AD_____

Award Number: W81XWH-06-1-0502

TITLE: Identification of Cytoplasmic Proteins Interacting with the Mammary Cell Transforming Domain of Ese-1

PRINCIPAL INVESTIGATOR: Arthur Gutierrez-Hartmann, MD

CONTRACTING ORGANIZATION: University of Colorado HSC
Aurora, CO 80045

REPORT DATE: April 2007

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01/4/2007	Annual	29 Mar 2006 – 28 Mar 2007

4. TITLE AND SUBTITLE

Identification of Cytoplasmic Proteins Interacting with the Mammary Cell Transforming Domain of Ese-1

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-06-1-0502

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Arthur Gutierrez-Hartmann, MD

E-Mail: a.gutierrez-hartmann@uchsc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Colorado HSC
Aurora, CO 80045

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: The Ets family of transcription factors contains several members that are important components of the cellular pathways leading to tumorigenesis. The Ese-1 gene is an Ets member that is particularly relevant to breast cancer. Ese-1 is located on chromosome 1q32.1, in a region that is amplified in 50% of early breast cancers. Ese-1 mRNA is over-expressed in human breast ductal carcinoma in situ (DCIS). We recently discovered that Ese-1 transforms MCF-12A cells via a novel cytoplasmic mechanism in which a unique 40-amino acid (AA), serine- and aspartic acid rich (SAR) domain is necessary and sufficient for transformation. Furthermore, we reported that Ese-1 protein is abundantly expressed in the cytoplasm of human ductal carcinoma in situ (DCIS) specimens. However, identification of the cytoplasmic partners of the Ese-1 SAR motif and the precise mechanism by which cytoplasmic signaling mediated by the Ese-1 SAR motif occurs remain unknown. In this application, we propose to take advantage of this novel paradigm. Specifically, we plan to apply innovative and powerful state-of-the-art molecular yeast two-hybrid and protein biophysical methods (MALDI-TOF and LC-mass spectrometry) to identify proteins that interact with the Ese-1 SAR transformation domain. We will then validate which partners of Ese-1 are functionally relevant to breast cancer. We have made diligent progress, having identified a putative interactor, which we are validating.

15. SUBJECT TERMS
ETS, Ese-1, Esx, MCF-12A, transformation, mammary, breast cancer

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	18	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

INTRODUCTION

The Ets family of transcription factors contains several members that are important components of the cellular pathways leading to tumorigenesis (1). For example, several Ets members are downstream targets of oncogenic Ras (2); dominant-negative Ets reverses the transformed phenotype (3,4); and, Ets proteins have been shown to regulate a repertoire of genes that govern cellular survival, proliferation and migration (1,6). Moreover, several Ets factors have been implicated in breast cancer (1,6). However, the ability of Ets factors to transform human breast cells, the identity of the precise Ets factor required for breast cell transformation, and the molecular mechanism by which such an Ets factor mediates breast cell transformation, all remain unknown. The Ese-1 gene is an Ets member that is particularly relevant to breast cancer. Ese-1 is located on chromosome 1q32.1, in a region that is amplified in 50% of early breast cancers. Ese-1 mRNA is over-expressed in human breast ductal carcinoma in situ (DCIS) (7-9). Also, there is a positive feedback loop between the HER2/neu proto-oncogene and Ese-1, in that HER2/neu activation induces Ese-1 expression, while Ese-1 activates the HER2/neu promoter via a putative ESX DNA binding site (7-9). Finally, HER2/neu and Ese-1 expression levels are positively correlated in human breast cancer cell lines (7-9). While some of the most important advances in our understanding and treatment of breast cancer have come about by studying regulatory proteins that reside in the cell nucleus and control gene expression, such as the estrogen and progesterone receptors (ER and PR), an increasing number of studies have shown that PR and ER mediate critical functions via nonnuclear mechanisms by activating cytoplasmic signaling components. We recently discovered that Ese-1 transforms MCF-12A cells via a novel cytoplasmic mechanism in which a unique 40-amino acid (AA), serine- and aspartic acid rich (SAR) domain is necessary and sufficient for transformation (10-12). Furthermore, we reported that Ese-1 protein is abundantly expressed in the cytoplasm of human ductal carcinoma in situ (DCIS) specimens (12). However, identification of the cytoplasmic partners of the Ese-1 SAR motif and the precise mechanism by which cytoplasmic signaling mediated by the Ese-1 SAR motif occurs remain unknown. Current data reveals that this 40-AA SAR domain functions autonomously, indicating that this motif is sufficient to recognize and activate cytoplasmic partner proteins, thus transforming MCF-12A human mammary epithelial cells (12). This is a paradigm-shifting observation that has important clinical relevance, since usual chemotherapies target nuclear events. Based on published observations and our own work indicating that Ese-1 is likely a key factor in some of the more aggressive forms of human breast cancer, we have chosen to place a significant amount of effort elucidating Ese-1’s mechanisms of action. The unifying hypothesis of this application is that the Ese-1 SAR motif is a modular functional domain that is required for breast cell transformation and that such a domain functions by interacting with specific cytoplasmic proteins. In this application, we propose to take advantage of this novel paradigm. Specifically, we plan to apply innovative and powerful state-of-the-art molecular yeast two-hybrid and protein biophysical methods (MALDI-TOF and LC-mass spectrometry) to identify proteins that interact with the Ese-1 SAR transformation domain. We will then validate which partners of Ese-1 are functionally relevant to breast cancer. We anticipate that the Ese-1 protein partners identified here, and the genes which these Ese-1 "transformation partner" combinations regulate, will provide several new drug targets to use in our battle against this dreaded malignancy.

BODY

Task 1: To purify and identify SAR-binding cytoplasmic proteins (SBCPs).

1. screen a human fetal brain cDNA library fused to the Gal4AD SBCPs, using a Gal4DBD-SAR fusion as bait.

We have made diligent progress towards accomplishing this aim. Specifically, we cloned the 40-AA SAR domain into the Gal4DBD "bait" vector and documented that it is expressed by Western blot analysis and that it does not contain any TAD activity in yeast. We completed a screen using a human fetal brain cDNA library fused to the Gal4 TAD domain, screening ~2.6 x 10^6 clones on His-minus plates. This yielded ~300 colonies of which ~30 grew rapidly and to a large size.

2. perform subsequent screens to rule out false-positives, and then sequence and identify true-positives.

We re-screened these 300 colonies on His-minus, X-Gal and quadruple nutrition marker drop-out plates, and identified 12 clones that pass the criteria to be "true-positives", because they grew rapidly and robustly on each of these three screens. DNA sequence analysis and BLAT searches identified three plausible candidates: Rho Guanine Dissociation Inhibitor (Rho GDI), Na/K ATPase β-1, and a proposed Breast Cancer Associated protein (BCA3).

3. affinity-purify SBCPs from MCF-12A cells transduced with retroviruses encoding either TAP-tagged NES-SAR and NES-mSAR.

We also proposed to use a mammalian tandem-affinity purification (TAP) tag fused to NES-SAR and a transformation-defective mutant, NES-mSAR, to purify SBCPs from MCF-12A cells. The TAP tag consists of two protein A IgG-binding domains and the Flag epitope, separated by a tobacco etch virus protease site. Retroviruses encoding TAP-NES-SAR or TAP-NES-mSAR have now been constructed, and transient transfection of plasmid DNAs into MCF-12A cells unfortunately failed to show any detectable protein expression. After several attempts. We concluded that the cloning vector must be corrupted. Thus, we have amino terminally-tagged these constructs with a single- and triple-Flag epitope. We are now optimizing transfection and characterizing expression levels of GFP- and Flag-rtagged versions of all of our SAR constructs. Once characterized, we will transfect breast cancer cells in large scale and affinity-purification will be accomplished by binding cytoplasmic extracts to IgG immobilized on magnetic beads. The beads will be eluted with excess Flag or GFP peptide, and eluted SAR-binding proteins submitted to directly LC/electrospray-ionization mass identification.

Task 2: To validate that the identified SBCP is a bona fide downstream effector of Ese-1-mediated cellular transformation.

a) perform co-immunoprecipitation studies to rigorously validate authentic in vivo interactions between endogenous (and recombinant) Ese-1 and identified SBCP(s).

Given that the yeast two-hybrid approach has provided the only SBCPs identified to date, we focused on these three poteins: Rho GDI, Na/K ATPase β-1, and BCA3. We have attempted multiple co-IP experiments using antibodies targeting epitope-tagged recombinant forms of these three putative SBCPs and recombinant and endogenous Ese-1. These studies indicate that RhoGDI may be the only relevant SBCP. Further studies are

underway to rigorously prove this interaction, and to determine whether it is functionally relevant to the Ese-1 transformation mechanism.

b) loss-of-function: RNAi knock-down of identified SBCPs to substantiate their biological relevance in mediating Ese-1- and SAR-induced transformation.

