Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 3130–3134, April 1992
Developmental Biology

Developmental and environmental regulation of a mammary gland-specific nuclear factor essential for transcription of the gene encoding β-casein

(murine transcription factor/lactation/protein phosphorylation/regulation of DNA binding)

MICHAEL SCHMITT-NEY*, BRIGITTE HAPP, ROLAND K. BALL, AND BERND GRONER†

Friedrich Miescher Institute, P.O. Box 2543, CH 4002 Basel, Switzerland

Communicated by Elwood V. Jensen, December 30, 1991 (received for review October 8, 1991)

ABSTRACT **During the lactation period, mammary epithelial cells secrete large amounts of milk proteins. The coordinate regulation of milk protein expression is effected by the lactogenic hormones. We have investigated the activity of a mammary gland-specific transcription factor (MGF), which mediates hormonal influences at the level of a milk protein gene promoter. MGF-binding sites are present in the promoters of the most abundantly expressed milk protein genes. Mutation of the MGF-binding site in the β-casein gene promoter completely abolishes responsiveness of the promoter to lactogenic hormones in cultured mammary epithelial cells. MGF activity is closely controlled *in vivo*. High MGF levels were found in mouse mammary gland nuclear extracts toward the end of pregnancy and during lactation. Withdrawal of suckling pups from their mothers during the lactation period caused a strong and rapid decrease of MGF activity. Readdition of pups to their mothers restored maximal MGF levels within 4 hr. We investigated MGF phosphorylation as a possible posttranslational modification responsible for regulation of the DNA-binding activity of MGF. Treatment of nuclear extracts from lactating mammary glands with potato acid phosphatase abolished MGF-binding activity. Casein kinase II phosphorylation of nuclear extracts from animals withdrawn from their pups for 24 hr enhanced MGF-binding activity. These results suggest that the reversible activation of MGF by suckling and withdrawal might be mediated by the action of kinases and phosphatases.**

Multiple extracellular signals are received and processed by mammary epithelial cells during growth and differentiation. These signals originate from neuronal input, interactions of epithelial cells with hormones, growth factors, extracellular matrix components, and other cell types (1–4). The cells pass through developmental and functional stages in which ductal and alveolar mammary epithelial cells proliferate (mammogenesis), acquire and sustain the ability to produce large amounts of milk (lactogenesis and lactation), and regress to a less differentiated state upon cessation of milk production (involution). Milk has a very high protein content of ≈30 mg/ml. The caseins are the most abundant milk proteins, and α-, β-, and γ-caseins together constitute ≈80% of total milk protein. Among caseins, β-casein is the most highly expressed (5). The caseins are phosphoproteins that form calcium-dependent micelles and, thus, establish very high concentrations of calcium, phosphates, and essential amino acids in milk (6). The milk proteins are coordinately controlled in their expression (7, 8).

Developmental and environmental cues are thought to set off signal-transduction pathways that influence the phenotype of cells through the activity of transcription factors and alterations in the pattern of gene transcription (9). We have identified a mammary gland-specific transcription factor (MGF) that is essential for the transcription of a milk protein gene promoter (10) and that is subject to complex hormonal and environmental regulation. MGF activity is regulated during the development of the gland. Low MGF levels can be found early in pregnancy, and high levels are found at the end of pregnancy and during the lactation period. MGF is also controlled environmentally through suckling of the pups. Withdrawal of the pups at the height of lactation strongly decreases MGF activity within 24 hr. Maximal MGF levels can be restored within several hours after returning pups to their mothers.

Transcription factor phosphorylation has been recognized as a link between the pathways induced by the interaction of extracellular signals with their cognate receptors and the activity of specific transcription factors (11). The activity of MGF can be modulated *in vitro* by the treatment of nuclear extracts with phosphatases and casein kinase II (CKII), suggesting that the lactogenic hormones might exert a posttranslational effect on MGF.

