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Expression and functional properties of transforming growth factor α and epidermal growth factor during mouse mammary gland ductal morphogenesis

(mammary epithelium/growth factors/immunohistochemistry/polymerase chain reaction/terminal end buds)

SUZANNE M. SNEDEKER*, CHARLES F. BROWN, AND RICHARD P. DIAUGUSTINE

Hormones and Cancer Workgroup, Laboratory of Biochemical Risk Analysis, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

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ABSTRACT **Primer-directed enzyme amplification was used to examine epidermal growth factor (EGF) and transforming growth factor α (TGF-α) mRNA transcripts in mammary glands of young virgin, mature virgin, midpregnant, and midlactating mice. Transcripts for both EGF and TGF-α mRNA were detected in virgin and pregnant mice, whereas transcripts for EGF mRNA but not TGF-α mRNA were expressed in 10-day lactating mice. TGF-α was localized in the epithelial cap-cell layer of the advancing terminal end bud and in the stromal fibroblasts at the base of the terminal end bud; EGF was localized in the inner layers of the terminal end bud and in ductal cells of mammary epithelium. Implantation of pellets containing EGF or TGF-α into the regressed mammary gland of ovariectomized mice stimulated the reappearance of end buds; contralateral glands implanted with pellets containing albumin or insulin were not affected. These results indicate that an EGF-receptor-mediated pathway remained intact in the mammary gland epithelium in the absence of ovarian steroids and that local availability of either EGF or TGF-α is sufficient to stimulate the pattern of normal ductal growth. The detection of EGF and TGF-α transcripts at different stages of mammary gland development and the different patterns of immunolocalization suggest that each polypeptide plays a different role in normal mammary gland morphogenesis.**

Unlike other organ systems, most of the development of mouse mammary gland occurs postnatally. Before onset of ovarian function, the postnatal mouse mammary gland consists of a primary duct and a few primitive branched ducts emanating from the nipple (Fig. 1*A*) (1, 2). Ovarian secretion of estrogens at about 4 weeks of age stimulates rapid ductal growth in the mouse mammary gland (1, 3) and the formation of bulbous, multicell layered terminal end buds (TEBs) that serve as growth points for elongation and branching of the ducts through the fatty stroma (Fig. 1*B*) (2–5). The growing ductal tree extends to fill the fat pad (Fig. 1*C*) and when the gland reaches the limits of the fat pad at 12–14 wk of age (Fig. 1*D*), the TEBs disappear (5, 6).

Estrogens are thought to directly stimulate mammary ductal morphogenesis by interaction with specific receptors in the gland (7, 8). The steps that succeed mammary gland estrogen-receptor binding and ultimately lead to cell proliferation have not been identified. Estrogens may stimulate cell division through an autocrine or paracrine mechanism (9) by stimulating the production of a peptide growth factor. Particular attention has been given to an autocrine role of transforming growth factor α (TGF-α) because expression of this growth factor has been identified in breast tumors (10, 11) and derived cell lines (11, 12). TGF-α is a 50-amino acid mitogenic polypeptide that is structurally and biologically homologous to epidermal growth factor (EGF) and competes for binding to the same receptor (13, 14). EGF stimulates proliferation of both normal mammary epithelial cells (15–18) and breast tumor cells *in vitro* (19). TGF-α expression also has been identified in normal cells, including human keratinocytes (20) and human (21) and rodent (22) mammary epithelial cells, suggesting that members of the EGF family have functional roles in normal mammary gland.

In the present study, mouse mammary gland gene expression of TGF-α and EGF was examined during different stages of mammary ductal morphogenesis. Immunolocalization was used to determine whether specific epithelial or stromal cells in the gland synthesized EGF or TGF-α. We also examined

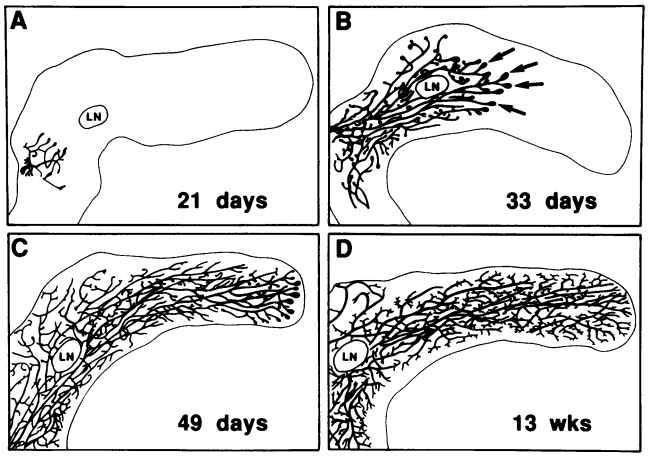

FIG. 1. Ductal morphogenesis in the fourth (abdominal) mammary gland of the virgin female BALB/c mouse. Tracings of the ductal tree were made from photographs of gland whole mounts; outlined area indicates the limits of the mammary fat pad. (*A*) Mammary gland of 21-day-old animal, depicting thin primitive ducts emanating from the primary duct that is attached to the nipple. Note some lateral branching and thickening of the ends of the ducts. At ≈4 weeks of age the ovaries start to function, and bulbous TEBs appear at the ductal tips. (*B*) Mammary gland of 33-day-old animal, showing extensively branched ductal system that has advanced beyond the lymph node (LN). Note the presence of large TEBs (arrows) at the ductal tips of the advancing gland. (*C*) Mammary gland of 49-day-old animal, showing the ductal tree approaching the limits of the fat pad. TEBs are still present in the advancing ducts. (*D*) Mammary gland of 13-week-old animal. Mammary ducts have reached the limits of the fat pad, and the TEBs are no longer present.

