GATA-6 Activates Transcription of Surfactant Protein A*

(Received for publication, August 2, 1999, and in revised form, October 7, 1999)

Michael D. Bruno‡, Thomas R. Korfhagen‡, Cong Liu‡, Edward E. Morrissey§, and Jeffrey A. Whitsett¶†

From the ‡Division of Pulmonary Biology, Children’s Hospital Medical Center, Cincinnati, Ohio 45229-3039 and the §Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Surfactant protein A (SP-A) is a member of the collectin family of innate host defense molecules expressed primarily in respiratory epithelial cells of the lung. SP-A concentrations are influenced by both cell-specific and ubiquitous nuclear proteins that regulate SP-A gene transcription in a cell-selective and temporally regulated manner. In this work, a consensus GATA-binding site (GBS) was identified at positions −69 to −64 of the mouse SP-A gene. The transcriptional activity of wild-type SP-A reporter constructs in HeLa cells was increased 5- to 10-fold when cotransfected with a GATA-6 expression plasmid. Deletion of the GBS completely blocked transactivation by GATA-6. Transfection of a construct expressing GATA-6-engrailed fusion protein inhibited basal expression of the SP-A/chloramphenicol acetyltransferase construct in MLE-15 cells. Nuclear extract proteins from MLE-15 cells bound to the GBS in the mouse SP-A gene, and a supershifted band was detected with a GATA-6-specific antibody. Transactivation of the wild-type SP-A constructs by GATA-6 increased transcriptional activity 7- to 10-fold, whereas thyroid transcription factor-1 (TTF-1) increased the activity of these constructs 12- to 18-fold. The effects of cotransactivating with both GATA-6 and TTF-1 expression constructs were additive. However, mutation of the TTF-1-binding sites alone or in combination decreased GATA-6 transactivation. Likewise, mutation of the GBS blocked TTF-1 activation of the SP-A promoter. In situ hybridization demonstrated GATA-6 mRNA in the peripheral epithelial cells of fetal mouse lung, consistent with the sites of SP-A expression. GATA-6 is expressed in respiratory epithelial cells and binds to a cis-acting element in the SP-A gene promoter, activating the transcriptional activity of the gene.

Surfactant protein A (SP-A) is a 28- to 36-kDa member of the collectin family of innate defense molecules (for review, see Refs. 1–3). In humans, SP-A is expressed at high levels by respiratory epithelial cells of the lung, being secreted by subtypes of tracheobronchial, bronchiolar, and alveolar cells in vivo. SP-A binds to phospholipids, contributing to tubular myelin formation, and influences surfactant phospholipid secretion and re-uptake in vitro (1–3). Targeted gene inactivation of mouse SP-A suggests that its primary role is in host defense. SP-A binds to the surface of various respiratory pathogens or directly activates macrophages, enhancing opsonization and clearance of bacteria and viruses from the lung (1–7). Although SP-A does not play a critical role in surfactant function or homeostasis in vivo, SP-A is required for the formation of tubular myelin in the alveolus (8–10).

SP-A gene expression is regulated in a complex manner at the level of gene transcription and mRNA stability (for review, see Ref. 11). Like surfactant proteins B and C, SP-A transcription is dependent upon the action of thyroid transcription factor-1 (TTF-1), a homeodomain protein of the Nkx family of transcription factors (12–14). TTF-1 regulates the mouse and baboon SP-A genes, enhancing reporter gene transcription in vitro (15, 16).

Recently, in situ hybridization studies demonstrated that GATA-5 and GATA-6 are expressed in developing mouse lung (17). The GATA family of transcription factors includes at least six polypeptides whose temporal and spatial expression is highly distinct. GATA-6 mRNA was detected in respiratory epithelial cells of developing lung tubules, consistent with a potential role in lung cell differentiation or gene regulation. Embryonic stem cells with a targeted disruption of the Gata-6 gene failed to contribute to bronchial epithelial cells in the lung, supporting the concept that GATA-6 may play a role in the differentiation of subsets of lung cells (18). The recent finding that GATA-6 activates TTF-1 reporter gene transcription provides further support for the concept that GATA-6 plays a role in the regulation of respiratory epithelial cell differentiation or function, either regulating TTF-1 expression or directly influencing transcription of surfactant proteins (19). This study was designed to test whether GATA family members influence mouse SP-A gene transcription in vitro. Cell transfection and electrophoretic mobility shift assay of native and mutant mouse SP-A gene sequences demonstrated that GATA-6 activates SP-A gene transcription in vitro.