MATERIALS AND METHODS

Cell Growth and Plasmids. HC11 mammary epithelial cells (12) were grown in RPMI 1640 medium/10% heat-inactivated fetal calf serum/bovine insulin at 5 μg/ml/murine epidermal growth factor (EGF) at 10 ng/ml/gentamycin at 50 μg/ml/2 mM glutamine. Before induction cells were maintained 3 days at confluency with EGF. The hormone induction was for 4 days in RPMI 1640 medium/10% fetal calf serum (charcoal treated)/0.1 μM dexamethasone (Sigma)/ovine prolactin (Sigma) at 5 μg/ml with insulin but without EGF. The plasmid pβc(−344/−1)CAT (β-casein promoter construct) has been described (10, 13). Site-directed mutagenesis was done by PCR (14). Two independent PCR reactions were done: (*i*) the upper-strand primer was 5′-TGTGGACTTCTTTTAATTAAGGGACTTTTG-3′ (positions −104 to −75 of the β-casein promoter with mismatches at positions −91 and −92) in association with a 5′ primer containing a *Bam*HI site 5′-ATCGGATCCTCTCTAAAGCTTGTGAAT-3′ (−343 to −324) and (*ii*) the lower-strand primer was 5′-CAAAAGTCCCTTAATTAAAAGAAGTCCACA-3′ (−104 to −75 with mismatches at positions −91 and −92) in association with a 3′ primer carrying a *Bam*HI site 5′-CAGGATCCGATGGTCTATCAGACTCTGT-3′ (−20 to −1). For both reactions the template was pβc(−344/−1) (10). The two products were gel-purified, mixed, and used in a third PCR reaction with the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MGF, mammary gland-specific transcription factor; CAT, chloramphenicol acetyltransferase; CKII, casein kinase II; EGF, epidermal growth factor.

*Present address: Institute of Human Genetics, University of Freiburg, D-7800 Freiburg, Germany.
†To whom reprint requests should be addressed.

5′ and 3′ primers described above. The product was cleaved with *Hin*dIII (position −335 of the β-casein promoter) and *Bam*HI and cloned between the *Hin*dIII and the *Bam*HI sites of pBLCAT 3 vector (15). Mutations were verified by DNA sequencing.

Transfection and Hormonal Induction. HC11 cells were transfected with 10 μg of β-casein promoter–chloramphenicol acetyltransferase (CAT) construct DNA and 1 μg of pSV2neo per 10-cm culture dish by using the calcium phosphate precipitation technique. Transfected cells were selected in G418 as described (13). At least 100 colonies of cells were pooled and used for the hormone induction experiments. Cellular extracts for determining CAT activity were prepared by lysis of cells in 0.25 M Tris·HCl, pH 7.8/0.5% Triton X-100, and the CAT assays were done as described (13, 16).

Nuclear Extract Preparations and Band-Shift Assays. Nuclear extract preparations and band-shift assays were done as described (10). Two different probes were used for the band-shift assays. The β-casein promoter fragment (−1 to −181) was labeled by a kinase reaction as described (10). This fragment contains a high-affinity MGF-binding site (−85 to −100) and a low-affinity MGF-binding site (−135 to −150). The second probe was a 30-base-pair (bp) double-strand oligonucleotide, representing the region from −75 to −104 in the β-casein promoter, containing only the high-affinity MGF-binding site. For preparation of this probe the upper-strand (5′-TGTGGACTTCTTGGAATTAAGGGACTTTTG-3′) oligonucleotide was hybridized to a 10-bp primer (5′ CAAAAGTCCC 3′), and elongation of the second strand was done with Klenow polymerase in the presence of [α-^{32}P]ATP.