RNA interference studies are being designed to target RhoGDI, to directly interrogate whether knock-down of this putative SBCP will decrease the transformation potency of Ese-1 in MCF-12A and MCF-10A nontransformed human mammary epithelial cells.

c) gain-of-function: Enhancement of Ese-1 and SAR-mediated transformation potency by SBCP over-expression in MCF-12A cells.

Over-expression of HA-Ese-1 ± recombinant RhoGDI in MCF-12A and MCF-10A nontransformed human mammary epithelial cells will be pursued in order to determine whether over-expression of this putative SBCP will increase the transformation potency of Ese-1.

Task 3: To determine the molecular mechanism of Ese-1/SBCP-mediated mammary cell transformation.

These studies have not yet been initiated, since they depend on the previous aims.

KEY RESEARCH ACCOMPLISHEMENTS

- Identification of RhoGDI as a putative SBCP and Ese-1-interacting protein.
- Generation of Flag-tagged SAR and Ese-1 expression vectors.

REPORTABLE OUTCOMES

Abstracts:

1. Walker D, Prescott JD, **Gutierrez-Hartmann A**. Identification of ESE1 SAR-Domain Interacting Proteins that Mediate a Novel Cytoplasmic Transformation Mechanism. 97th Annual Meeting of the AACR, Washinton DC, April, 2006.
2. Prescott JD, Walker D, Tentler JJ, Poczobutt J, Schedin P, **Gutierrez-Hartmann A**. Ese-1 Transforms Human Mammary Epithelial Cells via a Unique 40-AA Domain Acting Through a Novel Cytoplasmic Mechanism. Mammary Gland Gordon Research Conference, Il Ciocco, Italy, June, 2006.

Manuscripts

1. **Gutierrez-Hartmann A**, Duval DL, Bradford AP. ETS transcription factors in endocrine systems. *Trends Endocrinol Metab*, in press, 2007.

Reagents Developed

1. Flag-tagged GFP-SAR and GFP-Ese-1 expression vectors.
2. Cell lines stably expressing some of the above.

Doctoral training

1. Darius Walker, an URM working on this project, is completing his PhD thesis studies in the Molecular Biology Graduate Program.

CONCLUSIONS

We have just completed the fist year of this work, and we have identified RhoGDI as a putative SAR binding cytoplasmic protein using the yeast two hybrid approach, and preliminary co-IP studies suggest that this may be a relevant target. We plan to continue our studies, essentially as planned. We have initiated the co-IP affinity-purification approach, coupled with protein identification via mass spectrometry. We believe that we are on track for a timely completion of all of the proposed studies.

REFERENCES

1. Wasylyk, B, and Nordheim, A**.** 1997. Ets transcription factors: Partners in the integration of signal responses., p. 251-284. *In* A. Papavassiliou (ed.), Transcription Factors in Eukaryotes. Landes Bioscience.
2. Wasylyk, B, Hagman, J, and Gutierrez-Hartmann, A**.** 1998. Ets transcription factors: nuclear effectors of the Ras/MAP kinase signaling pathway. Trends Biochem. Sci. 23**:**213-216.
3. Sapi, E, Flick, M, Rodov, S, and Kacinski, B**.** 1998. Ets-2 transdominant mutant abolishes anchorage-independent growth and anchorage-stimulating factor-stimulated invasion by BT20 breast carcinoma cells. Cancer Res. 58**:**1027-1033.
4. Delannoy-Courdent, A, Mattot, V, Fafeur, V, Fauquette, W, Pollet, I, Calmels, T, Vercamer, C, Boilly, B, Vandenbunder, B, and Desbiens, X**.** 1998. The expression of Ets-1 transcription factor lacking its activation domain decreases uPA proteolytic activity and cell motility, and impairs normal tubulogenesis and cancerous scattering in mammary epithelial cells. J. Cell Sci. 111**:**1521-1534.
5. Galang, C, Garcia-Ramirez, J, Solski, P, Westwick, J, Der, C, Neznanov, N, Oshima, R, and Hauser, C**.** 1996. Oncogenic Neu/ErbB-2 increases, Ets, AP-1 and NF-kB-dependent gene expression, and inhibiting Ets activation blocks Neu-mediated cellular transformation. J. Biol. Chem. 271**:**7992-7998.
6. Janknecht, R, and Nordheim, A**.** 1993. Gene regulation by Ets proteins. Biochim. Biophys. Acta 1155**:**346-356.
7. Chang, C, Scott, G, Kuo, W, Xiong, X, Suzdaltseva, Y, Park, J, Sayre, P, Erny, K, Collins, C, Gray, J, and Benz, C**.** 1997. ESX: A structurally unique Ets overexpressed early during human breast tumorigenesis. Oncogene 14**:**1617-1622.
8. Oettgen, P, Alani, R, Barcinski, M, Brown, L, Akbarali, Y, Boltax, J, Kunsch, C, Munger, K, and Libermann, T**.** 1997. Isolation and characterization of a novel epithelium-specific transcription factor, ESE-1, a member of the *ets* family. Mol. Cell. Biol. 17**:**4419-4433.
9. Tymms, M, Ng, A, Thomas, R, Schutte, B, Zhou, J, eyre, H, Sutherland, G, Seth, A, Rosenberg, M, Papas, T, Debouck, C, and Kola, I**.** 1997. A novel epithelial-expressed ETS gene, ELF3: Human and murine cDNA sequences, murine genomic organization, human mapping to 1q.32.2 and expression in tissues and cancer. Oncogene 15**:**2449-2462.
10. Eckel KL, J.T. Tentler, G.J. Cappetta, S.E. Diamond, Gutierrez-Hartmann A. The epithelial-specific ETS transcription factor ESX/ESE-1/Elf-3 modulates breast cancer-associated gene expression. *DNA & Cell Biol*. 22:79-94, 2003.

11. Schedin PJ, Eckel KL, McDaniel SM, Prescott JD, Brodsky KS, Tentler JJ, Gutierrez-Hartmann A. ESX induces transformation and functional epithelial to mesenchymal transition in MCF-12A mammary epithelial cells. *Oncogene* 23:1766-1779, 2004.
12. Prescott JD, Koto K, Singh M, Gutierrez-Hartmann A. The ETS transcription factor ESE-1 transforms MCF-12A mammary epithelial cells via a novel cytoplasmic mechanism. *Mol Cell Biol* 24:5548-5564, 2004.

APPENDICES

Enclosed is a draft of our paper: **Gutierrez-Hartmann A**, Duval DL, Bradford AP. ETS transcription factors in endocrine systems. *Trends Endocrinol Metab*, in press.

ETS transcription factors in endocrine systems

Arthur Gutierrez-Hartmann[1], Dawn L. Duval[2,3] and Andrew P. Bradford[1]

[1]University of Colorado HSC, P. O. Box 6511, Mail Stop 8106, Aurora, CO 80045, USA
[2]University of Colorado HSC, 4200 E. 9th Ave., Campus Box C-238, Denver, CO 80262, USA
[3]Present address: Department of Clinical Sciences, Animal Cancer Center, Colorado State University, 300 West Drake Road, Fort Collins, CO 80523, USA
Corresponding author: Gutierrez-Hartmann, A. (a.gutierrez-hartmann@UCHSC.edu).

E26 transformation-specific (ETS) transcription factors have become increasingly recognized as key regulators of differentiation, hormone responses and tumorigenesis in endocrine organs and target tissues. The ETS family is highly diverse, consisting of both transcription activators and repressors that mediate growth factor signaling and regulate gene expression through combinatorial interactions with multiple protein partners on composite DNA elements. ETS proteins have a role in the endocrine system in establishing pituitary-specific gene expression, mammary gland development and cancers of the breast, prostate and reproductive organs.

Introduction: structure and function of ETS proteins

The E26 transformation-specific (ETS) family is restricted to metazoans and thus represents an evolutionarily recent class of transcription factors [1–3]. It is one of the largest transcription factor families, consisting of 27 *ETS* genes in humans, 26 in mice, 10 in *Caenorhabditis elegans* and 9 in *Drosophila* that can be structurally categorized into 11 subfamilies (ETS, ERG, ELG, ELF, ESE, ERF, TEL, PEA3, SPI, TCF and PDEF) [4,5] (Figure 1). The ETS family is defined by the ETS domain, which is a highly conserved DNA-binding domain (DBD) comprising ~85 amino acids (AAs) that is folded into a winged helix-turn-helix DNA-binding motif that binds to a 5′-GGA(A/T)-3′ DNA core motif [2]. All ETS proteins, with the exception of GA-binding protein (GABP)α, bind to DNA as a monomer and are auto-inhibited by virtue of two inhibitory regions that flank the DBD. Disinhibition, resulting in enhancement of ETS DBD activity and of target gene specificity, is achieved by three mechanisms: (i) flanking DNA sequences; (ii) partner–protein interactions; and (iii) kinase-mediated phosphorylation. ETS protein activation of target genes typically involves specific protein–protein interactions and such ETS–protein partner combinations frequently bind to bipartite DNA-binding sites [1–3]. Thus, although target gene selectivity of ETS factors is influenced by specific protein partners, the precise mechanism for achieving DNA-binding specificity, given the high level of redundancy, remains unclear [4,5]. Many ETS subfamilies (ETS, ERG, ELG, ESE, TEL and PDEF) contain the Pointed domain, which serves as a protein–protein interaction motif, several (TEL, ERF and TCF) contain a repressor domain and the majority (ETS, ERG, ELG, PEA3, ESE, SPI and TCF) contain a transcription activation domain (TAD) [1–3]. The activity of certain ETS factors is further regulated by subcellular localization. For example, YAN and TEL (members of the TEL subfamily) NET (also called SAP2, a member of the TCF subfamily) and ERF also contain a nuclear export sequence regulated by mitogen-activated protein kinases (MAPKs) or small ubiquitin-like modifiers (SUMOs), which controls their transcriptional repression activity [2]. By contrast, a cytoplasmic localization is required for ESE1 to transform MCF-12A and MCF-10A human mammary epithelial cell lines [6].

