Annu. Rev. Nutr. 1999. 19:407–36

REGULATION OF MILK PROTEIN GENE EXPRESSION

Jeffrey M. Rosen,[1] *Shannon L. Wyszomierski,*[1] *and Darryl Hadsell*[2]

Departments of [1]Cell Biology and [2]Pediatrics, Baylor College of Medicine, Houston, Texas 77030-3498; e-mail: jrosen@bcm.tmc.edu, sl035776@condor.bcm.tmc.edu, dhadsell@bcm.tmc.edu

KEY WORDS: casein, whey acidic protein, prolactin, insulin, transgenic and knockout mice

ABSTRACT

Studies using both transgenic mice and transfected mammary epithelial cells have established that composite response elements containing multiple binding sites for several transcription factors mediate the hormonal and developmental regulation of milk protein gene expression. Activation of signal transduction pathways by lactogenic hormones and cell-substratum interactions activate transcription factors and change chromatin structure and milk protein gene expression. The casein promoters have binding sites for signal transducers and activators of transcription 5, Yin Yang 1, CCAAT/enhancer binding protein, and the glucocorticoid receptor. The whey protein gene promoters have binding sites for nuclear factor I, as well as the glucocorticoid receptor and the signal transducers and activators of transcription 5. The functional importance of some of these factors in mammary gland development and milk protein gene expression has been elucidated by studying mice in which some of these factors have been deleted.

CONTENTS

INTRODUCTION 408
COMPOSITE RESPONSE ELEMENTS 410
STAT5 412
C/EBPs 412
NFI 413
GR 414
YY1 and the Milk Box Region 414
Protein:Protein Interactions 415

0199-9885/99/0715-0407$08.00

HORMONAL AND DEVELOPMENTAL REGULATION 416
Hormonal and Developmental Regulation of STAT5 416
Hormonal and Developmental Regulation of C/EBPs 417
The Role of Chromatin Structure 419
Hormonally Regulated Signal Transduction Pathways: JAK/STAT 420
Synergistic Actions of PRL and HC 421
Insulin and IGF in Mammary Gland Development 422
Insulin and IGFs and Lactation 423
Insulin-Induced Transcription 423
Insulin-Dependent JAK/STAT Activation 424
Glucose-Dependent Gene Transcription 425
Cell:Substratum Interactions 425
CONCLUSIONS 428

INTRODUCTION

In addition to their importance in nutrition, the caseins and whey proteins provide molecular markers for functional differentiation in the mammary gland (150). The genes encoding these proteins are regulated by the complex interplay of peptide and steroid hormones, predominantly the lactogenic hormones—prolactin (PRL), insulin, and hydrocortisone—and cell-cell and cell-substratum interactions (34, 146). In addition, these genes display both tissue-specific and developmental patterns of expression (49). Our interest in the regulation of milk protein gene expression began as a result of the pioneering studies of Yale Topper and his colleagues more than three decades ago (59). At that time, few model systems were available for studying the mechanisms by which peptide hormones regulate gene expression. These investigators demonstrated that it was possible to study the regulation of milk protein gene expression in mammary explant cultures in a serum-free, chemically defined medium. Explant cultures retained the necessary cellular organization and cell-substratum interactions required for lactogenic hormone action and were useful for defining the hormonal regulation of endogenous milk protein gene expression (49). However, because of the inefficiency of gene transfer into these cultures, explant cultures were not optimal models for defining the sequences critical for hormonal, cell-specific, or developmental regulation.

The advent of recombinant DNA technology led to the cloning of various casein and whey protein genes from a number of different mammalian species (reviewed in 10). The principal whey proteins in rodents, ruminants, and man are whey acidic protein (WAP), β-lactoglobulin, and α-lactalbumin, respectively. Each is encoded by a relatively small, single-copy gene. In contrast, the predominant milk proteins, the caseins, are encoded by a cluster of single-copy genes. In bovine, the αs1-, β-, αs2-, and κ-casein genes are clustered in this order in a 250-kb region on chromosome 6 (121). The mouse locus is comparable in size, approximately 260 kb, and is located on chromosome 5. An extra αs2-like casein gene, δ-casein, has been identified in the mouse locus, which

is closely related and linked to the γ-casein gene (124). The human casein locus is approximately 350–370 kb, about 100 kb larger than that of bovine and mice (123). In all three mammalian casein loci analyzed, the αs1- and β-casein genes are closely linked and arranged in $5' \rightarrow 3'$ and $3' \rightarrow 5'$ transcriptional orientations respectively. The three genes encoding the calcium-sensitive caseins (αs1-, β-, and αs2-) have evolved from a common ancestral gene (11, 44) and share common regulatory motifs in the proximal and distal $5'$ flanking regions (43, 168, 177). The κ-casein gene is, however, not evolutionarily related to these genes, although its expression pattern is similar and its protein product is essential for micelle formation and stability (4).

Two general approaches have been employed to identify functional promoter and enhancer elements required for the hormonal, developmental, and cell-specific regulation of milk protein gene expression. In the first, identification of regions of DNase I hypersensitivity to determine sites of protein-DNA interaction was followed by DNA footprinting and electrophoretic gel mobility shift assays. The functional importance of these sites was then (72) determined by the analysis of wild-type and mutant constructs in transgenic mouse models (13, 68, 74, 114, 122, 143, 153). This strategy has allowed the evaluation of specific mutations in relationship to gene copy number in transgenics expressing whey protein gene constructs. This analysis was, however, problematic for the casein genes. With these genes, a more conventional approach was useful.

In contrast to the whey protein genes, β-casein gene regulatory elements were defined primarily by analyzing constructs in stably transfected mammary epithelial cells (138, 139). The clonal HC11 mammary epithelial cell line (7) and CID9 cells (138) were both derived from the COMMA-D cells originally isolated from the mammary glands of mice in midpregnancy (26). In both cases, endogenous β-casein gene expression was regulated appropriately by lactogenic hormones. However, although HC11 cells deposited their own laminin matrix after being cultured for several days at confluence (18), the CID9 cells needed to be grown on a laminin-rich extracellular matrix (138). These stable transfectants provided useful models in which to define the factors involved in the hormonal (139) and cell-substratum (138) regulation of β-casein gene expression.

More recently, nonmammary cells, such as the CHOK1 hamster ovary cell line, into which the PRL receptor has been transfected, have been used to define regulatory regions in several milk protein genes (58, 156). Once several of the components of the lactogenic hormone–regulated signal transduction pathways such as the signal transducers and activators of transcription (STAT) proteins were cloned, it was also possible to overexpress these factors along with the PRL receptor in other nonmammary cells, such as COS, African green monkey kidney cells, in order to study milk protein gene regulation (156). However,

because milk protein gene expression is influenced by the complex interplay of both positive and negative transcription factors and hormone-regulated signaling pathways, none of these cell systems is ideal. In fact, the precise contribution of specific transcription factors and their binding sites can be determined only in vivo using transgenic and knockout mice. The effects of the deletion of some of these transcription factors, specifically STAT5 and CAAT/enhancer binding protein (C/EBP)β, on milk protein gene expression can be studied in the mammary gland and in primary mammary epithelial cultures derived from these knockout mice (81, 127, 140). This approach has confirmed the importance of these factors in the regulation of milk protein gene expression and has provided new insight into their role in mammary gland development and differentiation.

Our laboratory has studied the rat β-casein and WAP genes as representative molecular markers of mammary epithelial cell differentiation. Studies using transgenic mice and transfected mammary epithelial cells have established that hormonal and developmental regulation of these genes requires a complex DNA element referred to as a composite response element (CoRE). In the following sections, a more detailed description of the protein-protein and protein-DNA interactions regulating milk protein gene expression, the influence of hormone-induced signaling pathways, and the effects of these pathways on mammary gland development are presented.

COMPOSITE RESPONSE ELEMENTS

Milk protein gene expression is controlled by promoter regions containing CoREs (Figure 1). CoREs are clusters of transcription factor binding sites containing both positive and negative regulatory elements that integrate signal transduction pathways through protein:DNA and protein:protein interactions. They confer unique temporal and spatial patterns of gene expression (56). In most cases, the level of transcriptional activation from a CoRE is much higher than the combined activation from each transcription factor alone, and frequently, CoREs recruit transcription factors from different families to the same promoter region (61 and references therein). The CoREs in the milk protein gene promoters exemplify these principles. None of the transcription factors used in the milk protein CoREs are mammary-specific or even restricted in the mammary gland to lactation. For WAP, nuclear factor (NF)-I, glucocorticoid receptor (GR), and STAT5 are crucial (73, 74). For β-casein, STAT5, GR, and C/EBPβ all participate in transcriptional activation, and Yin Yang (YY)-1 is involved in repression (33, 67, 86, 118, 119, 139, 140, 156). The unique combination of these particular transcription factors results in mammary gland–specific, lactation-dependent expression of the milk protein genes.

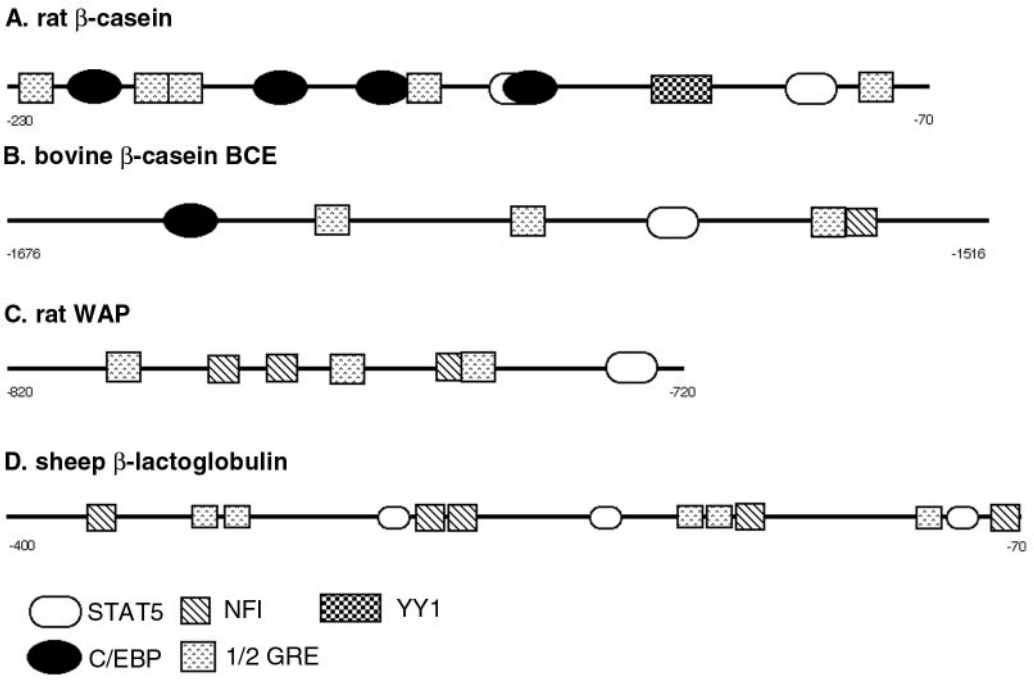

Figure 1 Diagrammatic representations of well-studied milk protein gene promoters. (*A*) Rat β-casein promoter. Most transcription factor binding sites have been verified functionally, as described in text. (*B*) Bovine β-casein enhancer element (BCE). CCAAT/enhancer binding protein (C/EBP) site and STAT5 (signal transducers and activators of transcription 5) sites have been extensively analyzed. Half-palindromic glucocorticoid response elements (GREs) and NFI binding site are putative based on sequence identity. (*C*) Rat whey acidic protein (WAP) promoter. Most transcription factor binding sites have been verified functionally, as described in text. (*D*) Sheep β-lactoglobulin promoter. STAT5 and NFI sites have been verified functionally. Half-GREs are putative based on sequence identity. Not all putative half-GREs in the β-lactoglobulin promoter are shown.

