely that one common component of the β4 and α3 promoters is a *cis* element that interacts with Sp1 family members. Mutagenesis of a consensus AP2 site within the rat α3 promoter did not affect its activity in PC12 cells (Yang et al., 1995).

Transfection analyses suggest that the rat α3 minimal promoter may contain some cell-type-specific information as this promoter shows somewhat greater activities in cell lines that express the endogenous α3 gene compared to α3-negative cell lines (Boyd, 1996). However, the rat promoter still show significant activity in α3-negative lines (Boyd, 1996); Yang and Deneris, unpublished) which suggests that other cell-type specific elements are likely to be required to establish strict neuronal cell-type specificity. Indeed, a positive *cis* element, β43′, outside the minimal promoter region has been identified and shown to influence its cell-type-specific activity (Yang et al., 1997).

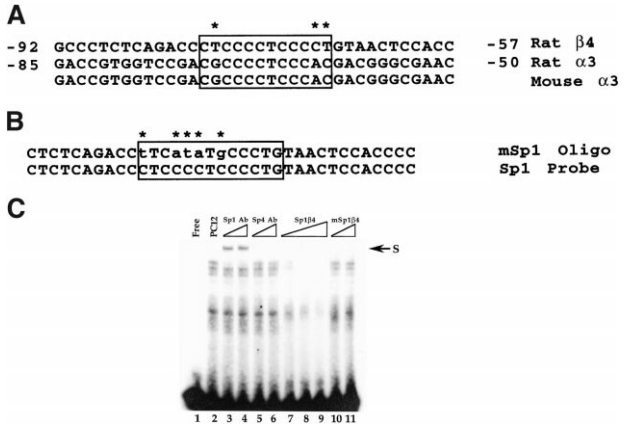

Fig. 1. Sp1 binding motif in the rat β4 promoter. (A) Rat α3 and β4 minimal promoter sequence comparison. GA motif is boxed and differences are indicated by asterisks. (B) Sequences of $Sp1_{\beta4}$ probe and $mSp1_{\beta4}$ oligonucleotide that were used for EMSA in (C). Substituted bases of $mSp1_{\beta4}$ are indicated by asterisks. (C) The β4 GA motif can bind Sp1 protein present in PC12 cell nuclear extracts. EMSA was performed with $Sp1_{\beta4}$ probe and PC12 extract in the presence or absence of increasing amounts of antisera or competitors. Lane 1, probe alone; lanes 2–10, 5 μg nuclear extract; lanes 3, 4, 0.02 and 0.04 μg anti-Sp1 antibody; lanes 5, 6, 0.02 and 0.04 μg anti-Sp4 antibody; lanes 7–9, 150-, 300-, or 600-fold molar excess of unlabeled $Sp1_{\beta4}$ competitor; lanes 10–11, 150- or 300-fold molar excess of unlabeled $mSp1_{\beta4}$ competitor. S indicates a supershift complex.

3.2. Activation of the rat α3 and β4 promoters by the POU factor, SCIP

Several POU domain transcription factors are expressed together with neuronal nicotinic receptor genes in the medial habenula. The α3 promoter is activated in a neural cell-type-specific manner by one of these POU domain factors termed SCIP/Tst-1/Oct-6 (Yang et al., 1994). The β4 promoter can also be activated by SCIP in transient co-transfection experiments (Table 1). These data led to the hypothesis that α3 and β4 are in vivo targets of SCIP (Yang et al., 1994). It is not clear, however, whether they are targets of SCIP as the levels of α3 in the medical habenula of SCIP null mice are not different from wild-type controls (Bermingham et al., 1996). Perhaps other POU factors expressed in medical habenula are able to maintain levels of α3 in the absence of SCIP.

