Transactivation of lung lysozyme expression by Ets family member ESE-1

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Lei W, Jaramillo RJ, Harrod KS. Transactivation of lung lysozyme expression by Ets family member ESE-1. Am J Physiol Lung Cell Mol Physiol 293: L1359–L1368, 2007; doi:10.1152/ajplung.00130.2007.—Epithelial-specific Ets (ESE) transcription factors, consisting of ESE-1, ESE-2, and ESE-3, are constitutively expressed in distinct epithelia of mucosal tissues, including the lung. Each ESE member exhibits alternative splicing and yields at least two isoforms (a and b) with transcriptional targets largely unidentified. The studies described herein define a novel role for ESE transcription factors in transactivation of the human lysozyme gene (LYZ), an essential component of innate defense in lung epithelia. Of the six ESE isoforms, ESE-1a and ESE-1b transactivated LYZ promoter in reporter gene assays, whereas only ESE-1b dramatically upregulated transcription of endogenous LYZ in both nonpulmonary and pulmonary epithelial cells. Importantly, ESE-1a and ESE-1b could transactivate the LYZ promoter in cultured primary airway epithelial cells. ESE-2 and ESE-3 isoforms were unable to substantially transactivate the lysozyme promoter or upregulate transcription of endogenous LYZ. Two functional consensus Ets sites located in the proximal 130-bp LYZ promoter were responsive to ESE-1a as identified by site-directed mutagenesis and DNA binding assays. Short hairpin RNA attenuation of endogenous ESE-1b mRNA levels in lung epithelia resulted in decreased LYZ transcription. Furthermore, ESE-1 antibody specifically enriched the 130-bp proximal LYZ promoter in chromatin immunoprecipitation analyses. These findings define a novel role for ESE transcription factors in regulating lung innate defense and suggest distinct regulatory functions for ESE family members.

cosmal epithelia are essential components of innate immunity, not only providing an anatomic barrier, but also contributing actively to host defense by secreting a plethora of proteins such as lysozyme and lactoferrin (3, 12, 28). Transcriptional mechanisms coordinating the expression of these host defense proteins in mucosal epithelia are poorly understood. Various reports of Ets family members regulating expression of host defense genes are suggestive, but definitive elucidation of mechanisms in the lung epithelium are not defined yet.

Herein, LYZ is identified as a transactivation target of ESE-1 but not ESE-2 and ESE-3 isoforms. Specifically, ESE-1b transactivates the LYZ promoter through two consensus Ets binding sites in the proximal promoter region. We also provide evidence that ESE isoforms have differential regulating potentials on the LYZ promoter. These studies provide a foundation for elucidating the transcriptional networks in mucosal tissues such as regulating coordinated host defense gene expression.

MATERIALS AND METHODS

Cell culture, transfection, and reporter gene assays. This study was reviewed and approved by the Lovelace Respiratory Research Institute (LRRI) Institutional Biosafety Committee and the LRRI Institutional Review Board for human research. Normal human bronchial epithelial cells were purchased from Lonza and kept in bronchial epithelial basal medium (BEBM, CC-3171; Cambrex) without retinoic acid; air-liquid interface (ALI) culture of NHBE cells were carried out with protocols provided in mixture of BEBM and DMEM-high glucose (D6429; Sigma) at the ratio of 1:1. The epithelial cell lines A549, H292, and H441, immortalized or transformed cells of human lung epithelial origin, were maintained in RPMI 1640 (Invitrogen) medium containing 10% FBS; human cervical epithelial cells (HeLa) were maintained in Advanced MEM (Invitrogen) containing 10% FBS. Cells were seeded onto 48-well plates (2 × 10⁴ cells per well) 24 h before the experiment and transfected with FuGENE 6 (Roche) as recommended by the manufacturer. Typically, 100 ng of reporter plasmid, 25 ng of ESE expression vector, and various amounts of empty expression vector were added to a total 200 ng per well. For competition assays, 25 ng each of ESE-1b and ESE-2 or ESE-3 isoform expression vector were cotransfected. Twenty-four hours after transfection, cells were washed once with cold PBS, followed by adding 100 μl of 1× lysis buffer (Promega) per well, and kept at −70°C for 1 h. After 30 min of rotation at room temperature, 5 μl of lysates was mixed with 25 μl of substrate (Promega) to detect luciferase activity. Because ESE factors affect transcriptional activity of cytomegalovirus (CMV) or thymidine kinase promoters, we omitted internal control plasmid based on these promoters and carried out the transfections in duplicate; each experiment was repeated at least three times.

