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Activation of the Tyrosine Hydroxylase Promoter by NeuroD in Neural Stem Cells

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ABSTRACT

Transcription of the rat tyrosine hydroxylase (TH) gene is controlled by enhancer sequences in its 5' flanking region; these enhancers include the AP1-, AP2-, dyad-, POU/Oct- binding domain, and cAMP response element (CRE) motifs. Of cis-regulatory elements, a putative E-box is located at -190 bp on the 5' flanking region of TH. In this study, we investigated whether the TH gene expression is regulated by bHLH transcription factors such as NeuroD or neurogenin 1. We used mouse neural stem cells, A3B1L6, immortalized with *v-myc*. In A3B1L6 cells, protein kinase A (PKA) increased TH activity. The stimulatory effect of PKA was dependent on a CRE located at -45 bp from the site of transcription initiation, suggesting that A3B1L6 provides an appropriate system for the study of TH gene expression. Importantly, coexpression of NeuroD or neurogenin 1 with a ubiquitous bHLH partner E47 could transactivate the TH promoter. The data indicate that expression of the TH gene may be regulated by bHLH transcription factors probably through the E-box at -190.

Key words: tyrosine hydroxylase (TH), neural stem cell, NeuroD, PKA, CREB

INTRODUCTION

Tyrosine hydroxylase (TH) catalyzes the rate-limiting step in catecholamine synthesis, by hydroxylating tyrosine to dihydroxyphenylalanine (DOPA), which is sequentially converted to dopamine, norepinephrine and epinephrine (Nagatsu et al., 1964). TH gene is tightly regulated in catecholamine-synthesizing and secreting cells, including dopaminergic, noradrenergic and adrenergic neurons in the central nervous system, or sympathetic ganglia and adrenal chromaffin cells in the peripheral nervous system.

Tissue specific expression of the TH gene is the consequence of several transcription factors through cis-regulatory elements for AP1, AP2 (Fung et al., 1992; Cambi et al., 1989), dyad (Yoon et al., 1992), SP1 (Yang et al.,1998; Cambi et al., 1989), Nurr1 (Yoon et al., 1992) as well as CRE (Cambi et al., 1989; Piech-Dumas et al., 1999; Yang et al.,1998). In particular, cAMP has been shown to stimulate the TH gene expression by activation of PKA-CREB pathway. PKA is a tetramer composed of two

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regulatory (R) subunits and two catalytic (C) subunits. Binding of cAMP to R subunits leads to dissociation of the R2C2 complex, resulting in the release of active C-subunits. PKA activity has been implicated in cell-type specification in invertebrate and vertebrate embryos (Harootunian et al., 1993; Meinkoth et al., 1993). Coexpression of the catalytic subunit of PKA (PKA/Cα) dramatically increased the transcriptional activity of the rat TH gene in a dose-dependent manner, while coexpression of the specific inhibitor (PKI) blocks cAMP-stimulated induction and reduces basal transcriptional activity (Kim et al., 1993b). In cell lines including human neuroblastoma, SK-N-BE(2)C (Kim et al., 1996; Miner et al., 1992), rat adrenal pheochromocytoma, PC12, mouse CATH.a (Suri et al., 1993) derived from a TH-expressing mouse brainstem tumor, and PATH.a (Suri et al., 1993) derived from adrenal tumors (Kim et al., 1993a; Kim et al., 1993b; Lazaroff et al., 1995), CRE was required for induction of both basal and cAMP-inducible transcription of the rat TH gene. CREB binds to a consensus CRE motif located at -38 bp upstream of transcription initiation site, and then activates the transcription of TH gene (Schimmel et al., 1999). Taken together, PKA mediates cAMP-inducible transcription of the TH gene via the CRE.

In vivo studies have shown that in addition to CRE element at -38 bp, the AP1 element located at -205 bp is also important for normal patterns of TH gene expression (Trocme et al., 1998). In addition, several putative cis-acting elements such as POU/OCT, SP1, and E-box are located between -365 and -38 bp (Schimmel et al., 1999).

A putative E-box, CANNTG, is found at -190 bp. It is well known that E-box provides a binding site for basic helix loop helix (bHLH) transcription factors, including NeuroD and neurogenin. NeuroD or neurogenin binds to a ubiquitous bHLH factor, E47, and the heterodimers ultimately induce neuronal differentiation or regulate neuroendocrine-specific genes. Until now it is not clear whether E-box mediates the gene expression of TH.

