A GCM Motif Protein Is Involved in Placenta-specific Expression of Human Aromatase Gene*

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A new cis-element, trophoblast-specific element 2 (TSE2) is located in the placenta-specific enhancer of the human aromatase gene that dictates its tissue-specific expression. In the minimum enhancer region, an element similar to the trophoblast-specific element (TSE), originally described for the human chorionic gonadotropin a-subunit gene, also exists (Yamada, K., Harada, N., Honda, S., and Takagi, Y. (1995) J. Biol. Chem. 270, 25064–25069). The co-presence of TSE and TSE2 is required to direct trophoblast-specific expression driven by a heterologous thymidine kinase promoter. A 2562-base pair cDNA clone encoding a 436-amino acid protein that binds to TSE2 was isolated from a human placental cDNA library using a yeast one-hybrid system with the TSE2 as a reporter sequence. The protein was revealed to be identical to hGCMa, a mammalian homologue of the glial cell missing gene (gcm) located in the enhancer region of the human glycoprotein hormone a-subunit (12–15). It is noteworthy that a common trans-factor is involved in the expression of two hormone related genes that are characteristic of human placenta. Despite this fact, the compositions of the two trophoblast-specific enhancers are quite different. A composite enhancer (−180/−111 bp) of the glycoprotein a-subunit gene promoter, featuring two cAMP response elements and an upstream regulatory element (URE), is responsible for its placental expression. URE has been further subdivided into three overlapping sites, an a-activator element, TSE, and URE1 (15). It was also found that human GATA-2 and GATA-3 bind to the a-activator element, with TSE/URE1 forming an overlapping element that may be bound by two functionally interchangeable proteins, TSEBP and URE-binding protein (15, 16). In contrast, the trophoblast-specific enhancer region of the aromatase gene does not contain cAMP response element or GATA binding domains. There are some recognition sites for transcription factors like PEA, Sp1, and AD4 (17), but none of them has proven to be functional. Furthermore, TSE does not exhibit enhancer activity by itself, as shown for both the hCG a-subunit gene (13) and the aromatase gene (11).

In the present report, we document identification of a new regulatory element, tentatively designated trophoblast-specific element 2 (TSE2), in the minimum enhancer region for placenta-specific transcription of the aromatase gene and cloning of a binding protein for the element by a yeast one-hybrid strategy. The cDNA we isolated encodes a protein previously reported as hGCMa (18), a mammalian homologue of the Drosophila protein that is the product of glial cell missing gene (gcm).
protein exhibits binding specificity identical to that of the binding activity for TSE2 in JEG-3 cells and is expressed solely in the placenta. These results are the first demonstration of intrinsic targets of a mammalian GCM protein.

MATERIALS AND METHODS

Oligonucleotides—The sequences of the double-stranded oligonucleotides used in electrophoretic mobility shift assays are as follows (only leading sequences are shown): C3, 5'-CATAAAGCTCTTATTCAAAGG-3' (different from that shown in Fig. 1 in Ref. 11); C3n, 5'-CATGCTATCATATTATTCAAGG-3'; C3l, 5'-CATGAGTGGTTTCTCTGGATTTAG-3'; C3t, 5'-CATAAAGCTCTTATTCAAAGG-3'. C3, 5'-CATGACGCTTCAGG-3'. C4, 5'-CA

Reporter Gene Construction—FB (−307/-142) and HB (−217/-142) (11) fragments were generated by polymerase chain reaction and cloned into plasmid pGV2313 at the BglII site of pBluescript KS-. They were digested by BamHI and HindIII, and subcloned into pBLCAT2 employing BamHI/HindIII sites. Modified FB and HB CAT constructs were generated by polymerase chain reaction with mutagenic primers and FB pBLCAT2 as a template. C3-, 2

Reporter Constructs for Library Screening with the Yeast One-hybrid System—Yeast one-hybrid (24, 25) screening was performed according to the manufacturer's instructions. Five tandem repeats of modified C3 (C3B), cloned into the Smal site of pBluescript KS-, were further subcloned into pBLCAT2 at BamHI/HindIII. The orientation and the positions of the insertion and mutations are shown in Fig. 3E. Inserted portions of each CAT construct were verified by sequencing.

Methods for cell culture, transient transfections, and CAT assays were as described previously (11). pRSVLuc (23) was cotransfected in all experiments as an internal control for transfection efficiency. Preparation of nuclear extracts from mammalian cells and details of electrophoretic mobility shift assay (EMSA) were as described previously (11).