Construction of ESE protein expression vectors. ESE-1 and ESE-3 cDNAs were isolated from H441 cells; ESE-2a and ESE-2b cDNAs were isolated from human lung RNA samples. All acquired cDNAs were FLAG-tagged through PCR. RNAs were extracted from H441...
cells and human lung tissue with RNase mini kits (Qiagen) according to the protocol provided. First-strand cDNA was synthesized with SuperScript First Strand Synthesis System (Invitrogen) and amplified with AccuPrime Taq polymerase (Invitrogen). The primers used are as follows (restriction enzyme sites are shown in italics, and the antisense of FLAG-tag coding sequences are underlined): ESE-1, upstream, 5’-aagagcatgtcgtcgtcatccttgtaatcgttttcattttctctccatc-3’ (HindIII downstream, 5’-tcacttatcgtcgtcatccttgtaatcgttttcattttctctccatcctc-3’ (Xhol); ESE-2, upstream, 5’-agagacatgtcgtcgtcatccttgtaatcgttttcattttctctccatc-3’ (BamHI); ESE-2b, upstream, 5’-agagacatgtcgtcgtcatccttgtaatcgttttcattttctctccatc-3’ (BamHI); ESE-3, downstream, 5’-agtgcctgttgacacacaggtgtggtaaatcgttttcattttctctccatc-3’ (Xhol). The cDNAs of alternative spliced isoform ESE-1a and ESE-3a were acquired through overlapping/splicing by PCR. PCR products were cloned into pGEM-T vector (Promega) and verified through sequencing. Corresponding cDNAs were subsequently transferred into pcDNA3 (Invitrogen) to achieve an expression vector for each ESE protein. Overexpressed FLAG-tagged ESE-1 proteins were detected with immunoblotting as described in Ref. 18.

Construction of LYZ reporter plasmids and site-directed mutagenesis. DNA was extracted from H441 cells with TRIzol (Invitrogen) according to the protocol provided by the manufacturer and used as the template in the PCR with primers hLysoS (5’-aagagcatgtcgtcgtcatccttgtaatcgttttcattttctctccatc-3’ and hLysoAS (5’-aagagcatgtcgtcgtcatccttgtaatcgttttcattttctctccatcctc-3’) to isolate the 3-kb promoter sequence of LYZ (KpnI and HindIII sites are underlined). After cloning into the pGEM-T vector, the LYZ promoter was verified through sequencing, and the corresponding DNA fragment was inserted into pGL3-Basic (Promega) between the KpnI and HindIII sites to get the reporter construct E. Deletions within the truncated LYZ promoter conferring expression of the luc gene were acquired through enzyme digestion and treatment (4’-3’5’Hlyso at PvuII, 0.5’Hlyso at Smal, 0.35’Hlyso at NruI, and 0.13’Hlyso was constructed through PCR). Transcription factor motifs were analyzed using Match 10.0 software provided within the TRANSFAC database and altered through PCR-based site-directed mutagenesis. In brief, 200 ng of purified 32P-labeled Lysosome plasmid was amplified using Pfx DNA polymerase (Invitrogen) with corresponding mutation primer pairs as listed in Table 1 for 14 cycles. Purified PCR products were digested with DpnI for 4 h, and the precipitated DNAs were used to transform XL2-Blue competent cells (Stratagene). The mutated sites were verified through sequencing.

