Prolactin-inducible Proteins in Human Breast Cancer Cells*

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The mechanism of action of prolactin in target cells and the role of prolactin in human breast cancer are poorly understood phenomena. The present study examines the effect of human prolactin (hPRL) on the cell line, T-47D, in serum-free medium containing bovine serum albumin. [35S]Methionine-labeled proteins were analysed by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis and fluorography. Treatment of cells with hPRL (1–1000 ng/ml) and hydrocortisone (1 pg/ml) for 36 h or longer resulted in the synthesis and secretion of three proteins having molecular weights of 11,000, 14,000, and 16,000. Neither hPRL nor hydrocortisone alone induced these proteins. Of several other peptide hormones tested, only human growth hormone, a hormone structurally and functionally similar to hPRL, could replace hPRL in causing protein induction. These three proteins were, therefore, referred to as prolactin-inducible proteins (PIP). Each of the three PIPs was purified to homogeneity by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and specific antibodies were generated to them in rabbits. By immunoprecipitation and immunoblotting (Western blot) of proteins secreted by T-47D cells, it was demonstrated that the three PIPs were immunologically identical to one another. In addition, the 16-kDa and 14-kDa proteins (PIP-16 and PIP-14), and the 11-kDa protein (PIP-11), incorporated [3H]glucosamine. Furthermore, 2-deoxyglucose (2 mM) and tunicamycin (0.5 μg/ml), two compounds known to inhibit glycosylation, blocked the production of PIP-16 and PIP-14, with a concomitant increase in the accumulation of PIP-11. These results indicate PIP-16 and PIP-14 are glycosylated variants of PIP-11. Finally, in vitro translation of poly(A)* messenger RNA followed by immunoprecipitation revealed a 12.5-kDa protein, possibly the precursor form of PIPs. In addition, T-47D cells treated with hPRL plus hydrocortisone contained 10-fold more mRNA for PIPs than control cells, suggesting that the hormones’ action is at the level of gene expression. Our finding represents a first demonstration of an induction of specific proteins via regulation of gene expression by prolactin in human target cells.

The role that the pituitary hormone, prolactin, plays in promoting the growth of mammary tumors in experimental animals is well known (1). However, its significance in human mammary tumorigenesis is unclear (2). In addition, large gaps still exist in our knowledge concerning the molecular mechanism of action of prolactin in target cells (3). In order to facilitate these studies, a prolactin-responsive human cell model is needed. Our past efforts have established that many human breast cancer cell lines maintained in tissue culture possess specific cell membrane receptors for prolactin (4). Furthermore, prolactin synergizes with glucocorticoid in causing cell rounding, loss of adhesion, and increased lipid synthesis in one human breast cancer cell line, T-47D (5). In the present study, we show for the first time that prolactin, in the presence of glucocorticoid, increased the accumulation of mRNA for, and the synthesis of, unique secretory proteins by the T-47D cells. This finding represents a first demonstration of an induction of specific proteins via regulation of gene expression by prolactin in human target cells.

MATERIALS AND METHODS

Cell Line—The human breast cancer cell line, T47D, was derived from the pleural effusion of a patient with disseminated carcinoma of the breast (6). This cell line contains the highest concentration of prolactin receptors of 11 breast cancer cell lines tested (4, 7). The nontumor human breast cell line, HBL-100, was generated from human breast milk (8). Cell lines were routinely maintained in Dulbecco’s modified Eagle’s medium supplemented with insulin (10 μg/ml), glutamine (4 mM), glucose (4.5 g/liter), streptomycin (50 μg/ml), penicillin (50 units/ml), and fetal bovine serum (10%, v/v). This medium is referred to as complete medium (CM). All the above reagents were purchased from Gibco. Cells were kept in a humidified atmosphere of 95% air, 5% CO₂ at 37 °C.

Hormones and Antiserum—Purified human prolactin (hPRL) was kindly provided by Drs. Henry G. Friesen and Ian G. Worzley, University of Manitoba. Purified human growth hormone (hGH), ovine growth hormone, ovine prolactin, and human luteinizing hormone were gifts from the National Pituitary Agency. National Institutes of Health. Hydrocortisone was purchased from Sigma. Antiserum to human α-lactalbumin and casein were gifts of Drs. C. Kleinberg, Columbia University, and Dr. J. Kulski, University of Western Australia. All the antisera were raised in rabbits. In addition, antisera were generated to three prolactin-inducible proteins which will be described in a later section.

