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Role of Ets2 in cyclic AMP regulation of the human chorionic gonadotropin β promoter

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Abstract

Activation of the human chorionic gonadotropin β (hCGβ) by the protein kinase A (PKA) pathway has been shown to occur through an activating protein-2 (AP-2)-dependent mechanism. However, in HepG2 cells, which are deficient in AP-2, the PKA catalytic subunit is still able to stimulate the hCGβ promoter. Ets2 plays a critical role in placental development as revealed by placental abnormalities in Ets2 knockout mice. Transfection of Ets2 into JEG-3 placental cells causes a slight, but reproducible, increase in hCGβ promoter basal activity. However, cotransfection with the PKA catalytic subunit causes a strong synergistic increase in hCGβ promoter activity. Ets2 synergistic activation of the hCGβ promoter is specific for the PKA pathway, as activation of the ras pathway, which also acts through Ets2, does not activate the hCGβ promoter. c-Jun-mediated repression of hCGβ is inhibited by Ets2 cotransfection, indicating that protein-protein interactions may be responsible for Ets2 activation of the hCGβ promoter. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Humanchorionic gonadotropin β (hCGβ promoter); Ets2; Protein kinase A (PKA)

1. Introduction

Protein kinase-A (PKA) activation of the human chorionic gonadotropin β (hCGβ) promoter has been shown to occur through an AP-2 (activating protein-2) dependent mechanism (Johnson et al., 1997). However, mutation of the three proposed AP-2 binding sites within the PKA-responsive region of hCGβ reduces, but does not completely eliminate, PKA activation (Johnson and Jameson, 1999). Also, the PKA catalytic subunit still activates the hCGβ promoter in HepG2 cells, which are deficient in AP-2 protein (Williams et al., 1988). These results suggest that factors other than AP-2 may be involved in the stimulation of hCGβ by PKA.

We considered other transcription factors involved in placental development as candidate regulators of the hCGβ promoter. One of these factors, Ets2, causes a deficiency in placental cell proliferation when deleted in the mouse genome (Yamamoto et al., 1998). In situ hybridization experiments in mouse embryos indicated that Ets2 mRNA was highly expressed in trophoblastic tissues, namely the ectoderm and the trophectoderm (Yamamoto et al., 1998). These data indicated that Ets2 plays a role in the development and differentiation of trophoblast tissue.

Ets2 belongs to the Ets family of transcription factors, which is characterized by a common DNA binding domain (winged helix–turn–helix) (Wasylyk et al., 1998). This common DNA binding domain binds to the core sequence, GGAA, within an Ets response element. The pointed Ets subfamily (Ets1, Ets2 and Pointed P2) are highly homologous (Wasylyk et al., 1997). Ets1 and Ets2 share extensive homology in their activation and DNA-binding domains. Both Ets1 and Ets2 are nuclear phosphoproteins that are activated by the ras/raf/MAPK pathway, which phosphorylates a conserved threonine residue within the amino terminus (Ets1 T38, Ets2 T72) (Yang et al., 1996).

In humans, Ets2 is located on chromosome 21. The Ets2 locus is duplicated in Down syndrome (trisomy 21) patients in whom there is only a partial duplication

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of chromosome 21 (Rahmani et al., 1990). Additionally, mice that overexpress Ets2 mimic many of the developmental defects of human Down syndrome patients (Sumarsono et al., 1996). High levels of hCG are used as a pre-term marker for increased risk of Down syndrome (Eldar-Geva et al., 1995). The specific increase in hCGβ subunit expression in Down syndrome, the Ets2 knockout mice and their defects in extraembryonic tissue prompted a closer examination of Ets2 transcriptional activities on hCG expression.

Transient transfections indicate that Ets2 stimulates basal hCGβ expression and contributes to the PKA activation of hCGβ. PKA-Ets2 synergistic activation is specific for hCGβ since the human α-subunit promoter is not affected by PKA and Ets2 cotransfection.

2. Materials and methods

2.1. Transient transfection reactions

Transient transfections were carried out in 12-well plates for JEG-3 cells and HepG2 cells by the calcium phosphate method (Graham and Eb, 1973). Typical reactions for JEG-3 and HepG2 cells consisted of 500 ng of reporter plasmid added to 150 μl of HBS (21 mM Hepes [pH 7.8], 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2PO_4, 6 mM dextrose: final pH 7.05) with 5 μl of 2 M $CaCl_2$ added drop-wise while vortexing. Expression vectors (20 ng) were included when appropriate (all amounts are per well of cells.)

