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Review

Genetics of the hydrophobic surfactant proteins

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Abstract

The hydrophobic surfactant proteins, SP-B and SP-C, serve important roles in surfactant function and metabolism. Both proteins are encoded by single genes, located on human chromosomes 2 and 8 respectively, which have been characterized and extensively studied. Mutations in the SP-B gene have been shown to cause severe lung disease, and polymorphisms in the SP-B gene may be associated with the development of RDS in premature infants. In contrast, mutations in the SP-C gene have not yet been identified or shown to cause lung disease, although given the apparent importance of SP-C in surfactant function, this remains a possibility.

Keywords: Respiratory distress syndrome; Surfactant protein; Alveolar proteinosis; Hyaline membrane disease; Bronchopulmonary dysplasia; Interstitial lung disease; Polymorphism; Prematurity

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Abbreviations: RDS, respiratory distress syndrome; SP, surfactant protein; TTF-1, thyroid transcription factor 1; HNF, hepatocyte nuclear factor; PAP, pulmonary alveolar proteinosis; GM-CSF, granulocyte macrophage colony stimulating factor; RFLP, restriction fragment length polymorphism

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1. Introduction

Pulmonary surfactant is the mixture of lipids and specific proteins needed to reduce alveolar surface tension and prevent end-expiratory collapse. An inability to produce sufficient amounts of surfactant due to pulmonary immaturity is the principal cause of the respiratory distress syndrome observed in infants born prematurely [1]. Pulmonary surfactant contains at least four distinct specific proteins which have been identified to date. These include SP-A and SP-D, which are larger, hydrophilic proteins, and two smaller, extremely hydrophobic proteins, called SP-B and SP-C. The hydrophobic proteins appear to be critical for surfactant's ability to lower surface tension by enhancing adsorption and spreading of surfactant phospholipid to the air-liquid interface [2]. Both SP-B and SP-C are important components of mammalian derived exogenous surfactant preparations used to treat infants with RDS such as Survanta™, Curosurf™, and Infasurf™. The importance of SP-B in normal surfactant function is indicated by the observations that infants or experimental animals unable to produce SP-B develop lethal neonatal respiratory disease [3], and polymorphisms in the SP-B gene were observed more frequently in infants with RDS than in a control population [4]. Similar abnormalities in the SP-C gene have not yet been observed. This review will focus on the contribution of variations in the SP-B and SP-C genes and their established and potential roles in causing lung disease.

2. Surfactant protein B

SP-B is encoded on a single gene located on the short arm on human chromosome 2, and has been mapped by in situ hybridization to 2p12→2p11.2 [5]. The complete human, as well as murine, and rabbit sequences, have been characterized [6]. It is a relatively small gene, spanning approximately 9500 bases. It is comprised of 11 exons, of which the 11th exon is untranslated. The organization of the murine and rabbit genes are similar in terms of the number of exons, and relative size, although the overall size of the gene is somewhat smaller in both the mouse and rabbit due to the shorter size of several introns. The production of SP-B is lung specific, and within the lung its expression is limited to nonciliated bronchiolar epithelial cells (Clara cells) and alveolar type II cells. Although SP-B was the second surfactant associated protein to be described, the name of the SP-B locus is referred to as *sftp3*.

The 5′ flanking region of the SP-B gene has been extensively characterized, and contains sequences that are important in conferring cell and tissue specificity of expression [7]. Proximal (nucleotides −80 to −110) and more distal (−331 to −439) cis-acting elements that interact with the transcription factors TTF-1 and members of the HNF-3 family have been identified that appear to be particularly important for SP-B expression [8,9]. Additional sequences in this region with both non-specific and cell specific, and positive and negative effects on SP-B gene transcription, have been identified [10].

