Review Article

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STRUCTURE, FUNCTION AND DEFICIENCY DISEASES OF LUNG SURFACTANT PROTEIN-C

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Abstract: Pulmonary surfactant is a mixture of lipids and hydrophobic surfactant proteins B (SP-B) and SP-C and hydrophilic proteins SP-A and SP-D. Pulmonary surfactant reduces the surface tension at the air-water interface of the lung alveoli by forming a surface active film. In this way, it prevents alveoli from collapsing and facilitates the process of breathing. SP-C cooperates with SP-B to enhance the surface active properties of surfactant phospholipids. Reports on the association of lung disease with SP-C deficiency have led to new insights into the importance of SP-C for proper surfactant homeostasis. In most animal species the major form of SP-C is a 35-residue peptide chain which contains two thioester-linked palmitoyl groups, giving a total molecular mass of 4.2 kDa. Though the function of SP-C in vivo remains unclear, but effects of SP-C on the adsorption, spreading, and stability of lipid films at an air/water interface have been documented in a number of in vitro studies. Genetic disorders disrupting normal surfactant metabolism (surfactant dysfunction disorders) have been recognized in the neonatal and pediatric populations. Although rare, these disorders cause significant mortality and morbidity, including acute respiratory distress and failure in full-term neonates, and interstitial lung disease (ILD) in older infants, children, and adults. The genes involved in these disorders are critical for surfactant production and function in the lung and include SP-C gene (*SFTPC*) besides others. More than 40 mutations in the *SFTPC* gene have been identified in people with dysfunction of lung surfactant. Mutations in the *SFTPC* gene result in the reduction or absence of mature SP-C and accumulation of abnormal forms of SP-C, resulting into breathing problems in newborns and onset of breathing difficulties in children and adults. *SFTPC* gene mutations associated with surfactant dysfunction affect the processing of the SP-C protein. Many of these mutations occur in a region of gene called the BRICHOS domain, which appears to be involved in the processing of SP-C protein. It is not known which of these outcomes causes the symptoms of SP-C dysfunction. Recent reports suggest that abnormally processed SP-C proteins form altered three-dimensional shape and accumulate inside lung cells. These misfolded proteins may trigger a cellular response that results in cell damage and death. Mutations in BRICHOS domain of SP-C have been associated to ER stress, proteasome dysfunction, and apoptosis. This article reviews information on structure and function of SP-C and the associated mutations in *SFTPC* gene in relation to SP-C deficiency diseases.

Keywords: Surfactant protein-C; SP-C; palmitylation; interstitial lung disease; BRICHOS domain; surface films.

INTRODUCTION

Pulmonary surfactant, a lipo-protein complex, is a highly surface-active material found in the fluid lining the air-liquid interface of the alveolar surface. Surfactant plays a dual role in preventing alveolar collapse during breathing cycle and protection of lungs from injuries and infections caused by foreign bodies and pathogens. Thus, pulmonary surfactant reduces the surface tension at the air-water interface of the lung alveoli by forming a surface active film, and in this way, it prevents alveoli from collapsing and facilitates the process of breathing. Surfactant membranes contain two hydrophobic lipoproteins (SP-B and SP-C), two hydrophilic lipoproteins: SP-A and SP-D, and approximately 90 wt % of lipids, which consist of equimolar amounts of saturated and unsaturated phospholipid species and cholesterol. Surfactant proteins B and C are small and very hydrophobic proteins, mainly involved in surface activity. SP-B is essential for breathing, and full-term infants unable to produce SP-B, develop lethal neonatal respiratory distress. However, SP-B deficient infants lack not only SP-B but also mature SP-C. Recent studies indicate that children with selective deficiency of SP-C develop acute and chronic lung disease in infancy. SP-C mutations seem to result into conformational diseases that occur as a consequence of mis-folded pro-SP-C but not due to deficiency. The other two proteins associated with surfactant, SP-A and SP-D belong to the collectin family, so named because all members contain a collagen-like domain, and lectin domains (Gupta, 2012; Gupta and Gupta, 2012a, b, c). Lung collectins have supratrimeric assembly in the native state. The extent of oligomerization of lung collectins SP-A and SP-D may have consequences in various diseases and may vary among individuals (Gupta, 2012).