Phosphatase and Kinase Treatment of Nuclear Extracts. Potato acid phosphatase (Boehringer Mannheim) was resuspended at a concentration of 1 mg/ml (60 units per ml) in 10 mM Pipes [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid]/50 mM KCl/5% (vol/vol) glycerol. Mammary gland nuclear extracts (2 μg of protein) in 20 mM Hepes, pH 7.9/100 mM KCl/0.2 mM EGTA/0.2 mM EDTA/2 mM dithiothreitol/20% (vol/vol) glycerol were incubated with 1 μg of phosphatase for 20 min at 37°C. Band-shifts assays were done with the dephosphorylated extracts as described (10), except that incubation with the probe was for 20 min at room temperature. The phosphorylation reactions were done with mammary gland nuclear extracts from animals withdrawn from their pups for 24 hr. The extracts (2 μg) were treated with 0.09 unit of CKII for 30 min at 37°C in 50 mM Tris·HCl, pH 7.5/10 mM $MgCl_2$/100 mM NaCl with either 1 mM ATP or 1 mM GTP. CKII (100 units per mg) was provided by F. Meggio and L. Pinna (Department of Biological Chemistry, University of Padova, Italy) (17).

RESULTS

MGF Binding Is Essential for β-Casein Promoter Activity. Binding experiments of nuclear proteins from mammary epithelial cells to the promoter region of the β-casein-encoding gene have revealed several specific DNA–protein interactions in the electrophoretic mobility-shift assay. DNA footprinting and methylation interference experiments have shown that a predominant nuclear binding protein (MGF) recognizes the promoter region at position −100 to −85 (10). This sequence is conserved, at the same position, in the 5′-flanking regions of at least six different casein genes from mouse, rat, and cow (18, 19). The sequence conservation is suggestive of a functional role for this DNA motif and its nuclear-binding factor. The availability of a cell culture system allowed us to test the functional significance of the MGF-binding site in gene-transfer experiments. The cellular system used is the HC11 cell line (12); this line is a clonal derivative of spontaneously immortalized mammary epithelial cells from mid-pregnant BALB/c mice (20). HC11 cells retain important features of mammary epithelial differentiation—most notably the ability to respond to lactogenic hormone stimulation with increased transcription from the β-casein gene promoter (13).

We stably transfected a β-casein promoter–CAT gene construct into HC11 cells (Fig. 1) and measured the CAT activity of cellular protein extracts after exposing the cells to different hormonal treatments. Simultaneous action of the lactogenic hormones dexamethasone and prolactin caused a 70-fold induction in transcriptional activity of the wild-type version of the β-casein promoter. A mutant promoter–CAT gene was constructed in which the MGF recognition sequence was altered. The β-casein promoter sequence between −100 and −85 was changed to 5′-GACTTCTTT-TAATTAA-3′. This sequence exhibits an ≈100-fold reduced affinity for MGF (data not shown). Lactogenic hormone treatment of HC11 cells transfected with the mutated promoter–CAT construct failed to induce CAT activity (Fig. 1). This experiment indicates that MGF binding is absolutely necessary for activity of the β-casein gene promoter.

MGF Activity Is Induced in the Mammary Gland During Pregnancy and Maintained by Suckling During the Lactation Period. Specific binding of MGF to the β-casein gene promoter and the characteristic migration properties of the DNA–protein complex in the electrophoretic mobility-shift assay allowed us to quantitate occurrence of MGF activity in mammary epithelial tissue as a function of the developmental state of the gland. Mammary gland cell nuclear extracts were prepared from mice pregnant and lactating for increased times. Fig. 2 shows that only low levels of MGF activity can

