Activating Transcription Factor 4 Overexpression Inhibits Proliferation and Differentiation of Mammary Epithelium Resulting in Impaired Lactation and Accelerated Involution*

The basic leucine zipper containing activating transcription factors (ATFs) modulates the expression of growth-regulating genes. In this study, we sought to determine specifically the consequences of ATF4 expression on mammary gland development in transgenic mice. Overexpression of ATF4 severely impaired normal development of the mammary gland, which was associated with reduced proliferation and differentiation of mammary alveolar epithelium and up-regulation of p21WAF1 and p27KIP1. In addition, there was also impaired lactation accompanied by decreased expression of α-lactalbumin, whey acidic protein, and β-casein, possibly because of the down-regulation of STAT5a tyrosine phosphorylation. Mammary gland involution in ATF4-transgenic mice was accelerated, compared with wild type littermates by whole mount analysis. In addition, day 18 of lactation in transgenic mice was phenotypically equivalent to day 3 of involution in wild type mice, as determined by the TUNEL assay and expression of Bax. The concentration of the proapoptotic molecule caspase-3 was increased during lactation in ATF4-transgenic animal. Mammary glands from ATF4-transgenic mice also showed significant nuclear translocation of activated STAT3 and up-regulation of one of its target genes, insulin-like growth factor-binding protein-5, which is thought to facilitate apoptosis by sequestering insulin-like growth factor. Together, these findings suggest that ATF4 may play a role during mammary gland development and that down-regulation of ATF4 may be important for the onset of involution in the mammary gland.

Among the main regulatory elements that contribute to transcriptional regulation of extracellular signals are the cAMP-responsive element (CRE) and activation protein (AP-1) sequence motifs. It is increasingly accepted that the CRE site (TGACGTCG) is recognized by a family of basic leucine zipper-containing proteins known as CRE-binding proteins (CREB) or activating transcription factors (ATFs), including ATF4. Because ATF binding sites are present in several growth-regulating gene promoters, ATFs are believed to be involved in different regulatory circuits, allowing cells to integrate signals from distinct pathways. The mammalian ATF4 protein has been demonstrated to form heterodimers with members of the AP-1 and C/EBP family of proteins, including Fos (1), Jun (1–3), JunD (4), and several C/EBP proteins (C/EBPα, C/EBPβ, C/EBPδ, and C/EBPγ) (5–8). ATF4 acts both as a transcriptional activator (7, 9–14) and as a transcriptional repressor (4, 15–19), presumably by sequestering other regulatory factors away from promoters. ATF4 also interacts with the coactivator CREB-binding protein and components of the general transcription machinery, such as the TATA-binding protein, TFIIB and the RAP30 subunit of TFIIF (20).

Overexpression of ATF4 in murine NIH3T3 fibroblasts reduces the ability of the ectopically expressed Ras oncogene to transform cells as judged by cellular morphology and foci formation and has thus been proposed to reduce transcription ability of Ras promoter (18). ATF4-knockout mice display abnormal lens formation (21, 22) that is at least partially the result of p53-mediated apoptosis because deletion of the p53 gene in the ATF4-knockout background reduces the apoptotic phenotype allowing normal lens formation (21). However, the potential role of ATF4 in mammary gland development remains unknown.

We have shown previously that heregulin, a mesenchymal growth factor in the mammary gland, up-regulates the expression and transactivation function of ATF4 in breast cancer cells (14). The present study was undertaken to analyze the consequences of ATF4 overexpression on mammary gland development in transgenic animals. We showed that overexpression of ATF4 in the mammary epithelium of ATF4-transgenic mice decreased proliferation and impaired differentiation of alveolar epithelial cells during pregnancy and lactation. In addition, ATF4 overexpression induced apoptosis and accelerated involution of the mammary gland, suggesting a role for ATF4 in the regulation of normal mammary gland involution.

**EXPERIMENTAL PROCEDURES**

**Generation of Transgenic Mice**—A FLAG-tagged ATF4 cDNA was digested with SalI, blunt ended, and subcloned into the EcoRI site of the pBJ41 vector under the control of an ovine β-lactoglobulin promoter

---

* This work was supported by National Institutes of Health Grant CA 90970, Cancer Center Core Grant CA16672, and the Breast Cancer Research Program of the University of Texas M. D. Anderson Cancer Center (to R. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence may be addressed: Dept. of Molecular and Cellular Oncology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030.

