Characterization of ESE-2, a Novel ESE-1-related Ets Transcription Factor That Is Restricted to Glandular Epithelium and Differentiated Keratinocytes*

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Epithelial cell differentiation is tightly controlled by distinct sets of transcription factors that regulate the expression of stage-specific genes. We recently isolated the first epithelium-specific Ets transcription factor (ESE-1). Here we describe the characterization of ESE-2, a second epithelium-restricted ESE-1-related Ets factor. Like ESE-1, ESE-2 is induced during keratinocyte differentiation. However, whereas ESE-1 is expressed in the majority of epithelial cell types, ESE-2 expression is restricted to differentiated keratinocytes and glandular epithelium such as salivary gland, prostate, mammary gland, and kidney. In contrast to ESE-1, full-length ESE-2 binds poorly to DNA due to the presence of a negative regulatory domain at the amino terminus. Furthermore, although ESE-1 and the amino-terminally deleted ESE-2 bind with similar affinity to the canonical E74 Ets site, ESE-2 and ESE-1 differ strikingly in their relative affinity toward binding sites in the c-MET and PSMA promoters. Similarly, ESE-1 and ESE-2 drastically differ in their ability to transactivate epithelium-specific promoters. Thus, ESE-2, but not ESE-1, transactivates the parotid gland-specific PSP promoter and the prostate-specific PSA promoter. In contrast, ESE-1 transactivates the keratinocyte-specific SPRR2A promoter Ets site and the prostate-specific PSMA promoter significantly better than ESE-2. Our results demonstrate the existence of a unique class of related epithelium-specific Ets factors with distinct functions in epithelial cell gene regulation.

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1 The abbreviations used are: ESE-1 and -2, epithelium-specific Ets factor 1 and 2, respectively; ESE-2a and -2b, ESE-2 isoform a and b, respectively; SPRR, small proline-rich protein; RACE, rapid amplification of cDNA ends; GAPDH, glyceraldehyde phosphate dehydrogenase; EMSA, electrophoretic mobility shift assay; UTR, untranslated region; SAM, sterile alpha motif; PSP, parotid secretory protein; PSMA, prostate-specific membrane antigen; PSA, prostate-speciﬁc antigen; EST, expressed sequence tag; PCR, polymerase chain reaction; RT, reverse transcriptase; kb, kilobase(s); CRISP, cysteine-rich secretory protein; Endo A, extra-endosomal cytoskeletal protein A.

Normal epithelial cell development, proliferation, and differentiation are induced by mesenchymal-epithelial interactions and involve cell-cell interactions, extracellular matrix, and soluble growth and differentiation factors. These interactions trigger the activation or expression of a distinct set of transcription factors leading to a specific pattern of gene expression along a tightly controlled pathway. Abnormalities in this process due to deregulated gene expression can lead to the development of benign adenomas or malignant carcinomas that make up the majority of solid tumors. In order to understand tumor development, it is therefore critical to understand normal epithelial cell differentiation and proliferation. Whereas rapid progress in understanding immune system development and gene regulation has led to the discovery and characterization of a whole set of genes involved in leukemia and lymphoma development, relatively little is known about epithelial cell differentiation and organ development and the mechanisms involved in solid tumor formation. Most of the genes involved in chromosomal translocations in leukemias and lymphomas encode transcription factors that under normal physiological conditions coordinate the correct spatial and temporal expression of genes (1). We therefore postulate that similar mechanisms of oncogenesis play a role in epithelium-derived tumors as well. It is thus surprising that many aspects of epithelium-specific gene expression have not been explored in detail up to now, and very few distinctly epithelium-specific transcription factors have been identified.

We and others have recently isolated a unique epithelium-specific transcription factor, ESE-11 (ESX/ELF3/ERT/Jen), the first member of a novel subset of the Ets transcription factor oncogene family (2–8). The Ets family encodes a group of more than 30 transcription factors that share a highly conserved DNA binding domain, the Ets domain (9–13), with limited homology outside the DNA binding domain; however, certain subclasses can be distinguished according to additional homology domains shared by a subset of Ets factors (9, 10, 14). Ets factors have been shown to be critical determinants of metazoan development and play crucial roles in transcriptional reg-

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ulation of genes involved in tissue development, cellular differentiation, cell cycle control, and cellular proliferation (9–11, 14). An ever increasing number of Ets family members have also been implicated in the pathogenesis of various types of human cancer (15–22).

ESE-1 is exclusively expressed in a variety of epithelial cells under normal physiological conditions, with highest expression being observed in the epithelium lining the gastrointestinal tract and in fetal lung (7). Most other tissues containing epithelial cells including, among others, prostate, mammary gland, liver, kidney, pancreas, and skin also express high levels of ESE-1, suggesting that ESE-1 is expressed in both simple and stratified epithelium. Expression of ESE-1 is differentially regulated during differentiation of various epithelial cells. Thus, undifferentiated keratinocytes both in vitro and in vivo express very little ESE-1; however, expression of ESE-1 can be induced in cultured keratinocytes that are induced to differentiate in the presence of calcium (4, 7). In situ hybridization also demonstrated increased expression of ESE-1 in the granular layer, with little or no expression in the basal layers of the skin, suggesting a role for ESE-1 in terminal differentiation of keratinocytes (4, 7).

Expression of ESE-1 correlates with the up-regulation of several terminal differentiation markers of the skin such as the small proline-rich proteins SPRR2A, SPRR1, and SPRR3 as well as transglutaminase 3 and profilaggrin (4, 7, 23–25). The regulatory elements of each of these genes contain conserved binding sites for Ets factors that are critical for epithelial differentiation. ESE-1 was induced during terminal differentiation might regulate the expression of a whole set of terminal differentiation genes (4, 7, 23–25). The induction of the SPRR2A gene has recently been shown to be upregulated in vivo, in addition to ESE-1, the ubiquitously expressed transcription factor AP-1 (23).

ESE-1 appears to play a similarly important role during mammary gland and prostate development. ESE-1 is expressed in the mammary gland, but expression is extinguished during lactation and reappears during involution, suggesting that ESE-1 is involved in remodeling of the mammary gland (30). Expression of ESE-1 is furthermore up-regulated by growth factors such as heregulin and epidermal growth factor (8) and appears to be increased in certain cancers of epithelial origin including lung and breast cancer (2, 5, 30).

