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Regulatory sequences in the 3' Untranslated region of the human cGMP-Phosphodiesterase β-Subunit gene

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Abstract

PURPOSE—Rod cGMP-phosphodiesterase, a key enzyme in visual transduction, is important for retinal integrity and function. Mutations in the gene encoding its β-subunit (PDEβ) cause retinal degeneration in animals and humans. Here we tested the hypothesis that elements in the 3'UTR of the PDEβ gene are involved in the regulation of PDEβ expression.

METHODS—Involvement of the 3' untranslated region (3'UTR) of PDEβ mRNA in the regulation of PDEβ expression was assessed by transfecting Y-79 retinoblastoma cells or the heads of *Xenopus laevis* tadpoles with constructs containing the SV40 or PDEβ promoter, the luciferase cDNA and either the SV40 or PDEβ 3'UTR (or fragments of its sequence).

RESULTS—Compared to the SV40 3'UTR (used as control), the entire PDEβ 3'UTR decreased reporter gene expression in Y-79 retinoblastoma cells as well as in SY5Y neuroblastoma and 293 human embryonic kidney cell lines. However, we observed that two 100 nt fragments from the PDEβ 3'UTR increased while its non-canonical polyadenylation signal abolished reporter gene expression both in Y-79 retinoblastoma cells and in *ex vivo* experiments using *Xenopus* tadpole heads. In particular, an 11 nt element (EURE) in one of the 100 nt fragments was responsible for up-regulation of luciferase expression.

CONCLUSION—Our studies indicate that the 3'UTR of the PDEβ mRNA is involved in the very complex regulation of this gene's expression in the retina. Moreover, they show that the PDEβ poly-A signal has a dominant inhibitory effect over two other regions in the 3'UTR that stimulate gene expression.

INTRODUCTION

Cyclic GMP-phosphodiesterase (PDE), an enzyme involved in the conversion of light energy into an electrical signal in retinal photoreceptor cells, is composed of at least four subunits, two with catalytic function (α and β) and two with inhibitory activity (γ).

Mutations in the coding region of the PDEβ subunit gene are known to cause retinal degeneration in mice and dogs[1,2] and, most importantly, result in autosomal recessive retinitis

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pigmentosa[3-7] and congenital stationary night blindness[8] in humans. A normal PDEβ subunit is, therefore, necessary to maintain the structure and function of photoreceptor cells.

Elements present in the 5' flanking region of the human PDEβ gene are necessary for efficient, cell-specific expression of PDEβ or reporter genes in cell cultures[9-13] and in *Xenopus*[9] retinas. Transcription factors binding to these elements and activating or repressing transcription are also very important for retinal integrity and function. When mutated, these proteins may cause retinal degeneration (e.g., NRL[14], CRX[15,16], and Sp4[17]). In the last few years, considerable effort has been placed on characterizing these factors. In contrast, no information is currently available about the existence of regulatory elements in the 3'UTR of PDEβ.

The 3'UTR of several genes has been implicated in post-transcriptional regulation[18,19]. Specifically, it has been demonstrated that the 3'UTR plays a role in message stability[20], translation[21] and intracellular transport[22], and that it is involved in polyadenylation[23]. Furthermore, the 3'UTR has been acknowledged as a vital component of genes in the search for human mutations causing disease[24].

We have previously shown that mouse PDEβ gene expression is also regulated at the translational level[25] and that the 3'UTR of its mRNA increases the efficiency of protein synthesis[26]. In this paper, we further analyze the function of this 3'UTR and show that it contains specific sequences causing up- or down-regulation of expression of a reporter gene and that these events can be measured in transfections using different cell lines or the head of *Xenopus laevis* embryos[27]. This *ex-vivo* transfection system has been employed to characterize regulatory sequences in the 5' flanking region of the PDEβ[9] and other retina-specific genes[27,28]. In addition, we report the identification of a novel 11-nucleotide element, EURE (Eleven nucleotide Untranslated Region Element) that may regulate PDEβ gene expression.

METHODS

Preparation of Luciferase constructs

Several PDEβ 3'UTR constructs were cloned into the pGL3-Promoter vector (Promega, Madison Wisconsin) between the luciferase reporter gene and the SV40 late poly-A signal. Transcription of the luciferase gene in this vector is controlled by the SV40 promoter.

- Construct p689 had the full-length PDEβ 3'UTR (653 nt + 36 nt from the poly-A signal to the end of the poly-A tail). This was amplified from human retinal total RNA using the 3'RACE Kit (that provides the 3' primer AUAP, Invitrogen, Carlsbad, California), and 5' primer "a" (Table 1).
- Construct p228 had the terminal 228 nt of PDEβ 3'UTR (starting at the first putative poly-A signal) amplified by PCR with primers "b" (Table 1) and AUAP (3'RACE Kit).
- Construct p431 contained nt 31−461 of PDEβ 3'UTR (Figure 1), and was obtained by RT-PCR of total RNA from Y-79 cells using primers "c" and "d" (Table 1).
- Seven ~100 nt PDEβ 3'UTR fragments (F1-F7, Figure 1) were generated by PCR with sequence-specific primers 1/2; 3/4; 5/6; 7/8; 9/10; 11/12; 13/14, respectively (Table 1). These fragments were used to generate constructs pF1-to pF7- SV40 (see Figure 6). In addition, construct pF7-PDEβ had F7 and the PDEβ poly-A signal. Primers 13/15 were used to obtain this fragment (see Figure 5).
- A construct with the SV40 promoter in the pGL3 vector replaced by the PDEβ promoter (−93/+53) was used as backbone for insertion of F1, F5, or the 653 nt PDEβ 3'UTR up to the poly-A signal (see Figure 8).

- To generate construct pEURE, containing the 11 nt element EURE identified in F5, the pGL3 Promoter vector and primers 16/17 (Table 1) were used. Primer 16 has a sequence specific to the vector plus the EURE sequence, GTTTTTATAAA, and primer 17 is only from the pGL3 vector sequence. This resulted in a product with the 11 nt of PDEβ located immediately 5' to the SV40 3'UTR that was cloned back into the pGL3 Promoter Vector (Figure 9).
- For comparative studies, constructs with the full length 3'UTRs of cone PDEα' (265 nt), cone arrestin (96 nt), and the SRB7 component of mammalian RNA polymerase II holoenzyme (306 nt) were prepared in the pGL3 Promoter vector replacing the SV40 3'UTR and poly A signal.

