Communication

Transferrin mRNA Level in the Mouse Mammary Gland Is Regulated by Pregnancy and Extracellular Matrix*

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We have isolated an almost full length cDNA to transferrin from a mouse mammary tumor virus-induced tumor cDNA library. On Northern blots of RNA isolated from liver and mammary glands, the cDNA hybridized to a single band of 2.4 kilobases. The authenticity of the probe is shown further by 83% sequence homology to human cDNA and identical amino acid sequence to a small cDNA probe isolated from a mouse liver library. The level of transferrin mRNA is very low in the glands from virgin mice, but is as abundant in the glands from pregnant and lactating mice as in the liver. We further show that the steady-state transferrin mRNA level in culture is relatively insensitive to lactogenic hormones compared to that of β-casein mRNA. Culturing the cells on extracellular matrix, however, markedly affects its expression. These findings raise the possibility that the composition of the basement membrane in the mammary gland may play a role in regulation of transferrin mRNA levels in vivo.

A number of tissues in higher organisms synthesize transferrin. However, liver is usually considered to be the major source of body transferrin. The regulation of transferrin appears to be tissue-specific, and in some cases physiological factors that modulate its expression have been identified. In liver its synthesis is stimulated by iron depletion and by steroid hormones (1). In chicken, transferrin is synthesized by oviduct and its transcription is induced by estrogens (2). In rat Sertoli cells, transferrin synthesis and secretion are regulated by testosterone and insulin (3). Moderate synthesis in some cases physiological factors that modulate its expression have been identified: Twenty-six positive clones of various sizes but similar restriction analysis and the subcloning were done by standard procedures (12).

Sequencing—pMTf-5 was subcloned in phage vectors mp18 and mp19 and partially sequenced using the method of dideoxynucleotide chain termination (13).

RNA Isolation and Analysis—Total cellular RNA was isolated as described (14). The RNA was electrophoresed in 1.0% agarose gel containing 2.2 M formaldehyde in MOPS buffer (0.2 M MOPS, 50 mM sodium acetate, 5.0 mM EDTA, pH 7.0). RNA was transferred to GeneScreen Plus (Du Pont-New England Nuclear) membrane as described (15). The membranes were fixed by baking for 2 h in a vacuum oven and prehybridized in 45% formamide, 1 X SSC (1 X SSC is 0.15 M NaCl, 0.015 M sodium citrate), 1 X Denhardt’s solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin), 20 mM Na2HPO4, 10% dextran sulfate, 100 μg/ml of single-stranded calf thymus DNA, 0.1% sodium dodecyl sulfate, and hybridized in the same solution containing 5 X 106 cpm (specific activity 0.5–2 X 108 cpm/μg DNA) of the 32P nick-translated probes.

RESULTS

Isolation and Characterization of Transferrin cDNA Clones—cDNA clones representing mouse transferrin were obtained by screening a mouse mammary tumor virus-induced tumor library using a partial rat transferrin cDNA clone. Twenty-six positive clones of various sizes but similar restriction pattern were isolated. We focused our attention on a cDNA clone with the longest insert (2.3 kilobases (kb)). This clone (designated pMTf-5) was further characterized by subcloning into plasmid Puc18 and subjected to restriction analysis. A partial restriction map of pMTf-5 is shown in Fig. 1A. We subsequently cloned the cDNA insert of pMTf-5 into M13 phage for partial sequencing. The overall homology between the sequenced region of pMTf-5 and the recently described

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The abbreviations used are: ECM, extracellular matrix; kb, kilobases; EHS, Engelbreth-Holm-Swarm tumor; PMME, primary mouse mammary epithelial cells; MOPS, morpholinospropanesulfonic acid.
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FIG. 1. Partial restriction map of pMTf-5 (A) and the alignment of human serum transferrin (HTF) cDNA (18) and pMTf-5 sequences and the deduced amino acid sequences (B). The homology between pMTf-5 and HSTf is 83% at cDNA level and 81% at amino acid level. Boxes indicate amino acid residues that are conserved among all transferring protein families. The scale in A is in kb.

FIG. 2. The identity of mammary gland transferrin (Tf) mRNA and its modulation by pregnancy and lactation. Total cellular RNA (10 μg) extracted from mammary glands of virgin (lanes 1 and 2), pregnant (lanes 3 and 4), and lactating (lanes 5 and 6) mice, mammary glands (lanes 1, 3, and 5), and liver (lanes 2, 4, and 6) were separated on formaldehyde-agarose gel, blotted to Genescreen Plus membrane, and hybridized with nick-translated pMTf-5 as described under "Materials and Methods." A small 0.4-kb cDNA from a mouse liver library kindly provided by Pentacost and Teng (17) hybridized to the same bands (not shown).

human serum transferrin (16) was 83% at the DNA level and 81% at amino acid level (Fig. 1B). Most of the conserved amino acid residues for the transferrin family (including transferrin of human and the lactoferrin of human, mouse, mare, and chicken conalbumin) are preserved in this cDNA from mouse mammary gland (Fig. 1B). The deduced sequence of pMTf-5 is identical to that of a fragment of transferrin cDNA isolated from mouse liver (17) between amino acid 292 and 347, for which the sequences are available.

