Review

Regulation of surfactant protein gene transcription

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Abstract

Surfactant protein concentrations are precisely maintained during fetal development and postnatally controlled, at least in part, by the regulation of gene transcription and/or mRNA stability. Together, these mechanisms contribute to the unique temporal-spatial distribution of surfactant protein synthesis that is characteristic of the mammalian lung. Surfactant proteins A, B and C are expressed primarily in subsets of respiratory epithelial cells, wherein their expression is modified by developmental, physiological, humoral and inflammatory stimuli. Cell specific and humoral regulation of surfactant protein transcription is determined by the interactions of a number of nuclear transcription proteins that function in combination, by binding to cis-acting elements located in the 5′ regulatory regions of each of the surfactant protein genes. The unique combination of distinct and shared cis-acting elements and transcriptional proteins serves to modulate surfactant protein synthesis in the lung. The present review will summarize efforts to identify the mechanisms contributing to the regulation of surfactant protein gene transcription in the lung, focusing to the nuclear transcription factor, TTF-1 (or thyroid transcription factor-1), a member of the Nkχ2 family of nuclear transcription proteins. A complete review of regulatory aspects of surfactant homeostasis is beyond the scope of the present summary. © 1998 Elsevier Science B.V. All rights reserved.

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Contents

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1. Expression of surfactant proteins A, B and C

The cloning of cDNAs encoding the surfactant proteins provided the reagents with which to define the precise temporal-spatial patterns of surfactant protein gene expression in the lung (see [1,2] for review). The isolation and characterization of the genes encoding each of the proteins in turn has provided insights into the molecular mechanisms that govern their transcription. Study of gene transcription in vitro has been facilitated by the development of pulmonary adenocarcinoma cell lines (H441 and MLE cells) that are now widely used for the analysis of surfactant protein gene transcription. These cell lines express surfactant proteins A, B or C in vitro and are amenable to transfection of DNA [3]. In a second advance, the use of transgenic mice, wherein the cis-acting elements of surfactant protein genes have been permanently inserted into the mouse genome, has facilitated the study of the transcriptional elements from the surfactant protein and CCSP (Clara cell secretory protein) gene in vivo [4].

2. Expression of surfactant protein genes in Type II cells is regulated by extracellular signals

Rapid advances are being made in determining how specific transcription factor complexes associate with target binding sites to effect cell specific expression of surfactant protein genes. The maintenance of Type II cell specific expression of surfactant protein genes is also highly dependent on signals provided by cell-cell, cell-extracellular matrix, and growth factor stimulation in the alveolar microenvironment. Isolated Type II cells grown by conventional tissue culture techniques rapidly loose expression of SP-A, SP-B and SP-C. Culture under conditions that main-

tain Type II cell shape prolongs surfactant protein gene expression and Type II cell differentiation in vitro. Isolated Type II cells cultured on a three dimensional substrata of fetal lung fibroblasts imbedded in rat tail collagen matrix and maintained as a free floating gel maintain a cuboidal Type II cell phenotype and sustain expression of SP-A, SP-B and SP-C mRNA [5]. The precise cell shape and contact requirements provided in these experiments suggest that organization of the Type II cell cytoskeleton may influence surfactant protein gene expression. Disruption of microfilaments or microtubules in these cultures reduced SP-A, SP-B and SP-C mRNA levels [6]. Primary cultures of Type II cells supplemented with keratinocyte growth factor (KGF) increased SP-A, SP-B and SP-D mRNA levels but not SP-C mRNA [7,8]. When purified epithelial rudiments from fetal rat lungs were cultured in media supplemented with KGF, SP-C mRNA expression was detected by in situ hybridization suggesting that KGF influences differentiation of Type II cells in vitro [9].