The SP-B gene is transcribed into a 2000 base mRNA, which directs the synthesis of a 381 amino acid preproprotein. A 23 amino acid signal peptide, encoded in the first exon, is removed co-translationally. The resulting proprotein undergoes proteolysis in at least two distinct steps to yield the mature 79 amino acid, 8700 Da protein found in the airspaces [7]. Mature SP-B is encoded in exons 6 and 7 of the SP-B gene. The first proteolytic processing step removes the aminoterminal portion of the proprotein, whereas the second removes the carboxyterminal portion (Fig. 1). These latter processing steps are specific to alveolar type II cells [11]. The enzymes and the genes responsible for these processing steps have not yet been identified. The protein sequences of mature porcine and bovine SP-B have been directly determined [12,13], along with deduced amino acid sequences determined from cDNA sequences from a number of species [6,14], and are highly con-

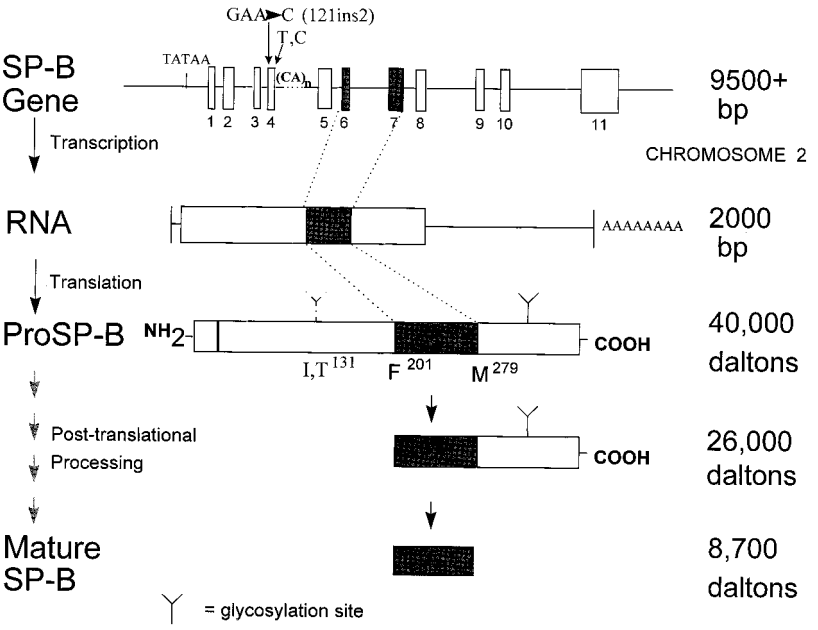

Fig. 1. SP-B gene, mRNA, proprotein and processing. Exons and translated portions of the mRNA are represented by boxes; introns and untranslated portions are represented by lines. The location of the 121ins2 mutation, an exon 4 coding polymorphism, and the intron 4 CA repeat polymorphism are shown. Amino acids encoded by the exon 4 polymorphism, and the start and end of mature SP-B are indicated by their single letter abbreviation.

served with $>80\%$ homology. The structure and properties of SP-B are reviewed elsewhere in this issue by Dr. S. Hawgood.

3. Hereditary SP-B deficiency

3.1. History and clinical aspects

Infants who are unable to produce SP-B develop fatal lung disease, demonstrating the importance of SP-B in normal lung function. The disease was first recognized in 1993 in a family in which three full-term infants died from lung disease of neonatal onset. Both parents and three siblings were free of pulmonary symptoms suggesting an autosomal recessive pattern of inheritance [3]. In all three affected infants, SP-B protein was undetectable in lung tissue by immunologic methods, while SP-A and the precursor protein of SP-C were present in amounts comparable to those in control tissues. SP-B mRNA was also undetectable by Northern blot analysis, supporting the hypothesis that the primary cause of disease was an inability to produce SP-B. SP-B deficiency was established as the cause of disease when the affected infants were shown to be homozygous for a frameshift mutation in exon 4 of the SP-B gene that accounted for the lack of SP-B protein [15].

Since the initial description of the disorder, more than 35 families with infants affected by hereditary SP-B deficiency have been identified (L. Nogee, unpublished observation). Affected infants have generally been born at or near term, and developed severe respiratory symptoms shortly after birth. The presenting clinical signs and symptoms are those of surfactant deficiency, including tachypnea, grunting, flaring, retractions and need for supplemental oxygen. Pulmonary hypertension is a frequent component of the clinical picture, and it is not uncommon for SP-B deficient infants to be treated with extracorporeal life support when available. The radiographic appearance most often resembles that of RDS in premature infants, although some affected infants have not had prominent infiltrates on chest radiographs early on in the course of the illness, and some have had more of an interstitial rather than alveolar pattern. The clinical diagnosis most often ascribed to such infants has been RDS, although affected infants have been thought to have persistent pulmonary hypertension, presumed sepsis with neg-