ETS factors are *trans*-acting phosphoproteins that have important roles in epithelial, hematopoietic, neuronal, endothelial and endocrine systems with key roles in cell migration, proliferation, differentiation and oncogenic transformation [1–3,7]. The founding member is encoded by the v-*ets* oncogene in the E26 retrovirus, which causes hematopoietic malignancies in chickens [1–3]. In humans, ETS factors are also associated with several malignancies. For example, chromosomal translocations involving *ETS* genes are found in 95% of human Ewing sarcoma cases and in several human hematopoietic malignancies [1]. In addition, amplification or upregulation of one or more *ETS* genes, including *ETS1*, *ETS2*, *ER81*, *ERM*, *PEA3*, *PDEF* and *ESE1*, is associated with a variety of epithelial cancers (e.g. lung, breast, colon and prostate) [1,2,7]. In mammalian cells, ETS proteins are key nuclear targets of growth factor and *Ras* oncogene signaling pathways, typically acting through the activating protein-1–ETS bipartite Ras response element (RRE) to regulate a repertoire of genes that control cell survival (anti-apoptosis), proliferation and motility [1–3]. Here, we focus on ETS factors in endocrine systems (Figure 2), with particular emphasis on their role in the regulation of pituitary-specific gene expression and tumorigenesis.

ETS factors regulating pituitary function

ETS factors have crucial roles in pituitary gonadotrope and lactotrope biology. Gonadotropes and lactotropes are two of the five hormone-secreting cell types that populate the anterior pituitary gland. Gonadotropes synthesize and secrete the glycoprotein hormones luteinizing hormone (LH) and follicle stimulating hormone (FSH), which regulate gonadal function. Lactotropes produce the protein hormone prolactin (PRL), which stimulates breast development and lactation. Hypothalamic gonadotropin-releasing hormone (GnRH) regulates the production and secretion of LH and FSH in pituitary gonatotropes. Stimulation by GnRH leads to MAP kinase activation and

ETS factor-mediated induction of the α-subunit gene through a GnRH response element [8]. The α-subunit is a common heterodimeric component of the gonadotrope-specific LH and FSH glycoprotein hormones. Activation of ETS factors in response to GnRH stimulation also induces the expression of the immediate-early gene *Egr-1* through serum response elements [9]. Egr-1, in turn, is a primary activator of the LHβ-subunit gene in response to GnRH stimulation [8]. Thus, ETS factors are crucial mediators of the signaling pathways that regulate the responses of pituitary gonadotropes to hypothalamic GnRH stimulation (Figure 3a).

Pituitary lactotropes exhibit even greater dependence on ETS transcription factors for cellular growth, differentiation and PRL gene expression. Lactotrope cell growth and PRL synthesis and secretion are under tonic inhibitory regulation through hypothalamic-secreted dopamine acting on D2 receptors. The ERF repressor factor is expressed in pituitary lactotrope cell lines and it might be a key contributor to the negative effects of dopamine on PRL gene transcription and lactotrope cell growth [10]. Specifically, dopamine-mediated inhibition of extracellular-signal-regulated kinase (ERK) 1 and ERK2 activity increases ERF repressor action on the rat (r) PRL promoter, whereas growth factor-stimulated ERK activity reverses the repressor function of ERF through ERF phosphorylation [10].

ETS factors also regulate lactotrope differentiation. Pit-1 is a POU homeodomain, pituitary-specific transcription factor that governs the ontogeny of somatotrope, lactotrope and thyrotrope cell types and regulates growth hormone (GH), PRL and thyroid-stimulating hormone β gene transcription [11,12]. In GHFT pituitary precursor cells, which express low levels of Pit-1 but fail to express endogenous PRL or GH, *PRL* gene expression and the lactotrope phenotype is induced only by fibroblast growth factor-2 (FGF-2), and in an ETS-dependent fashion [13]. Specifically, this FGF-2 differentiating response was inhibited by a dominant-negative ETS construct and was augmented by the expression of ETS1. In the GH4 rat somatolactotrope cell line, FGF-2- and FGF-4-mediated induction of rPRL promoter activity is ETS dependent [14], and GH4 cells stably expressing FGF-4 produce tumors that are faster growing and more invasive when injected into Wistar rats [15]. Interestingly, the cooperation of ETS factors with Ikaros, a zinc-finger transcription factor, also regulates expression of the FGF-R4 isoform of the FGF receptors in GH4 somatolactotrope cells [16]. In addition, stable expression of a dominant-negative ETS construct (which encodes only the ETS-2 DBD) in GH4 cells was found to decrease PRL expression but had no effect on GH expression [17]. Furthermore, targeted expression of this same dominant-negative ETS construct to pituitary lactotropes in transgenic mice resulted in diminished lactotrope cell numbers (J. Tentler and A.G.H., unpublished). Taken together, these data reveal that ETS factors might represent a crucial determinant in both dopamine-mediated inhibitory lactotrope regulation and in growth factor-induced ontogeny of pituitary lactotropes.

One of the most characterized systems that best exemplifies the roles of various ETS factors in endocrine gene regulation is the lactotrope-specific *PRL* gene (Figure 3b). The proximal rPRL promoter contains four regions that bind to nuclear proteins derived from GH4 or GH3 rat pituitary somatolactotrope cells, with three of these regions [footprint (FP) I, III and IV] binding to Pit-1 [11,12]. An additional site was identified in promoter mutagenesis studies as the basal transcriptional element (BTE) [11,12]. The BTE site contains an ETS-binding site that is crucial for both basal activity of the PRL promoter and for responses to growth factors, including FGF-2, FGF-4, insulin, insulin-like growth factor, epidermal growth factor (EGF) and thyrotropin-releasing hormone [11,12,18]. Although electrophoretic mobility shift analyses have shown that the BTE site can bind to a variety of ETS factors, including ELK1, SAP1 (also known as ELK4), ETS1 and GABPα (together with its non-DNA-binding partner GABPβ1), BTE DNA-affinity purification of nuclear proteins from GH3 pituitary tumor cells identified the heterodimeric ETS factor GABPα–β1 as the key functional ETS factor binding to the BTE site [18]. Subsequent downregulation of the GABPα and GABPβ1 subunits using small interfering RNA strategies in GH3 cells reduced the expression of endogenous PRL and established the role of GABP as a crucial regulator at the BTE in the PRL promoter [18].

A second crucial ETS regulatory site in the proximal rPRL promoter is a composite RRE, which is constituted by an ETS-binding site adjacent to a Pit-1 binding site, FP IV [3,19]. The *rPRL* RRE binds to ETS1 and Pit-1 and primarily mediates Ras activation of the *rPRL* promoter, but it also contributes to basal activity. Oncogenic V12Ras activates the Raf–MAPK kinase–MAPK signaling cascade, with MAPK directly phosphorylating chicken ETS1 [3]. Site-specific mutation of the ETS1 Thr82 MAPK phosphorylation site to Ala results in the loss of ETS1 enhancement of the Ras response [3]. Mutation of either the ETS1 or Pit-1 site diminishes the Ras response of the *rPRL* promoter in transient transfections of GH4 cells [19]. Similarly, expression of a dominant-negative ETS construct or Pit-1β (an alternatively spliced isoform that functions as a dominant-negative effector in pituitary cells) represses Ras activation of the *rPRL* promoter [3]. Contributing to this ETS–Pit-1 combinatorial code is the physical interaction of the TAD of ETS1 with the Pit-1 homeodomain (AAs 199–291), as demonstrated using NMR approaches [20] (Figure 4). In addition, Pit-1 phosphorylation at Thr220, within the homeodomain, regulates the binding of Pit-1 to both ETS1 and the monomeric Pit-1 binding site within the RRE [20]. However, Pit-1 is also a unique cell-specific target for Ras signaling, and mutation analyses of Pit-1 have localized Ras responsiveness to AAs 60–80, at the C-terminus of the TAD [21]. This Pit-1 TAD region contributes to the Ras response through the p160 steroid receptor coactivator-1 [21]. Thus, the binding of Pit-1 and ETS1 to the composite RRE in the proximal *rPRL* promoter seems to generate a unique binding platform for Ras-stimulated coactivator complexes.