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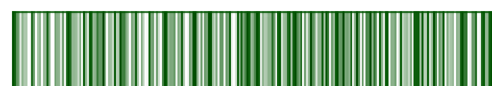

Respiratory distress syndrome (RDS) caused by lack of pulmonary surfactant, affects 65000 infants annually in only USA. Surfactant replacement therapy reduces the morbidity and mortality associated with RDS. Human SP-C, the important component of pulmonary surfactant, is expressed as a proprotein (proSP-C), which becomes post-translationally modified with palmitate and undergoes several rounds of proteolytic cleavage. Reports on the association of development of lung disease with SP-C deficiency have led to the new insights into the importance of SP-C for proper surfactant homeostasis. In addition, new information has become available on the role of the palmitoyl chains of SP-C in surface activity. Developments in the processing and function of SP-C, with particular emphasis on the signals for and role of palmitoylation of SP-C have been reviewed (ten Brinke *et al.,* 2002c) and suggest that SP-C has roles beyond modulation of alveolar surface tension. From data on similarly modified proteins, it has been proposed that the properties of SP-C, including the covalent addition of palmitic acid, render it capable of being targeted to and interacting with specific cell membranes (Beers and Fisher, 1992). SP-C cooperates with SP-B to enhance the surface active properties of surfactant phospholipids. SP-B deficiency is associated with the aberrant processing and secretion of an immature SP-C peptide, may contribute to the respiratory failure associated with hereditary SP-B deficiency (Vorbroker *et al.,* 1995a). SP-C can be regarded as a specific marker for type II cells (Kalina *et al.,* 1992; Wohlford-Lenane *et al.,* 1992). This review describes the latest information on structure and functions of SP-C and the associated lung diseases in relation to SP-C structure.

MOLECULAR PROPERTIES OF SP-C

A 35-residue peptide chain: In most animal species the major form of SP-C is a 35-residue peptide chain which contains two thioester-linked palmitoyl groups, giving a total molecular mass of 4.2 kDa. Several minor variants of SP-C, which are formed from N-terminal truncation, lysine palmitoylation, methionine oxidation and C-terminal esterification, are known. The primary structure of SP-C is evolutionarily conserved and appears to be the only constituent which is unique to pulmonary surfactant, indicating important and specific functions. Alveolar mature SP-C (SP-$C_{3.7}$, Mr 21,000) precursor is a 35 amino acid peptide that is synthesized and processed from a 191-197 amino acid precursor (proSP-C21). Although its solubility in organic solvents and avidity for lipid membranes impart properties important for its biophysical activity, SP-C represents a challenging protein for the alveolar type II cell that synthesizes and traffic the peptide through the regulated secretory pathway. Results consider proSP-C21 as a hybrid molecule incorporating structural and functional features both of bitopic integral membrane proteins as well us more classically recognized propeptide hormones (Solarin *et al.,* 2001).

Glasser *et al.,* (1988a) characterized the NH_2-terminal amino acid sequence of pulmonary proteolipids from extracts of bovine, canine, and human surfactant. Two distinct peptides identified, were termed surfactant proteolipid SPL (pVal) and SPL (Phe). The SPL (pVal) of 20870 D contains a unique polyvaline domain. The human mRNA demonstrated a Mr of 22,000 D precursor protein, whereas active hydrophobic peptide was produced by proteolytic processing to Mr of 5,000-6,000 D. Human SPL (pVal) mRNA was more abundant in the adult than in fetal lung. The SPL (pVal) gene locus was assigned to chromosome 8. However, ovine SP-C cDNA contains 809 bp, predicting a protein of 190 amino acids (Pietschmann and Pison, 2000).

The SP-C from human, canine, and bovine sources is highly hydrophobic protein, composed of 33-35 amino acids, the differences being due to NH2-terminal heterogeneity. A COOH-terminal leucine is conserved throughout. The cysteines in each species were found to be present as thioesters of palmitic acid. Acylation of recombinant SP-C with palmityl coenzyme A, followed by characterization before and after release of the acyl group with 1, 4-dithiothreitol, provided corroborating evidence for the native structure (Stults *et al.,* 1991). Two forms of SP-C, isolated exhibited similar secondary structures at the air/water interface. Both forms of SP-C were able to induce the insertion of phospholipids into a monolayer. In contrast to the palmitoylated monomeric form of SP-C, the non-acylated dimeric form of SP-C did not require calcium ions to insert phospholipids into a monolayer without the negatively charged phosphatidylglycerol (Creuwels *et al.,* 1995b).