ative blood cultures, meconium aspiration syndrome, and transient tachypnea of the newborn. The hypoxic respiratory failure in SP-B deficient infants is severe and progressive, and almost all affected infants have been treated with mechanical ventilation, and often with high frequency ventilation. Affected infants may transiently improve following treatment with exogenous surfactant preparations containing SP-B, but the response is not sustained. Repeated doses (almost 80) were ineffective in preventing respiratory failure and death in an antenatally diagnosed infant in whom surfactant replacement therapy was initiated at birth [16]. Variable improvement has been seen in some children with corticosteroid therapy [17]. The disease is ultimately fatal, although with aggressive supportive therapy SP-B deficient infants may survive for many months. The costs of caring for these children are high, both in economic terms and for the stresses imposed on the family.

3.2. Pathology and pathophysiology

The lung pathology of SP-B deficient infants is variable. The predominant findings are often nonspecific changes due to lung injury, including alveolar cell hyperplasia and interstitial fibrosis [3,18]. One particularly striking finding on histopathologic examination of the lung tissue of many SP-B deficient infants is the accumulation of granular, eosinophilic material that stains positively with periodic acid-Schiff reagent filling distal air spaces. Foamy macrophages and desquamated alveolar cells are also observed trapped in the alveolar material. This appearance has been referred to as alveolar proteinosis, because of the similarity in appearance to that of adults and older children with this disorder. The nature of the proteinosis material is different, in that it lacks SP-B, whereas the alveolar proteinosis material in older children or adults contains abundant SP-B, as well as SP-A, SP-C, and surfactant phospholipid. The amount of proteinaceous material observed is variable and may be minimal in some cases. It is unknown whether the variability in the amount of proteinaceous material is related to effects of treatment, such as mode of ventilation or surfactant therapy, duration of survival, or natural biologic variability of the disease. Additionally, mechanisms other than SP-B deficiency can result in the histopathological picture of alveolar proteinosis. Mice in whom the genes for granulocyte macrophage colony stimulating factor (GM-CSF) or its receptor were inactivated developed these histopathologic findings [19,20], and a deficiency of a component of the GM-CSF receptor has recently been implicated in the pathogenesis of alveolar proteinosis in human infants [21], indicating that other genetic mechanisms can produce similar pathology. Thus the term 'hereditary SP-B deficiency' more accurately describes infants genetically unable to produce SP-B, rather than the term 'congenital alveolar proteinosis', which may result from mechanisms other than SP-B deficiency.

The incidence of SP-B deficiency is unknown, although the disease appears to be quite rare. A disproportionate number of families have had more than one child affected with this recessive disorder, suggesting that in the absence of a family history of neonatal respiratory disease cases have gone unrecognized. The extent of phenotypic variation is also unknown. The report of an infant with unexplained chronic lung disease associated with reduced production of SP-B supports the notion that milder forms of the disease may be observed [17].

Experimental animals in whom antibodies directed against mature SP-B were instilled intratracheally developed severe lung disease, supporting the role of the mature peptide in normal lung function [22,23]. However, the pathophysiology of SP-B deficiency appears to be more complicated than simply related to the lack of the mature protein. Unexpectedly, a number of secondary alterations in the content and metabolism of other surfactant components have been observed in SP-B deficient infants, suggesting additional roles for SP-B or its precursor protein in surfactant metabolism.

The lung tissue of all SP-B deficient infants studied to date contains abundant 6000 to 12 000 Da peptides that are strongly immunoreactive with antisera directed against the SP-C proprotein. These peptides are secreted into the lung fluid, and result in intense immunohistochemical staining of the proteinaceous material with anti-proSP-C antisera. Using antisera directed against different epitopes of the SP-C proprotein, Vorbroker et al. concluded that these peptides contain the aminoterminal portion of the

proSP-C molecule, and likely result from incomplete processing of the proprotein [24].

The mechanism whereby the lack of SP-B interferes with SP-C processing remain unknown. Possible mechanisms could include a direct role for SP-B, or proSP-B or one of its domains in the proteolysis of proSP-C. Another possibility may relate to other secondary abnormalities within the type II cell. Ultrastructural analysis of the lung tissue of SP-B deficient infants revealed reduced numbers of and poorly formed lamellar bodies, the intracellular storage organelles of surfactant [25]. As the final processing steps of SP-C take place in a distal cellular compartment (multivesicular bodies or lamellar bodies), the lack of the processing organelle could lead to disruption of SP-C processing.