ETS proteins in mammary gland development and breast cancer

During embryogenesis, ETS1, PEA3, ERM, ER81 and ESE1 are expressed in the mammary gland, and the PEA3 subfamily members PEA3, ERM and ER81 have been shown to be expressed throughout mammary gland development [7,22–24]. *In situ* hybridization approaches have revealed that ERM and PEA3 are expressed in epithelial cells of the developing terminal end buds, suggesting that they have a role in branching morphogenesis [24]. *PEA3* knockout mice show an increased number of proliferating cells and increased terminal end buds, implying that PEA3 functions as a repressor of mammary epithelial cell growth [24].

In postnatal mammary glands, ETS factors have been shown to have key roles in pregnancy-induced, PRL-mediated mammary gland lobuloalveolar development and milk production and in breast tumorigenesis. In the early phase of pregnancy, a proliferative phase of mammary alveolar morphogenesis mediated by PRL and signal transducer and activation of transcription (STAT)5 is initiated, and the PRL–STAT5 pathway is responsible for the development of lobuloalveoli and the induction of lactation. Studies using *elf5* and *prlr* gene knockout mice and rescue by targeted re-expression of *elf5* in *prlr* nullizygous mammary epithelium revealed that the ETS factor Elf5 (also known as Ese-2) is the crucial downstream effector of the PRL–STAT5 signaling pathway [25,26]. Thus, Elf5 (Ese-2) is necessary and sufficient for lactation-competent mammary gland development during pregnancy.

Several different lines of evidence suggest that ETS factors have a particularly relevant role in breast cancer. Although many ETS factors have been shown to be overexpressed in breast cancer, most of these studies have focused on mRNA rather than protein expression, and few have examined compartment-specific expression [7,22–24]. A recent comprehensive quantitative PCR and subtractive tissue analyses of 25 different ETS factors in normal and malignant murine mammary gland tissues and cell lines revealed that multiple ETS factors are expressed in lymph, stromal and epithelial mammary compartments [4]. For example, ELK4, ELF1 and ETS2 are the most abundant ETS factors expressed in the normal mammary gland, whereas PDEF, PEA3, ESE1, ESE2, ESE3, TEL (also known as ETV6),and NERF (also known as ELF2) mRNAs displayed significantly elevated expression in the epithelial cell compartment of mammary tumors [4]. Important data revealing a direct role of ETS proteins in mammary tumorigenesis have been reported, showing that a dominant-negative ETS2 DBD can block the anchorage-independent growth and cellular invasiveness of the NmuMG, MMT and BT20 breast cancer cell lines [27,28]. However, some of the most convincing reports stem from transgenic mice studies, showing that compound *ets2* knockout MMTV–polyoma middle T (PyMT) mice, in which one e*ts2* allele has been knocked out, are more resistant to PyMT-induced breast tumorigenesis than are their wild-type counterparts [29]. Similarly, the generation of female mice expressing a homozygous targeted *ets2* gene, *ets2(A72/A72)*, which precludes ERK phosphorylation of Thr72, blocked mammary tumors caused by transgenic-targeted oncogenes and seemed to do this exclusively through a stromal location [30,31]. Additionally, using immunohistochemical and western blotting approaches, several studies have revealed that specific ETS proteins, including members of the ETS, PEA3 and ESE subfamilies, are upregulated in breast cancer tumors and cell lines [7,22–24]. Although increased PEA3 and PDEF (also called PSE) mRNAs have been associated with breast cancer [7,22–24], their precise role in mammary tumorigenesis has been controversial. Studies testing their ability to transform mammary epithelial cells reveal that PEA3 and PDEF actually inhibit breast cancer cell growth [32–35]. Thus, although most reports implicate ETS factors in breast cancer, only the ETS factor ESE1 has been shown to confer an epithelial-to-mesenchymal transition phenotype and actually to transform human mammary epithelial cells [6,36–38].

ESE1 (also known as ESX or ELF3) is an epithelial-specific ETS protein that is particularly relevant to breast cancer because the *ESE1* gene maps to human chromosome 1q32.1, in a region that is overrepresented in 50% of early breast cancers [7]. ESE1 mRNA and protein is overexpressed in human breast ductal carcinoma *in situ*, an early cancer stage that also overexpresses HER2 (also known as neu) [7]. Furthermore, a positive feedback loop between the *HER2* proto-oncogene and ESE1 seems to exist [7]. ESE1 contains several unique features among ETS factors. For example, its mRNA expression is restricted to the mammary ductal epithelia and terminal ductal–lobular units, which are the most terminally differentiated, epithelial-derived cells [7]. Unique to ESE1 among ETS proteins are a 40-AA serine- and aspartic rich (SAR) motif and an HMG-like AT-hook domain, in addition to the Pointed, TAD and ETS DBD domains found in most ETS factors [7] (Figure 5a). Recent work revealed that ESE1 is required for the transformed phenotype of $HER2^+$ T47D breast cancer cells [36], that it transforms the immortalized but nontransformed MCF-12A and MCF-10A human mammary epithelial cells and that it does so through an autonomously functioning, unique 40-AA SAR domain acting through a novel cytoplasmic mechanism [6,37] (Figure 5b). A subsequent study screening ~250 cDNAs implicated in breast cancer independently identified ESE1 as being able to confer a motile phenotype upon MCF-10A cells, resulting in disorganized organoids in 3D culture [38], as previously reported [37]. The discovery that ESE1 functions in the cytoplasm to transform mammary epithelial cells established novel paradigms for ETS factor function and mechanisms by which transcription factors induce cell transformation.

ETS factors in the prostate

The expression of several ETS factors in the normal and/or cancerous prostate has been reported, including ETS1, ETS2, ELF1, ESE2 (also called ELF5), ER81, ERG, PDEF and PEA3, with ETS fusions having become one of the most common genetic markers of prostate cancer [1,39].

The ETS factor PDEF/PSE was originally isolated as a transcriptional regulator of the prostate-specific antigen gene [7]. Subsequently, PDEF/PSE mRNA was found to be increased in breast and prostate cancer, yet PDEF/PSE protein expression was found to be decreased as the prostate malignancy grade progressed [7]. These data have been interpreted to indicate that PDEF might actually function as a tumor suppressor and that a translation control mechanism seems to regulate PDEF protein expression in both prostate and breast cancers [7,32,35].

The first clinically relevant candidates for dominant oncogenes in prostate cancer are ETS fusion genes resulting from chromosomal translocation of the 5′ untranslated region of a prostate-specific, androgen-responsive, transmembrane serine protease gene (*TMPRSS2*) to *ERG*, *ER81* (also known as *ETV1*) and *PEA3* (also known as *ETV4*) ETS transcription factor genes [39]. Deletion of genomic DNA between *TMPRSS2* (21q21.3) and *ERG* (21q21.2) results in the TMPRSS2:ERG fusion protein in ~50% of prostate cancers and chromosomal rearrangements between *TMPRSS2* (21q21.3) and *ER81* (7p21.2) or *PEA3* (17q21) occur in another ~30%, indicating that *TMPRSS2–ETS* gene fusions might be the most common genetic abnormality defined to date in human malignancies [39]. These fusions result in androgen-mediated, robust induction of these various ETS factors, which are then thought to activate a repertoire of ETS-responsive genes, leading to prostate cell transformation [39].

ETS factors in reproductive tissues

The ETS family of transcription factors has crucial roles in the regulation of reproduction and embryogenesis. In the male, ERM is required in testicular Sertoli cells for spermatogonial stem cell self-renewal and transcriptional regulation of the stem cell niche [40]. Testicular germ cell tumors show allelic imbalance in the chromosomal region encoding TEL [41], and increased expression of ETS1 was associated with metastasis and angiogenesis [42]. PEA3 expression was increased in testicular seminomas, correlating with enhanced matrix metalloprotease (MMP) 2 levels [43].

In the female, uterine expression of ETS1 and the PEA3 subfamily have been implicated in endometrial angiogenesis and implantation [44,45]. ETS1 is also a crucial regulator of the uterine decidual PRL promoter and is dramatically upregulated during decidualization of the endometrial stroma [46]. In early postimplantation development, ETS2 expression is restricted to placental trophoblasts and is essential for placental function. Deletion of the DNA-binding domain of ETS2 in transgenic mice resulted in embryonic death before day 8.5, owing to defects in extra-embryonic tissue, including deficient MMP9 activity and failure of ectoplacental cone proliferation [47]. Subsequent analysis indicated that ETS2 is necessary for the expression of extra-embryonic ectoderm (EXE) markers and anteroposterior patterning mediated by EXE-derived signals [48]. Deletion of a second trophoblast-specific ETS-related gene, *elf-5*, also results in conceptuses lacking EXE [49].

