Synergistic effects of nuclear factors – GATA, VBP and ER in potentiating vitellogenin gene transcription

Bee Yen Teo, Nguan Soon Tan, Toong Jin Lam, Jeak Ling Ding*

National University of Singapore, Marine Biotechnology Laboratory, Department of Biological Sciences, 10 Kent Ridge Crescent, Singapore 117543, Singapore

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Abstract **The *Oreochromis aureus* vitellogenin (OaVtg) gene contains three imperfect oestrogen response elements (EREs) and GATA and VBP (vitellogenin binding protein) binding sites. An analysis of the promoter indicates that the 5′-flanking region up to position −625 is sufficient to mediate E_2 control. Furthermore, transfection of deletion and mutagenised promoters indicates that both GATA and VBP synergise with ER, and thus contribute to the regulation of the endogenous OaVtg gene. These findings support the notion that the interplay of promoter elements mediates proper hormone-dependent and tissue-specific expression of the OaVtg gene, regardless of non-consensus sequence context of EREs and VBP.**

Key words: *Oreochromis aureus*; Vitellogenin gene promoter; Imperfect estrogen response element; Estrogen receptor; GATA; Vitellogenin binding protein

1. Introduction

The liver-specific expression of genes is controlled to a large extent at the transcriptional level and appears to require multiple transcription factors acting positively or negatively in well defined combinations. A prominent example is the oestrogen (E_2)-controlled hepatic vitellogenesis in adult oviparous vertebrate females [1]. Vitellogenin (Vtg) is a yolk protein precursor produced abundantly during oogenesis as a result of the co-operativity between two hormone-dependent processes, transcriptional vitellogenin gene induction and post-transcriptional vitellogenin mRNA stabilisation [1,2]. To date, only one Vtg gene, named OaVtg, has been isolated and characterised in the tilapia fish, *Oreochromis aureus*. Analysis of its promoter led to the identification of three imperfect oestrogen response elements (EREs): ERE_d, ERE_p and ERE_{exon2}. Our earlier studies have shown that ERE_p contributes to most of the E_2-induced activity in the promoter. However, only a maximum 14-fold increase in reporter protein was observed [3]. This is far from the physiological situation where a >100-fold increase on yolk production was observed. Sequence analysis of the OaVtg promoter revealed various transcription factor binding sites, which could be responsible for tissue-specific expression of the OaVtg gene. In this work, we investigate the roles of two such factors, namely GATA and vitellogenin binding protein (VBP), in the regulation of OaVtg gene in relation to oestrogen receptor (ER).

In the GATA family of transcription factors, GATA-6 is reported to synergise with ER in an oestrogen response unit (ERU) in the chicken VTGII gene [4]. Since multiple GATA binding sites (WGATAR) are mapped within the promoter of the OaVtg gene [3], we envisage that GATA-6 plays a crucial role in regulating this gene. VBP also plays a pivotal role in the E_2-dependent regulation of the *Xenopus* and chicken Vtg genes [5]. Since the tilapia Vtg gene contains an imperfect binding site for VBP, we postulate that VBP plays a crucial role in regulating the OaVtg gene. We report here a synergistic effect of nuclear transcription factors – GATA, VBP and ER to strongly transactivate the OaVtg promoter. Using site-directed mutagenesis, crucial elements that control transcriptional activity of the OaVtg promoter have been identified. Transfection of OaVtg promoter constructs into a liver cell line suggests that in addition to GATA and VBP, other liver-specific transcription factors such as C/EBP also play an important role in potentiating the OaVtg gene. To mimic the physiological level of Vtg transactivation, these studies were performed over a range of E_2 concentrations.

2. Materials and methods

2.1. Construction of reporter vectors

Fig. 1A,B shows the OaVtg-CAT constructs used in this study. Mutagenesis of the constructs was carried out using the Transformer site-directed mutagenesis kit (Clontech). In particular, the GATA site mutation involved changing the GAT core motif to TGG; and the proximal imperfect ERE was changed from GGGCAcacTGACA to GGTCAcacTGACC, which made this a consensus ERE (Fig. 1B).

The control plasmid pTK-CAT and the E_2-dependent reporter gene pB1 ERU-TKCAT were gifts from Prof. W. Wahli (University of Lausanne, Switzerland). The expression plasmid pCI-neo chicken ER (linked to the CMV enhancer/promoter) was a gift from Prof. P. Chambon (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Strasbourg, France). The plasmids pRSV-GATA-6 and pRSV-VBP contain the coding region of the chicken GATA-6 and VBP, respectively, downstream of the RSV promoter (gift from Prof. John B.E. Burch, Fox Chase Cancer Center, Philadelphia, PA, USA). pSEAP-control was obtained from Clontech. Each of these reporters and expression supercoiled plasmids was isolated on a CsCl gradient [6].

2.2. Cell culture and transient expression assays

COS-1 cells were cultured as previously described [3]. Twenty-four hours prior to transfection, cells were replated into six-well tissue culture plates at 3×10^5 cells per well. The cells were then co-transfected with a total of 1 μg DNA mixture containing OaVtg-CAT construct:pCI-neo chicken ER:pRSV chicken GATA-6:pRSV chicken VBP:pSEAP-control vectors in a ratio of 6:1:1:1:1. Liposome-mediated transfection was carried out with 6 μl of Lipofectamine reagent (Gibco BRL) and the cells were incubated at 37°C for 5 h.