The study of α3 activation by SCIP has been important in another respect as it has revealed a potential alternative mechanism of POU domain factor function. In general, POU transcription factors are single polypeptides composed of two functional domains that are necessary but not by themselves sufficient for transcriptional activation. One is the DNA binding domain, called the POU domain, that serves to bring the factor to specific regulatory sequences in the regulatory regions of certain genes. The second domain is called the activation domain. This domain makes contact with components of the basal transcription complex to modulate the rate of gene transcription. Thus, to activate transcription, the DNA-binding domain of these POU factors brings the activation domain to particular promoters through a high affinity interaction with specific DNA sequences. This mechanism of transactivation has been demonstrated for several POU factors, including SCIP. In non-neural cells SCIP has been shown to work this way. However, activation of α3 by SCIP in PC12 cells is likely to occur through a different mechanism as neither binding sites for SCIP in the α3 promoter nor the SCIP amino-terminal activation domain is required for activation (Fyodorov and Deneris, 1996; Yang et al., 1994). Surprisingly, the DNA binding POU domain of SCIP is, by itself, sufficient for activation of α3 (Fyodorov and Deneris, 1996). These findings raise the possibility that SCIP has the potential to regulate gene expression through more than one type of transcriptional mechanism. Whether this mode of transactivation occurs in vivo is not yet known. Interestingly, however, the phenotypic effects on Schwann cell development of a transgene expressing a SCIP truncation comprising little more than the SCIP POU domain and originally predicted to act as a dominant negative factor (Weinstein et al., 1995) is dramatically different from the Schwann cell phenotype observed in homozygous SCIP null mutant mice (Bermingham et al., 1996). These results indicate that the SCIP POU domain is not behaving as a dominant negative factor in Schwann cells. Rather the data are consistent with the POU domain of SCIP functioning as a positive transcriptional modulator in vivo.

3.3. Transgenic analysis of rat α3 upstream region

The region between the rat β4 and α3 genes has been the focus of our research because it was suspected that the relatively small rat β4/α3 intergenic region, which is the α3 upstream region, might contain important *cis* regulatory elements (Yang et al., 1997). The rat β4/α3 intergenic region is contained in a 2.8-kb *Sac*I fragment and includes the 240-bp α3 promoter region, the 1.4-kb β4/α3 intergenic region and 1.3-kb of the upstream β4 gene (Fig. 2). The β4 sequences in this fragment are entirely 3′-untranslated exon (Yang et al., 1997).

Table 1
Comparative activation of α3 versus β4 promoters by SCIP/Tst-1 in PC12 cells
Data represent the average and range of duplicate PC12 cell transfections in which 10 μg of the α3 reporter −1607/+47 or the β4 reporter −2800/+137 were electroporated with 1 μg empty CGS vector (−SCIP/Tst-1) or with 1 μg CGS-SCIP effector (+SCIP/Tst-1). Data are presented as mean ± SEM, $n = 4$.

Promoter	Relative activity		Fold activation
	−SCIP/Tst-1	+SCIP/Tst-1	
α3	24,000 ± 5000	304,000 ± 22,000	13
β4	10,000 ± 1300	102,000 ± 12,000	10

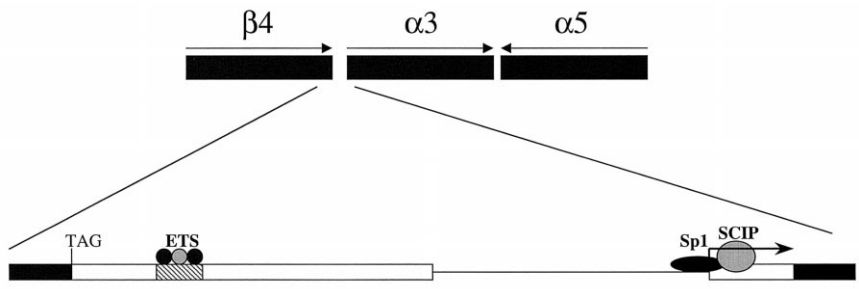

Fig. 2. Genomic organization of the clustered β4α3α5 genes. Upper schematic shows relative positions of the three genes and their respective directions of transcription (arrows). Lower schematic shows an expanded view of the 3.5-kb region between the rat β4 and α3 protein coding sequences. The human intercoding region is about 1 kb longer (Battaglioli et al., 1998). Filled portions, protein coding sequences of β4 and α3; unfilled portions, untranslated sequences; thin line between untranslated sequences, 1.4 kb β4/α3 intergenic region. Region bounded by α3 promoter and β43′ enhancer was tested for the presence of neuron-specific elements in transgenic mice (Yang et al., 1997). The transcription start site region of the α3 promoter is shown by a black arrow. Cross hatched portion of the β4 3′-untranslated exon indicates the location of the β43′ enhancer. Sites of potential ETS, Sp1, and SCIP transcription factor interactions are indicated. TAG, β4 translation stop codon.