Several ETS factors have been investigated as prognostic markers in gynecological tumors. In cervical cancer, overexpression of ETS1, ERM (also called ETV5), ERG or ETS2 was associated with higher grade, metastasis and poor prognosis and correlated with elevated levels of MMP-1 and increased angiogenesis [50,51]. A polymorphism of the MMP-1 promoter, which generates an additional ETS-binding site, was also linked to advanced clinical stage and reduced survival in cervical and endometrial cancer [52,53]. Loss of the ETS repressor protein NET (also called SAP2) resulted in enhanced c-Fos expression in cervical cancer cells, a key event in transformation [54]. By contrast, expression of PEA3 suppressed the invasiveness of cervical carcinoma cells [55].

In endometrial tumors, increased expression of ETS1 and ELF1 is associated with advanced surgical stage, enhanced invasion and angiogenesis, and poor prognosis linked to elevated levels of MMPs [56,57]. ERM was also upregulated in endometrial carcinoma in tumor stages associated with myometrial invasion [58]. Accordingly, treatment of progesterone receptor B-expressing Ishikawa endometrial cancer cells with progesterone resulted in inhibition of cell growth and invasion, concomitant with decreased levels of ETS1 and MMPs [59], whereas estrogen-stimulated growth and invasion was associated with increased ETS1 and MMP expression [60]. Overall, evidence indicates that ETS factors, by virtue of their transcriptional regulation of MMPs and angiogenic genes, are crucial mediators of angiogenesis, invasion and metastasis in the development and progression of gynecological malignancies.

Increases in ETS1 and ELF1 are also linked to the malignant potential of ovarian cancer, being associated with higher grade and poor prognosis [61,62]. Over expression of ETS1 in ovarian cancer cells also conferred resistance to chemotherapy [63]. Similarly to cervical cancer, expression of ETS1 and PEA3 correlated with elevated MMPs 1, 2 and 9 and increased angiogenesis, suggesting a central role for these ETS factors in the progression of ovarian carcinoma [64,65]. However, consistent with its inhibition of cervical cancer cell invasion, PEA3 is also reported to downregulate *Her2* gene expression and reverse the transformed phenotype of ovarian cancer cells *in vitro* [66].

ETS factors in thyroid and pancreatic malignancies

Evidence suggests a role for ETS1 and ETS2 in the pathogenesis of thyroid cancer. Expression of ETS1 is increased in papillary and follicular thyroid carcinomas relative to benign nodules or normal tissue [67]. ETS1 and ETS2 expression and transcriptional activity were also increased in thyroid cancer cells. A dominant-negative ETS construct suppressed anchorage-independent growth and induced apoptosis in thyroid carcinoma but not in normal thyroid cell lines, implying a requirement for ETS1 and ETS2 to maintain the transformed phenotype [68]. In pancreatic adenocarcinoma, particularly in late-stage invasive tumors, elevated ETS2 protein was detected and PEA3 was shown to activate the mucin 4 promoter, an epithelial marker of pancreatic ductal carcinoma not

present in normal tissue, suggesting that ETS factors might also be important in pancreatic cancer [69,70]. Finally, ETS1 has been implicated in ectopic expression of the endocrine ligand parathyroid hormone-related peptide, which is associated with bone metastases and hypercalcemia in several cancers, including breast cancer [71].

Conclusion

In summary, ETS factors have been shown to have crucial roles in development, differentiation and tumorigenesis in several endocrine systems. Studies in the pituitary, mammary and prostate glands have provided compelling insights into the functional role of specific ETS factors in these endocrine tissues. Emerging data in many other endocrine organs suggest a broader role for ETS transcription factors in endocrine pathophysiology. Future studies, targeting select endocrine tissues, by generating tissue-specific knockouts of individual ETS genes or expressing a dominant-negative ETS transgene, will provide an even greater understanding of the role of ETS factors in endocrine biology.

Acknowledgements

The authors wish to acknowledge grant support from the NIH, including R01 DK 376676 and R01 DK 46868 to A.G.H.; K01 DK 02946 to DLD; and, RO1 CA 104875 to A.P.B.. Also, A.G.H. acknowledges BCRP BC051589 grant support from the DOD.

References

1 Seth, A. and Watson, D.K. (2005) ETS transcription factors and their emerging roles in human cancer. *Eur. J. Cancer* 41, 2462–2478

2 Sharrocks, A.D. (2001) The ETS-domain transcription factor family. *Nature* 2, 827–837

3 Wasylyk, B. *et al.* (1998) Ets transcription factors: nuclear effectors of the Ras/MAP kinase signaling pathway. *Trends Biochem. Sci.* 23, 213–216

4 Galang, C.K. *et al.* (2004) Changes in the expression of many Ets family transcription factors and of potential target genes in normal mammary tissue and tumors. *J. Biol. Chem.* 279, 11281–11292

5 Hollenhorst, P.C. *et al.* (2004) Expression profiles frame the promoter specificity dilemma of the ETS family of transcription factors. *Nucleic Acids Res.* 32, 5693–5702

6 Prescott, J.D. *et al.* (2004) The ETS transcription factor ESE-1 transforms MCF-12A human mammary epithelial cells via a novel cytoplasmic mechanism. *Mol. Cell. Biol.* 24, 5548–5564

7 Feldman, R.J. *et al.* (2003) The epithelial-specific Ets factors occupy a unique position in defining epithelial proliferation, differentiation and carcinogenesis. *Anticancer Res.* 23, 2125–2131

8 Jorgensen, J.S. *et al.* (2004) Multiple and overlapping combinatorial codes orchestrate hormonal responsiveness and dictate cell-specific expression of the genes encoding luteinizing hormone. *Endocr. Rev.* 25, 521–542

9 Duan, W.R. *et al.* (2002) GnRH regulates early growth response protein 1 transcription through multiple promoter elements. *Mol. Endocrinol.* 16, 221–233

10 Liu, J.C. *et al.* (2002) Activation of Go-coupled dopamine D2 receptors inhibits ERK1/ERK2 in pituitary cells. A key step in the transcriptional suppression of the prolactin gene. *J. Biol. Chem.* 277, 35819–35825

11 Gourdji, D. and Laverriere, J-N. (1994) The rat prolactin gene: a target for tissue-specific and hormone-dependent transcription factors. *Mol. Cell. Endocrinol.* 100, 133–142

12 Dasen, J.S. and Rosenfeld, M.G. (2001) Signaling and transcriptional mechanisms in pituitary development. *Annu. Rev. Neurosci.* 24, 327–355

13 Lopez-Fernandez, J. *et al.* (2000) Differentiation of lactotrope precursor GHFT cells in response to fibroblast growth factor-2. *J. Biol. Chem.* 275, 21653–21660

14 Schweppe, R. *et al.* Functional components of fibroblast growth factor signal transduction in pituitary cells. *J. Biol. Chem.* 272, 30852–30859

15 Shimon, I. *et al.* (1996) Heparin-binding secretory transforming gene (hst) facilitates rat lactotrope cell tumorigenesis and induces prolactin gene transcription. *J. Clin. Invest.* 97, 187–195

16 Yu, S. *et al.* (2002) Fibroblast growth factor receptor 4 is a target for the zinc-finger transcription factor Ikaros in the pituitary. *Mol. Endocrinol.* 16, 1069–1078

17 Tentler, J.J. *et al.* (2003) Selective repression of rat prolactin gene by stable expression of dominant-negative Ets in GH4 pituitary cells. *Endocrine* 20, 3–12

18 Schweppe, R.E. *et al.* (2003) Purification and mass spectrometric identification of GA-binding protein (GABP) as the functional pituitary Ets factor binding to the basal transcription element of the prolactin promoter. *J. Biol. Chem.* 278, 16863–16872

19 Bradford, A.P. *et al.* (1996) GHF-1/Pit-1 functions as a cell-specific integrator of Ras signaling by targeting the Ras pathway to a composite Ets-1/GHF-1 response element. *J. Biol. Chem.* 271, 24639–24648

20 Augustijn, K.D. *et al.* (2002) Structural characterization of the Pit-1-Ets-1 interactions: phosphorylation regulates Pit-1/Ets-1 binding. *Proc. Natl. Acad. Sci. U. S. A.* 99, 12657–12662

21 Duval, D.L. *et al.* (2007) Differential utilization of transcription activation subdomains by distinct coactivators regulates Pit-1 basal and Ras responsiveness. *Mol. Endocrinol.* 21, 172–185

22 Coletta, R.D. *et al.* (2004) Transcriptional control of the cell cycle in mammary gland development and tumorigenesis. *J. Mammary. Gland Biol. Neoplasia.* 9, 39–53