Abnormalities in surfactant phospholipids have also been observed in SP-B deficient infants. Amniotic fluid analyses from two affected infants demonstrated very abnormal phospholipid profiles at term, with lecithin:sphingomyelin ratios of <1.0 (normal >2.0 at 38 weeks gestation), and an absence of phosphatidylglycerol [16,26]. Phosphatidylglycerol was also markedly reduced in the lung fluid and tissue of infants who underwent lung transplantation [26]. SP-A metabolism may also be altered in the absence of SP-B. While the total amount of SP-A in lung fluid and tissue did not differ markedly from that of controls, immunohistochemical staining for SP-A in lung tissue of deficient infants demonstrated intense staining of the extracellular proteinaceous material, however epithelial cell staining for SP-A staining was sparse compared that of control lung tissues [18]. The functional significance of alterations in SP-A metabolism remains unknown.

The proteinaceous material that accumulates in the airspaces of SP-B deficient infants thus contains abundant amounts of SP-A and aberrant SP-C, and a relative paucity of surfactant phospholipid. Mature SP-C protein was not detected in lung or amniotic fluid from SP-B deficient infants [24]. Thus the respiratory failure in SP-B deficient infants may result not only from the lack of SP-B, but possibly from the lack of SP-C, the accumulation of non-surface active proteins, and derangement of type II cell intracellular metabolism. The mechanisms underlying these secondary changes in surfactant metabolism remain to be elucidated. One hypothesis is that SP-B, or other domains of proSP-B, may be necessary for the proper formation of lamellar bodies. In the absence of the proper development of this intracellular organelle, surfactant phospholipid and proteins cannot be packaged properly. Clarification of the mechanisms involved in derangement of surfactant metabolism in SP-B deficiency should provide insights into type II cell function.

3.3. Genetics of SP-B deficiency

The common mutation responsible for SP-B deficiency involves a substitution of GAA for C in codon 121, a net 2 base pair insertion that causes a frameshift, and premature signal for the termination of translation, accounting for the lack of SP-B protein [15]. This mutation, termed 121ins2, has accounted for approximately 2/3 of the mutant alleles identified to date. It has been found principally in families of Northern European, Anglo-Saxon, and Scottish-Irish descent. However affected infants have been identified in other ethnic groups, including families of Middle Eastern, Mediterrean, and Asian descent. It is not known whether the common mutation resulted from a founder effect, or due to a mechanism causing a recurrent de novo mutation at the same site. While the exact population frequency of the 121ins2 mutation is also unknown, it appears to be low. Thus identification of this mutation in patients with clinical features of SP-B deficiency can be useful diagnostically [26]. Two other mutations have been described, both of which result in only a single amino acid change, and allow for production of proSP-B [17,27]. Clinical disease associated with production of abnormal SP-B peptides is possible. Other mechanisms that could result in SP-B deficiency, which have not yet been observed, include deletions or rearrangements of the SP-B gene, mutations in sequences important for SP-B gene regulation, or mutations in other genes encoding proteins needed for SP-B gene transcription. As proSP-B undergoes proteolytic processing to yield the mature peptide, mutations in the gene needed for SP-B processing could also potentially result in disease.