Analysis of Proteins Synthesized by Cells—Human breast cancer cells, T-47D (1 × 10⁶/35-mm culture dish), were seeded in CM. Two days later, the medium was aspirated, the cells were washed with insulin-free and fetal bovine serum-free medium (referred to as DM), and each dish finally received 2 ml of DM. Hormones were added as 100 × concentrates (20 μl/2 ml) in DM that contained 1% (w/v) bovine serum albumin (BSA). For those dishes that received only one hormone or no hormone, 20 or 40 μl of DM/BSA was added. All dishes contained the same amount (200 μg/ml) of BSA. The cells

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‡ The abbreviations used are: hPRL, human prolactin; hGH, human growth hormone; BSA, bovine serum albumin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PIP, prolactin-inducible proteins.
were incubated for another 6 days at which time 100 μCi of [35S] methionine (>1200 Ci/mmol, Amersham Corp.), or 15 μCi of d-1[6,3H]glucosamine hydrochloride (30–60 Ci/mmol, New England Nu-clear) was added to each dish. Eighteen hours later, the medium from each dish was collected and centrifuged at 500 × g for 5 min to remove any cells present. The medium was then extensively dialyzed at 4 °C against glass-distilled water and lyophilized. The dry proteins were dissolved in an SDS mixture (10 mM sodium phosphate buffer, pH 7.2, containing 2% sodium dodecyl sulfate, 5% β-mercaptoethanol, 10% glycerol, and trace amount of bromphenol blue). Each sample was then heated in a boiling water bath for 5–10 min. Proteins in each sample were separated by SDS-polycrylamide slab gel electropho-eresis (9). All reagents for electrophoresis were obtained from Bio- rad. The radiolabeled proteins on the gel were detected by fluorogra-phy (10). The molecular mass marker 14C-proteins were: myosin (200,000), phosphorylase (94 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), and cytochrome c (12 kDa).

**Purification of Prolactin-inducible Proteins and Production of Spe-cific Antisera—** Twenty T-150 culture flasks were seeded with 106 T-47D cells in CM. Two days later, the cells were washed twice with DM, and each flask was replaced with DM. Human prolactin (or human growth hormone) and hydrocortisone were added, each to a final concentration of 1 μg/ml. After 5 days of hormone treatment, three of the flasks received [35S]Met (50 μCi/ml). The next day, the media from all the flasks (600 ml) were collected, pooled, dialyzed against distilled water at 4 °C, and lyophilized. The lyophilized prolactin- reteins were redissolved in a solution of phosphate-buffered saline (PBS) containing 8 M urea. The dissolved proteins were fractionated by gel filtration on a Sephacryl S-1000 column equilibrated with phosphate-buffered saline, 8 M urea, which was also used for elution. This gel filtration step was essential to separate the bulk of the proteins (e.g. BSA which was introduced with the hormones) from the prolactin-induced proteins. The prolactin-induced proteins in each fraction were monitored by SDS-PAGE and fluorography, as described earlier. The prolactin-induced proteins eluted after the main BSA peak. The fractions that contained the prolactin-induced proteins were pooled, dialyzed against water, lyophilized, redissolved in SDS mixture (1–2 ml), and, finally, heated. The entire sample was run on preparative slab SDS-PAGE (0.3 mm thick). The gel was stained with 0.1% Coomassie Blue in 7% acetic acid and, finally, destained with 10% methanol in 7% acetic acid. The prolactin-induced proteins, 16-, 14-, and 11-kDa bands, were prominently stained. Each band was excised with a sharp blade, after which each polycrylamide gel segment was cut into small fragments (1–2 mm). The fragments from each band were soaked with agitation at room temperature for 10 min in a 100 μl of SDS mixture (minus bromphenol blue), and this solution discarded. A second 2-ml buffer was added, and the contents were heated in a boiling water for 10 min. After boiling, the entire content was packed into a cylindrical glass tube with the bottom end plugged with glass wool and wrapped with a closed dialysis tubing (M, cut-off 3,500). The protein was electrophoresed (5 h at 10 mA/ tube) out of the gel into the reservoir made up of the dialysis tubing. The content of each dialysis bag was dialyzed against water at room temperature overnight. This procedure resulted in the complete puriﬁcation of all the three prolactin-induced proteins. The homogeneity of each protein was established by analytical SDS-PAGE (see “Results”). Using the above procedure, 40–60 μg of each prolactin-induced protein could be obtained from 1 liter of conditioned medium.