In this study, we inquired whether the tissue specific expression of TH gene could be regulated through E-box using immortalized mouse neural stem cells (NSCs), A3B1L6. For this we first verified the validity of A3B1L6 for the study of TH gene expression recapitulating the stimulatory effect of cAMP. Then we analyzed the effect of NeuroD and neurogenin on the TH promoter.

MATERIALS AND METHODS

PC12 cell differentiation by Fsk

PC12 was obtained from Korean Cell Line Bank (KCL, No. 21721) and plated in a density of 6×10^5/60 mm-diameter culture dishes coated with poly-L-ornithine (Sigma, USA) in the presence of RPMI 1640 (Hyclone, USA) containing 10% horse serum (HS; Gibco-Invitrogen, USA) and 5% FBS, 100 unit/mL penicillin, and 100μg/mL streptomycin. To determine the effect of cAMP, the medium was replaced with low-serum medium containing 0.5% FBS for 6 hours and then forskolin (TOCRIS, UK) was added to a final concentration of 30μM for the indicated times.

TH mRNA expression with RT-PCR

The growth medium (15% serum) was replaced with low-serum medium (0.5%). Total RNA was isolated cells using RNAzol (TelTest Inc., USA) and cDNA was made using First strand cDNA synthesis kit for RT-PCR (Roche, Germany). PCR was carried

Table 1.

Genes	Sequences (5' → 3')	PCR	Product sizes
TH	(R) GCATAGAGGCCCTTCAGCGTG (F) GTTCTCAACCTGCTCTTCTCC	(94°C, 30 sec; 62°C, 30 sec; 72°C, 1 min), 26 cycles	490 bp
NFH	(R) GGGACTTGGCCTCTTCTTTT (F) GAGTGTCGGATTGGCTTTGG	(94°C, 30 sec; 58°C, 30 sec; 72°C, 1 min), 35 cycles	349 bp
GAPDH	(R) GTTGCTGTTGAAGTCACAGGAGAC (F) TCCATGACAACTTTGGCATCGTGG	(94°C, 30 sec; 55°C, 30 sec; 72°C, 1 min), 25 cycles	340 bp

using specific primers for TH, NFH, and GAPDH (Table 1).

Transfection and Chloramphenicol acetyltransferase (CAT) assay

A3B1L6 was previously described (Kim et al., 2003). HAD was established by introducing *v-myc* and maintained in Dulbecco's modified Eagle's medium with high glucose (DMEM; Gibco-Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone, USA), 100 unit/mL penicillin (Gibco-Invitrogen, USA), and 100μg/ml streptomycin (Gibco-Invitrogen, USA). Transfection was performed by the calcium phosphate method (Graham et al., 1973). When cells reached to 50% confluence, 1μg of reporter plasmids were used with and without 0.2 μg of expression vector for the catalytic subunit of PKA. To investigate the efficacy of E-box mediated on TH promoter, 0.3μg of reporter plasmids were used. Expression vectors for NeuroD, neurogenin 1, E47 were used 0.3μg each for transfection. The total amount of DNA per transfection was kept constant with pUC19. In all experiments, pCMV-β-gal plasmid was included as an internal control for normalizing the transfection efficiencies between experiments. Forty eight hours after incubation, cells were harvested in phosphate- buffered saline (PBS). Extracts were prepared by resuspending cells in 0.25 M Tric-HCl (pH 8.0), subject freezing-and-thawing 3 times, and then heated at 65°C for 10 min to inactivate endogenous acetylase. CAT reaction was carried out using cell extracts 35μg protein with 0.5μCi of [^{3}H] chloramphenicol (NEN, USA) and n-butyryl coenzyme A (Sigma, USA) at 37°C for 30 min. Then, [^{3}H]-butyryl chloramphenicol was extracted with Xylene : TMPD (1 : 1) (Sigma, USA) and then CAT activity was measured with β-counter. Co-transfection of pCMV-β-gal plasmid was performed to correct for any variation in transfection efficiency.

Luciferase assay

A3B1L6 cells were transfected with pFA-CREB and pFR-luc (Stratagene, CA); and pPKA/Cα (Ghil et al., 2000), using lipofectamine method (Gibco-Invitrogen, USA). At 48 hours after transfection, cell extracts were prepared with lysis buffer and assayed following the manufacturer's instruction (Promega, USA). The CREB activity was determined using a luminometer. Simultaneously, the activity of β-galactosidase was also assayed to correct for any variation in transfection efficiency.

