

Developmental and Hormonal Regulation of Surfactant Protein C (SP-C) Gene Expression in Fetal Lung

ROLE OF TRANSCRIPTION AND mRNA STABILITY*

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Pulmonary surfactant protein C (SP-C) gene expression is developmentally and hormonally regulated in fetal lung. In the present study, we investigated the role of transcriptional and posttranscriptional mechanisms in the developmental, cAMP, and dexamethasone induction of SP-C mRNA. We found that developmental induction of SP-C mRNA was not coincident with induction of SP-C gene transcription. SP-C mRNA levels reached ~90% of levels in adult lung on day 24 of gestation, whereas SP-C gene transcription was only ~4% of level in adult lung and did not increase until day 28 of gestation (term in rabbit = 31 days). Treatment of fetal lung tissues *in vitro* with dibutyl cyclic AMP (Bt₂cAMP) and dexamethasone increased SP-C mRNA accumulation by different mechanisms. Increase in SP-C mRNA accumulation by Bt₂cAMP was the result of increased SP-C gene transcription, whereas increased SP-C mRNA accumulation by dexamethasone was due to stabilization of RNA. In control tissues the SP-C mRNA half-life ($t_{1/2}$) was 11.2 h, and after dexamethasone treatment it increased to 30 h. These data show that both transcriptional and mRNA stabilization mechanisms regulate induction of SP-C gene expression during fetal lung development and by cAMP and dexamethasone in fetal lung *in vitro*.

Pulmonary surfactant prevents collapse of alveoli during expiration by reducing net contractile forces at the alveolar surface (1). Surfactant is synthesized and secreted by alveolar type II epithelial cells of lung and is composed of lipids (90%) and proteins (5–10%) (2). Deficient production of surfactant, as found in premature infants, is associated with the development of respiratory distress syndrome of the newborn (3), the leading cause of neonatal morbidity and mortality in developed countries.

Four lung-specific surfactant-associated proteins have been isolated to date: surfactant protein A (SP)¹-A, SP-B, SP-C, and SP-D (4, 5). SP-B and SP-C are extremely hydrophobic proteins that appear to be essential for maintenance of biophysical properties and physiological activity of surfactant (6). Although SP-C in concert with SP-B has been implicated in maintaining surface active properties of surfactant and SP-C alone enhances the adsorption rate of phospholipids *in vitro*, its precise

role in surfactant function remains to be defined. During fetal lung development SP-C mRNA can be detected prior to the appearance of morphologically identifiable alveolar type II epithelial cells (7), and in adult lung its expression is confined to type II epithelial cells (7–9). These data suggest that SP-C, in addition to enhancing surface activity of surfactant, also serves some undefined function in development and differentiation of the lung.

We previously isolated and characterized SP-C cDNAs in the rabbit and found that SP-C mRNA accumulation is induced during fetal lung development (10). We also found that cyclic AMP analogs and dexamethasone added *in vitro* to 21-day gestational age fetal lung tissues increased accumulation of SP-C mRNA in a time-dependent manner. Molecular mechanisms that mediate developmental, cAMP, and glucocorticoid regulation of SP-C mRNA accumulation have not been defined. In the present study we used transcription run-on assays and Northern blot analysis to investigate the role of transcriptional and posttranscriptional mechanisms in the developmental, cAMP, and glucocorticoid induction of SP-C mRNA accumulation. We found that increases in SP-C mRNA accumulation during development were not coincident with increases in SP-C gene transcription; SP-C mRNA levels reached 90% of levels found in adult lung by day 24 of gestation but an increase in SP-C gene transcription was not found until day 28 of gestation suggesting significant stabilization of SP-C mRNA. In fetal lung *in vitro*, the effect of dexamethasone to increase SP-C mRNA accumulation was found to be due solely to an increase in the half-life of SP-C mRNA, whereas the effect of cAMP was found to be due to an increase in SP-C gene transcription. These findings have been reported in preliminary form (11).

MATERIALS AND METHODS

Organ Culture of Fetal Lung Tissues—Lung tissues of 21-day gestational age fetal rabbits were maintained in organ culture in serum-free Waymouth's medium MB 752/1 (Life Technologies, Inc.) according to Snyder *et al.* (12). Lung explants were maintained in culture for up to 3 days in either control medium or medium containing Bt₂cAMP (1 mM) or dexamethasone (10⁻⁷ M) or a combination of the two agents. Dexamethasone was dissolved in ethanol before addition to the culture medium. The concentration of ethanol in culture medium was 0.01%; at this concentration ethanol had no effect on SP-C mRNA levels. Actinomycin D (actinomycin D-mannitol, water-soluble, Sigma) (5 µg/ml) was added in experiments designed to measure the rate of SP-C mRNA degradation. Culture medium was changed every 24 h.