EMSA. Because dozens of Ets proteins were expressed in all kinds of epithelial cells, HeLa cells were transfected with pcDNA3 or ESE-1a and ESE-1b expression vector using DreamFect (OZ Biosciences) with the ratio of plasmid to DreamFect at 1:5. Nuclear proteins were extracted with NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce) and quantified with Bradford Reagent purchased from Bio-Rad. Oligos were tagged with a Biotin 3’ end DNA Labeling Kit (Pierce) and annealed. EMSA was performed by preincubating each DNA-binding reaction, consisting of 5 mM Tris, 0.5 mM DTT, 0.05 μg/μl poly[dI-dC], 5 mM MgCl2, 80 mM KCl, 0.05% Nonidet P-40, 10 mM EDTA, 2.5% glycerol, and 10 μg of extracted nuclear proteins, at room temperature for 20 min followed by adding 2 μl of biotin-labeled oligos and incubating at room temperature for an additional 20 min. For supershift assay and competition assays, 1 μl of anti-FLAG monoclonal antibody (Sigma) or 100× unlabeled oligos were added into the corresponding binding reaction during preincubation. Twenty microliters of binding mixture containing 1× loading buffer was separated on 5% polyacrylamide gel in 1× Tris-borate-EDTA (TBE) at 100 V, and oligos were electrotransferred to a Hybond nylon membrane (GE Healthcare) in 0.5× TBE. After cross-linking with UV Stratalinker (Stratagene), the biotin-labeled oligos were detected with a LightShift Chemiluminescent EMSA Kit (Pierce).

RT-PCR, semi-quantitative RT-PCR, and quantitative PCR analysis. To analyze the transcription of the endogenous LYZ, RNAs were extracted directly from NHBE, HeLa, A549, H292, and H441 cells with the RNase mini kit (Qiagen) and used for RT-PCR analysis. One well of ALI cells cultured 2 wk were used for RNA extraction with TRIzol (Invitrogen) according to the manufacturer’s instructions. One microgram of total RNA was used to synthesize the first-strand cDNA with QuantiTect Reverse Transcription Kit (Qiagen). FastStart DNA Polymerase (Roche) was used to amplify full LYZ coding sequence (CDS) with primers FF (ctgctagcatggatcc) and FR (ctggtaactccccagcaac) in parallel, full GAPDH CDS was amplified as an internal control with primer GS (atcactgccacagcaag) and primer GR (ttactctgggagcttgcttg). To assess the effects of ESE proteins on the endogenous LYZ, 2 × 105 plasmids per well of cells were seeded into six-well plates 1 day before transfection with 500 ng of empty pcDNA3 or corresponding ESE expression vectors. RNAs were extracted 24 h after transfection with TRIzol and reverse-transcribed with QuantiTect Reverse Transcription Kit (Qiagen). Because multiple transcripts containing partial LYZ coding sequence were detected from the epithelial lineage cells, a semi-quantitative RT-PCR procedure was employed as previously reported (20) to coamplify full LYZ CDS and GAPDH with optimized primers at 300 nM each of FF and FR vs. 50 nM each of QGF (cgtggaaggactcatgcc) and QGR (gcctacagcatc). Densities of ethidium-stained PCR bands were analyzed with software provided by Bio-Rad and normalized to corresponding internal controls. QuantiTect SYBR Green PCR Kit (Qiagen) was used to detect mRNA levels of ESE-1 and used in chromatin immunoprecipitation (ChIP) assay with A1L 7300 real-time PCR system (Applied Biosystems). The quantitative PCR results were analyzed with software provided. All assays were repeated at least three times.

Lentivirus-mediated delivery of ESE-1 short hairpin RNA. The 293TN packaging cells (System Biosciences) were cotransfected with lentiviral vector pGIPZ (empty) or V2LHS_17990 [encoding ESE-1 hairpin RNA (shRNA); Open Biosystem] and packaging plasmids pPAX2/pMD2.G (Addgene) according to Ref. 34. The packaged lentiviral vectors were transduced into A549 cells in the presence of polybrene (6 μg/ml; Millipore) for 6 h, followed by selecting with puromycin (3 μg/ml). Total RNAs were extracted from the survived A549 cells and used for quantitative RT-PCR to detect ESE-1 mRNA levels with primer EQF (gccagatctcgcagcaac) and EQR (ggacacagctgcttg) or used for semi-quantitative RT-PCR to detect lysozyme mRNAs as described above.