We report here the identification and characterization of a second ESE-1-related epithelium-specific Ets factor, ESE-2, with an even more restricted expression pattern than ESE-1. We demonstrate that ESE-2 is functionally distinct from ESE-1 in terms of both its DNA binding specificity and transactivation of epithelium-specific promoters, indicating that a unique set of related Ets factors might play critical but distinct roles in epithelial cell gene regulation and differentiation.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—CV-1 (green monkey kidney), A431 (human vulvar carcinoma), HaCAT (keratinocyte line), A549 (lung carcinoma), C-33A (human cervical carcinoma), U-937 (human leukemia), A549 (lung carcinoma), C-33A (human cervical carcinoma), and NIH 3T3 (human fibroblast) cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Raji (human B cells) were grown in Dulbecco's modified Eagle's medium/F-12 medium supplemented with 10% fetal calf serum. LNCaP (human prostate cancer) cells were grown in T-medium (Life Technologies, Inc.) with 10% fetal calf serum.

**Isolation and Analysis of cDNA Clones Encoding a Novel Ets-related Protein**—To search for novel members of the Ets family, a human EST DNA database was searched for sequences homologous to known Ets members as described (13). The translated amino acid sequence of one 0.4-kb cDNA clone from a human colon carcinoma cDNA library demonstrated significant protein sequence homology to the pointed domain shared by other Ets factors including FLI-1, ESE-1, and Tel.

**5'- and 3'-RACE**—Primers flanking the open reading frame were used to amplify the 5'- and 3'-ends of the partial ESE-2 cDNA as described (13). Amplified DNA fragments were subcloned and sequenced as described (13). The sequence of the ESE-2 cDNAs was confirmed by repeating amplification using primers specific for both ends of the longest RACE products obtained in the first two rounds of PCR amplification.

**RNA Isolation and Northern Blot Analysis**—Poly(A)⁺ mRNAs were isolated as described by Libermann et al. (32). Total cellular RNA was isolated from keratinocyte cultures, Raji cells, salivary gland, and the salivary gland adenoma T98-87 using guanidine isothiocyanate nucleic acid extraction and cesium chloride gradient ultracentrifugation (33).

Northern blots and dot blots containing poly(A)⁺-selected mRNA derived from different human tissues (CLONTECH) were hybridized with random prime-labeled ESE-2, ESE-1, and GAPDH cDNA in QuickHyb solution (Stratagene) as described (13) and washed at 50 °C with 0.2× SSC, 0.2% SDS.

**RT-PCR Analysis**—cDNAs were generated from 1 μg of mRNA isolated from different cells or tissues using oligo(dT)₁₂–₁₈ priming (Life Technologies) and Moloney murine leukemia virus reverse transcriptase (Life Technologies) in deoxyribonucleosone I (Life Technologies)-treated samples. Each PCR used equivalent amounts of 0.1 ng of cDNA, a 4 ng/μl concentration of each primer, 0.25 units of Tag polymerase (Promega, Madison, WI), a 150 μM concentration of each dNTP, 3 mM of MgCl₂, reaction buffer, and water to a final volume of 25 μl and were covered with mineral oil.

The sequences of the ESE-2 primers were as 5'-CTGCTTCTTCTGT-CCTTGAAAGCC-3' (sense) and 3'-ATGAGATGATGATAGATGCCGC-5' (antisense) with an expected size of 480 bp. The sequences of the ESE-2 primers were 5'-CTGAGCAAGAGCTACTGGAGGACGTGC-3' (sense) and 5'-CTCATGTTGCGGACAGGCTCGGAC-5' (antisense) with an expected amplification product of 188 bp. The sequences of the primers for ELF-1 were 5'-ATGGCTCTGTGGTTGTGCCAAC-3' (sense) and 5'-CATTAGTGGGCGCAACAGCCTCCTGAGGAC-5' (antisense) with an expected amplification product of 800 bp. The sequences of the primers for GAPDH were 5'-CATGGATGCTTATGGGACC-3' (sense) and 5'-CATGGAGAAGAGGCTGGG-5' (antisense) with an expected amplification product of 200 bp. The sequences of the primers for the ESE-2a- and ESE-2b-specific isoforms were 5'-GGCTCCTGATTGTTGACTGTA-3' (ESE-2a sense), 5'-TGACCTAGGCGACCTGCGGC-3' (ESE-2b sense), and 5'-GAGCTGTGATGTCATGTA-3' (ESE-2a and -2b antisense) with expected amplification products of 318 and 180 bp for ESE-2a and ESE-2b, respectively.

**RT-PCR amplifications were carried out using a Perkin-Elmer thermal cycler 480 as follows: 20–30 cycles of 1 min at 94 °C, 1 min at 56 °C, and 1 min at 72 °C followed by 15 min at 72 °C. Lower numbers of cycles were used to verify linearity of the amplification signal. 10 μl of the amplification product was analyzed on a 2% agarose gel.**

**In Vitro Transcription/Translation**—Full-length ESE-2a cDNA encoding the whole open reading frame was inserted downstream of the T7 promoter into the pCR8 TA cloning vector (InVitrogen). The ESE-2a Δ42 deletion mutant was generated by fusing a Scal–SalI fragment of ESE-2a that deletes the amino-terminal 42 amino acids in frame with an optimized ATG translation initiation site into the whole open reading frame inserted downstream of the T7 promoter into the pCR8 TA cloning vector (InVitrogen). The ESE-2a Δ42 deletion mutant was generated by fusing a Scal–SalI fragment of ESE-2a that deletes the amino-terminal 42 amino acids in frame with an optimized ATG translation initiation site inserted downstream of the β-globin UTR into the Xbal-BamHI sites of pB8 KS⁺.

5'-CTGATTGCGAGCCAGCTGAAATTACGCGTCGAGTAGATCTACC
ATGGG-3'

3'-TAAGCTCTTGGAGTCTATGGGAACTGCTAGTATGAGGACC
CCTAG-5'

**SEQUENCE 1**

Coupled in vitro transcription/translation reactions were performed (Promega) as described (12). **Electrophoretic Mobility Shift Assay—EMSA**s were performed as
described (12, 31) using 2 μl in vitro translation product and 0.1–0.2 ng of 32P-labeled double-stranded oligonucleotide probes (5000–20,000 cpm) in the presence or absence of competitor oligonucleotides (1 and 10 ng) and run on 4% polyacrylamide gels, containing as buffer 0.5× Tris/glycine/EDTA as described (13).