3.4. Treatment

Treatment options for SP-B deficient infants re-

main limited. The disease is invariably fatal, and currently the only available treatment options for SP-B deficient infants are provision of compassionate care for the infant and family, or lung transplantation. Experience with lung transplantation for SP-B deficiency has been reported in three infants [26]. One infant died of adenoviral infection shortly after the procedure, however two others have had encouraging short-term results. Experience with lung transplantation in infants is limited however, and the long-term outlook for these children is unknown. It is unlikely that protein replacement with the mature peptide would be sufficient to adequately treat the disease. SP-B replacement by administration of multiple (>80) doses of exogenous surfactants containing bovine SP-B was attempted in an antenatally diagnosed SP-B deficient infant beginning at birth [13]. While this child's initial lung disease was less severe than that of a previous sibling, his lung disease still progressed and he died awaiting lung transplantation. Post-mortem analysis of the lung tissue did not exhibit alveolar staining for SP-B suggesting that the exogenously administered SP-B was not incorporated intracellularly. Multinucleated giant cells within granulomata that stained positively for SP-B were observed in lung tissue, suggesting that the exogenously administered SP-B was not metabolized normally. Additionally, the defect in SP-C processing was not corrected. It is possible that the amounts of SP-B used to treat this infant were insufficient, or that there was toxicity due to the other surfactant components administered. Purified preparations of SP-B for aerosolization or intratracheal administration are not available. It is likely however that intracellular synthesis of SP-B will be necessary to correct the metabolic alterations in surfactant processing within the type II cell. This consideration, along with the tissue specificity of SP-B expression, the rapidly fatal nature of the disease, and lack of treatment alternatives, make SP-B deficiency an important disease model for gene therapy in the lung. Preliminary experience with viral vectors capable of directing SP-B synthesis in tissue culture and experimental animals has been reported, but considerably more work needs to be done before they are ready for clinical use [28–30].

3.5. Animal models of SP-B deficiency

Clark and colleagues developed SP-B 'knock-out' mice by targeted disruption of the SP-B gene [31]. Homozygous SP-B deficient mice lacked SP-B specific mRNA and protein, and did not expand their lungs and died within minutes of birth. This mouse model resembles the human disease in that SP-C is also not processed appropriately, and lamellar body formation is disrupted. These animals provide an invaluable tool for studying the pathophysiology of the disease, and for studying potential therapeutic options. Akinbi and colleagues generated a transgenic mouse line who carried an SP-B gene construct which encoded the aminoterminal portion and mature SP-B peptide, but lacked the carboxyterminal portion of proSP-B, under the control of the SP-C promoter [32]. These mice expressed SP-B from the transgene, and were crossbred with mice heterozygous for the disrupted SP-B allele to eventually obtain mice that expressed SP-B only from transgene in the null background. These animals survived, indicating that they could be rescued by reconstituting SP-B expression. Additionally, these experiments indicate that the carboxyterminal portion of proSP-B is not essential for survival.

4. SP-B gene polymorphisms and respiratory distress syndrome

In addition to the mutations in the SP-B gene associated with acute respiratory failure in full-term infants, a number of polymorphisms have been identified in the SP-B gene. One coding polymorphism at the end of exon 4 has been recognized, although not commented on, in previous reports [6]. One allele encodes isoleucine at codon 131, the other threonine. The substitution of isoleucine for threonine would abolish a consensus signal for N-linked glycosylation at this site. It is unlikely that glycosylation at this location is essential, as this glycosylation site is not present in other mammalian species [14]. The distribution of these two alleles in the population is not known, nor whether either allele could predispose to or possibly be associated with disease.

A highly polymorphic region containing a variable nucleotide tandem repeat (VNTR) sequence is located within intron 4 of the SP-B gene. The variation in the size of these repeats accounts for a previously recognized SP-B restriction fragment length polymorphism (RFLP) for *Bam*H1 [5]. The different alleles due to variations in this region have been extensively characterized by Floros and coworkers [4]. At least 12 different alleles have been identified, each containing a variable number (from 3 to 18) of motifs consisting of a conserved 20 bp element followed by a variable length of $(CA)_n$ repeats. Differences in the distribution of alleles in different ethnic groups were observed. The most common allele contains 11 motifs, and accounted for 90% of the alleles in Caucasian individuals, but for a lesser percentage in African-Americans (75%) and Nigerian (83%) individuals. The second most common allele (5%) in the Caucasian group had five motifs deleted, whereas insertions of motifs were more common in the African-American and Nigerian groups [33].

The functional significance of these small intronic insertions and deletions is unknown. Floros et al. examined the association of SP-B alleles that differed at this locus with the development of RDS in premature infants. They found that 32% of infants with RDS had a polymorphism (either insertion or deletion of extra motifs as opposed to the most commonly observed allele), whereas only 9.5% of the control group had a polymorphism [4]. However, the RDS infants were significantly more premature in terms of their gestational age, a known risk factor for RDS, so that the association could also be related to prematurity. In a subsequent study, the frequency of an SP-B polymorphism at this locus in combination with an SP-A gene polymorphism, was significantly higher in babies with RDS than in a control group comparable for gestational age and ethnic background [34].