RESULTS

Transactivation of the TH promoter by PKA in immortalized neural stem cell and adrenal chromaffin cell lines

Previous studies have demonstrated that the 2.4 kb of rat TH promoter sequence is sufficient to recapitulate the temporal, spatial, and inducible expression pattern of endogenous TH. The 2.4 kb promoter region of the rat TH gene contains DNA elements for many transcription factors including AP2, AP1, E-box, POU/Oct, and CREB, putative transcriptional regulatory elements are found between -365 bp and -38 bp (Fig. 1A). In particular, CREB was reported to mediate basal and cAMP- induced TH transcription in various cultured cells. Activation of CREB by phosphorylation on Ser133 has been shown to mediate PKA-dependent or -independent induction of TH transcription (Patankar et al., 1997; Kim et al., 1993b). A rat pheochromocytoma cell line, PC12, express TH gene. Activation of PKA induces neuronal differentiation in PC12 cells (Sanchez et al., 2004). Thus, we added 30μM Fsk to PC12 cells and determined TH gene expression. The cells in the presence of 15% serum had refractile cell bodies with very short processes. In the presence of 0.5% FBS the cells tended to stop growing and exhibited neurites extended from the cell body after 96 hours (Fig. 1B). The expression level of TH was determined by RT-PCR. The level of TH mRNA transcript was higher in low serum medium, which was not significantly altered by addition of Fsk (Fig. 1C). The expression of neurofilament heavy chain (NFH), a neuronal marker, was increased in the presence of Fsk (Fig. 1C). The results suggest that PC12 are already specified to express TH and activation of PKA further increase it.

In order to verify the validity of *in vitro* system, we first compared immortalized mouse NSCs, A3B1L6, and human adrenal chromaffin cells, HAD. A3B1L6 has been shown to possess the ability to differentiate into neurons, astrocytes, and oligoden-