Ten micrograms of each protein was emulsified with complete Freund’s adjuvant, and injected intradermally at multiple sites in a rabbit. Two and four weeks later, the same amount of protein was given via the same route in complete Freund’s adjuvant. The rabbits were bled 8–10 weeks after the final injection. Antisera were obtained after removal of the clots and cells. Pre-immune serum was also obtained by bleeding each of the rabbits before immunization with the protein.

**Immunoprecipitation—** Each sample containing [35S]Met-labeled proteins was allowed to incubate at 4 °C overnight with 5 μl of antibodies precomplexed in 0.05 M Tris-HCl, pH 7.8, containing 0.1 M NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 100 units/ml trysl and, 0.5% Nonidet P-40 (referred to as immunoprecipitation buffer) in a final volume of 100 μl. The next day, 50 μl of Pansorbin™ (Staphylococcus aureus suspension, Calbiochem-Behring), washed previouly with 4 μl urea in immunoprecipitation buffer and resus-pended in immunoprecipitation buffer, was added. One hour later, the bacteria-antibody-antigen complexes were washed once with 4 M

![Fig. 1. Effect of hormones on [35S]methionine-labeled proteins secreted by T-47D cells.](image-url)
cancer cells that were exposed to hydrocortisone alone (Fig. 1, lane B) were essentially similar to those produced by untreated cells, with the exception that three high molecular mass proteins (300–440 kDa) were reduced. Upon the treatment of cells with hPRL plus hydrocortisone, (Fig. 1, lane C), three proteins, 160, 75, and 36 kDa, disappeared (or were substantially reduced). In addition, three new proteins were induced: 16, 14, and 11 kDa with the 14-kDa protein being the most prominent. hGH plus hydrocortisone (Fig. 1, lane D) produced an identical protein pattern as hPRL plus hydrocortisone. In contrast, in the absence of hydrocortisone, treatment produced an identical protein pattern as hPRL plus hydrocortisone. Furthermore, the observation that hGH was equipotent with hPRL plus hydrocortisone is consistent with our previous observations that hGH and hPRL bind to the same receptors, and that these two "lactogenic" hormones are equipotent in their ability to induce other biological responses in the T-47D cells (4, 5).

The hormone specificity and the dose-response of induction of 16-, 14-, and 11-kDa proteins were also examined. Fig. 2 shows that, in the presence of hydrocortisone, 1 ng/ml hGH (lane D) caused a significant induction of the proteins; maximal induction was achieved with 0.1–1 µg/ml hGH (lanes A and B). When the radioactivity in the protein bands was quantitatively determined (see legend to Fig. 2), it was found that 1 ng/ml and 1µg/ml hGH caused a 2- and 10-fold induction, respectively, of each of the three proteins. In the presence of hydrocortisone, hPRL produced a similar dose response (not shown). Thus, the induction of proteins in T-47D cells occurs at physiological concentrations of the hPRL and hGH, suggesting that this phenomenon is a physiologically relevant event. In contrast, in the presence of hydrocortisone, human luteinizing hormone (lane F), ovine growth hormone (lane E), and ovine prolactin (not shown), at 1 µg/ml, failed to induce the three proteins. Since the three proteins were induced specifically by hPRL and by hGH acting through the prolactin receptors (4, 5), we propose to call them "prolactin-inducible proteins" or "PIPS"—coded as PIP-11 (11 kDa), PIP-14 (14 kDa), and PIP-16 (16 kDa).

Hormone treatment of 36 h was found to be required to observe a significant increase in protein induction. The maximum rate of synthesis of PIPs occurred, however, 72 h after the addition of hormones, and this rate could be maintained for 1 week, the longest time interval tested (data not shown).