Oligonucleotides used as probes for competition studies are shown below as follows: Drosophila E74 wild type oligonucleotide (Sequence 2), Drosophila E74 mutant oligonucleotide (Sequence 3), human SPRR2A promoter wild type oligonucleotide (Sequence 4), human SPRR2A promoter mutant M1 oligonucleotide (Sequence 5), human MP6 promoter oligonucleotide site A (Sequence 6), human MP6 promoter oligonucleotide site B (Sequence 7), human PSA promoter oligonucleotide site A (Sequence 8), human PSA promoter oligonucleotide site B (Sequence 9), human PSA promoter oligonucleotide site C (Sequence 10), human CRISP-1 promoter oligonucleotide (Sequence 11), human CRISP-3 promoter oligonucleotide (Sequence 12), human PSP94A promoter oligonucleotide (Sequence 13), human PSP94B promoter oligonucleotide (Sequence 14), murine MP6 promoter wild type oligonucleotide (Sequence 15), murine MP6 promoter mutant oligonucleotide (Sequence 16), murine EndoA enhancer oligonucleotide (Sequence 17), human MET promoter, site A (MET A) (Sequence 18), human MET promoter site B (Sequence 19), human PSMA promoter oligonucleotide (Sequence 20).

5'–TCGAGTAACCGGAAGTACATCGAG–3'
3'–CATTGGCCCTTCTAGATGCTAGCCT–5'

SEQUENCE 2

5'–TCGACTGTAACCGGAAGTACATCGAG–3'
3'–CATTGGCCCTTCTAGATGCTAGCCT–5'

SEQUENCE 3

5'–TCGAGCTGTAACCGGAAGTACATCGAG–3'
3'–CATTGGCCCTTCTAGATGCTAGCCT–5'

SEQUENCE 4

5’–TCGAGCTACCTAAGGAGTAGGAAAGC–3’
3’–GATGTCATTCCTTCACTTTCTGAGCT–5’

SEQUENCE 5

5’–TCGAGCTACCTAAGGAGTAGGAAAGC–3’
3’–GATGTCATTCCTTCACTTTCTGAGCT–5’

SEQUENCE 6

5’–AGAGAAGAGCCACATGGTTTAAATAGGAGGTGAAGC–3’
3’–CTCTTCTCTCTGGATAGAATATCTCTCCCTATCTTGAGCT–3’

SEQUENCE 7

5’–TCGACACTGTAACCGGAAGTACATCGAG–3’
3’–GATGTCATTCCTTCACTTTCTGAGCT–5’

SEQUENCE 8

5’–TCGACACTGTAACCGGAAGTACATCGAG–3’
3’–GATGTCATTCCTTCACTTTCTGAGCT–5’

SEQUENCE 9

5’–TCGACACTGTAACCGGAAGTACATCGAG–3’
3’–GATGTCATTCCTTCACTTTCTGAGCT–5’

SEQUENCE 10

5’–TCGACACTGTAACCGGAAGTACATCGAG–3’
3’–GATGTCATTCCTTCACTTTCTGAGCT–5’

SEQUENCE 11

5’–TCGACACTGTAACCGGAAGTACATCGAG–3’
3’–GATGTCATTCCTTCACTTTCTGAGCT–5’

SEQUENCE 12

5’–TCGACACTGTAACCGGAAGTACATCGAG–3’
3’–GATGTCATTCCTTCACTTTCTGAGCT–5’

SEQUENCE 13

5’–TCGACGTTCCTCCTCCTCCTGCTCCCT–3’
3’–GCAAGAGGAAAGGACCCGAGAGCGCT–5’

SEQUENCE 14

5’–TCGACGTTCCTCCTCCTCCTGCTCCCT–3’
3’–GCAAGAGGAAAGGACCCGAGAGCGCT–5’

SEQUENCE 15

5’–TCGACGTTCCTCCTCCTCCTGCTCCCT–3’
3’–GCAAGAGGAAAGGACCCGAGAGCGCT–5’

SEQUENCE 16

5’–TCGACGTTCCTCCTCCTCCTGCTCCCT–3’
3’–GCAAGAGGAAAGGACCCGAGAGCGCT–5’

SEQUENCE 17

5’–TCGACGTTCCTCCTCCTCCTGCTCCCT–3’
3’–GCAAGAGGAAAGGACCCGAGAGCGCT–5’

SEQUENCE 18

5’–TCGACGTTCCTCCTCCTCCTGCTCCCT–3’
3’–GCAAGAGGAAAGGACCCGAGAGCGCT–5’

SEQUENCE 19

Expression Vector and Luciferase Reporter Gene Constructs—An 885-bp PSP promoter fragment from –889 to –4 of the PSP gene (34) was cloned from murine genomic DNA by PCR and cloned into the pCRII TA cloning vector. The EcoRI-linked PSP promoter was transferred into the EcoRI site of the pGL3 luciferase vector. Synthetic wild type PSP promoter ets site oligonucleotides as described above containing Snf1 and XhoI ends were inserted into the Snf1 site of the Δ56-e-fos-pXP2 plasmid. The Δ56-e-fos-pXP2 plasmid was created by inserting a blunted XbaI–SalI fragment encoding the Δ56-e-fos minimal promoter into the SalI site of the pXP2 luciferase vector (13). PSA and PSMA promoter reporter constructs were kindly provided by Dr. Gary Quinn. The SPRR2A enhancer reporter construct was described before (7). The full-length ESE-2a cDNA was inserted into the EcoRI site of the pCI (Promega) eukaryotic expression vector downstream of the cytomegalovirus promoter.

DNA Transfection Assays—Co-transfections of 3 × 105 COS, CV-1, or HSG cells were carried out with 2 μg of reporter gene construct DNA and 3 μg of expression vector DNA using 12.5 μl of LipofectAMINE (Life Technologies) as described (13). The cells were harvested 16 h after transfection and assayed for luciferase activity (35). Transfections for every construct were performed independently in duplicates and repeated three times with two different plasmid preparations with similar results. Co-transfection of a second plasmid for determination of transfection efficiency was omitted because potential artifacts with this technique have been reported (36) and because many commonly used viral promoters contain potential binding sites for Ets factors.

Keratinocyte Culture and Differentiation—Monolayer cultures of primary human foreskin keratinocytes were prepared from a pool of neonatal foreskins obtained from routine circumcisions using a modified version of the protocol of Rheinwald (37, 38). Keratinocytes were isolated using dispase incubation of foreskin tissue to allow for dermal-epidermal separation. Epidermal specimens were trypsinized and isolated and characterized of two alternative splice products of the human Ets-related cDNA, ESE-2.—A human cDNA data base was searched for sequences homologous to Ets family transcription factors. One EST, originating from a cDNA clone isolated from a human colon carcinoma cDNA

RESULTS

Isolation and Characterization of Two Alternative Splice Products of the Human Ets-related cDNA, ESE-2.—A human cDNA data base was searched for sequences homologous to Ets family transcription factors. One EST, originating from a cDNA clone isolated from a human colon carcinoma cDNA
library, showed significant homology to the Pointed domain shared by a subset of Ets family members and usually found close to the amino terminus of Ets factors. The cDNA contained an open reading frame throughout the entire sequence, suggesting that part of the 5’- and 3’-ends were missing. Using sequence specific primers for this EST and RT-PCR, the novel gene was determined to be expressed in human prostate tissue. To isolate the full-length cDNA for the gene, we used the RACE method (see “Experimental Procedures”) and Marathon RACE-ready Prostate cDNA (CLONTECH) as a substrate, resulting in the identification of a novel epithelium-specific Ets gene, ESE-2.