LMH/2A cells are a subline of chicken LMH hepatoma cells that expresses high levels of ER [7,8]. It has been useful for promoter analysis of Vtg and apolipoprotein genes [9]. LMH/2A cells were maintained in phenol red-free Waymouth's medium (Hyclone Laboratories, USA) supplemented with 10% charcoal/dextran-treated foetal bovine serum, 100 U/ml of penicillin and 100 μg/ml streptomycin. The cells were routinely cultured in gelatin-coated flasks (Iwaki Glass,

*Corresponding author. Fax: (65) 779 2486.
E-mail: dbsdjl@nus.edu.sg

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Japan) at 37°C in a humidified incubator supplied with 95% air/5% CO_2. Cells growing as monolayer were trypsinised and replated into gelatin-coated six-well tissue culture plates (Iwaki Glass, Japan) at 3×10^5 cells per well for 24 h prior to transfection. Co-transfection was carried out with 1 μg of DNA mixture containing OaVtg-CAT construct:pRSV chicken GATA-6:pSEAP-control:pBluescript II SK(±) (Stratagene) vectors in a ratio of 6:1:1:2. Liposome-mediated transfection was carried out with 15 μl of Lipofectamine.

For both cell lines, hormonal induction was performed using either 10^{-7} M or a range of E_2 concentrations (10^{-12}–10^{-5} M). Transfection efficiencies were normalised with pSEAP-control vector. Forty-eight hours after induction, cell extracts were assayed for CAT enzyme using CAT ELISA (Boehringer Mannheim). SEAP activity was measured as described by Tan et al. [10]. The data presented represent the means ± S.D. obtained from three independent transfection assays.

3. Results

3.1. The OaVtg promoter up to position −625 is sufficient to confer E_2 inducibility

Eight constructs (Fig. 1A) were transiently transfected into ER-deficient COS-1 cells together with the chicken oestrogen receptor (cER) expression plasmid. Significant induction of CAT was observed for five of these promoter constructs: pOaVtg1.7-TKCAT (5.5-fold), pOaVtg1.3-TKCAT (4.9-fold), pOaVtg0.6-TKCAT (3.6-fold), pOaVtg1.1-TKCAT (2.8-fold) and pOaVtg2.1-TKCAT (2.7-fold) in the presence of E_2. Constructs like pOaVtg1.0-, 0.7- and 0.5-TKCAT gave low or no response to E_2 (Fig. 2).

3.2. The ER acts synergistically with GATA to transactivate the OaVtg gene

Multiple binding sites for GATA were identified in the OaVtg promoter (Fig. 1A). Given the proximity of some of the GATA sites to the imperfect EREs, we speculate that GATA factor contributes to the regulation of this gene. Transient co-transfection of the eight OaVtg-CAT reporter constructs with cER expression vector into COS-1 cells in combination with expression vector for chicken GATA-6 was monitored in the presence or absence of E_2. GATA alone did not alter the basal expression from these constructs. However, this factor potentiated the E_2-dependent expression from most OaVtg-CAT constructs (Fig. 2). In the absence of E_2, co-transfection of both the GATA and cER had the same effect on pOaVtg1.7-, 1.3- and 0.6-TKCAT plasmids as the transfection by ER alone, whereas addition of E_2 conferred a synergistic enhancement of CAT activity that was 10-, 7- and 6-fold higher, respectively. Similarly, co-transfection of both GATA and cER activated pOaVtg2.1- and 1.1-TKCAT by ~4-fold over that of basal promoter activity (Fig. 2). Furthermore, although no synergism was observed for pOaVtg1.0- and 0.7-TKCAT, the presence of GATA resulted in a slight enhancement of E_2-induced transcription (Fig. 2). pOaVtg0.5-TKCAT, however, did not respond to E_2 even in the presence of GATA.

3.3. Co-operativity between VBP and ER

The strategic location of VBP_{-289} in the OaVtg promoter, albeit imperfect in sequence context, led us to determine its role in the E_2-dependent regulation of the OaVtg gene. Of the eight OaVtg-CAT reporter constructs, only pOaVtg2.1-, 1.7-, 1.3-, 1.1- and 0.6-TKCAT contain VBP_{-289} (Fig. 1A). We co-transfected these five constructs and cER expression vector into COS-1 cells in combination with expression vector for chicken VBP, and assayed for CAT protein after treatment with E_2. VBP dramatically increased the E_2-dependent expression of CAT by 6–9-fold from all five OaVtg-CAT constructs (Fig. 2).

In similar experiments where the five OaVtg-CAT reporter constructs containing VBP_{-289} and cER expression vector were co-transfected into COS-1 cells in combination with expression vector for chicken GATA and VBP, we were able to determine if there was any functional interactions among the three nuclear transcription factors. In the absence of E_2, co-transfection of GATA and VBP expression vectors consistently resulted in a suppression of CAT transcription as compared to that of GATA or VBP alone (Fig. 2). This may reflect the in vivo situation where Vtg gene expression is suppressed in the absence of E_2, although the mechanisms underlying this suppression remains unknown. In the presence of E_2, co-transfection of GATA and VBP expression vectors did not further enhance CAT transcription as compared to that of GATA or VBP alone (Fig. 2). Thus, no synergism is observed between GATA and VBP.