STAT5

STAT5 is the primary transcription factor responsible for signaling by PRL in the mammary gland. STAT5 was originally identified as a binding activity in the lactating mammary gland, known as mammary gland factor (MGF) (139, 157, 165) or milk protein binding factor (MPBF) (163). MGF was cloned originally from sheep mammary gland and was found to be a new STAT protein family member and designated STAT5 (156). Subsequently, STAT5 has been cloned from rats, mice, and humans (51, 60, 77, 79). Molecular cloning from mice revealed the existence of two STAT5 genes that are extremely similar and probably the result of a gene duplication event (79). These were designated STAT5a and STAT5b. These display 93% identity at the amino acid level, with most of the differences occurring in the carboxy-terminus, which encodes the transactivation domain of the protein.

The cloning of STAT5 resulted in several unexpected findings. First, STAT5 is not mammary specific; it is expressed in many different tissues (60, 79, 156). Second, expression of STAT5 in the mammary gland is not limited to lactation. Finally, in addition to PRL, a variety of hormones, growth factors, and cytokines can activate STAT5 (5, 8, 37, 40, 51, 57, 77, 107, 133, 170).

Both STAT5a and STAT5b encode different isoforms, some of which may arise by alternative splicing. Carboxy-truncated STAT5 isoforms have been identified for both gene products and designated STAT5a2 and STAT5bΔ40C (60, 125). Both are generated by insertions, which introduce premature stop codons. These naturally occurring isoforms cannot activate transcription independently and may act as dominant negative inhibitors of STAT5-dependent transcription (90, 158).

The genomic structure of the STAT genes (173) suggests that there may be additional alternatively spliced forms of STAT5. Accordingly, three novel isoforms of STAT6 have been identified recently (108). Carboxy-truncated STAT5 isoforms, thought to be generated by proteolytic processing, that are slightly smaller than STAT5a2 and STAT5bΔ40C have also been identified (6).

All milk protein genes identified to date contain at least one STAT5 binding site. In tissue culture, STAT5 binding is necessary for hormonal induction of β-casein (139) and αs1-casein (58, 113) gene expression. One or more STAT5 binding sites are necessary for efficient expression of WAP (74) and β-lactoglobulin (13) in transgenic mice.

C/EBPs

C/EBPs are a family of transcription factors containing highly conserved, basic leucine zipper (bZIP) motifs at their carboxy-termini. These motifs mediate dimerization and DNA binding. The amino-terminal transactivation domains differ between family members (15, 167). Of the known C/EBP family members, C/EBPβ and -δ appear to play important roles in mammary gland

development and milk protein gene expression. The β-casein proximal promoter contains four C/EBP binding sites (between nucleotides -240 and -82). Three of these are within the minimal promoter region needed for hormonal responsiveness. Mutational analysis revealed that these sites are important for transcriptional activation of the β-casein promoter. In HC11 cells, C/EBPβ and C/EBPδ bind to these sites (33).

Although they lack introns, like most C/EBP genes, the C/EBPα and C/EBPβ mRNAs encode multiple protein isoforms. This is accomplished through the use of alternative translational start sites. In addition to a full-length (42 kDa) form, C/EBPα encodes a 30-kDa form that lacks some of the activities of the full-length form (76, 105). Three C/EBPβ isoforms are generated by a leaky ribosome-scanning mechanism. Two of these are known as LAPs (liver-enriched activating proteins) and seem to function identically. The third C/EBPβ is a 20-kDa isoform known as LIP (liver-enriched inhibitory protein) (28). LIP acts as a dominant negative transcription factor. It lacks the amino-terminal transactivation domain but can bind to target sequences with higher affinity than can LAP. LIP can also heterodimerize with LAP and other C/EBPs. For these reasons, LIP is inhibitory at substoichiometric amounts, and the LAP-to-LIP ratio is likely more important in controlling gene expression than is the absolute level of C/EBPβ (28). LIP is capable of inhibiting β-casein gene expression in transient cotransfection assays (SL Wyszomierski & JM Rosen, unpublished observations).

NFI

NFI (nuclear factor I) was originally identified as a host-encoded protein required for efficient adenovirus DNA replication in vitro (92). The NFI gene family consists of four highly related genes in vertebrates that are differentially expressed during mouse development (20). Although the amino-terminal DNA binding and dimerization domain is well conserved between all NFI proteins, the carboxy-terminal sequences diverge substantially. Alternative splicing leads to alterations in the carboxy-terminal domain affecting the regulatory properties of different NFI isoforms. More than a dozen NFI isoforms have been identified, and further diversity exists because of the possibility of heterodimerization. These isoforms function in one of three ways: by altering chromatin topology, direct activation, or repression (23, 103, 104).

There are several known examples of tissue-specific NFI isoforms. Cooperative interaction between these isoforms and nuclear hormone receptors occurs in CoREs found within the vitamin D response element of c-*fos* (14, 78) and the mouse mammary tumor virus long terminal repeat (21). In these examples, NFI may tether nuclear receptors via a combination of protein:protein and protein:DNA interactions.

Mammary-specific NF-1 isoforms are important components of a CoRE that regulates WAP gene expression (72, 73). DNase I mapping studies of transgenic mice identified a region 830 to 720 bp 5′ to the transcription start site, which was critical for transgene expression. DNAse I footprinting identified several NFI binding sites. To determine the functional importance of these sites, point mutations were introduced into the NFI binding sites, and several independent lines of transgenic mice were analyzed (74). Transgene expression was totally abrogated when the palindromic NFI site or both NFI binding sites were mutated. These results indicate that NFI plays a critical role in the regulation of WAP gene expression. NFI also plays important roles in the regulation of several other whey protein genes (75, 117, 163) (G Bjursell, personal communication). There are several NFI isoforms, ranging in size from 46 to 114 kDa in the mammary gland. During lactation, two isoforms of 46 and 68 kDa are present. With mammary involution, these smaller isoforms are lost and a 74-kDa isoform appears (38). The precise nature of these developmentally regulated NFI isoforms has not yet been determined.

GR

Despite the importance of glucocorticoids to milk protein gene expression, most of these genes do not contain consensus glucocorticoid response elements (GREs). The WAP promoter contains a cluster of half-palindromic GREs (half-GREs) in both its distal regulatory (nucleotides −853 to −729) and proximal regulatory (nucleotides −160 to −70) regions. The half-GREs in the distal element have been mapped by in vitro footprinting (73). The β-casein proximal promoter also contains several half-GREs that have been mapped by in vitro footprinting (166). Mutation of the three downstream half-GREs in the minimal hormone response region (between nucleotides −176 and −70) completely abolishes the cooperative effects of hydrocortisone (HC) and PRL at the β-casein promoter in HC11 mammary epithelial cells (67). Mutation of the upstream GREs (between nucleotide −282 and −177) severely diminishes the cooperative effects of HC and PRL (67), and deletion of this region abolishes them entirely (66). Based solely on sequence similarity, half-GREs may also exist in other casein promoters (119).

YY1 and the Milk Box Region

The β-casein promoter contains a negative regulatory region between nucleotides −150 and −110 (139). This bipartite element represses transcription in the absence of lactogenic hormones. Simultaneous mutation of both transcription factor binding sites leads to extremely high basal transcription. Hormonal induction is still possible with this negative regulatory region mutated (139). The downstream site in this region of the β-casein promoter interacts

with YY1 (86, 118). The proximal promoters of rat α- and γ-casein genes contain putative YY1 binding sites as well (118, 177).

YY1 is a multifunctional protein that can either activate or repress transcription. It is expressed ubiquitously. YY1 contains an activation domain at its amino terminus and two repression domains—one with a high glycine content in the middle of the protein and one with C2H2 Zn-fingers in the carboxy-terminal region. These two repression domains may interact with each other. Interactions with other proteins may be crucial in modulating the activity of YY1 (141). YY1 acts as a repressor of β-casein gene expression.

YY1 interaction with the β-casein promoter was demonstrated in an analysis of YY1 binding to a large promoter fragment using the electrophoretic gel mobility shift assay. Mutation of the YY1 site led to a stronger lactation-associated activation complex in extracts from the lactating mammary gland (118). These effects of YY1 were counteracted by STAT5. Similarly, mutation of the STAT5 binding site, which is crucial for the formation of the lactation-associated activation complex, caused an increase in YY1 DNA binding (118). YY1 represses β-casein gene expression in the absence of lactogenic hormones, but it exhibits little or no effect following hormonal induction (86). The YY1 site in the β-casein promoter is a low-affinity site and may be markedly influenced by protein-protein interactions (118). In mammary epithelial cells, the level of YY1 is not changed by lactogenic hormones (86, 118).

The identities of factors binding to the upstream repressor site are not clear. Analysis of the nucleotide sequence reveals two potential overlapping binding sites: a C/EBP site and a STAT site that binds STAT5 weakly and STAT6 more strongly (89). C/EBPs bind to this site (33, 119). An oligonucleotide corresponding to this site bound C/EBPs in extracts from mammary epithelial cells but did not recognize other nuclear factors (33). C/EBPβ interacts with YY1 (9). Therefore, we propose that the protein:protein interaction between C/EBPβ and YY1 bound to the milk box region of the promoter causes β-casein gene repression.

Protein:Protein Interactions

As mentioned, the transcriptional activation from a CoRE is generally greater than the sum of its parts (61). The transcription factors act cooperatively, and protein:protein interactions between them are probably as important as is the interaction of each transcription factor with its response element. STAT5 and GR exhibit a high degree of transcriptional synergy at the β-casein promoter (144), as is discussed more extensively later. C/EBPβ and GR are also capable of protein:protein interaction (98) and exhibit transcriptional synergy at a promoter unrelated to the milk protein genes, that of the α-1 acid glycoprotein gene (3, 98). An interaction among these proteins may stabilize the binding of

each individual transcription factor to its response element and, thereby, create a stable activation complex. Finally, the transcription factors at the CoREs may act cooperatively to recruit coactivators and corepressors.

HORMONAL AND DEVELOPMENTAL REGULATION

Hormonal and Developmental Regulation of STAT5

STAT5 is expressed at all stages of mammary gland development, with only slight changes in gene expression observed between virgin, pregnant, lactating, and postweaning mice (60, 79). STAT5 is activated by tyrosine phosphorylation (see section on JAK/STAT) following stimulation by PRL. Tyrosine phosphorylation of STAT5 also changes dramatically during mammary gland development (80). The kinetics of STAT5 tyrosine phosphorylation parallel the developmental profile of milk protein gene expression in the mammary gland (80). When STAT5a and STAT5b are tyrosine phosphorylated in the normal mammary gland, heterodimers of the two proteins are observed (80). STAT1 and STAT3 also are expressed during all stages of mammary gland development, but they are phosphorylated in a pattern reciprocal to that of STAT5. STAT3, in particular, is highly phosphorylated during involution, which suggests it may play a role in this process (80, 112). The roles of STAT1 and STAT3, as well as the identity of their target genes during mammary gland development, are interesting, unanswered questions.

Analysis of STAT5a-deficient, STAT5b-deficient, and STAT5a/STAT5b double knockout mice has demonstrated the importance of STATs to mammary gland development and revealed some surprises concerning the role of STATs in lactation. STAT5a knockout mice are unable to lactate, and they exhibit decreased lobulo-alveolar development. In the STAT5a knockout mice, STAT5b tyrosine phosphorylation was dramatically reduced, and the level of STAT5b protein was somewhat reduced (81). STAT5b null mice also have decreased lobulo-alveolar development, although the phenotype is not as severe as that of STAT5a null mice (149, 151). STAT5a/STAT5b-deficient mice have been generated. The phenotype in the mature virgin gland appears similar to the phenotype seen with a single STAT5 knockout; fewer terminal end buds are present.