Mature human SP-C (SP-$C_{3.7}$) corresponding to residues 24–58 of the 197-residue integral membrane protein proSP-C, is generated via multiple proteolytic cleavages (Beers *et al.,* 1994). The final cleavage of proSP-C to SP-C likely occurs in the lysosome-like lamellar bodies in which surfactant lipids and proteins are packed together, and from which they are secreted by exocytosis into the alveolar space (Vorbroker 1995b; Johnson *et al.,* 2001). The mature SP-C is anchored in the membrane by a hydrophobic domain that comprises the 20-amino-acid-residues-long hydrophobic core of the mature SP-C peptide. The N-terminus remains in the cytoplasm, which leads to a type II transmembrane orientation of the precursor protein. The hydrophobic domain acts as both signal sequence and membrane-anchoring domain. The correct membrane insertion of SP-C precursor is itself a prerequisite for further processing and intracellular transport of the mature SP-C (Keller *et al.,* 1991). The secondary structure of native and depalmitoylated

porcine SP-C adopts mainly an α-helical conformation which is oriented parallel to the lipid acyl chains (Vandenbussche *et al.,* 1992).

ProSP-C processing: The proprotein (proSP-C), which becomes posttranslationally modified with palmitate, undergoes several rounds of proteolytic cleavage. In comparison to human integral membrane protein of proSP-C of 197 amino acids, (Johansson *et al.,* 2009a,b), rat SP-C is synthesized by the alveolar type II cells as a proprotein of 194 aa (SP-C21). Contained within the propeptide is the 35-aa mature SP-C protein (SP-$C_{3.7}$), which is flanked by propeptides of 23 amino acids at the N terminus and 136 residues at the C-terminus. After translation, SP-C21 is translocated to the ER and inserted into the ER membrane as a bitopic protein in a type II membrane orientation, which is anchored by the α-helical domain Leu^{36}-Leu^{55} contained within the mature SP-C sequence (Fig. 1) (Kabore *et al.,* 2001; ten Brinke *et al.,* 2002c). The proSP-C C-terminus contains conserved cysteine residues, which could participate in disulfide mediated folding in the oxidizing environment of the ER lumen. The proSP-C contains four regions; a short N-terminal segment (residues 1–23) facing the cytosol and important for intracellular trafficking, a transmembrane (TM) region constituting the main part of mature SP-C (residues 24–58) eventually secreted with phospholipids into the alveoli, a linker region (residues 59–89), and a BRICHOS domain (residues 90–197), which is localized to the ER lumen (Mulugeta and Beers, 2006). The linker region plus the BRICHOS domain form the C-terminal part of proSP-C (CTC). Maturation of proSP-C involves proteolytic processing in several steps at different intracellular locations, following insertion of the SP-C part as a TM helix (Beers *et al.,* 1994). Though mature SP-C (PDB ID: 1SPF) (Johansson *et al.,* 1994) adopts α TM helical conformation, its valine-rich sequence (Fig. 2A) is far from optimal for TM helix formation. In model TM segments, poly-Leu variants insert into the ER membrane in a helical conformation, while poly-Val variants get trapped in an extended conformation, suggesting that helix propensities influence the ability to insert into the ER membrane (Mingarro *et al.,* 2000). Consistent with this hypothesis, engineered SP-C with a poly-Leu repeat rather than the native poly-Val variant yields a stable TM helix, whereas wild-type (WT) SP-C is metastable and forms β-sheet aggregates and amyloid-like fibrils in vitro (Kallberg *et al.,* 2001). The reason for using such a discordant sequence for a segment destined to end up as a TM helix is not known, but this sequence is highly conserved throughout proSP-Cs (Hedlund *et al.,* 2009). The typical amino acid sequence of the TM domain of proSP-C plays a role in membrane formation and morphology, which may be relevant under physiological conditions (ten Brinke *et al.,* 2003) (Fig. 1 and 2A).