ChIP analyses. ChIP analyses were carried out with ChIP-IT Express Enzymatic Kit (Active Motif) according to the protocol provided by the manufacturer.

Table 1. Sequences of oligos used for EMSA and mutation of putative Ets sites in LYZ proximal promoters

<table>
<thead>
<tr>
<th>Site</th>
<th>Wild-Type Oligos</th>
<th>Mutant Oligos</th>
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<tbody>
<tr>
<td>I</td>
<td>Sense</td>
<td>aaggggaagaaggtgatttaagatatg</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>cattcttttaactctctctctctctctct</td>
</tr>
<tr>
<td>II</td>
<td>Sense</td>
<td>caatagacagctgctgctgctgctgtcctga</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>gttggagaaaaaaggtgctgtctgctgttgg</td>
</tr>
</tbody>
</table>

The changed nucleotides are shown in bold. Ets, E26 transforming specific protein; LYZ, human lysozyme gene.
provided by manufacturer. In brief, three 15-cm dishes of A549 cells (90% confluence) were fixed with 1% formaldehyde for 10 min at room temperature and neutralized with 0.125 M glycine. Released nuclei from collected cells were enzymatically treated at 37°C for 10 min in 1-ml volume, and 50 μl of sheared chromatin were used for immunoprecipitation with normal rabbit serum or polyclonal antibody against ESE-1 (Orbigen). The 110-bp LYZ proximal promoter was amplified with primers LF (gacctaggtgctatgacagtcttcg) and LPR (tagtgctcagggtgtgacctgg). The 130-bp secretoglobin 3A2 (SCGB3A2) promoter contains no consensus Ets sites and was amplified with primers SPF (acccctcaatgttggtggaa) and SPR (gcacacataattatatgccattgag) as negative control to verify the specific enrichment of LYZ proximal promoter fragments by ESE-1 antibody. All ChiP experiments were repeated in triplicate. Specific enrichment of LYZ promoter by ESE-1 antibody was quantified as described above and normalized to the nonspecific precipitation of SCGB3A2 promoter, which does not contain Ets sites.

RESULTS

LYZ promoter (3.0 kb) is active in various epithelial cells. Lysozyme is constitutively expressed in some subtypes of mucosa epithelium (3, 11, 28). To determine if the human lysozyme gene (LYZ, GenBank acc. no. NM_000239) is expressed in NHBE and cell lines derived from nonpulmonary (HeLa) as well as pulmonary epithelia (A549, H292, and H441), RT-PCR was carried out with RNAs extracted from these cells as templates. The full LYZ CDS could not be detected in undifferentiated NHBE and HeLa cells but was readily detected in A549, H441, and H292 cells (Fig. 1A). Interestingly, the differentiated NHBE cells express higher levels of LYZ mRNAs as detected after 2 wk of ALI culture (Fig. 1B). To assess LYZ promoter function across different epithelial cells, a reporter construct containing 3.0 kb of the LYZ promoter (3.0hLyso) was constructed to confer the expression of firefly luciferase gene (luc). This reporter construct exhibited a strong basic transcriptional activity in all cells studied (Fig. 1C), indicating there are positive regulators for LYZ promoter activity in pulmonary and nonpulmonary epithelial cells. These findings suggest that other mechanisms beyond transcriptional activation may regulate LYZ expression across various epithelial cells. Furthermore, these findings provide a basis for studying transcription factor-mediated regulation of LYZ.

Transactivation of the LYZ promoter by ESE-1. ESE proteins are potential candidates for regulating transcription of LYZ due to the coexpression pattern in many mucosal epithelia and numerous putative Ets binding sites located upstream of the LYZ transcription start site. Expression vectors for the six ESE family isoforms were constructed as detailed in MATERIALS AND METHODS and used for cotransfection studies with 3.0hLyso to assess their effects on the LYZ promoter. As shown in Fig. 2A, ESE-1a and ESE-1b dramatically transactivated the LYZ promoter in NHBE, A549, and HeLa cells with ESE-1a exhibiting weaker transactivating capacities, whereas ESE-2 and ESE-3 isoforms differentially transactivated this reporter construct in different cells yet never reaching the levels of transactivation induced by ESE-1.