Surprisingly, the absence of either STAT5a or STAT5b does not dramatically alter β-casein expression in the mammary gland. WAP expression is reduced in each case, however (81, 149). This suggests that both STAT5a and STAT5b can induce β-casein expression in the mammary gland and that the low level of activated STAT5b in the STAT5a-null mammary gland is sufficient for β-casein expression.

Hormonal and Developmental Regulation of C/EBPs

C/EBPs play important functional roles in mammary development and lactation. In mice, C/EBPβ mRNA levels are low in the mammary gland from nulliparous mice and increase during pregnancy (127). They decrease slightly during lactation and are elevated again after 24 and 48 h of involution (39). Because the C/EBPβ mRNA encodes multiple protein isoforms, analysis of protein levels is essential. In rats and mice, C/EBPβ-LAP levels are threefold higher in the pregnant than the virgin and pregnant gland, whereas C/EBPβ LIP levels are over 100-fold higher. This results in a high LIP-to-LAP ratio during pregnancy. When lactation begins, LIP levels drop dramatically, and LAP levels are decreased by a lesser magnitude. Therefore, during pregnancy, the activities of LAP as well as other C/EBPs are likely to be inhibited by LIP. This is probably one mechanism of inhibiting casein gene expression early in pregnancy. Down-regulation of LIP with the onset of lactation likely results in the relief of transcriptional inhibition and allows C/EBPα and LAP to activate the milk protein gene promoters (119, 140).

C/EBPδ is expressed in the mammary gland during pregnancy and lactation in a pattern similar to that of LAP. It increases slightly in pregnancy and decreases slightly during early lactation. A small increase in C/EBPδ protein is seen during late lactation (119). C/EBPδ mRNA is dramatically increased during the earliest stages of involution. It is detectable 12 h after forced involution by pup removal, peaks at 18 h, and then decreases steadily after 24, 48, and 72 h. This surge of C/EBPδ expression precedes other markers of involution, which suggests that C/EBPδ may be responsible for the transcriptional regulation of genes needed for apoptosis during involution (39). Additionally, C/EBPδ has been implicated in cell cycle arrest in mammary cells. In the COMMA-1D mammary epithelial cell line, C/EBPδ mRNA and nuclear C/EBPδ protein were elevated in cells that were growth arrested in G_o phase of the cell cycle by serum withdrawal. The level of C/EBPδ decreased when the cell cycle was reinitiated by the addition of serum. When C/EBPδ expression was decreased using antisense mRNA expression, the cells were delayed in entering G_o following serum withdrawal (102).

C/EBPα mRNA is present in mouse mammary glands during all stages of mammary gland development (140). C/EBPα mRNA levels decrease by 50% in mammary glands during the transition from mature virgin to pregnancy, but the level of C/EBPα mRNA did not change during pregnancy, lactation, or involution (39, 140).

In many tissues, C/EBPs exhibit a sequential cascade of expression. Generally, C/EBPβ and C/EBPδ are expressed first and correlate with proliferation, whereas C/EBPα is expressed later and correlates with differentiation events

(15, 176). As demonstrated above, this is clearly not the case in the mammary gland. C/EBPα and C/EBPβ are both expressed during pregnancy (a proliferative stage) and lactation (a differentiated stage) and C/EBPδ and β are both expressed during involution (a remodeling stage characterized by extensive apoptosis). As with STAT5, creation of C/EBPα and C/EBPβ knockout mice has greatly enhanced our understanding of their role in milk protein gene expression and mammary gland development. Homozygous deletion of the C/EBPα gene resulted in mice that died shortly after birth as a result of metabolic defects. Transplantation of the mammary anlage from newborn mice into the cleared fat pad of syngeneic hosts was necessary to examine the effect of the absence of C/EBPα on mammary glands. Comparison of grafts from knockout mice with those from wild-type mice indicated that C/EBPα was unnecessary for mammary gland development and β-casein expression (140). These results, coupled with conflicting reports (119, 136) concerning mammary gland expression of C/EBPα, call into question the importance of C/EBPα in milk protein gene expression and mammary gland development.

In contrast, deletion of C/EBPβ resulted in a dramatic mammary phenotype (127, 140). In virgin animals, the absence of C/EBPβ delayed the outgrowth of the virgin mammary gland. At maturity, the virgin C/EBPβ knockout mammary tissue consists of abnormally large primary ducts with few secondary branches. This defect was intrinsic to the mammary epithelium, as transplantation into wild-type stroma did not rescue the phenotype. Furthermore, wild-type mammary epithelium develops normally in C/EBPβ-deficient stroma. Because the knockout animals are sterile as a result of ovarian defects, pregnancy was either simulated by treatment with estrogen and progesterone or rescued by ovarian transplants. Similar results were seen following each procedure. Lobular-alveolar development was impaired in the C/EBPβ knockout mice. During pregnancy or following treatment with estrogen and progesterone, normal mice develop a full, branched, ductal tree with alveoli distributed evenly along the ducts. The C/EBPβ knockout mice, in contrast, developed only large ducts with severely reduced side branching, resulting in sparse alveoli. In the alveoli formed, the cells lacked secretory vesicles and fat globules (127, 140).

The role of C/EBPβ in expression of milk protein genes also was examined. The absence of C/EBPβ reduced β-casein gene expression by 85% to 100%, and WAP expression was undetectable (127, 140). For the β-casein promoter, this effect is consistent with the presence of C/EBP binding sites. The WAP promoter, however, does not contain a known, functionally important C/EBP binding site. The effect on WAP expression, therefore, may be indirect, resulting from the cells not being able to reach a fully differentiated state in the absence of C/EBPβ.

The Role of Chromatin Structure

The acetylation state of histones is correlated with transcriptional activation. Acetylated histones modulate an open chromatin configuration and promote transcription. Deacetylated histones are associated with a closed chromatin conformation and repression of transcription. Many proteins initially identified as coactivators contain histone acetyltransferase activity. Transcription factors target the histone acetyltransferases to specific genes, creating an open chromatin conformation at the promoter that facilitates transcriptional activation (reviewed in 55, 169).

Using this information as a framework, we propose the following model for the regulation of β-casein gene expression (Figure 2*A*). STAT5, GR, and

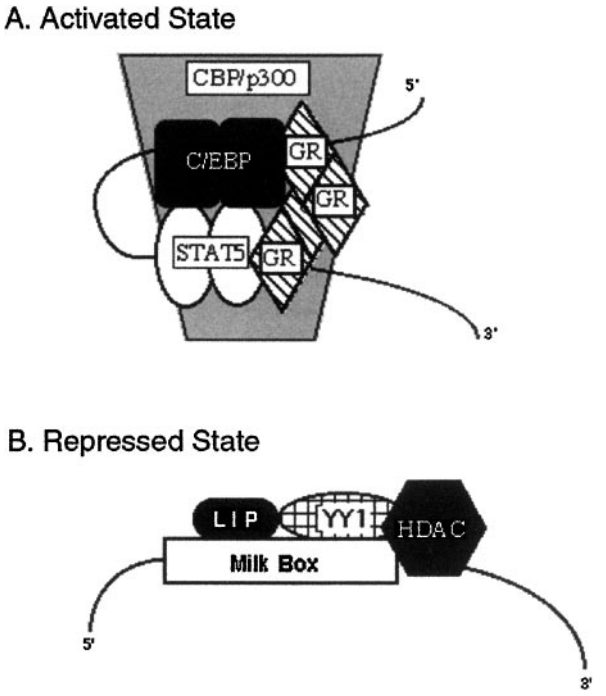

Figure 2 Model for protein:protein interactions mediating the chromatin structure of the β-casein promoter. (*A*) In the activated state, protein:protein interactions between STAT5 (signal transducers and activators of transcription 5), CCAAT/enhancer binding protein (C/EBP) β, and glucocorticoid receptor (GR) may create a stable activation complex. The three proteins together may effectively recruit p300/CBP to β-casein promoter, causing histone acetylation and a high level of transcriptional activation. (*B*) In the repressed state, LIP (liver-enriched inhibitory protein) may tether Yin Yang (YY)1 to the weak YY1 binding site in the β-casein promoter. YY1 may then recruit histone deacetylases (HDACs), resulting in histone deacetylation and repression of transcription.

C/EBPβ all interact with p300/CBP (17, 88, 111). We theorize that the protein:protein interactions between STAT5, GR, and C/EBPβ are needed to form a stable activation complex on the β-casein promoter. The interaction of each transcription factor with p300/CBP is then needed to recruit and effectively retain CBP/p300 at the β-casein promoter. Nuclear receptor coactivators also may play a role in this complex. The coactivator TIF1β was found in a complex interacting with both GR and C/EBPβ on the α-1 acid glycoprotein promoter (19).

Just as histone acetylases are involved with local chromatin remodeling to activate genes, histone deacetylases (HDACs) modulate local chromatin structure and repress transcription (reviewed in 109). YY1, which represses the β-casein promoter, interacts with all the human HDACs identified to date (174, 175). The following working model, therefore, has been proposed for the regulation of β-casein gene expression (Figure 2*B*). YY1 represses the β-casein promoter by bringing to the promoter HDACs which create a repressive chromatin configuration. Because YY1 is capable of interacting with C/EBPβ and because the YY1 binding site in the β-casein promoter is a weak binding site, a LIP:YY1 complex may be responsible for tethering both YY1 and the HDAC to the promoter. When LIP levels decrease at the end of pregnancy, the complex may then dissociate. The importance of histone acetyltransferases and HDACs in modulating the activity of the β-casein promoter has been demonstrated for the bovine β-casein element 1 (see below).

Hormonally Regulated Signal Transduction Pathways: JAK/STAT

The importance of PRL for milk protein gene expression is underscored by the fact that milk protein gene expression and mammary gland development are severely affected in the heterozygotes of both PRL and PRL receptor (PRL-R) knockout mice (50, 101). PRL binds to the extracellular portion of the PRL-R and initiates events in the JAK/STAT signal transduction cascade (reviewed in 27). Specifically, following PRL treatment, JAK2, which is associated with the PRL-R in the absence of ligand, is activated by transphosphorylation when two JAK2 molecules are brought together by ligand-induced dimerization of the receptor (134). Activated JAK2 tyrosine phosphorylates PRL-R, creating docking sites for proteins containing SH2 domains, including STAT5 (110), which subsequently are tyrosine phosphorylated, dimerize, and translocate to the nucleus. Tyrosine phosphorylation of a specific residue (Y700 in rats/Y694 in sheep) of STAT5 is essential for dimerization and transactivation (41). Activated STAT5 binds to gamma interferon activation sequence (GAS) sites and modulates the activity of target genes such as the milk protein genes (41, 156).

It appears that STATs are inactivated by a nuclear tyrosine phosphatase and recycled back to the cytoplasm (47, 90, 100, 142).

Synergistic Actions of PRL and HC

Although glucocorticoids are essential lactogenic hormones, the mechanisms by which they regulate milk protein gene expression synergistically with PRL and insulin have not been defined completely. In mammary epithelial cells, WAP and β-casein respond differently to stimulation by HC. When HC11 cells are treated with only insulin and HC, little induction of β-casein gene expression is observed; addition of PRL leads to a marked induction. In contrast, WAP expression is induced when the cells are treated with only insulin and HC. Addition of PRL enhances WAP expression an additional two- to three-fold (32, 49). The effects of HC on WAP gene transcription seem to be direct. The interaction of GR with the distal regulatory region of the WAP promoter changes the chromatin structure and makes the NF-I binding sites accessible (73). STAT5 then enhances WAP transcription following PRL treatment (73).