3. Regulation of surfactant protein gene expression by inflammatory mediators

Decreased surfactant function is a common complication in recovery from inflammatory lung diseases that include ARDS and bacterial infections. Surfactant is inactivated by the leakage of serum proteins into the alveoli. Increased alveolar-capillary permeability and the release of various cytokines may influence surfactant function and expression. Intratracheal instillation of a single dose of TNF decreased SP-A, SP-B and SP-C mRNA levels in vivo. Likewise, TNF-α and phorbol esters reduced the SP-A and SP-B mRNA [10] in pulmonary adeno-
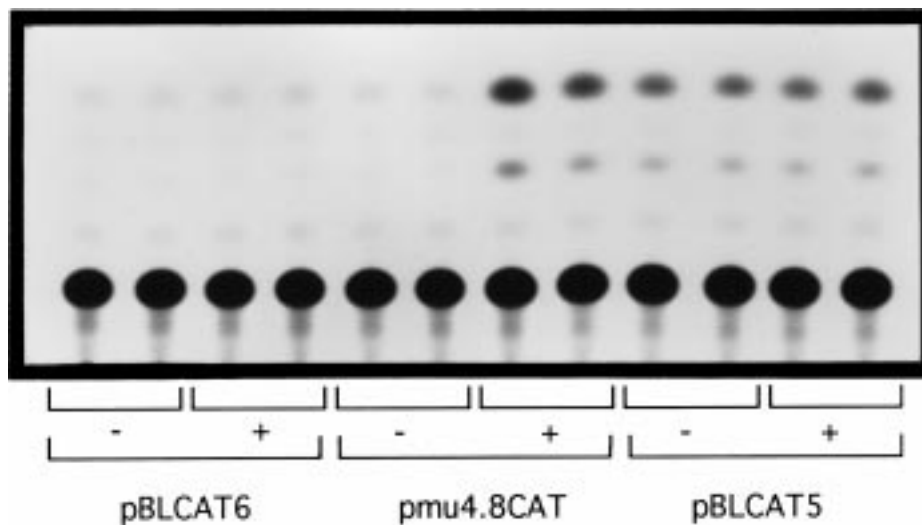
Fig. 1. The murine SP-C promoter is activated by the transcription factor TTF-1. Cat assays of extracts from cells that were transiently co-transfected with chloramphenicol acetyl transferase (CAT) reporter plasmids under control of different promoters in the presence (+) or absence (−) of a TTF-1 producing plasmid demonstrate that TTF-1 selectively activates the SP-C promoter. Acetylated reaction products (upper spots) are separated from the unreacted chloramphenicol substrate (lower spots) by thin layer chromatography. Assays were normalized for transfection efficiency to β-galactosidase activity produced by a control plasmid. The pBLCAT6 plasmid is the promoterless CAT reporter vector. CAT activity from pBLCAT6 extracts demonstrates minimal reporter activity which is not affected by co-expression of TTF-1. Specific activation of the SP-C promoter by TTF-1 is demonstrated in the next two sets of CAT assays. A pBLCAT6 plasmid containing 4.8 kb of the murine SP-C promoter and upstream sequence (pmu4.8CAT) drives low level expression in the absence of TTF-1 while co-expression of TTF-1 stimulates a 10-fold increase in SP-C-CAT activity. The selective response of the SP-C promoter to TTF-1 transactivation is further demonstrated by the assays labeled pBLCAT5, where the CAT reporter gene is under control of the herpes simplex virus thymidine kinase gene promoter (HSV-TK). Basal levels of HSV-TK driven expression is higher than unstimulated 4.8 SP-C expression but the HSV-TK promoter is unresponsive to stimulation by TTF-1. Assays of duplicate transfections are shown side by side.

carcinoma cells by mechanisms independent of NFkB activation [11]. In MLE cells, TNF-α decreased SP-C mRNA levels and decreased the transcriptional activity of SP-C promoter constructs. The cis-acting element mediating inhibitory effects of TNF-α on SP-C gene transcription was mapped to within 320 bp of the start of transcription [12].