‡‡ Generation of Transgenic Mice—A FLAG-tagged ATF4 cDNA was digested with SalI, blunt ended, and subcloned into the EcoRI site of the pBJ41 vector under the control of an ovine β-lactoglobulin promoter
ATF4 Regulation of Involution and Development

(23). Transgenic mice with a B6DF1/J genetic background were produced by injecting purified DNA fragments into the embryos of fertilized oocytes in B6DF1/J mice as described previously (23). The founder mice were genotyped using Southern blotting with 20 μg of tail DNA digested with BamHI. Mice with exogenous genes were bred with the same strain but recorded as different lines. Mice from the F5–F6 generation were used for phenotype analysis. PCR analysis was performed to amplify a 350-bp sequence between the FLAG tag and the ATF4 cDNA using the forward primer 5'-GACTACAAGGACGAGCTAGCAGAAGT-3' and the reverse primer 5'-AAAGATCTACATGGTGTCACCCAGAG-3'. To determine the pregnancy stage, the mice were mated and inspected for the presence of vaginal plugs in the morning. The day of the vaginal plug was counted as day 0 of pregnancy.

Histological and Morphological Analysis—For histological analysis, mammary gland tissue was fixed in 10% neutral buffered formaldehyde and embedded in paraffin by standard methods. Sections, each 4 μm thick, were stained with hematoxylin and eosin. For whole mount analysis, mammary glands were stained with carmine and aluminum as described previously (23). Briefly, the glands were fixed with acetic acid/ethanol (1:3) for 2 h and stained with 0.5% carmine and 0.2% aluminum potassium sulfate for 15 h. After briefly being rinsed with distilled water, the mammary glands were dehydrated using graded ethanol, and lipids were removed with two changes of acetone. Finally, the glands were preserved in methyl salicylate.

RT-PCR and Northern Blot Analysis—To investigate whether ATF4 has any role in normal mammary gland development, we first examined the level of ATF4 and ATF2 protein in wild type mammary glands using Western blot analysis. As shown in Fig. 1A, top panel, ATF4 expression levels were high in virgin and pregnant glands but were dramatically lower during lactation and higher after weaning. ATF2 was expressed in pregnant glands (Fig. 1A, middle panel). Paxillin was used as a control (Fig. 1A, bottom panel).

Generation and Analysis of ATF4-transgenic Lines—A FLAG-tagged ATF4 mouse cDNA was cloned into the pBHiJ4 vector under the control of an ovine β-lactoglobulin promoter, which has been shown to confer tissue-specific, hormonally regulated expression of heterologous genes in the mammary epithelial cells of transgenic mice during pregnancy and lactation (28) (Fig. 1B). Six founders with transgene integration were identified by PCR, and results were confirmed by Southern blot. These founders were then bred with the wild type mice to generate females to test expression of ATF4. A representative Southern blot derived from BamHI-digested genomic DNA are shown for the three founders (TG23, TG24, TG24) in Fig. 1C. The mice from founders TG23, TG24, and TG25 expressed the transgene protein product, as detected by Western blotting of mammary extracts with FLAG antibody, which specifically recognizes the FLAG epitope tag present in the transgene product and does not react with endogenous ATF4 (Fig. 1D). These lines of mice varied in their levels of ATF4 expression, thus permitting dose responsiveness of the phenotype to be assessed. One line, TG23, was selected for further analysis because it had the highest levels of protein expression.

The level of ATF4 expression in the various stages of mammary gland development was determined using semiquantitative RT-PCR. RNA expression was compared between aged-matched wild type and transgenic glands (TG23) at days 10 and 15 of pregnancy, days 2 and 12 of lactation, and days 1, 3, and 7 of involution (Fig. 1E). The ratio of transgene ATF4 to endogenous ATF4 was 2-fold at day 10 of pregnancy, 1.6-fold at day 15 of pregnancy, increased to 8.2-fold at day 2 of lactation and 5.2-fold at day 12 of lactation, and reduced at day 7 of involution (Fig. 1E).

To establish the cellular localization of ATF4, we performed immunohistochemistry on sections of mammary glands from wild type and ATF4-transgenic animals using anti-FLAG antibody to detect the expression of FLAG-tagged ATF4 transgene. Samples from day 2 of lactation showed ATF4 expression in both the nucleus and the cytoplasm of epithelial cells lining the alveoli (Fig. 1F).

