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REGULATION OF LUNG SUFACTANT PROTEIN GENE EXPRESSION

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1. ABSTRACT

Surfactant, a complex mixture of lipids and proteins, produced by the alveolar type II cells of the lung epithelium maintains alveolar integrity and plays important roles in the control of host defense and inflammation in the lung. Surfactant protein (SP) A, B, C and D genes are expressed in a cell-type restricted manner by the Clara and/or alveolar type II cells of the lung. Surfactant protein genes are independently regulated during fetal lung development and by hormones, cytokines and other agents. Transcriptional and/or posttranscriptional (mRNA stability) mechanisms control multifactorial regulation of surfactant protein gene expression. *In vitro* cell culture and transgenic animal studies have shown that relatively short promoter sequences control cell/tissue-specific expression and developmental regulation of surfactant protein genes. Surfactant protein promoter function is dependent on the combinatorial actions of multiple transcription factors, and thyroid transcription factor 1 (TTF-1/Nkx2.1) is a common positive regulator of surfactant protein promoter activity.

2. INTRODUCTION

The prime function of the lungs is gas exchange that allows diffusion of oxygen from inhaled air into the venous blood and diffusion of carbon dioxide into the expired air. The respiratory tract of the lung consists of a series of branching airways that terminate in air-sacs or alveoli. Gas exchange occurs across the alveolar epithelium that is exceedingly thin and has a total surface area of 50 - 100 square meters. The containment of such a large surface area within the limited thoracic cavity is achieved by the creation of an enormous number of alveoli that are surrounded by blood capillaries. In the human lung there are about 300 million alveoli encompassing a total surface area of approximately 100 square meters yet accounting for only 4-6 liters volume (1). In other words, the lung can be considered as a collection of 300 million bubbles whose stability is vital for normal respiration. During respiration, contractile forces that are generated on the alveolar surface due to the surface tension of the aqueous layer of alveolar lining fluid tend to collapse the alveoli. The stability of the alveoli is maintained by the action of surfactant (2), a phospholipid-rich lipid-protein complex that reduces surface tension at the alveolar air-liquid interface (3). In the absence of surfactant, the collapse of the alveoli will lead to respiratory distress, a condition characterized by increased alveolar-capillary permeability and the need for ventilatory support. Inadequate levels of surfactant due to premature birth is linked to the development of newborn respiratory distress syndrome (4), the major cause of neonatal morbidity and mortality in developed countries. Altered levels and abnormalities of surfactant occur in association with acute respiratory distress syndrome (5) and pulmonary infections caused by a wide variety of pathogens including bacteria, virus and fungi (6).

Alveolar type II epithelial cells synthesize and store surfactant in intracellular inclusion organelles that have characteristic lamellated structures called lamellar bodies (7). Alveolar type II cells secrete lamellar bodies into the alveolar lumen where they are transformed into a quadratic lattice like structure called tubular myelin (8) that has been suggested to serve as an intermediate in the formation of the monolayer lipid film on the alveolar surface. Surfactant contains 90% lipids and 5-10% proteins; of the lipids 80-90% is phospholipid and dipalmitoylphosphatidylcholine (DPPC) constitutes the major surface active phospholipid (9). Four distinct lung-specific surfactant associated proteins have been isolated and characterized to date. These proteins, termed surfactant protein (SP)-A, SP-B, SP-C (10) and SP-D (11), have been shown to play diverse and important roles in the biophysical properties, function and metabolism of surfactant and in the control of host defense and inflammation in the lung. SP-A (28,000-36,000 Da) and SP-D (43,000 Da) are hydrophilic proteins that are characterized by the presence of amino-terminal collagenous and carboxy-terminal lectin-like domains and are members of the collectin family of proteins. SP-A and SP-D appear to play important roles in innate immune defense in the lung (12) and in the control of surfactant lipid metabolism and homeostasis (13). Their involvement in influencing the surface tension reducing capabilities of surfactant phospholipids is less clear. SP-B (8,700 Da) and SP-C (4000 Da) are extremely hydrophobic proteins that are produced through proteolytic processing of higher molecular weight precursor proteins (14). A number of studies have indicated that SP-B and SP-C enhance the spreading and adsorption of phospholipids to an air-liquid interphase and promote the reduction of surface tension (14). In particular, SP-B has been suggested to stabilize the phospholipid monolayer by interacting with DPPC (15). Inherited deficiency of SP-B in infants with congenital alveolar proteinosis inevitably leads to fatal respiratory failure highlighting the important role of SP-B in lung function (16). Targeted disruption of SP-B causes respiratory failure in newborn mice further supporting the important role of SP-B in lung function (17). The precise roles of SP-C in surfactant and lung function are less clear. A mutation in SP-C gene that gives rise to altered SP-C precursor protein may be associated with the onset of interstitial lung disease (18). Studies of SP-C deficient mice have indicated that although SP-C deficiency did not affect lung function, the SP-C deficient surfactant displayed reduced surface activity at low-end expiration or small alveolar volumes (19). Based on these data it was suggested that SP-C may play important roles in stabilizing the phospholipid layers that form during film compression at low lung volumes (19).