**Purification of PIPs and the Generation of Antibodies**—In an attempt to establish the relationship of the three PIPs with each other and possibly with other proteins produced by T-47D cells (see Fig. 1), as well as to understand the mode of induction of the PIPs by prolactin, we decided to generate specific antibodies to each of the three PIPs. This was accomplished first by purifying each of them to homogeneity in two steps (gel filtration chromatography and preparative SDS-PAGE; see "Materials and Methods"). Each of the three purified PIPs was shown to be homogeneous (Fig. 3). Each of the PIPs was used to immunize a rabbit, and the resulting antisera were used for the following studies.

**Fig. 2. Dose response of hGH and the effect of unrelated hormones.** T-47D cells were plated and treated with hormones in the manner described under "Materials and Methods." [35S]Metionine-labeled proteins were analyzed by a 15% polyacrylamide gel which improved the separation of the 16- and 14-kDa proteins, but sacrificed the resolution of the high molecular weight proteins. Therefore, only the part of the gel that contained the 16-, 14-, and 11-kDa proteins is shown. For each sample, 30,000 cpm of radiolabeled proteins were analyzed. Lane A, hydrocortisone plus hGH (11 µg/ml); lane B, hydrocortisone plus hGH (100 ng/ml); lane C, hydrocortisone plus hGH (10 ng/ml); lane D, hydrocortisone plus hGH (1 ng/ml); lane E, hydrocortisone plus human luteinizing hormone (1 µg/ml); lane F, hydrocortisone plus ovine growth hormone (1 µg/ml); lane G, hydrocortisone only. Hydrocortisone was used at 1 µg/ml in all the situations. To quantitate the radioactivity in each protein band, the positions of the radiolabeled protein bands on the dry polyacrylamide gel were marked. The gel segments were excised and allowed to swell in water for 1 h. The swollen gel segments were dissolved in 0.5 ml of hydrogen peroxide (30%) at 65°C. After the addition of 10 ml of scintillation fluid (ACS, Amersham Corp.), the radioactivity in each sample was determined.

**Fig. 3. Analysis of purified prolactin-inducible proteins.** Aliquots of proteins purified by preparative SDS-PAGE (see "Materials and Methods") were run on a 15% SDS-polyacrylamide slab gel. Lane A, PIP-11 (1370 cpm); lane B, PIP-14 (850 cpm); lane C, PIP-16 (1240 cpm).
Characterization of the Three anti-PIP Antisera—Identical aliquots of [35S]methionine-labeled proteins secreted by T-47D cells treated with hPRL (or hGH) plus hydrocortisone were reacted with each of the antisera, and the immunoprecipitated products were analyzed by SDS-PAGE and fluorography (see “Materials and Methods”). Fig. 4A shows that all three PIPs were precipitated by each of the antisera. Anti-PIP-14 antiserum appeared to have the highest titer of antibodies. To exclude the possibility that the PIPs were tightly associated with one another, resulting in their coprecipitation by any one antiserum, the proteins secreted by T-47D were first separated by SDS-PAGE and then electrophoretically transferred to nitrocellulose paper (Western blot). The transferred proteins were reacted with anti-PIP-14 antiserum and visualized using an Immune-Blot assay system (Bio-Rad). The result of the Western blot was shown in Fig. 4B: all three PIPs were detected by the anti-PIP-14 antiserum. Thus, the data of Fig. 4, A and B, indicate that all three PIPs were immunoologically related, possibly variant species of the same protein.

Based on two-dimensional gel electrophoretic data (not shown), charge heterogeneity of PIP-14 and PIP-16, but not PIP-11, was evident. Taken together with the immunological similarity of the three PIPs, it was suspected that PIP-14 and PIP-16 were glycosylated forms of PIP-11. To test this possibility, T-47D cells were labeled with [3H]glucosamine, and the proteins in the media were analyzed. Fig. 5 shows that PIP-14 and PIP-16, but not PIP-11, were labeled. In addition, [35S]methionine-labeled proteins made by hormone-treated T-47D cells in the presence of 2-deoxyglucose and tunicamycin, two compounds known to effectively inhibit glycosylation (13, 14), were analyzed after immunoprecipitation with anti-PIP-14 antiserum. Fig. 6 shows that both 2-deoxyglucose and tunicamycin were able to block the synthesis of PIP-14 and PIP-16. Concomitantly, there was an increased accumulation of PIP-11. It is concluded that PIP-14 and PIP-16 are glycosylated forms of PIP-11.