Studies indicate that the correct intracellular trafficking of proSP-C depends on the N-terminal propeptide. Deletion of the region from residues Met^{10} to Thr^{18} of proSP-C results in retention of the peptide in

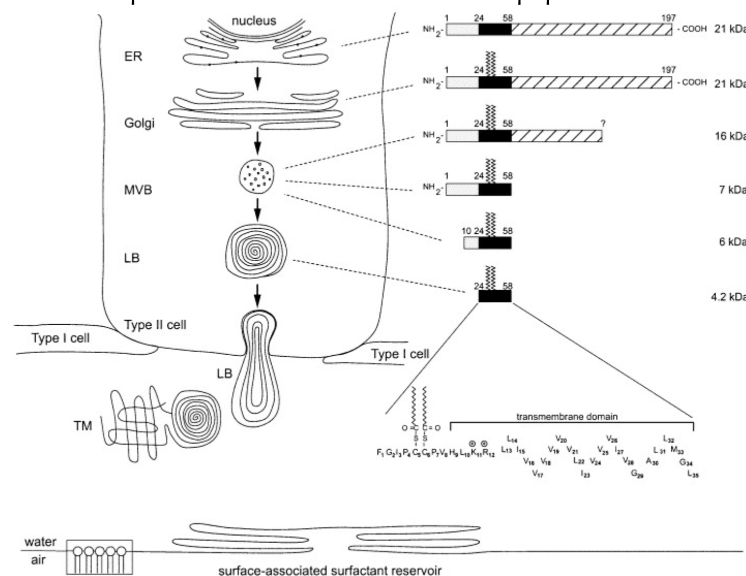

Fig.1: Processing and sorting of proSP-C. To the right of the figure, proSP-C and its processing intermediates are depicted, as well as the amino acid sequence of mature SP-C. In the left part of the figure, the localization of the processing intermediates in the different organelles of the type II cell is represented. SP-C is synthesized by the alveolar type II cells as a proprotein of 194-197 aa (SP-C21). Within the propeptide is the 35-aa mature SP-C protein (SP-C3.7), which is flanked by propeptides of 23 amino acids at the N terminus and 136 residues at the C terminus. After translation, SP-C21 is translocated to the endoplasmic reticulum (ER) and inserted into the ER membrane as a bitopic protein in a type II membrane orientation, which is anchored by the α-helical domain Leu^{36}-Leu^{55} contained within the mature SP-C sequence. The proSP-C C terminus contains conserved cysteine residues, which could participate in disulfide mediated folding in the oxidizing environment of the ER lumen. Proteolytic removal of the C- and N-flanking domains is Brefeldin A-sensitive, indicating that processing is dependent upon delivery of SP-C21 to post-Golgi processing compartments. Within the mature peptide, aa residues Cys^{28}, Cys^{29}, and Lys^{34} (CCK) have been shown to contain covalent palmitic acid (ER: endoplasmic reticulum; MVB: multivesicular body; LB: lamellar body; TM: tubular myelin (with permission from ten Brinke *et al.,* 2002c; Kabore *et al.,* 2001;).

The ER (Johnson *et al.,* 2001; Conkright *et al.,* 2001). In contrast, truncation mutants of proSP-C, which lack the C-terminal propart, are directed to distal compartments in transfected epithelial cells (Conkright *et al.,* 2001). Pulse-chase labeling of type II cells demonstrated proSP-C processing intermediates of 19, 16, and 13 kDa that contained the NH2-terminal of proSP-C. The processing of proSP-C begins in a late Golgi or post-Golgi compartment and that proSP-C processing takes place in the trans-Golgi and multi-vesicular bodies before SP-C incorporation into lamellar bodies (Vorbroker *et al.,* 1995b). Beers and Lomax (1995) suggest that initial synthetic processing events

for SP-C include post-translational cleavages of the C-terminus of proSP-C21 yielding two intermediates (16 and 6 kDa) in subcellular compartments of type II cells which are distal to the trans-Golgi network. Beers *et al.,* (1998) suggested that SP-C expressed in A549 cells is directed to cytoplasmic vesicles where it is proteolytically processed in a manner similar to native type II cells and that amino acids Cys186-Ile194 located at the C- terminus of proSP-C21 are necessary for correct intracellular targeting and subsequent cleavage events.