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Abbreviations: TGF-α, transforming growth factor α; EGF, epidermal growth factor; TEB, terminal end bud.

*To whom reprint requests should be addressed at: P.O. Box 12233, Mail Drop D4-01, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

the capacity of these polypeptides *in vivo* to stimulate normal growth of the regressed gland in ovariectomized mice. An understanding of the growth-regulatory pathways in the mammary gland may enable us to identify the biochemical basis for the high frequency of breast cancer in women.

MATERIALS AND METHODS

RNA Isolation and cDNA Synthesis. Mammary glands were excised from anesthetized BALB/c or CD-1 [Crl:CD-1-(ICR)BR] mice (Charles River Breeding Laboratories), frozen in liquid N_2, weighed, and stored at −70°C. Mouse kidney and rat hypothalamus were used as controls for EGF and TGF-α mRNA expression, respectively. RNA was isolated from a pooled sample of 4–8 g of frozen tissue after homogenization in guanidinium thiocyanate (23). Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose affinity chromatography (24). Integrity of the RNA was checked by agarose gel electrophoresis, and the quantity was determined spectrophotometrically. The cDNA was synthesized by incubating 100 μg of RNA at 37°C for 60 min with 500 units of Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories), 2.5 μg of $(dT)_{12-18}$ primers (Pharmacia), 10 mM dNTP mix (Pharmacia), 75 mM KCl, 10 mM dithiothreitol, and 3 mM $MgCl_2$ in 50 mM Tris·HCl, pH 8.3 (total vol = 50 μl). The sample was denatured by heating at 94°C for 2 min and cooled to 4°C; 500 units of Moloney murine leukemia virus reverse transcriptase was added, and the reaction was incubated at 37°C for 30 min. The reaction was terminated by heating for 2 min at 94°C, and the sample was stored on ice.

Enzymatic Amplification of cDNA. The cDNA obtained by reverse transcriptase was amplified with *Thermus aquaticus* (*Taq*) DNA polymerase (AmpliTaq, Perkin–Elmer/Cetus) (25). The polymerase chain reaction contained 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (wt/vol) gelatin, each dNTP at 200 μM, 2 μM of each primer, 5 units of AmpliTaq, cDNA derived by reverse transcription of 33 μg of RNA, and 10 mM Tris·HCl, pH 8.3 (final vol = 50 μl). Samples were overlaid with 100 μl of paraffin oil and were amplified for 60 cycles (1 cycle = 94°C for 1 min, 50°C for 2 min, and 72°C for 2 min) in a Perkin–Elmer/Cetus thermocycler. TGF-α primers corresponded to the rat TGF-α precursor sequence (26), amino acids 54–60 (5′-GGACAGCTCGCTCTGCTAGCG-3′) and 189–183 (5′-CTTCTCGTGTCTGCAGACGAG-3′). EGF primers embraced the carboxyl-terminal region of the mouse EGF precursor (27), amino acids 1143–1149 (5′-ATCTGACTTCATGGAGACAG-3′) and 1216–1210 (5′-AACTGGCTCTGTCTGCCGTG-3′). β-Actin primers were made to the mouse sequence (28), amino acids 35–41 (5′-GTGGGCCGCTCTAGGCACCA-3′) and 116–108 (5′-CGGTTGGCCTTAGGGTTCAGGGGGG-3′). Unincorporated nucleotides were removed by spin dialysis with Centricon-30 microconcentrators (Amicon). Aliquots of the amplified products were analyzed by electrophoresis through 2% agarose in 1× TAE (1× TAE = 40 mM Tris/20 mM sodium acetate/1 mM EDTA, pH 7.2) and visualized by ethidium bromide staining.

Immunohistochemical Localization. Immunolocalization of EGF and TGF-α was determined on trypsinized paraffin sections of perfusion-fixed mammary glands (29) from CD-1 mice by the double peroxidase–antiperoxidase technique (30). Mouse kidney and rat hypothalamus sections served as positive controls for EGF and TGF-α localization, respectively. Rabbit anti-mouse EGF (lot 53, Biomedical Technologies, Stoughton, MA) and rabbit anti-rat TGF-α (lot 010384-4, Peninsula Laboratories) were used as primary antisera. Controls included dilution of the primary antisera (1:1000 to 1:5000), competition with excess EGF (Collaborative Research) or synthetic rat TGF-α 34–50, and substitution of normal rabbit serum for the primary antiserum.