Isolation of RNA and Northern Blot Analysis—Total RNA from fetal lung tissues was extracted with TRI reagent (Molecular Research Center, Inc., Cincinnati, OH) according to the manufacturer's instructions. RNA (5–10 µg) was electrophoresed in agarose gels (1.2%) containing 20 mM MOPS and 1.1% formaldehyde (13). RNAs were transferred to Hybond N⁺ (Amersham Corp.) membrane by capillary action using 50 mM sodium hydroxide. Membranes were hybridized to ³²P-labeled rabbit SP-C cDNA (~0.9 kb) and human β actin cDNA (~1.7 kb) at 60 °C, and final washes were done in 1 × SSC at 60 °C. Membranes were also hybridized to a ³²P-labeled antisense oligonucleotide (30-mer) of ribosomal 18 S RNA to assess the integrity of RNA preparations, loading of equivalent amounts of RNA, and quantitative transfer of RNA. Autoradiographic signals were quantified by scanning densitometry (Milli-

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¹ The abbreviations used are: SP, surfactant protein; Bt₂cAMP, dibutyl cyclic AMP; MOPS, 3-(N-morpholino)propanesulfonic acid; kb, kilobase(s).

pore BioImage Analyzer). Densities of SP-C and actin mRNA signals were normalized to the density of 18 S rRNA signal to correct for the amount of RNA loaded on the gels.

Isolation of Nuclei and Transcription Run-on Assay—Methods for isolation of nuclei, transcription run-on assay, and hybridization of RNA have been described previously (14). RNA from nuclei was extracted with TRI reagent by modification of the method for extraction of RNA from cells/tissue. Nuclei (20×10^6 in 200 μ l) were lysed by adding 1.4 ml of TRI reagent to the reaction mixture, vigorously vortexed for 30–60 s, and stored at room temperature for 10 min. Chloroform (0.28 ml) was added to the lysed nuclei, and the samples were shaken vigorously for 15 s and stored at room temperature for 5 min. Samples were centrifuged for 15 min at $12,000 \times g$ at 4°C . The upper aqueous phase was mixed with 100 μ g of yeast or *E. coli* tRNA that served as a carrier, and total RNA was precipitated with an equal volume of isopropanol for 10 min at room temperature. RNA was collected by centrifugation at $12,000 \times g$ for 10 min, rinsed with 1.0 ml of 70% ethanol, dissolved in water, and reprecipitated by addition of 0.3 M NaOAc, pH 5.2, and 2.5 volumes of ethanol at -20°C . The final RNA precipitate was dissolved in 200 μ l of hybridization buffer, and radioactivity of RNA was determined by liquid scintillation spectrometry. The final RNA preparations were checked for presence of unincorporated [^{32}P]UTP.

This method for isolation of RNA does not involve many steps, and the entire procedure can be completed in a relatively short time. It is especially convenient for extraction of RNAs from a large number of samples. RNA extracted by our method produced hybridization results similar to those RNAs obtained by digestions with DNase I, proteinase K, and multiple phenol and chloroform extractions. A similar method for isolation of RNA from labeled nuclei was described recently (15).

Equal amounts of radioactive RNAs were hybridized to 10 μ g of linearized plasmid DNA containing rabbit SP-C cDNA or human β actin cDNA that had been bound to nitrocellulose membranes. In some experiments RNAs were also hybridized to membranes containing antisense SP-C cDNA (0.9 kb) or antisense SP-C gene fragments in M13. Linearized plasmid DNAs were denatured by treatment with 0.3 M NaOH for 30 min at 37°C and neutralized with 1 M NH_4OAc . M13 DNA and denatured plasmid DNAs were applied to nitrocellulose membranes using a dot blot apparatus, and the DNAs were fixed to the membrane by baking at 80°C for 2 h under vacuum. Hybridizations were carried out in a final volume of 200 μ l at 45°C for 3 days. Washed membranes were subjected to autoradiography using an intensifying screen, and signals were quantified by scanning densitometry (Mili-pore BioImage Analyzer). After autoradiography membranes were counted in a liquid scintillation counter to determine the amount of bound radioactivity. Usually we detected no degradation of RNA during hybridization. Transcription rates determined by either quantification of autoradiographic signals or by quantification of bound radioactivity were similar. Signal/radioactivity bound to linearized pUC DNA/M13 DNA (background) was subtracted from radioactivity obtained with plasmids containing SP-C/actin cDNAs or M13 containing antisense SP-C cDNA/antisense SP-C gene fragments.

Measurement of Half-life of SP-C and Actin mRNAs—Half-lives of SP-C and actin mRNAs were determined by measuring their rate of degradation after inhibition of RNA synthesis with actinomycin D (5 $\mu\text{g}/\text{ml}$). Actinomycin D at this concentration inhibited total RNA synthesis by $\sim 80\%$ after 3 h of incubation. Tissues were incubated in the presence or absence of dexamethasone (10^{-7} M) for 24 h, and incubation was continued for an additional 24 h in the presence or absence of dexamethasone in medium containing actinomycin D (5 $\mu\text{g}/\text{ml}$). Tissues were collected at 0, 6, 9, 12, 18, and 24 h after addition of actinomycin D, and SP-C and actin mRNA levels were determined by Northern blot analysis. Half-life of RNA was calculated from the equation $t_{1/2} = \ln 2/k$ ($t_{1/2}$ = half-life; degradation rate constant $k = -(2.303)$ (slope)) (16).