3.4. Functional analysis of a GATA factor-dependent ERU

In the presence of GATA, the construct pOaVtg0.6-TKCAT, harbouring a 600 bp (−625 to −5) fragment of the OaVtg promoter, is sufficient to stimulate a six-fold increase in E_2-induced CAT production (Fig. 3). This construct contains $GATA_{-478}$ and ERE_p (Fig. 1B). To map this fragment in detail and ascertain the functional relevance of these sites, we introduced mutations into the wild-type pOaVtg0.6-TKCAT construct (Fig. 1B). These were then transfected into COS-1 cells, where the ER and GATA interactions can be studied independently of other liver-specific factors. Interestingly, replacement of ERE_p with a consensus ERE in pOaVtg0.6-TKCAT did not significantly alter E_2-dependent expression (Fig. 3). However, when $GATA_{-478}$ was destroyed (pOaVtgΔ$GATA_{-478}$-TKCAT construct; Fig. 3), E_2-dependent expression was compromised, resulting in a two-fold drop in CAT production. Furthermore, this mutant harbouring a consensus ERE in place of ERE_p was not sufficient to rescue E_2-dependent inducible expression (Fig. 3). This suggests that the imperfect ERE_p and the flanking $GATA_{-478}$ site comprise a functional ERU that exhibits estrogen-dependent enhancer property. To test this hypothesis, a deletion construct (pOaVtg0.25-TKCAT) containing a 250 bp (−625 to −362) region of the OaVtg promoter was cloned (Fig. 1B). This construct contains both ERE_p and $GATA_{-478}$, but lacks both VBP_{-289} and a single half-ERE_{-179} when compared to pOaVtg0.6-TKCAT (Fig. 1B). For the wild-type pOaVtg0.25-TKCAT plasmid, E_2 induction of CAT required the presence of both cER and GATA. A strong synergistic effect is ob-

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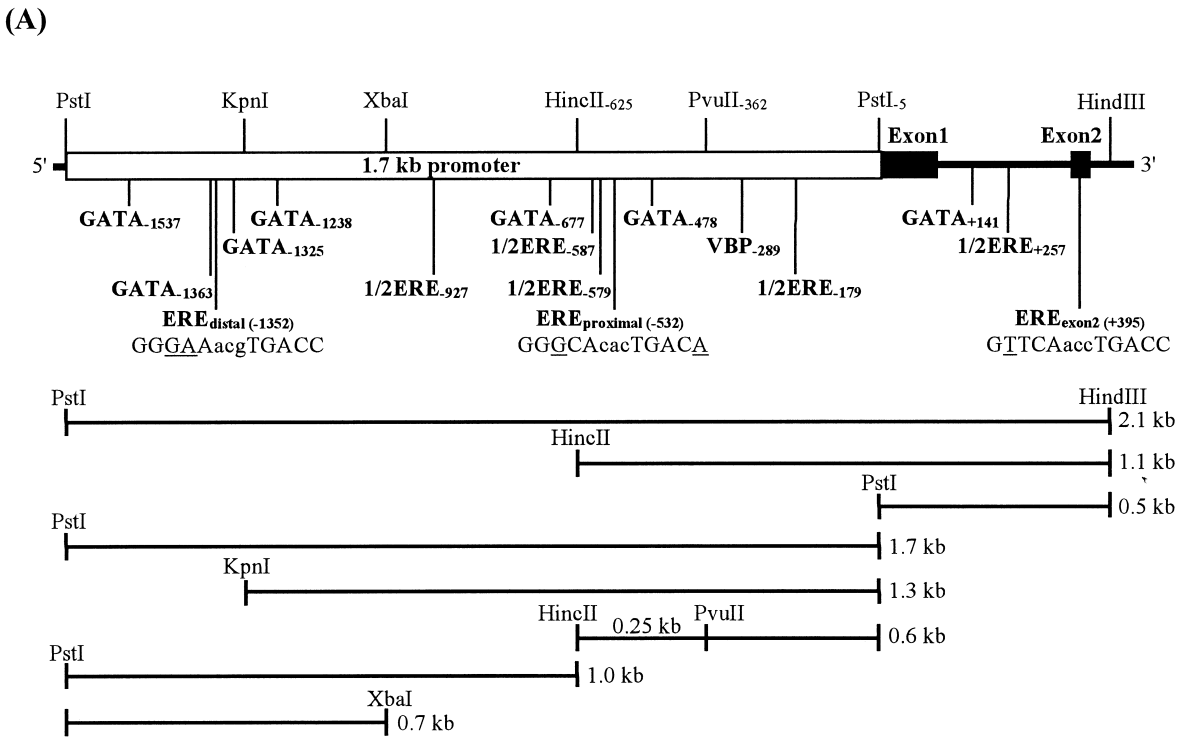
Fig. 1. A: Schematic illustrations of the various promoter regions of the OaVtg gene used in this study. B: The wild-type and mutated (Δ) fragments of OaVtg 0.6- and 0.25-kb regions are illustrated. Regulatory elements of interest are labelled. Numbers in subscripts refer to the nucleotide position relative to the transcription start site. All OaVtg fragments are cloned upstream of the TK promoter in the pTK-CAT reporter plasmid.