4. Transcription factors determine surfactant protein gene expression

Transcription proteins exert their effects on gene expression by binding directly or indirectly to cis-acting elements often located near or within the gene locus in the chromosome. Proteins binding to DNA may influence chromatin structure generally or specifically, by altering protein-DNA interactions that influence function of the basal transcriptional complex usually located near the start of transcrip-

tion in pol II dependent promoters. Analysis of the mechanisms underlying surfactant protein B gene expression has been particularly useful in identifying the role of several transcription factors controlling surfactant protein gene expression [13]. TTF-1, a 38 kDa homeodomain containing transcription factor and a member of Nkχ2 family of nuclear transcription proteins, binds to and activates promoters of the surfactant proteins A [14], B [13], C [15] and CCSP (Clara cell secretory protein) [16] genes (Figs. 1 and 2). In the thyroid, TTF-1 activates the transcription of thyroglobin, thyroperoxidase and the sodium-iodide (Na/I) transport protein [17–19].

4.1. TTF-1 binds to complex cis-acting elements

TTF-1 binds to consensus elements CAAG located at multiple sites in the 5′ flanking regions of the target genes presently identified [13–15]. Sites are
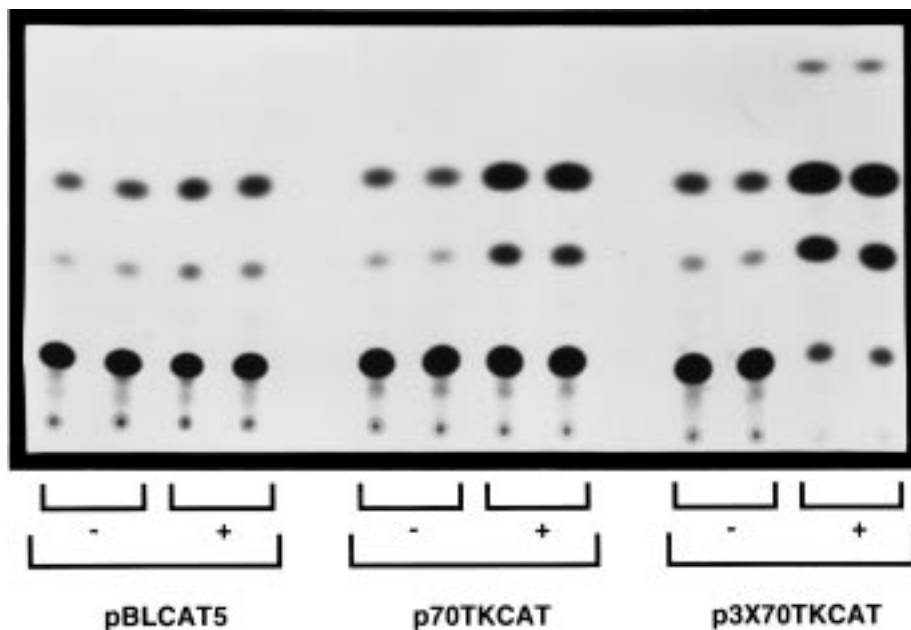
Fig. 2. Fusion of SP-C sequences to HSV-TK promoter constructs defines the TTF-1 responsive region. The TTF-1 responsive region of the SP-C promoter was mapped by deletion analysis to −320 bp of the transcription start site [15]. This region was then tested for its ability to confer TTF-1 responsiveness on the HSV-TK promoter. In transient co-transfection experiments the unaltered HSV-TK promoter (pBLCAT5) was not stimulated by co-expression of TTF-1. When a 72 bp fragment of SP-C DNA spanning the candidate TTF-1 elements was fused to the TK promoter (p 70 TK-CAT), TTF-1 (+) stimulated CAT expression over expression levels obtained in the absence of TTF-1 (−). Oligomerization of three copies of the 72 bp region on the HSV-TK promoter plasmid increased the magnitude of TTF-1 transactivation (p 3×70 TK-CAT). The TTF-1 responsive region of the SP-C promoter was further subdivided and analyzed by site specific mutagenesis and biochemical analysis to define sites of TTF-1 binding and activation.

often clustered in groups of two or more binding sites that serve as cell specific elements or enhancers (Fig. 3). TTF-1 sites are often found in close proximity to binding sites for other nuclear transcription proteins, including the retinoic acid receptor element (RARE), HNF-3 (hepatocyte nuclear factor family members), AP-1, CREB, myb, etc. Binding of these various nuclear transcription factors, singly or in combination, may (1) stabilize higher order complexes with TTF-1, (2) interfere with or enhance TTF-1 binding or function, or (3) alter chromatin structure in the region to change DNA binding protein interactions that influence gene transcription positively or negatively.