RESULTS

Developmental Regulation of SP-C Gene Transcription and SP-C mRNA Levels—We found previously that SP-C mRNA accumulation is induced during fetal lung development (10). To determine if the induction of SP-C mRNA levels is regulated at the transcriptional level, we analyzed SP-C gene transcription and SP-C mRNA levels in fetal lung tissues (19–30-day gestational ages) and adult rabbit lung (Fig. 1A). We found a marked lag between the temporal induction of SP-C gene transcription and elevation of SP-C mRNA levels. The increases in SP-C mRNA accumulation were not associated with similar increases in SP-C gene transcription. SP-C mRNA level in 24-day

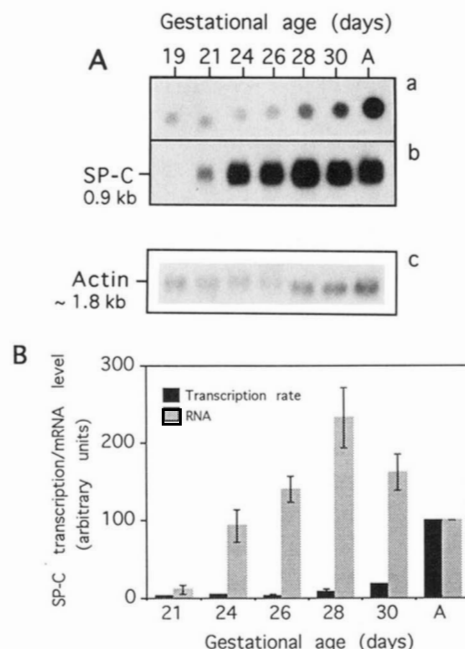


FIG. 1. Developmental regulation of SP-C gene transcription and SP-C mRNA accumulation in fetal rabbit lung. A, nuclei from lung tissues of fetal rabbits of gestational age 19–30 days and of adult animals were isolated and SP-C gene transcription was analyzed by transcription run-on assay. Approximately 6×10^6 cpm were hybridized to filters containing rabbit SP-C cDNA in pUC (panel a). RNAs (5.0 μ g) isolated from lung tissues of fetal and adult rabbits were analyzed by Northern blotting with ^{32}P -labeled rabbit SP-C cDNA (panel b) or human β actin cDNA (panel c). B, autoradiographic signals obtained after transcription run-on assay and Northern blotting were quantified by scanning densitometry. In transcription run-on assays, signals obtained with filters containing pUC were subtracted from signals obtained with filters containing SP-C cDNA in pUC. SP-C mRNA levels were normalized to 18 S rRNA levels. Data of transcription analysis represent mean \pm S.D. of analysis of transcription rates in lungs from two separate litters of animals. Data of Northern blotting represent mean \pm S.E. of analysis of RNA levels in lungs from four separate litters of animals.

fetal lung was $\sim 90\%$ of the level in adult lung, whereas SP-C gene transcription level was only $\sim 4\%$ of the level in adult lung (Fig. 1B). An increase in SP-C gene transcription was not observed until day 28 of gestation, and after day 28 of gestation it increased to reach peak levels in adult lung tissue. Total RNA transcription in nuclei of different gestational age fetal lungs did not change significantly, but a decrease of $\sim 50\%$ was consistently found in nuclei from adult lungs. SP-C gene transcription rates were similar or identical whether measured with double-stranded SP-C cDNA or single-stranded antisense SP-C cDNA probes.

cAMP and Glucocorticoid Regulation of SP-C Gene Transcription and SP-C mRNA Levels—In previous studies we found that cyclic AMP analogs and dexamethasone increases the SP-C mRNA accumulation in 21-day fetal lung tissues *in vitro* in a time-dependent manner. Induction by cAMP analogs occurred consistently after only 48 h of incubation, whereas induction by dexamethasone was found after 24 h of incubation. In tissues treated with a combination of Bt_2cAMP and dexamethasone, the effects on SP-C mRNA levels were less than additive after 24 h of incubation, whereas after 48–72 h of incubation they were additive. To determine if the changes in SP-C mRNA accumulation are due to changes in SP-C gene transcription, we analyzed SP-C gene transcription and SP-C mRNA levels in tissues treated with Bt_2cAMP or dexamethasone or a combination of the two agents (Fig. 2). Total RNA transcription rate was unaffected by treatment with hormonal agents.