After mixing, the $CaCl_2$-HBS-DNA mixtures were incubated at room temperature for 20 min. After room temperature incubation, the $CaCl_2$-HBS-DNA mixture was added directly to the medium in the wells covering the JEG-3 cells. Alternatively, in HepG2 cells, the medium was removed from the cells and the $CaCl_2$-HBS-DNA mixture was added directly onto the cells; after 20 min at room temperature new medium was added back to the wells.

Both JEG-3 and HepG2 cells were then incubated at 37°C for 4 to 6 h after addition of the $CaCl_2$–HBS–DNA mixture. Following incubation the medium was removed, the cells were washed in PBS and new growth medium was added. When specified, growth factors were added 18–24 h after the transfection. Transient transfections used 8-bromoadenosine 3′:5′-cyclic monophosphate (8Br-cAMP) (Sigma, St Louis MO) when specified. Transfected cells were assayed for luciferase activity 18–24 h after refeeding or after 8Br-cAMP addition (Nordeen, 1988).

2.2. Luciferase assay conditions

Medium was removed from the cells and the cells were lysed in GME (25 mM glycylglycine [pH 7.8], 15 mM $MgSO_4$, 4 mM EDTA), 1% Triton X-100, 1 mM DTT). Cell lysate was added to 364 μl of GME mix (300 μl GME, 60 μl 100 mM K_2HPO_4, 0.4 μl 1 M DTT and 4 μl 200 mM ATP). The mixture was assayed with an automatic luminometer (Autolumat LB953 CG + G Berthold Wallace, Gathersburg, MD), which injects 100 μl luciferin solution (4 ml GME, 1 ml of 1 mM luciferin and 10 mM DTT) into each reaction tube. All transient transfections were performed in multiples of three or six wells, with the relative light units being averaged for the triplicate transfections. Error bars represent standard error of the means.

2.3. Reporter genes and plasmid constructions

Reporter plasmids were constructed with established protocols (Sambrook et al., 1989; Ausubel, 1992). CGβ and α-subunit luciferase constructs have been reported elsewhere (Albanese et al., 1991; Pestell et al., 1994). Site-directed mutations were introduced according to previously published work (Johnson et al., 1997) and were sequenced to verify the inserted mutations.

2.4. Expression vectors

cDNAs for AP-2, Ets2 and Ets2-DBD were all transferred into the expression vector pcDNA3.1 (Stratagene). E1A12s and c-Jun expression vectors have been described elsewhere (Pestell et al., 1994, 1996). All plasmids were purified using Qiagen columns.

2.5. Tissue culture conditions

JEG-3 cells (American Type Culture Collection, HTB-36) were maintained in DMEM (Sigma) supplemented with 10% fetal bovine serum (FBS) (Sigma) and 1% penicillin-streptomycin (Gibco BRL Gathersburg, MD). HepG2 cells (American Type Culture Collection, HB 8065) were maintained in MEM (Sigma) supplemented with 10% FBS (Sigma), 1% penicillin-streptomycin (Gibco BRL) and 0.5 mM sodium pyruvate (Gibco BRL). JEG-3 and HepG2 cells were grown at 37°C in a 5% CO_2 incubator.

3. Results

3.1. PKA activation of the hCGβ promoter requires factors other than AP-2

An hCGβ reporter construct in which all three AP-2 binding sites had been mutated was still partially responsive to PKA induction in JEG-3 cells (Johnson and Jameson, 1999). This construct (-AP2) was also stimulated by cotransfection with the PKA catalytic subunit in HepG2 cells, which are deficient in endogenous AP2

(Williams et al., 1988) (Fig. 1). Expression of the full-length hCGβ reporter construct was increased 8-fold by the PKA catalytic subunit and 9-fold when transfected with an AP-2 expression vector. Cotransfection of the PKA catalytic subunit and AP-2 induced a greater than additive effect on hCGβ expression (27-fold). The mutant construct shows a similar response to the PKA catalytic subunit (7.8-fold), but reduced response to AP-2. Activity after cotransfection of PKA and AP-2 was not significantly different than activity with the PKA catalytic subunit alone (3-fold). The residual effect of AP-2 on the mutant constuct (-AP2) may be due to an interaction between AP-2 and Sp1 (Pena et al., 1999). The PKA induction of the hCGβ promoter in HepG2 cells was unexpected, suggesting that PKA activation of hCGβ promoter requires factors other than AP-2.