Cell Cultures and Transient Transfection

Y-79 human retinoblastoma (Y-79), 293 human embryonic kidney (HEK), and SY5Y human neuroblastoma cells were obtained from the American Type Culture Collection (ATCC, Manassa, Virginia). Y-79 cells were propagated in suspension in RPMI 1640 media (with L-glutamine and no sodium phosphate) supplemented with 15% fetal bovine serum (FBS). 293 HEK cells were propagated in Dulbecco's Modified Eagle's Medium/F-12 supplemented with 10% FBS and pen/strep mix (Invitrogen). SY5Y cells were maintained in a 1:1 mixture of Eagle's minimum essential medium with non-essential amino acids and Ham's F12 medium with 10% FBS. Y-79 cells were plated as described previously[12] at a density of 10^6 cells/ 60mm plate. Likewise, 293 HEK and SY5Y cells were plated at a density of 10^6 cells/plate.

Transfections of Y-79 cells were carried out with 20 μg of the pGL3 construct/plate and the calcium phosphate precipitation method[12]. For 293 HEK and SY5Y cell transfections, as well as Y-79 cell transfections with constructs having EURE, 8.5 μg of the appropriate pGL3 construct/plate and lipofectamine were used (Invitrogen's protocol). In all experiments, the pSV-β-galactosidase control vector (Promega) containing the bacterial lacZ gene driven by the SV40 early promoter was co-transfected with the construct being tested as an internal control for variations in transfection efficiency. 15 μg/60-mm plate of pSV-β-galactosidase plasmid were used for calcium phosphate transfections and 6.3 μg/60-mm plates for lipofectamine transfections. Each construct was transfected in triplicate plates. Luciferase and β-galactosidase assays were performed and the relative luciferase activity was calculated as previously described[10].

Ex Vivo Transfections in *Xenopus* Embryos

All experiments using *Xenopus* adhered to the ARVO statement for the Use of Animals in Ophthalmic and Vision Research. *Xenopus in vitro* fertilizations and transfections were carried out as described by Batni et al[27]. Briefly, freshly laid *Xenopus* eggs were fertilized with crushed testicular tissue *in vitro*. Embryos grew until they reached stages 24−28, and groups of 10−12 dissected heads were transfected with 10 μg of DNA and 30 μl of DOTAP (Roche, Basel, Switzerland), each plasmid transfected in triplicate. After incubation for 72−80 hr post-fertilization, heads were assayed for luciferase activity using the Luciferase Assay System (Promega). The activity measured in these transfections has been shown to be quite accurate and thus a normalization control[27] is not needed. Results are expressed as Relative Luciferase Activity/ Head (RLU/ Head), obtained by dividing total luciferase activity by the number of heads used for each plasmid tested.

RESULTS

Cloning and Sequence Analysis of the Human PDEβ 3'UTR

3' RACE was performed on the retinal RNA of a human donor eye to obtain the full length PDEβ 3'UTR (Figure 1). The resulting 689 nt product was longer than the previously reported PDEβ 3'UTR[29] by 44 nt and contained the putative poly-A signal at position 654 and the poly-A tail. Its sequence (accession number FJ417399, Figure 1) was compared with that of the reported PDEβ cDNA[29] and with the PDEβ genomic sequence[30]. A few nucleotide differences were observed with the published sequences, however, none fell within regions that we found to be conserved in different species (doubly underlined). The *alu* element (underlined) previously described[30] is not present in mouse, dog or bovine PDEβ genes suggesting its evolutionarily recent insertion into the human gene. In addition to the poly-A signal starting at position 654, another potential poly-A signal previously suggested[29] (dashed underlined) starts at position 461. However, no transcripts shorter than the sequence shown in Figure 1 have been found in human retinal mRNA samples. Further work is needed to determine whether both or only one poly-A signal is responsible for PDEβ polyadenylation.

Effects of the PDEβ 3'UTR on Luciferase Expression

PDEβ has been shown to be present in Y-79 retinoblastoma cells, indicating that these cells have the regulatory machinery necessary for PDEβ expression[31]. Therefore, Y-79 cells were chosen to analyze the effect of the 3'UTR of PDEβ on gene expression. Transfection of construct p689 (containing the luciferase reporter gene driven by the SV40 promoter and the full-length 3'UTR of PDEβ) into Y-79 cells resulted in luciferase activity that was ~ 1/8 of that produced by the pGL3 vector (Figure 2). Moreover, transfection of the p228 construct containing the SV40 reporter, the luciferase reporter and the terminal 228 nt of PDEβ 3'UTR resulted in almost complete abolishment of luciferase expression (Figure 2).

To determine whether this inhibitory effect is a feature of the PDEβ 3'UTR, constructs with the SV40 promoter and the complete 3'UTRs from three different genes [cone arrestin and cone PDEα' (both photoreceptor specific transcripts[32,33]) and the SRB7 hallmark component of the mammalian RNA polymerase II holoenzyme (a ubiquitously expressed mRNA)[34]] were produced and transfected into Y-79 cells for comparison with p689 and pGL3. The luciferase activity of cells transfected with the PDEβ full-length 3'UTR construct was ~1/5, 1/8 and 1/24 of that of cells with the transfected SRB7, cone arrestin and PDEα' 3'UTR constructs, respectively (Figure 3).

We also tested whether the PDEβ 3'UTR down-regulation of luciferase expression was specific to Y-79 cells by transfecting the p689 construct into 293 HEK and SY5Y neuroblastoma cells. In both cell lines, the PDEβ 3'UTR-containing construct decreased gene expression by ~ 75% when compared to that produced by pGL3 (Figure 4).