MATERIALS AND METHODS

Plasmid Construction and Site-directed Mutagenesis—The SP-A constructs used in this study are presented in Fig. 1. The mouse SP-A sequences used to generate the various constructs were isolated and cloned as described previously (20). The murine SP-A gene promoter construct pCPA0.9 (−907 to +45) was made by isolating a 952-base pair Spsl-PstI fragment from pCPA1.4 and using linker ligation to create a 5′-HindIII site. The product was cloned into pCPA-0 to generate pCPA0.9. The SP-A promoter construct pCPA0.45 was generated by PCR using linker primers containing 5′-HindIII and 3′-PstI sites to create fragment −399 to +45. The promoter constructs pCPA0.1 (−70 to +45) and pCPA0.1m (−60 to +45) were generated by PCR using linker primers containing 5′-HindIII and 3′-PstI sites. The TTF-1 site-directed mutants were generated by PCR using pCPA0.3 (−256 to +45)
as template, as described previously (15). Oligomers were made to each of the three of the TTF-1-binding sites, replacing each with a restriction enzyme sequence. The TTF-1 site located at positions 2159 to 2154 was changed to a SalI site; the site located at positions 2135 to 2130 was changed to a NcoI site; and the TTF-1 site at positions 2125 to 2120 was changed to a BamHI restriction site. These oligomers were then used in PCRs with pCPA0.3 as template. These products were digested as appropriate and cloned into pCPA-0. The mutation of the GATA-binding site was generated by PCR using pCPA0.45 as template and replacing the 6-base pair GATA-binding site (GBS), TGATAG, with an EcoRI site, GAATTC. Additional constructs from positions 2907 and 399 to 145 were generated from the 5'9'-end by digesting the linker-ligated HindIII site and from the 3'9'-end using a BstEII site within the flanking region 5' to the TTF-1-binding elements (TBEs).

Cell Culture, Transfections, and Reporter Gene Assays—HeLa cells were cultured in Dulbecco’s modified Eagle’s medium and 10% fetal calf serum. MLE-15 cells were cultured in HITES medium (RPMI 1640 containing 10 mM HEPES, 5 μg/ml insulin, 10 μg/ml transferrin, 3 x 10⁻⁸ μM sodium selenite, 1 x 10⁻⁸ μM β-estradiol, and 200 mM l-glutamine) and transferred to Dulbecco’s modified Eagle’s medium and 10% fetal calf serum as described previously (15). MLE-15 cells express TTF-1, GATA-6, SP-A, and surfactant protein B and C mRNAs. Cells were transfected using calcium precipitates prepared with 7.5 pmol of test plasmid and 4 pmol of pCMV/β-galactosidase. For GATA-6 transactivation experiments in HeLa cells, each 1-cm dish was treated with a precipitate prepared using 7.5 pmol of promoter/CAT fusion plasmid, 4 pmol of pCMV/β-galactosidase, and transferred to Dulbecco’s modified Eagle’s medium and 10% fetal calf serum as described previously (15). MLE-15 cells express TTF-1, GATA-6, SP-A, and surfactant protein B and C mRNAs. Cells were transfected using calcium precipitates prepared with 7.5 pmol of test plasmid and 4 pmol of pCMV/β-galactosidase. For GATA-6 transactivation experiments in HeLa cells, each 1-cm dish was treated with a precipitate prepared using 7.5 pmol of promoter/CAT fusion plasmid, 4 pmol of pCMV/β-galactosidase, and 1 pmol of either the empty expression vector (pCMV1.1) or an expression vector containing the entire GATA-6 open reading frame (pCMV/GATA-6), a kind gift from Dr. J. Molkentin, Children’s Hospital Medical Center, Cincinnati, OH). Cells were maintained at 37 °C for 48–72 h, and the lysates were assayed for β-galactosidase and CAT activities as described previously (15). MLE-15 cells express TTF-1, GATA-6, SP-A, and surfactant protein B and C mRNAs. Cells were transfected using calcium precipitates prepared with 7.5 pmol of test plasmid and 4 pmol of pCMV/β-galactosidase. For GATA-6 transactivation experiments in HeLa cells, each 10-cm dish was treated with a precipitate prepared using 7.5 pmol of promoter/CAT fusion plasmid, 4 pmol of pCMV/β-galactosidase, and 1 pmol of either the empty expression vector (pCMV1.1) or an expression vector containing the entire GATA-6 open reading frame (pCMV/GATA-6), a kind gift from Dr. J. Molkentin, Children’s Hospital Medical Center, Cincinnati, OH). Cells were maintained at 37 °C for 48–72 h, and the lysates were assayed for β-galactosidase and CAT activities as described previously (15).