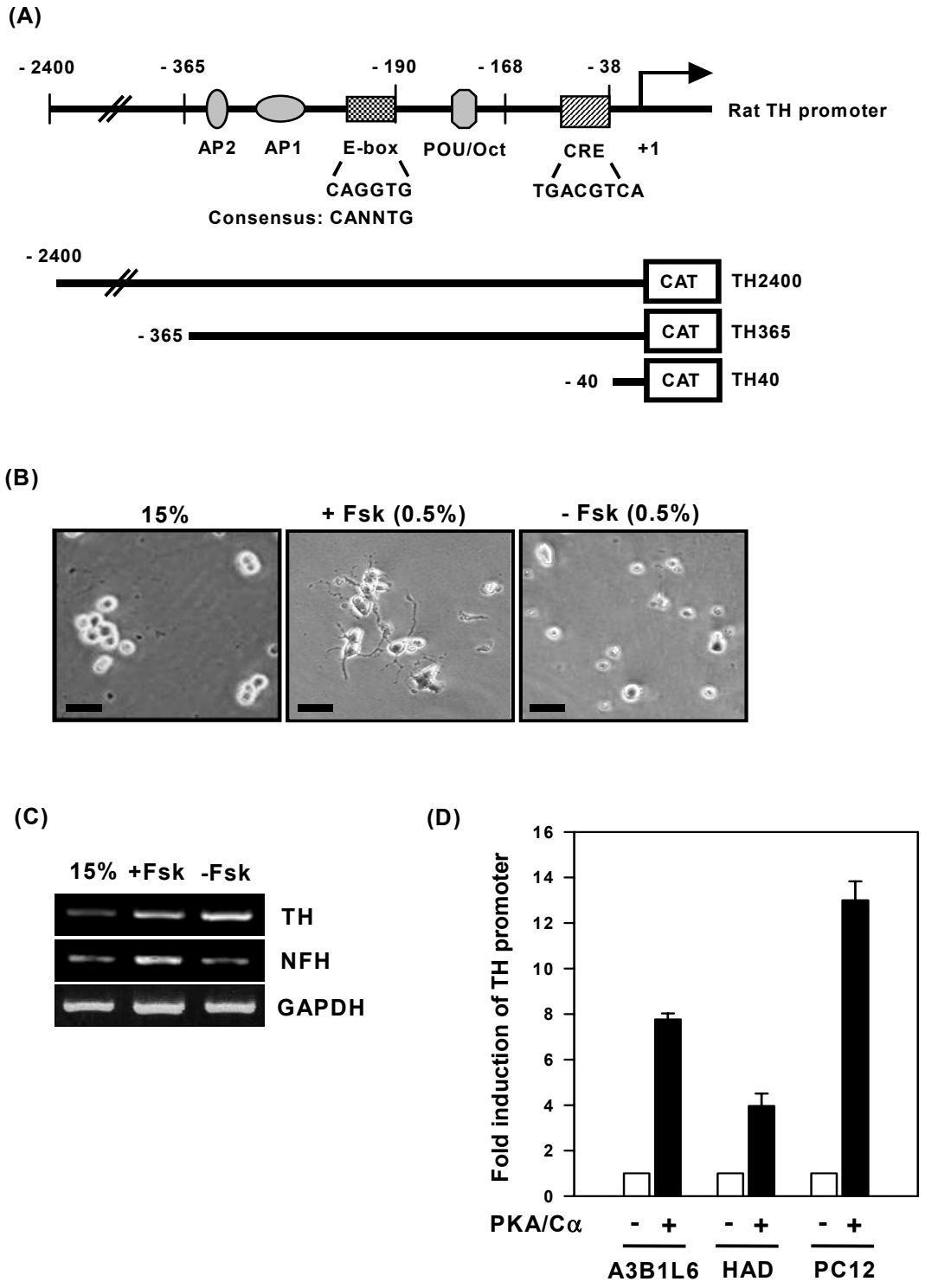

Fig. 1. PKA leads to increased activity of TH promoter in immortalized neural stem cells, A3B1L6, as well as adrenal chromaffin cells, HAD and PC12. (A) A schematic presentation of TH reporter genes. CRE, a putative E-box, and cis-domains such as AP2 and 1, and POU/Oct are located between -365 bp and -38 bp from the transcription initiation site. (B) The effect of forskolin in PC12 cells. PC12 cells grown in the presence of 15% serum (left). PC12 cells in low serum medium stopped growth (right). The processes were extended from PC12 following the treatment with 30μM forskolin for 4 days (middle). Scale bar, 50μm. (C) Expression of endogenous TH was increased with in 0.5% low-serum using RT-PCR. (D) Promoter activity of TH2400 was increased by PKA and in PC12 cells, immortalized neural stem cells (A3B1L6) and HAD.

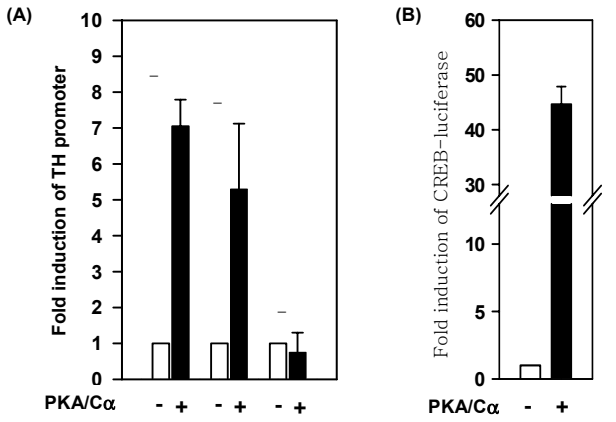

Fig. 2. Transactivation of TH promoter by PKA in A3B1L6 cells. (A) In A3B1L6 cells, both TH2400 and TH365 promoters were transactivated by PKA/Cα, but TH lacking CRE. (B) The transactivation by PKA was mediated by CREB as determined by pFR-luc and pGAL4-DBD.