PDEβ poly-A signal

We studied the effect of the non-canonical PDEβ poly-A signal at position 654 on gene expression because both the p689 and p228 constructs that contain the full length (653 nt and poly-A signal and poly-A tail) and terminal 228 nt (192 nt and poly-A signal and poly-A tail), repectively, have this sequence and decrease luciferase expression (Figure 2). For these experiments, we compared luciferase activity from two constructs: pF7-SV40, prepared by replacing the SV40 3'UTR with F7 (the last 110 nt of the PDEβ 3'UTR before the poly-A signal) but keeping the SV40 poly-A signal, and pF7-PDEβ in which the SV40 poly-A signal was substituted with the PDEβ poly-A signal. In both constructs, the luciferase reporter gene was driven by the SV40 promoter (Figure 5). The relative luciferase activity produced in Y-79 cells by construct pF7-PDEβ was approximately 1/6 of that produced by pF7-SV40. This

activity was not significantly different from that generated by the p689 construct that has the entire PDEβ 3'UTR with its poly-A signal, whereas the luciferase activity produced by the pGL3 Promoter vector was similar to that obtained with pF7-SV40 (Figure 5).

Fragments of the PDEβ 3'UTR

Although the p689 construct dramatically reduced luciferase expression compared with that obtained with the pGL3 Promoter vector, the p431 construct up-regulated the expression of luciferase by almost 3-fold (Figure 6).

To narrow down the region(s) of the PDEβ 3'UTR segment responsible for the observed effect on luciferase expression, we generated seven ~ 100 nt fragments of the 3'UTR and subcloned each fragment into the pGL3 Promoter vector (Figure 6). When transfected into Y-79 cells, Fragment 1 (pF1) and Fragment 5 (pF5, Figure 1), increased luciferase expression to the level produced by p431 (Figure 6). Fragments in pF2, pF3, pF4, pF6 and pF7 had no significant effect when compared to pGL3.

To determine if the up-regulation caused by F1 and F5 also is observed in other cell lines, 293 HEK and SY5Y neuroblastoma cells were transfected with the pF1 and pF5 constructs (Figure 7). In 293 HEK cells both constructs increased expression, as they did in Y-79 cells. However, in SY5Y cells, pF5 increased expression over control levels whereas pF1 did not.

Potential combined effect of the PDEβ promoter and 3'UTR

The basal promoter of the PDEβ gene (−93/+53) has previously been characterized and shown to produce high levels of rod-specific PDEβ expression both in Y-79 cells and in the developing retinas of *Xenopus* embryo heads maintained *ex vivo*[9]. To determine if there is a combined effect of the PDEβ promoter and the 3'UTR on luciferase expression, constructs depicted in Figure 8 were generated with this promoter cloned upstream of the luciferase reporter gene and the SV40 3'UTR (construct pβ-3'SV40-SV40), fragments of the PDEβ 3'UTR (F1, construct pβ-F1-SV40; F5, construct pβ-F5-SV40), or the full-length PDEβ 3'UTR (construct pβ-3'β-PDEβ). When transiently transfected into dissected *Xenopus* embryo heads, construct pβ-3'β-PDEβ produced much lower luciferase activity than the other constructs, and ~ 1/10 the activity generated by the control pβ-3'SV40-SV40. This activity is similar to that obtained by transfecting Y-79 cells with the SV40 promoter-containing p689 (Figure 2). In contrast, both pβ-F1-SV40 and pβ-F5-SV40 showed an ~ 3-fold increase in expression of luciferase compared to pβ-3'SV40-SV40, similar to the results obtained by transfecting Y-79 cells with pF1-SV40 and pF5-SV40 which have the SV40 promoter (Figure 6). Therefore, the data from the *Xenopus* transfections suggest that there is no combined effect of the PDEβ promoter and 3'UTR that would significantly affect PDEβ gene expression.

An 11 nt element in F5 is responsible for the increase in reporter gene expression

Sequence analysis demonstrated that an 11 nt sequence (EURE) in F5 is highly conserved across mouse, dog, cow, and human PDEβ 3'UTR sequences (Figure 9A). We have found that EURE is present in the 3'UTR of several genes, including ADP-ribosylation-like factor 6 interacting protein 5, ubiquitin conjugating enzyme E2D1, ethanolamine kinase transcript variant 1. Insertion of EURE between the luciferase cDNA and the SV40 3'UTR of pGL3 led to ~ 2-fold higher luciferase activity in Y-79 cells than that obtained with the pGL3-Promoter vector (Figure 9B). This suggests that the increase in luciferase expression following transfection of *Xenopus* heads with the F5 construct (Figure 8) is due to the presence of the EURE sequence in that fragment of the PDEβ 3'UTR.

DISCUSSION

It is currently accepted that the 3'UTR of mRNA is important in post-transcriptional events[18-21] and in translational control[22-23]. However, while many transcription enhancers and repressors have been already identified, few sequence elements have been described in the 3'UTR controlling gene expression. With an increasing interest in 3'UTRs[24], tools to assay function of such regions are becoming very important.

We worked out a systematic approach that led to the discovery of at least two enhancers and one repressor in the 689 nt 3'UTR of the human PDEβ mRNA. To determine the location of these sequences we created seven ~ 100 nt segments of the PDEβ 3'UTR that were introduced into reporter gene vectors, and then tested each one of them in Y79 cells for its effect on luciferase activity. Since the length of the 3'UTR has been reported to have an effect on gene expression[35], we were careful to keep a similar number of nucleotides in our fragment constructs. We showed that two of these constructs containing F1 and F5 increased the luciferase reporter gene activity, while none of the other five constructs did. Therefore, specific sequences in F1 and F5 must be responsible for the increased gene expression.

When the sequences from human, cow, dog, and mouse PDEβ 3'UTRs were compared, an 11 nt stretch was found to be conserved in all of them. This 11 nt sequence, EURE, is present in F5. Transfection of Y-79 cells with pEURE, this EURE-containing-pGL3 vector doubled the reporter gene activity of pGL3, similar to the upregulation of luciferase expression observed from the pF5 construct (Figure 6). Thus, these 11nt define a novel PDEβ 3'UTR enhancer.