3. CELL/TISSUE-SPECIFIC EXPRESSION OF SURFACTANT PROTEIN GENES

In the lung SP-A (20-22) and SP-D (23) are expressed in a cell type-specific manner by the alveolar type II and bronchiolar (Clara) epithelial cells. In the adult mouse the expression of SP-D but not of SP-A is also detected in cells of tracheal epithelium and tracheal submucosal glands (23). However, the expression of SP-A and SP-D is not confined to lung, and their expression has been detected in tissues other than lung. Specifically, SP-A and SP-D expression was detected in the intestine (24-27) and the Eustachian tube (28, 29) and SP-D but not SP-A expression was found in the stomach (30). Additionally, immunoreactive SP-A has been detected in the prostrate (31, 32), synovial intima and mesothelial cells of pleura, pericardium and peritoneum (33) and immunoreactive SP-D has been detected in salivary and lacrimal glands (34). In these tissues, as in the case of lung, SP-A and SP-D are thought to be involved in the control of innate immune response to microbial pathogens. In contrast to the expression of SP-A and SP-D, the expression of SP-B and SP-C appear to be restricted to lung although recent studies have shown that SP-B is also expressed in the Eustachian tube (35). In the lung, whereas SP-B expression is restricted to alveolar type II and Clara epithelial cells (21, 22), SP-C expression is restricted solely to alveolar type II epithelial cells (36, 37).

4. DEVELOPMENTAL REGULATION OF SURFACTANT PROTEIN GENE EXPRESSION

The expression of surfactant protein mRNAs is induced during fetal lung development in rats (38), rabbit (39), sheep (40) and humans (41). The induction of surfactant protein mRNAs occurs in concert with an increase in surfactant phospholipid synthesis indicating the importance of both the protein and lipid components of surfactant in pulmonary function. Both increased cellular expression and increased numbers of cells (Clara and alveolar type II epithelial cells) expressing surfactant protein mRNAs contribute to the developmental induction of surfactant protein mRNAs. Even though the expression of all surfactant protein mRNAs is increased during fetal lung development, subtle differences exist with regard to the time-course of induction of each mRNA. By Northern blot analysis the expression of SP-A mRNA was first detected in fetal rabbit lung on day 26 of gestation and its levels increased during development to reach maximal levels on day 28 of gestation (42). The developmental induction of SP-A mRNA must be primarily due to an increase in the transcription rate of the gene because both SP-A gene transcription rate and SP-A mRNA accumulation increase by similar degree during fetal lung development (43). In situ hybridization analysis of mRNA expression, during fetal rabbit lung development, detected SP-A mRNA in epithelial cells of type II identity on gestational day 26 and in epithelial cells of bronchioles on gestational day 28 (22) indicating differential regulation of induction in the two cell types. In the human, SP-A and SP-A mRNA were detected in bronchiolar cells and pre-type II cells lining terminal airways at 19-20 weeks of gestation (44) and SP-A increased dramatically in the 3rd trimester of pregnancy (45). The levels of SP-A in the amniotic fluid increase with advancing gestation in humans (45).

During fetal lung development in the rabbit, SP-B mRNA was first detected by Northern blot analysis on gestation day 26 and its levels increased to reach maximal levels on gestation days 28-30 (46). Although SP-B gene transcription rate increased during fetal lung development, the increases were significantly less compared to increases in SP-B mRNA levels suggesting that both transcriptional and post-transcriptional (mRNA stabilization) regulation of SP-B mRNA contribute toward developmental induction of SP-B mRNA (46). In situ hybridization analysis of SP-B mRNA expression during fetal lung development in the rabbit detected SP-B mRNA for the first time in cuboidal

Table 1. Multifactorial regulation of surfactant protein gene expression in fetal lung tissues and lung cell lines *in vitro*

	Transcription				**mRNA stability**			
	SP-A	**SP-B**	**SP-C**	**SP-D**	**SP-A**	**SP-B**	**SP-C**	**SP-D**
Dexamethasone	↑/↓	↑	↑	↑	↓	↑	↑	
Cyclic AMP	↑	↑	↑	↔	↔	↔	↔	
TNF-alpha	↓	↓	↓			↓		
TGF-beta	↓	↓	↓					
Phorbol ester	↓	↓						
Insulin	↓	↓						
Retinoic acid	↑/↓	↑	↑/↓					

↑, induction; ↓, inhibition; ↔, no effect

epithelial cells of pre-alveolar region and in bronchiolar epithelial cells on days 24 and 28 of gestation respectively (22). The concentration of SP-B mRNA in the two cell types increased during development to approximately equal levels in the adult lung (22). In the human, Pro-SP-B and SP-B mRNA were detected in bronchi and bronchioles at 15 weeks of gestation and after 25 weeks, pro-SP-B, active SP-B peptide and SP-B mRNA were detected in non-ciliated bronchiolar and type II epithelial cells (47). The levels of SP-B in amniotic fluid increase with advancing gestation in humans (45).

During fetal rabbit lung development, SP-C mRNA expression was first detected by Northern blot analysis on gestation day 19 and its levels increased to maximal levels on gestation day 28 (48). By in situ hybridization SP-C mRNA expression was first detected in epithelial cells of pre-alveolar region in gestational day 19 fetal rabbit lungs and its levels increase with advancing gestation and by day 27 of gestation SP-C mRNA expression was confined to alveolar type II cells (36). Similar to the induction of SP-B gene expression, the increases in SP-C mRNA levels were not correlated with corresponding increases in SP-C gene transcription suggesting that mRNA stabilization also plays an important role in the developmental induction of SP-C mRNA (49). In contrast to other surfactant protein mRNAs, SP-C mRNA is expressed at a much earlier stage in gestation. In the human, Pro-SP-C and SP-C mRNA were detected by 15 weeks of gestation and their expression increased with advancing gestation (47).