Pellet Implants. Plastic pellets used for the sustained release of growth factors *in vivo* were prepared according to the method of Murray *et al.* (31) by using a vinylacetate polymer (ELVAX 40-W, DuPont) and bovine albumin as carrier. Pellets were cut into 1-mm^3 pieces weighing 0.8–1.2 mg and contained 5.5 μg of EGF per mg (Collaborative Research), 4.4 μg of TGF-α per mg (Penisula Laboratories), or insulin (Sigma) at equimolar concentration to EGF, or albumin alone. Virgin female mice were ovariectomized at 5 weeks of age and were used 2–3 mo later. Growth factor pellets were hydrated in saline and were delivered by a trocar into the right third gland through a 4-mm incision at the base of the mammary fat pad on the dorsal side of anesthetized animals; albumin pellets were implanted into the contralateral gland. Animals were killed 4 days later, and glands were processed for whole mounts (32), examined, and photographed.

RESULTS

Expression of Mammary Gland TGF-α and EGF mRNA. TGF-α mRNA transcripts were detected in mammary glands of 33- and 49-day-old mice, mature (13 week) virgin female mice, and midpregnant (14 day) BALB/c mice (Fig. 2*A*). A faint band was also observed for the 21-day-old virgin mice. The visualized band corresponded to the size predicted by the TGF-α primers [408 base pairs (bp)]. Amplification of mRNA transcripts from glands of lactating (10 day) animals did not generate any product of the targeted cDNA region that could be visualized on agarose gels (Fig. 2*A*). We also examined the same stages of mammary gland development in the BALB/c mouse for EGF mRNA transcripts. Amplified product (221 bp), as predicted by EGF cDNA primers, was detected throughout ductal morphogenesis and in midpregnant animals (Fig. 2*B*). In contrast to the pattern for TGF-α

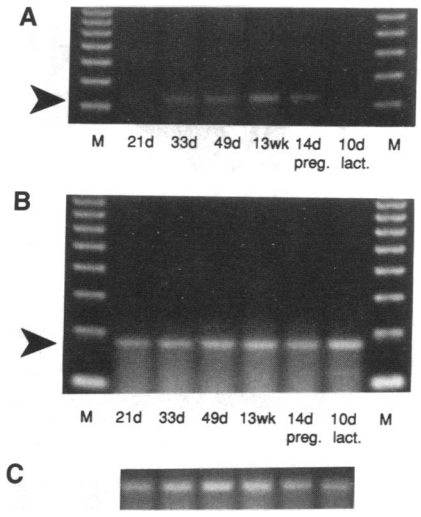

FIG. 2. Detection of TGF-α and EGF transcripts in BALB/c female mouse mammary glands during different stages of development. Agarose gel (2%) electrophoresis was used to detect reaction products of primer-directed enzymatic amplification. Each reaction mixture contained cDNA as the reverse transcript from 33 μg of total RNA. Marker lane (M) contains a 123-bp DNA ladder. (*A*) TGF-α transcripts (arrow), as predicted (408 bp) by the TGF-α primers, were detected in mammary glands obtained from 33- and 49-day, 13-week-old virgin and 14-day pregnant (preg.) mice. A faint band was seen in glands from 21-day-old animals. TGF-α transcripts were not visualized in mammary glands from 10-day lactating (lact.) animals. (*B*) EGF transcripts (arrow), as predicted (221 bp) by the EGF primers, were detected in mammary glands from 21-, 33-, and 49-day and 13-week-old virgin, 14-day pregnant and 10-day lactating mice. (*C*) Amplified product (245 bp) obtained with β-actin primers was seen in all samples.

expression, EGF mRNA transcripts were also detected during midlactation (Fig. 2*B*), which is in accord with the known expression of prepro-EGF mRNA seen in mammary gland throughout lactation (29, 33). Amplified product (245 bp) was visualized when reverse transcripts from each stage of gland development were analyzed with primers to mouse β-actin cDNA (Fig. 2*C*).

CD-1 mice gave a similar pattern of expression for TGF-α/EGF mRNA transcripts as did BALB/c mice; however, pooled glands from 21-day-old CD-1 mice exhibited a more prominent mRNA transcript for both growth factors (data not shown). This difference might be explained by an earlier onset of ductal morphogenesis in the CD-1 compared with BALB/c mice. At 3 weeks of age, CD-1 female mice display a compact array of thick branched ducts terminating in numerous TEBs, compared with the sparse number of thin ducts and few, if any, TEBs in 3-week-old female BALB/c mice (Fig. 1*A*). Transcripts of both growth factors also were detected in mammary glands from BALB/c or CD-1 mice that had been ovariectomized either for 2 weeks or for 4 mo (unpublished observations).

Except for the EGF-specific transcript (4.7 kb) seen during lactation, we could not detect specific transcripts for TGF-α or EGF by conventional Northern (RNA) blot analysis of poly(A)$^+$ RNA isolated from whole mammary glands at various stages of development.