Two alternative splice forms, ESE-2a and ESE-2b, were identified, which differ in their 5’-end sequences (Fig. 1), indicating the possible existence of two different promoters. The length of the ESE-2a (2451 bp) and ESE-2b (2317 bp) full-length cDNAs correlate well with the estimated sizes of the mRNA species as detected by Northern blot analysis (see Fig. 3). A highly repetitive sequence element, the Alu sequence, was found in the 3’-UTR of both ESE-2 cDNAs.

Predicted Amino Acid Sequence of ESE-2—Sequence analysis revealed an open reading frame encoding a 265-amino acid protein with a predicted molecular mass of 31.3 kDa for ESE-2a and an open reading frame encoding a 255-amino acid protein with a predicted molecular mass of 30.1 kDa for ESE-2b (Fig. 1). The 5’-end of ESE-2b including the 5’-UTR is divergent from ESE-2a because of alternative 5’ exons, indicating the presence of two different transcription start sites possibly derived from a proximal and a distal promoter. As a result of this alternative splicing, the ESE-2a isoform has a 10-amino acid extension at the amino terminus in comparison with ESE-2b. The ATG initiation codon for ESE-2a is the sole ATG present in frame, and several in-frame termination codons are found upstream of the ATG. The putative ATG initiation codon for ESE-2b is the sole ATG in frame, but no in-frame termination codon has been detected in the 5’-UTR. Additional 5’-RACE with ESE-2b-specific primers did not reveal any additional sequence, suggesting that we have indeed reached the 5’-end. The ESE-2 cDNAs contain a poly(A) tract, which is preceded by a classical polyadenylation site (Fig. 1) at an appropriate distance. An ATTAA motif, associated with rapid mRNA turnover, is found just after the polyadenylation site.

A hydropathy plot of the predicted amino acid sequences of ESE-2 reveals a primarily hydrophilic protein. The deduced amino acid sequence of ESE-2a and ESE-2b predicts proteins rich in glutamic acid (8%), serine (9%), and leucine (9%). Potential phosphorylation sites present in ESE-2 include four potential tyrosine kinase sites, three potential protein kinase C sites, seven potential casein kinase II sites, and one potential Jun NH2-terminal kinase/p38/extracellular signal-regulated kinase kinase phosphorylation site (S/TP) within the Ets DNA binding domain.

Sequence Comparison of ESE-2 with Other Members of the Ets Family—Comparison of the deduced amino acid sequence of ESE-2 with those of other members of the Ets family revealed the highest degree of homology to ESE-1, the prototype of a new subclass of the Ets family (7). Homologies are clustered in two primary regions, i.e., a potential protein-protein interaction domain A (Pointed domain) at the amino terminus and the putative DNA binding domain B (Ets domain), which extends over 82 amino acids at the carboxyl terminus. The remainder of the ESE-2 amino acid sequence shows only limited homology to ESE-1.

The homology within the Ets DNA binding domain between ESE-2 and ESE-1 (65%) is much lower than for other subsets of Ets factors such as ELF-1, NERF, and MEF, which share close to 90% homology within the DNA binding domain (Fig. 2A). However, the homology of ESE-2 to other Ets family members is only in the range of 34–45%, with ETS-1, ETS-2, and ER71 showing the lowest homologies. Similarly to ESE-1, homology within the carboxyl-terminal part of the ESE-2 Ets domain is relatively high, whereas the amino terminus of the Ets domain is less conserved. ESE-2 does not have an additional DNA binding domain such as the A/T hook domain found in ESE-1 (7).

Like ESE-1, ESE-2 contains the amino-terminal Pointed domain, which is present in a subset of Ets factors (Fig. 2B) and has been shown to encode a dimerization domain in the Ets factor Tel (40). The Pointed domain is a subclass of the SAM domain family found in a variety of different classes of proteins including Polycomb proteins and Eph receptors (41–43). Recent crystallization of the Eph receptor SAM domain and protein-protein interaction studies of polycomb proteins have demonstrated that the SAM domain is a protein-protein interaction domain leading to both homo- and heterodimerization or possibly higher order complexes of SAM domain-containing proteins (42, 43).

ESE-2 Expression in Human Tissues Is Highly Restricted—To determine the expression pattern and size of the ESE-2 transcript, poly(A+) mRNAs derived from various adult human tissues were analyzed by Northern blot hybridization with ESE-2 cDNA as a probe (Fig. 3). For comparison, the Northern blots were rehybridized with a probe for ESE-1 and in order to control for amounts of RNA with GAPDH. The results demonstrate the predominance of one transcript size of about 2.4–2.6 kb. Expression of ESE-2 was exclusively detected in a subset of tissues with a high content of epithelial cells, namely in kidney and prostate, but in no other tissue, indicating that ESE-2 expression is even more restricted than ESE-1 (Fig. 3). ESE-1 was expressed in almost all tissues with high epithelial cell content, including kidney, prostate, small intestine, colon, ovary, pancreas, liver, and placenta, with particularly high expression in the gastrointestinal tract (Fig. 3).

Because of this highly restricted expression pattern, a whole set of additional tissues was examined for expression of ESE-2 by dot blot hybridization analysis (Fig. 4). Strikingly, examination of 50 different adult and fetal human tissues revealed that ESE-2 is indeed exclusively expressed in tissues with high epithelial cell content. By far the highest level of ESE-2 mRNA was detected in salivary gland, followed by mammary gland, fetal kidney, and trachea. Adult kidney, prostate, and lung expressed moderate amounts of ESE-2, and fetal lung showed weak expression as well. None of the other tissues demonstrated detectable levels of ESE-2. These tissues are similar, in that they all are particularly enriched in glandular epithelium, suggesting that ESE-2 expression is limited to glandular epithelium. The same dot blot analysis has been performed for ESE-1, demonstrating that ESE-1 is expressed in the same tissues as ESE-2, but in addition in all other tissues with high epithelial cell content (4). A dramatic difference between ESE-2 and ESE-1 is the expression in the gastrointestinal tract. ESE-1 is highly expressed in colon and small intestine, whereas ESE-2 is absent (7). In addition, liver expresses significant levels of ESE-1 but no ESE-2.