To test if the ESE proteins could transactivate endogenous LYZ, an individual ESE expression vector was transfected into A549 and HeLa cells. We optimized a semiquantitative RT-PCR protocol based on the primer FF and FR to coamplify the full-length LYZ CDS and internal control GAPDH with QGF and QGR. As shown in Fig. 2B, ESE-1b markedly upregulated transcription of endogenous LYZ in both A549 and HeLa cells. ESE-2 and ESE-3 isoforms could not upregulate the basal transcription of endogenous LYZ in either cell line. The differential transactivating capacities of ESE-1a and ESE-1b are not related to their protein levels as detected by immunoblotting (Fig. 2C). These findings using both promoter-driven reporter
assays and endogenous gene expression indicate that ESE-1 isoforms, particularly ESE-1b, can induce LYZ expression in mucosal epithelial cells.

**Attenuation of ESE-1b transactivation by other ESE members.** Ets transcription factors are known to bind overlapping consensus DNA motif (27), suggesting that competition between coexpressed ESE members may have biological significance such as the ablation of LYZ transcription in A549 cells. To test this in LYZ promoter regulation, ESE-2 and ESE-3 isoform expression vectors were cotransfected in conjunction with the ESE-1b expression vector and 130hLyso. Coexpression of ESE-2a, ESE-3a, or ESE-3b reduced the transactivation of the LYZ promoter by ESE-1b in both A549 and HeLa cells (Fig. 3). Interestingly, coexpression of ESE-2b with ESE-1b did not alter transactivation of the LYZ promoter in A549 cells but was able to attenuate ESE-1b transactivation in HeLa cells, suggesting ESE-2b may have unique cell-specific mechanisms for transactivation of the LYZ promoter. The overall findings suggest that competition by multiple ESE family members for distinct Ets consensus sites may not be a critical mechanism in ESE-mediated transcriptional regulation.

Identification of the proximal promoter sequences responsive to ESE-1b. To map the corresponding ESE-1b-responsive cis element(s) in the LYZ promoter, reporter plasmids containing truncated LYZ promoters conferring the luc reporter gene were constructed (Fig. 4A). Each of the truncated promoter constructs was functional in HeLa, A549, H292, and H441 cells (data not shown). The 0.13-kb truncated promoter was sufficient for transactivation by ESE-1b in both A549 and HeLa cells (Fig. 4B). Interestingly, the 3.0- and 1.7-kb promoters exhibited diminished transactivation by ESE-1b in HeLa cells but not in A549 cells, suggesting that there are potential inhibitory elements functional in the 1.7- to 3.0-kb upstream region in HeLa but not A549 cells. Regardless, the findings from these experiments indicate that the proximal 130-bp LYZ promoter contains cis element(s) responsive to ESE-1b in both pulmonary and nonpulmonary cell lineages.

Two putative Ets sites within the 130-bp proximal LYZ promoter were identified computationally using Match 10.0 of the TRANSFAC database, and these core motifs were designated as motifs I and II, with motif I being the most proximal to the transcription start site (Fig. 4C). To assess whether these sites are responsive to ESE-1b, site-directed mutagenesis was used to alter the core-binding sequence in each putative site with oligos shown in Table 1. Mutation of either motif I or II abolished the transactivation by ESE-1b in A549 cells (Fig. 4D). Similar results were obtained in HeLa cells, with the mutation of motif II showing somewhat less functionality in ESE-1b transactivation than that for motif I. Mutation of either Ets sites resulted in decreased basic transcriptional activities and loss of ESE-1b transactivation in NHBE cells (Fig. 4E), strongly supporting a role for ESE-1b transactivation of the LYZ promoter in primary lung epithelial cells. These findings indicate that both Ets sites located at 39 bp (site I) and 84 bp (site II) upstream of the transcription start site are important for ESE-1b transactivation of the LYZ promoter in pulmonary or nonpulmonary epithelial cells.