SP-D mRNA and SP-D were first detected in lung homogenates at 21 days of gestation in the rat (50) and their content increased during fetal development and the postnatal period (50, 51). The increased expression of SP-D with advancing gestation was correlated with increased amounts of SP-D in the amniotic fluid (50). In the human, immunoreactive SP-D was detected on airway surfaces by 10 weeks of gestation and the staining increased in distal airways with advancing gestation (52).

Molecular mechanisms responsible for the developmental induction of surfactant protein gene expression are not completely understood. In the developing lung, the expression of TTF-1 and HNF-3beta occur at the onset of lung morphogenesis, preceding the expression of surfactant protein gene expression, and then overlap with the expression of surfactant protein gene expression (53, 54). These data are suggestive of the potential regulatory roles of TTF-1 and HNF-3beta in the developmental induction of surfactant protein gene expression.

5. MULTIFACTORIAL REGULATION OF SURFACTANT PROTEIN GENE EXPRESSION

A number of hormones, growth factors, cytokines and other agents influence fetal lung development and differentiation (55-58) and in turn modulate surfactant synthesis (59). Among hormones, glucocorticoids, and agents that act by increasing intracellular cyclic AMP levels have profound stimulatory effects on fetal lung maturation and surfactant synthesis (55, 60). Glucocorticoid, thyroid and beta-adrenergic receptors are present in the lung and glucocorticoid and thyroid hormone levels increase during development concomitant with an increase in surfactant synthesis. These data suggest that there may be a causal relationship between increasing glucocorticoid and thyroid hormone levels and surfactant synthesis. Regulation of surfactant protein gene expression by cyclic AMP, glucocorticoids and other agents is summarized in table 1.

5.1. Regulation by cyclic AMP

Cyclic AMP analogs (42, 61-67) and agents that increase intracellular cAMP levels such as beta-adrenergic agonists (68) and prostaglandin E2 (PGE2) (69) and vasoactive intestinal peptide (VIP) (Boggaram, V., and Mendelson, C. R., unpublished observations) increase the expression of SP-A and SP-A mRNA in fetal lung tissues *in vitro*. Cyclic AMP mediated increase in SP-A mRNA expression appears to be due solely to an increase in SP-A gene transcription (43). The cyclic AMP analog, 8-bromo-cAMP modestly increased SP-B and SP-C mRNA levels in fetal human lung tissues in vitro (70). Adenylate cyclase activators such as forskolin and terbutaline increase SP-B but not SP-C mRNA levels in fetal human lung tissues in vitro (71). Similarly cyclic AMP analogs and forskolin increase SP-B and SP-C mRNA expression in fetal rat (72, 73) and rabbit (74-76) lung tissues *in vitro*. These data indicate that the effects of cAMP analogs to alter SP-C mRNA expression in fetal lung tissues *in vitro* may be species-specific. These data also indicate that cAMP has significantly stronger inductive effects on SP-A gene expression than on SP-B and SP-C gene expression.

5.2. Regulation by glucocorticoids

Glucocorticoids have both stimulatory and inhibitory effects on SP-A gene expression in fetal lung tissues *in vitro* that appears to be species-specific and dependent on the differentiation status of the tissue at which treatment was initiated (42, 43, 77). In mid-trimester human fetal lung tissues (15-18 week gestation) *in vitro* glucocorticoids have dose-dependent effects on SP-A mRNA levels - at 10^{-10} and 10^{-9} M glucocorticoids increase SP-A mRNA levels whereas at higher concentrations they decrease SP-A mRNA levels (77, 78). The inhibitory effects of glucocorticoids on SP-A mRNA levels are reversible and blocked by the glucocorticoid receptor antagonist RU486 (79). The stimulatory and inhibitory effects of glucocorticoids were found to be due to their differential actions on SP-A gene transcription and SP-A mRNA stability - at 10^{-10} and 10^{-9} M concentration, glucocorticoids increase SP-A mRNA by increasing gene transcription whereas at concentrations of 10^{-8} M and higher they decrease SP-A mRNA by exerting a dominant effect to decrease SP-A mRNA stability (78, 79). In variance with these findings glucocorticoids were found to have a dominant effect to reduce SP-A mRNA levels by inhibiting gene transcription and mRNA stability (80). The inhibitory effects of glucocorticoids are blocked by cycloheximide indicating the importance of labile protein factors in mediating the inhibitory effects (80). The reasons for the discrepancy between the two studies are not clear, but may be related to the gestational age of fetal lungs and differences in the explant culture system. In human fetal lung *in vitro* one study found that dexamethasone preferentially reduces SP-A2 mRNA levels without affecting SP-A1 mRNA levels (81) while an other study reported that dexamethasone reduces SP-A1 and SP-A2 mRNA levels equally (82). As in the case of human fetal lung tissues, glucocorticoids reduce SP-A mRNA levels in a dose-dependent manner in fetal baboon lung tissues *in vitro* and antagonize the stimulatory effects of cAMP (83). Maternal administration of glucocorticoids increases the expression of SP-A and SP-A mRNA in rats (84), rabbits (85) and sheep (86). The extent of the stimulatory effects of glucocorticoids depends on the gestational age at which they are administered and the maturational status of the fetal lungs assayed. Glucocorticoids are also effective in increasing SP-A (84-86) and SP-A mRNA levels in adult animals (39, 84, 85). Adrenelectomy do not significantly alter SP-A, SP-B and SP-C mRNA levels (87) indicating that glucocorticoids may not play a primary role in the maintenance of steady-state levels of surfactant protein mRNAs.