Since salivary gland expressed the highest level of ESE-2, we decided to confirm that ESE-2 has a similar size transcript in salivary gland by Northern blot analysis (Fig. 5). The ESE-2 transcript was easily detectable in human salivary gland with a similar size to the prostate transcript (2.4 kb). In contrast, neither Raji B cells or a salivary gland adenoma (T95-87) expressed ESE-2.

ESE-2 Is Exclusively Expressed in a Subset of Epithelial
Cells—To confirm that ESE-2 expression is indeed restricted to cells of epithelial origin, several cell types of epithelial and nonepithelial origin were tested for ESE-2 expression by RT-PCR (Fig. 6A). Only cells of epithelial origin such as LNCaP prostate cancer cells, HEK293 human embryonic kidney cells, and HSG human salivary gland cells, all cells derived from tissues that express ESE-2 by dot blot and Northern hybridization, as well as human foreskin expressed ESE-2 mRNA.
whereas cells of nonepithelial origin including endothelial cells, fibroblasts, macrophages, and B and T cells were devoid of ESE-2 mRNA. Interestingly, none of the epithelial carcinoma cell lines, including HeLa cervical carcinoma, A431 vulvar carcinoma, C-33A squamous carcinoma of the cervix, and A549 lung carcinoma, or HaCaT keratinocytes express ESE-2, although most of these cell lines except C-33A cells express ESE-1 (7). These results suggest that ESE-2 expression is restricted to a subset of epithelial cell types, correlating with the expression pattern observed by dot blot and Northern blot hybridization.

The Two ESE-2 Isoforms, ESE-2a and ESE-2b, Are Differentially Expressed in Different Tissues—Isolation of two alternative splice forms of ESE-2 encoding two ESE-2 isoforms, ESE-2a and ESE-2b, which have distinct 5' ends, suggested that these two isoforms might be derived due to the usage of two different promoters. In addition, it appeared that the transcript size in the kidney is slightly higher (2.6 kb) than in the prostate (2.4 kb) (Fig. 1). To determine whether the different ESE-2 splice products are differentially expressed in different tissues, we designed isoform-specific PCR primers (see "Experimental Procedures") and tested their relative expression levels in different tissues and cell lines by RT-PCR (Fig. 6B). Surprisingly, kidney expressed only the ESE-2a isoform and no ESE-2b, correlating with the slightly higher transcript on Northern blots. Prostate, on the other hand, expressed both isoforms but expressed significantly more ESE-2b than ESE-2a and, therefore, possibly the slightly lower mRNA size on Northern blots. Interestingly, LNCaP cells, which are derived from a prostate cancer, expressed exclusively the ESE-2b isoform. These results suggest that expression of the ESE-2a transcript in prostate might be restricted to different types of epithelial cells than ESE-2b or that malignant transformation of prostate epithelial cells leads to extinction of ESE-2a expression. Further experiments will clarify these hypotheses. HSG salivary gland ductal cells (44) and differentiated primary human foreskin keratinocytes also expressed both isoforms, but again with significantly stronger expression of the ESE-2b isoform. These data indicate that the ESE-2a isoform is predominantly expressed in kidney epithelium, whereas the ESE-2b isoform is more strongly expressed in prostate, salivary gland, and keratinocytes.

Induction of ESE-2 mRNA Expression during in Vitro Differentiation of Primary Human Keratinocytes—Since ESE-2, in
addition to glandular epithelial cells, was also detected in the skin and since we and others had previously demonstrated that ESE-1 expression is induced during terminal differentiation of the skin, we were interested to know if ESE-2 expression might be regulated during keratinocyte differentiation as well. Relatively few models of epithelial cell differentiation using primary cells exist. We had previously used a primary human foreskin keratinocyte monolayer differentiation system as a model for epithelial differentiation (7). In this culture system, keratinocytes grown in the presence of low calcium stay undifferentiated but can be induced to differentiate by the addition of calcium and serum concomitant with the expression of various terminal differentiation markers of the skin. We had shown that ESE-1 is inducible in this culture system, which correlated well with the expression of ESE-1 during normal keratinocyte differentiation in the skin by in situ hybridization (7). Examination of ESE-2 expression in this keratinocyte differentiation system by RT-PCR demonstrates that in undiffer-

**Fig. 3.** Comparison of expression of ESE-2 with ESE-1 in different human adult tissues. Northern blot analysis of poly(A)⁺ mRNAs from indicated human adult tissues is shown. PBL, peripheral blood leukocytes. The blot was sequentially probed with ESE-2 (upper panel), ESE-1 (middle panel), and GAPDH cDNA probes (lower panel) under stringent conditions as described under “Experimental Procedures.” Numbers on the right indicate sizes of major mRNA bands. The sizes of molecular weight markers are indicated on the left.

**Fig. 4.** Dot blot analysis of ESE-2 expression in different human fetal and adult tissues. Dot blot analysis of ESE-2 expression in selected human tissues (left) with indicated human tissues and controls (right).
entiated keratinocytes, similar to ESE-1, there is relatively little expression of ESE-2 (Fig. 7). Upon induction of keratinocyte differentiation by the addition of calcium and serum, ESE-2 mRNA expression is induced. However, ESE-2 mRNA induction occurs only 48 h after calcium addition and is further enhanced at 72 h, whereas ESE-1 mRNA is already induced at 12 h, indicating that ESE-2 expression is indeed up-regulated during keratinocyte differentiation but at a later stage than ESE-1. Another Ets factor, ELF-1, in contrast, is constitutively expressed throughout keratinocyte differentiation, and the levels do not appear to change significantly during keratinocyte differentiation. Similarly, levels of the housekeeping gene GAPDH were equal in all samples, suggesting similar amounts and equal quality of cDNA. In summary, both ESE-1 and ESE-2 represent a novel subset of Ets family members that are epithelium-specific and are induced during keratinocyte differentiation.