The mechanism(s) whereby these polymorphisms could be associated with disease remain unclear, as this type of polymorphic variation is not one that would obviously affect SP-B regulation. It is possible that the polymorphic markers could be linked to abnormalities elsewhere in the gene affecting SP-B production or processing, although the association was with having any polymorphism, and not a particular allele. Individuals who are carriers for SP-B deficiency, and who have one allele incapable of producing SP-B, have in general not had histories of pulmonary disease, although systematic studies examining pulmonary function in such individuals have not been performed. Interestingly, mice heterozygous for one abnormal SP-B allele developed air trapping as adults [35], suggesting that having one abnormal SP-B allele may be a risk factor for the development of lung disease. Finally, the recent report of an infant with a mutation on one allele resulting in a single amino acid substitution, and one apparently normal allele, who initially had undetectable SP-B levels in his lung fluid, but whose SP-B levels increased as his lung disease improved [27], also supports the notion that one abnormal allele could be associated with lung disease in some circumstances.

5. Surfactant protein C

Human SP-C is encoded by a single gene located on the short arm of chromosome 8, centromeric to 8p23.1, and the locus is referred to as *sftp2* [36]. It is a fairly small gene, spanning only 3500 bases. The gene contains six exons and five introns; the last exon is untranslated [37]. The SP-C gene is located in close proximity to the gene for bone morphogenetic protein-1 (BMP-1), whose transcription initiation site is located only 706 bases downstream of the polyadenylation site of the SP-C gene [38]. The gene is transcribed into a 0.86 kb mRNA, which directs the synthesis of a 22 000 Da proprotein. Alternative splicing at the beginning of exon 5 leads to two different transcripts, which differ by 18 bases in length [37]. The resulting proproteins thus differ by six amino acids. The functional significance of this alternative splicing is unknown. The proprotein is further proteolytically processed to yield the extremely hydrophobic 35 amino acid SP-C protein found in the airspaces (Fig. 2). The DNA sequence from which the mature SP-C peptide is derived is located in exon 2. Two cysteine residues contained in the mature SP-C peptide are posttranslationally palmitoylated, and SP-C is thus a proteolipid [39]. The enzymes involved in the posttranslational processing of proSP-C to mature SP-C have not been characterized. The structure and properties of

SP-C are reviewed by Johansson elsewhere in this issue.

The SP-C gene promoter contains sequences directing lung specificity and cell specificity to the distal respiratory epithelium, and SP-C expression is restricted to alveolar type II cells in human lung. A 3.7 kb portion of the sequence 5′ to the SP-C gene has been used extensively to target a number of transgenes to the distal respiratory epithelium in mice [40].

The initial characterization of the SP-C gene revealed two groups of clones which differed considerably in their DNA sequences at multiple locations, including single nucleotide substitutions, insertions, and deletions, primarily in intronic sequences [37]. Southern blot analysis however, was consistent with a single SP-C gene [36], and the observed sequence differences were thus likely due to different SP-C alleles. Variations in SP-C cDNA sequences had been observed previously, including coding sequence changes at codons 138 (Asn or Thr) and codon 186 (Asn or Ser) [41]. It is not known whether these variants have any correlation with disease. While the SP-C gene sequences that have been reported to date differ considerably from one another, the extent of allelic variation and distribution of different SP-C alleles in the general population remain unknown. A single base deletion in intron 5 creates an *Eco*R1 RFLP, and a highly polymorphic $(CA)_n$ repeat region is located in close proximity to the SP-C gene (within the first intron of the BMP-1 gene) [38]. These polymorphic markers may be useful in studies attempting to link the SP-C gene with disease.