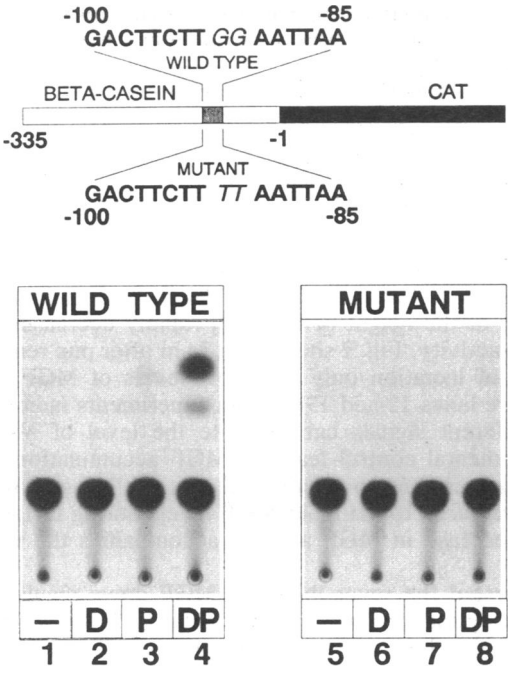

FIG. 1. The MGF-binding sequence is essential for the transcriptional activity of the β-casein gene promoter. Wild-type rat β-casein promoter (−335 to −1) or a mutant promoter, in which two nucleotides crucial for MGF-binding activity have been replaced, were linked to the coding region of the CAT reporter gene. These two constructs were transfected in the HC11 mammary epithelial cells. Transfected cells were induced with either no hormone (−), dexamethasone (D), prolactin (P), or dexamethasone and prolactin (DP). Cellular extracts were prepared, and the enzymatic CAT activity was determined. The wild-type promoter is transcriptionally active in the presence of both hormones (lane 4). It is not active without hormones (lane 1) or with only one of the hormones (lanes 2 and 3). The mutated promoter has lost its hormonal responsiveness (lane 8).

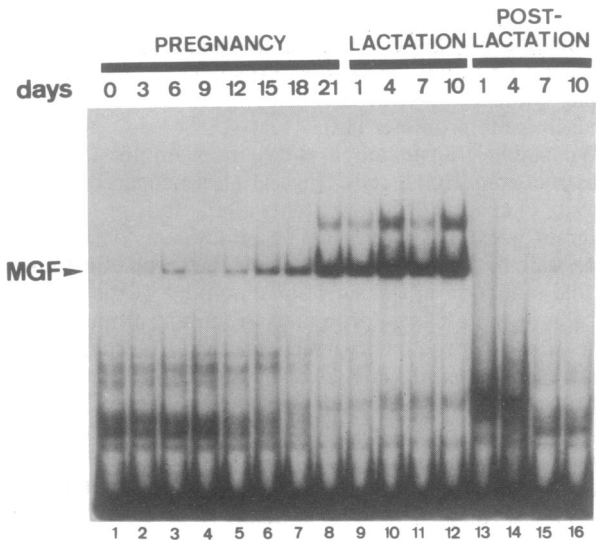

FIG. 2. Detection of MGF in mammary tissue during pregnancy, lactation, and postlactation by the electrophoretic mobility-shift assay. Extracts were prepared from mammary gland cell nuclei of mice pregnant for 0, 3, 6, 9, 12, 15, 18, or 21 days (lanes 1–8) and from mice lactating for 1, 4, 7, or 10 days (lanes 9–12). After 10 days of lactation pups were removed, and mammary cell nuclear extracts were prepared from animals 1, 4, 7, and 10 days (lanes 13–16) in the postlactation period. MGF-binding activity was determined by incubating the nuclear proteins with a radioactively labeled β-casein promoter fragment (position −1 to −181) and electrophoretic separation of DNA from DNA–protein complexes. Migration position of MGF–DNA complex is indicated. A second binding site for MGF is present in this probe (see text and ref. 10). Occupancy of both sites causes the slightly slower migrating band seen in lanes 8–12. For each point three BALB/c mice were sacrificed by cervical dislocation, and their mammary glands were dissected and pooled. The glands were frozen in liquid nitrogen and stored at −70°C until nuclear extract was prepared. Probe preparation and mobility-shift assays have been described (10).

be detected in early and mid-pregnancy. A drastic increase in MGF activity can be seen at the end of pregnancy. This increase parallels the accumulation of milk protein mRNA (7, 8). A high level of MGF activity is maintained during the period of lactation. Removal of pups from their lactating mothers at the height of lactation rapidly decreases MGF-binding activity. Fig. 2 shows that 24 hr after pup removal at day 10 of lactation only very low levels of MGF remain (compare lanes 12 and 13). These experiments indicate that two different signals can regulate the level of MGF: (*i*) developmental control leads to MGF accumulation late in pregnancy and (*ii*) environmental control through pup suckling is required to maintain MGF levels during lactation. A rapid decrease in MGF activity accompanies the weaning period.