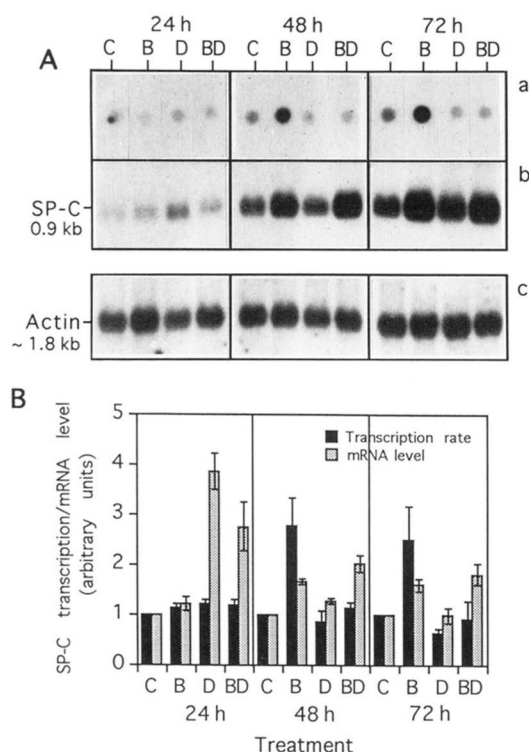


FIG. 2. Effects of Bt₂cAMP (B) and dexamethasone (D) on SP-C gene transcription and SP-C mRNA accumulation in fetal rabbit lung *in vitro*. A, 21-day gestational age fetal rabbit lung tissues were maintained in the absence (C) or presence of Bt₂cAMP (1 mM) or dexamethasone (10⁻⁷ M) or a combination of the two agents for 24–72 h. Nuclei were isolated from fetal lung tissues in culture and SP-C gene transcription rates were determined by run-on assay as described under “Materials and Methods.” ~5.0 × 10⁶ cpm were hybridized to nitrocellulose filters containing SP-C cDNA in pUC, and an autoradiogram was obtained (panel a). RNA (5.0 µg) isolated from lung tissues in culture was analyzed by Northern blotting with ³²P-labeled SP-C cDNA (panel b) or ³²P-labeled human β actin cDNA (panel c). B, autoradiographic signals obtained after transcription run-on assay and Northern blot analysis were quantified by scanning densitometry. SP-C mRNA levels were normalized to 18 S rRNA levels to correct for loading equal amounts of RNA. Data represent mean ± S.E. of eight separate experiments. In transcription run-on assays, signals obtained with filters containing pUC was considered as background and subtracted from signals obtained with filters containing SP-C cDNA in pUC. Data represent mean ± S.E. of four separate experiments.

We found that Bt₂cAMP increased SP-C gene transcription and SP-C mRNA levels after 48–72 h of incubation. The increases in SP-C mRNA accumulation were associated with similar or higher levels of SP-C gene transcription (Fig. 2B). In tissues incubated with dexamethasone, induction of SP-C mRNA accumulation after 24 h was not associated with an increase in SP-C gene transcription (Fig. 2B). After 48 h of incubation with dexamethasone, SP-C gene transcription was reduced compared to levels in tissues maintained in control medium. In tissues treated with a combination of Bt₂cAMP and dexamethasone for 48–72 h, SP-C gene transcription was reduced significantly compared to levels in tissues treated with Bt₂cAMP alone. Actin mRNA levels and gene transcription were not significantly affected by treatment with Bt₂cAMP, dexamethasone, or a combination of the two agents. These data suggest that the dexamethasone-dependent increase in SP-C mRNA accumulation is mediated at posttranscriptional level, whereas the cAMP-dependent increase is due primarily to an increase in transcription.

We also observed that SP-C gene transcription and SP-C mRNA accumulation increased during culture of fetal lung tissues.

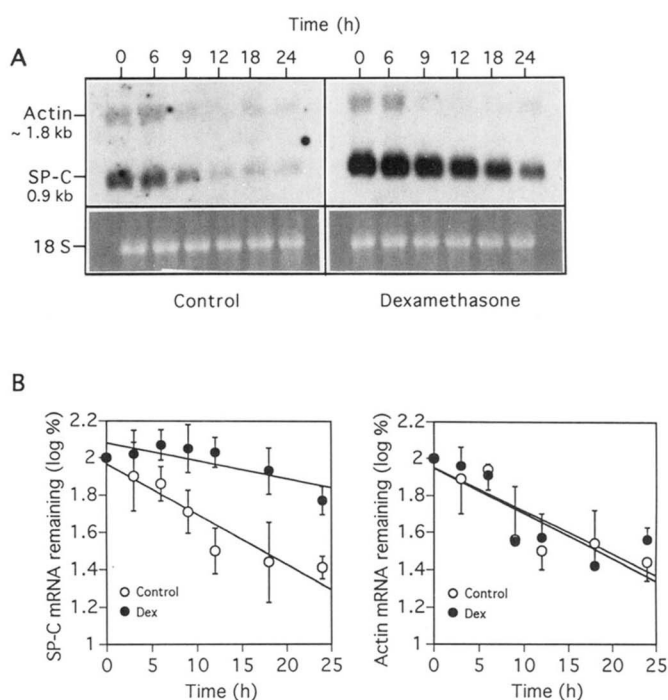


FIG. 3. Effect of dexamethasone on the turnover of SP-C and actin mRNAs in fetal rabbit lung *in vitro*. A, 21-day fetal rabbit lung tissues were maintained *in vitro* in control or dexamethasone containing medium for 24 h. Afterward, lung tissues were incubated in the presence of actinomycin D (5 µg/ml) in control or dexamethasone containing medium for up to 24 h. Lung tissues were collected at indicated times, and RNA was isolated. RNA (5 µg) was analyzed by Northern blotting with ³²P-labeled SP-C cDNA and human β actin cDNA (1.8 kb) and an autoradiogram was obtained. B, SP-C and actin mRNA signals were quantified by scanning densitometry and their levels were normalized to 18 S rRNA levels to correct for loading equal amounts of RNA. Data from four separate experiments were analyzed and fitted using linear regression analysis. Treatment of tissues with dexamethasone increased the half-life (*t*_{1/2}) of SP-C mRNA by >2.5-fold; in control tissues *t*_{1/2} was 11.4 h, and in dexamethasone-treated tissues it was 30 h. Dexamethasone treatment had no significant effect on the half-life of actin mRNA; in control tissues *t*_{1/2} was 12.2 h, and in dexamethasone-treated tissues *t*_{1/2} was 14.2 h.