3.2. *Ets2 activates the hCGβ promoter in a PKA dependent pathway*

The Ets2 transcription factor was transiently transfected with the hCGβ (−345– +114) reporter construct into JEG-3 placental choriocarcinoma cells. Ets2 alone caused a small (4-fold), but reproducible stimulation of the hCGβ reporter construct (Fig. 2). The PKA catalytic subunit alone stimulated hCGβ expression 19-fold, whereas cotransfection of PKA catalytic subunit with the Ets2 expression vector stimulated hCGβ expression 106-fold.

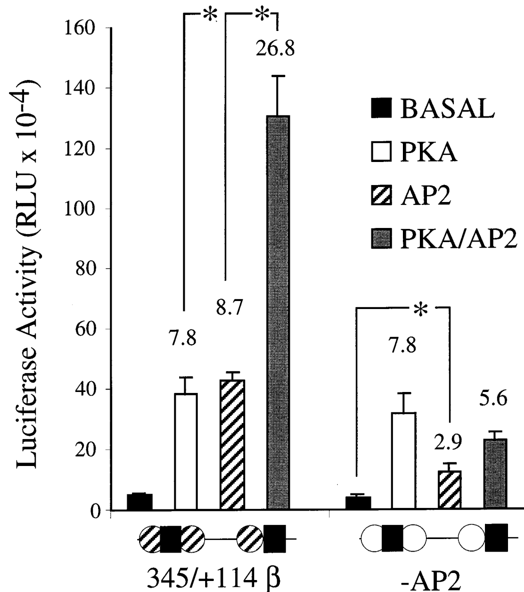


Fig. 1. PKA activation of the hCGβ promoter in HepG2 cells. HepG2 cells were transiently cotransfected with 500 ng of reporter constructs containing wild type hCGβ promoter (−345/+114) or the hCGβ promoter in which the three known AP-2 binding sites had been mutated (-AP2), along with 20 ng of expression vectors for the PKA catalytic subunit (PKA) and/or AP-2. Reporter constructs are shown graphically with AP-2 binding sites as hatched circles, Sp1 binding sites as filled squares, and open circles indicating AP-2 binding sites no longer able to bind proteins. Numbers above each bar represent fold activation over basal activity. Results represent mean ± SE of three individual transfections. Asterisk indicates a significant difference (ANOVA $P < 0.05$) from indicated bars.

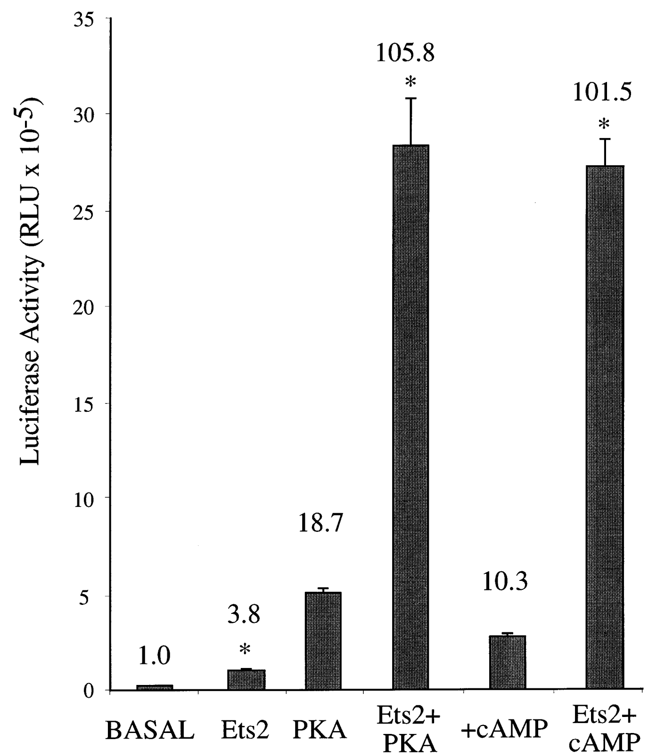


Fig. 2. Ets2 activation of the hCGβ promoter. JEG-3 cells were transiently cotransfected with 500 ng of hCGβ −345/+114 reporter construct and 20 ng of expression vectors for Ets2 and/or the PKA catalytic subunit (PKA). Luciferase assays were performed 48 h post-transfection to allow for 24 h 8Br-cAMP (cAMP) treatment of transiently transfected JEG-3 cells. The numbers above each bar represent fold activation over basal activity. Results represent mean standard error of three individual transfections. Asterisks indicate a significant difference (ANOVA $P < 0.05$) from Basal, PKA and cAMP controls as compared to those with addition of the Ets2 expression vector.