TGF-α and EGF Immunolocalization. TGF-α and EGF immunoreactivities were examined in sections from 33-day-old mice to assess whether these growth factors were translated during mammary gland ductal growth. TGF-α immunoreactivity was seen in the cap-cell layer of TEBs (open arrows, Fig. 3 *A* and *B*) and in loosely arranged fibrocytes in the region where the base of the TEB narrowed to form a subtending duct (Fig. 3*B*); epithelial cells in the interior layers of TEBs did not stain for TGF-α. This pattern of TGF-α cap-cell localization was confirmed by examining serial sections near the solid tip (Fig. 3*A*) and midway through the advancing TEB (Fig. 3*B*). In contrast, EGF immunoreactivity was conspicuously absent from the cap-cell layer of the TEB (solid arrows, Fig. 3*C*) but was observed in epithelial cells of interior layers of the TEBs (Fig. 3*C*). Few cells in the adipocytes near the advancing TEBs (Fig. 3*C*) or in the stromal fibroblast tunic flanking the TEB showed positive EGF staining. The most prominent EGF staining was seen in selected luminal cells of ductal epithelium (Fig. 3*D*). This intense pattern of staining frequently extended to the lumen of ducts. Myoepithelial cells and surrounding periductal stroma (Fig. 3*D*) did not exhibit EGF staining. A positive reaction with EGF antisera was observed in clusters of small adipocytes and fibroblasts (preadipocytes) located in the region between the nipple and the lymph node of gland 4, whereas large mature adipocytes distant from the site of the end buds did not stain for EGF (data not shown).