Effect of Dexamethasone on the Stability of SP-C mRNA—Control of mRNA stability appears to play a major role in post-transcriptional regulation of gene expression (17). We determined the effect of dexamethasone on the stability of SP-C mRNA (Fig. 3) since dexamethasone acted posttranscriptionally to increase SP-C mRNA accumulation. We initially attempted to measure SP-C mRNA turnover by label-chase analysis of cellular RNA with [³H]uridine, but we found that the amount of ³H-labeled SP-C mRNA recovered on filters after hybridization was too low to accurately assess the rate of degradation of SP-C mRNA. We therefore employed the widely used method of measuring mRNA turnover after inhibition of cellular RNA synthesis with actinomycin D. We found that the half-life of SP-C mRNA was 11.4 h in tissues in control medium, and it increased to 30 h in tissues treated with dexamethasone (Fig. 3A). The half-life of actin mRNA was not altered by dexamethasone treatment (control = 12 h, dexamethasone-treated = 14 h).

DISCUSSION

The temporal induction of SP-C mRNA during fetal lung development is remarkably different from that of SP-B and SP-A mRNAs. In rat (18), rabbit (7, 10), and human fetal lungs (19, 20), SP-C mRNA is detected prior to the appearance of morphologically identifiable alveolar type II epithelial cells. Differentiated alveolar type II cells are first observed in rabbit

lung tissue at approximately day 26 of gestation (21). In rabbit fetal lung, SP-C mRNA is detected at day 19 of gestation, the earliest time point examined (7, 10, 22). SP-C mRNA levels increase as a function of gestation, and in neonatal and adult lung tissues its level decreases significantly compared to levels found in lung tissues of 28–31-day gestational age fetal rabbits.

We found a marked discrepancy between the temporal induction of SP-C gene transcription and SP-C mRNA accumulation. The lag between SP-C gene transcription and SP-C mRNA accumulation suggested that significant stabilization of SP-C mRNA can account for the accumulation of SP-C mRNA during prenatal lung development. In adult lung, however, transcription appears to regulate SP-C mRNA accumulation. Because of experimental difficulties involved, we did not attempt to measure SP-C mRNA turnover *in vivo* during fetal lung development.

The temporal induction of SP-C gene transcription and mRNA during fetal rabbit lung development is quite different from the temporal induction of SP-A gene transcription and mRNA. SP-A gene transcription increases as early as day 24 of gestation and continues to increase to reach highest levels in 28-day lung (14). SP-A gene transcription in neonatal lung is decreased compared to levels in 28-day lung. The temporal induction of SP-A gene transcription coincides with temporal induction of SP-A mRNA (14), suggesting that our observed differences between temporal induction of SP-C gene transcription and SP-C mRNA accumulation represent a specific phenomenon, not a pleiotropic effect.

Snyder and co-workers (7) used *in situ* hybridization analysis to detect SP-C mRNA in epithelial cells of prealveolar region of 19-day rabbit fetal lung. They found that the SP-C mRNA content increased as a function of gestational age, and by day 27 of gestation and thereafter expression of SP-C mRNA was restricted to epithelial cells with morphologic characteristics of alveolar type II cells (7). Taken together, the data of Snyder and co-workers and our finding that SP-C gene transcription does not increase until day 28 of gestation suggest that an increase in SP-C gene transcription is linked to differentiation of alveolar type II cells.

The number of identifiable type II cells increases as a proportion of total epithelial cells in lung after day 26 of gestation in the rabbit (21, 23). This makes accurate assessment of increases in SP-C gene transcription rate during development difficult. SP-C mRNA content of prealveolar and alveolar epithelial cells increase during fetal rabbit lung development to reach highest levels at term (7). This suggests that an increase in SP-C mRNA content of type II epithelial cells during development must be the result of increases in SP-C gene transcription/SP-C mRNA stability. These data suggest that increases in SP-C gene transcription rate during development must be due both to an increase in the number of type II epithelial cells and to enhanced transcription/stability of SP-C mRNA.

The physiological significance of high levels of SP-C mRNA in embryonic lung is unclear. A similar pattern of accumulation was also noted for SP-A and SP-B mRNAs (22). The transition from an aqueous to a gaseous environment that accompanies birth may necessitate an acute requirement for surfactant proteins and lipids. Elevated levels of surfactant protein mRNAs may reflect such a need.

Glucocorticoids and cAMP play major roles in promoting fetal lung development and enhance surfactant synthesis (24). Glucocorticoids increase SP-C mRNA accumulation in human fetal lung *in vitro* (19, 20) and in rat lung both *in vitro* (25) and *in vivo* (26, 27). The effects of maternal administration of glucocorticoids on SP-C mRNA levels in fetal lung are not clear. Maternal administration of glucocorticoids to pregnant rabbits

on day 26 of gestation resulted in a decrease in SP-C mRNA level in lungs of fetal rabbits delivered on day 27 (28). A recent study showed that glucocorticoids administered to pregnant rabbits on days 25 and 26 of gestation increased SP-C mRNA levels 2-fold compared to uninjected control animals (29). In the same study injection of saline to pregnant animals increased SP-C mRNA levels in fetal lung tissues 2-fold, leading the authors to suggest that maternal stress related factors alone can increase SP-C mRNA levels. Glucocorticoid regulation of SP-C mRNA levels *in vivo* might depend on the developmental stage of the fetus when the hormone was administered maternally.