Transgenic mice were generated with a reporter (28 Z) carrying the lacZ gene fused downstream of the 2.8-kb *Sac*I fragment to determine whether this region of the cluster contains *cis* regulatory elements important for neuron-specific transcription in vivo. Expression of the 28 Z transgene was found to be restricted to neurons of the CNS; no expression was detected in non-neural tissues. LacZ positive cells were detected, virtually exclusively, in a subset of CNS nuclei that transcribe the endogenous α3 gene including the retinal ganglion cells. Some overlap was seen with the β4 gene, e.g. in retinal ganglion cells, but almost none with the α5 gene (Yang et al., 1997). These results suggest that neuron-specific *cis* elements are positioned between the α3 and β4 coding regions and are likely to be important for establishing part of the restricted CNS patterns of the cluster. However, the transgenic analysis indicated as was expected that additional elements outside the *Sac*I fragment are probably also needed for controlling the clustered genes; 28 Z was expressed in only a subset of regions in the brain that express the clustered genes, no expression was detected in peripheral neurons, and expression was influenced by position effects.

3.4. Rat β43′ enhancer

To determine the location of possible neuron-specific *cis* elements in the 2.8-kb *Sac*I fragment deletion analysis was performed in PC12 cells and other lines that do not express the endogenous clustered genes. This analysis revealed a *cis* element, β43′, exhibiting the properties of an enhancer at the β4 end of the *Sac*I fragment (McDonough and Deneris, 1997). The enhancer is therefore positioned in the β4 3′-untranslated region (Fig. 2). The transcriptional activity of β43′ is quite strong as a single copy of it can activate a TATA box minimal promoter 60-fold in PC12 cells. These results support the idea that the cluster has been maintained in order to preserve the organization and function of transcriptional regulatory elements.

One mechanism that may operate to produce the highly restricted neuronal expression patterns of β4, α3, α5 is positive modulation of transcription through the action of enhancers that are active in only particular neuronal cell types. To determine whether β43′ might be an enhancer with this property its activity was assayed in different cell types. Interestingly, the activity of β43′ in different cell lines is indeed correlated with the expression of the cluster. Of particular importance was the finding that β43′ had little activity in neural lines that do not express the cluster (McDonough and Deneris, 1997).

More recently the activity of β43′ has been assayed by transient transfection of dissociated primary sympathetic and retinal cultures containing both neurons and non-neuronal cells (Francis and Deneris, 1998). In these assays, the activity of the enhancer placed upstream of a minimal promoter such as the SV40, α3 or adenoviral major late promoter is compared to similar reporters that carry the SV40 enhancer instead of β43′. Transfections are performed by either calcium phosphate precipitation or electroporation. Reporter expression is then determined by one of two methods. To assess cell-type-specific expression of the reporter in the mixed neuron/non-neuronal transfected cells, the cultures are fixed and then co-stained with an anti-luciferase antibody to detect reporter expression and a monoclonal antibody against a neuron-specific form of tubulin (betaIII) to identify neurons. The second method for measuring reporter expression is simply to determine luciferase enzyme activities using transfected cell extracts as done with cell-line transfections. Typical numbers of immunopositive cells in a single transfection is about 100 for sympathetic cultures and several hundred positive retinal neurons. Fig. 3 illustrates this type of assay for retinal cultures. The cultures were transfected with luciferase reporters carrying the SV40 promoter and its own enhancer. Co-staining revealed roughly equal numbers of luciferase-positive non-neuronal cells and neurons. In contrast, when a similar reporter was used except that the SV40 enhancer was replaced with the β43′ enhancer the