In contrast to their differential effects on SP-A gene expression in human fetal lung *in vitro*, glucocorticoids generally increase SP-B and SP-C mRNA expression in human (70, 88-92), rat (72, 73) and rabbit (46, 48) fetal lung tissues *in vitro*. The increases in the SP-B and SP-C mRNA levels in human fetal lung tissues *in vitro* are associated with increases in the levels of SP-B and SP-C proteins (89, 90). Protein synthesis inhibitors, such as cycloheximide and puromycin, block glucocorticoid induction of SP-B and SP-C mRNA expression indicating the requirement for continued protein synthesis or labile protein factors for glucocorticoid action (92, 93). Glucocorticoid induction of SP-B mRNA expression in fetal rabbit (46) and human (91) lung tissues *in vitro* were not associated with similar increases in gene transcription rates suggesting that post-transcriptional mechanisms also play important roles in the glucocorticoid induction. Indeed analysis of the effects of glucocorticoids on the turnover of SP-B mRNAs showed that glucocorticoids stabilize SP-B mRNA (46, 91). Glucocorticoid induction of SP-C mRNA expression in fetal rat (73) and fetal human lung (91, 93) tissues *in vitro* was associated with similar increases in gene transcription rates suggesting that transcription plays a primary role in the induction process. However, in fetal rabbit lung tissues *in vitro* glucocorticoid induction of SP-C mRNA was found to be primarily due to enhanced mRNA stability (49). Thus increased transcription rates and mRNA stability contribute to the glucocorticoid induction of SP-B and SP-C mRNA expression.

Maternal administration of glucocortcoids increases fetal expression of SP-B mRNA in rabbits (39) and rats (84) and that of SP-C mRNA in rats (84) and SP-B protein in sheep (86). Glucocorticoids also increase SP-B and SP-C mRNA levels in adult rats (87). These data indicate that glucocorticoids have similar effects to induce SP-B and SP-C gene expression in vivo as in explant culture.

Glucocorticoids increase the expression of SP-D mRNA in fetal rat (94, 95) and human lung explants (96). Maternal administration of glucocorticoids increases fetal expression of SP-D mRNA (94, 95) and SP-D protein (97) in rats. Glucocorticoids increase SP-D transcription (95) and promoter activity (98) indicating that transcriptional mechanisms are involved in the glucocorticoid induction of SP-D gene expression. Intratracheal instillation of lipopolysaccharide to rats increases the expression of SP-D and its mRNA in lungs indicating that inflammatory stress up-regulates SP-D expression (99).

Molecular mechanisms underlying transcriptional and post-transcriptional (mRNA stability) effects of glucocorticoids on SP-A gene regulation are not fully understood. Deletion mapping of human SP-A1 genomic regions identified -32/+63 bp region to be necessary for partial inhibition of SP-A promoter activity by glucocorticoids in NCI-H441 cells (100). The -32/+63 bp region of SP-A1 gene contained sequence elements similar to negative glucocorticoid response elements found in other genes and bound H441 nuclear proteins induced by glucocorticoid treatment. The identity of glucocorticoid inducible proteins interacting with SP-A1 -32/+63 bp region is not known. In transient transfection experiments in NCI-H441 cells, human SP-A 3' untranslated region(s) were found to reduce CAT expression by glucocorticoids indicating that the 3' untranslated region may contain elements necessary for post-transcriptional regulation (101). Cis-acting elements that are involved in the transcriptional and post-transcriptional regulation of SP-B and SP-C gene expression by glucocorticoids have not yet been identified. In H441 cells glucocorticoids (10^{-10} M - 10^{-6} M) had no effect on the promoter activity of constructs containing -5000/+39 bp and -2200/+39 bp of rabbit SP-B 5' flanking DNA (Boggaram, V., unpublished observations). Additionally, glucocorticoids had no effect on heterologous promoter expression from constructs containing rabbit SP-B intron 6 and intron 10 sequences that contained copies of the glucocorticoid response element (GRE) half-site, TGTTCT (Boggaram, V., unpublished observations). These data indicate that SP-B glucocorticoid response elements may be located outside of 5 kb of 5' flanking DNA and introns 6 and 10. In transient transfection assays in H441 cells, reporter constructs containing +3000 bp of human SP-D 5' flanking DNA displayed a five-fold increase in promoter activity in response to glucocorticoids and deletion to 161 bp although decreased response to glucocorticoids was still higher by two fold compared to controls (98). The glucocorticoid response region did not contain DNA elements with sequence similarity to glucocorticoid response elements suggesting that glucocorticoid regulation may not be mediated through GREs.