Transfections of DNA constructs into *Xenopus* embryos' heads have been used to characterize both *Xenopus* and human gene promoters (i.e. *Xenopus* rhodopsin[27] and human PDEβ[9]). We corroborated the results of our transfections into Y-79 cells using *ex vivo* transfections into tadpole heads. We found that constructs containing the full-length PDEβ 3'UTR produced a similar decrease in luciferase activity in either *Xenopus* embryo's heads or Y-79 cells, and that constructs with fragments F1 and EURE-containing F5 of the PDEβ 3'UTR increased reporter gene expression in both transfection systems. This implies that similar PDEβ translational mechanisms are present in both Y-79 retinoblastoma cells and *Xenopus* retina.

However, constructs containing F1 or F5 produced different results when transfected into various human cell types. This suggests the presence of two separate modules in the PDEβ 3'UTR that control the increase in gene expression by distinct mechanisms, one of them responding to F1 and the other to F5. While the module responding to F1 is absent or inhibited in neuroblastoma cells, both the F1 and F5 modules are present in Y-79 and 293 HEK cells. Since it has been reported that 3'UTRs regulate gene expression by binding trans-acting factors[20], it is possible that elements in F1 and F5, such as EURE, are involved in these interactions. Moreover, EURE may be rod PDEβ-specific in the retina because in a recent BLAST search we did not find it in any characterized retinal genes.

In transfections of the construct containing the entire 3'UTR of PDEβ mRNA into Y-79, 293 HEK and neuroblastoma cells, as well as in *Xenopus* heads, a substantial decrease in expression of the luciferase reporter gene was observed. However, none of the individual 100 nt fragments of PDEβ 3'UTR produced this effect and neither did the 3'UTRs of other genes (the photoreceptor-specific cone PDEα' and cone arrestin and the ubiquitously expressed SRB7 component of mammalian RNA polymerase II). Further experiments showed that the non-canonical poly-A signal of the PDEβ 3'UTR was responsible for this inhibition of expression: constructs differing exclusively in the poly-A signal produced much less luciferase activity when they had the PDEβ than the SV40 poly-A signal (Figure 5). The mechanism of this down-regulation may involve the binding to the poly-A signal of ubiquitous proteins found in every

cell type, which may inhibit translation. Another possibility is that this sequence is important for mRNA processing. Mutations in the poly-A signal have been reported to disrupt RNA processing[36]. The non-canonical PDEβ poly-A signal could have the same effect.

If the cis-elements of the 3'UTR of PDEβ are important regulators of gene expression, it is conceivable that mutations in these sequences could lead to degeneration of the photoreceptor cells. In addition, mutations in the genes encoding trans-acting factors binding to these elements may also cause retinal degeneration, similar to what is observed with mutated transcription factors such as NRL, CRX and Sp4 bind to photoreceptor-specific promoters[14-17].

In summary, our studies illustrate that the 3'UTR of the PDEβ mRNA is involved in the very complex regulation of this gene's expression in the retina. Moreover, they show that the PDEβ poly-A signal has a dominant inhibitory effect over two other regions in the 3'UTR that stimulate gene expression. However, we think that the involvement of these regions in the regulation of PDEβ expression depends on the levels of transacting proteins that potentially bind those sequences. The level of these factors could vary, for example, between developing and fully differentiated photoreceptors, leading to up- or down- regulation of PDEβ expression.

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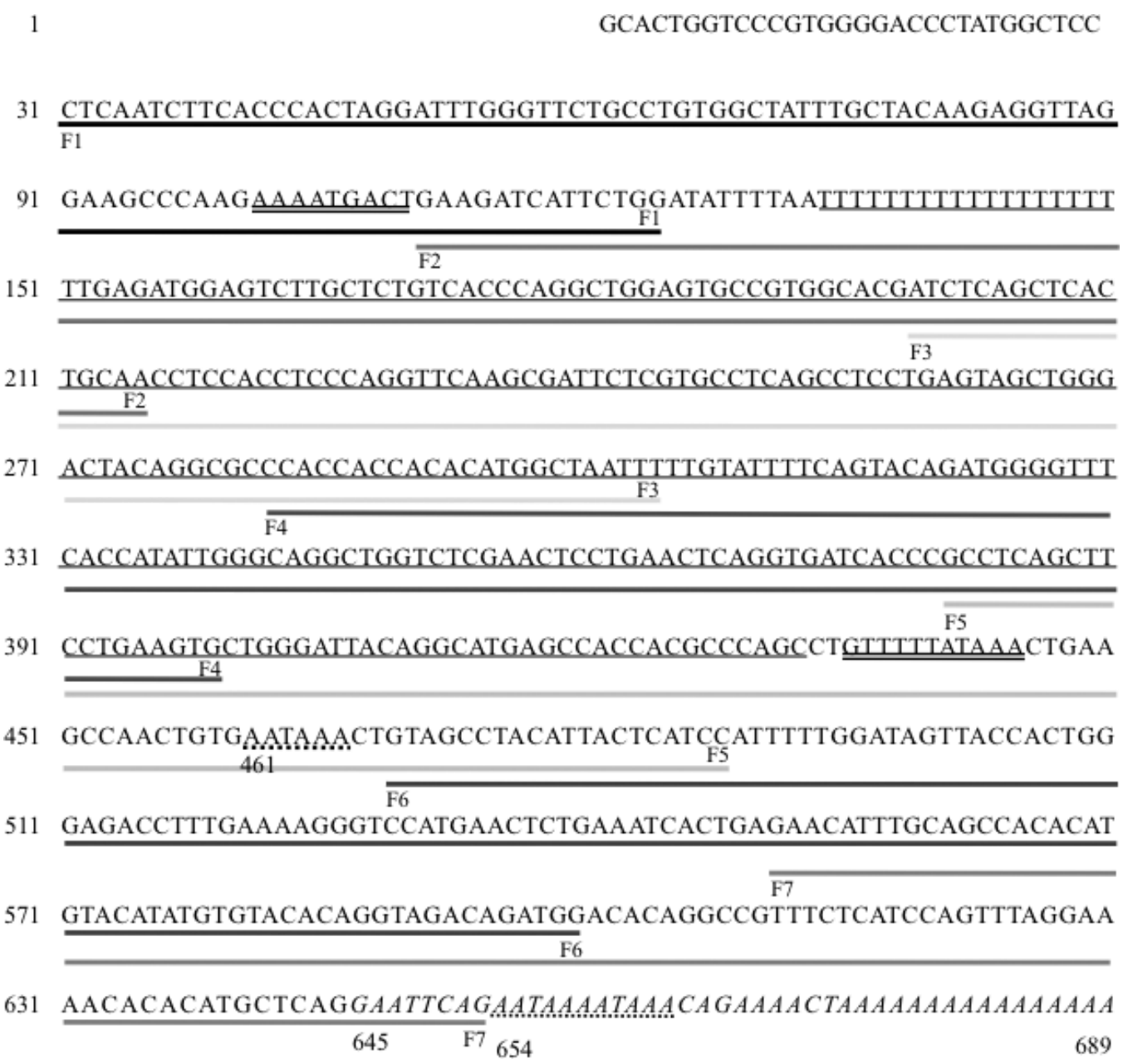