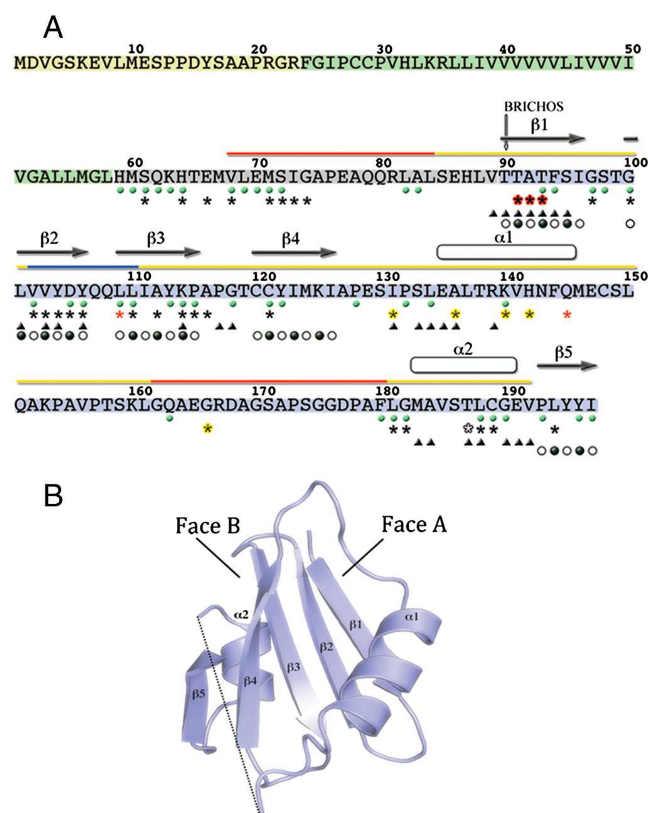

Fig. 2: ProSP-C sequence and 3D structure of its BRICHOS domain. (A) Sequence of full-length human proSP-C. The N-terminal, situated in the cytosol, is presented in yellow. The TM and mature SP-C parts are in green. The C terminal part of proSP-C (CTC) is shown in gray for the linker and blue for the BRICHOS domain. HDX rate constants in CTC are shown as colored lines above the sequence where red is fast, yellow is intermediate and blue is slow exchange. Secondary structure elements are shown as rectangles (helices) and arrows (β-strands). Starting position of the BRICHOS domain is labeled. Green dots, below the sequence, represent strictly conserved residues. Asterisks mark ILD mutations; the black are point mutations, the highlighted red correspond to the Δ91-93 deletion, the highlighted yellow are frameshift mutations, the two red asterisks correspond to start and end points of the Δexon 4 deletion, and the unfilled asterisk corresponds to an 18 base pair insertion. Residues in the trimer interface are labeled with black triangles. Open and filled circles identify residues on face A and B of the β-sheet, respectively. (B) Ribbon diagram representation of one subunit, with secondary structure elements β1-β2-β3-β4-α1-α2-β5 labeled. A dashed line indicates the missing region between helices α1 and α2 (with permission from Willander *et al.,* 2012).

ProSP-C is palmitoylated and undergoes additional post-translational modification that is blocked by an inhibitor of fatty acid synthesis. Results indicate that Nedd4-2 mediated ubiquitination regulates lumenal relocation of SP-C leading to processing and, ultimately, secretion of SP-C (Conkright *et al.,* 2009). Kotorashvili *et al., (*2009) indicated that PPDY mediated interaction with Nedd4 E3-ligases is required for trafficking of proSP-C. It is speculated that the Nedd4/ proSP-C tandem is part of a larger protein complex containing a ubiquitinated component that further directs its transport (Kotorashvili *et al.,* 2009).

Intermediate precursor of lung SP-C (SP-Ci): In both humans and mice, a deficiency of SP-B is associated with a decreased level of mature SP-C and accumulation of a larger SP-C peptide, denoted by SP-Ci. But SP-Ci is not observed under normal conditions. The intermediate SP-Ci, corresponding to residues 12-58 of proSP-C, lacks the surface activity of SP-C. Determination of the SP-Ci covalent structure revealed a 12-residue N-terminal peptide segment, followed by a 35-residue segment that is identical to mature SP-C. The SP-Ci structure determined herein is similar to that of a proposed late intermediate in the processing of proSP-C, suggesting that SP-Ci is the immediate precursor of SP-C (Li *et al.,* 2004a). However, the SP-Ci α-helical structure does not unfold and aggregate in contrast to the metastable nature of SP-C helix. Li *et al.,* (2004b) showed that a 12 residue N-terminal propeptide locks the metastable polyvaline part in a helical conformation in SP-Ci. In an acidic environment, SP-Ci unfolds and aggregates into amyloid fibrils like SP-C. Observations suggest that the propeptide part of SP-Ci prevents unfolding by locking the N-terminal part of the helix, and that acidic pH results in structural disordering of the region that is proteolytically cleaved to generate SP-C (Li *et al.,* 2006). Reports indicated that the early postnatal fatal RDS seen in SP-B-deficient children is combined with the near absence of active variants of SP-C.