(A)
PstI
KpnI
XbaI
HincII-625
PvuII-362
PstI-5
HindIII
Exon1
Exon2
5'
1.7 kb promoter
3'
GATA-1537
GATA-1238
GATA-1325
GATA-1363
1/2ERE-927
GATA-677
1/2ERE-587
1/2ERE-579
GATA-478
VBP-289
1/2ERE-179
GATA+141
1/2ERE+257
ERE distal (-1352)
GGGAAacgTGACC
ERE proximal (-532)
GGGCAcacTGACA
ERE exon2 (+395)
GTTCAaccTGACC
PstI
HindIII
2.1 kb
HincII
1.1 kb
PstI
0.5 kb
PstI
1.7 kb
KpnI
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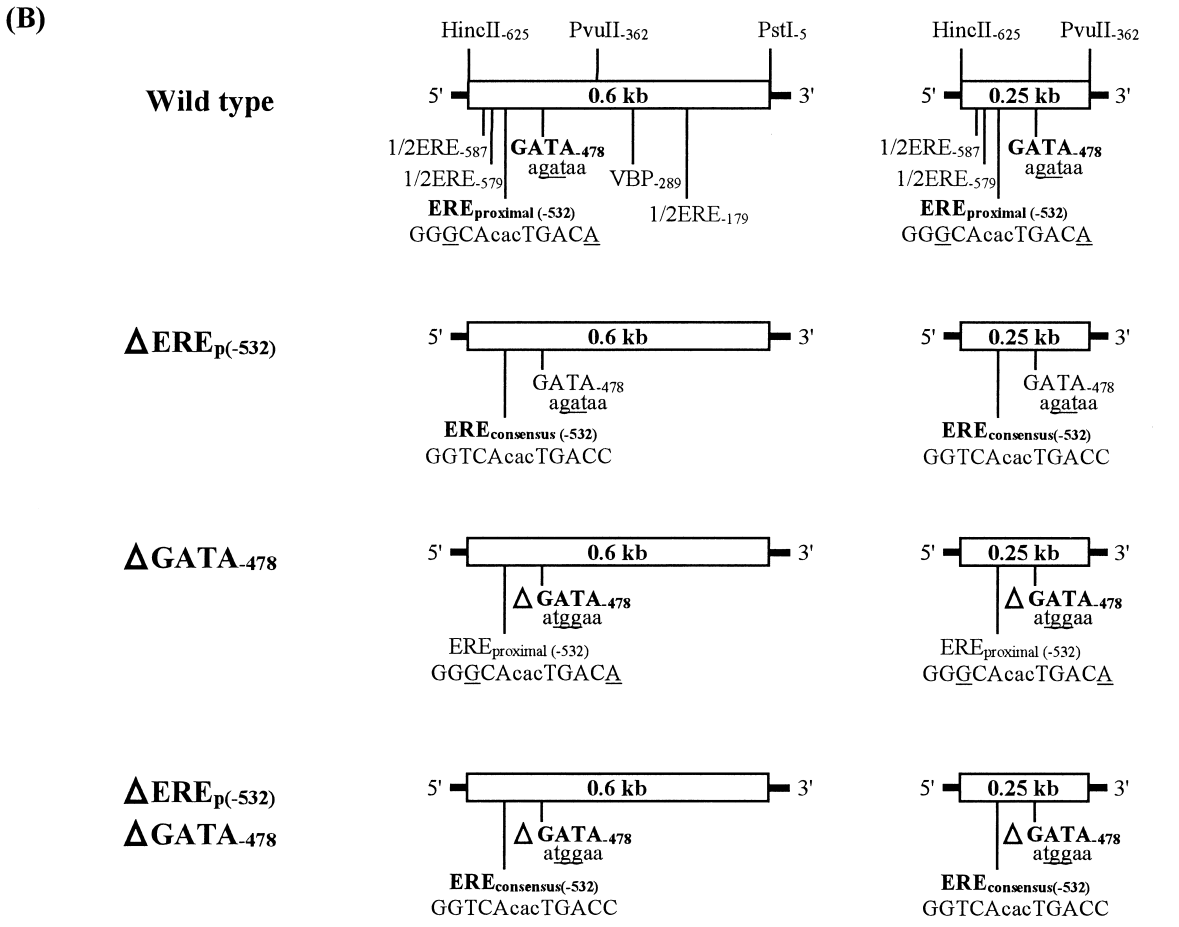
HincII
0.25 kb
PvuII
0.6 kb
PstI
1.0 kb
XbaI
0.7 kb