5.3. Regulation by other agents

Interferon-? amma increases SP-A but not SP-B and SP-C mRNA expression in human fetal lung tissues *in vitro* (102). The pro-inflammatory cytokine TNF-alpha and phorbol ester inhibit the expression of SP-A mRNA in H441 cells and fetal lung explants primarily by decreasing gene transcription (103-106). Phorbol ester inhibition of SP-A promoter activity may be mediated by an AP-1 DNA element located in the intronic region of SP-A gene (108). Recent studies have indicated that TNF-alpha inhibits SP-A mRNA expression in H441 cells via p38 signal transduction pathway (108). The inhibition of SP-B mRNA by TNF-alpha (103, 105, 109) and phorbol ester (104, 106) is mediated by transcriptional and posttranscriptional (mRNA stabilization) (106, 110, 111) mechanisms. TNF-alpha ?11) and Phorbol ester (112) response elements map within minimal SP-B promoter regions and specifically to TTF-1 and HNF-3 binding sites. The phorbol ester and TNF-alpha inhibition of SP-B mRNA expression occurs independently of NF-kappa B activation (111, 113) and is associated with decreased DNA binding activities of TTF-1 and HNF-3 elements (111, 112). The decreased nuclear TTF-1 and HNF-3 levels in phorbol ester treated H441 cells could be due to trapping of the proteins in the cytosol (112). The mechanisms responsible for cytoplasmic trapping of TTF-1 and HNF-3 are not known. TNF-alpha inhibits SP-C mRNA expression in mouse lung and lung epithelial cell lines by decreasing gene transcription and the TNF-alpha response elements are located within -320 bp of human SP-C promoter (114). Thus the inhibition of surfactant protein gene expression by TNF-alpha and other agents that act via protein kinase C may contribute to lung injury that occurs during inflammation.

TGF-beta family of proteins inhibits SP-A protein (115) and SP-A, SP-B and SP-C mRNA expression in fetal human lung tissues *in vitro* (116). TGF-beta also inhibits SP-A and SP-C mRNA expression in H441 cells (117) and in isolated type II cells (118) respectively. These data imply that TGF-beta family of proteins exerts regulatory influences on fetal lung maturation and surfactant homeostasis. TGF-beta inhibition of SP-B promoter activity was mapped to -112/-72 bp region of the human SP-B promoter that contains binding sites for TTF-1 and HNF-3 transcription factors that serve as positive regulators of transcription (119). Recent studies have indicated that in H441 cells TGF-beta inhibits SP-B promoter activity by promoting SMAD3 interactions with TTF-1 and HNF-3 that disrupts TTF-1 and HNF-3 binding to the SP-B promoter (120).

Infants born to diabetic mothers have increased incidence of RDS compared to similar gestational age infants born to non-diabetic mothers (121). As the fetus of the diabetic mother is frequently found to be hyperinsulinemic, elevated levels of insulin may have negative effects on fetal lung maturation and surfactant levels contributing to the increased incidence of RDS in such infants. Fetal rats under hyperinsulinemic conditions were found to have delayed lung maturation as assessed by lamellar body and surfactant phospholipid contents (122). Indeed the amniotic fluid levels of SP-A are reduced in diabetes (123) and insulin treatment of fetal human lung explants (124-126) and H441 cells (127) results in reduced SP-A, SP-B and SP-C mRNA expression. In a pregnant rat model of streptozotocin-induced diabetes, the developmental expression of SP-A (128), SP-B and SP-C (129) mRNAs was delayed implying that the increased incidence of RDS in infants born to diabetic mothers could be due to reduced surfactant protein expression. Insulin treatment of H441 cells reduced SP-A and SP-B gene transcription rates without any effect on the stability of mRNAs indicating that the inhibitory effects of insulin are mediated at the transcriptional level (127).

Retinoic acid, a biologically active derivative of vitamin A has divergent effects on surfactant protein gene expression. In human fetal lung *in vitro*, all-trans retinoic acid (0.3 ? M) and 9-cis-retinoic acid (1 ? M) inhibits SP-A and SP-C mRNA levels but increase SP-B mRNA levels (130, 131). In fetal rat lung *in vitro*, at 10^{-10} M all-trans retinoic acid stimulated SP-A mRNA levels without any significant effect on SP-C mRNA, whereas at $>10^{-9}$ M it inhibited SP-A mRNA but stimulated SP-B and SP-C mRNA levels (132). All-trans retinoic acid increases SP-B mRNA stability (133) and SP-B promoter activity (134) in H441 indicating that transcriptional and posttranscriptional mechanisms are necessary for retinoic acid action. Recent studies have supported a model in which RAR/retinoid X receptor, TTF-1 and co-activators such as p160 and CBP form a transcriptional complex at the enhancer that is necessary for retinoic acid stimulation of SP-B promoter activity (135, 136).

Intra-amniotic infection is associated with increased levels of IL-1 activity and increased incidence of preterm labor (137) but with decreased incidence of respiratory distress (138) in premature infants. The decreased incidence of respiratory distress in such infants indicates enhancement of lung maturation and up regulation of surfactant synthesis. Indeed intra-amniotic administration of IL-1 increases expression of SP-A and SP-B mRNAs and improves lung function in premature rabbits (139) and sheep (140). The function of IL-1 to alter the expression of surfactant protein mRNAs in fetal rabbit lung tissues *in vitro* appears to be dependent on the maturity of the lung. In immature lung (day 19 of gestation), IL-1 increases the expression of SP-A, SP-B and SP-C mRNAs but in mature lung (gestational day 27-30) it decreases the expression of surfactant protein mRNAs (141).