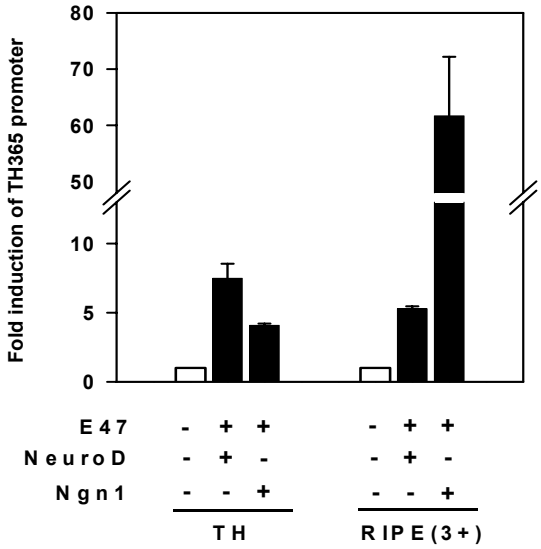

Fig. 3. Expression of TH gene is upregulated by neurogenic bHLH proteins in A3B1L6 cells. A putative E-box is located at -190 bp of transcription initiation site on TH promoter. Coexpression of NeuroD/E47 or neurogenin 1/ increased the promoter activity of TH365. This result indicates a possible regulation of TH gene as a by neurogenic bHLH transcription factor, RIPE(3+) was used as a positive control which contained three copies of E-box.

drocytes *in vitro* (Kim et al., 2003). Reporter genes containing various length of the TH promoter sequence were transiently transfected with the expression vector for the catalytic subunit, PKA/Cα. The CAT activity was determined using [^{3}H] chloramphenicol and butyryl-CoA as substrates. The CAT activity was significantly increased by coexpression of PKA (Fig. 1D). The results showed that immortalized cell lines provide useful system for the study of the TH gene regulation. Because of its high transfection efficiency, A3B1L6 was used for the subsequent experiments.

We next investigated whether CRE would be essential for TH gene induction by PKA/Cα in NSCs as previously reported (Trocme et al., 1998). We used reporter genes carrying various length of the TH promoter. Promoter activities of TH2400 and TH365 were dramatically increased by PKA/Cα. In contrast, the promoter activity of TH40 was not increase by PKA/Cα (Fig. 2A). In the same condition, PKA also increased the luciferase activity by phosphorylating CREB (Fig. 2B). Thus, it is very reasonable to conclude that PKA enhanced the TH promoter activity through CRE site in A3B1L6 and deletion of CRE abolished the effect of PKA. The results also suggest that A3B1L6 might be useful for the study of the TH gene regulation.

Sequence analysis indicated putative E-box at -190 bp of TH 5' upstream region

It is well known that E-boxes serve as binding sites for neurogenic bHLH proteins such as NeuroD and neurogenin. Heterodimers of NeuroD/E47 or neurogenin/E47 induce neuronal differentiation. A putative E box was found at -190 bp by sequence analysis. Therefore, the TH365CAT plasmid was cotransfected with expression vectors for NeuroD, neurogenin 1, and E47. Simultaneously, RIPE(3+), a reporter gene containing three copies of E-box of the rat insulin gene, was used as a positive control as shown previously (Cho et al, 2001). Both NeuroD and neurogenin 1 increased promoter activity of TH365CAT by 7.5- or 4-fold, respectively (Fig. 3). The result indicates that neurogenic bHLH transcription factor may be involved in expression of the TH gene in dopaminergic neurons or adrenal chromaffin cells.

DISCUSSION

In this study, we show that NeuroD or neurogenin 1 with a ubiquitous bHLH partner E47 trans-

activated the activities of TH promoter probably through the E-box at -190. TH can first be detected at embryonic day (E) 8.5 in the mouse (Thomas et al., 1995), and then express in the neural tube and the somites at E10.5, prosencephalon and mesencephalon at E11.5, and the dorsal root ganglia of thoracic level at E13.5 (Schimmel et al., 1999). During the similar periods, neurogenin 1 and 2 become strongly expressed in regions, almost consistent with expressing TH (Schimmel et al., 1999). In contrast, NeuroD expression is widely distributed in essentially all of the brain areas, peripheral ganglia, and sense organs that express neurogenin 1, 2, or 3 (Sommer et al., 1996). The similar localizations of TH, NeuroD, or neurogenin in embryo brain region provide us with the possibility that NeuroD or neurogenin 1 may be one of the transcriptional regulators of the TH gene and is supported by the colocalization of these bHLH proteins in the future dopaminergic area using *in vitro* NSC culture system.