**ESE-1b binding of functional Ets sites.** EMSAs were carried out to assess whether ESE-1b could directly bind the two putative Ets sites identified by site-directed mutagenesis. Nuclear proteins from HeLa cells transfected with ESE-1b expression plasmid formed a higher shifted complex with site I or
site II oligos (Fig. 5, arrow; sequence of oligos are shown in Table 1). Competition with 100
extra unlabeled wild-type (wt) site I and site II oligos abolished all the bands shifted as expected, whereas competition with the mutant (mut) site I or site II oligos had little effect on the larger shifted complex. In the absence of available antibodies for supershift analyses, the ESE-1b expression vector was constructed as detailed in MATERIALS AND METHODS with a FLAG peptide tagged on the COOH terminus. Addition of anti-FLAG antibody specifically abolished binding of the wt oligo due to the localization of the FLAG epitope adjacent to the DNA binding domain on the COOH terminus of ESE-1b. ESE-1a showed similar binding

Fig. 4. Cis elements responsive to ESE-1 transactivation are located in the proximal LYZ promoter. A: schematic structure of the truncated LYZ promoter constructs conferring expression of the luc reporter gene. B: the proximal LYZ promoter (130 bp) contains the cis element(s) responsive to ESE-1b transactivation in both A549 and HeLa cells. C: 2 putative Ets sites in the LYZ proximal promoter were identified by TRANSFAC software analysis, and each core motif was labeled as I and II (see MATERIALS AND METHODS). D: both site I (Mut I) and site II (Mut II) are required for transactivation by ESE-1b in A549 and HeLa cells as assessed by site-directed mutagenesis. E: core-motif mutation of either site I or site II resulted in decreased basic transcriptional activities of LYZ promoter and lost or decreased response to ESE-1b transactivation in NHBE cells. Values represent fold changes (means ± SE).
patterns on site I and site II to that of ESE-1b (data not shown), suggesting that the reduced transactivation of the LYZ promoter by ESE-1a is likely not related to its DNA binding activities.

**Endogenous ESE-1b transactivates LYZ.** High levels of ESE-1b but not ESE-1a mRNAs could be detected in A549 cells (data not shown). To assess if endogenous ESE-1b could regulate endogenous LYZ expression, small interfering RNA-mediated knockdown studies were performed. A549 cells were transduced with empty or ESE-1 shRNA encoded lentiviral vectors as detailed in MATERIALS AND METHODS. The shRNA

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**Fig. 5.** Binding of ESE-1b proteins with site I and site II is shown. The FLAG-tagged ESE-1b expression vector was transfected into HeLa cells, and nuclear proteins were extracted for gel shift assay. The additional band specifically shifted by ESE-1b is shown next to the arrow. Binding of wild-type (wt) oligos was wrestled away by excess unlabeled site I or site II oligos but not by mutated excess unlabeled oligos. Sequence of oligos is shown in Table 1. The anti-FLAG antibody specifically abolished the binding of ESE-1b. mut, mutant oligos.
recognizes both ESE-1a and ESE-1b mRNA targets; however, ESE-1b has much greater expression than ESE-1a in A549 cells (data not shown). As shown in Fig. 6A, the LYZ mRNA levels decreased concomitantly with knockdown of ESE-1 mRNA, indicating endogenous LYZ is a transcriptional target of ESE-1. To further assess ESE-1b binding to the endogenous LYZ promoter, ChIP was employed as detailed in MATERIALS AND METHODS. The rabbit polyclonal antibody against ESE-1 (α-ESE-1), but not the normal rabbit sera, specifically enriched the 110-bp LYZ promoter containing the ESE-1b-responsive elements. The 110-bp proximal SCGB3A2 promoter contains no consensus Ets sites and was used as a negative control, and no differences could be detected, indicating the specific enrichment of LYZ proximal promoter by ESE-1 antibody. These findings together support that endogenous LYZ is a target for endogenous ESE-1b transactivation in lung epithelial cells.