We tested the reversibility of MGF down-regulation by restoring pups to their mothers after a 24-hr withdrawal period. Fig. 3 shows that readdition of the pups rapidly increases MGF activity. Levels of MGF as high as in maximally lactating glands were observed 4 hr after pup addition (compare lanes 1 and 3). This experiment shows that suckling of the pups can induce MGF activity, analogous to the developmental regulation seen in late pregnancy.

DNA-Binding Activity of MGF Can Be Modulated by the Action of Potato Acid Phosphatase and CKII. Rapid reversal of the loss of MGF-binding activity after readdition of pups suggested that a posttranslational modification of MGF might play a role in regulating its DNA-binding activity. Protein phosphorylation has been shown to be a mechanism by which transcription factors can be reversibly converted into forms with increased or decreased DNA-binding affinity (11). To

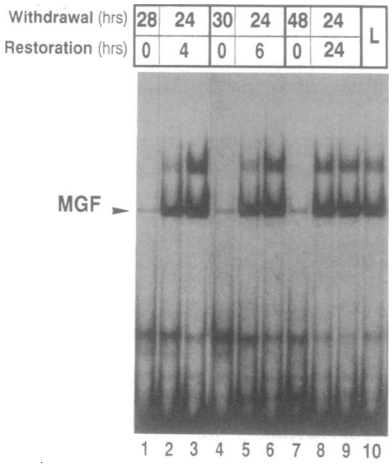

FIG. 3. Detection of MGF in mammary tissue of lactating mice after pup withdrawal and pup restoration to mothers. Lactating BALB/c mice were kept with five to nine pups in individual cages. Ten to 13 days after birth pups were removed, and mothers were kept alone for 24 hr. Then five to nine pups from another litter and removed from their own mothers 2 hr earlier were added for 4 hr (lanes 2 and 3), 6 hr (lanes 5 and 6), or 24 hr (lanes 8 and 9). Control animals were not reassociated with pups (lanes 1, 4, and 7). The nuclear extract used in lane 10 is derived from an animal at day 10 of lactation (L). Mothers were sacrificed, and the fourth mammary glands were isolated and quickly frozen. Nuclear extract preparation and the mobility-shift assay were done as described (10).

test whether phosphorylation can change the DNA-binding properties of MGF, we treated a nuclear extract from mammary cells of lactating mice with potato acid phosphatase