We found that cAMP and dexamethasone regulate SP-C mRNA levels in fetal lung *in vitro* by distinct mechanisms; the effects of cAMP are mediated at the transcriptional level, whereas the effects of dexamethasone are mediated solely at the posttranscriptional level. We also found that after 48 h of incubation dexamethasone inhibited both basal and Bt₂cAMP-dependent increases in SP-C gene transcription. This effect may explain why we did not observe a substantial increase in SP-C mRNA levels in tissues treated with a combination of Bt₂cAMP and dexamethasone for 48–72 h, even though dexamethasone increased the half-life of SP-C mRNA after 24 h and Bt₂cAMP increased SP-C gene transcription after 48 h. Mechanisms by which dexamethasone inhibited SP-C gene transcription are not known. Glucocorticoids were found to negatively regulate human glycoprotein α -subunit gene expression by interfering with a cAMP responsive element (30). Whether dexamethasone inhibits cAMP-induced SP-C gene transcription by a similar mechanism remains to be investigated.

We found previously that adenosine 3',5'-cyclic monophosphorothioate (Sp-diastereomer) (0.1–0.25 mM), an analog of cyclic AMP, increases SP-C mRNA accumulation in fetal rabbit lung *in vitro* by a magnitude similar to that of Bt₂cAMP (1 mM) (10). This suggested that the effects of Bt₂cAMP to increase SP-C mRNA accumulation are not due to butyric acid, a by-product of metabolism of Bt₂cAMP. Butyric acid modulates globin gene expression in erythroid cells (31, 32). Recently, butyric acid was found to modulate surfactant protein gene expression in fetal rat lung *in vitro* with significant inhibitory effects on SP-A and SP-B mRNA accumulation (33). The effects of butyric acid on SP-C mRNA levels were complex; butyric acid inhibited SP-C mRNA accumulation below control levels after 6 h of incubation but increased to control levels after 24 h of incubation (33). Both transcription and mRNA stability were found to mediate butyric acid-induced changes in SP-C mRNA accumulation. The authors have suggested that elevated levels of butyric acid analogs as found in diabetic mothers and fetuses might lead to increased incidence of newborn respiratory distress syndrome. It remains to be determined whether butyric acid has similar effects on surfactant protein gene expression in fetal lungs of other species. Incubation of fetal rabbit lung explants with butyric acid for 5 days did not inhibit levels of immunoreactive SP-A (34).

Glucocorticoids and cAMP influence expression of a number genes in different cell types of fetal lung (35). This might confirm our analysis of the effects of these agents on SP-C gene transcription rate in fetal lung explants. In fetal rabbit lung *in vitro* SP-A (14), SP-B,² and SP-C (10) genes are regulated independently by both cAMP and glucocorticoids. By *in situ* hybridization dexamethasone was found to increase SP-B mRNA content of both alveolar and bronchiolar epithelial cells of fetal rat lung explants (36). This suggests that dexamethasone-dependent increase in SP-B mRNA levels in fetal rat lung explants must be due to increased accumulation of SP-B mRNA in alveolar and bronchiolar epithelial cells of fetal lung. By dele-

² R. K. Margana and V. Boggaram, submitted for publication.

tion mapping and transfection analysis a region of SP-A gene, -378 to +20, was shown to mediate cAMP induction of SP-A-chloramphenicol acetyltransferase fusion gene expression in purified alveolar type II epithelial cells (37). This suggests that the inductive effects of cAMP on SP-A gene transcription in fetal rabbit lung *in vitro* are indeed due to activation of SP-A gene transcription in type II cells. These data suggest that glucocorticoids and cAMP regulate surfactant protein gene expression by altering their expression by specific cell types of lung and that changes in surfactant protein gene expression are not the result of pleiotropic effects of hormones.

We investigated the effects of dexamethasone on SP-C mRNA stability since stabilization of RNA plays a major role in post-transcriptional regulation of gene expression (17). We found that dexamethasone increased the half-life of SP-C mRNA by >2.5-fold. The action of dexamethasone that results in increased SP-C mRNA levels in rabbit fetal lung *in vitro* is different from that found in rat (25) and human fetal lung (38) tissues. In both rat and human fetal lung tissues *in vitro*, glucocorticoids increase SP-C mRNA levels by increasing gene transcription. However, mRNA stabilization and its effect on SP-C mRNA levels in rat and human lung *in vitro* cannot be ruled out, because the time course of the effects of glucocorticoid on SP-C gene transcription and SP-C mRNA accumulation were not investigated. The lack of effect of dexamethasone on SP-C gene transcription in rabbit fetal lung might be species-specific. SP-A gene expression appears to be regulated differently by glucocorticoids in rabbit (14) and human (39) fetal lung *in vitro*. In rabbit fetal lung *in vitro*, glucocorticoids regulate SP-A gene expression predominantly at the transcriptional level (14), whereas in human fetal lung *in vitro*, they exert dose-dependent effects on transcription and mRNA stability (40).

SP-C gene transcription and SP-C mRNA accumulation increased during explant culture in the absence of either serum or hormones. Several other reports indicate increases in mRNAs for rabbit SP-A (14), SP-C (10), and human SP-B (19) during explant culture of fetal lung tissues in serum-free defined medium. Increases in SP-A mRNA in rabbit fetal lung *in vitro* appeared to be coincident with increase in gene transcription (14). Increased expression of surfactant protein mRNAs during explant culture is probably due to an increased number of type II epithelial cells as a result of differentiation of fetal tissues (12). The factors and mechanisms that mediate spontaneous differentiation of fetal lung tissues *in vitro* are not well understood. Prostaglandins acting through cAMP may promote differentiation of type II cells and increase SP-A gene expression in human fetal lung tissues *in vitro* (41).