ATF4 Regulates Proliferation and Differentiation of the Mammary Alveolar Epithelium—To evaluate whether ATF4
affected alveolar development, we studied mammary gland structures in pregnant wild type and transgenic mice. Analysis of both whole mounts and hematoxylin and eosin-stained sections during pregnancy revealed that ductal branching and alveolar development were already impaired by day 10 of pregnancy (Fig. 2, A and B). The wild type mammary glands had sprouted many new side branches of alveoli (Fig. 2A). In contrast, the mammary glands from ATF4-transgenic mice had developed few new lateral branches with little alveolar development (Fig. 2B). The morphological differences between wild type and transgenic animals were increased at days 15 and 18 of pregnancy and consistently showed decreased lobuloalveolar development in ATF4-transgenic mice (compare Fig. 2, A and C with B and D). The wild type mammary glands at day 18 of pregnancy contained copious amount of lipid, whereas those of ATF4 mice did not (Fig. 2, K and L).

The alveolar density in the fat pads of transgenic mice from three different founder lines was severely impaired (Fig. 3A). A significantly greater area was occupied by adipocytes in glands from ATF4-TG23 (24 ± 4%) compared with the wild type mice (4 ± 1%) during pregnancy day 15 (Fig. 3B).

In addition to the abnormalities observed at day 18 of pregnancy, decreased lobuloalveolar development was observed in ATF4 females at day 2 of lactation (compare Fig. 2, M and N).

To explore the mechanism of impairment of mammary gland development we examined rates of BrdUrd incorporation on days 10 and 15 of pregnancy, when proliferation is high. Wild type and ATF4-transgenic mice from three independent founder lines at different developmental stages were pulse labeled with BrdUrd and then sacrificed. The percentage of BrdUrd-positive epithelial cells was determined by quantitative analysis of anti-BrdUrd-stained sections (Fig. 3D). Cell proliferation was reduced in ATF4-transgenic mice throughout pregnancy (Fig. 3C). At day 10 of pregnancy, it was reduced significantly up to 85% of the rate observed in wild type mice, and it remained low until day 15 (Fig. 3D).

**ATF4 Overexpression Impairs Lactation**—At parturition, litters were adjusted to eight in number. Pups were weighed daily to monitor growth; if losses occurred, replacement pups were added to maintain litter size. Littermates from ATF4-transgenic mice exhibited a 40–50% lower body weight than did wild type mice (Fig. 5A). At day 12, the mean weight of transgenic mice was 1.7 ± 0.3 g, and that of wild type mice was 5.0 ± 0.63 g. At 3 weeks, mean weights were 4.88 ± 0.25 g for ATF4-transgenic mice and 9.76 ± 0.8 g for wild type mice.

The dramatic changes in epithelial differentiation which occur in the mammary gland during lobuloalveolar development are reflected at the molecular level by tightly regulated and temporally ordered milk protein expression (33). To determine whether ATF4-transgenic mice manifested a defect in epithelial differentiation, we determined mRNA expression levels for a panel of markers of mammary gland differentiation (lacto-
those from matched wild type mice at day 12 of lactation. The lower in mammary glands from ATF4-transgenic mice than D, the STAT5a tyrosine phosphorylation was significantly of STAT5a, we examined immunohistochemical localiza-

tion of WAP, albumin, and β-casein in mammary glands from ATF4-transgenic and wild type mice during lactation. Northern blot analysis showed significantly lower levels of α-lactalbumin, WAP, and β-casein mRNAs in the mammary glands from the transgenic than in those from the wild type mice at day 12 of lactation (Fig. 5B). In contrast, expression levels of the epithelial cell marker keratin 18 did not differ significantly between mammary glands from wild type and ATF4-transgenic mice.

Phosphorylation of STAT5a also has been shown to be tightly linked to mammary gland differentiation and milk protein expression (34, 35). To determine whether the inability to transcribe milk protein genes correlated with reduced phosphorylation of STAT5a, we examined immunohistochemical localization of phosho-STAT5a in the lactating mammary gland in transgenic and wild type mice (36). As shown in Fig. 5, C and D, the STAT5a tyrosine phosphorylation was significantly lower in mammary glands from ATF4-transgenic mice than those from matched wild type mice at day 12 of lactation. The similar results were obtained by Western blotting using phos-
pho-STAT5a antibody (Fig. 5E).

Accelerated Mammary Gland Involution in ATF4-transgenic Mice—In addition to the anomalies observed during pregnancy of ATF4-transgenic mice, decreased lobuloalveolar development was observed at lactation. Fig. 6 shows hematoxylin and eosin-stained sections of ATF4 and wild type mammary glands during lactation and involution. At days 2, 12, and 18 of lactation, the majority of the wild type gland was composed of alveoli lined by epithelial cells that secrete milk components into the alveolar lumina (Fig. 6, A, C, and E). The well organized, secretory lobuloalveolar structures characteristic of lactating animals remained intact for the 1st day after weaning (Fig. 6G). By days 2 and 12 of lactation, the mammary glands of ATF4-transgenic mice had fewer large alveolar distanced structures compared with those of wild type mice, and some of the structure was in a collapsing state and had smaller luminal spaces (Fig. 6, B and D). By day 18 of lactation and the 1st day of involution, the glands from transgenic mice had extensive tissue remodeling; some of the lobuloalveolar structure had collapsed, and the adipocytes reappeared (Fig. 6F).