Preparation of Nuclear Extracts—MLE-15 and HeLa nuclear extracts were prepared using a modified mini-extract procedure. Nuclear extraction procedures were performed with ice-cold reagents. Confluent monolayers from six 10-cm-diameter dishes were washed twice with 10
Fig. 3. Conservation of a GATA-binding site in the rat and mouse SP-A genes. Region −70 to −1 of the mouse SP-A gene was 90% identical to region −70 to −1 of the rat SP-A gene (21). A consensus GATA-binding site is depicted in boldface, and the TATAAA sequence is boxed. Nucleotide differences are underlined.

**RESULTS**

Cotransfection of HeLa cells with the pcMV/GATA-6 expression vector and SP-A/CAT expression constructs containing sequences from positions −907 to +45 of the mouse SP-A gene (20) resulted in activation of reporter gene expression in all constructs (Fig. 2). Comparison of the mouse (20) and rat (21) SP-A sequences demonstrated a conserved GBS at positions −69 to −64 of the mouse gene (Fig. 3). Deletion of region −69 to −64 abrogated GATA-6-dependent activity, suggesting that the stimulatory effects of GATA-6 were dependent on an intact GBS located at positions −69 to −64 (Fig. 2, see pCPA0.1m).

Although both GATA-6 and GATA-5 expression vectors stimulated the activity of the pCPA0.9 construct, GATA-6 was consistently more active than GATA-5 (data not shown). HeLa cells do not express GATA-6 mRNA, likely accounting for the more marked activation of the SP-A promoter construct (5–10-fold) by GATA-6 in HeLa cells. The pCPA0.9 and pCPA0.45 constructs were also cotransfected with pcMV/GATA-6 in MLE-15 pulmonary adenocarcinoma cells. Whereas the basal activity of the SP-A/CAT constructs was considerably higher in MLE-15 cells than in HeLa cells, SP-A promoter activity was stimulated only 2–3-fold by cotransfection with the GATA-6 expression vector (data not shown), a finding likely related to the presence of endogenous GATA-6 activity in this cell line (19).

**GATA-6-engrailed Repressor**—To test for endogenous GATA activity in MLE-15 cells, the 0.45-kilobase pair SP-A/CAT construct was cotransfected into MLE-15 cells with pcMV/GATA-6-engrailed. The latter contains the GATA-6 DNA-binding domain, but the activation domain has been replaced by the repressor domain of engrailed, thereby inhibiting GATA-6-dependent transcription. pcMV/GATA-6-engrailed inhibited SP-A/CAT activity in the MLE-15 cells in a dose-dependent manner, supporting the concept that endogenous GATA activity, at least in part, contributed to the higher activity of SP-A/CAT reporter constructs in the MLE-15 cell line (Fig. 4).

**EMSA of the GBS**—Inspection of the 5′-flanking region of the mouse and rat SP-A genes revealed the presence of an extensive region of homology that included the sequence located at positions −69 to −64 (TGATAG) (Fig. 3). This sequence is homologous to a consensus GATA-binding site. To more precisely define the function of this element and to determine whether GATA-6 bound to the region, EMSA was performed with extracts prepared from adult mouse type II epithelial cells and MLE-15 cells. Migration of the 32P-labeled GATA-6 oligonucleotide was retarded by either mouse type II...
cell or MLE-15 cell nuclear extracts. Co-incubation of nuclear extracts with antibody to GATA-6 caused a clear supershift, consistent with the binding of GATA-6 to the EMSA probe containing the GBS (264 to 269) (Fig. 5).