The pMTf-5 Recognizes a Single mRNA Species in Liver and Mammary Gland—To establish whether the mRNA species recognized by pMTf-5 are similar in the liver and the mammary gland, we isolated RNA from tissues of virgin, pregnant, and lactating mice and hybridized the 32P nick-translated pMTf-5 to the electrophoresed RNA on Northern blots (Fig. 2). pMTf-5 detected one RNA species of about 2.4 kb, present both in the liver and in the mammary gland (Fig. 2). The size of this mRNA agrees with the expected size for the transferrin message. The cDNA to a fragment of transferrin isolated from mouse liver (17) between amino acid 292 and 347, for which the sequences are available.

The pMTf-5 Recognizes a Single mRNA Species in Liver and Mammary Gland—To establish whether the mRNA species recognized by pMTf-5 are similar in the liver and the mammary gland, we isolated RNA from tissues of virgin, pregnant, and lactating mice and hybridized the 32P nick-translated pMTf-5 to the electrophoresed RNA on Northern blots (Fig. 2). pMTf-5 detected one RNA species of about 2.4 kb, present both in the liver and in the mammary gland (Fig. 2). The size of this mRNA agrees with the expected size for the transferrin message. The cDNA to a fragment of transferrin isolated from a mouse liver library, mentioned above and kindly provided by B. Pentacost and T. Teng (17; Research Triangle Park, North Carolina), cross-hybridized to the same mRNA (not shown), indicating further that the mammary gland synthesizes authentic transferrin mRNA. A comparison of the mRNA levels in glands of virgin, pregnant, and early lactating mice indicated that the steady-state level of transferrin mRNA is increased by more than 40-fold in
glands of pregnant as compared to that of virgin mice (Fig. 2); the level is decreased slightly in the lactating gland. This indicates that modulation of transferrin levels observed previously (5) are at the level of transferrin mRNA.

Response to Lactogenic Hormones in Culture—In order to define the factors that regulate transferrin gene expression in the mammary gland during pregnancy, we cultured mammary epithelial cells from pregnant mice. It has been demonstrated in the same system that the expression of mRNA for another major milk protein, β-casein, as well as its synthesis and secretion, are dependent upon the presence of prolactin, hydrocortisone, and insulin (8, 18). Transferrin mRNA level is partially affected by the addition and/or subtraction of these three hormones (Fig. 3A), yet its response is distinct from that of β-casein. Elimination of either prolactin or insulin reduced the expression of transferrin mRNA but did not abolish it. Hydrocortisone, on the other hand, had little or no effect on transferrin expression while its removal decreased β-casein gene expression considerably (compare ratios of β-casein mRNA to transferrin mRNA in lanes 1 and 2 as opposed to lanes 3–6 in Fig. 3, panel C).

Extracellular Matrix Influence on Transferrin Gene Expression—PMME cells were seeded on either plastic, plastic-coated with EHS extracts, or on rat tail collagen gels coated with EHS extract and subsequently floated as described previously (8). The fold induction of transferrin on EHS matrix is dependent on the density of the culture and the quality of the EHS preparation as was found previously for β-casein (8). The level of transferrin mRNA was highest when PMME were cultured on floating EHS-coated rat tail collagen (20-fold increase in comparison to plastic), followed by EHS matrix coated as a thin film on plastic (5-fold over plastic in this experiment) (Fig. 4; this ratio has been as high as 20 in recent experiments). The level of transferrin (and β-casein) mRNA on floating EHS/collagen gels was as high as the lactating gland (Fig. 4, compare lanes 1 and 4).

**DISCUSSION**

We have obtained an almost full length cDNA clone to mouse transferrin from the mammary gland. Since the major iron binding protein in the mammary gland until recently was thought to be lactoferrin, it was of interest to compare the sequence of pMTf-5 with a cDNA clone (pT267) to mouse lactoferrin isolated by Pentacost and Teng (17). Maximum alignment of pMTf-5 and PT267 showed 66% homology for the two cDNA sequences and 58% homology for the deduced amino acid sequences (data not shown). On the other hand, pMTf-5 has 100% homology at the amino acid level to a small cDNA to liver transferrin isolated by the same investigators (17). pMTf-5 shares 83% homology with human serum transferrin and recognizes the same RNA species in the liver and mammary gland. It is therefore clear that the mouse mammary gland produces its own transferrin during pregnancy and lactation and that our isolated clone is indeed transferrin.

Two lines of evidence have suggested recently that transferrin may be essential for cell proliferation: the requirement for transferrin in chemically defined culture medium to support cell growth (19) and the widespread distribution of transferrin receptor on many different cell types (20). Studies by Ekkblom and Thesleff (21) have demonstrated that transferrin is necessary in metanephric mesenchyme differentiation. It also has been shown that transferrin or transferrin-like substances released from peripheral nerve promote the growth and the development of chicken embryo myoblasts in culture (22) and allow the regeneration of amphibian limb (23). Our data that mouse mammary gland expresses transferrin, and furthermore that its expression is regulated by pregnancy,
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add to the growing evidence that transferrin synthesis is associated with growth and functional differentiation in tissues other than the liver. The simplest explanation for this association might be that transferrin stimulates growth and differentiation by transporting iron, a substance that participates in reactions spanning all of biochemistry. Whether or not there is an additional role for transferrin in growth regulation and differentiation (distinct from an iron carrier) (21) is not clear at this time.

Mammary gland development and differentiation are influenced by the complex interplay of many factors including hormones and ECM. While the importance of cell-ECM interactions in modulation of mammary epithelial cell function has been addressed previously (24, 25), transferrin appears to be unique among skim milk proteins as it is more sensitive to ECM than to lactogenic hormones. The finding that transfer-

REFERENCES