Analysis of the 5′-upstream region of mouse P/Q-type Ca^{2+} channel \( \alpha_{1A} \) subunit gene for expression in pancreatic islet \( \beta \) cells using transgenic mice and HIT-T15 cells

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**ABSTRACT**

The \( \omega \)-agatoxin-IVA-sensitive P/Q-type Ca^{2+} channel plays a role in insulin release from the pancreatic islets of \( \beta \) cells. To dissect the molecular mechanisms underlying \( \beta \) cell expression of the P/Q-type channel, we characterized the 5′-upstream region of the mouse \( \alpha_{1A} \) subunit gene using transgenic mice and HIT insulinoma cells. The \( E. \ coli \) lacZ reporter gene was expressed in pancreatic acini and islets in transgenic mice carrying the 6·3 kb or 3·0 kb of the 5′-upstream region, although those with 1·5 kb or 0·5 kb of the 5′-upstream region failed to show reporter expression on histological examination. As the expression of \( \alpha_{1A} \) subunit gene could not be detected in acini using RT-PCR analysis, the reporter expression in acini might have been ectopic expression. When linked to the placental alkaline phosphatase reporter gene to examine promoter activity for \( \beta \) cell expression, the 6·3 kb and 3·0 kb fragment of the 5′-upstream region, but not the smaller 1·5 kb fragment, were able to drive reporter gene expression in HIT cells. The sequence between 3·0 and 1·5 kb upstream of the start codon enhanced thymidine kinase promoter activity in HIT cells, but not in fibroblast NIH3T3 cells. These results suggested that the \( \beta \) cell-specific elements of the \( \alpha_{1A} \) subunit gene are likely to be located in the distal upstream region (−3021 to −1563) of the 5′-upstream sequence and that the 6·3 kb fragment of the 5′-upstream region alone might be a lack of a negative cis-regulatory element(s) to suppress the \( \alpha_{1A} \) subunit gene expression in acini.

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**INTRODUCTION**

Glucose augments insulin release from the pancreatic islet \( \beta \) cells by altering the ATP/ADP ratio (Ashcroft et al. 1984, Newgard & McGarry 1995). An increase in ATP relative to ADP inhibits ATP-sensitive K\(^+\) (\( K_{\text{ATP}} \)) channel activity and gives rise to membrane depolarization (Longo et al. 1991, Larsson et al. 1996). ATP-dependent depolarization increases Ca\(^{2+}\) influx through voltage-activated Ca\(^{2+}\) channels and evokes insulin secretion (Larsson et al. 1996). Removal of extracellular Ca\(^{2+}\) abolishes glucose-stimulated insulin secretion (Curry et al. 1968, Hales & Milner 1968, Devis et al. 1975, Boyd 1992). This implies that the Ca\(^{2+}\) influx through voltage-activated Ca\(^{2+}\) channels is an important process for insulin release.

Several types of voltage-activated Ca\(^{2+}\) channels (L, P, Q, N and R) have been identified based on their pharmacological and physiological properties (Hess 1990, Tsien et al. 1991, Hofmann et al. 1994, Randall & Tsien 1995). These Ca\(^{2+}\) channels have been reported to be composed of at least three subunits, \( \alpha_{1}, \alpha_{2-\delta}, \) and \( \beta \) subunits (Zhang et al. 1993). The \( \alpha_{1} \) subunit is a pore-forming component, functions as a voltage sensor, and is capable of generating Ca\(^{2+}\) channel activity in heterogeneous expression systems (Mikami et al. 1989). Molecular cloning studies have revealed that \( \alpha_{1S}/\alpha_{1C}/\alpha_{1D}, \alpha_{1A}, \alpha_{1B} \) and \( \alpha_{1E} \) genes encode the \( \alpha_{1} \) subunits of the dihydropyridine (DHP)-sensitive L-type channels, \( \omega \)-agatoxin-IVA (\( \omega \)-Aga-IVA)-sensitive P/Q-type channels, \( \omega \)-conotoxin-GVIA (\( \omega \)-CgTx-GVIA)-sensitive N-type channels and DHP/\( \omega \)-Aga-IVA/\( \omega \)-CgTx-GVIA-insensitive R-type channels respectively (Mikami et al. 1989, Mori et al. 1991, Niidome et al. 1992, Williams et al. 1992).
L-type, P/Q-type and N-type channels have been reported to play roles in Ca\(^{2+}\)-influx-dependent insulin release (Stain et al. 1995, Ligon et al. 1998). The regulation of \(\alpha_1\) subunit gene expression is clinically important, and elucidation of the mechanisms of expression of \(\alpha_1\) subunit genes may facilitate understanding and treatment of \(\beta\) cell disorders such as diabetes and hyperinsulinemia. However, although recent studies have reported the mechanisms of expression of \(\alpha_1\) subunit genes in the neuronal cells (Kamp et al. 1995, Kim et al. 1997, Takahashi et al. 1999b), the mechanisms of transcription in the islet \(\beta\) cells have not been determined.