Site-directed Mutagenesis of the GBS—Mutation of the GBS (264 to 269) by substitution of the sequence GAATTC for TGATAG in the pCPA0.45 construct resulted in complete inhibition of the GATA-6-dependent activation of the SP-A reporter construct (Fig. 6, compare pCPA0.45-G with pCPA0.45). Thus, the element that mediates the stimulatory effects of GATA-6 is located at positions 264 to 269 in the 5'9-region of the SP-A gene.

Effects of GATA-6 and TTF-1 on SP-A Gene Transcription—Since Nkx2 and GATA family members are known to participate in the regulation of a number of genes in target tissues, we tested whether TTF-1 (Nkx2.1) influences GATA-6 activation of the SP-A/CAT reporter constructs in vitro. Cotransfection of TTF-1 and GATA-6 expression plasmids in HeLa cells markedly increased the activity of the cotransfected pCPA0.45/CAT constructs (25-fold) (Fig. 7). The effect of cotransfection with TTF-1 and GATA-6 expression plasmids was approximately additive (Fig. 7). To further clarify the nature of the effects of GATA-6 and TTF-1, SP-A/CAT constructs containing mutations in TTF-1-binding sites were cotransfected with pCMV/GATA-6 in HeLa cells. Mutation of three TTF-1 sites, singly or in combination, markedly reduced GATA-6- and TTF-1-dependent transactivation of the pCPA0.45 constructs, supporting the concept that optimal GATA-6-dependent activity required the binding of TTF-1 to TTF-1-binding elements in this region of the promoter (Fig. 8A). Likewise, mutation of the GBS blocked TTF-1 transactivation of the pCPA0.45 constructs (Fig. 8B). Thus, TTF-1 and GATA-6 transactivation depends on both intact GATA- and TTF-1-binding sites.

GATA-6 Transactivation Requires a Single GBS at Positions 269 to 264—Since other potential GATA-binding sites were...
noted upstream of positions -69 to -64, the larger SP-A construct, containing additional consensus GATA-binding sites, was tested. Cotransfection of pCMV/GATA-6 with pCPA0.9 alone or with a GBS mutation at gene positions -69 to -64 demonstrated that GATA-6 activity was mediated by the proximal GBS (Fig. 9).

Localization of GATA-6 mRNA in Fetal Mouse Lung—GATA-6 mRNA was localized in developing mouse lung by in utero hybridization (Fig. 10). On day 17.5, GATA-6 mRNA was detected in peripheral airway epithelial cells overlapping with the sites of expression of SP-A and TTF-1 mRNAs, providing further support for the potential role of GATA-6 in the regulation of expression of SP-A in prenatal lung development. Unlike TTF-1 or SP-A, GATA-6 mRNA was not confined to respiratory epithelial cells in the lung, being detected in epithelial, mesenchymal, and vascular tissues at this time in development.

DISCUSSION

GATA-6 mRNA was detected in respiratory epithelial cells in fetal lung at sites coincident with those of SP-A mRNA and protein. GATA-6 bound to an element (GBS) located at positions -64 to -69 in the 5'-flanking region of the SP-A gene, enhancing the activity of the SP-A gene reporter constructs. Mutation of the GBS or transfection of MLE-15 cells with a construct expressing a GATA-6-engrailed fusion protein inhibited the activity of the SP-A promoter constructs. Mutagenesis of the GBS inhibited TTF-1-dependent activation of the SP-A promoter, and the activity of the GBS was influenced by mutations in any of three distinct TTF-1 regulatory elements located within the 5'-flanking region of the SP-A gene. Taken together, these findings support a role for GATA-6 in the regulation of the transcriptional activity of the mouse SP-A gene.

GATA-6 was consistently more active than GATA-5 in the activation of the SP-A promoter construct. Although both GATA-5 and GATA-6 are detected in developing lung (18), only GATA-6 is expressed in respiratory epithelial cells, prior to and coincident with SP-A mRNA and protein. Previous studies demonstrated that GATA-6 and surfactant protein mRNAs are expressed in freshly isolated adult type II epithelial cells (19). In contrast, GATA-5 is expressed primarily in the mesenchyme of developing lung, as assessed by in situ hybridization, at sites that do not overlap with SP-A (17). In the present study, GATA-6 was also detected by EMSA in MLE-15 cells and mouse type II cells. Type II epithelial cells isolated from adult mice and immortalized MLE-15 cells express TTF-1, GATA-6, and various surfactant protein mRNAs (19). Early in lung morphogenesis, the distribution of GATA-6 and TTF-1 mRNAs is similar, with both being detected in progenitor cells of the respiratory epithelium. TTF-1 plays a critical role in lung morphogenesis and in the regulation of surfactant proteins in respiratory epithelial cells. Therefore, GATA-6 may influence SP-A expression indirectly, by enhancing TTF-1 gene transcription, or directly, by binding to and activating the promoters of the surfactant protein genes (19). Recent studies from this laboratory demonstrated that GATA-6 also directly influences both surfactant protein B and C transcription.2