(B)
Wild type
HincII-625
PvuII-362
PstI-5
5'
0.6 kb
3'
1/2ERE-587
1/2ERE-579
GATA-478
agataa
VBP-289
1/2ERE-179
ERE proximal (-532)
GGGCAcacTGACA
HincII-625
PvuII-362
5'
0.25 kb
3'
1/2ERE-587
1/2ERE-579
GATA-478
agataa
ERE proximal (-532)
GGGCAcacTGACA
ΔERE p(-532)
5'
0.6 kb
3'
GATA-478
agataa
ERE consensus (-532)
GGTCAcacTGACC
5'
0.25 kb
3'
GATA-478
agataa
ERE consensus(-532)
GGTCAcacTGACC
ΔGATA-478
5'
0.6 kb
3'
ΔGATA-478
atggaa
ERE proximal (-532)
GGGCAcacTGACA
5'
0.25 kb
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ΔGATA-478
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ERE proximal (-532)
GGGCAcacTGACA
ΔERE p(-532)
ΔGATA-478
5'
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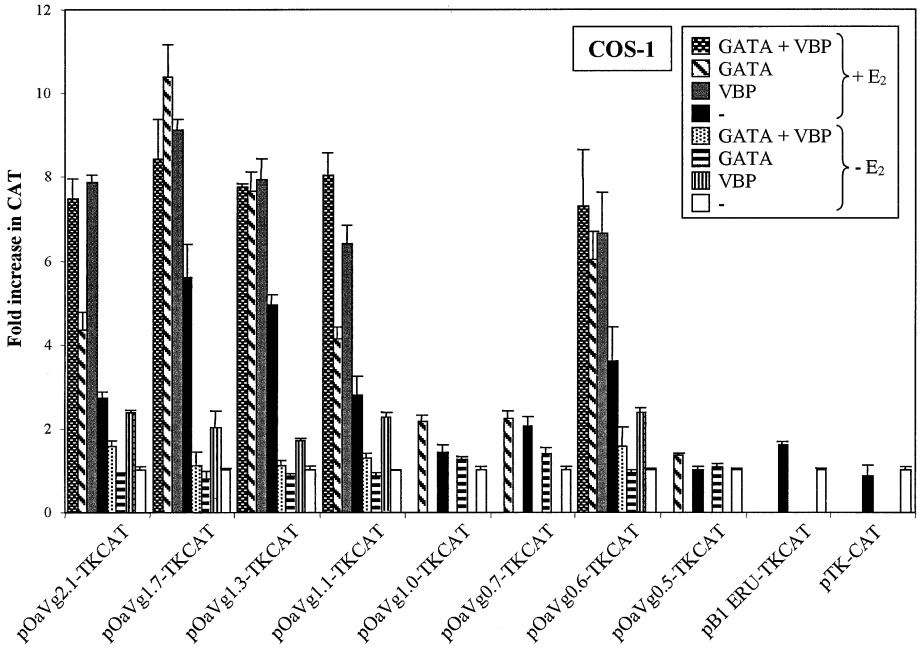

Fig. 2. A 600 bp region (−625 to −5) of the OaVtg promoter is sufficient to confer E_2 inducibility. Furthermore, ER exhibits synergism with GATA and VBP in the context of the OaVtg promoter. OaVtg-CAT constructs and cER expression plasmid were co-transfected into COS-1 cells. GATA and VBP expression vectors were also co-transfected as indicated.

served between them, resulting in five-fold higher CAT production than that of ER or GATA alone. Again, in the absence of GATA, mutant construct containing consensus ERE in place of ERE_p could not render the promoter E_2-dependent (Fig. 3). This substitution could only weakly rescue E_2-dependent expression in a construct in which the flanking $GATA_{-478}$ site was destroyed (Fig. 3).

3.5. *Characterisation of the tissue specificity of OaVtg promoter*

E_2-induced expression of the OaVtg promoter in COS-1 cells (a monkey kidney cell line) yielded only a 10-fold increase in CAT production. This is not sufficient to account for the ~80% yolk representation in plasma during oogenesis. Vitellogenesis occurs strictly in the liver of adult oviparous vertebrate females [1]. Coincidentally, in the OaVtg promoter, sequences corresponding to binding sites for liver-enriched factors like C/EBP and HNF5, as well as for the ubiquitous factor CTF/NF-1, were found. Hence, a more detailed characterisation of the strict liver-specific expression of the OaVtg gene was pertinent. The OaVtg-CAT reporter constructs were transfected into chicken LMH/2A hepatoma cells, either alone