Here, we describe the functional characterization of the 6·3 kb 5′-upstream promoter region of the P/Q-type Ca\(^{2+}\) channel \(\alpha_1\text{D}\), \(\alpha_1\text{A}\) and \(\alpha_1\text{B}\) subunit genes in the neuronal cells (Kamp et al. 1995, Kim et al. 1997, Takahashi et al. 1999b), the regulation of insulin release (Stain 1981). Syrian hamster pancreatic cells have established by simian virus 40 transformation of transgenic mice and HIT-T15 insulinoma cells (Santerre et al. 1981).

**MATERIALS AND METHODS**

**Isolation between mouse pancreatic acini and islets**

Isolation between pancreatic acini and islets was carried out using a modified collagenase digestion procedure (Gotoh et al. 1985). Briefly, Hanks’ balanced salt solution (HBSS) containing 1·0 mg/ml collagenase (Sigma, St Louis, MO, USA) was injected into the pancreas through the duct. The distended pancreas was removed and digested in HBSS at 37°C for 10 min. The digested pancreas was dispersed using a pipette. The dispersed tissues were divided between acini and islets by hand picking under a microscope or by centrifugation on a Ficoll (Amersham International plc, Amersham, Bucks, UK) gradient.

**Total RNA preparation and RT-PCR**

Total RNAs from HIT-T15 insulinoma (HIT) cells, mouse brain, pancreatic acini and islets were isolated with TRIzol Reagent (Gibco-BRL, Gaithersburg, MD, USA) according to the manufacturer’s protocol. First strand cDNA was synthesized with 1 μg total RNA using the SuperScript preamplification system (Gibco-BRL). Following first strand cDNA synthesis, PCR was carried out for 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, and a final extension of 72°C for 7 min. The following primers were used to amplify the \(\alpha_1\text{A}\) subunit, amylase or insulin cDNA fragments: \(\alpha_1\text{A}-f\) (5′-GAGATGATGGCCATTGTTGCCCAC-3′, sense, +5484 to +5508, Genbank accession number, U76716) and \(\alpha_1\text{A}-r\) (5′-TCAGAGATGAGTACTGAGGCTC-3′, antisense, +6041 to +6062) for \(\alpha_1\text{A}\) subunit cDNA fragment; amylase-f (5′-AGTTCGGTCTCTGCTGTCTCCCC-3′, sense, +852 to +874, Genbank accession number, M16540) and amylase-r (5′-AGTTCGGATGGATTATGAACTACA-3′, antisense, +1368 to +1393) for amylase cDNA fragment; insulin-f (5′-CTCTGGCTCTCTGACCCG-3′, sense, +4217 to +427, Genbank accession number, J00747) and insulin-r (5′-GCTGGTGCGACACTGATCCAAT-3′, antisense, +4452 to +4465) for insulin cDNA fragment. The reaction product was subcloned into pT7Blue(R)T-Vector (Novagen, Madison, WI, USA) and sequenced with a Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) using U19 and T7 primers with an ABI Prism 377 DNA sequencer (Applied Biosystems). Computer analysis of potential transcription factor binding sites was carried out using GENETYX software (Soft Development, Tokyo, Japan).