At day 3 of involution, most of the lobuloalveolar structures in the mammary glands of wild type mice had also collapsed, leaving mainly ducts, vessels, and clusters of epithelial cords, some with small lumina. Adipocytes, which constitute most of the tissue in a resting gland, reappeared (Fig. 6I). In contrast, the alveolar structures in ATF4-transgenic mice were remodeled with occasional epithelial cords and ducts remaining, and surrounding stroma and adipocytes (Fig. 6J). Taken together, our results suggest that the mammary glands from ATF-trans-

genic mice at day 18 of lactation were phenotypically similar to those of wild type mice at day 3 of involution (compare Fig. 6, I and F). Thus, involution is accelerated in ATF4-transgenic mice.

Involution is characterized by apoptosis of epithelial cells that can be distinctly identified by condensed chromatin (37). Apoptotic cells are shed into the lumina and the lobuloalveolar structure, where they usually decrease in size and with their condensed chromatin becoming encapsulated into apoptotic bodies that are phagocytosed by neighboring cells. To assess whether the observed acceleration of involution in the mammary gland of ATF4-transgenic mice was caused by apoptotic cell death, we performed TUNEL assays on lactating and in-

voluting mammary epithelia from wild type and ATF4-transgenic mice. Apoptotic cells were nearly absent on days 2, 12, and 18 of the lactating mammary gland of wild type mice (Fig. 7, A, C, and E). The mean percentage of apoptotic cells in ATF4-transgenic mice by day 2 of lactation (5.74 ± 2.7) was similar to that of wild type mice at day 2 of involution (5.68 ± 1.2) (Fig. 7G). Apoptosis peaked at day 3 of involution in the mammary gland of wild type mice (6.45 ± 2) and at day 2 of involution in the mammary gland of ATF4-transgenic mice (Fig. 7G). In wild type mice the number of apoptotic cells decreased at day 7 of involution when most of the gland had been remodeled; in transgenic mice, apoptosis was decreased by day 3 of involution (Fig. 7G).

The increased apoptosis in ATF4-transgenic mice prompted us to investigate the levels of apoptosis-regulating proteins. Bax, an inducer of apoptosis (38), is up-regulated at the start of involution and is thought to act as an apoptotic signal for epithelial cells (57). Bax levels were increased in mammary glands from ATF4-transgenic mice compared with wild type mice at lactation days 12 and 18 (Fig. 8A). Compared with relative levels of keratin 18, the expression level of Bax at day 12 of lactation in ATF4-transgenic mice was 100–200-fold higher in ATF4-transgenic mice than in age-matched wild type
mice. Bcl-2 levels promote cell survival and tumor formation in transgenic mice (38). The levels of Bcl-2 were lower during lactation and involution in mammary glands from ATF4-transgenic compared with the levels present in age-matched mammary glands from wild type mice (Fig. 8B). In addition, during lactation, when milk production was most strongly impaired in ATF4-transgenic mice, caspase-3 activity was increased significantly (Fig. 9).

**Activation of STAT3 in ATF4-transgenic Mice**

Earlier studies have shown that STAT3 has a role in the normal programming of apoptosis and involution in the mammary gland and that it targets IGFBP-5 during induction of involution (39). Once phosphorylated on the specific tyrosine residue (Tyr-705), STAT3 translocates to the nucleus and interacts with consensus promoter sequences to regulate transcription from target genes (40). STAT3 is activated at the start of involution (41), which is characterized by removal of epithelial cells by apoptosis (37, 42). On the basis of this information, we next sought to determine the status of STAT3 phosphorylation during lactation in ATF4-transgenic mice. Mammary glands from ATF4-transgenic mice showed elevated levels of phosphorylated STAT3 at day 18 of lactation compared with the wild type mice (Fig. 10A). Immunostaining of mammary glands at day 18 of lactation also revealed a significant elevated level of phosphorylated STAT3 in the nucleus of epithelial cells lining the alveoli of ATF4-transgenic mice compared with the alveoli of wild type mice (Fig. 10, B and C).