23 Shepherd, T. and Hassell, J. (2001) Role of Ets transcription factors in mammary gland development and oncogenesis. *J. Mammary Gland Biol. Neoplasia* 6, 129–140

24 Kurpios, N.A. *et al.* (2003) Function of PEA3 Ets transcription factors in mammary gland development and oncogenesis. *J. Mammary Gland Biol. Neoplasia* 8, 177–190

25 Harris, J. *et al.* (2006) Socs2 and elf5 mediate prolactin-induced mammary gland development. *Mol. Endocrinol.* 20, 1177–1187

26 Zhou, J. *et al.* (2005) Elf5 is essential for early embryogenesis and mammary gland development during pregnancy and lactation. *EMBO J.* 24, 635–644

27 Sapi, E. *et al.* (1998) Ets-2 transdominant mutant abolishes anchorage-independent growth and macrophage-stimulating factor-stimulated invasion by BT20 breast carcinoma cells. *Cancer Res.* 58, 1027–1033

28 Delannoy-Courdent, A. *et al.* (1998) The expression of Ets-1 transcription factor lacking its activation domain decreases uPA proteolytic activity and cell motility, and impairs normal tubulogenesis and cancerous scattering in mammary epithelial cells. *J. Cell Sci.* 111, 1521–1534

29 Neznanov, N. *et al.* (1999) A single targeted Ets2 allele restricts development of mammary tumors in transgenic mice. *Cancer Res.* 59, 4242–4246

30 Man, A.K. *et al.* (2003) Ets2-dependent stromal regulation of mouse mammary tumors. *Mol. Cell. Biol.* 23, 8614–8625

31 Tynan, J.A. *et al.* (2005) Ets2-dependent microenvironmental support of mouse mammary tumors. *Oncogene* 24, 6870–6876

32 Ghadersohi, A. *et al.* (2006) Prostate-derived Ets transcription factor (PDEF) downregulates survivin expression and inhibits breast cancer cell growth *in vitro* and xenograft tumor formation *in vivo*. *Breast Cancer Res. Treat.* DOI: 10.1007/s10549-006-9314-9 (http://springerlink.metapress.com)

33 Xing, X. *et al.* (2000) The Ets protein PEA3 suppresses Her-2/neu overexpression and inhibits tumorigenesis. *Nat. Med.* 6, 189–195

34 Yu, Z. *et al.* (2006) Antitumor activity of an Ets protein, PEA3, in breast cancer cell lines MDA-MB-361DYT2 and BT474M1. *Mol. Carcinog.* 45, 667–675

35 Feldman, R.J. *et al.* (2003) Pdef expression in human breast cancer is correlated with invasive potential and altered gene expression. *Cancer Res.* 63, 4626–4631

36 Eckel, K.L. *et al.* (2003) The epithelial-specific ETS transcription factor ESX/ESE-1/Elf-3 modulates breast cancer-associated gene expression. *DNA Cell Biol.* 22, 79–94

37 Schedin, P.J. *et al.* (2004) ESX induces transformation and functional epithelial to mesenchymal transition in MCF-12A mammary epithelial cells. *Oncogene* 23, 1766–1779

38 Witt, A.E. *et al.* (2006) Functional proteomics approach to investigate the biological activities of cDNAs implicated in breast cancer. *J. Proteome Res.* 5, 599–610

39 Tomlins, S.A. *et al.* (2005) Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* 310, 644–648

40 Chen, C. *et al.* (2005) ERM is required for transcriptional control of the spermatogonial stem cell niche. *Nature* 436, 1030–1034

41 Bergthorsson, J.T. *et al.* (2006) A genome-wide study of allelic imbalance in human testicular germ cell tumors using microsatellite markers. *Cancer Genet. Cytogenet.* 164, 1–9

42 Adam, M. *et al.* (2003) Angiogenetic protooncogene ets-1 induced neovascularization is involved in the metastatic process of testicular germ cell tumors. *Eur. Urol.* 44, 329–336

43 Gashaw, I. *et al.* (2005) Gene signatures of testicular seminoma with emphasis on expression of ets variant gene 4. *Cell. Mol. Life Sci.* 62, 2359–2368

44 Fujimoto, J. *et al.* (2003) Expression of ETS-1 related to angiogenesis in uterine endometrium during the menstrual cycle. *J. Biomed. Sci.* 10, 320–327

45 Koo, T.B. *et al.* (2005) Differential expression of the PEA3 subfamily of ETS transcription factors in the mouse ovary and peri-implantation uterus. *Reproduction* 129, 651–657

46 Brar, A.K. *et al.* (2002) An Ets motif in the proximal decidual prolactin promoter is essential for basal gene expression. *J. Mol. Endocrinol.* 29, 99–112

47 Yamamoto, H. *et al.* (1998) Defective trophoblast function in mice with a targeted mutation of Ets2. *Genes Dev.* 12, 1315–1326

48 Georgiades, P. and Rossant, J. (2006) Ets2 is necessary in trophoblast for normal embryonic anteroposterior axis development. *Development* 133, 1059–1068

49 Donnison, M. *et al.* (2005) Loss of the extraembryonic ectoderm in Elf5 mutants leads to defects in embryonic patterning. *Development* 132, 2299–2308

50 Fujimoto, J. *et al.* (2002) Clinical implications of expression of ETS-1 related to angiogenesis in uterine cervical cancers. *Ann. Oncol.* 13, 1598–1604

51 Simpson, S. *et al.* (1997) Altered expression of Erg and Ets-2 transcription factors is associated with genetic changes at 21q22.2-22.3 in immortal and cervical carcinoma cell lines. *Oncogene* 14, 2149–2157

52 Lai, H.C. *et al.* (2005) Matrix metalloproteinase 1 gene polymorphism as a prognostic predictor of invasive cervical cancer. *Gynecol. Oncol.* 96, 314–319

53 Nishioka, Y. *et al.* (2003) A relationship between matrix metalloproteinase-1 (MMP-1) promoter polymorphism and cervical cancer progression. *Cancer Lett.* 200, 49–55

54 van Riggelen, J. *et al.* (2005) Loss of net as repressor leads to constitutive increased c-fos transcription in cervical cancer cells. *J. Biol. Chem.* 280, 3286–3294

55 Iwasaki, M. *et al.* (2004) E1AF/PEA3 reduces the invasiveness of SiHa cervical cancer cells by activating serine proteinase inhibitor squamous cell carcinoma antigen. *Exp. Cell Res.* 299, 525–532

56 Fujimoto, J. *et al.* (2005) Sex steroid-dependent angiogenesis in uterine endometrial cancers. *J. Steroid Biochem. Mol. Biol.* 93, 161–165

57 Takai, N. *et al.* (2003) Clinical relevance of Elf-1 overexpression in endometrial carcinoma. *Gynecol. Oncol.* 89, 408–413

58 Planaguma, J. *et al.* (2005) Up-regulation of ERM/ETV5 correlates with the degree of myometrial infiltration in endometrioid endometrial carcinoma. *J. Pathol.* 207, 422–429

59 Saito, T. *et al.* (2004) Overexpressed progesterone receptor form B inhibit invasive activity suppressing matrix metalloproteinases in endometrial carcinoma cells. *Cancer Lett.* 209, 237–243

60 Mizumoto, H. *et al.* (2002) Acceleration of invasive activity via matrix metalloproteinases by transfection of the estrogen receptor-alpha gene in endometrial carcinoma cells. *Int. J. Cancer* 100, 401–406

61 Fujimoto, J. *et al.* (2004) Clinical implications of expression of ETS-1 related to angiogenesis in metastatic lesions of ovarian cancers. *Oncology* 66, 420–428

62 Takai, N. *et al.* (2003) The significance of Elf-1 expression in epithelial ovarian carcinoma. *Int. J. Mol. Med.* 12, 349–354

63 Wilson, L.A. *et al.* (2004) Role of the transcription factor Ets-1 in cisplatin resistance. *Mol. Cancer Ther.* 3, 823–832

64 Behrens, P. *et al.* (2001) The Ets-1 transcription factor is up-regulated together with MMP 1 and MMP 9 in the stroma of pre-invasive breast cancer. *J. Pathol.* 194, 43–50

65 Davidson, B. *et al.* (2003) Coordinated expression of integrin subunits, matrix metalloproteinases (MMP), angiogenic genes and Ets transcription factors in advanced-stage ovarian carcinoma: a possible activation pathway? *Cancer Metastasis Rev.* 22, 103–115

66 Hung, M.C. and Wang, S.C. (2000) Suppressing HER2/neu-mediated cell transformation by transcriptional repressors. *Breast Dis.* 11, 133–144

67 Fuhrer, D. *et al.* (2005) Evaluation of insulin-like growth factor II, cyclooxygenase-2, ets-1 and thyroid-specific thyroglobulin mRNA expression in benign and malignant thyroid tumours. *Eur. J. Endocrinol.* 152, 785–790