ESE-2, but Not ESE-1, Contains an Amino-terminal Negative Regulatory Domain That Inhibits DNA Binding—To evaluate whether ESE-2 can bind sequence specifically to DNA, full-length ESE-2a and a truncated ESE-2aD42, lacking the first 42 amino acids at the amino terminus, were synthesized by in vitro translation. An EMSA was performed using equivalent amounts of in vitro translated protein for the full-length and truncated ESE-2 proteins to determine their relative ability to bind to an oligonucleotide encoding the Drosophila E74 Ets binding site, which has previously been shown to bind to several members of the Ets family (45). The E74 oligonucleotide formed several higher molecular weight complexes with both the control reticulocyte lysate (Fig. 8, lane 1) and reticulocyte lysate expressing full-length ESE-2a protein (lane 2), which were competitively blocked by both the wild type E74 and mutant E74 oligonucleotides, suggesting that these complexes are nonspecific (Fig. 8, lanes 3–7). There was no strong evidence of any specific complex, although a very faint faster migrating complex seemed to be specific but co-migrated with a nonspecific background band. In contrast to full-length ESE-2a, when the truncated ESE-2aD42 protein was used, a strong faster migrating protein-DNA complex was specifically formed (Fig. 8, lane 8), which was absent from control lysate and which was competitively blocked by the wild type E74 and mutant E74 oligonucleotides, suggesting that these complexes are nonspecific (Fig. 8, lanes 3–7). There was no strong evidence of any specific complex, although a very faint faster migrating complex seemed to be specific but co-migrated with a nonspecific background band. In contrast to full-length ESE-2a, when the truncated ESE-2aD42 protein was used, a strong faster migrating protein-DNA complex was specifically formed (Fig. 8, lane 8), which was absent from control lysate and which was competitively blocked by the wild type E74 and mutant E74 oligonucleotides, suggesting that these complexes are nonspecific (Fig. 8, lanes 3–7). There was no strong evidence of any specific complex, although a very faint faster migrating complex seemed to be specific but co-migrated with a nonspecific background band. In contrast to full-length ESE-2a, when the truncated ESE-2aD42 protein was used, a strong faster migrating protein-DNA complex was specifically formed (Fig. 8, lane 8), which was absent from control lysate and which was competitively blocked by the wild type E74 and mutant E74 oligonucleotides, suggesting that these complexes are nonspecific (Fig. 8, lanes 3–7). There was no strong evidence of any specific complex, although a very faint faster migrating complex seemed to be specific but co-migrated with a nonspecific background band.

ESE-2 Binds to Ets Sites in the Promoters of Several Salivary Gland- and Prostate-specific Promoters and to the SPRR2A Promoter Ets Site—Since expression of ESE-2 is particularly high in salivary gland and other glandular epithelium as well as in differentiated skin, we were interested to know whether ESE-2 could interact with potential Ets binding sites found in...
with ESE-2aΔ42, which was absent with the control lysate. Only the wild type PSP Ets oligonucleotide competed efficiently with the specific complex, whereas an oligonucleotide with a mutation in the core of the Ets site was unable to compete. Similar results were obtained with the keratinocyte terminal differentiation-specific SPRR2A promoter Ets site (Fig. 10). The SPRR2A Ets site formed a specific complex with ESE-2aΔ42 that was not formed by the control translation and was specifically competed by the wild type but not mutant SPRR2A oligonucleotide. These results demonstrate that the PSP and SPRR2A genes might indeed be targets for ESE-2 in epithelial cells.

**ESE-2 and ESE-1 Differ in Their DNA Binding Specificity**—To directly compare the DNA binding properties of ESE-2aΔ42 and full-length ESE-1, we performed EMSA using a few selected Ets sites found in various epithelium-specific promoters. The E74 Ets site was used as a control because both ESE-1 and ESE-2 bind to it with similar affinity. However, ESE-2, even when truncated, was much more restricted in its DNA binding specificity than ESE-1 (Fig. 11). ESE-1 was able to bind specifically to all five Ets sites tested, whereas ESE-2 interacted only with the E74 and CRISP-1 Ets sites with high affinity and weakly with the MP6A Ets site. In contrast, a PSMA promoter Ets site and a c-MET promoter Ets site (47) were only bound by ESE-1 but not ESE-2. Table I summarizes the results obtained by EMSA analysis, including some data not shown, indicating the relative binding affinity of the different sites for ESE-2 and ESE-1 and the DNA sequence of the binding core. Based on this experiment, we have compiled putative high affinity consensus binding sites for ESE-2 and ESE-1 (bottom of Table I) that are similar to the consensus recognition sequences for other Ets factors in the core binding site GGA(A/T) but diverge slightly in the flanking sequences. Comparison of the relative DNA binding affinity of ESE-2 with the binding of ESE-1 to the different sites also reveals that indeed ESE-2 appears to have a more limited set of potential binding sites than ESE-1. Whereas ESE-2 can only interact with sequences that contain a GGAA core, ESE-1 also expresses affinity for GGAT. The ability to bind to GGAT has been observed for a subset of Ets factors such as ETS-1 and ETS-2 but not for ELF-1 and Pu.1. An additional difference is the capacity of ESE-1 to bind with high affinity to sites containing a G at the –2-position relative to GGAA, whereas ESE-2 can only interact with sites that contain a C or A. Although these differences are subtle, they may have significant implications for target gene specificity. These data suggest that ESE-2 has a distinct, probably more restricted, DNA binding specificity than ESE-1, although the DNA binding domains of ESE-1 and ESE-2 are closely related.

**ESE-2 Acts as a Transactivator of the PSP Promoter**—To determine whether ESE-2 can act as a positive or negative regulator of transcription despite the inability of full-length, *in vitro* translated ESE-2 to bind DNA and to further evaluate whether the PSP gene is a target for ESE-2 in parotid gland, we tested the ability of ESE-2 to transactivate the isolated PSP promoter Ets site fused to a heterologous promoter. Full-length ESE-2a was inserted into the eukaryotic expression vector pCI and was co-transfected into COS cells together with a pXP2 luciferase reporter gene construct containing two copies of the PSP Ets site inserted upstream of the minimal c-fos promoter Δ56. Cotransfection with pCI ESE-2a resulted in a 7-fold activation of the PSP promoter Ets site compared with the parental pCI vector, whereas the empty pXP2 vector was not activated by ESE-2a (Fig. 12). Thus, the PSP gene contains a high affinity binding site for ESE-2a, which can be transactivated by ESE-2a, demonstrating that ESE-2a is a positive regulator of
transcription and that PSP may be a relevant glandular epithelium-specific target for ESE-2.

To determine whether the PSP promoter itself is a target for ESE-2, we cloned the PSP promoter region upstream of the luciferase gene into the pGL3 vector. The PSP promoter construct was co-transfected with the ESE-2a expression vector into human salivary gland cell line HSG, since the PSP promoter is only active in salivary gland cells. ESE-2 induced a 4–5-fold increase in PSP promoter activity (Fig. 13), supporting the notion that the PSP gene might be a target for ESE-2 in salivary gland.
Transactivate Several Epithelium-specific Promoters—To determine whether the differences of ESE-1 and ESE-2 with regard to DNA binding are also reflected in their transactivation capacities, we tested their ability to transactivate several epithelium-specific promoters. The results in Fig. 13 demonstrate that there are significant differences in ESE-1 and ESE-2 transactivation. As shown above, the PSP promoter is transactivated by ESE-2, but only marginally by ESE-1. In contrast, only ESE-1, but not ESE-2, can positively transactivate the prostate epithelium-specific PSMA promoter. Furthermore, whereas ESE-1 represses the prostate epithelium-specific PSA promoter, ESE-2 up-regulates PSA promoter activity. Finally, ESE-1 transactivates the Ets site within the SPRR2A gene promoter significantly more than ESE-2. These results most vividly indicate that ESE-2 and ESE-1 have different specificities in both DNA binding and transactivation and, therefore, are expected to play distinct roles in epithelial cell gene regulation and differentiation.