Both direct and indirect mechanisms appear to be responsible for HC regulation of β-casein gene expression. PRL and HC act by kinetically distinct mechanisms in mammary epithelial cells (31). Pretreatment with glucocorticoids is essential for PRL induction of the β-casein gene transcription. This effect is gradually increased with longer glucocorticoid pretreatments, is rapidly reversed when glucocorticoids are withdrawn, and requires ongoing protein synthesis (31, 115). Pretreatment of HC11 mammary epithelial cells with HC leads to a marked increase in the LAP/LIP ratio (119) and may, therefore, facilitate the lactogenic hormone-mediated relief of repression of β-casein transcription (139). Thus, glucocorticoids may modulate β-casein gene transcription through alterations in the levels of different C/EBPβ isoforms in a manner that is indirect and requires new protein synthesis. Whether other pathways also mediate the indirect effects of HC on β-casein expression is unknown. In contrast to the indirect effects of HC, STAT5 and GR rapidly, directly, and synergistically regulate transcription of the β-casein gene.

STAT5 and GR interact in transiently transfected COS cells (144), mammary epithelial cells (16), and tissue extracts from mammary gland at all stages of development (16). STAT5 and GR activate transcription from the β-casein promoter in a synergistic fashion in transiently transfected COS cells (144). GR binding to the half-GREs in the β-casein promoter appears to enhance but is not absolutely required for the STAT5/GR transcriptional synergy (67, 145). The protein:protein interactions of STAT5 and GR may be necessary, however, for GR to bind to the half-GREs. The protein:protein interaction

between STAT5 and GR protects STAT5 from inactivation by dephosphorylation. This allows STAT5 to stay bound to the DNA for an increased amount of time, which increases transcriptional activation by STAT5 (171). Therefore, the transcriptional synergy exhibited by STAT5 and GR at the β-casein promoter is probably conferred by multiple mechanisms. Cooperation of the transactivation domains of STAT5 and GR is one mechanism. The strong transactivation domain in GR may supplement the relatively weak transactivation domain in STAT5 (144, 145). Enhancement of STAT5 DNA binding is a second mechanism. Recruitment of p300/CBP, coactivators with histone acetylase activity (55), to the promoter is likely to be a third mechanism by which STAT5 and GR synergistically regulate transcription. This direct effect of STAT5 and GR may play a role in transcriptional activation of the WAP gene as well.

Insulin and IGF in Mammary Gland Development

The role of insulin in regulating the mammary gland has been extensively studied for many years. Studies published almost 40 years ago (36, 126) first showed that high concentrations of insulin (5 μg/ml) in conjunction with HC could maintain the alveolar structure of mammary tissue explants. These studies were later extended to show that these high concentrations of insulin could also stimulate DNA polymerase activity (82). In vivo experiments conducted at approximately the same time established the requirement of growth hormone, PRL, and ovarian steroids (93) for mammary gland development in rodents and suggested that insulin (2) was capable of cooperating with ovarian steroids to promote mammary ductal development. More recent in vivo studies (62, 63, 131, 132) support the conclusion that insulin-like growth factor (IGF)-I is capable of cooperating with ovarian steroids to stimulate mammary ductal development. This cooperation appears to involve a mechanism similar to that of the original somatomedin hypothesis (137), which described the effects of growth hormone on bone growth. Growth hormone released from the pituitary, or administered exogenously, stimulates expression of IGF-I within the mammary gland (62, 131). Locally produced IGF-I in turn stimulates mammary ductal development in the presence of estrogen. In transgenic mice (46, 95) that overexpress IGF-I during pregnancy and lactation, IGFs appear to maintain the mammary epithelial cell population. In organ cultures in vitro (120), IGF-I stimulates mammary ductal development and can act as a survival factor. In addition, in situ hybridization studies (120) have demonstrated uniform expression of the IGF-I receptor in the developing mammary epithelium and localized expression of IGF-I and -II in the terminal end bud. From these results, it is clear that during early mammary gland development, IGF-I and/or insulin is capable of stimulating the growth of the mammary epithelium by increasing

progression through the cell cycle and/or decreasing apoptosis. Nevertheless, the effects of insulin and IGF on the lactating mammary gland appear to differ significantly from those that occur during early development.

Insulin and IGFs and Lactation

In intact animals, insulin is required for successful lactation; treatment of lactating mice with diabetogenic agents inhibits milk secretion (65) and causes a loss of mammary tissue DNA (64). Early in vitro studies showed that in addition to a role in maintaining mammary tissue in culture, insulin stimulated mammary phosphoprotein synthesis (154). Later studies demonstrated specific stimulation of α-lactalbumin and β-casein mRNAs and demonstrated that the effect of insulin on casein was mediated at the level of gene transcription (96, 116). These studies also showed that IGF-I and IGF-II, although capable of stimulating milk protein gene expression, were no more than one-tenth as potent as insulin. In HC11 mammary epithelial cells stimulation of β-casein gene expression by insulin and IGF-I also is associated with inhibition of apoptosis (87). In this system, both ligands inhibited apoptosis equally, despite the fact that insulin was more potent than IGF-I at inducing endogenous β-casein gene expression. In proliferating HC11 cells, both ligands also stimulated DNA synthesis equally, and both were capable of stimulating transient activation of $p42^{ERK2}$. This effect on the mitogen activated protein (MAP) kinase pathway, however, is unnecessary for activation of a minimal β-casein promoter in these cells (162). Beyond these observations, the molecular mechanism by which insulin and/or IGF-I cooperates with glucocorticoids and PRL to induce transcription of the milk protein genes is ill defined.

Insulin-Induced Transcription

Transcriptional activation by insulin seems to involve multiple mechanisms. Insulin-dependent transcription of the C/EBPα and C/EBPβ genes has been described in 3T3-L1 adipocytes (83). The identity of the specific transcription factors that mediate this effect is unknown. Furthermore, the insulin effect is apparently independent of the MAP kinase cascade (48). For the c-*fos* (42) and PRL (52, 106) genes, insulin appears to regulate transcription through activation of the ETS-related transcription factors ELK-1, SAP1a, GABPα, and/or GABPβ1. With the PRL gene, induction depends on c-*raf* activity, but it is independent of p21*ras*, phosphatidylinositol-3 kinase, or ribosomal S6 kinase (53). On the other hand, c-*fos* transcription depends on MAP kinase activation. For the regulation of collagenase gene expression, insulin stimulates transcription through AP-1 (135). This effect probably depends on the initial induction of c-*fos* transcription. In contrast, insulin induces transcription of GAPDH and fatty acid synthase (FAS) through an ETS-independent mechanism. For

GAPDH, a sequence termed IRE-A interacts with an SRY-related high mobility group (HMG) box protein that is likely mouse a4/SOX4 (94). For FAS, the insulin effect is mediated through interaction of a basic-helix-loop-helix/zipper protein, upstream stimulatory factor (USF), with an E box present within the proximal promoter (159, 160). Recently, the insulin-dependent induction of FAS has been demonstrated to require phosphatidylinositol 3-kinase (161). Unfortunately, the link between insulin-dependent phosphatidylinositol 3-kinase activation and USF-dependent FAS expression is unclear. Likewise, the pathways that link insulin stimulation to the other transcriptional events described above are only beginning to be delineated. Hence, the role of these factors and signaling pathways in insulin/IGF–dependent milk protein gene transcription is untested.

Of all the transcription factors identified above as insulin responsive, only three—C/EBPα, C/EBPβ, and USF—have been examined for a potential function in the regulation of milk protein gene expression. As described earlier, C/EBPβ is required for β-casein and WAP gene expression (127, 140). The effect on β-casein occurs through direct interactions with specific binding sites within the proximal promoter (33, 119). Unfortunately, the potential for C/EBPβ to mediate the insulin effects on the milk protein gene expression is untested. An essential role for C/EBPα, however, appears unlikely, as this factor appears not to be required for β-casein gene expression (140). In preliminary studies (G Abdel-Fattah & DL Hadsell, unpublished observations) both USF-1 and -2 appear to bind to DNA in a manner that is dependent on lactation and more specifically on the presence of insulin and PRL. However, binding sites for USF were not detected by EMSA in the β-casein or α-lactalbumin promoters. Thus, if USF is involved with milk protein gene expression, its role is probably indirect.

In addition to the pathways just described, at least two other mechanisms may explain the insulin-dependent component of milk protein gene transcription: (*a*) direct modulation of the JAK/STAT pathway, and (*b*) an indirect effect mediated through insulin-dependent changes in nutrient availability and/or metabolism. These are addressed below.

Insulin-Dependent JAK/STAT Activation

Insulin-dependent stimulation of transcription of milk protein genes may involve modulation of PRL signaling and the JAK/STAT pathway. This hypothesis has not been tested in mammary epithelial cells. However, growth factors, such as colony-stimulating factor-1 (8), platelet-derived growth factor (172), and epidermal growth factor (133), activate the JAK/STAT pathway in a cytokine receptor-independent fashion. Analysis of insulin signaling in nonmammary cells has revealed that insulin receptor interacts directly with and tyrosine

phosphorylates STAT5B (22). IR and IGF-IR can stimulate tyrosine phosphorylation, activation of JAK-1 and JAK-2 (45) and STAT 5 (99).

Glucose-Dependent Gene Transcription

Another indirect mechanism by which insulin could stimulate milk protein gene transcription is through modulation of nutrient uptake and/or metabolism. This mechanism mediates the effect of insulin on expression of the L-pyruvate kinase (30) and S14 (54) genes in liver. In the case of L-pyruvate kinase, insulin directly stimulates glucokinase, which in turn converts glucose into a metabolically active form through phosphorylation. Metabolism of this active glucose through the pentose-phosphate shunt is believed (29) to then produce a metabolite, which acts directly on USF to activate transcription (152). Other glucose-responsive genes include those for insulin (97, 164), GLUT 2 (155), and acetyl-coenzyme A carboxylase (12). Mechanisms that mediate regulation of these genes, however, appear to involve factors other than USF. Glucose-dependent transcription of acetyl-coenzyme A carboxylase requires the interaction of the transcription factor SP1 with GC-boxes found within the acetyl-coenzyme A carboxylase promoter (24). This effect also involves glucose-dependent dephosphorylation of SP1 by a type 1 phosphatase (25). The insulin and GLUT 2 genes, on the other hand, require the homeobox protein Ipf1/Pdx1 (1, 85). As for the regulation of milk protein gene expression, little is known concerning the possibility of glucose-dependent transcription. In preliminary studies (DL Hadsell & KF Trivers, unpublished observations) on primary cultures of mouse mammary epithelial cells, expression of α-lactalbumin and probably β-casein appear to be dependent on glucose. This glucose effect in mammary cells is relatively specific for milk protein genes and occurs over the normal range of blood glucose concentrations found in mice (DL Hadsell & T Alexeenko, unpublished data). However, the mechanism for this dependence and the relationship between glucose dependence and insulin dependence are subjects that require further study. The mechanisms described above are illustrated diagramatically in Figure 3.