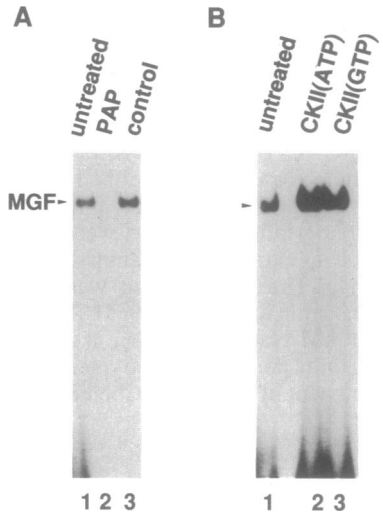

FIG. 4. MGF-binding activity is regulated by state of phosphorylation. (*A*) Nuclear proteins (2 μg) from mammary tissue of lactating mice were probed for MGF-binding activity in an electrophoretic mobility-shift assay (lane 1). Equivalent amounts of nuclear proteins were incubated with (lane 2) or without (lane 3) potato acid phosphatase (PAP) for 20 min at 37°C and then introduced into the electrophoretic mobility-shift assay. (*B*) Nuclear proteins from mammary tissue of lactating mice separated from pups for 24 hr were probed for MGF-binding activity (lane 1). Extracts were treated with CKII in the presence of 1 mM ATP (lane 2) or 1 mM GTP (lane 3) before the electrophoretic mobility assay. The probe used in *A* and *B* was a double-strand oligonucleotide of 30 bp containing only one MGF-binding site and labeled to a high specific activity with Klenow polymerase. Although signals in *A*, lane 1 and *B*, lane 1 appear similar in intensity, they correspond to different MGF activities due to different exposure times of autoradiograms. The signal in *A*, lane 1 is equivalent to the activity shown in Fig. 2, lanes 8–12. The signal shown in *B*, lane 1 is equivalent to the activity shown in Fig. 2, lane 13.

(21). Fig. 4*A*, lane 2 shows that the treatment greatly decreased DNA-binding activity of MGF. Identical results were obtained when alkaline phosphatase was used (data not shown).

CKII (22) has been implicated in the regulation of transcription factor activities. The serum response factor (SFR), a mammalian transcription factor that binds to an enhancer sequence in c-*fos* promoter, for example, has been shown to be phosphorylated and to increase its DNA-binding activity after CKII treatment (23, 24). Nuclear extracts from mammary glands of mice withdrawn from their pups for 24 hr were treated with CKII. These extracts contain reduced levels of MGF-binding activity compared with extracts prepared from mammary gland of lactating animals. After treatment with CKII in the presence of ATP or GTP the binding was enhanced ≈5- to 7-fold. The values were determined by quantitative evaluation of the autoradiographic signals in Fig. 4*B*. This experiment suggests that MGF is present in a latent form in withdrawn extracts and can be converted to a DNA-binding form by phosphorylation *in vitro*.

DISCUSSION

One view of development and differentiation states that numerous extracellular signals are integrated at the level of the activity of specific transcription factors, which control the expression of sets of genes and determine the phenotype of cells (see, for example, ref. 25). We have shown here that a MGF, crucial for the expression of an abundantly expressed milk protein gene, fulfills this description. MGF responds to the complex developmental inputs of ovarian, pituitary, and placental hormones that govern the terminal differentiation and the onset of lactation in mammary epithelial cells, and it responds to the environmental stimulus of suckling. The rapid regulation of milk protein synthesis through suckling is apparently mediated through MGF activity. This regulation seems sensible and necessary when we consider that lactating mice can produce ≈10% of their body weight in milk per day.

The molecular connection between suckling and MGF activity is not known, but it seems reasonable to consider direct and indirect mechanisms. Accumulation of milk in the ducts of the mammary gland could directly act back on the epithelial cells and the levels of MGF. The elevated intramammary pressure could distort the alveoli, and the cell deformation could then generate intracellular, inhibitory signals (26, 27). A direct effect could also be exerted by several growth factors detected in milk. EGF, transforming growth factor α, and transforming growth factor β are present in milk and have been shown to inhibit lactogenic hormone induction of β-casein synthesis *in vitro* (28–31). More complex, indirect mechanisms might involve neuroendocrine events. Suckling induces, for example, the responsiveness of pituitary cells to stimulatory secretagogues and subsequently the release of prolactin into the circulation (32, 33). Suckling also increases the release of adrenocorticotropin and subsequently the circulating levels of glucocorticoids (34, 35).

Regulation of the DNA-binding activity of MGF in the mammary gland provides an example for a strategy in which multiple, seemingly unrelated extracellular signals are integrated to affect a transcription factor central to the function of a highly differentiated cell. MGF most likely confers the coordinated regulation to the abundantly expressed casein genes that contain an MGF-binding sequence at nearly identical positions in their promoters (18, 19). The gel-retardation experiments provide a first hint at the mechanism of this multihormonal regulation. This method allows measurement of the DNA-binding form of MGF but not quantitation of the protein concentration. The loss of binding activity after phosphatase treatment of mammary nuclear extracts from lactating animals and the gain of binding activity after CKII treatment of extracts withdrawn from animals suggest that the phosphorylation state might play a role in regulating MGF activity. This conclusion was confirmed when we rephosphorylated potato acid phosphatase-treated extracts with CKII. A partial restoration of the MGF-binding activity could be observed (data not shown).