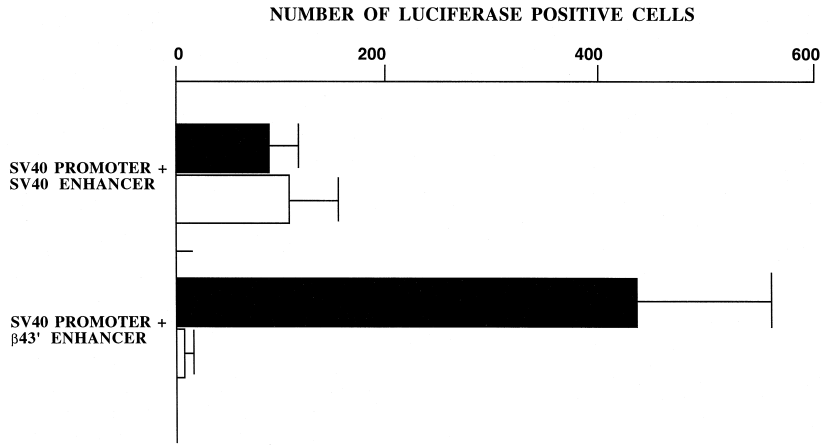

Fig. 3. Transient transfection analysis of the β43′ enhancer in primary dissociated retinal cultures. Dissociated retinae were transfected by calcium phosphate precipitation with luciferase reporters controlled by either the SV40 promoter and SV40 enhancer or the SV40 promoter and the β43′ enhancer. Reporter expression in neurons (filled bars) and non-neuronal cells (open bars) was measured by counting cells immunostained with the anti-βIII-tubulin antibody to detect neurons and anti-luciferase antibody to detect luciferase. Error bars represent mean ± SEM, $n = 4$ separate transfections.

vast majority of cells expressing luciferase were neurons. This shows that by simply switching from the SV40 enhancer to the β43′ enhancer reporter expression is largely limited to retinal neurons. Similar results have been obtained in transfected sympathetic and cortical neuron cultures, however the activity of β43′ is much weaker in these neuronal cell types than in retinal neurons (Francis and Deneris, 1998).

The rat β43 enhancer is composed of two 37-bp direct repeats that are separated by a 6-bp spacer (McDonough and Deneris, 1997). Sequence analysis of the enhancer identified three consensus binding sites (5′-A/TTCCT/G) for the ETS class of transcription factors (Wasylyk et al., 1993). One is located in the spacer region between the two repeats while the other two are located in each of the repeats (Fig. 2). This suggests that ETS-like factors expressed in PC12 cells are positive modulators of the enhancer. To analyze ETS sequences expressed in PC12 cells a degenerate RT-PCR screen was performed. This screen resulted in the identification of a previously unknown member of the family that we have named Pet-1 (Fyodorov et al., 1998). Pet-1 is currently hypothesized to be a β43′-interacting ETS factor as it is expressed in adrenal medulla and can activate transcription in a β43′-dependent manner (Fyodorov et al., 1998).

The distinctive characteristics of β43′ make it an attractive candidate component of the *cis* regulatory information needed to restrict transcription of one or more of the clustered genes to particular neuronal cell types. It may act selectively on α3 to control its pattern of expression but not that of β4 or α5. On the other hand, β43′ may be a special type of *cis* element as its position between the β4 and α3 promoters may enable it to coordinate the expression patterns of these genes. Interestingly, the differential activity of β43′ in central and peripheral neurons correlates with 28 Z transgene expression described above. 28 Z expression was detected in retinal ganglion cell layer but not in sympathetic neurons (Yang et al., 1997). As 28 Z contains the β43′ enhancer the greater activity of the enhancer in retinal neurons versus sympathetic neurons may be responsible for transgene expression in retinal ganglion cell layer but not in sympathetic ganglia.

Acknowledgements

This work was supported by Public Health Service grant NS29123 from National Institute of Neurological Disorders and Stroke.

References

Battaglioli, E., Gotti, C., Terzano, S., Flora, A., Fornasari, F., 1998. Expression and transcriptional regulation of the human α3 neuronal nicotinic receptor subunit in T lymphocytes cell lines. J. Neurochem. 71, 1261–1270.

Bermingham, J.R.J., Scherer, S.S., O'Connell, S., Arroyo, E., Kalla, K.A., Powell, F.L., Rosenfeld, M.G., 1996. Tst-1/Oct-6/SCIP regulates a unique step in peripheral myelination and is required for normal respiration. Genes Dev. 10, 1751–1762.