68 de Nigris, F. *et al.* (2001) Induction of ETS-1 and ETS-2 transcription factors is required for thyroid cell transformation. *Cancer Res.* 61, 2267–2275

69 Fauquette, V. *et al.* (2005) The antagonistic regulation of human MUC4 and ErbB-2 genes by the Ets protein PEA3 in pancreatic cancer cells: implications for the proliferation/differentiation balance in the cells. *Biochem. J.* 386, 35–45

70 Ito, Y. *et al.* (2002) Ets-2 overexpression contributes to progression of pancreatic adenocarcinoma. *Oncol. Rep.* 9, 853–857

71 Richard, V. *et al.* (2005) PTHrP gene expression in cancer: do all paths lead to Ets? *Crit. Rev. Eukaryot. Gene Expr.* 15, 115–132

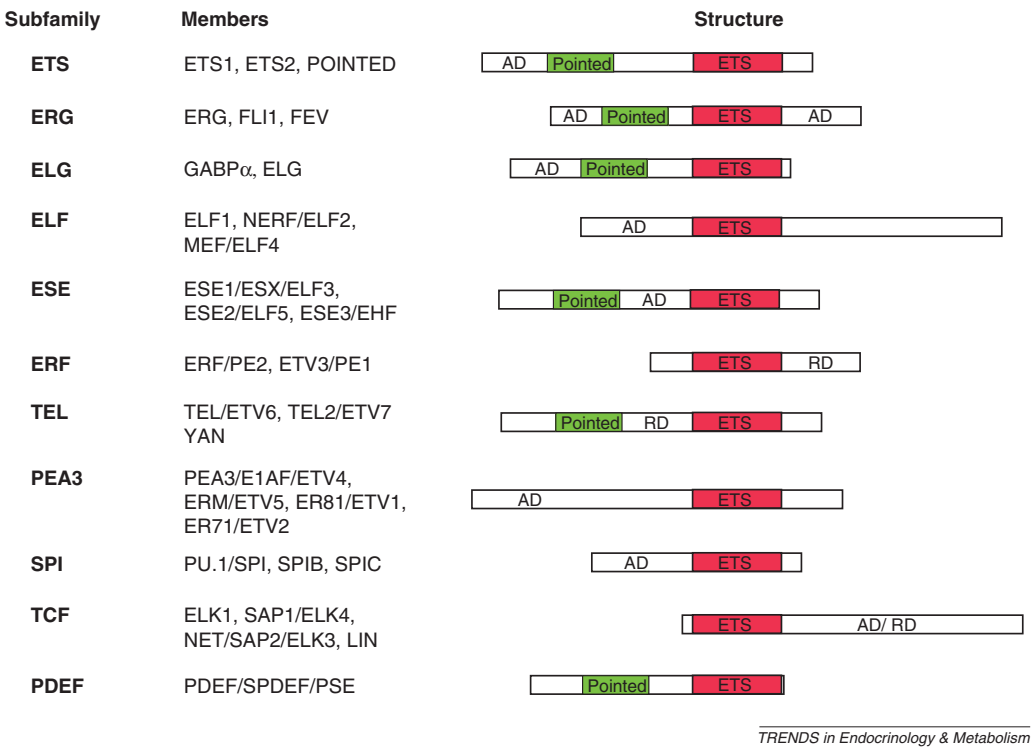

Figure 1. The ETS family of transcription factors. The main functional domains characteristic of members of each ETS sub family are depicted; alternative names for each member are given. Domains: AD, transcriptional activation domain; ETS, DNA binding domain; Pointed, basic helix–loop–helix pointed domain; RD, transcriptional repressor domain. Protein abbreviations: E1AF, E1A enhancer binding protein; EHF, ETS homologous factor; ELF, E74-like factor; ELG, ETS like gene; ER81, ETS related protein 81; ERF, ETS repressor factor; ERG, v-ets avian erythroblastosis virus E26 oncogene related; ERM, ETS related molecule; ESE, Epithelial specific ETS; ETS, v-ets erythroblastosis virus E26 oncogene homolog; ETV, ETS variant gene; FLI1, Friend leukemia virus integration 1; FEV, Fifth Ewing variant; GABP, GA repeat binding protein; LIN, abnormal cell lineage; MEF, myeloid ELF1-like factor; NERF, New ETS-related factor; PEA3, polyomavirus enhancer activator-3; PDEF, prostate derived ETS transcription factor; PSE, prostate epithelium-specific ETS; SAP, Serum response factor accessory protein; SPDEF, SAM pointed domain containing ETS transcription factor; SPI, spleen focus forming virus proviral integration oncogene; TEL, translocation, Ets, leukemia; TCF, Ternary complex factor.

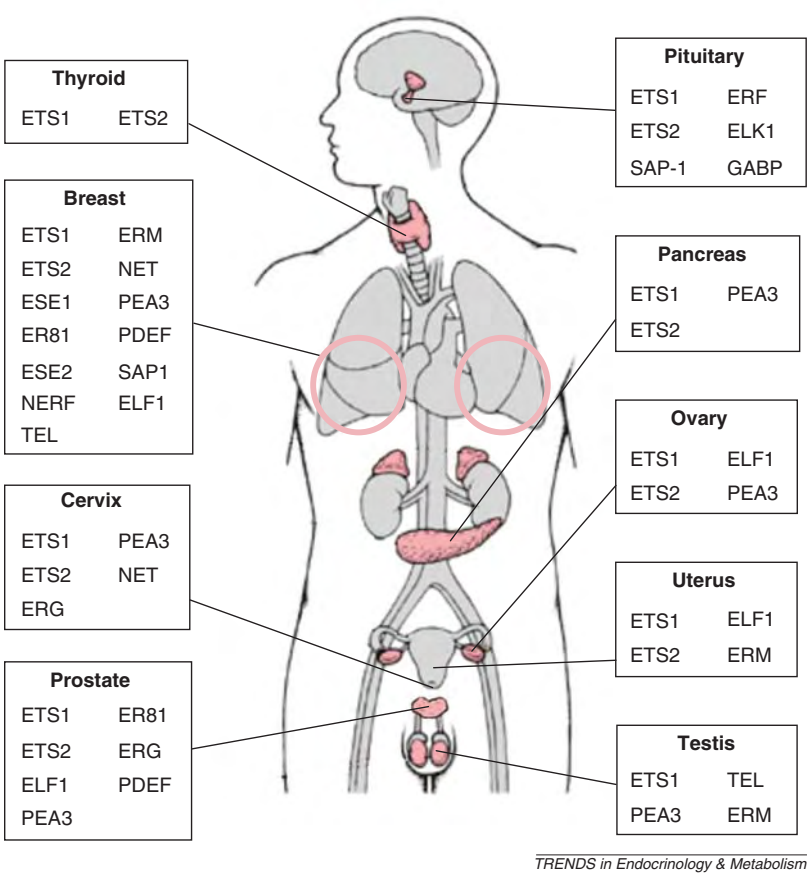

Figure 2. Mammalian ETS factor expression in endocrine organs and target tissues. A list of ETS factors implicated in normal function and/or tumorigenesis is shown for each human tissue. See text for discussion and corresponding references.