The relationship, if any, of PIPs to other proteins made by T-47D cells in the absence of various hormone combinations was also studied. Fig. 7 (lanes 1–4) shows that anti-PIP-14 antiserum was only able to precipitate the three PIPs in the culture medium of T-47D cells treated with hGH plus hydrocortisone (lane 4); very little of them was secreted by cells not treated with hormone (lane 1), or by cells exposed to only hydrocortisone (lane 2) or to hGH alone (lane 3). No other high molecular weight proteins were recognized by the antiserum. Similar results were obtained when intracellular proteins were analyzed (lanes 5–8). The presence of PIP-11 and PIP-14 was evident (although weakly) only in cells exposed to hGH plus hydrocortisone (lane 8). PIPs were absent from control cells (lane 5), or in cells treated only with hydrocortisone (lane 6) or hGH (lane 7). No other intracellular proteins in control or hormone-treated cells were recognized by antibodies to PIP-14 (all the proteins with M, > 20,000 in lanes 5–8 were nonspecifically brought down because these proteins were also present when preimmune rabbit serum was used instead of anti-PIP-14). Thus, the data of Fig. 7 indicate

![Fig. 4. Immunoprecipitation of [35S]Met-labeled PIPs (A) and immunoblot (Western blot) of PIPs (B). A, labeled culture medium from hPRL plus hydrocortisone-treated T-47D cells was dialyzed, lyophilized, and reconstituted in immunoprecipitation buffer. Identical aliquots were reacted with various preimmune and antisera, and the immunoprecipitated proteins were analyzed by a 15% polyacrylamide gel (procedures described under “Materials and Methods”). Lane 2, anti-PIP-11 serum; lane 4, anti-PIP-14 serum; lane 6, anti-PIP-16 serum. The odd-numbered lanes represent the corresponding preimmune sera. B, culture medium from hormone-treated cells was dialyzed, lyophilized, and redissolved in SDS mixture as described. Equal aliquots were applied on a 15% polyacrylamide gel. The procedures of the Western blot and the immunoblotting of PIPs have been described under “Materials and Methods.” Lane 1, preimmune serum; lane 2, anti-PIP-14 antiserum.

![Fig. 5. Incorporation of [3H]glucosamine into proteins secreted by T-47D cells. The treatment of cells with hormones in the presence of [3H]glucosamine and the separation and visualization of the labeled proteins on a 5–20% gradient polyacrylamide gel have been described under “Materials and Methods.” Lane A, cells treated with hGH plus hydrocortisone; lane B, cells treated with hPRL plus hydrocortisone; lane C, cells treated with hydrocortisone alone.](Image)
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Fig. 6. Effect of 2-deoxyglucose and tunicamycin on the synthesis of PIPs. After 5 days of incubation of T-47D cells with hGH plus hydrocortisone in serum-free medium (DM) that contained 1 g/liter instead of 4.5 g/liter glucose, 2-deoxyglucose or tunicamycin was added. One hour after the addition of the inhibitors, [35S]methionine was added. Eighteen hours later, the labeled proteins in the media were dialyzed, lyophilized, and reconstituted in immunoprecipitation buffer, and aliquots of each sample were incubated with either preimmune serum (data not shown) or anti-PIP-14 antiserum. The processed products of some other large proteins, and (2) that camycin (0.5 μg/ml) was added. One hour after the addition of the inhibitors, [35S]methionine was added. Eighteen hours later, the labeled proteins in the media were dialyzed, lyophilized, and reconstituted in immunoprecipitation buffer, and aliquots of each sample were incubated with either preimmune serum (data not shown) or anti-PIP-14 antiserum. The immunoprecipitated proteins were analyzed on a 15% polyacrylamide gel. All the above procedures have been described under "Materials and Methods." Lane A, cells not exposed to inhibitor; lane B, cells treated with 2-deoxyglucose (2 mM); lane C, cells treated with tunicamycin (0.5 μg/ml).

1) that the PIPs were not related to, and, therefore, not processed products of, some other large proteins, and (2) that hGH plus hydrocortisone stimulated the synthesis, and not secretion, of PIPs because PIPs were not found in the intracellular compartment of control cells or cells treated with hydrocortisone or hGH alone.