GATA-6 is a member of the zinc finger family of transcription factors. The family members GATA-1 and GATA-2 were initially identified as important factors in hematopoietic precursors.2

M. D. Bruno, T. R. Korfhagen, C. Liu, E. E. Morrisey, and J. A. Whitsett, unpublished observations.
sor cells, where they play a role in differentiation and gene expression during erythropoiesis. More recently, other GATA family members were identified in non-hematopoietic tissues during embryonic development. GATA-4, -5, and -6 mRNAs were detected in various tissues in the embryo, including heart, gastrointestinal tract, and lung (22, 23). In the present work, both GATA-6 and TTF-1 mRNAs were detected at overlapping sites in respiratory epithelial cells of developing lung (24), although GATA-6 mRNA was detected in both epithelial and mesenchymal compartments in the lung. GATA-6 bound to the SP-A promoter in a region of the SP-A gene containing a number of TTF-1-binding sites that were previously shown to be

FIG. 8. GATA-6 and TTF-1 do not transactivate SP-A promoter constructs with site-specific mutations of TTF-1- or GATA-binding sites. A, constructs were cotransfected into HeLa cells with pCMV/GATA-6. The relative CAT activity of the respective constructs is presented as fold induction compared with constructs transfected without pCMV/GATA-6. pCPA0.45 is the wild-type construct, and pCPA0.45T designates constructs mutagenized in the respective TBEs (see Fig. 1). B, constructs were cotransfected into HeLa cells with the expression plasmid pCMV/TTF-1. The relative CAT activity of the respective constructs is presented as fold activation after transfection with pCMV/TTF-1 compared with constructs transfected without pCMV/TTF-1. Data are representative of at least three separate experiments.

FIG. 9. The proximal GBS is required for transactivation by GATA-6. Constructs were cotransfected into HeLa cells with pCMV/GATA-6. The relative CAT activity of the respective constructs is presented as fold induction compared with constructs transfected without pCMV/GATA-6. pCPA0.9 contains sequences from positions −907 to +45 of the mouse SP-A promoter. pCPA0.9-G contains the mutation of the GBS at positions −69 to −64. pCPA0.9-TG contains mutations of the GBS at positions −69 to −64 and mutations in TBE-1, -3, and -4 (see Fig. 1). Data are representative of two separate experiments, each performed in triplicate.

FIG. 10. GATA-6 mRNA in embryonic day 17.5 mouse lung. In situ hybridization analyses were performed with radiolabeled antisense mouse GATA-6 (A) or sense riboprobe (B) and exposed to film for 6 weeks. Specific hybridization was observed in peripheral lung buds, acinar epithelium, and blood vessels. No hybridization was observed in the larger conducting airways. Magnification is approximately ×200.
involved in SP-A gene transcription (15). Mutation of any of the TTF-1-binding sites in the proximal region of the promoter inhibited GATA-6 stimulation of the SP-A/CAT constructs, suggesting that the effects of GATA-6 were dependent upon TTF-1 and its interaction with TTF-1-binding elements in the SP-A gene.

It is unclear whether TTF-1 and GATA-6 factors function independently or are involved in a higher order DNA-protein complex that may be involved in SP-A gene regulation. In analogous models, GATA-4 and Nkx2.5 appear to directly interact to regulate expression of the atrial natriuretic peptide analogues. In independently or are involved in a higher order DNA-protein complex that may be involved in SP-A gene regulation. In the present study, mutation of either the GBS or TTF-1-binding sites in the mouse SP-A gene reduced transactivation by the other factor. It remains unclear whether direct or indirect interactions between TTF-1 and GATA-6 mediate their cooperative effects on SP-A promoter activity. We were unable to demonstrate direct binding or coprecipitation of TTF-1 and GATA-6 in MLE-15 cell extracts using antibodies to GATA-6 or TTF-1 (data not shown). It is possible that the respective antibodies bind epitopes required for protein-protein interactions.