Several other modifications of the TTF-1 protein have been identified that influence TTF-1 function and may add to the complexity of surfactant protein regulation. TTF-1 has the ability to form covalently linked dimers and oligomers that have reduced DNA binding affinity [20]. TTF-1 binding activity is also affected by redox regulation. Oxidation of TTF-1

reduced TTF-1 binding affinity [20]. A diversity of TTF-1 molecules may also be generated at the level of transcription. Analysis of TTF-1 genomic sequences provides evidence that at least two distinct transcription start sites can generate alternative TTF-1 mRNA isoforms. cDNA clones encoded by transcription from both TTF-1 promoters have been recently identified and partially characterized [21,22]. It is unknown if the TTF-1 encoded by these longer transcripts has altered binding and activation activity.

In general, mutations of TTF-1 binding sites in the proximal promoter regions of the surfactant protein genes, markedly inhibit lung cell specific transcriptional activity. For example, mutagenesis of a proximal element located −80 to −100 of the SP-B promoter completely blocked SP-B gene transcription in vitro. On the other hand, mutation of the TTF-1 binding sites located in the enhancer element −400 in the SP-B gene inhibits, but does not ablate, cell selective activity of the SP-B promoter [13,23]. Thus

the proximal element serves to maintain cell selective expression and the distal element serves to act as a lung cell selective enhancer. Mutation of a HNF-3 binding site located near the TTF-1 site in the SP-B gene promoter also markedly inhibited gene transcription [13]. This element is located near a site of DNAse hypersensitivity, suggesting that the binding of HNF-3β alters chromatin structure, allowing unique combinatorial interactions among various transcription factors at the site. In general, complex combinatorial interactions among a number of nuclear transcription proteins and their binding sites, achieves control of surfactant protein gene expression in a cell selective manner.

4.2. Phosphorylation of TTF-1

TTF-1 is phosphorylated by both cAMP and calcium activated protein kinase in both thyroid and respiratory epithelial cells [24,25]. Phosphorylation sites have been mapped by mutagenesis studies, identifying the regions in both the amino and carboxy terminal domains that are phosphorylated [24,25]. TTF-1 is also phosphorylated by the rat homolog of the MST kinases [26]. MST (mammalian sterile) kinases are a distinct family of ubiquitous serine/threonine kinases. The MST kinases are not members of the mitogen activated protein kinases nor of the stress activated kinases. Thus, TTF-1 is a unique target of a constitutive protein kinase. Cyclic AMP increases the surfactant protein A and B gene expression in fetal lung explants and in adenocarcinoma cells in vitro. Co-transfection of pulmonary adenocarcinoma cells with a plasmid expressing the catalytic subunit of cAMP dependent protein kinase, cat-β activates SP-B expression in association with phosphorylation of TTF-1 [25]. Effects of cAMP on gene transcription are also modulated by binding of CREB to CRE elements in a number of target genes. However in pulmonary adenocarcinoma cells, co-transfection with CREB inhibited SP-B gene transcription. Thus, the effects of cAMP, at least in part, are regulated by a TTF-1 dependent mechanism rather than through CRE occupancy. C kinase activity also phosphorylates TTF-1; however, the precise regulatory role of this phosphorylation remains unclear.

4.3. Nuclear localization of TTF-1

Staining for TTF-1 is confined to the nuclei of cells in the central nervous system, thyroid and respiratory epithelial cells [27]. Site directed mutagenesis of the TTF-1 polypeptide identified the regions involved in nuclear transport, residing in the amino terminus and homeodomain regions of the molecule [28]. Since TTF-1 concentrations influence the occupancy of the nuclear complexes at TTF-1 binding sites and target genes, changes in the nuclear concentration of TTF-1 may strongly influence surfactant protein gene expression. Recent work demonstrated decreased nuclear staining of both TTF-1 and HNF-3β in adenocarcinoma cells treated with phorbol esters. These studies suggest that cytoplasmic trapping of the transcription factors perhaps mediated by changes in phosphorylation, contribute to changes in surfactant protein gene expression in this model [29].