JEG-3 transient cotransfections were performed with minimal amounts (20 ng) of expression vectors. A possible explanation for the synergistic activation of hCGβ may be an interaction between the Ets2 transcription factor and the PKA catalytic subunit expression vector, rather than a direct interaction on the hCGβ promoter. JEG-3 cells transiently transfected with Ets2 expression vectors were therefore treated with 8Br-cAMP to determine whether Ets2 synergistic activation was specific for hCGβ promoter or required cotransfection of the PKA catalytic subunit. JEG-3 cells treated with 0.5 mM 8Br-cAMP for 24 hrs showed a 10-fold increase in hCGβ expression (Fig. 2). Combining 8Br-cAMP treatment and Ets2 transient transfection showed a synergistic effect on hCGβ expression that equaled the effect shown by PKA cata-

lytic subunit and Ets2 (102- vs. 106-fold). The similar effects of 8Br-cAMP and the PKA catalytic subunit on Ets2-mediated activation of the hCGβ promoter indicate that Ets2 functions in a PKA-dependent pathway to activate hCGβ expression.

3.3. Ets2-PKA synergistic activation is specific for the hCGβ subunit promoter

The hCGβ and α-subunit genes are both expressed at high levels during the first trimester. The α-subunit continues to be expressed throughout pregnancy, whereas β-subunit expression declines in the second and third trimesters (Pierce and Parsons, 1981). The PKA-dependent activation of the α-subunit occurs through two tandem CREs within the proximal promoter (Delegeane et al., 1987; Deutsch et al., 1987; Silver et al., 1987) that bind the transcription factor CREB (Hoeffler et al., 1988). Transient transfections in JEG-3 cells indicate that the transcription factor Ets2 and the PKA catalytic subunit synergize to mediate PKA-dependent activation of hCGβ, in contrast to experiments with the α-subunit promoter (−846–+44) which showed that PKA catalytic subunit alone stimulated α-subunit expression 15-fold (Fig. 3A). Cotransfection of Ets2 and the PKA catalytic subunit showed no significant difference in expression as compared to the PKA catalytic subunit alone (16- vs. 15-fold, respectively). Thus, synergistic activation of Ets2 and the PKA catalytic subunit appears to be specific for hCGβ promoter and is not seen with the human α-subunit promoter.

Ets1 was also cotransfected with the PKA catalytic subunit in JEG-3 cells (Fig. 3B). Ets1, like Ets2, shows minimal stimulation of the hCGβ promoter basal activity. Ets1 also synergizes with the PKA catalytic subunit, although to a lesser extent than Ets2.