RESULTS

A New Element in the Placenta-specific Enhancer—As we showed previously, the upstream region −307 to the transcription start site was sufficient to dictate the expression of the placenta-specific exon I of the aromatase gene. The region spanning −307 to −142 (designated as FB) was capable of dictating trophoblast-specific expression under a heterologous TK promoter. A smaller region that was designated as HB (−217/-142) also retained the cell type-specific enhancer activity although it was reduced to about 1/5 of that of FB (11). In EMSAs, the labeled HB fragment gave two specific binding complexes (indicated as an arrowhead and a double arrowhead in Fig. 1, lane 2) with nuclear extract prepared from JEG-3 cells. The major complex was displaced by a 400-fold molar excess of the indicated DNA fragment was added as a competitor. Positions of the DNA fragments in the promoter region are shown at the bottom. The numbers show the position relative to the transcription start site of Exon Ia (11).

Expression of Candidate Clones in Sf21 Cells—Positive clones were further subcloned into pVL1923 (Invitrogen) and transfected into Spodoptera frugiperda SF21 cells with linearized baculovirus DNA using a BaculoGold transfection kit (Pharmenon). Recombinant virus amplification and infection were conducted following the manufacturer's instructions. The infected SF21 cells were maintained at 27 °C in Grace's insect cell culture medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum as described earlier (26). Three days postinfection, cells were harvested in Dulbecco's phosphate-buffered saline and collected by centrifugation. Cell pellets were resuspended in the buffer for preparation of nuclear extracts. They were processed as for extracts from mammalian cells, except leupeptin (1 µg/ml) and pepstatin A (2 µg/ml) were added to the homogenate and extraction buffer.

Northern Blot Analysis of TSE2-binding Protein (TSE2BP)/hGCMa Expression in Human Tissues—A Northern blot containing 2 µg of poly(A) RNA/lane was purchased from CLONTECH. The blot was probed with a 506-bp fragment (483 bp/988 bp) of TSE2BP/hGCMa (Fig. 6A). Hybridization was performed at 65 °C for 3 h in Express Hyb hybridization solution (CLONTECH), and washing was carried out twice with 0.1× SSC/0.05% SDS at 65 °C. The membrane was then stripped and reprobed with a β-actin probe.

Expression in Human Tissues—hGCMa is expressed in the placenta, ovary, and uterine tissues. A Northern blot containing 2 µg of poly(A) RNA/lane was purchased from CLONTECH. The blot was probed with a 506-bp fragment (483 bp/988 bp) of TSE2BP/hGCMa (Fig. 6A). Hybridization was performed at 65 °C for 3 h in Express Hyb hybridization solution (CLONTECH), and washing was carried out twice with 0.1× SSC/0.05% SDS at 65 °C. The membrane was then stripped and reprobed with a β-actin probe.

RESULTS

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FIG. 1. Two distinct binding sites exist in the minimal enhancer for placental aromatase transcription. EMSAs were performed with a JEG-3 cell nuclear extract (2.7 µg of protein/lane). The probes used were HB and C3 in lanes 1–4 and in lanes 5–12, respectively. A 400-fold molar excess of the indicated DNA fragment was added as a competitor. Positions of the DNA fragments in the promoter region are shown at the bottom. The numbers show the position relative to the transcription start site of Exon Ia (11).
Unexpectedly, however, the α-hCG TSE analogues inhibited the binding. Mutation of the TSE at C172 to T abolishes the competition to the TSE, whereas a mutant with T165 changed to C retains the activity (15). Both mutants were effective as a competitor for the binding to C3 probe (lanes 10 and 11). This result suggests that the 24-bp fragment TSE from α-hCG gene is an active competitor for binding to C3, although this competition is not due to the TSE recognition sequence itself. We tentatively named the core sequence in the C3 fragment the trophoblast-specific element 2 (TSE2), because, as we show later in this paper, this element is functional in the aromatase promoter, and its binding activity seems to be restricted in trophoblasts.

Core Sequence for the Binding in the C3 Fragment—To elucidate the recognition sequence for TSE2-binding protein in the C3 fragment, we planned a set of EMSAs in which a series of C3 analogues with sequential mutations were tested for their competition with the C3 probe. As shown in Fig. 2, mutations of AC or CC spanning −199 to −196 most affected competitive activity of the fragments. A 200-fold excess of mutant fragments was unable to compete with the C3 probe (lanes 4 and 5). On the other hand, mutations introduced into the following five bases gave less significant effects. TSE analogues were also found to be weak competitors, suggesting lower affinities for the putative TSE2BP. As illustrated in the lower part of Fig. 2A, the 24-bp TSE fragment (a portion of it shown as a reverse sequence) of the α-hCG gene, contains the most critical four bases, ACCC, for recognition of TSE2BP and the adjacent TNA in the reverse direction. The critical mutation in the recognition motif of TSE-binding protein, namely C shown as G with an asterisk to T at −172 resulted in a less significant change in the TSE2BP recognition context.