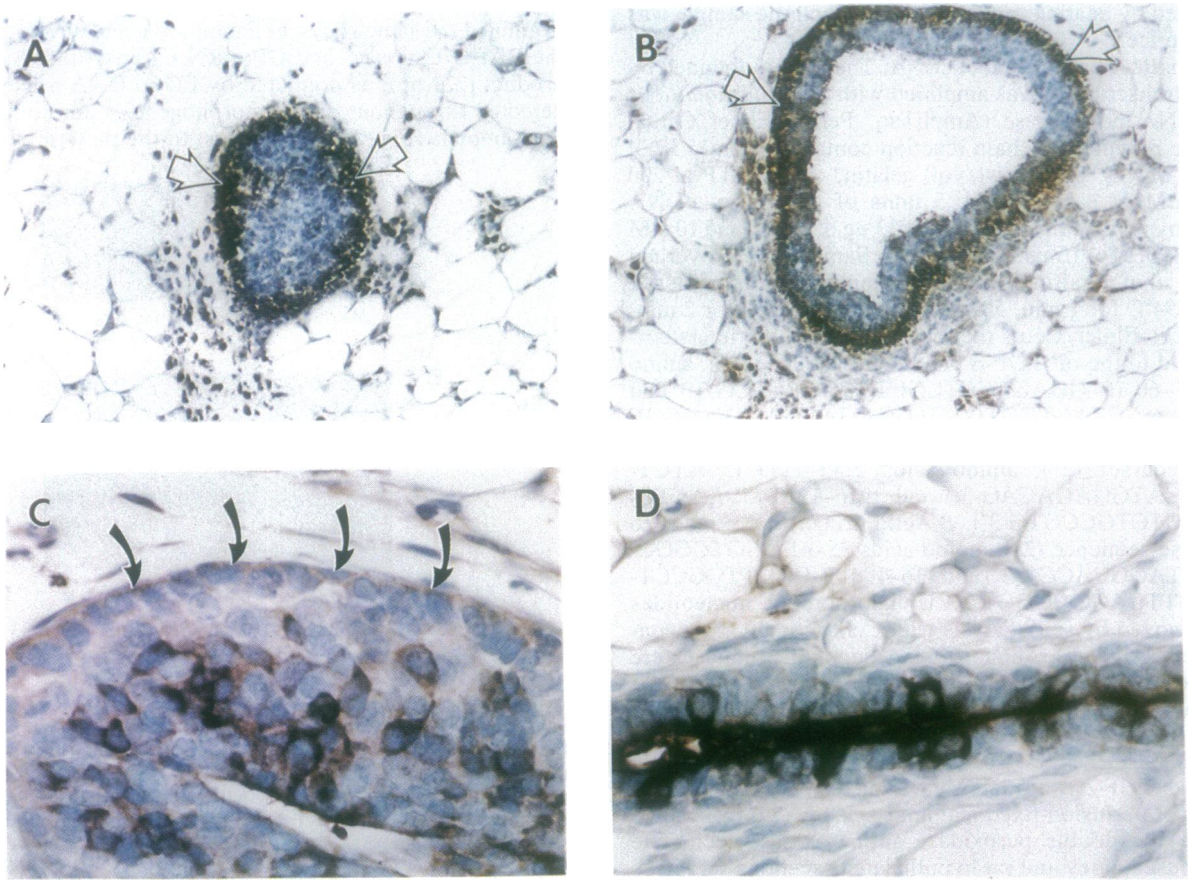

FIG. 3. Immunolocalization of TGF-α and EGF in trypsin-treated sections of the mammary gland of 33-day-old CD-1 mice by the peroxidase–antiperoxidase technique (toluidine blue counterstain). (*A*) TGF-α immunolocalization was observed in the cap-cell layer (open arrows) of a TEB sectioned across the growing tip of this structure. (×107.) (*B*) The same end bud was sectioned to about halfway through this structure, and TGF-α immunoreactivity was again detected in the cap-cell layer epithelium (open arrows). Some staining also occurred in loosely arranged stromal fibrocytes where the TEB narrows to form a subtending duct. (×107.) (*C*) EGF immunolocalization was present in the cytoplasm of epithelial cells in the interior and luminal layers of the TEB; the cap-cell layer (solid arrows) did not stain for EGF. (×428.) (*D*) Intense EGF immunostaining was detected in some luminal epithelial cells and in the lumen of the mammary ducts. EGF localization was not seen in the periductal stroma or in the ductal myoepithelial cells. (×428.)

Staining for TGF-α or EGF was not seen in the epithelium or stroma of the mammary gland when normal rabbit serum was substituted for primary antiserum. Incubation of primary antiserum with an excess of the corresponding peptide also blocked staining; however, EGF immunostaining in the ductal lumen and in the preadipocyte stroma was only partially blocked by incubation with excess EGF.

Stimulation of Growth *in Vivo* by EGF or TGF-α. Examination of mammary gland whole mounts from mice that had been ovariectomized for 2–3 mo revealed cessation of ductal growth (5), decreased ductal diameter, and disappearance of TEBs ("regressed" mammary glands). We observed the reappearance of end-bud structures 4 days after implanting pellets containing EGF into regressed mammary glands of ovariectomized BALB/c mice (Fig. 4 *A* and *C*). End-bud growth was seen at the ductal tips of the regressed gland up to 10–12 mm from the EGF implantation site. Similarly, implants containing TGF-α stimulated end-bud formation in the regressed gland (Fig. 4*E*). End-bud growth was limited to the gland implanted with the growth factor because contralateral glands implanted with pellets containing albumin alone did not show regeneration of end buds (Fig. 4 *B*, *D*, and *F*). Replacement of growth factor with an equimolar amount of insulin failed to stimulate growth in the regressed gland (data not shown). Regenerated end buds (Fig. 4 *A*, *C*, and *E*) were similar in appearance to TEBs present during the normal phase of rapid ductal growth (Fig. 1*B*).

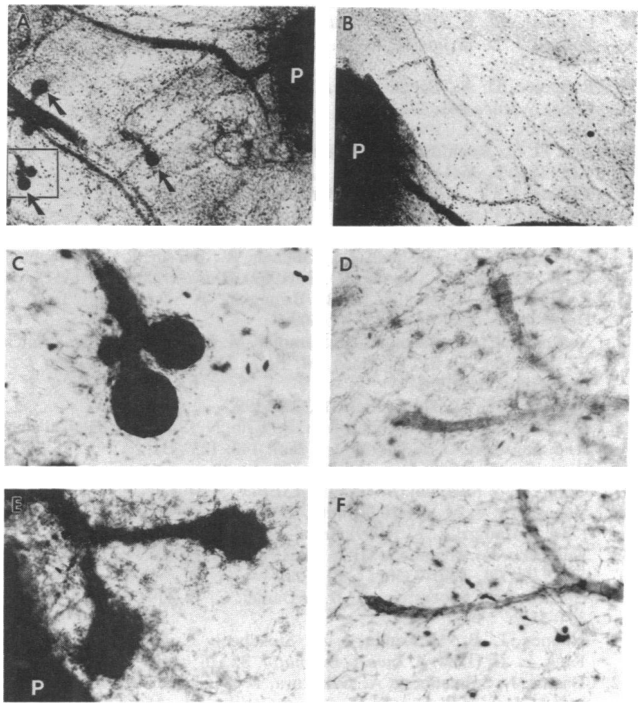

FIG. 4. Whole mounts of mammary gland 3 from ovariectomized BALB/c mice exposed to pellets containing EGF or TGF-α for 4 days. (*A*) Implantation of pellet (P) containing EGF into the right mammary gland stimulated the reappearance of end buds (arrows) at the ductal tips. (×12.) (*B*) Growth-quiescent mammary gland contralateral to *A* implanted with control pellet (P) containing bovine albumin. Terminal ducts are present with no regeneration of end buds. (×12.) (*C*) Enlargement of area enclosed in *A* showing the regeneration of end buds in mammary gland exposed to an EGF-containing pellet. (×60.) (*D*) Terminal ducts in regressed gland contralateral to *C* implanted with control bovine albumin pellet. (×60.) (*E*) Regenerated end buds in the right mammary gland of animal implanted with a pellet containing TGF-α. (×60.) (*F*) Regressed ducts in the mammary gland contralateral to *D*, implanted with control bovine albumin pellet. (×60.) The ductal tips of regressed glands, shown in *B*, *D*, and *F*, were within 6 mm of the placebo pellet.