Cell:Substratum Interactions

An extracellular matrix (ECM) performs many essential functions during all stages of mammary gland development. It is needed for end bud development, epithelial proliferation, and ductal branching in virgin glands. During pregnancy and lactation, it is needed for development and maintenance of the differentiated phenotype. The ECM is degraded during involution, resulting in massive levels of programmed cell death (reviewed in 130). One role of ECM during lactation is the regulation of milk protein gene expression. In primary mammary epithelial cells and many mammary epithelial cell lines, ECM and lactogenic hormones are required for a high level of expression of milk protein

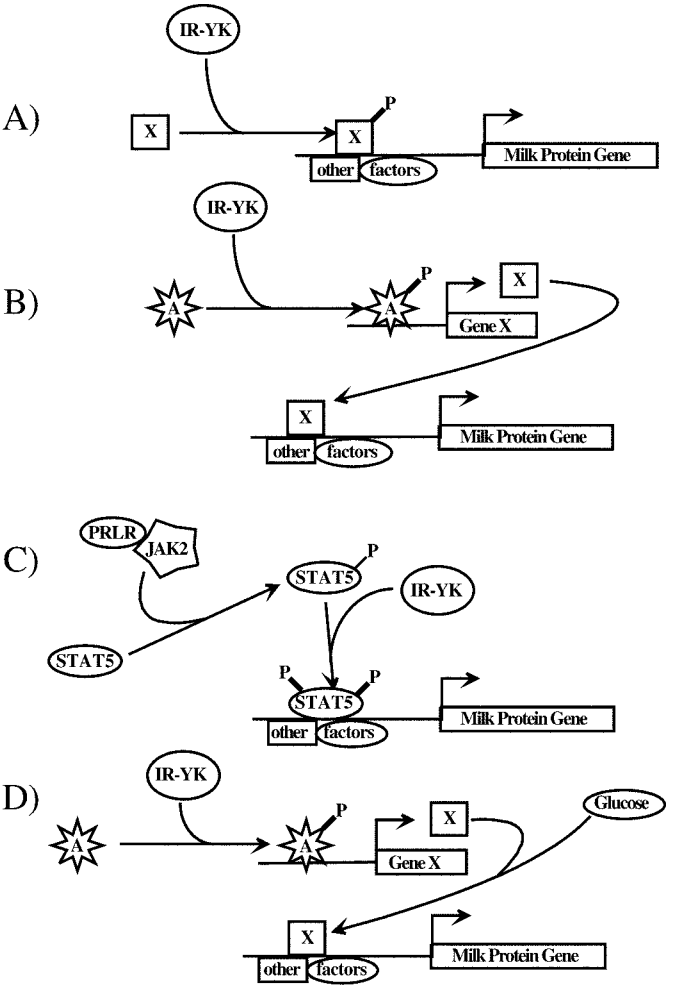

Figure 3 Proposed mechanisms for insulin-dependent induction of milk protein gene transcription. The insulin response is proposed to involve any or all of four hypothetical mechanisms. (*A*) Insulin-dependent activation of transcription factor *X*, which binds to and directly activates the milk protein genes. (*B*) Indirect effect of insulin through transcriptional activation of the gene that encodes for transcription factor *X*. This effect would be mediated independently through the direct effect of transcription factor *A* on gene *X*. (*C*) Modulation of prolactin-dependent JAK/STAT activation by insulin. (*D*) Insulin-dependent expression of a gene for a transcription factor, *X*, which is directly activated by nutrients such as glucose. Once activated, this nutrient-dependent factor would then directly activate milk protein gene expression.

genes (71, 147). ECM stimulates expression of most milk protein genes, and this effect has been extensively studied for β-casein and β-lactoglobulin. ECM has two separable functions needed for milk protein gene expression. First, ECM induces in the cells a morphological change that is necessary but not sufficient for β-casein gene expression. Many types of extracellular matrices are capable of performing this function (129). Then, the ECM induces a signal that in the presence of lactogenic hormones induces milk protein gene expression. Laminin is the extracellular matrix protein that generates this signal (148). Functional β1-integrins on the mammary epithelial cells also are necessary for this signaling (147). Integrin clustering, which would suggest signaling, was observed in mammary epithelial cells in the presence of ECM (129). A 160-bp element was identified in the bovine β-casein reporter (between nucleotides 1677 and 1517) that mediates the response of the β-casein gene to ECM in mammary epithelial cells. It was named β-casein element 1 (BCE), and is a mammary specific, ECM-dependent enhancer (138). The proximal promoter of the bovine β-casein gene exhibits only minimal responsiveness both to lactogenic hormones and to ECM. When this enhancer element is placed upstream of even a minimal promoter, the response of the promoter to lactogenic hormones in the presence of ECM is dramatically enhanced, and the response of the promoter to lactogenic hormones in the absence of ECM is increased slightly (138). Two transcription factor binding sites have been identified in the BCE, one for C/EBPs and one for STAT5. In the two mammary epithelial cell lines used for analysis, however, ECM did not change the binding of either STAT5 or any of the C/EBP isoforms to this element.

The first 406 bp of the β-lactoglobulin promoter also were identified as ECM responsive in primary mammary epithelial cells from transgenic mice with sheep β-lactoglobulin transgenes. The minimal transcription observed when the cells were cultured on plastic and treated with lactogenic hormones was dramatically increased when the cells were cultured on ECM. This promoter region contains STAT5 and NFI binding sites. NFI DNA binding was increased by ECM. Mutation of the STAT5 binding sites in the promoter reduced the level of transcriptional activation from the promoter in general and eliminated transcriptional enhancement by ECM. After two days of treatment with lactogenic hormone, STAT5 DNA binding was present only in cells cultured on ECM (148).

In primary mammary epithelial cells, PRL-R phosphorylation, JAK2 phosphorylation/activation, and STAT5 phosphorylation and DNA binding caused by PRL were all dependent on ECM. In primary mammary epithelial cells cultured in the absence of ECM, PRL-R signaling was not initiated (35). This effect has been observed only in primary mammary epithelial cell cultures and in the intact mammary gland. Because ECM did not change STAT5 DNA binding in CID-9 cells but ECM enhancement of β-casein transcription was still

seen, modulation of the JAK/STAT pathway appears to be only one mechanism by which ECM regulates milk protein gene expression. ECM modulation of chromatin may provide an additional mechanism. For example, if histone deacetylase inhibitors are present, lactogenic hormones can induce transcription from the BCE in the absence of ECM. Histone deacetylase inhibitors also exerted only a small effect on transcriptional activation from the BCE in the presence of ECM. A role for modulation of chromatin structure was also indicated by the observation that the BCE only responds to ECM when stably integrated into the genome (91).

CONCLUSIONS

Enormous progress has been made in the past few years in our understanding of the basic mechanisms involved in the hormonal, tissue-specific, and developmental regulation of milk protein gene expression. Studies in cell culture systems have been complemented nicely by the use of transgenic and knockout mice. This has led to the elucidation of the importance of both the specific signal transduction pathways regulated by peptide and steroid hormones and the composite response elements that integrate these signals. Both are required for the appropriate regulation of milk protein gene expression, and no single factor or signaling component is sufficient to determine the precise temporal and spatial patterns of milk protein gene expression. The mammary gland has also provided a wealth of information about the cross talk between peptide and steroid hormones in regulating gene expression. Although details are still missing and most likely a few errors exist in the model, an overall picture has emerged that provides a framework for future studies.

Finally, these studies of milk protein gene regulation have led to several important developments in the fields of biotechnology, nutrition, and cancer biology. First, the application of transgenic technology has led to the development of a new field of biotechnology in which pharmaceuticals can now be produced in the milk of transgenic livestock (reviewed in 128). Second, as an offshoot of these studies, it is now possible to manipulate the composition of milk for nutritional reasons and to improve food processing (84). Finally, improved preclinical mouse models of breast cancer have been developed that more closely mimic the human disease (69, 70). The availability of these models should help in the design and evaluation of new targeted therapies for breast cancer.

ACKNOWLEDGMENTS

These studies were supported by grant CA16303 from the National Institutes of Health. We thank Ms. Alvenia Daniels for excellent secretarial assistance.

Literature Cited

1. Ahlgren U, Jonsson J, Jonsson L, Simu K, Edlund H. 1998. β-Cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. *Genes Dev.* 12:1763–68
2. Ahren K, Jacobsohn D. 1956. Mammary gland growth in hypophysectomized rats injected with ovarian hormones and insulin. *Acta Physiol. Scand.* 37:190–201
3. Alam T, An MR, Mifflin RC, Hsieh CC, Ge X, Papaconstantinou J. 1993. transActivation of the alpha 1-acid glycoprotein gene acute phase responsive element by multiple isoforms of C/EBP and glucocorticoid receptor. *J. Biol. Chem.* 268:15681–88
4. Alexander LJ, Stewart AF, Mackinlay AG, Kapelinskaya TV, Tkach TM, Gorodetsky SI. 1988. Isolation and characterization of the bovine κ-casein gene. *Eur. J. Biochem.* 178:395–401
5. Azam M, Erdjument-Bromage H, Kreider BL, Xia M, Quelle F, et al. 1995. Interleukin-3 signals through multiple isoforms of Stat5. *EMBO J.* 14:1402–11
6. Azam M, Lee C, Strehlow I, Schindler C. 1997. Functionally distinct isoforms of STAT5 are generated by protein processing. *Immunity* 6:691–701
7. Ball RK, Friis RR, Schoenenberger CA, Doppler W, Groner B. 1988. Prolactin regulation of β-casein gene expression and of a cytosolic 120-kd protein in a cloned mouse mammary epithelial cell line. *EMBO J.* 7:2089–95
8. Barahmand-Pour F, Meinke A, Eilers A, Gouilleux F, Groner B, Decker T. 1995. Colony-stimulating factors and interferon-γ activate a protein related to MGF-Stat 5 to cause formation of the differentiation-induced factor in myeloid cells. *FEBS Lett.* 360:29–33
9. Bauknecht T, See RH, Shi Y. 1996. A novel C/EBP β-YY1 complex controls the cell-type-specific activity of the human papillomavirus type 18 upstream regulatory region. *J. Virol.* 70:7695–705
10. Bawden WS, Passey RJ, Mackinlay AG. 1994. The genes encoding the major milk-specific proteins and their use in transgenic studies and protein engineering. *Biotechnol. Genet. Eng. Rev.* 12:89–137
11. Bonsing J, Mackinlay AG. 1987. Recent studies on nucleotide sequences encoding the caseins. *J. Dairy Res.* 54:447–61
12. Brun T, Roche E, Kim KH, Prentki M. 1993. Glucose regulates acetyl-CoA carboxylase gene expression in a pancreatic β-cell line (INS-1). *J. Biol. Chem.* 268:18905–11
13. Burdon TG, Maitland KA, Clark AJ, Wallace R, Watson CJ. 1994. Regulation of the sheep β-lactoglobulin gene by lactogenic hormones is mediated by a transcription factor that binds an interferon-gamma activation site-related element. *Mol. Endocrinol.* 8:1528–36
14. Candeliere GA, Jurutka PW, Haussler MR, St-Arnaud R. 1996. A composite element binding the vitamin D receptor, retinoid X receptor α, and a member of the CTF/NF-1 family of transcription factors mediates the vitamin D responsiveness of the c-fos promoter. *Mol. Cell. Biol.* 16:584–92
15. Cao Z, Umek RM, McKnight SL. 1991. Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells. *Genes Dev.* 5:1538–52
16. Cella N, Groner B, Hynes NE. 1998. Characterization of Stat5a and Stat5b homodimers and heterodimers and their association with the glucocorticoid receptor in mammary cells. *Mol. Cell. Biol.* 18:1783–92
17. Chakravarti D, LaMorte VJ, Nelson MC, Nakajima T, Schulman IG, et al. 1996. Role of CBP/P300 in nuclear receptor signalling. *Nature* 383:99–103
18. Chammas R, Taverna D, Cella N, Santos C, Hynes NE. 1994. Laminin and tenascin assembly and expression regulate HC11 mouse mammary cell differentiation. *J. Cell Sci.* 107:1031–40
19. Chang CJ, Chen YL, Lee SC. 1998. Coactivator TIF1β interacts with transcription factor C/EBPβ and glucocorticoid receptor to induce α 1-acid glycoprotein gene expression. *Mol. Cell. Biol.* 18:5880–87
20. Chaudhry AZ, Lyons GE, Gronostajski RM. 1997. Expression patterns of the four nuclear factor I genes during mouse embryogenesis indicate a potential role in development. *Dev. Dyn.* 208:313–25