**Up-regulation of IGFBP-5 during Lactation in ATF4-transgenic Mice**

During mammary involution, when serum prolactin levels decline, IGFBP-5 expression is dramatically up-regulated, and IGFBP-5 binds with high affinity to IGF-I, preventing IGF-I from interacting with the IGF-I receptor and acting as a survival factor (43). Recent study demonstrated that IGFBP-5 induces premature cell death in the mammary gland of transgenic mice (25). To establish whether IGFBP-5 levels were altered in ATF4-transgenic mice, we determined IGFBP-5 mRNA levels by Northern blotting in aged-matched mammary glands from wild type and transgenic mice during day 12 of lactation. The level of IGFBP-5 mRNA was signifi-
cantly up-regulated (3–5-fold) in ATF4-transgenic mice compared with wild type mice (Fig. 11A). Consistent with the up-regulation of IGFBP-5 mRNA in ATF4-transgenic mice, the transgenic also had higher levels of IGFBP-5 expression in epithelial cells during lactation than did wild type mice as assessed by immunostaining with an anti-IGFBP-5 monoclonal antibody (Fig. 11B).

**DISCUSSION**

The present study describes the phenotypes of transgenic mice with deregulated expression of ATF4 in the mammary gland during pregnancy and lactation. The data presented suggest that 1) expression of ATF4 in the mammary gland is regulated during development with the highest expression during virgin and pregnancy and the lowest expression during lactation; 2) ATF4 overexpression disrupts normal lobuloalveolar development during pregnancy and lactation, specifically, decreasing epithelial cell proliferation during pregnancy and impairing alveolar cell differentiation throughout pregnancy and lactation; and 3) deregulated expression of ATF4 accelerates involution accompanied by an increase in apoptosis of epithelial cells during lactation.

Results from BrdUrd incorporation experiments indicated that ATF4 overexpression in the mammary gland resulted in decreased cell proliferation at early and late phases of pregnancy. Proliferation of mammary epithelial cells during pregnancy reaches its maximum in the early phase, coinciding with high concentrations of serum hormones such as progesterone (44). The high levels of p21WAF1 and p27Kip1 expression suggested that activation of cyclin-dependent kinase inhibitors might be responsible for the attenuated cell proliferation in ATF4-transgenic mice during pregnancy. Thus the defect identified in ATF4-transgenic mice suggests that the function of ATF4 in pregnancy involves inhibiting the cell cycle progression of mammary epithelial cells. During the early stages of pregnancy the mammary tissue of ATF4 mice exhibits a decrease in the number of alveolar epithelial cells compared with that of their WT littermates. This reduction is accompanied by a significant defect in lactation such that ATF4 mice exhibit growth retardation. Furthermore, the ATF4-expressing lobuloalveoli are less differentiated than wild type glands, as evidenced by reduced expression of differentiation markers (β-casein, αs, and WAP) in ATF4-transgenic animals during lactation.

Several genes and signaling pathways that control alveolar development have been identified, including prolactin receptor, STAT5 (45), ErbB2 (46) and ErbB4 (36), cyclinD1 (47, 48), C/EBPβ (49, 50), the osteoclast differentiation factor RANKL, and its receptor RANK (51). Data from these mouse models...
suggest that alveologenesis is a complex process requiring the functional cooperation of numerous molecules. Interestingly, comparable phenotypes were observed in some of these mice, lack of alveolar development. ErbB2 resulted in condensed alveoli and reduced luminal secretion at parturition (46). ErbB4 dominant negative epithelium formed condensed alveoli and failed to expand at midlactation, which correlated with reduced expression of $\beta$-lactoalbumin and WAP and loss of STAT5 activity. The ErbB3 and ErbB4 ligand heregulin up-regulates ATF4 mRNA levels in breast cancer cells (14). Furthermore, targeted expression of a heregulin transgene causes persistence of terminal end bud and late development of mammary adenocarcinomas, suggesting that heregulin inhibits signals that normally lead to terminal differentiation (52). Because ATF4-overexpressing mammary epithelia remain poorly differentiated, it is possible that ATF4 acts downstream of the ErbB receptor signaling pathway to control proliferation and differentiation of the mammary alveolar epithelium. ATF4 also inhibited STAT5a phosphorylation at Tyr-694, suggesting that STAT5a is an important downstream mediator of ATF4.