6. GENOMIC ORGANIZATION OF SURFACTANT PROTEIN GENES

SP-A genes of human (142-144), rabbit (145), mouse (146), rat (147) and baboon (148) are encoded by five or six exons and are approximately 5 kb in size. SP-A is encoded by a single copy gene in rats, rabbits and mice. In the human SP-A is encoded by two genes SP-A1 and SP-A2 that are 94 % identical at the nucleotide level (143, 144). A major difference between SP-A1 and SP-A2 genes is that SP-A1 gene encodes 5 exons while SP-A2 gene encodes six exons. Based on the nucleotide sequences of cDNA and genomic clones several allelic variants of human SP-A1 and SP-A2 genes have been identified (144). A pseudo SP-A gene has also been described in the humans (149). The human SP-A1, SP-A2 and the SP-A pseudogene have been mapped to chromosome 10 (150) and the mouse SP-A has been mapped to chromosome 14 (151).

A single-copy gene encodes SP-B in humans (152), mice (153) and rabbits (154). The SP-B gene is comprised of 11 exons and the size of the gene varies between 6.8-9.8 kb in different species. Whereas the rabbit SP-B gene is ~ 6.8 kb in size, the human and mouse SP-B genes are 9.4 and 9.8 kb respectively. The human and mouse SP-B genes are larger than the rabbit SP-B gene due to larger sizes of introns 2, 4, 8 and 10 of human and mouse SP-B genes. SP-B gene has been mapped to chromosome 6 in the mouse (151) and chromosome 2 in the human (155).

Human (156), mouse (157) and rabbit (158) SP-C genes are approximately 3 kb in size and are encoded by 6 exons. A single-copy gene encodes SP-C in mouse (157) and rabbit (159). Based on the differences between the nucleotide sequences of two different genomic clones it was suggested that the human SP-C is encoded by two distinct genes (156). However, the data of Southern blot analysis of human genomic DNA appears to be consistent with the existence of a single SP-C gene (160). Therefore the observed differences between the DNA sequences must be due to two different alleles and not due to two distinct genes. SP-C gene has been assigned to chromosome 14 in the mouse (151) and to chromosome 8 in the human (160).

Human SP-D gene is encoded by a single gene whose coding regions spans ~ 11 kb in size containing 8 exons (161). The collagen domain is encoded by five exons, including four tandem homologous exons (161). Sequence determinations of the protein and cDNA and genomic clones have suggested the existence of allelic variants of SP-D gene that are characterized by amino acid substitutions in the coding region. Like the human SP-A gene, the SP-D gene is localized on human chromosome 10 (161, 162). The mouse SP-D gene is approximately 13 kb in size and is organized similar to the human SP-D gene (163). The mouse SP-D gene is localized on chromosome 14 and resides contiguously with SP-A, mannose-binding lectin (Mb11) within a 55 kb region (163).

7. REGULATION OF SURFACTANT PROTEIN PROMOTERS

7.1. Regulation of SP-A promoter activity

Deletion mapping and functional studies of rabbit (164), mouse (165), rat (166), human (167), and baboon (168) SP-A genes have identified DNA sequences within 300 bp upstream of start site of transcription to be necessary for basal promoter activity in lung epithelial cells in vitro. Sequences within 300 bp upstream of start site of transcription were also found to be necessary for cAMP induction of promoter activity (164, 167, 168). Mutational analysis of putative DNA regulatory elements and analysis of DNA binding proteins by electrophoretic mobility shift assays (EMSA) and DNase I footprinting have shown that SP-A promoter activity is dependent on the binding of an array of transcription factors including, TTF-1 (165, 168-170), proteins that bind to a cyclic AMP regulatory element (CRE)-like element but are distinct from those binding to the canonical CRE (167), Sp1 and nuclear proteins distinct from Sp1 that bind to a GT box element (171), USF1 (172), B-Myb (173), GATA 6 (174) and C/EBP (175) (figure 1). Mutations of individual DNA elements significantly reduce promoter activity indicating that combinatorial or cooperative interactions between the various transcription factors are necessary for promoter function. Cyclic AMP induction of SP-A promoter activity is dependent on transcription factors that bind to CRE-like (167, 176), GT box (171), E box (177) and TTF-1 (178) elements. It has been suggested that protein kinase A induced TTF-1 phosphorylation and DNA binding activity mediate cyclic AMP induction of SP-A gene expression (178). Recent studies have indicated that cAMP mediated TTF-1 phosphorylation facilitates TTF-1 interactions with co-activator proteins CBP and SRC-1, resulting in its hyperacetylation and enhanced binding to SP-A promoter and transcriptional activity (179).

7.2. Regulation of SP-B promoter activity

Deletion mapping studies of SP-B 5' flanking DNA regions of human (180, 181) and rabbit (154) SP-B genes have identified minimal promoter regions comprising as little as -218/+41 and -236/+39 bp of DNA that confer high level promoter activity in a cell-specific manner in NCI-H441 lung epithelial cells. In contrast to the rather short promoter sequences that are necessary for human and rabbit SP-B promoter activities, the mouse SP-B promoter activity in MLE-12 lung epithelial cells is dependent on sequences within the -842 bp region (153). Mutational analysis of putative DNA regulatory elements and EMSA and DNase I footprinting analyses of nuclear proteins interacting with the minimal promoter sequences have identified binding sites for TTF-1 (182, 183), HNF-3 (182-184), Sp1/Sp3 (183), AP-1 (185) and ATF/CRE (186) transcription factors (figure 1) to be essential for promoter activity. SP-B promoter function is dependent on the