21. Chavez S, Candau R, Truss M, Beato M. 1995. Constitutive repression and nuclear factor I-dependent hormone activation of the mouse mammary tumor virus promoter in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 15:6987–98
22. Chen J, Sadowski HB, Kohanski RA, Wang LH. 1997. Stat5 is a physiological substrate of the insulin receptor. *Proc. Natl. Acad. Sci. USA* 94:2295–300
23. Crawford DR, Leahy P, Hu CY, Chaudhry A, Gronostajski R, et al. 1998. Nuclear factor I regulates expression of the gene for phosphoenolpyruvate carboxykinase (GTP). *J. Biol. Chem.* 273:13387–90
24. Daniel S, Kim KH. 1996. Sp1 mediates glucose activation of the acetyl-CoA carboxylase promoter. *J. Biol. Chem.* 271:1385–92
25. Daniel S, Zhang S, DePaoli-Roach AA, Kim KH. 1996. Dephosphorylation of Sp1 by protein phosphatase 1 is involved in the glucose-mediated activation of the acetyl-CoA carboxylase gene. *J. Biol. Chem.* 271:14692–97
26. Danielson KG, Oborn CJ, Durban EM, Butel JS, Medina D. 1984. Epithelial mouse mammary cell line exhibiting normal morphogenesis in vivo and functional differentiation in vitro. *Proc. Natl. Acad. Sci. USA* 81:3756–60
27. Darnell JE Jr. 1997. STATs and gene regulation. *Science* 277:1630–35
28. Descombes P, Schibler U. 1991. A liver-enriched transcriptional activator protein, LAP, and a transcriptional inhibitory protein, LIP, are translated from the same mRNA. *Cell* 67:569–79
29. Doiron B, Cuif MH, Chen R, Kahn A. 1996. Transcriptional glucose signaling through the glucose response element is mediated by the pentose phosphate pathway. *J. Biol. Chem.* 271:5321–24
30. Doiron B, Cuif MH, Kahn A, Diaz-Guerra MJ. 1994. Respective roles of glucose, fructose, and insulin in the regulation of the liver-specific pyruvate kinase gene promoter. *J. Biol. Chem.* 269:10213–16
31. Doppler W, Hock W, Hofer P, Groner B, Ball RK. 1990. Prolactin and glucocorticoid hormones control transcription of the β-casein gene by kinetically distinct mechanisms. *Mol. Endocrinol.* 4:912–19
32. Doppler W, Villunger A, Jennewein P, Brduscha K, Groner B, Ball RK. 1991. Lactogenic hormone and cell type-specific control of the whey acidic protein gene promoter in transfected mouse cells. *Mol. Endocrinol.* 5:1624–32
33. Doppler W, Welte T, Philipp S. 1995. CCAAT/enhancer-binding protein isoforms β and δ are expressed in mammary epithelial cells and bind to multiple sites in the β-casein gene promoter. *J. Biol. Chem.* 270:17962–69
34. Edwards G, Streuli C. 1995. Signalling in extracellular-matrix-mediated control of epithelial cell phenotype. *Biochem. Soc. Trans.* 23:464–68
35. Edwards GM, Wilford FH, Liu X, Hennighausen L, Djiane J, Streuli CH. 1998. Regulation of mammary differentiation by extracellular matrix involves protein-tyrosine phosphatases. *J. Biol. Chem.* 273:9495–500
36. Elias JJ. 1959. Effect of insulin and cortisol on organ cultures of adult mouse mammary gland. *Proc. Soc. Exp. Biol. Med.* 101:500–2
37. Fujii H, Nakagawa Y, Schindler U, Kawahara A, Mori H, et al. 1995. Activation of Stat5 by interleukin 2 requires a carboxyl-terminal region of the interleukin 2 receptor β chain but is not essential for the proliferative signal transmission. *Proc. Natl. Acad. Sci. USA* 92:5482–86
38. Furlong EEM, Keon NK, Thornton FD, Rein T, Martin F. 1996. Expression of a 74-kDa nuclear factor 1 (NF1) protein is induced in mouse mammary gland involution. Involution-enhanced occupation of a twin NF1 binding element in the testosterone-repressed prostate message-2/clusterin promoter. *J. Biol. Chem.* 271: 29688–97
39. Gigliotti AP, DeWille JW. 1998. Lactation status influences expression of CCAAT/enhancer binding protein isoform mRNA in the mouse mammary gland. *J. Cell Physiol.* 174:232–39
40. Gouilleux F, Pallard C, Dusanter-Fourt I, Wakao H, Haldosen LA, et al. 1995. Prolactin, growth hormone, erythropoietin and granulocyte-macrophage colony stimulating factor induce MGF-Stat5 DNA binding activity. *EMBO J.* 14:2005–13
41. Gouilleux F, Wakao H, Mundt M, Groner B. 1994. Prolactin induces phosphorylation of Tyr694 of Stat5 (MGF), a prerequisite for DNA binding and induction of transcription. *EMBO J.* 13:4361–69
42. Griffiths MR, Black EJ, Culbert AA, Dickens M, Shaw PE, et al. 1998. Insulin-stimulated expression of c-fos, fra1 and c-jun accompanies the activation of the activator protein-1 (AP-1) transcriptional complex. *Biochem. J.* 335:19–26
43. Groenen MA, Dijkhof RJ, van der Poel JJ, van Diggelen R, Verstege E. 1992. Multiple octamer binding sites in the promoter region of the bovine alpha s2-

casein gene. *Nucleic Acids Res.* 20:4311–18
44. Groenen MA, Dijkhof RJ, Verstege AJ, van der Poel JJ. 1993. The complete sequence of the gene encoding bovine α s2-casein. *Gene* 123:187–93
45. Gual P, Baron V, Lequoy V, Van Obberghen E. 1998. Interaction of Janus kinases JAK-1 and JAK-2 with the insulin receptor and the insulin-like growth factor-1 receptor. *Endocrinology* 139:884–93
46. Hadsell DL, Greenberg NM, Fligger JM, Baumrucker CR, Rosen JM. 1996. Targeted expression of des(1–3) human insulin-like growth factor I in transgenic mice influences mammary gland development and IGF-binding protein expression. *Endocrinology* 137:321–29
47. Haspel RL, Salditt-Georgieff M, Darnell JE Jr. 1996. The rapid inactivation of nuclear tyrosine phosphorylated Stat1 depends upon a protein tyrosine phosphatase. *EMBO J.* 15:6262–68
48. Hemati N, Ross SE, Erickson RL, Groblewski GE, MacDougald OA. 1997. Signaling pathways through which insulin regulates CCAAT/enhancer binding protein α (C/EBPα) phosphorylation and gene expression in 3T3-L1 adipocytes. Correlation with GLUT4 gene expression. *J. Biol. Chem.* 272:25913–19
49. Hobbs AA, Richards DA, Kessler DJ, Rosen JM. 1982. Complex hormonal regulation of rat casein gene expression. *J. Biol. Chem.* 257:3598–605
50. Horseman ND, Zhao W, Montecino-Rodriguez E, Tanaka M, Nakashima K, et al. 1997. Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of the prolactin gene. *EMBO J.* 16:6926–35
51. Hou J, Schindler U, Henzel WJ, Wong SC, McKnight SL. 1995. Identification and purification of human Stat proteins activated in response to interleukin-2. *Immunity* 2:321–29
52. Jacob KK, Ouyang L, Stanley FM. 1995. A consensus insulin response element is activated by an Ets-related transcription factor. *J. Biol. Chem.* 270:27773–79
53. Jacob KK, Sap J, Stanley FM. 1998. Receptor-like protein-tyrosine phosphatase α specifically inhibits insulin-increased prolactin gene expression. *J. Biol. Chem.* 273:4800–9
54. Jacoby DB, Zilz ND, Towle HC. 1989. Sequences within the 5′-flanking region of the S14 gene confer responsiveness to glucose in primary hepatocytes. *J. Biol. Chem.* 264:17623–26
55. Janknecht R, Hunter T. 1996. Transcription. A growing coactivator network. *Nature* 383:22–23
56. Jiang J, Levine M. 1993. Binding affinities and cooperative interactions with bHLH activators delimit threshold responses to the dorsal gradient morphogen. *Cell* 72:741–52
57. Johnston JA, Bacon CM, Finbloom DS, Rees RC, Kaplan D, et al. 1995. Tyrosine phosphorylation and activation of STAT5, STAT3, and Janus kinases by interleukins 2 and 15. *Proc. Natl. Acad. Sci. USA* 92:8705–9
58. Jolivet G, L'Hotte C, Pierre S, Tourkine N, Houdebine LM. 1996. A MGF/STAT5 binding site is necessary in the distal enhancer for high prolactin induction of transfected rabbit α s1-casein-CAT gene transcription. *FEBS Lett.* 389:257–62
59. Juergens WG, Stockdale FE, Topper YJ, Elias JJ. 1965. Hormone-dependent differentiation of mammary gland in vitro. *Proc. Natl. Acad. Sci. USA* 54:629–34
60. Kazansky AV, Raught B, Lindsey SM, Wang YF, Rosen JM. 1995. Regulation of mammary gland factor/Stat5a during mammary gland development. *Mol. Endocrinol.* 9:1598–609
61. Kel OV, Romaschenko AG, Kel AE, Wingender E, Kolchanov NA. 1995. A compilation of composite regulatory elements affecting gene transcription in vertebrates. *Nucleic Acids Res.* 23:4097–103
62. Kleinberg DL. 1998. Role of IGF-I in normal mammary development. *Breast Cancer Res. Treat.* 47:201–8
63. Kleinberg DL, Ruan W, Catanese V, Newman CB, Feldman M. 1990. Nonlactogenic effects of growth hormone on growth and insulin-like growth factor-I messenger ribonucleic acid of rat mammary gland. *Endocrinology* 126:3274-76. Erratum. 1990. *Endocrinology* 127(4): 1977
64. Kumaresan P, Turner CW. 1965. Effect of insulin and alloxan on mammary gland growth in rats. *J. Dairy Sci.* 48:1378–81
65. Lau C, Sullivan MK, Hazelwood RL. 1993. Effects of diabetes mellitus on lactation in the rat. *Proc. Soc. Exp. Biol. Med.* 204:81–89
66. Lechner J, Welte T, Doppler W. 1997. Mechanism of interaction between the glucocorticoid receptor and Stat5: role of DNA-binding. *Immunobiology* 198:112–23
67. Lechner J, Welte T, Tomasi JK, Bruno P, Cairns C, et al. 1997. Promoter-dependent synergy between glucocorticoid recep-