Quantitation of Messenger RNA for PIPs—In order to gain insights into the mechanism of induction of PIPs in T-47D cells by hormones, we decided to quantitate the amount of translatable messenger RNA in control and hormone (hPRL plus hydrocortisone)-treated cells. Oligo(dT)-selected poly(A)+ RNAs were translated in vitro using a rabbit reticulocyte lysate system, and the products were immunoprecipitated, analyzed, and quantitated. Fig. 8 (top panel) shows that the only in vitro translated product that reacted with anti-PIP-14 antibodies was a peptide of 12.5 kDa. This is presumably the precursor form of PIP. Substantial amounts of translatable PIP mRNA were present in hormone-treated cells (lanes D–F), and very little was found in control cells (lane A–C). The intensity of the 12.5 kDa bands was quantitated by densitometric tracing as shown in Fig. 8, lower panel: a 10-fold increase of PIP-mRNA was present in hormone-treated cells as compared to control cells. It is concluded from this result that hormone treatment induced the accumulation of PIP-mRNA in T-47D human breast cancer cells.

Other Observations—We have also examined whether or not PIPs are milk components. Incubation of [35S]methionine-labeled proteins secreted by T-47D cells with antiserum to human whole milk, α-lactalbumin, and casein failed to immunoprecipitate any one of the PIPs (not shown). In addition, Western blot analysis of human whole milk using anti-PIP-14 antiserum failed to reveal the presence of PIPs in the milk sample (not shown). Finally, immunoprecipitation and Western blot analysis of proteins synthesized and secreted by a nontumor human breast epithelial cell line HBL-100 failed to detect the presence of PIPs (data not shown).

DISCUSSION

In the present study, we were able to demonstrate for the first time that hPRL, in the presence of glucocorticoid, was able to induce the synthesis of three unique proteins in the prolactin receptor-positive human breast cancer cell line, T-47D. These PIPs have molecular mass of 16, 14, and 11 kDa. Only hGH, a hormone evolutionarily, structurally, and functionally related to hPRL, was able to cause the induction of PIPs also. This observation is consistent with our previous findings that 1) hGH binds to the prolactin receptors in the...
T-47D cells, and 2) hGH is equipotent with hPRL in a number of measurable biological responses in T-47D cells (4, 5).

To facilitate characterization of the PIPs and to gain better insight into the molecular mechanism of induction of PIPs by prolactin, we decided to purify each of the three PIPs and to generate polyclonal antibodies to each of them. Using these specific antibodies, we were able to establish that the three PIPs were immunologically related. We also demonstrated that only the two larger PIPs, 16 and 14 kDa, incorporated [3H]glucosamine, and that 2-deoxyglucose and tunicamycin, inhibitors of protein glycosylation, inhibited the translation of poly(A)+ mRNA. This suggests that the 12.5-kDa protein is the precursor form of the PIPs.

Our observations clearly showed that the induction of the final PIP products required the synergistic actions of prolactin and cortisol. This same hormone combination was previously shown to be required for the induction of casein mRNA in the rodent mammary gland (15–17). However, the exact role played by prolactin and glucocorticoid and their respective degree of contribution to the regulation of casein gene expression in the rodent mammary gland remain to be determined (15, 17). Nevertheless, it is probable that the two hormones regulate casein gene expression both at the transcriptional and post-transcriptional levels. The exact role of prolactin and glucocorticoid in the regulation of PIP gene expression in the human breast cancer cells, T-47D, awaits studies using a cloned PIP cDNA probe. The observation that prolactin and cortisol stimulate casein gene expression in the rodent mammary gland prompted us to examine if the PIPs made by T-47D human breast cancer cells are milk components and if hormone-treated nontumor human mammary epithelial cells (HBL-100) produced PIPs. Our data indicated that PIPs are not present in normal human milk and that PIPs are not made by nontumor breast cells. Thus, the identity and function(s) of PIPs remain to be elucidated. Nevertheless, our findings represent a first demonstration of prolactin regulation of specific gene product in human target cells. Furthermore, the human breast tumor cell line T-47D appears to be an excellent model to study the molecular mechanisms of action of prolactin. The gene coding for the prolactin-inducible proteins will provide a unique marker for these studies.

The present study has also paved the path for the elucidation of the biological significance of the prolactin-inducible proteins, and the role of prolactin in human breast neoplasm.

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