4.4. Pattern of HNF-3β and TTF-1 expression in the respiratory epithelium supports their role in organogenesis and surfactant protein gene expression

TTF-1 is expressed early in pulmonary organogenesis, being detected in the nuclei of epithelial cells in the lung primordia at the onset of lung formation [27,30]. TTF-1 gene transcription is induced by HNF-3β [31], the latter gene being critical to formation of the dorsal-ventral axis and foregut endoderm [32]. TTF-1 is expressed in the developing lung buds and is increasingly restricted to the periphery of respiratory tubules during lung development. Expression of TTF-1 decreases in the postnatal lung, being detected primarily in Type II cells in the alveolus, and being absent in Type I cells in the postnatal lung [30,33]. Gene targeting of TTF-1 in transgenic mice caused severe pulmonary hypoplasia, associated with an absence of expression of surfactant proteins in the remaining epithelium [34]. While lung parenchyma was completely ablated in the TTF-1 (−/−) mice, the trachea and proximal bronchi were relatively intact. The temporal-spatial distribution of TTF-1 and its demonstrated critical role in the formation of the lung parenchyma supports its primary role as a determinant of lung morphogenesis, as well
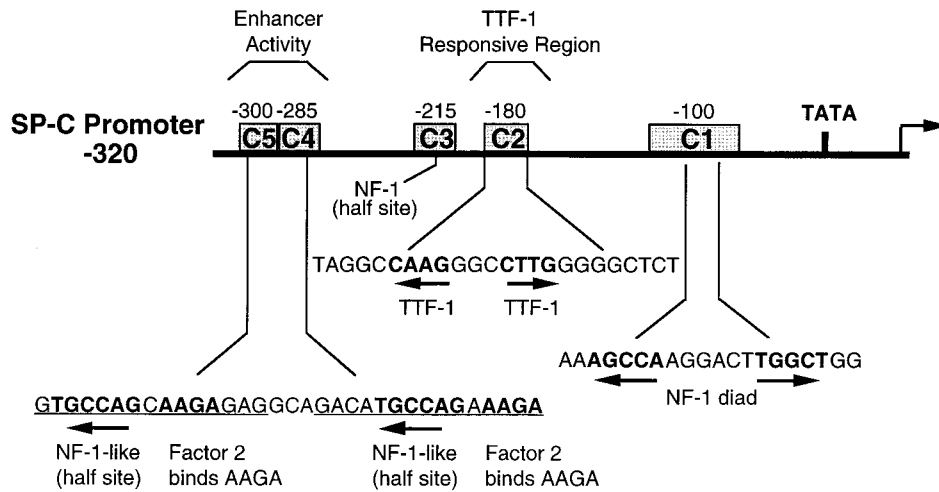
Fig. 3. Multiple protein to DNA interactions on the proximal SP-C promoter contribute to regulation of SP-C transcription. The configurations of known cis-active regulatory elements are diagramed relative to the transcription start site (arrow). Boxed regions labeled C1 to C5 denote sites of protein-DNA interaction determined by DNAse footprint analysis. Brackets indicate functionally defined regulatory regions (TTF-1 responsive and cell selective enhancer). Known transcription factor binding sites are indicated on SP-C sequence expanded below the indicated footprint sites. An extended NFI site in footprint C1 and NFI half site in C3 are required for basal SP-C promoter activity. Essential TTF-1 binding sites are indicated below footprint C2. Multiple protein interactions are detected in the SP-C enhancer which co-localizes to footprints C4–C5. In both C4 and C5 an NFI family member binds to a half site motif while an unknown factor(s) bind to an adjacent AAGA sequence. How the enhancer region interacts with proximal elements to confer precise cell specific transcription is unknown. Arrows indicate the relative orientation of consensus binding site motifs on the coding strand.
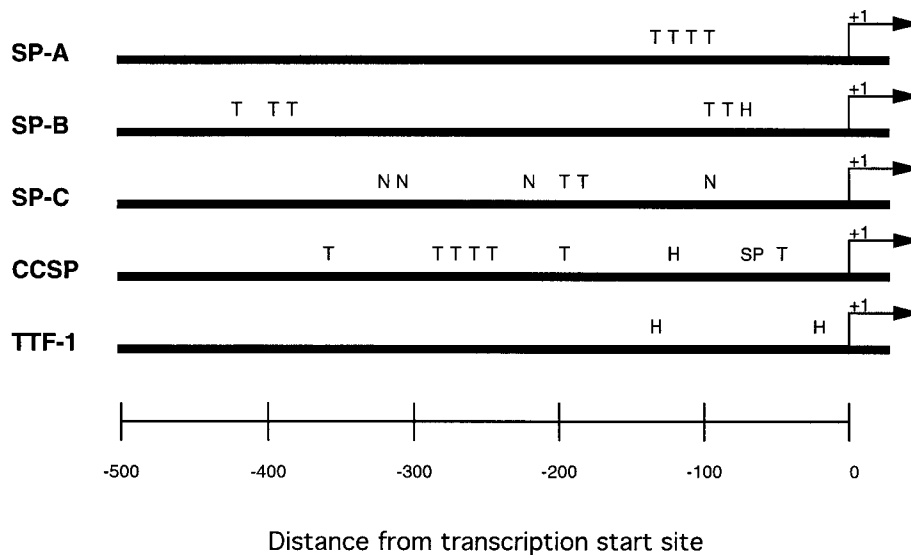