DISCUSSION

Ovarian steroids play a critical role in normal ductal development (3, 34) and tumor formation in the mammary gland (35, 36). TGF-α has been proposed as an autocrine growth factor in estrogen-responsive breast cancer cell lines (9); in the present study we demonstrate that the normal developing mammary gland can produce and respond to members of the EGF family *in vivo*. TGF-α and EGF mRNA transcripts were detected throughout the period of rapid ductal growth and in mammary glands of mature virgin mice, confirming that TGF-α expression is not an event unique to embryogenesis (37–39), neoplasia (10, 40), or transformation of cells (41, 42). Further support for a role for TGF-α in normal mammary tissue includes its recent identification in extracts of rat (22) and human mammary glands (12) and the detection of TGF-α mRNA transcripts in human mammary epithelial cells *in vitro* (21, 43).

Primer-directed enzyme amplification of reversed transcribed mRNA provided a sensitive means for detecting low levels of expression in virgin and midpregnant animals when transcripts could not be detected by Northern blots. Both low levels of expression and cell dilution may have contributed to the insensitivity of conventional hybridization techniques. Identification of EGF transcripts in glands during midlactation confirms earlier studies (29, 33) that showed prepro-EGF mRNA (4.7 kb) was abundant enough to be detected by Northern analysis. Rather than functioning as a local mitogen, the EGF precursor, which is synthesized and processed in the differentiated alveolar epithelium (29), may serve as the source for the abundant levels of EGF in milk (44). The lack of detectable TGF-α transcript in mouse lactating gland suggests that the influence of lactogenic hormones or the state of cell differentiation on the expression of TGF-α is distinct from that of EGF. The detection of TGF-α in rat lactating mammary gland suggests there may be species differences in TGF-α expression (45).

Localization of EGF and TGF-α in different cell populations suggests that these growth factors have different functional roles during mammary gland ductal morphogenesis. EGF was not detected in the cap-cell layer of the TEB but showed prominent staining in a subpopulation of luminal cells of the ductal epithelium known to exhibit relatively low rates of proliferation during ductal morphogenesis (46). This result suggests that EGF may not be a ductal mitogen at this stage of development but may function in other physiological roles, such as the regulation of fluid or ion flux (47, 48). TGF-α was localized in the cap-cell layer, which is considered a stem-cell population for both luminal and myoepithelial cells of the mammary ducts (4, 49). Because the TEB cap-cell layer is a proliferating cell population (4, 49) and is enriched with TGF-α/EGF receptors (50), TGF-α may serve as a positive growth regulator at this site. Function of TGF-α may not be limited to regulation of cell growth but may vary with the site of synthesis or developmental changes in the mammary gland.

TGF-α in cap cells may be functionally homologous with TGF-α in the epidermis (20). The epidermal basal-cell layer (51) and the TEB cap cells are enriched with TGF-α/EGF receptors (50) and have common antigenic markers (52). The cap-cell layer exists in close proximity to stromal cells, which might influence the expression or elaboration of TGF-α. Activation of the TGF-α/EGF receptor pathway could occur indirectly through the interaction of estrogens with stromal cells. The cap-cell layer is apparently devoid of estrogen receptors, which are present in the surrounding stromal cells (8). Studies have shown the importance of epithelial–stromal contact in estrogen-induced proliferation of normal cells *in vitro* (53–55). The TGF-α immunoreactivity in stromal fibroblasts at the TEB base is consistent with both localization of

TGF-α/EGF receptors at these sites (50) and reports that EGF-like growth factors can stimulate the synthesis of extracellular matrix components (22, 56, 57).

The formation of end buds in the regressed mammary glands of ovariectomized mice exposed to locally implanted pellets containing TGF-α or EGF indicated that the TGF-α/EGF receptor pathway remained intact and that these receptors are linked to a mitogenic pathway *in vivo*. These findings confirm and extend earlier studies (50) by showing that TGF-α, as well as EGF, is sufficient to stimulate the normal pattern of ductal growth. Because end-bud regeneration was observed in castrates, we conclude that synthesis of the TGF-α/EGF receptor and related postreceptor intermediates does not directly depend on ovarian steroids. Ovarian hormones probably are not required for the expression of TGF-α or EGF because we detected mRNA transcripts of both growth factors in glands of ovariectomized animals. Several earlier studies have shown that 17β-estradiol can increase the levels of TGF-α mRNA in breast cancer cells and the amount of TGF-α immunoreactivity in conditioned medium (12, 58, 59). Estrogens also augment expression of prepro-EGF mRNA in the mouse uterus (60). Because TGF-α and EGF may exist as membrane-bound precursors (29, 61), modulation of the proteolytic processing of precursor to mature peptide would affect the level of mature ligand available for binding to the receptor. One mechanism by which estrogens could stimulate this pathway would be through activating specific proteolytic enzymes that cleave the precursor, elaborating the mature peptide. Increasing availability of the bioactive ligand could upregulate both growth factor (20) and receptor (62). Systematic study of the functional roles of growth factors in glandular and stromal tissue during ductal morphogenesis may enable us to ultimately understand the basis for steroid-induced cell proliferation in the mammary gland.