DISCUSSION

The findings presented here indicate the LYZ promoter as a target for the highly tissue-restricted Ets transcription factor ESE-1 in lung epithelia. Interestingly, isoforms encoded by ESE-1 exhibited differential transactivating capacities, with ESE-1b showing greater transactivation activity than ESE-1a in lung epithelial cells. Transactivation of the LYZ promoter by ESE-1b was confined to the proximal 130-bp promoter region containing two functional Ets sites as identified by site-directed mutagenesis analysis and DNA binding assays. Isoforms encoded by ESE-2 or ESE-3 activated the LYZ promoter only marginally. Studies utilizing experimental approaches of both overexpression and endogenous ESE-1b proteins on regulating LYZ confirmed these findings. These studies provide strong evidence for the role of the ESE-1 in conferring LYZ expression in epithelial lineage cell types and extend our knowledge of transcriptional mechanisms regulating lung epithelial-specific gene expression.

The ESE transcription factors constitute an emerging subgroup of the Ets family of winged helix-turn-helix DNA-binding protein (27). Of the three ESE members, the first identified protein ESE-1 has been well-studied and shown to have multiple functions. ESE-1 can transactivate numerous genes including squamous differentiation marker SPRR1 (25), angiopoietin-1 (5), transforming growth factor-β type II receptor (9, 10, 16), inducible nitric oxide synthase (26), cyclo-
oxygenase 2 (13), and MIP-3α (17). Studies relating ESE-1 to tissue functions have primarily focused on regulating differentiation of keratinocytes (6) and corneal epithelia (32) as well as involution of mammary glands during development (22). The findings presented here indicate a role for ESE-1 in regulation of lung epithelial gene expression. Despite the nomenclature of ESE, recent reports indicate ESE family members are expressed in nonepithelial cell types (2, 13, 31), suggesting that their function may extend beyond tissues of epithelial origin. In light of this and the notion that lysozyme can be expressed by nonepithelial cells under certain activated epithelial origin, the expression of nonepithelial cells under certain activated conditions, other transcriptional mechanisms may exist for LYZ gene expression.

Although ESE members demonstrate different target gene specificity (15), the studies presented here also indicate that ESE isoforms probably have unique roles as well. For example, ESE-1b markedly upregulated transcription of endogenous LYZ and LYZ promoter activity in both pulmonary and non-pulmonary epithelial subsets, whereas ESE-1a only marginally upregulated transcription of endogenous LYZ in nonpulmonary epithelial cells. Compared with ESE-1a, ESE-1b has additional amino acids (23) following the acidic transactivation domain encoded by exon 4 (7). This additional stretch of peptide sequence appears to confer stronger transactivating capacities to ESE-1b on LYZ in multiple epithelial cell types. Compared with ESE-2 or ESE-3 isoforms, ESE-1 isoforms have an additional DNA binding domain designated as the “A/T hook” located between the NH2-terminal “pointed domain” and the COOH-terminal Ets domain (1, 23). The role of this A/T hook domain in the LYZ promoter requires further investigation; however, the findings presented here would suggest this region to be critically important in LYZ transactivation.

One critical element in defining the role of novel transcription factors is the delineation of their cis elements for future comparisons across multiple promoters. Ets proteins have a highly conserved DNA binding domain, suggesting these transcription factors may bind similar consensus cis element(s) (27). However, a recent report indicates that different Ets proteins can bind and transactivate unique sets of promoter (14). Our findings reiterate the importance of functional analysis specific to each gene target. In addition, our results are consistent with the notion that ESE transactivation is specific to certain family members with regards to LYZ transactivation, and such specificity may be defined by the binding of other transcription factors to flanking sequences adjacent to the Ets motifs. Indeed, DNA binding studies of Ets consensus motifs identified nuclear proteins that bound to elements aside from Ets sites. Future identification of ESE-1 responsive gene promoters will assist in the verification of specific consensus sites and interactions with other transcription factors.

The data presented here provide new insights into the role of ESE transcription factors in regulation of host defense genes in mucosal tissues. Further studies will be necessary to assess whether ESE-mediated regulation extends to other epithelial-specific host defense components such as defensins, chitinases, and other antimicrobial proteins. Additionally, further studies will be needed to assess the roles of ESE proteins in conjunction with other transcription factors in epithelial-specific regulatory networks.

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