Figure 1. Sequence of the PDEβ 3'UTR determined after 3'RACE

The sequence shows the terminal 44 nt that we have obtained after 3'RACE which complete the previously described PDEβ 3'UTR. The previously reported alu-element is underlined. Two regions of high homology between mouse, dog, bovine, and human are doubly underlined. A potential poly-A signal is dashed underlined, as is the poly-A signal closest to the poly-A tail. The beginning and ending nucleotides of fragments F1 through F7, used in luciferase activity studies, are labeled and lines of different shades of gray demark their sequences.

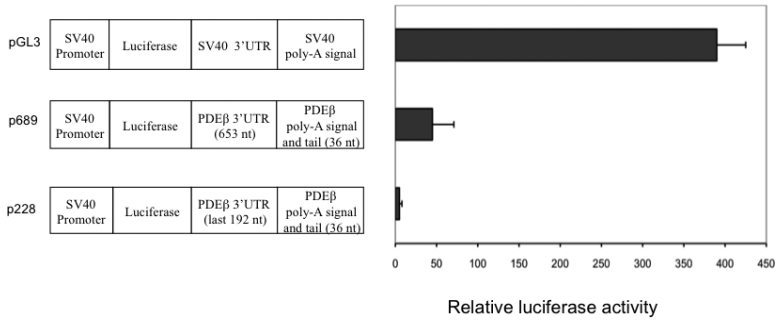

Figure 2. Both constructs with the full-length (689 nt) or terminal sequence (228 nt) of the PDEβ 3'UTR significantly reduce the expression of the luciferase reporter gene produced by the pGL3 Promoter vector

Luciferase activity was measured in lysates of Y-79 cells transfected with pGL3 (containing the SV40 3'UTR), p689 (containing the PDEβ full-length 3'UTR: 653 nt plus 36 nt poly-A signal and poly-A tail) or p228 (containing the last 228 nt of the PDEβ 3'UTR; 192 nt plus 36 nt poly-A signal and poly-A tail) and normalized to the corresponding β-galactosidase activity for each sample. Each transfection was done in triplicate and repeated 3−5 times. Results, expressed as relative luciferase activity, represent the mean normalized luciferase activity measured for each sample ± S.D. These results are statistically highly significant. Two-tailed p values are: pGL3 vs p689: $p < 0.0005$; pGL3 vs p228: $p < 0.0001$.

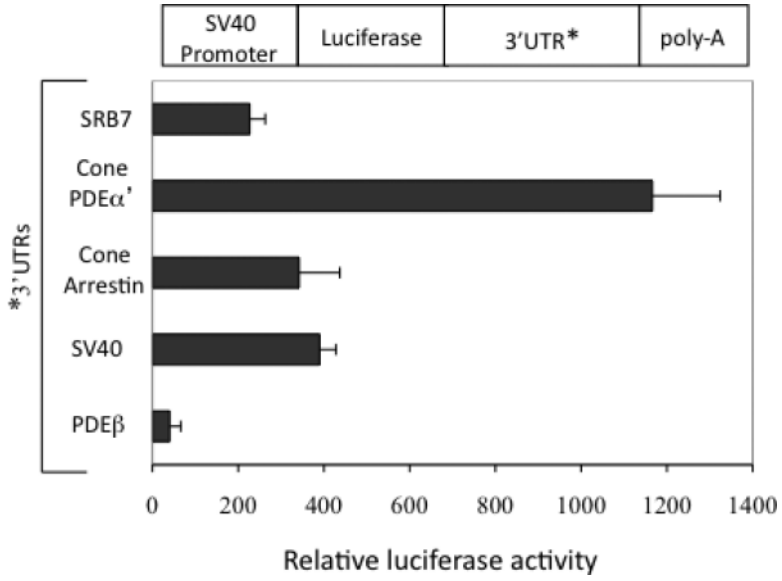

Figure 3. Comparison of the relative luciferase activity produced by constructs containing the PDEβ 3'UTR or other genes' 3'UTRs

Transfections with pGL3 constructs that had the SV40 3'UTR substituted by the 3'UTR of the SRB7 component of mammalian RNA polymerase II, cone arrestin or PDEα' were carried out in triplicate in Y79 cells and were repeated 3 times. The results represent the mean relative luciferase activity measured for each sample ± S.D. The full-length PDEβ 3'UTR shows down-regulation of luciferase expression when compared to the 3'UTR of any of the other genes studied.