**Plasmid constructions**

Plasmids for transient expression assay to identify transcriptional regulatory element(s) in the mouse \(\alpha_1\text{A}\) subunit gene were constructed by utilizing the placental alkaline phosphatase (PLAP) reporter gene. The plasmids \(\text{p} \alpha_1\text{A}6-3\text{-PLAP}\), \(\text{p} \alpha_1\text{A}3-0\text{-PLAP}\), \(\text{p} \text{TK-PLAP}\) and \(\text{p} 63-3-0 \alpha_1\text{A} \text{Thymidine Kinase (TK)}-\text{PLAP}\) were generated previously (Takahashi et al. 1999b). To construct \(\text{p} \alpha_1\text{A}1-5\text{-PLAP}\), \(\text{p} \alpha_1\text{A}3-0\text{-PLAP}\) was digested with NotI and StuI, the NotI–StuI fragment was removed and the plasmid was religated. pBS104 (Takahashi et al. 1999b) was digested with EcoRV and StuI, and the EcoRV–StuI fragment was inserted into the EcoRV site of pBluescript II KS− (Stratagene Inc., La Jolla, CA, USA) to yield pBS3·0·1·5. The plasmid p3·0·1·5\(\alpha_1\text{A} \text{TK-PLAP}\) was generated by inserting the SpeI–XhoI fragment of pBS3·0·1·5 into the SpeI–XhoI site of \(\text{p} \text{TK-PLAP}\). To construct pBS1·5·0·01, pBS104 was digested with StuI and SmaI, and the StuI–SmaI fragment was inserted into the EcoRV site of pBluescript II KS−. The plasmid p1·5·0·01\(\alpha_1\text{A} \text{TK-PLAP}\) was generated by inserting the SpeI–XhoI fragment of pBS1·5·0·01 into the SpeI–XhoI site of \(\text{p} \text{TK-PLAP}\).

**Cell culture, transient transfection and PLAP assay**

HIT cells were maintained in glucose-free Dulbecco’s modified Eagle’s medium (DMEM)
supplemented with 2 mM sodium pyruvate, 1% non-essential amino acids, 55 nM 2-mercapto-ethanol and 10% fetal calf serum (FCS). NIH3T3 cells were grown in DMEM with 10% FCS. All culture media were supplemented with 100 units/ml penicillin and 100 µg/ml streptomycin. To examine the expression of the mouse α1A subunit gene, we carried out transient expression assays using HIT and NIH3T3 cells. The cells were plated at a density of 1 × 10^5 cells in six-well dishes. Twenty-four hours after seeding, the cultures were washed extensively to remove non-adherent cells and the medium was replaced. On the second day, transfection was carried out with FuGENE 6 transfection reagent (Boehringer-Mannheim, Mannheim, Germany) according to the manufacturer’s protocol. One microgram of the plasmid containing the mouse α1A subunit gene promoter region fused to the PLAP reporter gene and 1 µg of the pSV-β-galactosidase control vector (Promega, Madison, WI, USA) were co-transfected into the cells.

RESULTS

P/Q-type Ca^{2+} channel α1A subunit mRNA expression in HIT cells and pancreas

To examine the endogenous expression of the P/Q-type Ca^{2+} channel α1A subunit gene, RT-PCR analysis was performed for HIT cells, mouse pancreatic acini and islets. Isolation between acini and islets was confirmed by the expression of amylase or insulin (Fig. 1) and sequencing of these PCR products confirmed the amylase or insulin sequence (data not shown).

PCR of mRNA without reverse transcription was performed as a negative control. RT-PCR of mRNA from mouse brain was performed as a positive control. RT-PCR using primers specific for α1A subunit gene yielded an amplified product corresponding to the predicted size from HIT cells, islets and brain, but not from acini (Fig. 1). Sequencing of these PCR products confirmed the α1A cDNA sequence corresponding nucleotides of carboxyl terminus (+5484 to +6062) (data not shown).