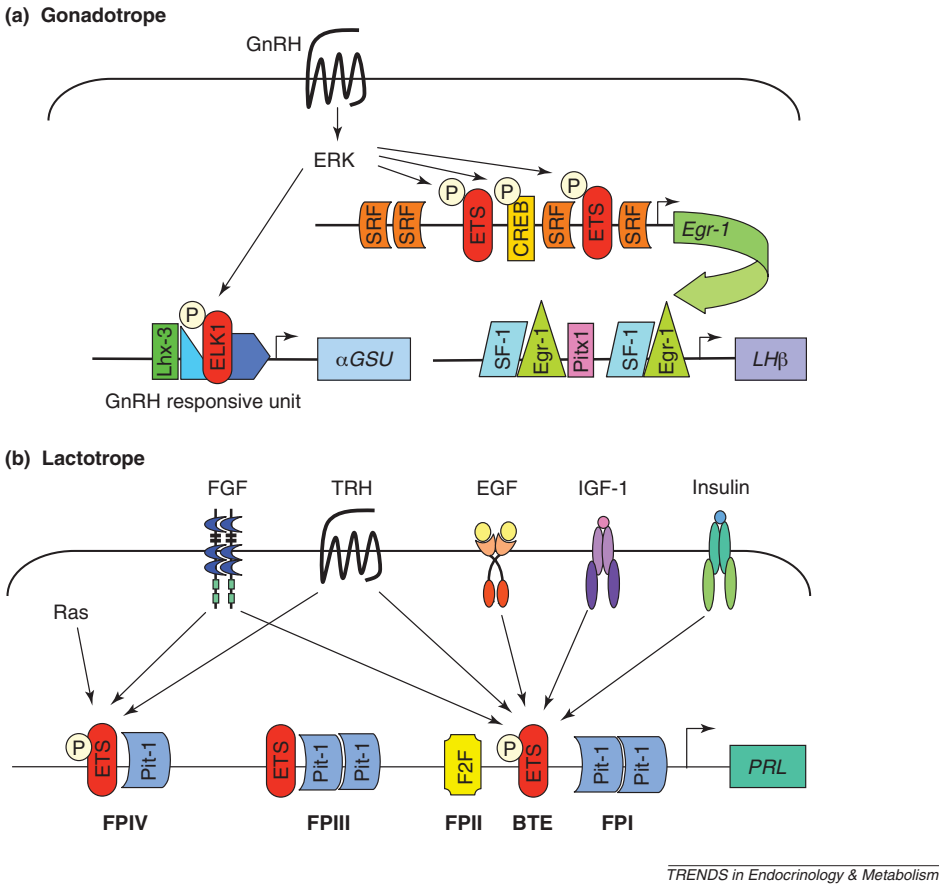

Figure 3. ETS factors as nuclear targets and integrators of signal transduction pathways regulating pituitary gonadotrope and lactotrope gene expression. **(a)** GnRH acting through the ERK pathway directly activates an ETS factor (ELK1) bound to the human α-glycoprotein subunit gene promoter (*αGSU*) within the GnRH responsive unit. Blue triangle, αBP1; hexagon, αBP2. GnRH also stimulates the expression of the human early growth response protein 1 gene (*Egr-1*) through ERK-dependent phosphorylation and activation of ETS factors (ETS) and the cyclic AMP response element binding protein (CREB). Upregulation of the transcription factor Egr-1 subsequently contributes to GnRH stimulation of the human *LHβ*-subunit gene promoter, through a composite response element consisting of binding sites for steroidogenic factor-1 (SF-1), the paired-like homeodomain transcription factor (Pitx1) and Egr-1. **(b)** ETS factors integrate multiple signaling pathways regulating *PRL* gene expression. Stimulation of the proximal –425 rat *PRL* gene promoter (*PRL*), in response to the indicated hormones, growth factors or oncogenic Ras, is mediated through ERK phosphorylation of distinct ETS factors (denoted by the circled P), described in the text, which bind to a composite ETS–Pit-1 binding site (FPIV) and/or an ETS binding site in the BTE. The binding of different ETS proteins to common response elements, and interactions with other transcription factors, provides a mechanism to confer highly specific responses to inductive signals and to coordinate and integrate hormonal and growth factor regulation of *PRL* gene expression. Abbreviations: FGF, fibroblast growth factors; IGF-1, Insulin like growth factor 1; Lhx-3, Lim-homeobox factor 3; SRF, serum response factor; TRH, thyroid-releasing hormone.

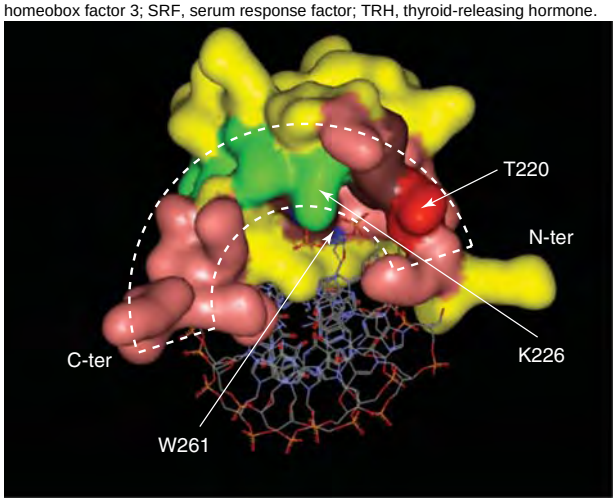

Figure 4. Chemical shift perturbations of the Pit-1 homeodomain induced by the binding of ETS1. A surface density representation of the Pit-1 homeodomain bound to DNA based on the crystal structure of the Pit-1 homeodomain. NMR shift perturbations of residues in the Pit-1 homeodomain (amino acids 199–291), in response to the binding of the Region III TAD (amino acids 190–257) of ETS1 [20], are indicated by color coding: red > purple > pink > green. Yellow residues are unaffected. The ETS interaction face on the Pit-1 homeodomain is indicated by a dashed white line. Amino acids highlighted in the ETS1 interaction face seem to affect the ETS1–Pit-1 interaction. W261 (blue) is in the hydrophobic DNA binding pocket, T220 (red) is a phosphorylation site and K226 (green) is a potential acetylation site. Adapted from Ref. [20]; copyright 2002 National Academy of Sciences USA.

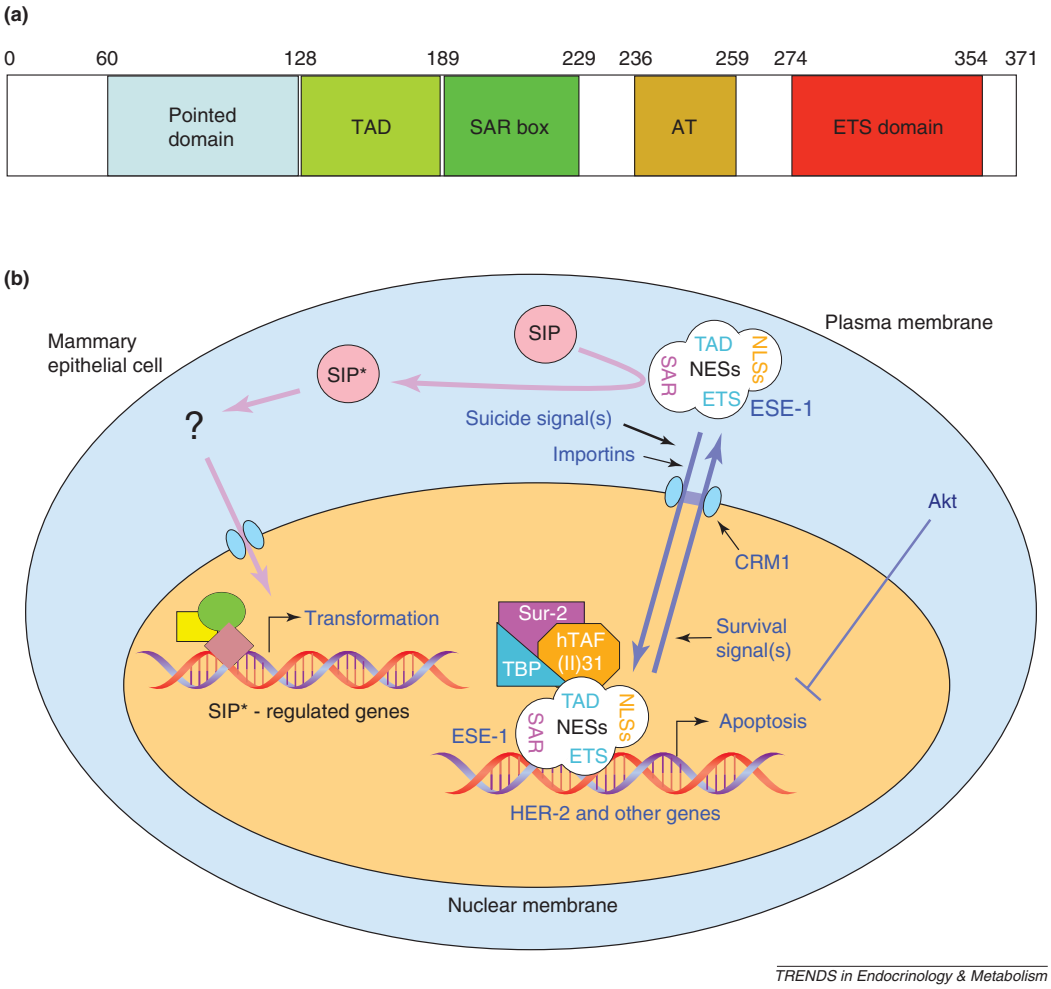

Figure 5. (a) Domain organization of ESE1. Shown is the 371 amino acid ESE1 protein, with the Pointed, TAD, SAR domain, AT-hook domain, and ETS DBD. Endpoints (residue numbers) are shown above each domain. **(b)** Model of ESE1 function in mammary gland biology. ESE1 is shown in the nucleus as binding to a target gene and activating an apoptotic pathway in normal epithelium, with nuclear localization governed by suicide signals. By contrast, survival signals are postulated to induce cytoplasmic localization of ESE1, through a Chromosomal region maintenance 1 (CRM1)-dependent mechanism, which is required for ESE1 to transform human mammary epithelial cells. The SAR domain is proposed to interact with SAR-interacting protein (SIP), converting it to the active form, SIP*, which then activates an unknown signal transduction pathway (shown as ?), resulting in specific gene(s) transcription that establishes the transformed phenotype. Abbreviation: NES, nuclear export sequence; NLS, nuclear localization sequence; TAF, transcription activating factor; TBP, TATA binding protein.