Our experiments do not allow definitive conclusions about the role of phosphorylation in the *in vivo* regulation of MGF. The *in vitro* experiments, in which the DNA-binding state of MGF is modulated through the action of potato acid phosphatase and CKII, and the *in vivo* regulation of MGF binding in pregnancy and through suckling are merely suggestive of a common mechanism. CKII activity *in vivo* has, so far, been related only to events of growth control. The activity of CKII is stimulated by growth factors like EGF, insulin, and insulin-like growth factor 1 *in vivo* (36, 37). Phosphorylation by CKII can enhance the DNA-binding activity of the serum response factor (refs. 23 and 24) *in vitro*, a factor that increases transcription of the c-*fos* gene. No activation of CKII through the prolactin receptor has yet been reported. It would be amusing if this enzyme had a physiological link to the origin of its name through the prolactin receptor and MGF. The prolactin receptor belongs to the cytokine receptor superfamily (38). The prolactin receptor could be connected to a protein kinase-mediated signal-transduction pathway; this relationship has been reported for other members of the cytokine receptor family. Phosphorylation events and associations with protein kinases have been observed upon activation of the growth hormone receptor and the interleukin 2 receptor (39, 40). However, the hormonally controlled activity changes of MGF seen *in vivo* may be mechanistically unrelated to those seen *in vitro*. Cloning of the MGF gene and derivation of specific antisera will provide the tools to address these questions more definitively.

We thank Paul Hofer for taking care of the animals; Drs. Flavio Meggio and Lorenzo Pinna (University of Padova) for providing purified CKII; Franz Fischer and Werner Zürcher for oligonucleotide synthesis; and Drs. Nancy Hynes, Witold Filipowicz, Brian Hemmings, and Yoshikuni Nagamine for critical reading of the manuscript. M.S.N. was supported by the Ludwig Institute for Cancer Research (Zürich). B.H. is the recipient of a European Molecular Biology Organization long-term fellowship.

1. Topper, Y. J. & Freeman, C. S. (1980) *Physiol. Rev.* **60,** 1049–1106.
2. Houdebine, L. M., Djiane, J., Dusanter-Fourt, I., Martel, P., Kelly, P. A., Devinoy, E. & Servely, J. L. (1985) *J. Dairy Sci.* **68,** 489–500.
3. Dembinski, T. C. & Shiu, R. P. C. (1987) in *The Mammary Gland: Development, Regulation and Function*, eds. Neville, M. C. & Daniel, C. W. (Plenum, New York), pp. 355–381.
4. Vonderhaar, B. K. & Ziska, S. E. (1989) *Annu. Rev. Physiol.* **51,** 641–652.
5. Blackburn, D. E., Hobbs, A. A. & Rosen, J. M. (1982) *Nucleic Acids Res.* **10,** 2295–2307.
6. Waugh, D. F. (1974) in *Lactation—A Comprehensive Treatise*, eds. Larson, B. L. & Smith, V. R. (Academic, New York), pp. 3–85.
7. Hobbs, A. A., Richards, D. A., Kessler, D. J. & Rosen, J. M. (1982) *J. Biol. Chem.* **257,** 3598–3605.
8. Hennighausen, L., Westphal, C., Sankaran, L. & Pittius, C. W. (1991) in *Transgenic Animals*, eds. First, N. & Haseltine, F. P. (Butterworth-Heinemann, Reed Publ., New York), pp. 65–74.
9. Mitchell, P. J. & Tjian, R. (1989) *Science* **245,** 371–378.
10. Schmitt-Ney, M., Doppler, W., Ball, R. K. & Groner, B. (1991) *Mol. Cell. Biol.* **11,** 3745–3755.
11. Bohmann, D. (1990) *Cancer Cells* **2,** 337–344.
12. Ball, R. K., Friis, R. R., Schönenberger, C. A., Doppler, W. & Groner, B. (1988) *EMBO J.* **7,** 2089–2095.
13. Doppler, W., Groner, B. & Ball, R. K. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 104–108.