Expression of PLAP reporter gene in HIT cells

To address whether the 6·3 kb fragment of the 5' upstream region of the mouse α1A subunit gene includes the regulatory sequences for expression in islet β cells, we made transfection gene constructs, pα1A6·3-PLAP (−6273 to +269), pα1A3·0-PLAP (−3021 to +269) and pα1A1·5-PLAP (−1563 to +269) (Fig. 2A). The promoterless construct basic-PLAP served as a background control and the pTK-PLAP construct, which contains the TK promoter driving a PLAP reporter gene, as a positive control. We measured PLAP activity after transient transfection into HIT cells. As shown in Fig. 2B, pα1A6·3-PLAP and pα1A3·0-PLAP, but not pα1A1·5-PLAP, drove PLAP expression in HIT cells efficiently.
Expression of lacZ reporter gene in the pancreas of transgenic mice

The expression patterns of the transgenes in the pancreas of 10-week-old transgenic mice were examined by histochemical staining. Incubation of pancreas sections from each transgenic mouse line with X-gal generated blue staining in the cells expressing the transgenes. Wild-type mice exhibited no positively stained cells in the pancreas. The expression patterns of the transgenic mouse lines are summarized in Table 1. The levels and distributions of transgene expression were not correlated with the transgene copy number (data not shown). No differences were observed in the expression patterns of the transgenes between males and females in any of the transgenic mouse lines (data not shown).

Different expression patterns were observed among the $\alpha_{1A}6\cdot3$-lacZ ($-6272$ to $+498$), $\alpha_{1A}3\cdot0$-lacZ ($-3021$ to $+498$), $\alpha_{1A}1\cdot5$-lacZ ($-1563$ to $+498$) and $\alpha_{1A}0\cdot5$-lacZ ($-497$ to $+498$) mouse lines in pancreatic acini and islets. The $\alpha_{1A}1\cdot5$-lacZ and $\alpha_{1A}0\cdot5$-lacZ mouse lines failed to express $\beta$-galactosidase in the acini or islets. In contrast, the $\alpha_{1A}6\cdot3$-lacZ and the $\alpha_{1A}3\cdot0$-lacZ mouse lines expressed the transgene in the islets, while the expression levels in the acini were relatively low (Fig. 3). Expression patterns of $\beta$-galactosidase were consistent among transgenic mouse lines carrying the same transgenes, suggesting that chromosomal effects could be excluded in these lines.

Islet $\beta$ cell-specific enhancer element in the 5′-upstream region of the P/Q-type Ca$^{2+}$ channel $\alpha_{1A}$ subunit gene

To examine whether the sequence between $3\cdot0$ and $1\cdot5$ kb 5′-upstream region ($-3021$ to $-1563$) contributes to the islet $\beta$ cell expression, we made fusion gene constructs p6·3–3·0$\alpha_{1A}$TK-PLAP ($-6273$ to $-3021$), p3·0–1·5$\alpha_{1A}$TK-PLAP ($-3021$ to $-1563$) and p1·5–0·01$\alpha_{1A}$TK-PLAP ($-1563$ to $-11$) (Fig. 4A). PLAP activity was measured after transient transfection into HIT and NIH3T3 cells. The presence of the sequence between $3\cdot0$ and $1\cdot5$ kb 5′-upstream of the start
DISCUSSION

The release of insulin from pancreatic islet β cells and insulin-secreting cell lines is evoked by closing intracellular ATP-inhibited K$_{ATP}$ channels, depolarizing the cells (Longo et al. 1991, Larsson et al. 1996) and increases in Ca$^{2+}$ influx through the DHP-sensitive L-type, ω-Aga-IVA-sensitive P/Q-type and ω-CgTx-GVIA-sensitive N-type voltage-activated Ca$^{2+}$ channels (Stain et al. 1995, Ligon et al. 1998). The majority of insulin release is dependent on L-type Ca$^{2+}$ channels and smaller portions are dependent on P/Q-type and N-type (Stain et al. 1995, Ligon et al. 1998). As the relationships among the roles of these Ca$^{2+}$ channels in insulin release are unclear, it is of interest to examine and compare the regulatory mechanisms underlying the β cell-specific expression of $a_1$ subunit genes. As a first step, we dissected the molecular mechanisms underlying the pancreatic islet β cell expression of the mouse P/Q-type Ca$^{2+}$ channel $a_1$ subunit gene. Transient transfection analysis in HIT cells indicated PLAP activity in p$\alpha_1A$6·3-PLAP and p$\alpha_1A$3·0-PLAP, but not p$\alpha_1A$1·5-PLAP. This result showed that the sequence between 3·0 and 1·5 kb upstream of the start codon contains positive cis-regulatory elements that function in HIT cells.