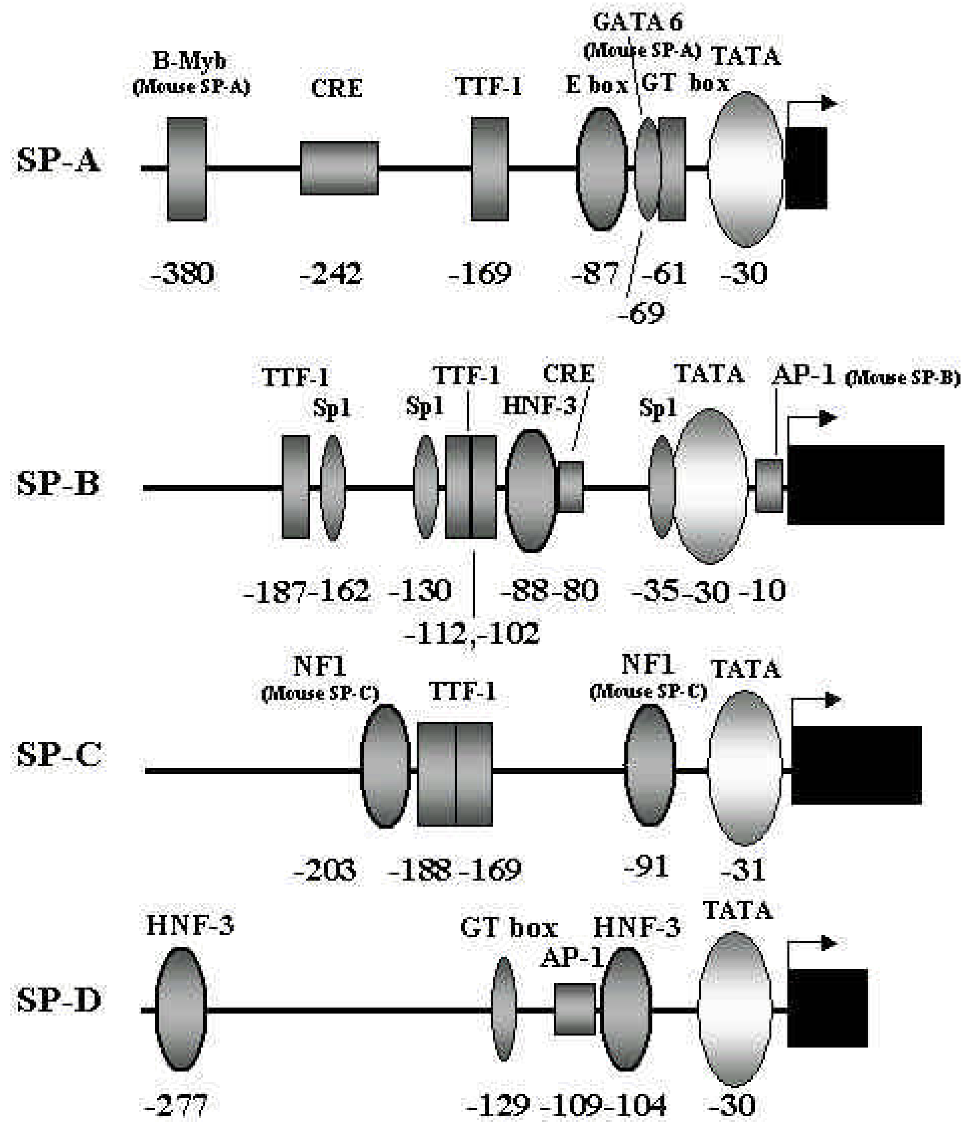

Figure 1. Schematic diagrams of minimal promoter regions of surfactant protein genes. The locations (in base pairs) of functionally important cis-DNA elements and the TATA sequence relative to the start site of transcription (arrow) are shown. The schematic diagrams of SP-A, SP-C and SP-D minimal promoters represent human surfactant protein genes and that of SP-B minimal promoter represents rabbit SP-B gene. Cis-DNA elements identified in mouse surfactant protein genes are also shown. Publications dealing with the identification of cis-DNA elements in surfactant protein promoters are referenced in section 7 of the manuscript.

functionality of each DNA element (183) and co-transfection experiments have indicated that the transcription factors interact in a combinatorial manner (187). The minimal promoter regions of rabbit, human and mouse SP-B genes are similar with regard to nucleotide sequence, placement and orientation of DNA regulatory elements. Indeed alteration of spacing between the DNA elements by insertion of half-helical and full-helical turns of DNA and alteration of orientations of DNA elements result in significant reduction of SP-B promoter activity indicating that correct helical phasing and orientation of DNA elements are necessary for promoter activity (187). Correct helical phasing and orientation of DNA elements may be necessary for the formation of a stereospecific trancriptional complex required for promoter activation (187).

7.3. Regulation of SP-C promoter activity

Deletion mapping studies of mouse SP-C 5' flanking DNA have identified DNA sequences within 320 bp upstream of start site of transcription to be necessary for expression in MLE-15 cells, a mouse lung cell line with characteristics of type II cells (188). DNase I footprinting analysis revealed multiple protein binding regions within the minimal promoter sequence indicating that the promoter regulation may be dependent on the binding of different transcription factors. The minimal promoter sequence contains multiple TTF-1 (188) and NF1 (189) binding sites that are essential for promoter activity (figure 1). Recent studies have shown that GATA-6, a member of the GATA family of zinc finger domain containing transcription factors, interacts directly with TTF-1 to activate SP-C promoter activity (190).