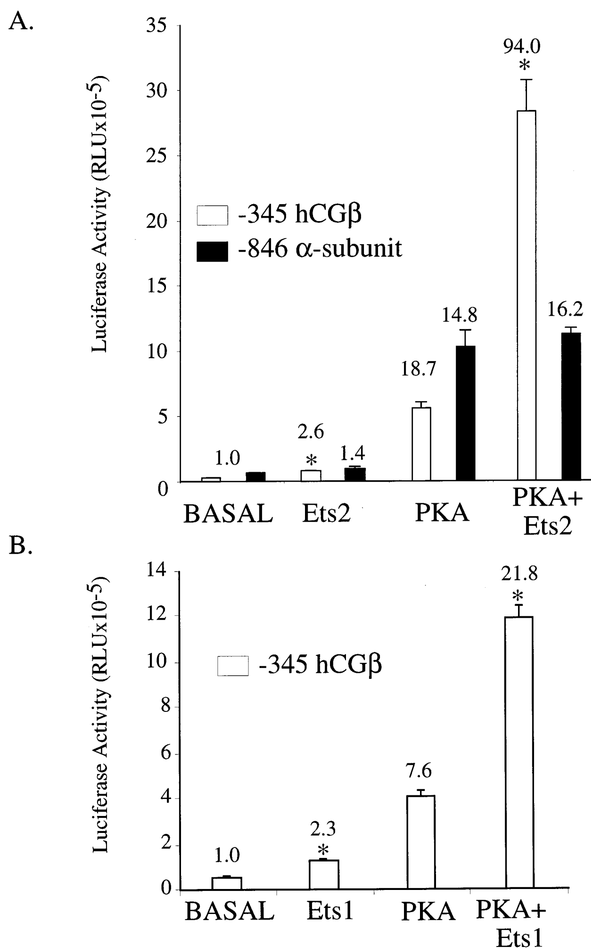


Fig. 3. Ets2 activates the hCGβ but not the α-subunit promoter. A. JEG-3 cells were transiently cotransfected with 500 ng of hCGβ −345/+114 or α-subunit −846–+44 reporter constructs, and 20 ng of expression vectors encoding Ets2 and/or the PKA catalytic subunit (PKA). Asterisk indicates a significant difference (ANOVA $P<0.05$) between Basal and PKA bars as compared to those with the addition of Ets2 expression vector. (B), JEG-3 cells were transiently transfected with 500 ng of hCGβ (−345/+114) reporter construct and 20 ng of the PKA catalytic subunit and/or Ets1 expression vectors. The numbers above each bar represent fold activation over basal activity. Results represent mean ± SE of three individual transfections. Asterisk indicates a significant difference (ANOVA $P<0.05$) between Basal and PKA bars as compared to those with the addition of Ets2 expression vector.

3.4. Ras does not synergize with Ets2 on hCGβ promoter

The Ras pathway activates Ets1 and Ets2. A mutation of Ras, RasL61, causes constitutive stimulation of the Ras/Raf/MAPK pathway and Ets2 activation (Galang et al., 1994; Albanese et al., 1995). The constitutively active RasL61 mutant was cotransfected with Ets2 expression vectors in HepG2 cells to test whether Ets2 activation of the hCGβ promoter was specific for the PKA pathway.

The PKA catalytic subunit stimulated the hCGβ (−345–+114) reporter construct 6-fold in HepG2 cells (Fig. 4). Ets2 alone stimulated hCGβ expression (5-fold), but when cotransfected with the PKA catalytic subunit, it had a synergistic effect (121-fold). Interestingly, RasL61 alone activates the hCGβ promoter 11-fold in HepG2 cells. Cotransfection of RasL61 with the Ets2 expression vector did not further stimulate the hCGβ promoter. Thus, the synergistic activation of Ets2 was specific for the PKA pathway. AP-2 and Ets2 together, with or without the PKA catalytic subunit, did not show superactivation (data not shown).

3.5. Ets2 domains necessary for hCGβ promoter synergistic activation

An Ets2 construct containing only the Ets2 DNA-binding domain (Ets2-DBD) has been shown to inhibit Ras-mediated activation by acting in a dominant nega-

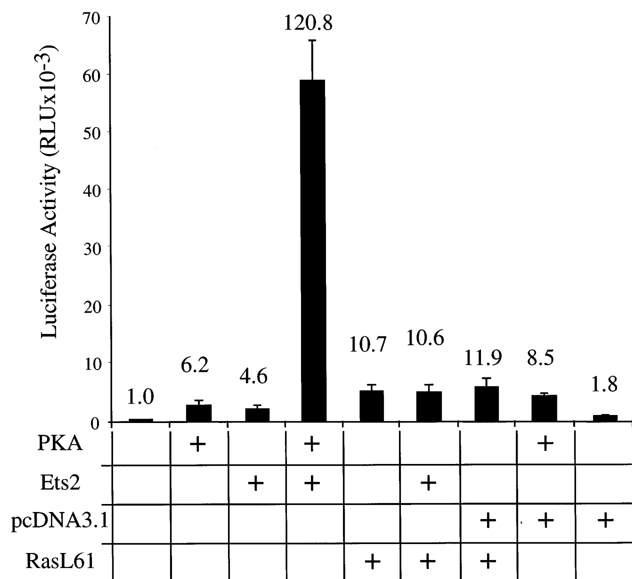


Fig. 4. Ras and PKA activation of Ets2 of the hCGβ promoter. HepG2 cells were transiently cotransfected with 500 ng of hCGβ (−345/+114) reporter constructs and 20 ng of expression vectors for Ets2 and/or the PKA-catalytic subunit (PKA) or RasL61. The numbers above each bar represent fold activation over basal activity for the respective cotransfections. Results represent mean ± SE of three individual transfections. pcDNA3.1 is the empty expression vector used for Ets2.