Similar to the models described here, C/EBPβ null mice possess undifferentiated alveolar epithelium; in contrast, branching morphogenesis was also impaired. In C/EBPβ mutant mice at term expression of the WAP, WDNM1, or $\beta$-casein was virtually undetectable. In ATF4-overexpressing mice, milk protein expression were down-regulated during midlactation. It has been shown that ATF4 can form heterodimers with C/EBPβ proteins (53). It is likely that the heterodimers are unable to bind the C/EBP consensus site and consequently inhibit C/EBP-mediated transcriptional activation. Thus, down-regulation of ATF4 during lactation may be essential to induce the expression of milk protein.

ATF4-overexpressing mice exhibited increased levels of apoptosis during lactation and accelerated involution compared with the wild type mice, suggesting that down-regulation of ATF4 during lactation acts an essential survival signal for the gland. Further support for a role of ATF4-mediated apoptosis in the mammary gland is the increased expression of Bax and decreased expression of Bcl-2 during late stages of lactation in ATF4-transgenic mice. Caspase-3 activity was increased significantly during lactation in transgenic mice, providing strong evidence that expression of ATF4 was able to promote inappro-
involution, epithelial cells synthesize and secrete high levels of IGFBP-5 (43). IGFBP-5 has been proposed to induce apoptosis by sequestering IGF-I to casein micelles, thus preventing it from binding to its receptor (25). Prolactin may act by suppressing the production of IGFBP-5 from the mammary epithelium and inhibit apoptosis (56). Recently, Tonner et al. (25) provided evidence for a causal relationship between IGFBP-5 and apoptosis by producing transgenic mice expressing IGFBP-5 from a mammary-specific promoter β-lactoglobulin. Overexpression of IGFBP-5 can lead to impaired mammary development, increased expression of proapoptotic molecule caspase-3, increased plasmin generation, and decreased expression of prosurvival molecules of Bcl-2 family. In addition, IGFBP-5 expression is reduced and involution delayed in STAT3 knockout mice, whereas in interferon regulatory factor-1 knockout mice involution and IGFBP-5 expression are both accelerated (39). We showed that IGFBP-5 is up-regulated during midlactation (day 12) in ATF4-transgenic animals. Morphologically, the mammary glands of ATF4-transgenic mice showed an accelerated involution that corresponded to acceleration in the onset of apoptosis. ATF4 overexpression in transgenic mice also increased apoptosis rates during lactation.

In light of these findings, we hypothesized that high levels of IGFBP-5 in the lactating mammary glands of ATF4-transgenic mice would decrease the biological potency of IGF-I, which in turn would induce apoptosis and accelerate involution. In this study we provide the first evidence that ATF4 inhibits cellular proliferation and induces cell death when expressed in the mammary gland of transgenic mice and may play a role in the normal program of apoptosis and involution in the mammary gland. We propose that one of the targets of ATF4 in the induction of involution could be IGFBP-5. Further studies will focus on the in vivo and in vitro models to unravel further the mechanism of action and targets of ATF4 in this process.

Acknowledgments—We thank Dr. Jeff Rosen for providing reagents, several helpful productive discussions, and critical reading of this manuscript. We also thank Dr. Rui-An Wang for taking care of the ATF4-transgenic mice during the early phase of this work.