In vivo, GATA-6 and SP-A mRNAs are co-localized in developing lung, and both are expressed in isolated adult type II epithelial cells (19). The early expression of GATA-6 in the development of respiratory tubes supports its potential role in lung morphogenesis and gene regulation. Similarly, GATA-6 mRNA are expressed at the highest levels in developing progenitor cells of the intestinal epithelium and developing cardiomyocytes (27, 28), suggesting a critical role of GATA-6 in developing tissues. Recent findings in chimeric mice generated from GATA-6−/− and GATA-6+/+ embryonic stem cells demonstrated that GATA-6−/− cells do not contribute to the formation of the bronchiolar epithelium in chimeric mice (18). In contrast, GATA-6+/− embryonic stem cells are fully competent to contribute to other fetal tissues. This finding suggests a unique role for GATA-6 in the morphogenesis of bronchiolar tissues. Further clarification of the role of GATA-6 in lung morphogenesis and gene regulation will require site-specific alterations in GATA-6 since mice lacking GATA-6 fail to survive to stages in which lung development is initiated (18).

GATA-6 Enhances SP-A Transcription

SP-A is subjected to precise temporal, spatial, and tissue-specific regulation mediated, at least in part, at the transcriptional level. Although TTF-1 plays a critical role in lung cell-selective expression of SP-A, mutations of the TBEs lead to enhanced SP-A reporter gene transcription in vitro. This enhanced gene transcription is also influenced by combinatorial interactions of other transcription factors. For example, B-Myb binds to the murine SP-A promoter and influences SP-A gene transcription, with the highest transactivation occurring with phosphorylated B-Myb (29). In contrast to findings with the rat and mouse SP-A genes, expression of rabbit, baboon, and human SP-A genes is enhanced by cAMP. Both basal and cAMP binding of the rabbit and primate SP-A genes require binding of TTF-1, upstream factors 1a and b, unknown members of the steroid receptor family, and Kruppel family members (16, 30–32). cAMP stimulates phosphorylation of TTF-1, enhancing transactivation of a baboon SP-A gene (30). It is therefore increasingly apparent that SP-A gene transcription is controlled by complex interactions among cis-acting elements and multiple transcription factors, including GATA-6.

This study demonstrates a role for GATA-6 in the regulation of the transcriptional activity of the mouse SP-A gene. Coexpression of GATA-6 and SP-A mRNAs during development and in adult type II epithelial cells supports a potential role for GATA-6 in the regulation of SP-A production, therefore influencing host defense and inflammatory responses in postnatal lung.

Acknowledgments—We appreciate the assistance of Dr. Susan Wert in situ hybridization, Iris Fink in tissue culture, and Ann Maher in manuscript preparation.