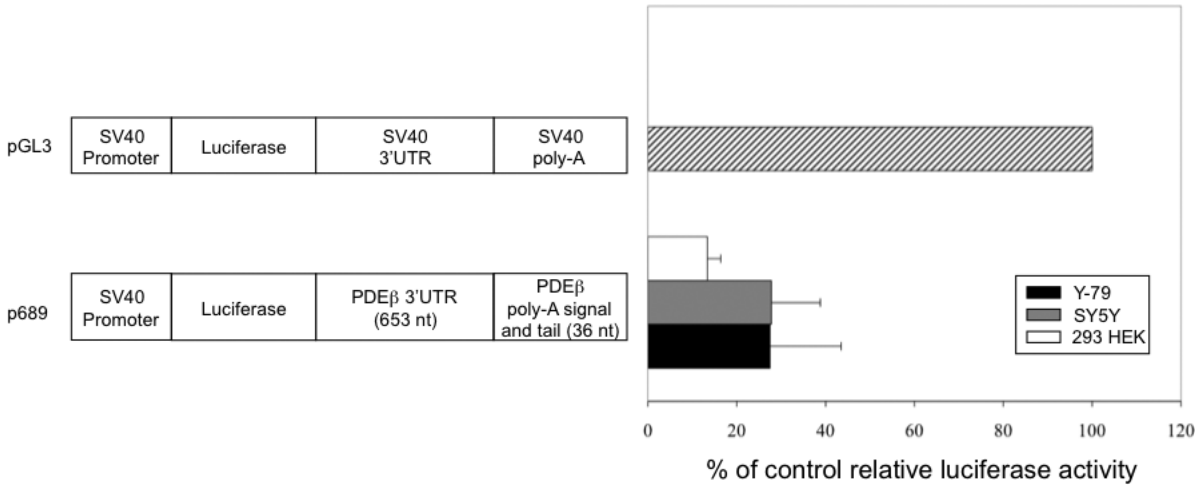

Figure 4. Comparison of the effect of PDEβ 3'UTR on reporter gene expression in different cell lines

The pGL3 Promoter vector used as control was transfected into Y-79, SY5Y (neuroblastoma) and 293 HEK (human embryonic kidney) cell lines and the luciferase activity obtained was considered 100% for each cell line (striped bar). Construct p689 was transfected in the same cell lines as pGL3 in triplicate and the experiment was repeated 3 times. The relative luciferase activity generated by p689 in Y-79 (black bar), SY5Y (grey bar) and 293 HEK cells (white bar) is expressed as percent of the control activity and demonstrates that the PDEβ 3'UTR reduces gene expression in these three cell lines. Error bars are S.D.

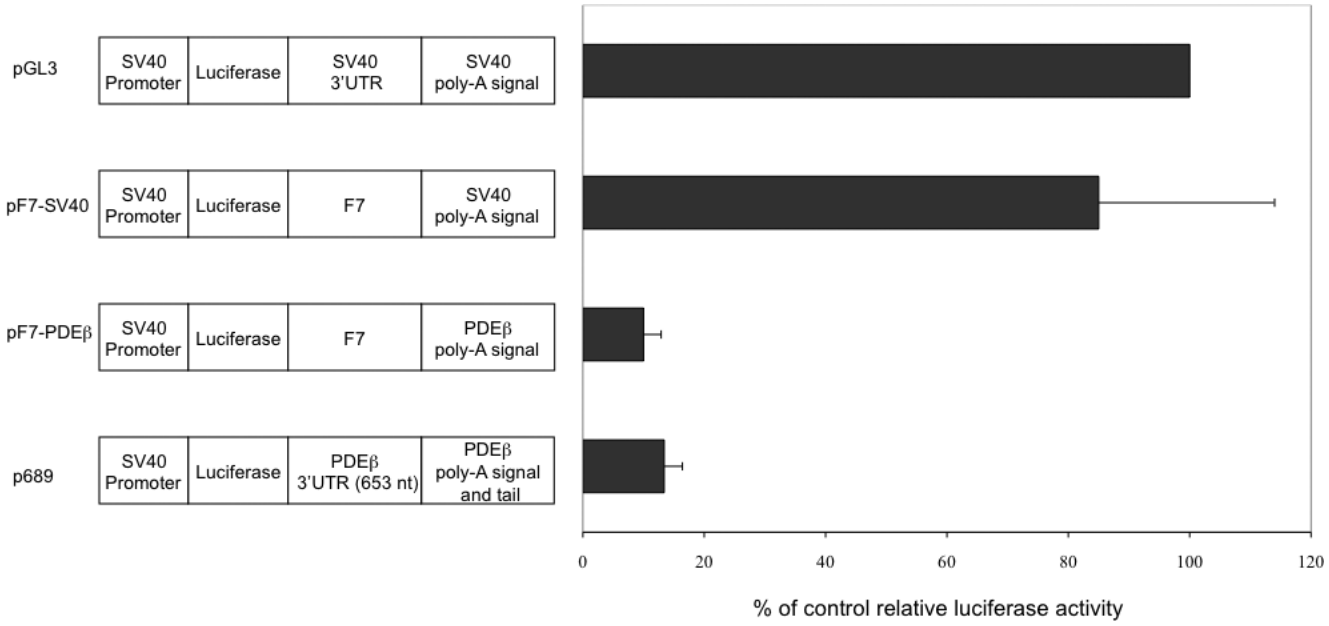

Figure 5. Effect of the PDEβ 3'UTR poly-A signal on gene expression

Relative luciferase activity generated by the control pGL3 Promoter construct was compared with that produced by constructs p689, pF7-SV40 and pF7-PDEβ (the latter two having F7 and the SV40 or PDEβ poly-A signal, respectively). Transfections in Y-79 cells were carried out in triplicate and repeated 3−5 times. The results, expressed as the mean percent of control relative luciferase activity ± S.D., show that the PDEβ 3'UTR poly-A signal reduces by ~ 85 −90% the relative luciferase activity produced by constructs that have the SV40 poly-A signal.