Binding to C3 Element Is Necessary for the Enhancer Activity—The functions of the TSE2 in the C3 region and the TSE-like element in C4 region (referred to as C4core hereafter) were examined. Two mutations were selected. One was a change of AG at −161/−162 to CT, shown as C4n in Fig. 3, expected to disrupt the C4core that would be recognized by TSEBP. The other change was CC to AA (C3l) shown in Fig. 2. C3 is a distinct element that does not compete with C3, but it contains an ACCC motif also seen in C3 as illustrated in Fig. 2. The TSE in the α-hCG promoter competes with C4(11) and weakly with C3 (Fig. 1, lane 9, and Fig. 2, lane 8). In the α-hCG TSE, the regions necessary for the competition to C3 or C4 are partly overlapping. Any mutation introduced in either C3 or C4 might result in a new binding sequence for the other site and thus an ambiguous result in the CAT assay. To rule out this possibility, the competition abilities of these mutated fragments were tested to confirm that the mutation specifically destroyed the competitive activity toward the intended binding site but had no effect on the other binding (Fig. 3A).

When either of these mutations was introduced into the HB CAT construct under a heterologous TK promoter, the enhancer activity was virtually lost in the CAT assay, (Fig. 3B). This result shows that the TSE2 in the C3 fragment and the C4core are both necessary for minimum enhancer activity of the trophoblast-specific promoter in the aromatase gene. TSE2 alone did not show any enhancer activity when one, two, four, or five copies were placed in tandem upstream of TK promoter. We previously showed that multiple copies of C2 that contain the binding site for TSEBP do not possess enhancer activity by themselves (11). The effect of C3 mutation was also examined in the basal enhancer carried by the FB fragment. The FB CAT reporter carrying the C3l mutation was about a half (48.6 ± 5.8%) as active as the wild type reporter.

Recently, a binding protein to TSE in the α-hCG gene was identified as a transcription factor of the AP2 family (27). Binding activities to C2 and C4 were both competed by a consensus AP2 binding site. With the nuclear extract used in this study, the binding activity to C2 or C4 was not inhibited by anti-AP2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). In the nuclear extract prepared in the presence of protease inhibitor mixtures, however, the binding complex appeared in a slower migrating position and was inhibited by the antibody (data not shown). The AP2 family protein thus seems to bind to the C2 and C4 region in the aromatase enhancer. TSE2 appears to be functionally involved in the expression of the placenta-specific aromatase transcript and, as we later show, occurrence of its binding activity seems to be restricted. A placenta-specific transcription factor might bind to this site. Since TSE2 does not contain any known cis-element of transcription factors, we applied yeast one-hybrid strategy (24, 25) to isolate a cDNA clone encoding the TSE2-binding protein.

Isolation of the cDNA Clone Encoding a TSE2-binding Protein—Five tandem copies of the C5B were ligated together and subcloned into the upstream region of the minimal promoter of either the pHISi-1 or pLaCZi reporter plasmids and integrated into the yeast genome of YC871. With the strategy described under “Materials and Methods,” we screened 1.0 × 106 cDNA plasmids and isolated 15 positive clones with high β-galactosidase activity. They encoded five different proteins with various types of DNA-binding domains. Apparently, the reporter sequence had ambiguously contained binding sites for multiple DNA binding proteins.

To identify the “true” TSE2-binding protein, we subcloned the positive clones into pVL1392 and expressed them in the
baculovirus/Sf21 insect cell system. Nuclear extracts were prepared from these virus-infected cells, and EMSA was performed. A cDNA encoding Caenorhabditis elegans protein kinase C in the reverse direction was used as a negative control.

As shown in Fig. 4, the virus containing clone B17 produced nuclear extract with one extra band at approximately 41 kDa on SDS-polyacrylamide gel electrophoresis. In EMSA, the nuclear extract showed multiple specific binding complexes with the C3 probe. Control nuclear extract did not produce any binding complexes. All the multiple binding complexes produced by the expressed B17 competed with C3 but not with mutated C3 (C3l). The binding specificity of the expressed protein derived from clone B17 was further examined with various DNA fragments as competitors (Fig. 4C). The binding complexes of the expressed clone were not inhibited by C3 mutants with effective mutations like C3l and C3p but weakly inhibited by C3s. They were weakly inhibited by TSE but not inhibited by C2 or C4. The specificity was essentially the same as that of the binding activity found in the JEG-3 nuclear extract, although the position of the bands appeared differently in gels.