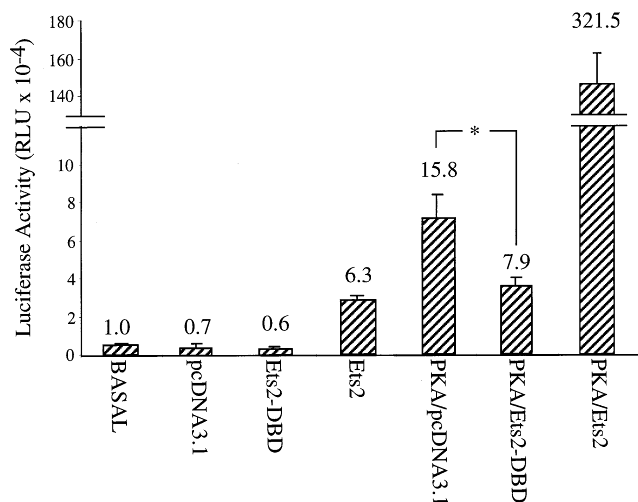


Fig. 5. Effect of Ets2 with and without its transactivation domain. JEG-3 cells were transiently cotransfected with 500 ng of hCGβ (−345/+114) reporter constructs and 20ng of expression vectors for Ets2 or Ets2-DBD and/or the PKA-catalytic subunit (PKA). The numbers above each bar represent fold activation over basal activity for the respective cotranfections. Results represent mean ± SE of three individual transfections. pcDNA3.1 is the empty expression vector used for both Ets2 and Ets2-DBD. Asterisk indicates a significant difference (ANOVA $P < 0.05$) between PKA/pcDNA 3.1 and PKA/Ets-2 DBD. No significant difference was observed between Basal, pcDNA 3.1 or Ets-2 DBD.

tive manner (Roberson et al., 1995; Foos et al., 1998). In contrast to the wild type Ets2 construct, the Ets2-DBD construct did not stimulate hCGβ (−345–+114) reporter expression (Fig. 5). Cotransfection of the PKA catalytic subunit with empty control vector (pcDNA3.1) produced a 16-fold activation of hCGβ reporter construct. Although the combination of Ets2 and PKA strongly stimulated the hCGβ promoter (322-fold over basal activity) in this experiment, cotransfection of Ets2-DBD with the PKA catalytic subunit inhibited PKA-mediated activation by 50% (8-fold stimulation vs. 16-fold). A possible explanation for this repression may involve inhibition of endogenous Ets2 from binding to, and stimulating, the hCGβ promoter. The synergistic activation by Ets2-PKA of the hCGβ promoter therefore requires the pointed transactivation domain of Ets2.

3.6. Localization of Ets2-PKA synergistic activation within the hCGβ promoter

A series of 5′ promoter deletion constructs was used to delineate the Ets2-PKA responsive element within the hCGβ promoter. The 5′ promoter deletion constructs, ranging from −315 to −87, each showed Ets2-PKA synergistic activation. There was a considerable range of inducibility (60- to 180-fold) (Fig. 6A) with a dramatic change in fold activation between the −248 and −187 constructs (see below). Each of the 5′ promoter deletion constructs contained 114 bp of 5′ UTR. Examination of the hCGβ promoter region between −87 and +114, the shortest hCGβ promoter tested, revealed two potential Ets binding sites (GGAA) within this minimal promoter region.

One putative Ets2 binding site was between −40 and −26 bp within the proximal promoter. The other was within the 5′ UTR between +65 and +79 bp, although in the opposite orientation with respect to the 5′ site (Fig. 6B). Additional 5′ deletions of the minimal promoter to −40 retained synergistic activation between Ets2 and the PKA catalytic subunit (Fig. 6C). However, deletion from −40 to −28 with concomitant removal of the putative 5′ Ets2 binding site reduced the synergistic activation by half (36- vs. 14-fold). Constructs starting at −28 bp and containing either +4, +70 or +114 bp of 5′ UTR did not show a significant difference in Ets2-PKA synergistic activity, indicating that the downstream putative Ets2 binding site alone is not sufficent to mediate the synergistic activation of the hCGβ promoter (data not shown).

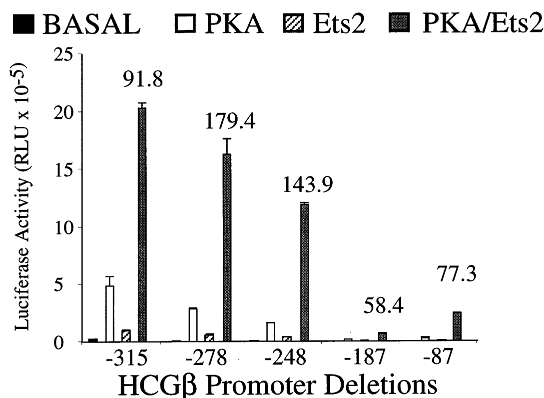
3.7. Jun-mediated repression of hCGβ expression is Ets2 dependent

c-Jun represses hCGβ expression, and this inhibition is localized between −250 and −200 bp in the hCGβ promoter (Pestell et al., 1994). Ets-2 interacts with the AP-1 complex through c-Jun, as determined by two-hy-

brid analysis (Basuyaux et al., 1997). Experiments were designed to determine if c-Jun repression of hCGβ was affected by Ets2. The adenovirus protein E1A12s, another repressor of hCGβ expression (unpublished data), was also used.