The $a_1A$6·3-lacZ and $a_1A$3·0-lacZ mouse lines, but not $a_1A$1·5-lacZ and $a_1A$0·5-lacZ, expressed lacZ reporter gene in the pancreatic acini and islets. In situ hybridization and immunocytochemical studies have shown that the $a_1$ subunit gene is expressed in the islets but not in the acini (Ligon et al. 1998). Our RT-PCR analysis also showed that the expression of $a_1$ subunit gene is detected in the islets but not in the acini. Our results showed that in transgenic mouse lines the ectopic expression of the reporter gene was detected in the acini, suggesting that the 6·3 kb 5′-upstream region alone is not sufficient for the authentic expression of the $a_1$ subunit gene. In the case of the tyrosine hydroxylase gene, authentic expression was not induced by the 5′-upstream region alone, however, the more exact expression was observed only after the insertion of additional 5′-upstream, intragenic, and 3′-downstream regions (Min et al. 1994). The blue staining in the acini in our transgenic mouse lines might have been due to the lack of a negative cis-regulatory element(s) in additional 5′-upstream, intragenic or 3′-downstream regions. The cells of

codon (−3021 to −1563), but not the sequences of between 6·3 and 3·0 kb and between 1·5 and 0·01 kb 5′-upstream, enhanced the activity of the TK promoter in HIT cells (Fig. 4B). In contrast, p6·3–3·0$a_1A$TK-PLAP, p3·0–1·5$a_1A$TK-PLAP and p1·5–0·01$a_1A$TK-PLAP did not enhance the activity of the TK promoter in NIH3T3 cells (Fig. 4B).

**FIGURE 2.** Promoter activity of the 5′-upstream region of the mouse P/Q-type Ca$^{2+}$ channel $a_1$ subunit gene. (A) Deletion constructs containing different lengths of the mouse $a_1A$ subunit gene promoter linked to the PLAP reporter gene were transiently transfected into HIT cells along with the pSV-β-galactosidase vector. (B) PLAP activity of different deletion constructs is shown relative to that of the vector pTK-PLAPI. All values are normalized to the pSV-β-galactosidase vector. Results represent the mean relative activities ± standard deviations of three independent experiments.
not less than half of the islets were stained with X-gal. This demonstrated that the sequence between 3·0 and 1·5 kb upstream contains positive regulatory elements of expression in the β cells, which comprise about 80% of islet cells. Thus, our results suggested that transcription of the αIA subunit gene might be under the same regulatory control in HIT cells in vitro and in the islet β cells of transgenic mice in vivo.

To examine whether the sequence between 3·0 and 1·5 kb upstream contains a positive cis-regulatory element for β cell-specific expression, we carried out deletion analysis by in vitro transfection assay with HIT cells and non-insulinoma NIH3T3 cells. The p3·0–1·5αIA TK-PLAP enhanced transcriptional activity of TK promoter, but not p6·3–3·0αIA TK-PLAP or p1·5–0·01αIA TK-PLAP in HIT cells. We previously confirmed a lack of expression of the αIA subunit gene and no promoter activity of pαIA 6·3-PLAP in NIH3T3 cells (Takahashi et al. 1999b). In this study, we confirmed that the sequence between 6·3 and 3·0 kb, between 3·0 and 1·5 kb, and between 1·5 and 0·01 kb upstream contains no positive cis-regulatory elements for β cell-specific expression in NIH3T3 cells. These results showed that the positive cis-regulatory element for β cell-specific expression is located in the sequence between 3·0 and 1·5 kb upstream of the mouse P/Q-type αIA subunit gene.

To understand the mechanisms responsible for expression of the αIA subunit gene in β cells, an analogy can be drawn with the insulin and gastrin

### Table 1. Expression of β-galactosidase activity in the pancreas of transgenic mice

<table>
<thead>
<tr>
<th>Lines</th>
<th>αIA0.5-lacZ</th>
<th>αIA1.5-lacZ</th>
<th>αIA3.0-lacZ</th>
<th>αIA6.3-lacZ</th>
<th>Wild-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas</td>
<td>1</td>
<td>2</td>
<td>10</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Acini</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Islets</td>
<td>−</td>
<td>−</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Percentages of stained cells: (+++) => 71%, (++) = 50–70%, (+) = 1–49%, (−) = no expression.