Identity of the Positive Clone B17—The complete nucleotide sequence of the B17 clone was determined by dideoxy sequencing (ABI). The complete nucleotide sequence and deduced amino acid sequences of B17 were deposited in GenBankTM. The 2652-bp clone has an open reading frame that encodes a protein of 436 amino acid with a predicted molecular mass of 49,213 daltons. A data base search revealed it to be essentially the same cDNA as the human cDNA hGCMa described by Akiyama et al. (18). There are two nucleotide differences in the coding region; C in hGCMa is T in B17 at 17 bp from the translation start site, and G in hGCMa is A in B17 at 1157 bp, resulting in two changes of amino acid residues, Ser6 and Gly386 in hGCMa, to Phe 6 and Glu386 in B17, respectively. hGCMa has been described as a mammalian protein with a conserved GCM motif that constitutes the binding domain of the Drosophila GCM protein (18). It is a transcription factor in Drosophila with a novel type of DNA-binding domain, termed the GCM motif in the amino-terminal region.

Two groups have determined binding sites for the Drosophila GCM protein by binding site selection assays (18, 28). When aligned, the two proposed GCM binding sites and TSE2 look similar (Fig. 5A). The first and the fifth guanines were put in TSE2 context (C3g1 and C3g2) and used as competitors in EMSA with C3 probe and the nuclear extract prepared from JEG-3 cells. As shown in Fig. 5B, they demonstrated good competition.

hGCMa/TSE2BP Expression—A 506-bp fragment of hGCMa/TSE2BP-specific region (483 bp/988 bp) was used as a probe for Northern blot analysis of various human tissues (Fig.
6). A major 3.0-kilobase band and a minor bigger band (4.2 kilobases) were detected in placenta. Other tissues did not show any signal even after prolonged exposure. Binding activity to the C3 probe was detected in the nuclear extract prepared from human placenta (Fig. 7), which showed a similar electrophoretic pattern of specific binding complexes to that of Sf21 cells expressing hGCMa/TSE2BP. The nuclear extracts prepared from Hep-G2 or HeLa cells did not give a specific binding complex with C3 probe.

Possible Involvement of hGCMa/TSE2BP in Other Placenta-specific Enhancers—Several cis-elements have been described to be a components of placenta-specific enhancers in various genes. Some of them, like PSE in the leukemia inhibitory factor receptor gene (19), PLE1 and PLE3 in the leptin gene (20), and the TEF-1 like element in the chorionic somatomammotropin gene (29, 30), seem to be recognized by cell-specific trans-factors of unknown identities. The core sequences of these elements were synthesized and used as competitors for the C3 probe in EMSAs with nuclear extracts from JEG-3 or B17/Sf21 cells (lanes 1–17) and JEG-3 (lanes 7–12). Added competitors (× 200) are shown at the top of the lanes.

DISCUSSION

In the present study, we characterized an element associated with the minimum enhancer region for placenta-specific transcription of the aromatase gene. By creating a series of mutant chimeric constructs, we found that this new element C3 (−205 to −184) and C4 (−177 to −153) were both necessary for minimum enhancer activity. Kamet al. (31) showed that the proximal 500 bp of the human placental aromatase gene is sufficient to direct placenta specific expression in mice. The importance of the HB region in the expression of placental aromatase transcripts has been noted by others. Toda et al. showed enhancer activity in the −242 to −166 fragment and the existence of a cell-specific trans-factor that bound to this region (32). Re-
We applied a yeast one-hybrid strategy to identify proteins that bind to TSE2. Several positive clones obtained in the screening were expressed in insect cells mediated by baculovirus and further screened for binding activity by EMSA with TSE2 probe. A clone was found to express a protein that gives specific binding complexes with the TSE2 probe. The nuclear extract derived from the clone showed the same binding properties as that of the nuclear extract from JEG-3 cells (Fig. 4C), although the specific complexes from the two sources differed in size. One possible explanation for the discrepancy is incomplete blocking of proteolytic activity in the JEG-3 cell case.

Sequence analysis of the clone revealed the cDNA to be the same as that previously described as hGCMa (18), a mammalian homologue of the Drosophila GCM. Drosophila GCM is the product of the gcm gene, whose mutation results in a disrupted central nervous system and disorganization in the peripheral nervous system. It functions as an important switch in early neurogenesis by committing cells to the glial differentiation in Drosophila (35, 36). GCM is a transcription factor in Drosophila with a novel type of DNA-binding domain, termed the GCM motif at the amino-terminal region. Several mammalian proteins with the motif have been reported (18, 37–39).