In addition to regulating gene expression at the transcriptional level, hormonal and developmental signals also modulate gene expression at the post-transcriptional level by altering RNA stability (42). Changes in mRNA stability occur during maturation of frog and mouse oocytes (43, 44). Both frog and mouse oocytes store a major fraction of maternal mRNA in a stable and translationally inactive form. During development oocyte-specific mRNAs are recruited for translation and later undergo degradation at an increased rate. Developmental changes in mRNA stability also play important roles in regulating gene expression in terminally differentiated cells. During red cell development, accumulation of globin is attributed to stabilization of globin mRNA and selective destabilization of non-globin mRNAs (45, 46). In cell lines that reflect different stages of B cell development, stabilization of immunoglobulin mRNA mediates accumulation of this mRNA in differentiated B cells (47, 48). Our investigation has revealed that changes in both mRNA stability and transcription regulate accumulation

of SP-C mRNA during development and differentiation of fetal lung.

Glucocorticoids regulate gene expression at posttranscriptional level by exerting both positive and negative effects on mRNA stability. Glucocorticoids increase stability of growth hormone (49), fibronectin (50), and phosphoenolpyruvate carboxykinase (51) mRNAs and decrease the stability of interleukin-1 β (52), interferon β (53), and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (54) mRNAs. In human fetal lung *in vitro*, they stabilize SP-B mRNA (38) and destabilize SP-A mRNA (40).

Molecular mechanisms underlying regulation of mRNA stability by hormonal and developmental signals are not well understood. 3'-Untranslated regions of mRNAs are required for regulation of mRNA stability, and alterations in RNA stability are mediated by the interaction of discrete protein factors (turnover factors) with specific domains and sequence elements (turnover elements) within mRNAs (42). A region located in the 3' untranslated region of phosphoenolpyruvate carboxykinase mRNA has been shown to confer glucocorticoid stabilization upon a heterologous mRNA when stably transfected into a rat hepatoma cell line (51). A glucocorticoid-inducible protein factor was suggested to interact with the 3'-untranslated region. SP-C mRNAs contain a 3'-untranslated region of 240–271 nucleotides. Sequence elements within SP-C mRNA and developmental- and glucocorticoid-specific putative protein factors that interact with RNA sequence elements to regulate RNA stability remain to be investigated.

Our investigation provides new information on the regulation of SP-C gene expression during fetal lung development and by glucocorticoid and cAMP in fetal lung *in vitro*. Results of our experiments reveal that, whereas post-transcriptional (mRNA stability) mechanisms play a major role in SP-C gene regulation in prenatal lung development, transcriptional mechanisms regulate its expression in adult lung. In fetal lung *in vitro* glucocorticoids and cAMP, agents that have profound effects on lung maturation and surfactant synthesis, regulate SP-C gene expression by diverse mechanisms. The effects of cAMP are exerted at the transcriptional level, whereas the effects of glucocorticoids occur at the level of mRNA stabilization.

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REFERENCES

- Goerke, J., and Clements, J. A. (1986) in *Handbook of Physiology: The Respiratory System* (Macklem, P. T., and Mead, J., eds) Vol. III, pp. 47–261, American Physiological Society, Washington, DC
- King, R. J. (1985) *Annu. Rev. Physiol.* **47**, 775–788
- Avery, M. E., and Mead, J. (1959) *Am. J. Dis. Child.* **97**, 517–523
- Possmayer, F. (1988) *Am. Rev. Respir. Dis.* **138**, 990–998
- Persson, A., Chang, D., Rust, K., Moxley, M., Longmore, W., and Crouch, E. (1989) *Biochemistry* **28**, 6361–6367
- Possmayer, F. (1990) *Am. Rev. Respir. Dis.* **142**, 749–752
- Wohlford-Lenane, C. L., Durham, P. L., and Snyder, J. M. (1992) *Am. J. Respir. Cell Mol. Biol.* **6**, 225–234
- Phelps, D. S., and Floros, J. (1991) *Exp. Lung Res.* **17**, 985–995
- Kalina, M., Mason, R. J., and Shannon, J. M. (1992) *Am. J. Respir. Cell Mol. Biol.* **6**, 594–600
- Boggaram, V., and Margana, R. K. (1992) *Am. J. Physiol.* **263**, L634–L644
- Boggaram, V., and Margana, R. K. (1994) *Am. J. Respir. Crit. Care Med.* **149**, A523 (Abstract)
- Snyder, J. M., Mendelson, C. R., and Johnston, J. M. (1981) *Dev. Biol.* **85**, 129–140
- Krocze, R. A., and Siebert, E. (1989) *Anal. Biochem.* **184**, 90–95
- Boggaram, V., and Mendelson, C. R. (1988) *J. Biol. Chem.* **263**, 19060–19065
- Fei, H., and Drake, T. D. (1993) *BioTechniques* **15**, 838
- Hod, Y., and Hanson, R. W. (1988) *J. Biol. Chem.* **263**, 7747–7752
- Brawerman, G. (1993) in *Control of Messenger RNA Stability* (Belasco, J., and Brawerman, G., eds) pp. 149–159, Academic Press, New York
- Schellhase, D. E., Emrie, P. A., Fisher, J. H., and Shannon, J. M. (1989) *Pediatr. Res.* **26**, 167–174
- Liley, H. G., White, R. T., Warr, R. G., Benson, B. J., Hawgood, S., and Ballard, P. L. (1989) *J. Clin. Invest.* **83**, 1191–1197
- Whitsett, J. A., Weaver, T. E., Clark, J. C., Sawtell, N., Glasser, S. W.,