**TABLE I**

Comparison of the relative binding affinities of ESE-2 and ESE-1 for Ets site by ESE-2. COS cells were cotransfected with the indicated ESE-2a expression vector construct or the parental pCI expression vector and luciferase constructs containing two copies of the PSP promoter Ets site (pXP2/\delta56/PSP) or the parental luciferase vector (pXP2). Luciferase activity in the lysates was determined 16 h later as described elsewhere (7). Data shown are means and S.D. for duplicate measurements from one representative transfection. The experiment was repeated three times with different plasmid preparations with comparable results.

| Enhancer | Binding site | ESE-2 | ESE-1 |
|----------|--------------|-------|-------|
| E74      | AACCGGAGTAAG | ++++  | ++++  |
| EndoA    | GACAGGAGTAG  | ++++  | ++++  |
| PSP      | TCCAGGAAAATG | ++++  | ++++  |
| CRISP-1  | AGCAGAGGATGC | ++++  | ++++  |
| MP6 A    | GTAAAGGAAGTA | ++++  | ++++  |
| SPRR2A   | AGCAGGAGGATA | ++++  | ++++  |
| CRISP-3  | ACAGAGAAACAT | ++++  | ND*   |
| PSP94 A  | TCCAGGAAAGCC | ++    | ND    |
| PSP94 B  | CAGAGGAAAGGA | −     | −     |
| MP6 B    | GAAAGGAAGGCG | −     | −     |
| MP6 C    | AGCAGGATGCAG  | −     | +     |
| PSA A    | TGGAGGAACATA  | −     | ND    |
| PSA B    | CTGTGGAAGGGG | −     | −     |
| PSA C    | CTGGGAAAAGAA | −     | −     |
| PSA E    | AGCAGGATGTGA  | −     | +     |
| PSMA     | ATGGAGAATCTT  | −     | −     |
| c-MET A  | GGGAGAAACCCG  | −     | −     |
| Consensus for ESE-2 | AACAGGAATTAN | G ac aaG | T cc |
| Consensus for ESE-1 | AACAGGAAGATAN | G Gc taG6 | T a cc |

* ND, not determined.

**FIG. 12.** Transcriptional activation of the PSP promoter Ets site by ESE-2. COS cells were cotransfected with the indicated ESE-2a expression vector construct or the parental pCI expression vector and luciferase constructs containing two copies of the PSP promoter Ets site (pXP2/\delta56/PSP) or the parental luciferase vector (pXP2). Luciferase activity in the lysates was determined 16 h later as described elsewhere (7). Data shown are means and S.D. for duplicate measurements from one representative transfection. The experiment was repeated three times with different plasmid preparations with comparable results.

**DISCUSSION**

Although several transcription factors involved in epithelium-specific gene expression have been characterized, such as IDX, CDX-2, IKLF, TCF-4, GLI-2, GLI-3, HNF-3α, HNF-3β, TTF-1, AP-2, LFB3, and Skn-1a, very few of these transcription factors are restricted to epithelial cells. We and others recently isolated the first epithelial cell-restricted member of the Ets transcription factor family, ESE-1 (ESEX/ERT/Jen/ELF3), the prototype of a new subfamily (2–8). ESE-2 represents the second member of the ESE subclass of Ets factors. Only a few members of the Ets family such as PU.1, SpiB, and SpiC are tissue-restricted. Interestingly, similar to ESE-1 and ESE-2, which belong to one subclass of Ets factors and are expressed exclusively in epithelial cells, Pu.1, SpiB, and SpiC belong to a distinct subclass and are restricted to the immune system. This suggests that structural similarities between different members of a particular subclass of Ets factors might reflect also a functional similarity. Indeed, both Erg and Fli-1 are Ets factors frequently translocated to the EWS gene in Ewing’s sarcoma, and both belong to the same subclass of Ets factors (15).

The ESE-2 gene is expressed as two alternative splice products, ESE-2a and ESE-2b, which encode two different isoforms. The alternative splice products have different 5’-untranslated regions and differ at their amino terminus by 10 extra amino acids for ESE-2a.2 Whether these 10 amino acids encode any specific function is unknown, since no homologies to any known protein domains or other proteins can be detected. We have preliminary evidence from the genomic structure of the ESE-2 gene that the ESE-2a and ESE-2b alternative splice forms are encoded by alternative exons with separate promoters. The differential expression of the ESE-2 isoforms supports the hypothesis that ESE-2a and ESE-2b have slightly divergent functions.

Although ESE-2 exhibits the highest degree of homology to ESE-1 in the DNA binding domain, the relative degree of homology in this region (65%) is not as high as for other Ets family subclasses such as ERP, SAP, and ELK or for NERF, ELF-1, and MEF, where the homology within the DNA binding domain is 80–90% (13, 48, 49). However, homology of ESE-2 to the Ets domain of other Ets family members is even further reduced. In addition to its function as a DNA binding domain, the Ets domain is also involved in protein–protein interactions with numerous other transcription factors (50–52). The relatively low degree of homology of the ESE-2 Ets domain to that of ESE-1 is supported by the differences in DNA binding specificity but may also determine differences in protein–protein interactions of the DNA binding domain with other transcription factors or other proteins.

Outside of the DNA binding domain, the only other region of significant homology is at the amino terminus, where ESE-2 is homologous to the Pointed domain found in several of the other Ets family members (21, 53, 54). The fact that the Pointed domain is not found in the Ets factors ELF-1 and NERF, which are the next most closely related to ESE-1 and ESE-2, further supports the notion that ESE-1 and ESE-2 represent a distinctively separate subfamily of the Ets family. The function of the Pointed domain is not clear, but due to its homology to the SAM domain and the fact that the Ets factor Tal homodimerizes, it has been suggested that it may be involved in either homo- or

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1. P. Oettgen, K. Kas, Y. Akbarali, and T. A. Libermann, unpublished data.
2. K. Kas, Y. Akbarali, F. Grall, and T. A. Libermann, manuscript in preparation.
heterodimerization (21, 40, 41, 53, 54). Nevertheless, various attempts to confirm that other Ets factors that contain the Pointed domain such as Ets-1 and Fli-1 can form dimers have failed so far. The Pointed domain of Tel is involved in chromosomal translocations in various human cancers leading to fusion proteins with several members of the tyrosine kinase family, suggesting a role for this domain in cellular transformation.