E1A12s and c-Jun each repress hCGβ PKA-stimulated expression in JEG-3 cells. The promoter activity was repressed 60 and 50% by Jun and E1A12s, respectively (Fig. 7). JEG-3 cells were cotransfected with the PKA catalytic subunit, Ets2, and either c-Jun or E1A12s, along with the hCGβ (− 345– + 114) reporter construct. E1A12s repressed Ets2-PKA synergistic activation of the hCGβ promoter (70% repression). In contrast, c-Jun did not repress the synergistic activation of Ets2-PKA, suggesting that c-Jun mediated repression can be overcome by increasing the amount of Ets2 present in JEG-3 cells. Western blot analysis of JEG-3 cells shows little native Ets2 protein in comparison to HeLa nuclear extracts or term human placental tissue (data not shown).

A.



B.

C.

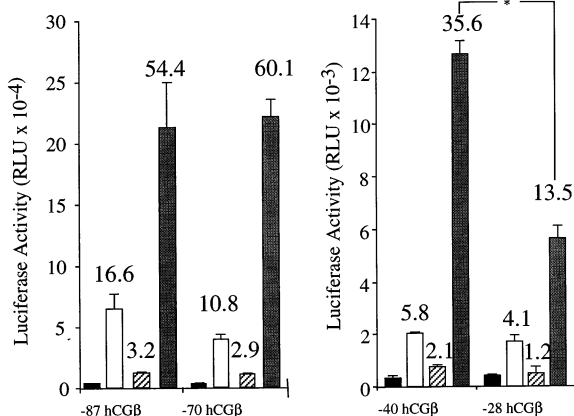


Fig. 6. Function of Ets2 and PKA using a minimal hCGβ promoter. A. JEG-3 cells were transiently cotransfected with 500 ng of hCGβ deletion reporter constructs and 20 ng of expression vectors for Ets2 and/or the PKA catalytic subunit (PKA). The numbers above the PKA/Ets-2 bar represent fold activation over basal activity for the respective deletion constructs. B. The two putative Ets2 binding sites within the proximal promoter are indicated. (C), JEG-3 cells were transiently as in panel A. The numbers above each bar represent fold activation over basal activity for the respective deletion constructs. Each of the proximal promoter constructs contains 114 bp of 5′ UTR. Results represent mean ± SE of three individual transfections. Asterisk indicates a significant difference (ANOVA $P < 0.05$) between PKA/Ets2 and the − 40 and − 28 HCGβ promoters. No significant difference was observed between Basal, PKA or Ets-2 for the reporter constructs.

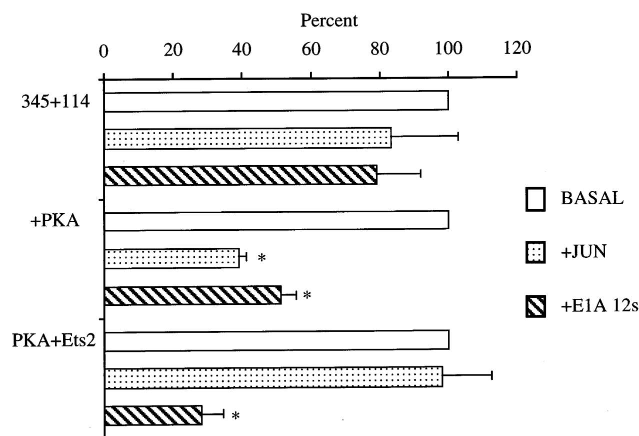


Fig. 7. Ets2 relieves c-Jun-mediated repression of hCGβ. JEG-3 cells were transiently cotransfected with 500ng of hCGβ (− 345/ + 114) reporter constructs and 20 ng of expression vectors for Ets2 and/or the PKA catalytic subunit (PKA) with addition of either 20 ng of c-Jun or E1A12s expression vectors. The basal levels for each group (Reporter alone, Reporter + PKA and Reporter + PKA + Ets2) were set at 100%. Addition of c-Jun or E1A12s expression vectors are presented relative percentages to basal controls. Results represent mean ± SE of three individual transfections. Asterisks indicate a significant difference (ANOVA $P < 0.05$) between controls and addition of c-Jun or E1A12s.