14. Higuchi, R., Krummel, B. & Saiki, R. K. (1988) *Nucleic Acids Res.* **16,** 7351–7367.
15. Luckow, B. & Schütz, G. (1987) *Nucleic Acids Res.* **16,** 5490.
16. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. eds. (1987) *Current Protocols in Molecular Biology* (Wiley, New York), p. 9.6.1–9.6.9.
17. Meggio, F., Perich, J. W., Reynolds, E. C. & Pinna, L. A. (1991) *FEBS Lett.* **279,** 307–309.
18. Gorodetsky, S. I., Tkach, T. M. & Kapelinskaya, T. V. (1988) *Gene* **66,** 87–96.
19. Yoshimura, M. & Oka, T. (1989) *Gene* **78,** 267–275.
20. Danielson, K. G., Oborn, C. J., Durban, E. M., Butel, J. S. & Medina, D. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3756–3760.
21. Boxer, L. M., Prywes, R., Roeder, R. G. & Kedes, L. (1989) *Mol. Cell. Biol.* **9,** 515–522.
22. Pinna, L. A. (1990) *Biochim. Biophys. Acta* **1054,** 267–284.
23. Manak, J. R., deBisschop, N., Kris, R. M. & Prywes, R. (1990) *Genes Dev.* **4,** 955–967.
24. Manak, J. R. & Prywes, R. (1991) *Mol. Cell. Biol.* **11,** 3652–3659.
25. Wuarin, J. & Schibler, U. (1990) *Cell* **63,** 1257–1266.
26. Ben-Ze'ev, A. (1991) *BioEssays* **13,** 207–212.
27. Watson, P. A. (1991) *FASEB J.* **5,** 2013–2019.
28. Taketani, Y. & Oka, T. (1983) *Endocrinology* **113,** 871–877.
29. Hynes, N. E., Taverna, D., Harwerth, I. M., Ciardiello, F., Salomon, D. S., Yamamoto, T. & Groner, B. (1990) *Mol. Cell. Biol.* **10,** 4027–4034.
30. Mieth, M., Böhmer, F. D., Ball, R. K., Groner, B. & Grosse, R. (1990) *Growth Factors* **4,** 9–15.
31. Taverna, D., Groner, B. & Hynes, N. E. (1991) *Cell Growth Differ.* **2,** 145–154.
32. Grosvenor, C. E., Mena, F. & Whitworth, N. S. (1979) *Endocrinology* **104,** 372–376.
33. Lamberts, S. W. J. & Macleod, R. M. (1990) *Physiol. Rev.* **70,** 279–318.
34. Voogt, J. L., Sar, M. & Meites, J. (1969) *Am. J. Physiol.* **216,** 655–658.
35. Koprowski, J. A. & Tucker, H. A. (1973) *Endocrinology* **93,** 645–651.
36. Klarlund, J. K. & Czech, M. P. (1988) *J. Biol. Chem.* **263,** 15872–15875.
37. Ackermann, P. & Osheroff, N. (1989) *J. Biol. Chem.* **264,** 11958–11965.
38. Cosman, D., Lyman, S. D., Idzerda, R. L., Beckman, M. P., Park, L. S., Goodwin, R. G. & March, C. J. (1990) *Trends Biochem. Sci.* **15,** 265–270.
39. Hatakeyama, M., Kono, T., Kobayashi, N., Kawahara, A., Levin, S. D., Perlmutter, R. M. & Taniguchi, T. (1991) *Science* **252,** 1523–1528.
40. Horak, I. D., Gress, R. E., Lucas, P. J., Horak, E. M., Waldmann, T. A. & Bolen, J. B. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1996–2000.