Boulter, J., Duvoisin, R.M., Connolly, J.G., Wada, E., Jensen, A., Gardner, P.D., Ballivet, M., Deneris, E.S., McKinnon, D., Heinemann, S., Patrick, J., 1990. α3, α5, and β4: three members of the rat neuronal nicotinic acetylcholine receptor-related gene family form a gene cluster. J. Biol. Chem. 265, 4472–4482.

Boyd, R.T., 1996. Transcriptional regulation and cell specificity determinants of the rat nicotinic acetylcholine receptor α3 gene. Neurosci. Lett. 208, 73–76.

Conroy, W.G., Berg, D.K., 1995. Neurons can maintain multiple classes of nicotinic acetylcholine receptors distinguished by different subunit compositions. J. Biol. Chem. 9, 4424–4431.

Couturier, S., Erkman, L., Valera, S., Rungger, D., Bertrand, S., Boulter, J., Ballivet, M., Bertrand, D., 1990. α5, α3, and non-α3: three clustered avian genes encoding neuronal nicotinic acetylcholine receptor-related subunits. J. Biol. Chem. 265, 17560–17567.

Francis, N., Deneris, E., 1998. The neuronal nAchR β43′ enhancer is neuron-selective and preferentially active in retinal versus sympathetic neurons. Soc. Neurosci. Abstr. 24, 1565.

Fyodorov, D., Deneris, E., 1996. The POU domain of SCIP/Tst-1/Oct-6 is sufficient for activation of an acetylcholine receptor promoter. Mol. Cell. Biol. 16, 5004–5014.

Fyodorov, D., Nelson, T., Deneris, E., 1998. Pet-1, a novel ETS domain factor that can activate neuronal nAchR gene transcription. J. Neurobiol. 34, 151–163.

McDonough, J., Deneris, E., 1997. β43′: an enhancer displaying neural-restricted activity is located in the 3′-untranslated exon of the rat nicotinic acetylcholine receptor β4 gene. J. Neurosci. 17, 2273–2283.

Raimondi, E., Rubboli, F., Moralli, D., Chini, B., Fornasari, D., Tarroni, P., De-Carli, L., Clementi, F., 1992. Chromosomal localization and physical linkage of the genes encoding human alpha3, alpha5, and beta4 neuronal nicotinic receptor subunits. Genomics 12, 849–850.

Vailati, S., Hanke, W., Bejan, A., Barabino, B., Longhi, R., Balestra, B., Moretti, M., Clementi, F., Gotti, C., 1999. Functional α6-containing nicotinic receptors are present in chick retina. Mol. Pharmacol. 56, 11–19.

Vernallis, A.B., Conroy, W.G., Berg, D.K., 1993. Neurons assemble acetylcholine receptors with as many as three kinds of subunits while maintaining subunit segregation among receptor subtypes. Neuron 10, 451–464.

Wasylyk, B., Hahn, S.L., Giovane, A., 1993. The Ets family of transcription factors. Eur. J. Biochem. 211, 7–18.

Weinstein, D.E., Burrola, P.G., Lemke, G., 1995. Premature Schwann cell differentiation and hypermyelination in mice expressing a targeted antagonist of the POU transcription factor SCIP. Mol. Cell. Neurosci. 6, 212–229.

Yang, X., Fyodorov, D., Deneris, E.S., 1995. Transcriptional analysis of acetylcholine receptor alpha 3 gene promoter motifs that bind Sp1 and AP2. J. Biol. Chem. 270, 8514–8520.

Yang, X., McDonough, J., Fyodorov, D., Morris, M., Wang, F., Deneris, E.S., 1994. Characterization of an acetylcholine receptor alpha 3 gene promoter and its activation by the POU domain factor SCIP/Tst-1. J. Biol. Chem. 269, 10252–10264.

Yang, X., Yang, F., Fyodorov, D., Wang, F., McDonough, J., Herrup, K., Deneris, E., 1997. Elements between the protein-coding regions of the adjacent β4 and α3 acetylcholine receptor genes directs neuron-specific expression in the central nervous system. J. Neurobiol. 32, 311–324.

Zoli, M., Lena, C., Picciotto, M.R., Changeux, J.-P., 1998. Identification of four classes of brain nicotinic receptors using β2 mutant mice. J. Neurosci. 18, 4461–4472.