To date, variations in the SP-C gene have not been associated with human disease. Hatzis et al. examined SP-C mRNA expression as assessed by in situ hybridization in eight infants who died from RDS and ten control infants who died from other causes [42]. They found similar levels of SP-C mRNA expression in both groups, and concluded that altered SP-C expression due to pretranslational mechanisms was unlikely to contribute to the pathogenesis of RDS. They also found that the SP-C gene sequences from four healthy adults without lung disease of different ethnic backgrounds (two Caucasian, two Nigerian), and from two premature Caucasian infants with RDS, did not differ from one another, although differences from previously reported sequences were

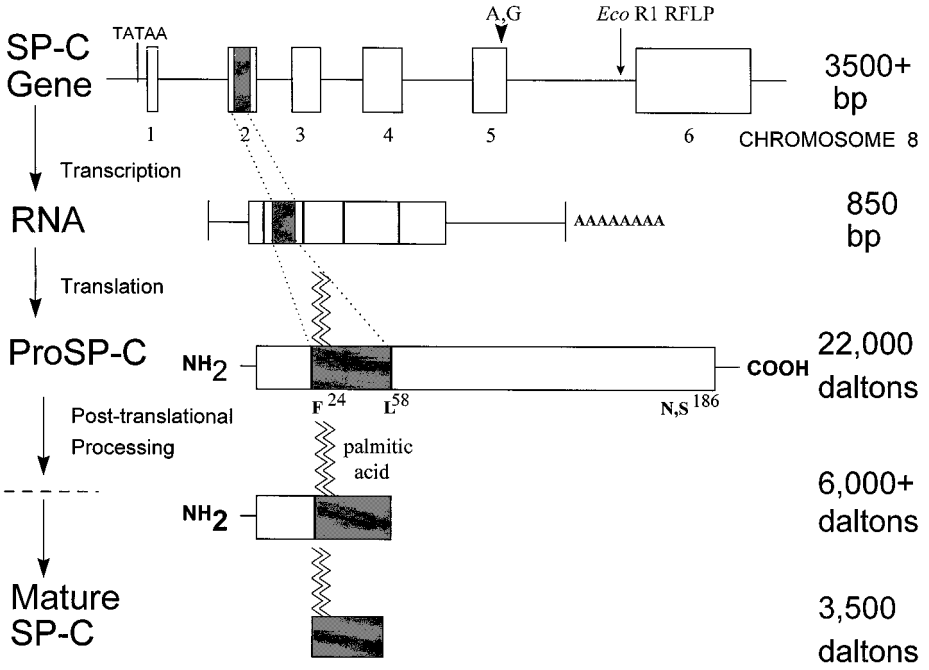

Fig. 2. SP-C gene, mRNA, proprotein and processing. Exons and translated portions of the mRNA are represented by boxes; introns and untranslated portions are represented by lines. The positions of a coding polymorphism in exon 5 and an *Eco*R1 RFLP are shown. Amino acids corresponding to the beginning and end of mature SP-C and the polymorphism are indicated by their single letter abbreviation. The proposed block that occurs in SP-C processing in the absence of SP-B is indicated by a dashed line.

observed. SP-C protein expression was not examined in this study. In contrast, in a recent preliminary report, Wert et al. observed markedly decreased staining for proSP-C in the lungs of 15 full-term infants who died from respiratory distress syndrome [43]. In three instances, more than one infant born to the same parents was affected, suggesting a genetic basis for their disease, although no SP-C gene mutations were identified in this study. These observations support a role for SP-C or proSP-C in the pathogenesis of some infants with neonatal respiratory failure.

It is not known whether specific mutations in the SP-C gene result in respiratory disease. Such mutations have not yet been reported, nor has targeted disruption of the SP-C gene in experimental animals been reported. However, given the small size of the SP-C gene, such mutations may be quite rare. It is possible that SP-C's role in surfactant and/or type II cell function may not be essential, with SP-B or other proteins able to compensate for a lack of SP-C, and that mutations in the SP-C gene will not cause respiratory disease. Alternatively, it may be that SP-C gene mutations could be lethal prenatally rather than causing lung disease postnatally, although this would be surprising given the restriction of SP-C expression to the distal respiratory epithelium.

6. Future directions

Much remains to be learned concerning the role of mutations and polymorphisms in the SB-B and SP-C genes and their contribution to human lung disease. The recognition of hereditary SP-B deficiency as a cause of lung disease has raised additional questions. The incidence of the disease remains to be determined. The mechanisms whereby the lack of SP-B disrupts other aspects of surfactant metabolism need to be elucidated. Better treatment options need to be developed, and the disease is an important model for gene therapy in the lung. Finally the contribution of more subtle mutations and polymorphisms in the SP-B (and possibly SP-C) gene(s) to the development of other lung diseases (such as chronic lung disease and recovery from lung injury) deserves more investigation.

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