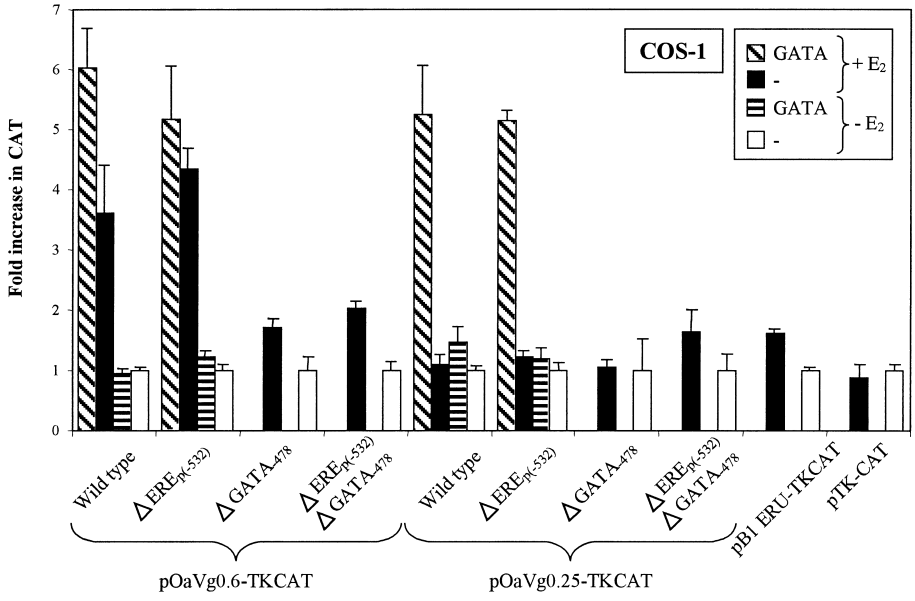

Fig. 3. ERE_p and $GATA_{-478}$ represents a functional ERU. Wild-type constructs: pOaVtg0.6-TKCAT and pOaVtg0.25-TKCAT and mutated constructs were transfected into COS-1 cells, together with cER and/or GATA expression vectors.

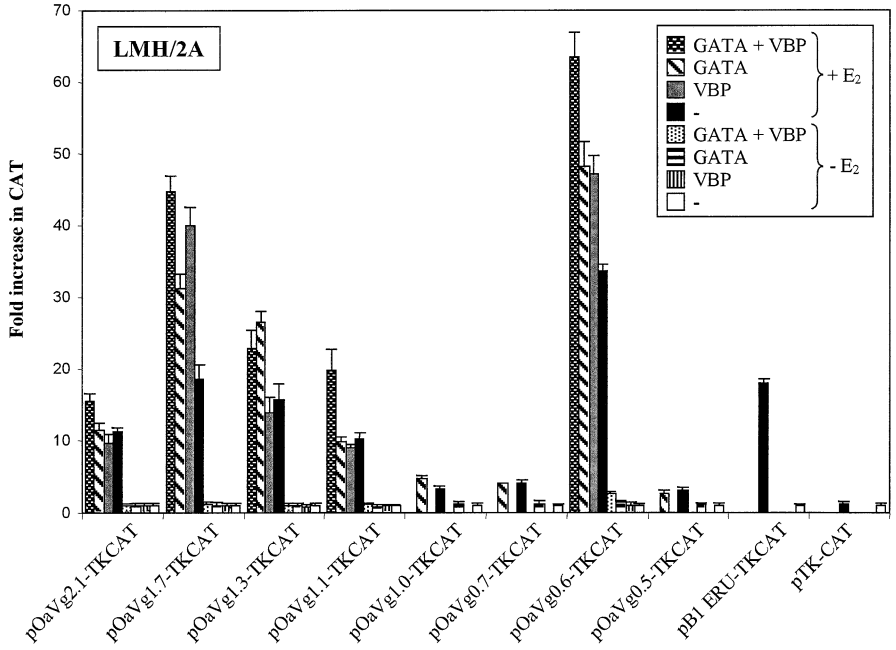

Fig. 4. Liver-specific factors are crucial in transactivation of the OaVtg gene. OaVtg-CAT constructs and cER expression plasmid were co-transfected into LMH/2A cells. GATA and VBP expression vectors were also co-transfected as indicated.

or in combination with a GATA-6 expression vector. VBP was not co-transfected as the LMH/2A cell line expresses VBP mRNAs at levels comparable to those seen in the liver [5].

In this liver cell line, all constructs exhibited significant E_2-induced CAT expression. In accordance with COS-1 cells, the pOaVtg1.7- and 0.6-TKCAT constructs responded very strongly, giving 18- and 33-fold increases, respectively, in E_2-induced CAT (Fig. 4). A similar result was obtained with other pOaVtg-TKCAT constructs: 1.3 (15-fold), 2.1 (11-fold), and 1.1 (10-fold). In contrast, constructs lacking ERE_p, VBP_{-289} and $GATA_{-478}$ (pOaVtg1.0-, 0.7- and 0.5-TKCAT) only exhibited three-fold expression of CAT (Fig. 4). In general, the magnitude of E_2 induction was much higher in LMH/2A cells than COS-1 cells. GATA caused a superactivation of pOaVtg1.7-, 1.3- and 0.6-TKCAT, reaching levels of 31-, 26- and 48-fold, respectively (Fig. 4). This dramatic enhancement caused by the co-expression of GATA is dependent on addition of E_2, suggesting a co-operative interaction between the endogenous ER, GATA and endogenous VBP.