The DNA sequence that binds Drosophila GCM has been determined by binding site selection assay by two groups (18, 28). The core sequence we determined independently for TSE2BP is similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar. Two C3 homologues having guanine in the first or the fifth position in the octamer recognition site for TSE2BP are similar.
pears to be involved in its placenta-specific expression, and cell-specific trans-factors of an unidentified nature have been suggested to bind them. PLE1 contains a GCM motif with critical guanines whose mutations were found to cause a 56% reduction in enhancer activity (20). As expected, in EMSA with expressed hGCMa/TSE2BP (Fig. 8), PLE1 competed with the C3 probe for binding and gave identical binding. It is thus very likely that hGCMa/TSE2BP is involved in the placental expres-
sion of the leitpin gene through PLE1.

Another possible functional site of hGCMa/TSE2BP is URE in the α-hCG promoter. In this region, since the binding to overlapping TSE is dominant, the URE binding site has only been revealed when a probe with a mutation at −172 was used (15). This is consistent with our finding that the affinity of hGCMa/TSE2BP for the TSE is rather low. The DNase I footprint for the URE binding protein (15) in the TSE region overlaps the critical three consecutive cytidines for hGCMa/
TSE2BP recognition. Putative URE-binding protein could be hGCMa/TSE2BP.

TEF-binding sites have been noted in the placenta-specific enhancer of the human chorionic somatomammotropin B gene (22, 29, 30). TEF group proteins recognize the binding site with the consensus 5′-(AT)G/A/G/A/G/ATG(C/T)(G/A)-3′ (41). While TEF-1 is a ubiquitous transcription factor, the expression of other TEF group proteins is more restricted (41). Binding activity to a TEF element has been attributed to chorionic somatomammotropin enhancer factor-1 that is apparently specific to choriocarcinoma, demonstrated as a fast moving complex in EMSA (22, 30). The DNA sequence of the C3 element partly overlaps the TEF element GT-IIIC, and the C3 complex also runs fast in EMSA. Furthermore, clones that encode a protein with a TEF domain were obtained in our one-hybrid screening with the C3 reporter sequence. Despite the apparent similarities, specific binding complexes of the nuclear extract prepared from JEG-3 cells with the GT-IIIC probe and TSE2 ran slightly differently in EMSA (data not shown). The C3 binding complexes with nuclear extracts from JEG-3 cells or SF21 cells expressing hGCMa/TSE2BP protein were not competed by GT-
IIIC (Fig. 8, lanes 6 and 13).

The trophoblast is the first cell lineage to differentiate in mammalian development (42). Establishment of the early pla-
cental structure is of the highest priority for the embryo that develops from the inner cell mass only after the structure is formed. Nevertheless, the molecules that control the underlying process are largely unknown. There are no genes that have been found to be essential for trophoblast commitment. Recently, two basic helix-loop-helix factors, Mash-2 and Hand 1 were identified in the mouse placenta and shown to be required for its development (43, 44), but their immediate target genes remain to be defined. A variety of cis-elements for known and unidentified trans-factors have been described in the placenta-
specific enhancers that have been analyzed, but not a single element has been found in common. TSE is associated with several genes like those for the α- and β-subunits, chorionic somatomammotropin, aromatase, and adenosine deaminase (34) and therefore could be a candidate for the master switch for placental cell differentiation. But there are exceptions such as leukemia inhibitory factor receptor and the leptin gene. Placenta may not possess a universal master switch for all placenta-specific genes, or a yet unknown “true master gene” controls the expression of these diverse transcriptional regulators. So far, while placenta-specific enhancers are located in genes for various proteins, none is located in genes for trans-
scription factors. Recently, transcription factors like AP-2 and hTEF-5 have been shown to be strongly expressed in the pla-
centa. The hGCMa described here may also fall into this cate-
gory. Further investigation of these placenta-specific transcrip-
tional regulators should hopefully give more information on the differentiation process of trophoblasts.

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FIG. 8. Binding of other placenta-
specific elements to hGCMa/TSE2BP.
EMSA was performed with the C3 probe
(lanes 1–16) or PLE1 (lanes 17 and 18)
using nuclear extracts from JEG-3 cells
(lanes 1–7) or B17/SF21 (lanes 8–18). The
indicated lanes contained a 400-fold mo-
lar excess of the competitor oligonucleo-
tides as shown.
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