REFERENCES

1. Hai, T., and Curran, T. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3720–3724
2. Chevray, P. M., and Nathans, D. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5789–5793
3. Kato, Y., Koike, Y., Tomizawa, K., Ogawa, S., Hosaka, K., Tanaka, S., and Kato, T. (1999) Mol. Cell. Endocrinol. 154, 151–159
4. Shimizu, M., Nomura, Y., Suzuki, H., Ichikawa, E., Takeuchi, A., Suzuki, M., Nakanura, T., Nakajima, T., and Oda, K. (1998) Exp. Cell Res. 239, 93–103
5. Gombart, A. F., Kawano, S., Chumakov, A., and Koefler, H. P. (1997) Proc. Am. Assoc. Cancer Res. 38, 185–189
6. Nishizawa, M., and Nagata, S. (1997) FEBS Lett. 399, 36–38
7. Vallejo, M., Ron, D., Miller, C. P., and Habener, J. F. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 4679–4683
8. Vinson, C. R., Hai, T., and Boyd, S. M. (1993) Genes Dev. 7, 1047–1058
9. Butcher, W. G., Powers, C., Olive, M., Vinson, C., and Gardner, K. (1998) J. Biol. Chem. 273, 552–560
10. Paweletz, T. W., Martindale, J. L., Guyton, K. Z., Hai, T., and Holbrook, N. J. (1999) Biochem. J. 339, 135–141
11. Gachon, F., Peleriaux, A., Thebault, S., Dick, J., Lemasson, I., Devaux, C., and Mesnard, J. M. (1999) J. Viral. 72, 8322–8337
12. Liang, G., and Hai, T. (1997) J. Biol. Chem. 272, 24088–24095
13. Reddy, R. T., Tang, H., Li, Z., and Wong-Staal, F. (1997) Oncogene 14, 2765–2779
14. Talukder, A. H., Vadlamudi, R., Mandal, M., and Kumar, R. (2000) Cancer Res. 60, 276–281
15. Jungling, S., Cibelli, G., Czardybon, M., Gerdes, H.-H., and Thiel, G. (1994) Eur. J. Biochem. 226, 925–935
16. Karpińska, B. A., Morle, G. D., Huggenvik, J., Uhler, M. D., and Leiden, J. M. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4820–4824
17. Lacoste, I., Segrel, E., Berlin-Torrent, C., Durant, H., Graus, L., Hai, T., Benarous, R., and Margottin-Gouguet, F. (2001) Mol. Cell. Biol. 21, 2192–2202
18. Meischner, L. M., Hughes, R. G., Chevray, P. M., and Pruitt, S. C. (1996) Biochem. Biophys. Res. Commun. 228, 586–595
19. Pati, D., Meistrich, M. L., and Plon, S. E. (1999) Mol. Cell. Biol. 19, 5001–5013
20. Hai, T., and Hartman, M. G. (2001) Gene (Amst.) 273, 1–11
21. Hettmann, T., Barton, K., and Leiden, J. M. (2000) Dev. Biol. 222, 110–123
22. Tanaka, T., Tsujimura, T., Takeda, K., Sugihara, A., Maekawa, A., Terada, N., Yoshida, N., and Akira, S. (1998) Genes Cells 3, 801–810
ATF4 Regulation of Involution and Development

23. Lundgren, K., Montes de Oca Luna, R., McNeil, Y. B., Emerick, E. P., Spencer, B., Barfield, C. R., Lozano, G., Rosenberg, M. P., and Finlay, C. A. (1997) Genes Dev. 11, 714–725.

24. Chapman, R. S., Duff, E. K., Lourenco, P. C., Tonner, E., Flint, D. J., Clarke, A. R., and Watson, C. J. (2000) Oncogene 19, 6386–6391.

25. Tonner, E., Barber, M. C., Allian, G. J., Beattie, J., Webster, J., Whitelaw, C. B. A., and Flint, D. J. (2002) Development 129, 4547–4557.

26. Gabreil, Y., Sherman, Y., and Ben-Sasson, S. A. (1996) J. Cell Biol. 119, 493–501.

27. Wang, R.-A., Mazumdar, A., Vadlamudi, R. K., and Kumar, R. (2002) EMBO J. 21, 5437–5447.

28. Marte, B. M., Jeschke, M., Graus-Porta, D., Taverna, D., Hofer, P., Groner, B., Yarden, Y., and Hynes, N. E. (1995) Mol. Endocrinol. 9, 14–23.

29. Sherr, C. J., and Roberts, J. M. (1999) Genes Dev. 13, 1501–1512.

30. Hengst, L., and Reed, S. I. (1996) Science 271, 1861–1864.

31. Millard, S. S., Yan, J. S., Nguyen, H., Pagano, M., Kiyokawa, H., and Koff, A. (1997) J. Biol. Chem. 272, 7095–7098.

32. Sheaff, R. J., Groudine, M., Gordon, M., Roberts, J. M., and Clarman, B. E. (1997) Genes Dev. 11, 1464–1478.

33. Robinson, G. W., McKnight, R. A., Smith, G. H., and Hennighausen, L. (1995) Development 121, 2079–2090.

34. Liu, X., Robinson, G. W., Wagner, K. U., Garrett, L., Wynshaw-Boris, A., and Hennighausen, L. (1997) Genes Dev. 11, 179–186.

35. Teglund, S., McKay, C., Schuetz, E., Van Deursen, J. M., Stravopodis, D., Wang, D., Brown, M., Bodner, S., Grosveld, G., and Ihle, J. N. (1998) Cell 93, 841–850.

36. Jones, F. E., and Stern, D. F. (1999) Oncogene 18, 3481–3490.

37. Walker, N. I., Bennett, R. E., and Kerr, J. R. (1989) Am. J. Anat. 185, 19–32.

38. Adams, J. M., and Cory, S. (1998) Science 281, 1322–1326.

39. Chapman, R. S., Lourenco, P. C., Tonner, E., Flint, D., Selbert, S., Takeda, K., Akira, S., Clarke, A. R., and Watson, C. J. (1999) Genes Dev. 13, 2894–2916.