REFERENCES

1. Mason, R. J., Greene, K., and Voelker, D. R. (1998) Am. J. Physiol. 275, L1–L13
2. Crouch, E. C. (1998) Am. J. Respir. Cell Mol. Biol. 19, 177–201
3. McCormack, F. X. (1998) Biochim. Biophys. Acta 1408, 109–131
4. Wright, J. R. (1997) Physiol. Rev. 77, 931–962
5. LeVine, A. M., Bruno, M. D., Huesman, K. M., Ross, G. F., Whitsett, J. A., and Jobe, A. H. (1998) J. Clin. Invest. 102, 1015–1021
6. LeVine, A. M., Kurak, K. E., Bruno, M. D., Stark, J. M., Whitsett, J. A., and Korthagen, T. R. (1998) Am. J. Respir. Cell Mol. Biol. 19, 700–708
7. LeVine, A. M., Owezdz, J., Stark, J., Bruno, M., Whitsett, J., and Korthagen, T. R. (1999) J. Clin. Invest. 103, 1015–1021
8. Korfhagen, T. R., Bruno, M. D., Ross, G. F., Huesman, K. M., Ikegami, M., Jabe, A. H., Wert, S. E., Stripp, B. R., Morris, E. R., Glasser, S. W., Bachurski, C. J., Iwamoto, H. S., and Whitsett, J. A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5954–5959
9. Ikegami, M., Korfhagen, T. R., Whitsett, J. A., Bruno, M. D., Wert, S. E., Wada, K., and Jabe, A. H. (1998) Am. J. Physiol. 275, L247–L254
10. Ikegami, M., Korfhagen, T. R., Bruno, M. D., Whitsett, J. A., and Jabe, A. H. (1997) Am. J. Physiol. 272, L479–L485
11. Mendelson, C. R., Gao, E., Li, J., Young, P. P., Michael, L. F., and Alcorn, J. L. (1998) Biochim. Biophys. Acta 1408, 132–149
12. Bohinski, R. J., DiLauro, R., and Whitsett, J. A. (1994) Mol. Cell. Biol. 14, 5671–5681
13. Yan, C., Sevor, Z., and Whitsett, J. A. (1995) J. Biol. Chem. 270, 24852–24857
14. Kelly, S. E., Bachurski, C. J., Burbano, M., and Glasser, S. W. (1996) J. Biol. Chem. 271, 6881–6888
15. Bruno, M. D., Bohinski, R. J., Huesman, K. M., Whitsett, J. A., and Korfhagen, T. R. (1995) J. Biol. Chem. 270, 6651–6656
16. Li, J., Gao, E., and Mendelson, C. R. (1998) J. Biol. Chem. 273, 4592–4600
17. Morrissey, E. E., Ip, H. S., Zhang, Z. L., Mu, M., and Parmacek, M. S. (1997) Dev. Biol. 183, 21–36
18. Morrissey, E. E., Tang, Z., Sigrist, K., Lu, M. M., Jiang, F., Ip, H. S., and Parmacek, M. S. (1998) Genes Dev. 12, 3579–3590
19. Shaw-White, J. R., Bruno, M. D., and Whitsett, J. A. (1999) J. Biol. Chem. 274, 2668–2674
20. Korfhagen, T. R., Bruno, M. D., Glasser, S. W., Ciracolo, P. J., Whitsett, J. A., Lattier, D. L., Wikenheiser, K. A., and Clark, J. C. (1992) Am. J. Physiol. 263, L1456–L1541
21. Lacaze-Masmonteil, T., Fraslon, C., Bourbon, J., Raymondjean, M., and Kahn, A. (1992) Eur. J. Biochem. 206, 613–623
22. Laverriere, A. C., MacNeill, C., Mueller, C., Poeleman, R. E., Burch, J. B., and Evans, T. (1994) J. Biol. Chem. 269, 23177–23184
23. Morrissey, E. E., Ip, H. S., Lu, M. M., and Parmacek, M. S. (1996) Dev. Biol. 177, 309–322
24. Zeng, L., Lim, L., Costa, R. H., and Whitsett, J. A. (1996) J. Histochem. Cytochem. 44, 1183–1193
25. Durocher, D., Charron, F., Warren, R., Schwartz, J. R., and Nemek, N. (1997) EMBO J. 16, 5687–5696
26. Shiojima, I., Komuro, I., Oka, T., Hiroi, Y., Mizuno, T., Takimoto, E., Monzen, K., Akikawa, R., Akazawa, Y., Yamazaki, T., Kudoh, S., and Yazaki, Y. (1999) J. Biol. Chem. 274, 8231–8239
27. Segalovida, J. L., Belaguli, N., Nizami, Y., Chen, C., Nemer, M., and Schwartz, R. J. (1998) Mol. Cell. Biol. 18, 3405–3415
28. Gao, X., Sedgwick, T., Shi, Y., and Evans, T. (1998) Mol. Cell. Biol. 18, 2901–2911
29. Bruno, M. D., Whitsett, J. A., Ross, G. F., and Korfhagen, T. R. (1999) J. Biol. Chem. 274, 27523–27528
30. Gao, E., Wang, Y., Alcorn, J. L., and Mendelson, C. R. (1997) J. Biol. Chem. 272, 23388–23406
31. Young, P. P., and Mendelson, C. R. (1996) Am. J. Physiol. 271, L287–L299
32. Michael, L. F., Alcorn, J. L., Gao, E., and Mendelson, C. R. (1996) Mol. Endocrinol. 10, 159–170