Many of in vivo studies (Baker et al, 1993; Hiremagalur et al., 1993; Sabban et al., 1998) and in vitro studies using PC12 (Kilbourne et al., 1990; Lewis et al., 1983; Lewis-Tuffin et al., 2004) have repoted the transcriptional induction of TH by various stimuli including cAMP, depolarization, and hypoxia. The stress-activated protein kinases (SAPKs), in general, are activated by noxious environmental stimuli such as ultraviolet light, osmotic stress, inflammatory cytokines, and inhibition of protein synthesis (Conrad, 2001; Derijard et al., 1994; Han et al., 1994; Hibi et al., 1993) and then induced TH gene transcription, following to induction of transcription factors, hypoxia response element (HRE) or CREB (reviewed by Conrad, 2001; Lewis-Tuffin et al., 2004). Therefore, as shown in Fig. 1C, we suggest possibility that the higher expression of TH mRNA transcript in low serum medium may result from SAPK activation by environmental stress during prolonged period (4 days) and then leading to phosphorylation of CREB.

In conclusion, our study investigated a possibility that bHLH transcription factors are related to TH gene expression. Further studies are necessary to verify the E-box.

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REFERENCES

Baker H, Morel K, Stone DM and Maruniak JA (1993) Adult naris closure profoundly reduces tyrosine hydroxylase expression in mouse olfactory bulb. *Brain Res* 614:109-116

Cambi F, Fung B and Chikaraishi D (1989) 5' flanking DNA sequences direct cell-specific expression of rat tyrosine hydroxylase. *J Neurochem* 53:1656-1659.

Cho JH, Kwon IS, Kim S, Ghil SH, Tsai MJ, Kim YS, Lee YD and Suh-Kim H (2001) Overexpression of BETA2/NeuroD induces neurite outgrowth in F11 neuroblastoma cells. *J Neurochem* 77:103-109.

Conrad PW, Conforti L, Kobayashi S, Beitner-Johnson D, Rust RT, Yuan Y, Kim HW, Kim RH, Seta K and Millhorn DE (2001) The molecular basis of O2-sensing and hypoxia tolerance in pheochromocytoma cells. *Comp Biochem Physiol B Biochem Mol Biol* 128:187-204. Review.

Derijard B, Hibi M, Wu IH, Barrett T, Su B, Deng T, Karin M and Davis RJ (1994) JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* 76:1025-1037.

Fung BP, Yoon SO and Chikaraishi DM (1992) Sequences that direct rat tyrosine hydroxylase gene expression. *J Neurochem* 58:2044-2052.

Ghil SH, Kim BJ, Lee YD and Suh-Kim H (2000) Neurite outgrowth induced by cyclic AMP can be modulated by the alpha subunit of Go. *J Neurochem* 74:151-158.

Graham FL and van der Eb AJ (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 52:456-467.

Han J, Lee JD, Bibbs L and Ulevitch RJ (1994) A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* 265:808-811.

Harootunian AT, Adams SR, Wen W, Meinkoth JL, Taylor SS and Tsien RY (1993) Movement of the free catalytic subunit of cAMP-dependent protein kinase into and out of the nucleus can be explained by diffusion. *Mol Biol Cell* 4:993-1002.

Hibi M, Lin A, Smeal T, Minden A and Karin M (1993) Identification of an oncoprotein- and UV-responsive protein kinase that binds and potentiates the c-Jun activation domain. *Genes Dev* 7:2135-2148.

Hiremagalur B, Nankova B, Nitahara J, Zeman R and Sabban EL (1993) Nicotine increases expression of tyrosine hydroxylase gene. Involvement of protein kinase A-mediated pathway. *J Biol Chem* 268:23704-23711.

Kilbourne EJ and Sabban EL (1990) Differential effect of membrane depolarization on levels of tyrosine hydroxylase and dopamine beta-hydroxylase mRNAs in PC12 pheochromocytoma cells. *Brain Res Mol Brain Res* 8:121-127.

Kim JS, Chae HD, Choi SY and Kim KT (1996) Transcriptional enhancement of tyrosine hydroxylase by prostaglandin E2 in SK-N-BE(2)C cells. *Brain Res Mol Brain Res* 39:177-184.

Kim KS, Lee MK, Carroll J and Joh TH (1993a) Both the basal and inducible transcription of the tyrosine hydroxylase gene are dependent upon a cAMP response element. *J Biol Chem* 268:15689-15695.

Kim KS, Park DH, Wessel TC, Song B, Wagner JA and Joh TH (1993b) A dual role for the cAMP-dependent protein kinase in tyrosine hydroxylase gene expression. *Proc Natl Acad Sci USA* 90:3471-3475.

Kim TE, Lee HS, Lee YB, Hong SH, Lee YS, Ichinose H, Kim SU and Lee MA (2003) Sonic hedgehog and FGF8 collaborate to induce dopaminergic phenotypes in the Nurr1-overexpressing neural stem cell. *Biochem Biophys Res Commun* 305:1040-1048.