tor and Stat5 in the activation of β-casein gene transcription. *J. Biol. Chem.* 272:20954–60
68. Lee KF, DeMayo FJ, Atiee SH, Rosen JM. 1988. Tissue-specific expression of the rat beta-casein gene in transgenic mice. *Nucleic Acids Res.* 16:1027–41
69. Li B, Murphy KL, Laucirica R, Kittrell F, Medina D, Rosen JM. 1998. A transgenic mouse model for mammary carcinogenesis. *Oncogene* 16:997–1007
70. Li B, Rosen JM, McMenamin-Balano J, Muller WJ, Perkins AS. 1997. neu/ERBB2 cooperates with p53-172H during mammary tumorigenesis in transgenic mice. *Mol. Cell. Biol.* 17:3155–63
71. Li ML, Aggeler J, Farson DA, Hatier C, Hassell J, Bissell MJ. 1987. Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells. *Proc. Natl. Acad. Sci. USA* 84:136–40
72. Li S, Rosen JM. 1994. Distal regulatory elements required for rat whey acidic protein gene expression in transgenic mice. *J. Biol. Chem.* 269:14235–43
73. Li S, Rosen JM. 1994. Glucocorticoid regulation of rat whey acidic protein gene expression involves hormone-induced alterations of chromatin structure in the distal promoter region. *Mol. Endocrinol.* 8:1328–35
74. Li S, Rosen JM. 1995. Nuclear factor I and mammary gland factor (STAT5) play a critical role in regulating rat whey acidic protein gene expression in transgenic mice. *Mol. Cell. Biol.* 15:2063–70
75. Lidmer AS, Kannius M, Lundberg L, Bjursell G, Nilsson J. 1995. Molecular cloning and characterization of the mouse carboxyl ester lipase gene and evidence for expression in the lactating mammary gland. *Genomics* 29:115–22
76. Lin FT, MacDougald OA, Diehl AM, Lane MD. 1993. A 30-kDa alternative translation product of the CCAAT/enhancer binding protein α message: transcriptional activator lacking antimitotic activity. *Proc. Natl. Acad. Sci. USA* 90: 9606–10
77. Lin JX, Mietz J, Modi WS, John S, Leonard WJ. 1996. Cloning of human Stat5B. Reconstitution of interleukin-2-induced Stat5A and Stat5B DNA binding activity in COS-7 cells. *J. Biol. Chem.* 271:10738–44
78. Liu M, Freedman LP. 1994. Transcriptional synergism between the vitamin D3 receptor and other nonreceptor transcription factors. *Mol. Endocrinol.* 8:1593–604
79. Liu X, Robinson GW, Gouilleux F, Groner B, Hennighausen L. 1995. Cloning and expression of Stat5 and an additional homologue (Stat5b) involved in prolactin signal transduction in mouse mammary tissue. *Proc. Natl. Acad. Sci. USA* 92:8831–35
80. Liu X, Robinson GW, Hennighausen L. 1996. Activation of Stat5a and Stat5b by tyrosine phosphorylation is tightly linked to mammary gland differentiation. *Mol. Endocrinol.* 10:1496–506
81. Liu X, Robinson GW, Wagner KU, Garrett L, Wynshaw-Boris A, Hennighausen L. 1997. Stat5a is mandatory for adult mammary gland development and lactogenesis. *Genes Dev.* 11:179–86
82. Lockwood DH, Voytovich AE, Stockdale FE, Topper YJ. 1967. Insulin-dependent DNA polymerase and DNA synthesis in mammary epithelial cells in vitro. *Proc. Natl. Acad. Sci. USA* 58:658–64
83. MacDougald OA, Cornelius P, Liu R, Lane MD. 1995. Insulin regulates transcription of the CCAAT/enhancer binding protein (C/EBP) α, β, and δ genes in fully-differentiated 3T3-L1 adipocytes. *J. Biol. Chem.* 270:647–54
84. Maga EA, Murray JD. 1995. Mammary gland expression of transgenes and the potential for altering the properties of milk. *Bio-Technology* 13:1452–57
85. Marshak S, Totary H, Cerasi E, Melloul D. 1996. Purification of the β-cell glucose-sensitive factor that transactivates the insulin gene differentially in normal and transformed islet cells. *Proc. Natl. Acad. Sci. USA* 93:15057–62
86. Meier VS, Groner B. 1994. The nuclear factor YY1 participates in repression of the β-casein gene promoter in mammary epithelial cells and is counteracted by mammary gland factor during lactogenic hormone induction. *Mol. Cell. Biol.* 14:128–37
87. Merlo GR, Graus-Porta D, Cella N, Marte BM, Taverna D, Hynes NE. 1996. Growth, differentiation and survival of HC11 mammary epithelial cells: diverse effects of receptor tyrosine kinase-activating peptide growth factors. *Eur. J. Cell Biol.* 70:97–105
88. Mink S, Haenig B, Klempnauer KH. 1997. Interaction and functional collaboration of p300 and C/EBPβ. *Mol. Cell. Biol.* 17:6609–17
89. Moriggl R, Berchtold S, Friedrich K, Standke GJ, Kammer W, et al. 1997. Comparison of the transactivation domains of

Stat5 and Stat6 in lymphoid cells and mammary epithelial cells. *Mol. Cell. Biol.* 17:3663–78

90. Moriggl R, Gouilleux-Gruart V, Jahne R, Berchtold S, Gartmann C, et al. 1996. Deletion of the carboxyl-terminal transactivation domain of MGF-Stat5 results in sustained DNA binding and a dominant negative phenotype. *Mol. Cell. Biol.* 16:5691–700
91. Myers CA, Schmidhauser C, Mellentin-Michelotti J, Fragoso G, Roskelley CD, et al. 1998. Characterization of BCE-1, a transcriptional enhancer regulated by prolactin and extracellular matrix and modulated by the state of histone acetylation. *Mol. Cell. Biol.* 18:2184–95
92. Nagata K, Guggenheimer RA, Enomoto T, Lichy JH, Hurwitz J. 1982. Adenovirus DNA replication in vitro: identification of a host factor that stimulates synthesis of the preterminal protein-dCMP complex. *Proc. Natl. Acad. Sci. USA* 79:6438–42
93. Nandi S. 1958. Endocrine control of mammary gland development and function in the C3 11/HE Crgl mouse. *J. Natl. Cancer Inst.* 21:1039–62
94. Nasrin N, Buggs C, Kong XF, Carnazza J, Goebl M, Alexander-Bridges M. 1991. DNA-binding properties of the product of the testis-determining gene and a related protein. *Nature* 354:317–20
95. Neuenschwander S, Schwartz A, Wood TL, Roberts CT Jr, Henninghausen L, LeRoith D. 1996. Involution of the lactating mammary gland is inhibited by the IGF system in a transgenic mouse model. *J. Clin. Invest.* 97:2225–32
96. Nicholas KR, Sankaran L, Topper YJ. 1983. A unique and essential role for insulin in the phenotypic expression of rat mammary epithelial cells unrelated to its function in cell maintenance. *Biochim. Biophys. Acta* 763:309–14
97. Nielsen DA, Welsh M, Casadaban MJ, Steiner DF. 1985. Control of insulin gene expression in pancreatic β-cells and in an insulin-producing cell line, RIN-5F cells. I. Effects of glucose and cyclic AMP on the transcription of insulin mRNA. *J. Biol. Chem.* 260:13585–89
98. Nishio Y, Isshiki H, Kishimoto T, Akira S. 1993. A nuclear factor for interleukin-6 expression (NF-IL6) and the glucocorticoid receptor synergistically activate transcription of the rat α 1-acid glycoprotein gene via direct protein-protein interaction. *Mol. Cell. Biol.* 13:1854–62
99. Okajima Y, Matsumura I, Nishiura T, Hashimoto K, Yoshida H, et al. 1998. Insulin-like growth factor-I augments erythropoietin-induced proliferation through enhanced tyrosine phosphorylation of STAT5. *J. Biol. Chem.* 273:22877–83
100. Onishi M, Nosaka T, Misawa K, Mui AL, Gorman D, et al. 1998. Identification and characterization of a constitutively active STAT5 mutant that promotes cell proliferation. *Mol. Cell. Biol.* 18:3871–79
101. Ormandy CJ, Camus A, Barra J, Damotte D, Lucas B, et al. 1997. Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. *Genes Dev.* 11:167–78
102. O'Rourke J, Yuan R, DeWille J. 1997. CCAAT/enhancer-binding protein-δ (C/EBP-δ) is induced in growth-arrested mouse mammary epithelial cells. *J. Biol. Chem.* 272:6291–96
103. Osada S, Daimon S, Ikeda T, Nishihara T, Yano K, et al. 1997. Nuclear factor 1 family proteins bind to the silencer element in the rat glutathione transferase P gene. *J. Biochem.* 121:355–63
104. Osada S, Ikeda T, Xu M, Nishihara T, Imagawa M. 1997. Identification of the transcriptional repression domain of nuclear factor 1-A. *Biochem. Biophys. Res. Commun.* 238:744–47
105. Ossipow V, Descombes P, Schibler U. 1993. CCAAT/enhancer-binding protein mRNA is translated into multiple proteins with different transcription activation potentials. *Proc. Natl. Acad. Sci. USA* 90:8219–23
106. Ouyang L, Jacob KK, Stanley FM. 1996. GABP mediates insulin-increased prolactin gene transcription. *J. Biol. Chem.* 271:10425–28
107. Pallard C, Gouilleux F, Benit L, Cocault L, Souyri M, et al. 1995. Thrombopoietin activates a STAT5-like factor in hematopoietic cells. *EMBO J.* 14:2847–56
108. Patel BKR, Pierce JH, LaRochelle WJ. 1998. Regulation of interleukin 4-mediated signaling by naturally occurring dominant negative and attenuated forms of human Stat6. *Proc. Natl. Acad. Sci. USA* 95:172–77
109. Pazin MJ, Kadonaga JT. 1997. What's up and down with histone deacetylation and transcription? *Cell* 89:325–28
110. Pezet A, Ferrag F, Kelly PA, Edery M. 1997. Tyrosine docking sites of the rat prolactin receptor required for association and activation of stat5. *J. Biol. Chem.* 272:25043–50
111. Pfitzner E, Jahne R, Wissler M, Stoecklin E, Groner B. 1998. p300/CREB-binding

protein enhances the prolactin-mediated transcriptional induction through direct interaction with the transactivation domain of Stat5, but does not participate in the Stat5-mediated suppression of the glucocorticoid response. *Mol. Endocrinol.* 12:1582–93

112. Philp JAC, Burdon TG, Watson CJ. 1996. Differential activation of STATs 3 and 5 during mammary gland development. *FEBS Lett.* 396:77–80
113. Pierre S, Jolivet G, Devinoy E, Houdebine LM. 1994. A combination of distal and proximal regions is required for efficient prolactin regulation of transfected rabbit α s1-casein chloramphenicol acetyltransferase constructs. *Mol. Endocrinol.* 8:1720–30. Erratum. 1995. *Mol. Endocrinol*. 9(4):412
114. Pittius CW, Hennighausen L, Lee E, Westphal H, Nicols E, et al. 1988. A milk protein gene promoter directs the expression of human tissue plasminogen activator cDNA to the mammary gland in transgenic mice. *Proc. Natl. Acad. Sci. USA* 85:5874–78
115. Poyet P, Henning SJ, Rosen JM. 1989. Hormone-dependent β-casein mRNA stabilization requires ongoing protein synthesis. *Mol. Endocrinol.* 3:1961–68
116. Prosser CG, Sankaran L, Hennighausen L, Topper YJ. 1987. Comparison of the roles of insulin and insulin-like growth factor I in casein gene expression and in the development of α-lactalbumin and glucose transport activities in the mouse mammary epithelial cell. *Endocrinology* 120:1411–16
117. Rajput B, Shaper NL, Shaper JH. 1996. Transcriptional regulation of murine β-1,4-galactosyltransferase in somatic cells. Analysis of a gene that serves both a housekeeping and a mammary gland-specific function. *J. Biol. Chem.* 271:5131–42
118. Raught B, Khursheed B, Kazansky A, Rosen J. 1994. YY1 represses β-casein gene expression by preventing the formation of a lactation-associated complex. *Mol. Cell. Biol.* 14:1752–63
119. Raught B, Liao WS, Rosen JM. 1995. Developmentally and hormonally regulated CCAAT/enhancer-binding protein isoforms influence β-casein gene expression. *Mol. Endocrinol.* 9:1223–32
120. Richert MM, Wood TL. 1999. The insulin-like growth factors and IGF type I receptor during postnatal growth of the murine mammary gland: sites of mRNA expression and potential functions. *Endocrinology.* 140:454–61
121. Rijnkels M, Kooiman PM, de Boer HA, Pieper FR. 1997. Organization of the bovine casein gene locus. *Mamm. Genome* 8:148–52
122. Rijnkels M, Kooiman PM, Krimpenfort PJ, de Boer HA, Pieper FR. 1995. Expression analysis of the individual bovine β-, α(s2)- and κ-casein genes in transgenic mice. *Biochem. J.* 311:929–37
123. Rijnkels M, Meershoek E, de Boer HA, Pieper FR. 1997. Physical map and localization of the human casein gene locus. *Mamm. Genome* 8:285–86
124. Rijnkels M, Wheeler DA, de Boer HA, Pieper FR. 1997. Structure and expression of the mouse casein gene locus. *Mamm. Genome* 8:9–15
125. Ripperger JA, Fritz S, Richter K, Hocke GM, Lottspeich F, Fey GH. 1995. Transcription factors Stat3 and Stat5b are present in rat liver nuclei late in an acute phase response and bind interleukin-6 response elements. *J. Biol. Chem.* 270: 29998–30006
126. Rivera E, Bern HA. 1961. Influence of insulin on maintenance and secretory stimulation of mouse mammary tissues in organ-culture. *Endocrinology* 69:340–53
127. Robinson GW, Johnson PF, Hennighausen L, Sterneck E. 1998. The C/EBPβ transcription factor regulates epithelial cell proliferation and differentiation in the mammary gland. *Genes Dev.* 12:1907–16
128. Rosen JM, Li S, Raught B, Hadsell D. 1999. The mammary gland as a bioreactor: factors regulating the efficient expression of milk protein-based transgenes. *Am. J. Clin. Nutr.* 63:6275–325
129. Roskelley CD, Desprez PY, Bissell MJ. 1994. Extracellular matrix-dependent tissue-specific gene expression in mammary epithelial cells requires both physical and biochemical signal transduction. *Proc. Natl. Acad. Sci. USA* 91:12378–82
130. Roskelley CD, Srebrow A, Bissell MJ. 1995. A hierarchy of ECM-mediated signalling regulates tissue-specific gene expression. *Curr. Opin. Cell. Biol.* 7:736–47
131. Ruan W, Catanese V, Wieczorek R, Feldman M, Kleinberg DL. 1995. Estradiol enhances the stimulatory effect of insulin-like growth factor-I (IGF-I) on mammary development and growth hormone-induced IGF-I messenger ribonucleic acid. *Endocrinology* 136:1296–302
132. Ruan W, Newman CB, Kleinberg DL. 1992. Intact and amino-terminally shortened forms of insulin-like growth factor I induce mammary gland differentiation and development. *Proc. Natl. Acad. Sci. USA* 89:10872–76