3.6. Synergism of ER, GATA and VBP is E_2-dose-dependent

To determine whether the synergistic interaction between ER and GATA/VBP was dependent on the level of E_2, OaVtg-CAT plasmids were transfected into LMH/2A and COS-1 cells (co-transfected with cER) together with GATA and VBP expression vectors. Transfectants were then stimulated for 48 h with an increasing dose of E_2. The constructs pOaVtg2.1-, 1.7- and 0.6-TKCAT were selected as they promote very high CAT expression in all experiments. The synergistic transactivation amongst GATA, VBP and ER is dependent on the dose of E_2 (Fig. 5A–C). In both cell lines, although all constructs were responsive to a low concentration of E_2 (10^{-9} M), the interaction of GATA, VBP and ER was more pronounced at $\geq 10^{-7}$ M E_2. This indicates that a high E_2 environment similar to vitellogenesis may be necessary for synergism between GATA, VBP and ER.

4. Discussion

The *O. aureus* vitellogenin (OaVtg) gene contains three imperfect EREs: ERE_d, ERE_p and ERE_{exon2}, and the promoter contains four half-EREs. In addition, multiple binding sites for GATA (WGATAR) and VBP were also identified. Previously, only ERE_d and ERE_p have been shown to be important for the activation of the promoter in COS-1 cells co-transfected with cER expression plasmid. It was proposed that ERE_p contributes to most of the E_2-induced promoter activity in the OaVtg promoter [3]. In this paper, we document extensive studies to characterise the E_2- and liver-specific expression of the OaVtg gene.

Firstly, we were able to delineate the minimal region required for E_2 responsiveness in the OaVtg promoter to a 600 bp fragment (−625 to −5). Although pOaVtg2.1- and 1.1-TKCAT plasmids also contain this 600 bp region, lower induction was observed as they harbour an internal transcription initiation (+1) site which might interfere with or hinder the transcription of CAT from its own +1 site. Further deletion of the 600 bp to a 250 bp region (−625 to −362), viz. pOaVtg0.25-TKCAT, completely abolished E_2 inducibility, whereas pOaVtg0.6-TKCAT showed E_2 responsiveness in the absence of GATA. The obvious difference between pOaVtg0.6-TKCAT and pOaVtg0.25-TKCAT is that the latter lacks half-ERE_{-179} and VBP_{-289}. Hence, it seems that this particular half-ERE and VBP may interact with the ER and stabilise its binding to ERE_p. Thus, the ∼600 bp promoter is required to confer E_2 responsiveness to TK promoter. Plasmids lacking this 600 bp region (pOaVtg1.0-, 0.7- and 0.5-TKCAT) gave ≤2-fold induction of CAT. pOaVtg0.5-TKCAT containing only ERE_{exon2} was unresponsive to E_2.

Numerous studies suggest that many different transcription

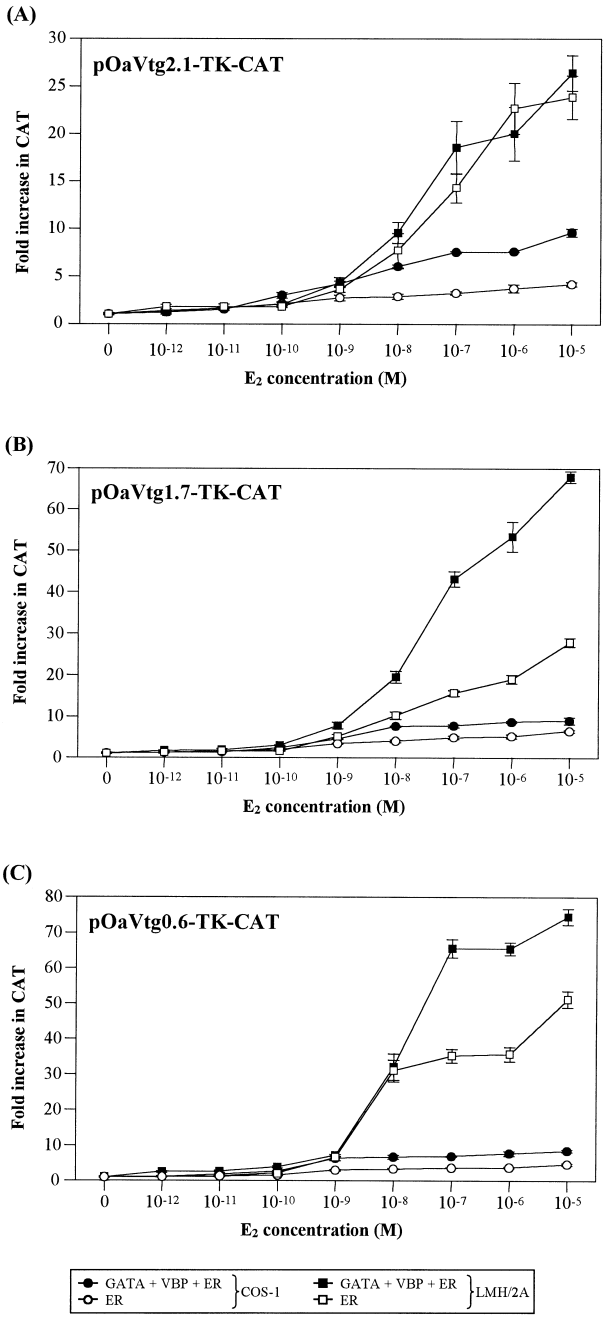

Fig. 5. Synergistic interaction of ER, GATA and VBP exhibit a dose-response profile. pOaVtg2.1-TKCAT (A), pOaVtg1.7-TKCAT (B) and pOaVtg0.6-TKCAT (C) were transfected into COS-1 and LMH/2A cells. cER, GATA and VBP expression vectors were also co-transfected as indicated.