4. Discussion

AP-2 binding sites within the hCGβ promoter are necessary for both basal and PKA activation. Deletion of AP-2 binding sites within JEG-3 cells reduces basal and PKA activation by 80 and 50%, respectively (Johnson et al., 1997; Johnson and Jameson, 1999). cAMP treatment of JEG-3 cells for 24 h induces AP-2 binding to DNA. In HepG2 cells, cotransfection of AP-2 and the PKA catalytic subunit show a greater than additive effect on hCGβ promoter activity. Taken together, these data indicate that AP-2 plays a major role in PKA induction of hCGβ. However, transfection of the PKA catalytic subunit alone in HepG2 cells, which are deficient in AP2, still stimulates the hCGβ promoter. This finding leads to the conclusion that other factors are involved in PKA activation of the hCGβ promoter.

Ets2 alone modestly stimulates hCGβ promoter activity (3–6-fold). On the other hand, cotransfection

experiments with Ets2 and the PKA catalytic subunit in JEG-3 cells result in a remarkable synergistic effect on hCGβ activity. This synergistic activation appears to be specific for the hCGβ promoter. The human α-subunit promoter was not stimulated by Ets2, nor was there synergistic activation by the combination of Ets2 and the PKA catalytic subunit. Despite the high homology between Ets2 and Ets1, the large synergistic activation was specific for Ets2. This is the first reported case of synergistic activation between the PKA pathway and the transcription factor Ets2.

Ets2 responds to the Ras/Raf/MAPK pathway through phosphorylation of a threonine residue in the amino-terminus of the pointed domain (Yang et al., 1996). An activating Ras mutant (RasL61) did not enhance the action of Ets2, indicating that the synergistic activation with Ets2 was selective for the PKA pathway. These experiments in HepG2 cells also indicate that Ets2-PKA synergistic activation is not cell-type dependent, nor is it dependent on AP-2.

Proximal promoter deletions of the hCGβ promoter showed that Ets2 and the PKA catalytic subunit could still activate a minimal promoter spanning −87–+114. Two putative Ets2 binding sites were identified within this region. Deletion constructs indicated that the 5′ Ets2 site might be functionally important. However, mutation of this site in the context of the hCGβ (−345–+4) reporter construct did not reduce Ets2-PKA synergistic activation (data not shown). A number of causes might account for this lack of effect by the Ets2 mutant. First, Ets2 may not bind to this region of the hCGβ promoter. EMSA and supershift assays failed to show Ets2 binding to these sites (data not shown). Second, Ets2 may not need to bind directly to the hCGβ promoter to affect transcription. The Ets family uses other neighboring transcription factors to stabilize their interactions with DNA (Block et al., 1996; Fitzsimmons et al., 1996; Wasylyk et al., 1998). Third, the mutation introduced into the 5′ putative Ets2 site may not fully disrupt Ets2 binding in the presence of other cooperative factors. Lastly, other distal sites within the hCGβ promoter may compensate for mutation of the proximal Ets2 binding site. There is a decrease in synergistic activation (from 144- to 58-fold) when the proximal promoter is deleted between −248 and −187 bp. This suggests that a distal Ets2 interaction site may be present between −248 and −187 of the hCGβ promoter. This site may be necessary for Ets2 binding or its interaction with other proteins; both possibilities are being explored further.

Further evidence to support the possibility that Ets2 may act at more than one site comes from the experiments examining c-Jun repression of hCGβ. Cotransfection of c-Jun into JEG-3 cells represses hCGβ expression through a specific region (−250–−220) within the hCGβ promoter (Pestell et al., 1994). As mentioned above, this region showed a nearly 3-fold reduction in Ets2 synergistic activity when deleted from reporter constructs. Cotransfection of c-Jun and the PKA catalytic subunit reveals that c-Jun represses hCGβ promoter activity by 60%. Inclusion of the Ets2 expression vector blocked c-Jun repression of hCGβ promoter activity, but not repression by E1A12s. Two-hybrid analysis shows that Ets2 can associate with c-Jun (Basuyaux et al., 1997). These data indicate that Ets2 may block c-Jun repression of the hCGβ promoter by directly interacting with c-Jun. We suggest therefore that Ets2-mediated synergistic activation of the hCGβ promoter occurs partially through a mechanism in which it removes or reverses the repressive effects of c-Jun. Other repressors of hCGβ, such as Oct 3, may also play a role in Ets-2 mediated activation.

These findings provide a novel Ets2-dependent pathway for PKA stimulation. Ets2 appears to act independently of AP-2, another factor involved in cAMP induction of the CGβ promoter. Ets2 also appears to counteract c-Jun-mediated repression. Thus, cAMP regulation of this promoter involves a complex interplay of signaling pathways and transcription factors that converge within the first 350 bp of the CGβ promoter.

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