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**Figure 3.** β-Galactosidase transgene expression patterns in the pancreas of αIA 6·3-lacZ-15. The section was counterstained with nuclear fast red. The transgene was expressed in the acinus and islets. Arrows indicate the transgene expression in the acinus. Bar=400 µm.
genes (Wang & Brann 1990, Moitoso de Vargas et al. 1997). When fused to the chloramphenicol acetyltransferase reporter gene, the 5′-upstream regions of the insulin and gastrin genes drove reporter gene expression in HIT cells. The sequence CATCTGG (Nir box) was demonstrated to be responsible for the β cell-specific expression (Wang & Brann 1990, Moitoso de Vargas et al. 1997). Our computer-assisted analysis indicated that the distal upstream region (-3021 to -1563) of the α1A subunit gene contains six putative Nir box-like sequences (-2355 CATCTTG -2347; -2325 CATATGG -2319; -2559 CATCTTG -2353; -2077 CATGTGG -2071; -1979 CATCTTG -1973; and -1866 CATGTGG -1800). It is likely that the Nir box-like sequence of the α1A subunit gene also contributes to specific expression of the α1A subunit gene in β cells.

In our previous study, the brain cell type-specific expression mechanisms of the α1A subunit gene was examined (Takahashi et al. 1999a). The sequence between 3′-0 and 1′-5 kb upstream contained positive cis-regulatory elements for the expression in amygdaloid nucleus, septum, habenula medial nucleus, substantia nigra, inferior colliculus, pontine nucleus and cerebellar granule cells. In contrast, the sequence between 6′-3 and 3′-0 kb upstream contained negative cis-regulatory elements for these neurons, except the inferior colliculus. Thus, the brain cell type-specific expression of the α1A subunit gene appeared to be controlled by the concerted action of positive and negative cis-regulatory mechanisms, except the inferior colliculus. On the other hand, we detected no negative cis-regulatory elements for β cell-specific expression in the 6′-3 kb 5′-upstream region. The Nir box-like sequence has not been reported to be responsible for specific expression of brain cells, including the inferior colliculus cells. It is likely that the expression mechanisms of the α1A subunit gene are different between β cells and brain cells.

The density of β-Aga-IVA-sensitive current in β cells varies considerably from cell to cell (Ligon et al. 1998). This heterogeneity likely reflects variations in α1A subunit gene expression levels. As between 50% and 70% of the cells in the islets showed blue staining, our α1Aβ3-lacZ and α1A3-0-lacZ transgenic mouse lines did not express detectable levels of the lacZ reporter gene in all β cells. Thus, it is likely that negative cis-regulatory elements might be active in β cells that were not stained with X-gal. Cell-specific regulation of transcription of the α1A subunit gene may be one of the mechanisms responsible for the diversity in the pharmacological properties of β cells. This phenomenon should be examined in future studies.

In summary, we have identified a region in the 5′-upstream region of the mouse P/Q-type α1A subunit gene (-3021 to -1563) that contains a positive cis-acting regulatory element likely to be

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Fragment analysis of the cell-specific expression of the P/Q-type Ca2+ channel α1A subunit gene. (A) Deletion constructs containing different fragments upstream of the start codon inserted into the pTK-PLAPI vector were transiently transfected into HIT and NIH3T3 cells along with the pSV-β-galactosidase vector. (B) PLAP activities of different deletion constructs are expressed relative to the vector pTK-PLAPI. All values are normalized to the pSV-β-galactosidase vector. Results represent the mean relative activities ± standard deviations of three independent experiments, and statistical significance was evaluated by Student’s t-test (*P<0.005).
responsible for the pancreatic islet β cell expression of the α1A subunit gene. On the other hand, the ectopic expression of reporter gene in acini has shown that the 6-3 kb of the 5’-upstream region alone might be a lack of a negative cis-regulatory element(s) to suppress α1A subunit gene expression in acini. Further studies, including deletion and additional sequence analysis and identification of trans-regulatory factors, should help to clarify the mechanism of β cell-specific expression of the P/Q-type α1A subunit gene.

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