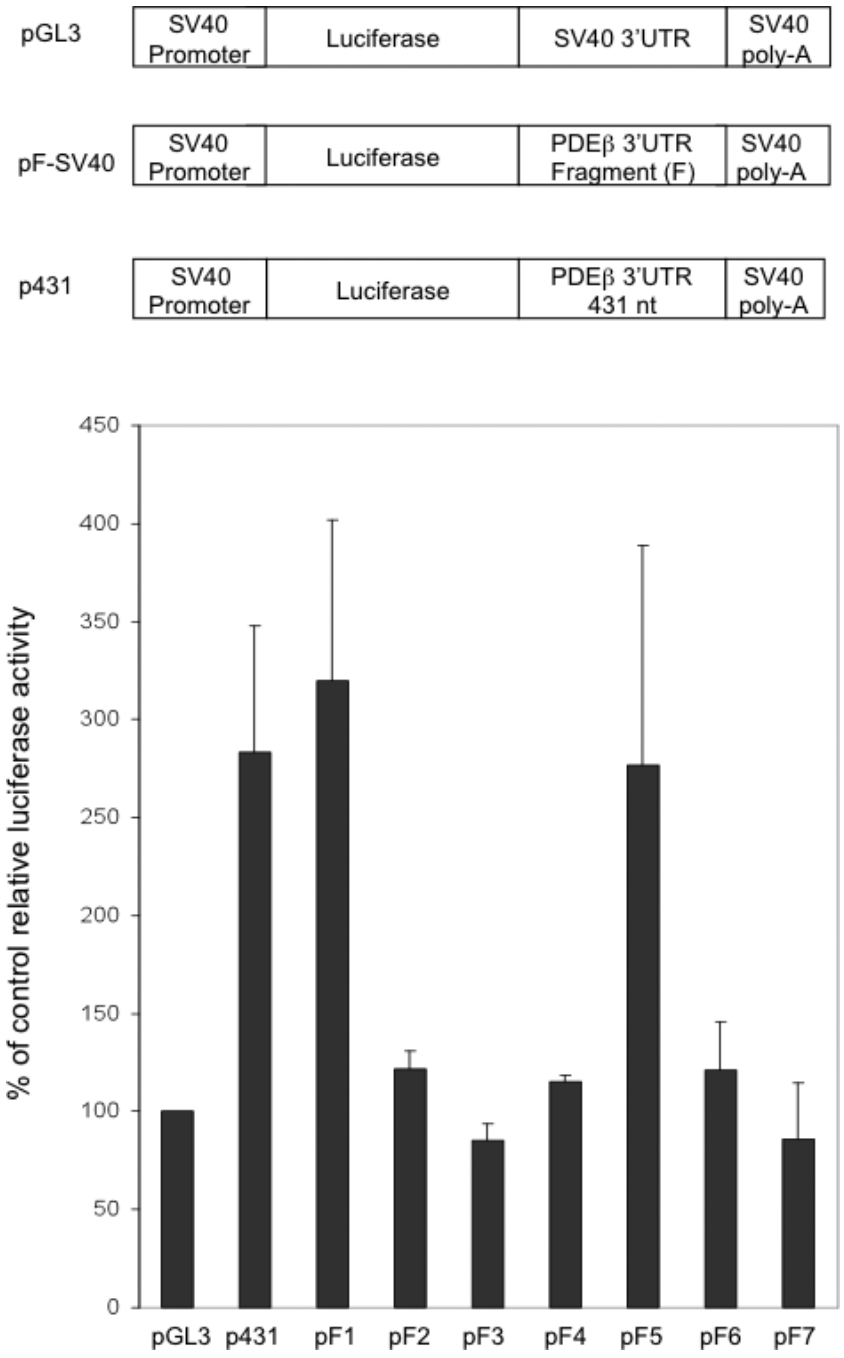

Figure 6. Effect of different segments of PDEβ 3'UTR on reporter gene expression

Fragments of PDEβ 3'UTR were cloned between the luciferase cDNA and the SV40 poly-A signal and each construct was transfected in triplicate into Y-79 cells. This experiment was repeated 3−5 times. Relative luciferase activity is shown as percent of control ± S.D. (the pGL3 Promoter vector with the SV40 3'UTR is used as 100%). Three constructs (pF1, pF5 and p431) increased reporter gene expression.

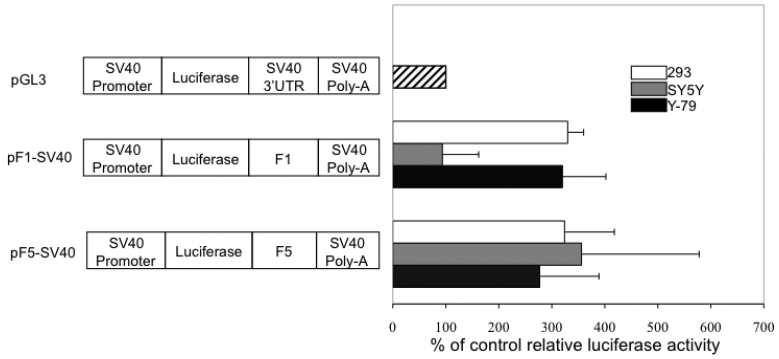

Figure 7. Comparison of the effect of F1 and F5 on reporter gene expression in different cell lines

The pGL3 Promoter vector used as control was transfected in Y-79, SY5Y and 293 HEK cells and the luciferase activity obtained was considered 100% for each cell line (striped bar). Constructs pF1 and pF5 were transfected in triplicate in the same cell lines and transfections were repeated 3 times. The relative luciferase activity generated by these in Y-79 (black bars), SY5Y (grey bars) and 293 HEK (white bars) cells by pF1 and pF5 is expressed as percent of control activity and shows that F1 increases gene expression only in Y79 and 293 HEK cells whereas F5 does increase expression in the three cells lines studied. Error bars are S.D.