Distance from transcription start site

Fig. 4. Summary of transcription factor binding sites associated with genes that are expressed in the lung. The location of transcription factor binding sites are shown relative to the basal promoter and transcription start site (+1) of surfactant proteins A, B, C, Clara cell secretory protein, and thyroid transcription factor 1 genes. Binding sites are identified for TTF-1 (T), hepatocyte nuclear factor 3β (H), SV40 promoter factors (SP) SP-1, SP-3, and nuclear factor 1 (N). The number and position of binding sites for a specific factor is different for each gene expressed selectively in the lung. These data suggest that the different combinations of factors effect the distinct patterns and levels of expression of each gene. Data has been obtained for the murine SP-A and SP-C genes, human SP-B and TTF-1 genes, and murine CCSP gene.

as respiratory epithelial cell differentiation and surfactant protein gene expression. TTF-1 expression is increased in regenerating respiratory tubules following lung injury, being detected at high levels in the respiratory epithelial buds in infants with cystic fibrosis, bronchopulmonary dysplasia and other forms of severe lung injury.

4.5. Transcriptional activation of surfactant protein gene expression by NFI

The nuclear factor I (NFI) family of transcription factors consists of four distinct genes, each of which is differentially spliced to produce multiple isoforms of NFI. NFI was originally identified as a transcription factor that activated adenoviral gene expression. NFI has subsequently been shown to be expressed in many organs and to activate a large number of mammalian genes. NFI expression in the developing mouse lung was examined by in situ hybridization using probes specific for each of four NFI genes [35]. NFI mRNA was detected by fetal day 11.5 and increased during lung development. The NFI-B gene was expressed at highest levels in the developing lung. Lower levels of NFI-A, NFI-C and NFI-χ mRNA expression were detected and localized to multiple cell types.

Multiple NFI consensus binding half sites (TGGCA) were associated with DNAse I protected regions of the SP-C promoter and suggested a role for NFI in activating SP-C gene expression [36]. Mutations of NFI sites in a region located -312 to -97 in the SP-C promoter inhibited activation by NFI. These results are summarized in Figs. 3 and 4. Differential splicing of four NFI genes can produce 18 distinct isoforms of NFI that have been identified to date. Furthermore, NFI proteins form homodimers or heterodimers that may influence surfactant protein gene expression.