7.4. Regulation of SP-D promoter activity

Deletion mapping studies of human SP-D 5' flanking DNA in H441 cells identified the presence of negative regulatory elements upstream of -698 bp and positive elements between -698 and -285 bp upstream of start site of transcription (98). The SP-D promoter contains AP-1, HNF-3, GT box and C/EBP elements (figure 1) that are important for promoter activity (191, 192). Although SP-D promoter region contains a sequence motif similar to SP-B TTF-1 binding element, EMSA did not show TTF-1 binding to the SP-D sequence. Co-transfection experiments showed that SP-D promoter is not activated by TTF-1 indicating that SP-D gene expression may not be regulated by TTF-1 (191).

8. REGULATION OF SURFACTANT PROTEIN PROMOTER EXPRESSION IN TRANSGENIC MICE

Transgenic animals serve as important tools to identify and analyze genomic regions necessary for cell/tissue-specific and developmental regulation of gene expression. By analyzing the expression of human growth hormone in transgenic mice carrying various lengths of rabbit SP-A 5' flanking DNA linked to human growth hormone reporter gene, it was determined that as little as -378 bp of 5' flanking DNA is sufficient for alveolar type II and Clara cell-specific and appropriate developmental regulation of expression of the transgene (193). In certain lines of transgenic mice carrying -378 bp of SP-A 5' flanking DNA, ectopic expression of the transgene was detected in heart, thymus and spleen indicating that sequences upstream of -378 bp may be required to suppress expression in tissues other than lung. Alternatively, the ectopic expression of the transgene could be due to the positional effects of transgene integration (193).

Deletion mapping analysis of rabbit SP-B 5' flanking DNA in transgenic mice showed that the -730/+39 bp region contains necessary information for lung cell-specific expression and developmental regulation of chloramphenicol acetyltransferase (CAT) reporter gene (194). The -730/+39 bp region expressed CAT in a tissue-restricted manner in the alveolar type II cells and Clara cells of the lung similar to the endogenous mouse SP-B. CAT expression was detected in gestational day 14 fetal mouse lung and increased during development to maximal levels on gestation day 18. The developmental induction of CAT was similar to that of the endogenous SP-B. The minimal promoter region, -236/+39 bp, identified to be necessary and sufficient for promoter expression in lung cells *in vitro* also supported expression of the CAT transgene in a cell/tissue-specific manner in alveolar type II and Clara cells in transgenic mice (194). However, the expression level of CAT gene from the -236/+39 bp region was significantly lower than from the -730/+39 bp region. These data indicate that the -730/-236 bp region of rabbit SP-B gene may contain tissue-specific enhancer elements (194). Further these data indicate that alveolar type II and Clara cell-specific expression of SP-B gene is controlled by shared cis-DNA elements. Deletion analysis of 5’ flanking regions of SP-B gene identified –236/+39 bp region to be necessary and sufficient for high level expression in H441 (154) and MLE-12 (194) cells further supporting that Clara and type II cell restricted expression of SP-B gene is controlled by shared cis-DNA elements. Transgenic mice carrying -1039/+431 bp of human SP-B gene expressed CAT reporter at high levels in the lung and the developmental regulation of CAT expression was similar to SP-B (195). The expression of CAT in the lung was localized to alveolar type II and bronchiolar (Clara) epithelial cells. In addition to lung, substantial expression of CAT was found in the thyroid, trachea and intestine.

Transgenic mice containing 3.7 kb of human SP-C genomic DNA upstream of start site of transcription expressed CAT reporter gene in a restricted manner in the lung (196). CAT expression was found in alveolar type II epithelial cells and in bronchiolar epithelial (Clara) cells in contrast to SP-C that is expressed solely in alveolar type II cells (196). Further mapping of SP-C genomic regions showed that deletion of the -1910/-215 bp region of SP-C 5' flanking DNA restricted the expression of CAT to alveolar type II cells (197).

9. PERSPECTIVES

Surfactant protein gene expression is subject to unique spatial and temporal control in the lung. Molecular mechanisms underlying spatial and temporal expression of surfactant protein genes are not completely understood. A number of important cis-DNA elements and interacting transcription factors necessary for surfactant protein promoter function in lung cells *in vitro* have been identified. The locations and the orientations of the cis-DNA elements are highly conserved among surfactant protein genes. The distinct organization of surfactant protein promoters could promote unique interactions between transcription factors leading to the differential spatial and temporal expression of surfactant protein genes. Glucocorticoids alter surfactant protein gene expression via posttranscriptional regulation by modulating the stability of surfactant protein mRNAs. Cis-elements and trans-acting factors that control glucocorticoid regulation of surfactant protein mRNA stability remains to be identified. TNF-alpha, an important mediator of lung inflammation down regulates surfactant protein gene expression indicating that reduced surfactant protein levels contribute to inflammation related lung injury. An increased understanding of molecular mechanisms underlying inhibition of surfactant protein gene expression by inflammatory agents will contribute to the development of novel therapies to treat lung injury associated with inflammatory diseases of the lung.

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