factors synergise with steroid receptors, especially when the promoter contains a weak non-consensus steroid response element [11]. In plasmids harbouring the 600 bp region of the OaVtg promoter, E_2 stimulation obtained with GATA and ER is synergistic. A single GATA binding site ($GATA_{-478}$) in this 600 bp fragment may serve as an important *cis* element. Even for constructs lacking this region, the presence of GATA slightly enhanced E_2-induced CAT transcription, suggesting that GATA is required to stabilise the binding of ER to the imperfect EREs. Furthermore, synergistic interaction was also observed for VBP and ER in the presence of E_2. This is a novel finding as such functional interaction between VBP and other transcription factor(s) has hitherto not been reported. Hence, we propose that GATA and VBP play pivotal roles in individually synergising with ER to regulate the OaVtg gene. However, no synergism exists between GATA and VBP.

In site-directed mutagenesis studies, functional interactions between ER and GATA were demonstrated for both pOaVtg0.6- and 0.25-TKCAT reporter constructs. However, pOaVtg0.6-TKCAT showed E_2 responsiveness in the absence of GATA. Thus, it appears likely that, depending on the presence or absence of the half-ERE_{-179}, synergism between ER and GATA confers E_2 inducibility as shown by pOaVtg0.25-TKCAT, or strongly amplifies the ER response as displayed by pOaVtg0.6-TKCAT. Furthermore, substitution of a perfect ERE in place of the imperfect ERE_p did not render this ERU independent of GATA-6. This is similar to the ERU of chicken VTGII [4]. Thus, perfect EREs may also have to reside within more complex ERUs to function as E_2-dependent enhancers. We propose that GATA stabilises weak interactions of ER to ERE, provided that the ERE is adjacent to a GATA binding site, as is the case for the OaVtg and chicken VTGII gene. In addition to such reciprocal stabilisation, it has been reported that ER interacts with the steroid receptor co-activator-1 (SRC-1) to significantly increase ER transcriptional activity [12]. Hence, the interaction of the ER-SRC-1 complex with GATA could further boost the transcription rate to a level comparable to the E_2-induced vitellogenesis in vivo. Recruitment of this co-activator on the promoter probably confers increased affinity of the transcription initiation complex for this promoter. Since the ER transactivation domains synergise with themselves and/or with other types of transactivation domains [13], this phenomenon undoubtedly contributes to the enhanced activities of ERUs compared to isolated EREs.

In the chicken liver hepatoma cells, only pOaVtg1.7-, 1.3- and 0.6-TKCAT exhibited E_2-dependent superactivation of transcription when GATA was present. The dramatic enhancement by GATA is dependent on addition of E_2, suggesting a co-operative interaction between the endogenous liver ER and GATA. Since E_2 has no effect on GATA alone, we conclude that this synergism is possible only if the ER is first activated by its own ligand. With other constructs, the addition of GATA yielded no further enhancement of CAT expression in the LMH/2A cells, as the synergistic effects of GATA/ER could have been masked by the transactivation capacity conferred by other transcription factors, like VBP. Taken together, our transfection studies in LMH/2A cells suggest that tissue-specific factor(s) such as C/EBP and HNF-5 are also required for maximal OaVtg expression. Thus, this liver cell line hosts the necessary ensembles of tissue-specific transcription factors to achieve an efficient hormonal control of OaVtg promoter, where synergistic interplay occurs between imperfect EREs, half-EREs and other *cis* elements.

The dose-response studies show that the synergistic interaction of ER, GATA and VBP requires a relatively high concentration of ligand-activated ER. This mimics the in vivo situation, where such synergistic interactions occur when the fish produces a high level of E_2 during vitellogenesis. In salmon, the gonadotropin IIβ subunit gene is transactivated through synergism between its ER and SF-1 only at high

concentration of E_2, close to 10^{-7} M [14]. In tilapia and trout, the level of E_2 observed in the plasma during vitellogenesis is $\sim 2\times10^{-8\text{ to }-7}$ M [15,16].

In summary, our findings establish the co-ordinated role of three transcription factors: ER, GATA and VBP, in regulating the OaVtg gene. The results suggest that E_2-dependent liver-specific activation of the OaVtg promoter is established by binding of ER to EREs, and this interaction is stabilised through co-operation with multiple liver-enriched factors via their nearby recognition sequences. Such interplay of DNA binding nuclear proteins keeps the promoter in a state that is accessible for the RNA polymerase complex. The specific mechanisms should not only provide an explanation for transcription activation but also for the complete suppression of transcription in the liver in the absence of E_2. The OaVtg promoter might be maintained in the silent state by interaction with nucleosomes. The liganded ERs, assisted by liver-specific transcription factors, would then primarily act to make the promoter region accessible for the RNA polymerase complex [17]. ER alone cannot overcome tissue-specific barriers and that one or several additional liver components participate in mediating tissue-specific expression of the Vtg genes [18].

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