Lazaroff M, Patankar S, Yoon SO and Chikaraishi DM (1995) The cyclic AMP response element directs tyrosine hydroxylase expression in catecholaminergic central and peripheral nervous system cell lines from transgenic mice. *J Biol Chem* 270:21579-21589.

Lewis EJ, Tank AW, Weiner N and Chikaraishi DM (1983) Regulation of tyrosine hydroxylase mRNA by glucocorticoid and cyclic AMP in a rat pheochromocytoma cell line. Isolation of a cDNA clone for tyrosine hydroxylase mRNA. *J Biol Chem* 258:14632-14637.

Meinkoth JL, Alberts AS, Went W, Fantozzi D, Taylor SS, Hagiwara M, Montminy M and Feramisco JR (1993) Signal transduction through the cAMP-dependent protein kinase. *Mol Cell Biochem* 127-128:179-186. Review.

Miner LL, Pandalai SP, Weisberg EP, Sell SL, Kovacs DM and Kaplan BB (1992) Cold-induced alterations in the binding of adrenomedullary nuclear proteins to the promoter region of the tyrosine hydroxylase gene. *J Neurosci Res* 33:10-18.

Nagatsu T, Levitt M and Udenfriend S (1964) Tyrosine hydroxylase. The initial step in norepinephrine biosynthesis. *J Biol Chem* 239:2910-2917.

Patankar S, Lazaroff M, Yoon SO and Chikaraishi DM (1997) A novel basal promoter element is required for expression of the rat tyrosine hydroxylase gene. *J Neurosci* 17:4076-4086.

Piech-Dumas KM and Tank AW (1999) CREB mediates the cAMP-responsiveness of the tyrosine hydroxylase gene: use of an antisense RNA strategy to produce CREB-deficient PC12 cell lines. *Brain Res Mol Brain Res* 70: 219-230.

Sanchez S, Jimenez C, Carrera AC, Diaz-Nido J, Avila J and Wandosell F (2004) A cAMP-activated pathway, including PKA and PI3K, regulates neuronal differentiation. *Neurochem Int* 44:231-242.

Schimmel JJ, Crews L, Roffler-Tarlov S and Chikaraishi DM (1999) 4.5 kb of the rat tyrosine hydroxylase 5' flanking sequence directs tissue specific expression during development and contains consensus sites for multiple transcription factors. *Brain Res Mol Brain Res* 74:1-14.

Sabban EL, Nankova BB, Serova LI, Hiremagalur B, Rusnak M, Saez E, Spiegelman B and Kvetnansky R (1998) Regulation of gene expression of catecholamine. biosynthetic enzymes by stress. *Adv Pharmacol* 42:564-567.

Sommer L, Ma Q and Anderson DJ (1996) Neurogenins, a novel family of atonal-related bHLH transcription factors, are putative mammalian neuronal determination genes that reveal progenitor cell heterogeneity in the developing CNS and PNS. *Mol Cell Neurosci* 8:221-241. Review.

Suri C, Fung BP, Tischler AS and Chikaraishi DM (1993) Catecholaminergic cell lines from the brain and adrenal glands of tyrosine hydroxylase-SV40 T antigen transgenic mice. *J Neurosci* 13:1280-1291.

Trocme C, Mallet J and Biguet NF (1997) AP-1 mediates trans-synaptic induction of tyrosine hydroxylase gene expression in adrenal medulla but not in superior cervical ganglia. *J Neurosci Res* 48:489-498.

Trocme C, Sarkis C, Hermel JM, Duchateau R, Harrison S, Simonneau M, Al-Shawi R and Mallet J (1998) CRE and TRE sequences of the rat tyrosine hydroxylase promoter are required for TH basal expression in adult mice but not in the embryo. *Eur J Neurosci* 10:508-521.

Yang C, Kim HS, Seo H and Kim KS (1998) Identification and characterization of potential cis-regulatory elements governing transcriptional activation of the rat tyrosine hydroxylase gene. *J Neurochem* 71:1358-1368.

Yoon SO and Chikaraishi DM (1992) Tissue-specific transcription of the rat tyrosine hydroxylase gene requires synergy between an AP-1 motif and an overlapping E box-containing dyad. *Neuron* 9:55-67.