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1. Vonderhaar, B. K. (1988) in *Breast Cancer: Cellular and Molecular Biology*, eds. Lippman, M. E. & Dickson, R. B. (Academic, Boston), pp. 251–266.
2. Turner, C. W. & Gomez, E. T. (1933) *Res. Bull. Mo. Agric. Exp. Stn.* **182**, 1–43.
3. Nandi, S. (1958) *J. Natl. Cancer Inst.* **21**, 1039–1063.
4. Daniel, C. W. & Silberstein, G. B. (1987) in *The Mammary Gland, Development, Regulation and Function*, eds. Neville, M. C. & Daniel, C. W. (Plenum, New York), pp. 3–36.
5. Williams, J. M. & Daniel, C. W. (1983) *Dev. Biol.* **97**, 274–290.
6. Faulkin, L. J., Jr., & DeOme, K. B. (1960) *J. Natl. Cancer Inst.* **24**, 953–963.
7. Richards, J. E., Shyamala, G. & Nandi, S. (1974) *Cancer Res.* **34**, 2764–2772.
8. Daniel, C. W., Silberstein, G. B. & Strickland, P. (1987) *Cancer Res.* **47**, 6052–6057.
9. Lippman, M. E., Dickson, R. B., Kasid, A., Gelmann, E., Davidson, N., McManaway, M., Huff, K., Bronzert, D., Bates, S., Swain, S. & Knabbe, C. (1986) *J. Steroid Biochem.* **24**, 147–154.
10. Derynck, R., Goeddel, D. V., Ullrich, A., Gutterman, J. U., Williams, R. D., Bringman, T. S. & Berger, W. H. (1987) *Cancer Res.* **47**, 707–712.
11. Bates, S. E., Davidson, N. E., Valverius, E. M., Freter, C. E., Dickson, R. B., Tam, J. P., Kudlow, J. E., Lippman, M. E. & Salomon, D. S. (1988) *Endocrinology* **2**, 543–555.
12. Perroteau, I., Salomon, D., DeBortoli, M., Kidwell, W., Hazarika, P., Pardue, R., Dedman, J. & Tam, J. (1986) *Breast Cancer Res. Treat.* **7**, 201–210.
13. Marquardt, H., Hunkapiller, M. W. & Todaro, G. J. (1984) *Science* **223**, 1079–1082.
14. Derynck, R., Roberts, A. B., Winkler, M. E., Chen, E. Y. & Goeddel, D. V. (1984) *Cell* **38**, 287–297.
15. Imagawa, W., Tomooka, Y., Hamamoto, S. & Nandi, S. (1985) *Endocrinology* **116**, 1514–1524.
16. Taketani, Y. & Oka, O. (1983) *FEBS Lett.* **152**, 256–260.
17. Tonelli, Q. J. & Sorof, S. (1980) *Nature (London)* **285**, 250–252.
18. Richards, J., Guzman, R., Konrad, M., Yang, J. & Nandi, S. (1982) *Exp. Cell Res.* **141**, 433–443.
19. Singletary, S. E., Baker, F. L., Spitzer, G., Tucker, S. L., Tomasovic, B., Brock, W. A., Ajani, J. A. & Kelly, A. M. (1987) *Cancer Res.* **47**, 403–406.
20. Coffey, R. J., Jr., Derynck, R., Wilcox, J. N., Bringman, T. S., Goustin, A. S., Moses, H. L. & Pittelkow, M. R. (1987) *Nature (London)* **27**, 817–820.
21. Zajchowski, D., Band, V., Pauzie, N., Tager, A., Stampfer, M. & Sager, R. (1988) *Cancer Res.* **148**, 7041–7047.
22. Liu, S. C., Sanfilippo, B., Perroteau, I., Derynck, R., Salomon, D. S. & Kidwell, W. R. (1987) *Mol. Endocrinol.* **1**, 683–692.
23. Han, J. H., Stratowa, C. & Rutter, W. J. (1987) *Biochemistry* **26**, 1617–1625.
24. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 1408–1412.
25. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
26. Lee, D. C., Rose, T. M., Webb, N. R. & Todaro, G. J. (1985) *Nature (London)* **313**, 489–491.
27. Scott, J., Urdea, M., Quiroga, M., Sanchez-Pescador, R., Fong, N., Selby, M., Rutter, W. J. & Bell, G. I. (1983) *Science* **221**, 236–240.
28. Alonso, S., Minty, A., Bourlet, Y. & Buckingham, M. (1986) *J. Mol. Evol.* **23**, 11–22.
29. Brown, C. F., Teng, C. T., Pentecost, B. T. & DiAugustine, R. P. (1989) *Mol. Endocrinol.* **3**, 1077–1083.
30. Ordronneau, P., Lindström, P. B.-M. & Petrusz, P. (1981) *J. Histochem. Cytochem.* **29**, 1397–1404.
31. Murray, J. B., Brown, L., Langer, R. & Klagsbrun, M. (1983) *In Vitro* **19**, 743–748.