Although ESE-1 and ESE-2 are highly related and restricted to epithelial cells, ESE-1 and ESE-2 are significantly different in various aspects. Whereas full-length ESE-1 binds with high affinity to DNA, full-length ESE-2 has very low affinity to DNA. Only upon deletion of the amino-terminal 42 amino acids does ESE-2 bind with high affinity to DNA, suggesting the existence of a negative regulatory domain at the amino terminus. This characteristic is not unique to ESE-2, since several other members of the Ets family such as ERP, Tel, and Ets-1 also contain inhibitory domains that interfere with DNA binding via intramolecular interactions (10, 12, 55). Nevertheless, these Ets factors including ESE-2 bind to DNA in vitro and transactivate genes, suggesting a mechanism of disengaging the negative regulatory function such as phosphorylation or protein-protein interaction. A further striking difference between ESE-2 and ESE-1 is the DNA binding specificity of ESE-1 and ESE-2, which is clearly more restricted for ESE-1. Based on our EMSA analysis, there are at least two nucleotides within the core binding site that show a broader range of variability for ESE-1 than ESE-2. Whereas ESE-1 can bind to a variety of different sites, even the truncated form of ESE-2 binds only to a subset of the sites recognized by ESE-1. Differences in the DNA binding domains of ESE-1 and ESE-2 most likely contribute to this distinction, and indeed there are several amino acid differences in the carboxyl-terminal α-helical and β-sheet regions that have been demonstrated in other Ets factors by x-ray crystallography to interact directly with DNA. We cannot exclude the possibility that ESE-2 may bind to a different set of binding sites that were not part of our investigation. However, we believe that this is less likely, because we have tested many additional Ets-related binding sites not reported here that do not bind ESE-2, and up to now Ets factors much more distantly related to ESE-1 than ESE-2 have similar binding specificities.

While ESE-1 and ESE-2 expression is restricted to epithelial cells, their specific expression patterns are remarkably distinct. Whereas ESE-1 is expressed in almost any type of epithelial cell, including the gastrointestinal tract and liver, ESE-2 is highly expressed mainly in tissues containing glandular epithelium including the prostate, salivary gland, mammary gland, trachea, lung, and kidney. Particularly high expression is observed in salivary gland, and Ets binding sites found in several salivary gland-specific genes bind specifically to ESE-2.

It is interesting that both ESE-1 and ESE-2 are not expressed in undifferentiated keratinocytes but are induced during keratinocyte differentiation. ESE-2, however, is clearly induced at a later time point than ESE-1 in an in vitro keratinocyte differentiation system. The terminal differentiation of epithelial cells proceeds along a tightly coordinated pathway requiring precisely timed regulation of specific sets of genes. The differential expression of the ESE genes may allow for the activation of different sets of epithelium-specific genes at different time points, suggesting that ESE-2 may induce genes required for later stages of terminal differentiation or modulate their expression. We have identified at least one potential target for both ESE genes, the SPRR2A gene (7), and indeed ESE-1 and ESE-2 have different effects on the transcription of the SPRR2A gene. SPRR2A belongs to a family of proline-rich proteins (56). In the skin, they are incorporated
into the cornified envelope, and their expression is tightly linked with terminal differentiation of keratinocytes. Although some of these proline rich proteins are restricted to the epidermis, others are expressed in the epithelium of the upper digestive tract, tongue, and sublingual epithelium (56).

Differences between ESE-2 and ESE-1 in DNA binding and expression also extend to differences in their abilities to transactivate various epithelium-specific target genes. This difference is particularly striking for the two prostate-specific genes, PSA and PSMA. ESE-1 and ESE-2 have opposing effects upon the regulation of the PSA gene promoter, with ESE-1 acting as a repressor and ESE-2 as an activator. The opposite is true for the PSMA gene promoter; whereas ESE-2 activates this promoter, ESE-1 has no effect. This suggests that despite the fact that ESE-1 and ESE-2 are coexpressed in the prostate epithelium and belong to the same subclass of Ets factors, ESE-1 and ESE-2 target different genes, or in some cases the same genes but with opposing actions. A similar scenario has been observed in the R7 retinal cells of Drosophila, where two Ets factors, Pointed and Yan, target the same genes in the same cell, but with opposing functions (57, 58). The distinctive behavior of ESE-1 and ESE-2 may be due to unique posttranslational modifications such as phosphorylation, differences in DNA binding, and differences in protein-protein interactions with transcription factors or co-activators/co-repressors interacting with other regulatory elements in a specific promoter.

Salivary gland expresses particularly high levels of ESE-2, suggesting an important function in salivary gland epithelium. The salivary glands are composed of the parotid, submandibular, and sublingual glands. Saliva is the major product produced by terminally differentiated acinar and ductal cells in these glands. Very little is known about the specific transcription factors regulating expression of genes in the salivary gland. Through transgenic approaches, regulatory regions necessary for tissue-specific expression of the PSP gene, the most abundantly expressed protein in the parotid gland, have been identified (34, 59–61). Despite the fact that the regulatory regions of this gene have been identified, the actual transcription factors controlling PSP gene expression are unknown. ESE-2 is the first transcription factor that has been shown to bind a site in the PSP gene and to transactivate the PSP promoter. This ESE-2 binding site is in the proximal promoter and may be required for the basal parotid gland-specific expression of the PSP gene, which is further enhanced by more distant enhancer elements. Because of the specific and high level expression of ESE-2 in salivary gland, it is likely that ESE-2 may be one of the transcription factors that is critical for PSP gene expression. In contrast to ESE-2, ESE-1 transactivates the PSP promoter only marginally, suggesting that although both genes are expressed in salivary gland, ESE-2 and ESE-1 have differential effects on the PSP gene.

In conclusion, ESE-2 represents the second member of an epithelium-specific subclass of the Ets transcription factor family. In addition to its role in regulating the later stages of terminal differentiation of keratinocytes, it appears to regulate a number of epithelium-specific genes found in tissues containing glandular epithelium such as the salivary gland and prostate. Despite their similarity, ESE-1 and ESE-2 have different DNA binding and transactivation properties in these tissues. Identification of two epithelium-specific members of the Ets family provides exciting opportunities to test the notion that epithelial cell differentiation is governed by the differential activation and expression of distinct members of the Ets transcription factor family. Since a large number of human cancers are of epithelial origin, we can also assume that deregulated ESE-2 expression may contribute to malignant transformation.

During the preparation of this manuscript, the identification of a novel Ets gene, the ELF-5 gene, was reported (62). The protein sequence presented for ELF-5 is identical to ESE-2b.
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