133. Ruff-Jamison S, Chen K, Cohen S. 1995. Epidermal growth factor induces the tyrosine phosphorylation and nuclear translocation of Stat 5 in mouse liver. *Proc. Natl. Acad. Sci. USA* 92:4215–18
134. Rui H, Lebrun JJ, Kirken RA, Kelly PA, Farrar WL. 1994. JAK2 activation and cell proliferation induced by antibody-mediated prolactin receptor dimerization. *Endocrinology* 135:1299–306
135. Rutter GA, White MR, Tavare JM. 1995. Involvement of MAP kinase in insulin signalling revealed by non- invasive imaging of luciferase gene expression in single living cells. *Curr. Biol.* 5:890–99. Erratum. 1995. *Curr. Biol*. 5(9):1072
136. Sabatakos G, Davies GE, Grosse M, Cryer A, Ramji DP. 1998. Expression of the genes encoding CCAAT-enhancer binding protein isoforms in the mouse mammary gland during lactation and involution. *Biochem. J.* 334:205–10
137. Salmon WD Jr, Daughaday WH. 1957. A hormonal controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. *J. Lab. Clin. Med.* 49:825–36
138. Schmidhauser C, Casperson GF, Myers CA, Sanzo KT, Bolten S, Bissell MJ. 1992. A novel transcriptional enhancer is involved in the prolactin- and extracellular matrix-dependent regulation of β-casein gene expression. *Mol. Biol. Cell* 3:699–709
139. Schmitt-Ney M, Doppler W, Ball RK, Groner B. 1991. β-casein gene promoter activity is regulated by the hormone-mediated relief of transcriptional repression and a mammary-gland-specific nuclear factor. *Mol. Cell. Biol.* 11:3745–55
140. Seagroves TN, Krnacik S, Raught B, Gay J, Burgess-Beusse B, et al. 1998. C/EBPβ, but not C/EBPα, is essential for ductal morphogenesis, lobuloalveolar proliferation, and functional differentiation in the mouse mammary gland. *Genes Dev.* 12:1917–28
141. Shi Y, Lee J-S, Galvin KM. 1997. Everything you have ever wanted to know about Yin Yang 1. . .. *Biochim. Biophys. Acta* 1332:F49–66
142. Shuai K, Liao J, Song MM. 1996. Enhancement of antiproliferative activity of γ interferon by the specific inhibition of tyrosine dephosphorylation of Stat1. *Mol. Cell. Biol.* 16:4932–41
143. Simons JP, McClenaghan M, Clark AJ. 1987. Alteration of the quality of milk by expression of sheep β-lactoglobulin in transgenic mice. *Nature* 328:530–32
144. Stocklin E, Wissler M, Gouilleux F, Groner B. 1996. Functional interactions between Stat5 and the glucocorticoid receptor. *Nature* 383:726–28
145. Stoecklin E, Wissler M, Moriggl R, Groner B. 1997. Specific DNA binding of Stat5, but not of glucocorticoid receptor, is required for their functional cooperation in the regulation of gene transcription. *Mol. Cell. Biol.* 17:6708–16
146. Streuli CH. 1993. Extracellular matrix and gene expression in mammary epithelium. *Semin. Cell. Biol.* 4:203–12
147. Streuli CH, Bailey N, Bissell MJ. 1991. Control of mammary epithelial differentiation: basement membrane induces tissue-specific gene expression in the absence of cell-cell interaction and morphological polarity. *J. Cell Biol.* 115:1383–95
148. Streuli CH, Edwards GM, Delcommenne M, Whitelaw CB, Burdon TG, et al. 1995. Stat5 as a target for regulation by extracellular matrix. *J. Biol. Chem.* 270:21639–44
149. Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, et al. 1998. Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. *Cell* 93:841–50
150. Topper YJ, Freeman CS. 1980. Multiple hormone interactions in the developmental biology of the mammary gland. *Physiol. Rev.* 60:1049–106
151. Udy GB, Towers RP, Snell RG, Wilkins RJ, Park SH, et al. 1997. Requirement of STAT5b for sexual dimorphism of body growth rates and liver gene expression. *Proc. Natl. Acad. Sci. USA* 94:7239–44
152. Vallet VS, Henrion AA, Bucchini D, Casado M, Raymondjean M, et al. 1997. Glucose-dependent liver gene expression in upstream stimulatory factor-2 $-/-$ mice. *J. Biol. Chem.* 272:21944–49
153. Vilotte J-L, Soulier S, Stinnarkre M-G, Massoud M, Mercier J-C. 1989. Efficient expression of bovine α-lactalbumin in transgenic mice. *Eur. J. Biochem.* 186:43–48
154. Voytovich AE, Owens IS, Topper YJ. 1969. A novel action of insulin on phosphoprotein formation by mammary gland explants. *Proc. Natl. Acad. Sci. USA* 63:213–17
155. Waeber G, Thompson N, Chautard T, Steinmann M, Nicod P, et al. 1998. Transcriptional activation of the macrophage migration-inhibitory factor gene by the corticotropin-releasing factor is mediated by the cyclic adenosine 3′,5′-monophosphate responsive element-binding protein CREB in pituitary cells. *Mol. Endocrinol.* 12:698–705

156. Wakao H, Gouilleux F, Groner B. 1994. Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. *EMBO J.* 13:2182–91. Erratum. 1995. *EMBO J.* 14(4):854–55
157. Wakao H, Schmitt-Ney M, Groner B. 1992. Mammary gland-specific nuclear factor is present in lactating rodent and bovine mammary tissue and composed of a single polypeptide of 89 kDa. *J. Biol. Chem.* 267:16365–70
158. Wang D, Stravopodis D, Teglund S, Kitazawa J, Ihle JN. 1996. Naturally occurring dominant negative variants of Stat5. *Mol. Cell. Biol.* 16:6141–48
159. Wang D, Sul HS. 1995. Upstream stimulatory factors bind to insulin response sequence of the fatty acid synthase promoter. USF1 is regulated. *J. Biol. Chem.* 270:28716–22. Erratum. 1996. *J. Biol. Chem*. 271(13):7873
160. Wang D, Sul HS. 1997. Upstream stimulatory factor binding to the E-box at −65 is required for insulin regulation of the fatty acid synthase promoter. *J. Biol. Chem.* 272:26367–74
161. Wang D, Sul HS. 1998. Insulin stimulation of the fatty acid synthase promoter is mediated by the phosphatidylinositol 3-kinase pathway. Involvement of protein kinase B/Akt. *J. Biol. Chem.* 273:25420–26
162. Wartmann M, Cella N, Hofer P, Groner B, Liu X, et al. 1996. Lactogenic hormone activation of Stat5 and transcription of the β-casein gene in mammary epithelial cells is independent of p42 ERK2 mitogen-activated protein kinase activity. *J. Biol. Chem.* 271:31863–68
163. Watson CJ, Gordon KE, Robertson M, Clark AJ. 1991. Interaction of DNA-binding proteins with a milk protein gene promoter in vitro: identification of a mammary gland-specific factor. *Nucleic Acids Res.* 19:6603–10
164. Welsh M, Nielsen DA, MacKrell AJ, Steiner DF. 1985. Control of insulin gene expression in pancreatic beta-cells and in an insulin-producing cell line, RIN-5F cells. II. Regulation of insulin mRNA stability. *J. Biol. Chem.* 260:13590–94
165. Welte T, Garimorth K, Philipp S, Doppler W. 1994. Prolactin-dependent activation of a tyrosine phosphorylated DNA binding factor in mouse mammary epithelial cells. *Mol. Endocrinol.* 8:1091–102
166. Welte T, Philipp S, Cairns C, Gustafsson JA, Doppler W. 1993. Glucocorticoid receptor binding sites in the promoter region of milk protein genes. *J. Steroid Biochem. Mol. Biol.* 47:75–81
167. Williams SC, Cantwell CA, Johnson PF. 1991. A family of C/EBP-related proteins capable of forming covalently linked leucine zipper dimers in vitro. *Genes Dev.* 5:1553–67
168. Winklehner-Jennewein P, Geymayer S, Lechner J, Welte T, Hansson L, et al. 1998. A distal enhancer region in the human ß-casein gene mediates the response to prolactin and glucocorticoid hormones. *Gene* 217:127–39
169. Wolffe AP, Pruss D. 1996. Targeting chromatin disruption: transcription regulators that acetylate histones. *Cell* 84:817–19
170. Wood TJ, Sliva D, Lobie PE, Pircher TJ, Gouilleux F, et al. 1995. Mediation of growth hormone-dependent transcriptional activation by mammary gland factor/Stat 5. *J. Biol. Chem.* 270:9448–53
171. Wyszomierski SL, Yeh J, Rosen JM. 1999. Glucocorticoid receptor/STAT5 interactions enhance STAT5 activation by prolonging STAT5 DNA binding and tyrosine phosphorylation. *Mol. Endocrinol.* 13:330–43
172. Yamamoto H, Crow M, Cheng L, Lakatta E, Kinsella J. 1996. PDGF receptor-to-nucleus signaling of p91 (STAT1 α) transcription factor in rat smooth muscle cells. *Exp. Cell Res.* 222:125–30
173. Yan R, Qureshi S, Zhong Z, Wen Z, Darnell JE Jr. 1995. The genomic structure of the STAT genes: multiple exons in coincident sites in Stat1 and Stat2. *Nucleic Acids Res.* 23:459–63
174. Yang WM, Inouye C, Zeng Y, Bearss D, Seto E. 1996. Transcriptional repression by YY1 is mediated by interaction with a mammalian homolog of the yeast global regulator RPD3. *Proc. Natl. Acad. Sci. USA* 93:12845–50
175. Yang WM, Yao YL, Sun JM, Davie JR, Seto E. 1997. Isolation and characterization of cDNAs corresponding to an additional member of the human histone deacetylase gene family. *J. Biol. Chem.* 272:28001–7
176. Yeh WC, Cao Z, Classon M, McKnight SL. 1995. Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. *Genes Dev.* 9:168–81
177. Yu-Lee LY, Richter-Mann L, Couch CH, Stewart AF, Mackinlay AG, Rosen JM. 1986. Evolution of the casein multigene family: conserved sequences in the 5′ flanking and exon regions. *Nucleic Acids Res.* 14:1883–902