32. Russo, I. H., Tewari, M. & Russo, J. (1989) in *Integument and Mammary Glands*, eds. Jones, T. C., Mohr, U. & Hunt, R. D. (Springer, Berlin), p. 239.
33. Rall, L. B., Scott, J., Bell, G. I., Crawford, R. J., Penschow, J. D., Niall, H. D. & Coghlan, J. P. (1985) *Nature (London)* **313**, 228–231.
34. Bresciani, F. (1968) *Cell Tissue Kinet.* **1**, 51–63.
35. Welsch, C. W. (1987) in *Cellular and Molecular Biology of Mammary Cancer*, eds. Medina, D., Kidwell, W., Heppner, G. & Anderson, E. (Plenum, New York), pp. 163–179.
36. Lippman, M. E. (1985) in *Williams Textbook of Endocrinology*, eds. Wilson, J. D. & Foster, D. W. (Saunders, Philadelphia), 7th Ed., pp. 1309–1326.
37. Rappolee, D. A., Brenner, C. A., Schultz, R., Mark, D. & Werb, Z. (1988) *Science* **241**, 1823–1825.
38. Twardzik, D. R. (1985) *Cancer Res.* **45**, 5413–5416.
39. Lee, D. C., Rochford, R., Todaro, G. J. & Villarreal, L. P. (1985) *Mol. Cell. Biol.* **5**, 3644–3646.
40. Malden, L. T., Novak, U. & Burgess, A. W. (1989) *Int. J. Cancer* **43**, 380–384.
41. Shankar, V., Ciardiello, F., Kim, N., Derynck, R., Liscia, D. S., Merlo, G., Langton, B. C., Sheer, D., Callahan, R., Bassin, R. H., Lippman, M. E., Hynes, N., Salomon, D. S. (1989) *Mol. Carcinog.* **2**, 1–11.
42. Rosenthal, A., Lindquist, P. B., Bringman, T. S., Goeddel, D. V. & Derynck, R. (1986) *Cell* **46**, 301–309.
43. Valverius, E. M., Bates, S. E., Stampfer, M. R., Clark, R., McCormick, F., Salomon, D. S., Lippman, M. E. & Dickson, R. B. (1989) *Mol. Endocrinol.* **3**, 203–214.
44. Carpenter, G. (1980) *Science* **210**, 198–199.
45. Liscia, D. S., Merlo, G., Ciardiello, F., Kim, N., Smith, G. H., Callahan, R. & Salomon, D. S. (1990) *Dev. Biol.* **140**, 123–131.
46. Bresciani, F. (1965) *Exp. Cell Res.* **38**, 13–32.
47. Green, J. & Muallem, S. (1989) *FASEB J.* **3**, 2408–2414.
48. Vehaskari, V. M., Hering-Smith, K. S., Moskowitz, D. W., Weiner, I. D. & Hamm, L. L. (1989) *Am. J. Physiol.* **256**, F803–F809.
49. Dulbecco, R., Henahan, M. & Armstrong, B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7346–7350.
50. Coleman, S., Silberstein, G. B. & Daniel, C. W. (1988) *Dev. Biol.* **127**, 304–315.
51. Nanney, L. B., McKanna, J. A., Stoscheck, C. M., Carpenter, G. & King, L. E. (1984) *J. Invest. Dermatol.* **82**, 165–169.
52. Daams, J., Sonnenberg, A., Sakakura, T. & Hilgers, J. (1987) in *Cellular and Molecular Biology of Mammary Cancer*, eds. Medina, S., Kidwell, W., Heppner, G. & Anderson, E. (Plenum, New York), pp. 1–8.
53. McGrath, C. M. (1983) *Cancer Res.* **43**, 1355–1360.
54. Haslam, S. Z. (1986) *Cancer Res.* **46**, 310–316.
55. Shyamala, G. & Ferenczy, A. (1984) *Endocrinology* **115**, 1078–1081.
56. Chen, L. B., Gudor, R. C., Sun, T.-T., Chen, A. B. & Mosesson, M. W. (1978) *Science* **197**, 776–778.
57. Lembach, K. J. (1976) *J. Cell. Physiol.* **89**, 277–288.
58. Salomon, D. S., Zwiebel, J. A., Bano, M., Losonczy, I., Fehnel, P. & Kidwell, W. R. (1984) *Cancer Res.* **44**, 4069–4077.
59. Dickson, R. B., Huff, K. K., Spencer, E. M. & Lippman, M. E. (1985) *Endocrinology* **118**, 138–142.
60. DiAugustine, R. P., Petrusz, P., Bell, G. I., Brown, C. F., Korach, K. S., McLachlan, J. A. & Teng, C. T. (1988) *Endocrinology* **122**, 2355–2363.
61. Teixidó, J., Gilmore, R., Lee, D. C. & Massagué, J. (1987) *Nature (London)* **326**, 883–885.
62. Clark, A. J. L., Ishii, S., Richert, N., Merlino, G. T. & Pastan, I. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8374–8378.