5. Role of glucocorticoids

Maternal corticosteroids are routinely used to prevent RDS in preterm infants and are used postnatally to enhance lung function in preterm infants with bronchopulmonary dysplasia. Glucocorticoids enhance surfactant protein B and C mRNA in fetal

lung explants and in cell culture in vitro and in vivo [37–40]. Both inhibitory and stimulatory effects of glucocorticoids have been observed on SP-A mRNA production and are mediated by changes in mRNA stability and/or gene transcription [41–44]. The effects of glucocorticoids on surfactant protein expression vary with cell type, dose and species. Effects of glucocorticoids in H441 cells are modulated primarily by changes in SP-B mRNA stability rather than by gene transcription [40]. Glucocorticoid dependent changes in surfactant protein gene transcription are modest and are likely mediated indirectly rather than by direct binding of the glucocorticoid receptor (GR) to the surfactant protein genes. Surfactant protein mRNA and immunostaining are virtually unaltered in GR $(-/-)$ mice generated by gene targeting [45]. These results demonstrate that the GR is not required for surfactant protein gene expression in the perinatal and postnatal periods. Mice with a targeted mutation of the corticotrophin releasing factor (CRF) gene were reported with immature lungs and a 44% reduction in SP-B mRNA [46]. These results are distinct from the direct mutation of the GR gene where the levels of SP-A, SP-B, SP-C mRNA expression and the tempo-spacial patterns of SP-B and SP-C expression were unchanged.

6. Respiratory adaptation and surfactant protein gene expression

Surfactant proteins play important roles in host defense (SP-A and SP-D), and surfactant function and metabolism (SP-B and SP-C). Evidence from mice with targeted disruption of SP-A or SP-B demonstrate the critical role of gene dosage in mRNA expression [47,48]. Heterozygous SP-A $(+/-)$ and heterozygous SP-B $(+/-)$ mice express approximately 50% of protein and mRNA in neonates and/or adults compared to wild-type littermates. Even a 50% reduction in surfactant protein B mRNA and protein caused discernable changes in lung function in vivo [49]. SP-B $(+/-)$ mice are susceptible to lung injury following oxygen exposure and have decreased lung compliance, under normal vivarium conditions [50]. Thus, changes in surfactant protein expression accompany lung differentiation, oxygen toxicity, ARDS, RDS, lung inflammation, and infection. It

is hoped that understanding mechanisms controlling surfactant protein production will provide the basis for strategies to optimize surfactant concentrations in clinical settings, whether by replacement of surfactant proteins or by modulation of surfactant protein gene transcription or mRNA stability.

7. Unresolved issues and future perspectives

The surfactant protein genes are expressed in distinct subsets of differentiated cells. SP-A, SP-B and SP-C are expressed in the alveolar Type II cells, while SP-A and SP-B are also expressed in distal airway epithelial cells. Our knowledge of how these genes are differentially expressed is incomplete. Functional analysis of the surfactant protein gene promoters in mouse lung epithelial cell lines have provided the first evidence as to how cell specific expression is achieved. Co-transfection experiments demonstrated that the transcription factor TTF-1 activated the surfactant protein gene promoters. Selective activation by TTF may be due to differential phosphorylation of TTF, distinct isoforms of TTF-1 and varying cellular concentrations of TTF-1. Specific activation by TTF-1 may also be conferred by the distinct arrays of TTF-1 binding sites consisting of different numbers, orientations and binding site affinities for TTF-1 in each promoter region examined. While specificity of expression can be generated by unique TTF-1 transcriptional complexes other factors play a central role in cell specific and precise temporal-spacial activation of surfactant protein genes. HNF-3β stimulates human SP-B expression and also stimulates TTF-1 gene expression. HNF-3β is expressed in the developing foregut endoderm and is one of a larger family of HNF and HFH (HNF homolog) transcription factors that are expressed in the developing lung. In addition, the SP-C promoter has been shown to be stimulated by NFI family members. All four NFI genes are expressed in the lung producing numerous distinct isoforms which could selectively modify patterns of expression. These data provide insights into a network of regulatory molecules involving variants of tissue restricted and ubiquitous transcription factors which bind and activate cell specific expression of surfactant protein genes. These in vitro experimental approaches will not elucidate the role of higher ordered chromatin structure in effecting regulated access to DNA binding sites or the dynamic positioning of distal enhancers relative to cell selective regulatory elements. The use of transgenic mice for in vivo promoter studies will be necessary to identify the chromatin dependent regulatory events that complement the transcription factor binding studies described in this report.

Acknowledgements

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