40. Jove, R. (2000) Oncogene 19, 2466–2467.

41. Philip, J. C., Burden, T. G., and Watson, C. J. (1996) FEBS Lett. 396, 77–80.

42. Strange, R., Li, F., Saurer, S., Burkhardt, A., and Friis, R. R. (1992) Development 115, 49–58.

43. Tonner, E., Barber, M. C., Travers, M. T., Logan, A., and Flint, D. J. (1997) Endocrinology 138, 5101–5107.

44. Talamantes, F., Marr, G., Dipinto, M. N., and Stetson, M. H. (1984) Am. J. Physiol. 247, E126–E129.

45. Miyoshi, K., Shillingford, J. M., Smith, G. H., Grimm, S. L., Wagner, K.-U., Oka, T., Rosen, J. M., Robinson, G. W., and Hennighausen, L. (2003) Cell Biol. 155, 531–542.

46. Jones, F. E., Welte, T., Fu, X.-Y., and Stern, D. V. (1999) J. Cell Biol. 147, 77–87.

47. Fantl, V., Stamp, G., Andrews, A., Rosewell, I., and Dickson, C. (1995) Genes Dev. 9, 2364–2372.

48. Sicinski, P., Donaher, J. L., Parker, S. B., Li, T., Fazeli, A., Gardner, H., Haslam, S. Z., Bronson, R. T., Eledge, S. J., and Weinberg, R. A. (1995) Cell 82, 621–630.

49. Robinson, G., Johnson, P. F., Hennighausen, L., and Sterneck, E. (1998) Genes Dev. 12, 1907–1916.

50. Seagraves, T., Krnacik, S., Raught, B., Gay, J., Burgess-Beusse, B., Darlington, G. J., and Rosen, J. M. (1998) Genes Dev. 12, 1917–1928.

51. Fata, J. E., Kong, Y. Y., Li, J., Sassaki, T., Irie-Sasaki, J., Moorehead, R. A., Elliott, R., Scully, S., Voura, E. B., Lacey, D. L., Boyle, W. J., Khokha, R., and Penninger, J. M. (2000) Cell 103, 41–50.

52. Kranz, I. M., and Leder, P. (1996) Oncogene 18, 1781–1788.

53. Produst, L. M., Krezel, A. M., and Kim, Y. (2001) J. Biol. Chem. 276, 505–513.

54. Hadsell, D. L., Greenberg, N. M., Fliiger, J. M., Baumrucker, C., and Rosen, J. M. (1996) Endocrinology 137, 321–320.

55. Neuenschwander, S., Schwart, A., Wood, T. L., Roberts, T. L., Jr., Hennighausen, L., and LeRoith, D. (1996) J. Clin. Invest. 97, 2225–2232.

56. Travers, M. T., Barber, M. C., Tonner, E., Quarrrie, L., Wilde, C. J., and Flint, D. J. (1996) Endocrinology 137, 1530–1539.

57. Heermeier, K., Benedict, M., Li, M., Furth, P., Nunez, G., and Hennighausen, L. (1996) Mech. Dev. 56, 197–207.
Activating Transcription Factor 4 Overexpression Inhibits Proliferation and Differentiation of Mammary Epithelium Resulting in Impaired Lactation and Accelerated Involution

Rozita Bagheri-Yarmand, Ratna K. Vadlamudi and Rakesh Kumar

J. Biol. Chem. 2003, 278:17421-17429.
doi: 10.1074/jbc.M300761200 originally published online February 28, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M300761200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 57 references, 30 of which can be accessed free at
http://www.jbc.org/content/278/19/17421.full.html#ref-list-1
Activating transcription factor 4 overexpression inhibits proliferation and differentiation of mammary epithelium resulting in impaired lactation and accelerated involution.

Rozita Bagheri-Yarmand, Ratna K. Vadlamudi, and Rakesh Kumar

The original versions of Figs. 1 (D and E), 4A, and 10A in the vinculin, endo-ATF4, K18, and phospho-Stat3 lanes contain errors. Lanes in the vinculin panel of Fig. 1D were duplicated. Images from lanes in the endo-ATF4 panel of Fig. 1E were combined from separate parts of the original image without indicating that the images had been spliced. Lane 2 of the K18 panel of Fig. 4A contained a band that was copied from a separate image. Lanes in the phospho-Stat3 panel of Fig. 10A were duplicated. We have now replaced these panels with results from new experiments using material from the original experiment. These revisions do not change the results or interpretations of this article.