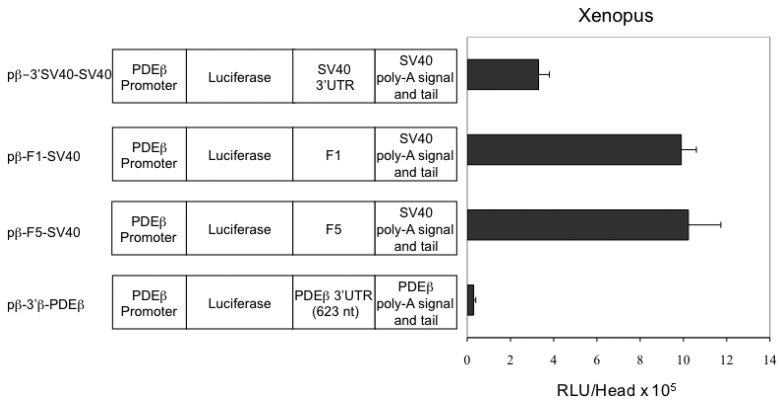

Figure 8. The PDEβ promoter does not modify the effect of the PDEβ 3'UTR or its fragments on reporter gene expression

The SV40 promoter in pGL3 and the pF1-SV40 and pF5-SV40 constructs of Figure 7 was replaced by the PDEβ promoter resulting in constructs pβ-3'UTR-SV40, pβ-F1-SV40 and pβ-F5-SV40. After transient transfection into *Xenopus* embryo heads (see Methods), the luciferase activity generated by pβ-F1-SV40 and pβ-F5-SV40, almost tripled that of the control pβ-3'UTR-SV40. Replacement of the SV40 3'UTR and poly-A signal of pβ-3'SV40-SV40 with the PDEβ full-length 3'UTR (construct pβ-3'β-PDEβ) decreased by 90% the relative luciferase activity of pβ-3'UTR-SV40. The results are expressed as relative luciferase (RLU) activity/head × 10^5, and are statistically highly significant. Two-tailed p values are: pβ-3'SV40-SV40 vs. pβ-3'β-PDEβ: p≡ 0.0005; pβ-3'SV40-SV40 vs pβ-F1-SV40: p=0.0002; pβ-3'SV40-SV40 vs pβ-F5-SV40: p=0.0016.

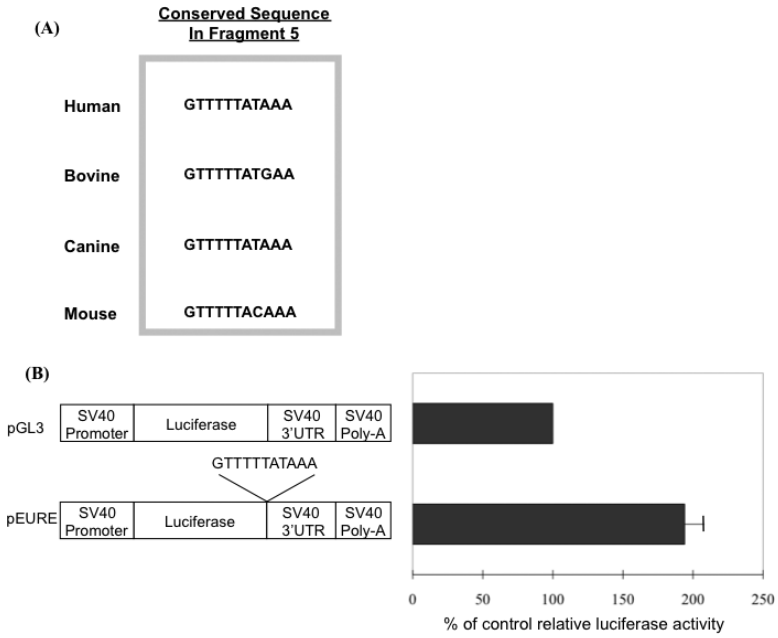

Figure 9. Insertion of the 11 nt EURE element found in F5 of PDEβ 3'UTR into the pGL3 Promoter vector doubles its reporter gene expression in Y-79 cells

(A) Homologous sequence found in Fragment 5 of the PDEβ 3'UTR of several species. (B) Transfections of pGL3 and pEURE in Y79 cells were carried out in triplicate and this experiment was repeated several times. The relative luciferase activity generated by the control pGL3 construct is considered 100%. Results are shown as percent of control relative luciferase activity ±S.D.

Table 1

Primers used for generating the full-length PDEβ 3'UTR or its fragments and for the amplification of other genes' 3'UTRs.

PRIMER	SEQUENCE	
a	5'	GCACTGGTCCCGTGGGGACCCTAT
b	5'	AATAAACTGTAGCCTACATTAC
c	5'	GCGCTCGAGCTCAATCTTCACCCACTAGGA
d	3'	GCGGATCCTCACAGTTGGCTTCAGTTTA
Human Cone Arrestin	5'	GCGCTCGAGGGAGCTGAGCACCTCGCTCTG
	3'	GCGGGATCCCACATCTGAACAAACTGATTTATTAG
Human Cone PDEα'	5'	CCGCTCGAGTATTATCTAACTGGTCTAACTGGTCTAAACTTC
	3'	CCGGATCCCAGGATTGCATGATTTTTT
Human SRB7	5'	CCGCTCGAGCCAGACTCATAGCATCAGTGG
	3'	CCGGATCCCATATGTTTCCTTATATTATGTTC
1	5'	ATCTAGACTCAATCTTCACCCACTAGG
2	3'	CGGATCCCAGAATGATCTTCAGTC
3	5'	GCTCTAGAGAAGATCATTCTGGATAT
4	3'	TAGGATCCTTGCAGTGAGCTGAGATC
5	5'	GATCTAGAATCTCAGCTCACTGCAACC
6	3'	CAGGATCCAAATTAGCCATGTGTGGTG
7	5'	ATCTAGACCACCACCACACATGGCTAA
8	3'	ATGGATCCCACTTCAGGAAGCTGAGGC
9	5'	CATCTAGAGCCTCAGCTTCCTGAAGTG
10	3'	CAGGATCCGGATGAGTAATGTAGGCTAC
11	5'	CTCTAGAGTAGCCTACATTACTCATCC
12	3'	TGGATCCCCCATCTGTCTACCTGTGTAC
13	5'	CTCTAGAGAACATTTGCAGCCACAC
14	3'	TGGATCCCTGAATTCCTGAGCATGT
15	3'	CGTCGACCTGTTTATTTTATTCTG
16	5'	GCTCTAGAGTTTTTATAAACGCTTCGAGCAGACATGATAA
17	3'	GCGTCGACTTTGTAGAGGTTTTACTTGCT