- Korfhagen, T. R., and Hull, W. M. (1987) *J. Biol. Chem.* **262**, 15618–15623
21. Snyder, J. A., and Magliato, S. (1991) *Anat. Rec.* **229**, 73–85
22. Durham, P. L., Nanthakumar, E. J., and Snyder, J. L. (1992) *Exp. Lung Res.* **18**, 775–793
23. Kikkawa, Y., Motoyama, E. K., and Gluck, L. (1968) *Am. J. Pathol.* **52**, 177–208
24. Mendelson, C. R., and Boggaram, V. (1991) *Annu. Rev. Physiol.* **53**, 415–440
25. Veletzka, S., Nichols, K. V., Gross, I., Lu, H., Dynia, D. W., and Floros, J. (1992) *Am. J. Physiol.* **262**, L684–L687
26. Schellhase, D. E., and Shannon, J. M. (1991) *Am. J. Respir. Cell Mol. Biol.* **4**, 304–312
27. Fisher, J. H., McCormack, F., Park, S. S., Stelzner, T., Shannon, J. M., and Hofmann, T. (1991) *Am. J. Respir. Cell Mol. Biol.* **5**, 63–70
28. Connelly, I. H., Hammond, G. L., Harding, P. G. R., and Possmayer, F. (1991) *Endocrinology* **129**, 2583–2591
29. Durham, P. L., Wohlford-Lenane, C. L., and Snyder, J. L. (1993) *Anat. Rec.* **237**, 365–377
30. Akerbloom, I. E., Slater, E. P., Beato, M., Baxter, J. D., and Mellon, P. L. (1988) *Science (Wash. DC)* **241**, 350–353
31. Ginder, G. D., Whitters, M. J., and Pohlman, J. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81**, 3954–3958
32. Glauber, J. G., Wandersee, N. J., Little, J. A., and Ginder, G. D. (1991) *Mol. Cell. Biol.* **11**, 4690–4697
33. Peterec, S. M., Nichols, K. V., Dynia, D. W., Wilson, C. M., and Gross, I. (1994) *Am. J. Physiol.* **267**, L9–L15
34. Mendelson, C. R., Chen, C., Boggaram, V., Zacharias, and Snyder, J. M. (1986) *J. Biol. Chem.* **261**, 9938–9943
35. Odom, M. W., Ertsey, R., and Ballard, P. L. (1990) *Am. J. Physiol.* **259**, L283–L293
36. Floros, J., Gross, I., Nichols, K. V., Veletzka, S. V., Dynia, D., Lu, H., Wilson, C. M., and Peterec, S. M. (1991) *Am. J. Respir. Cell Mol. Biol.* **4**, 449–454
37. Alcorn, J. L., Gao, E., Chen, Q., Smith, M. E., Gerard, R. D., and Mendelson, C. R. (1993) *Mol. Endocrinol.* **7**, 1072–1085
38. Venkatesh, V. C., Ianuzzi, D. M., Ertsey, R., and Ballard, P. L. (1993) *Am. J. Respir. Cell Mol. Biol.* **8**, 22–228
39. Boggaram, V., Smith, M. E., and Mendelson, C. R. (1989) *J. Biol. Chem.* **264**, 11421–11427
40. Boggaram, V., Smith, M. E., and Mendelson, C. R. (1991) *Mol. Endocrinol.* **5**, 414–423
41. Acarregui, M. J., Snyder, J. M., Mitchell, M. D., and Mendelson, C. R. (1990) *Endocrinology* **127**, 1105–1113
42. Williams, D. L., Sensel, M., McTigue, M., and Binder, R. (1993) in *Control of Messenger RNA Stability* (Belasco, J., and Brawerman, G., eds) pp. 161–197, Academic Press, New York
43. De Leon, V., Johnson, A., and Bachavarova, R. (1983) *Dev. Biol.* **98**, 400–408
44. Richter, J. D. (1991) *Bioessays* **13**, 179–183
45. Lodish, H. F., and Small, B. (1976) *Cell* **7**, 59–65
46. Aviv, H., Voloch, Z., Bastos, R., and Levy, S. (1976) *Cell* **8**, 495–503
47. Cox, A., and Emtage, J. S. (1989) *Nucleic Acids Res.* **17**, 10439–10454
48. Genovese, C., and Milcarek, C. (1990) *Mol. Immunol.* **27**, 733–743
49. Paek, I., and Axel, R. (1987) *Mol. Cell. Biol.* **7**, 1496–1507
50. Dean, D. C., Newby, R. F., and Bourgeois, S. (1988) *J. Cell Biol.* **106**, 2159–2170
51. Petersen, D. D., Koch, S. R., and Granner, D. K. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 7800–7804
52. Lee, S. W., Tsou, A.-P., Chan, H., Thomas, J., Petrie, K., Eugui, E. M., and Allison, A. C. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 1204–1208
53. Peppel, K., Vinci, J. M., and Baglioni, C. (1991) *J. Exp. Med.* **173**, 349–355
54. Simonet, W. S., and Ness, G. C. (1989) *J. Biol. Chem.* **264**, 569–573