Alternatively spliced forms of *SFTPC:* *SFTPC* gene is genomically small (3.5 kb with a 3.7kb promoter), located on human chromosome 8, and directs the synthesis of an alternatively spliced 191 or 197 amino acid proprotein (proSP-C) that undergoes sequential proteolytic cleavages to yield the 35 amino acid mature SP-C peptide (Beers and Mulugeta, 2005; Glasser *et al.,* 1988b). SP-C genes are encoded by DNA containing six exons and five introns. In both genes, the active hydrophobic region of the polypeptide was located in the second exon that encodes a peptide of 53 amino acids. The entire nucleotide sequences of two classes of SP-C genes differed by only 1%. Two cDNAs encoding SP-C were distinguished on the basis of an 18 nt deletion at the beginning of the fifth exon; no such deletion was detected within the two classes of SP-C

genes. Both classes of SP-C genes are actively transcribed and readily distinguished on the basis of their nucleotide sequences and restriction fragment analyses of their flanking DNA. Rabbit lung cDNA possesses considerable nucleic acid and predicted amino-acid homology with SP-C cDNAs from other species. The porcine *SFTPC* is a single-copy gene on pig chromosome 14. Two transcripts were found in a newborn pig lung cDNA library: a full-length clone and a clone missing exon 5. Full-length lung specific *SFTPC* transcript appeared in 50-day-old fetus that increased during lung development. Both *SFTPC* transcripts were significantly down-regulated in porcine lungs infected with *Actinobacillus pleuropneumoniae* (Cirera *et al.,* 2006).

THE FUNCTIONS OF SP-C

The function of SP-C in vivo remains unclear, but effects of SP-C on the adsorption, spreading, and stability of lipid films at an air ⁄ water interface have been documented in a number of in vitro studies. In addition, SP-C has been shown to recognize lipopoly-saccharide (LPS) (Augusto *et al.,* 2001).

Role of SP-C in DPPC enrichment of surface films

Action mechanism of SP-C in lung surfactant mono- and multilayers: In vitro, SP-C enhances the resistance to surfactant flow by regulating the ratio of solid to fluid phase in the monolayer, leading to a jamming transition at which the monolayer transforms from fluidlike to solidlike. The accompanying three orders of magnitude increase in surface viscosity helps minimize surfactant flow to the airways and likely stabilizes the alveoli against collapse (Alonso *et al.,* 2005). Binary lipid and lipid/SP-C systems indicated that the surfactant films possess a region of high film compressibility of the spread monolayer close to its equilibrium at surface pressure (pi = 50 mN/m) that was due to the exclusion of layered protrusions with each layer 5.5 to 6.5 nm thick. The protrusions contained the protein in high concentration. The more the film was compressed, the larger was the number of layers on top of each other (Amrein *et al.,* 1997). The formation of the multilayer structures was fully reversible in repeated compression-expansion cycles including the plateau region of the phase diagram. The ability of lipid/SP-C mixtures to form reversible multilayer structures during compression may be relevant to stability in lungs during expiration and inhalation (von Nahmen *et al.,* 1997; Kramer *et al.,* 2000).

Experimental evidence indicated that SP-C mixed with DPPC yielded higher elasticities and viscosities as compared with films formed by the single components. This behavior, likely, supports breathing cycles, especially for the turn from inspiration to expiration and vice versa (Wüstneck *et al.,* 2001). TOF-SIMS spectra revealed positive secondary ions (SI) characteristic for DPPC and SP-C, but not for DPPG. SI mapping results in images with domain structures in DPPC/DPPG and DPPG/SP-C, but not in DPPC/SP-C films (Bourdos *et al.,* 2000).

Results on porcine pulmonary SP-C, incorporated into bilayers of chain-perdeuterated dipalmitoyl-phosphatidylglycerol (DPPG-d62) and chain-perdeuterated dipalmitoyl-phosphatidylcholine (DPPC-d62) and into bilayers DPPC-d62 and DPPG, suggested that SP-C induced a nonrandom lateral distribution in the mixed lipid bilayer and influenced motions responsible for deuteron transverse relaxation in both the gel and liquid crystalline phases. The presence of Ca^{2+} in the aqueous phase substantially altered the effect of SP-C on transverse relaxation in the bilayer (Dico *et al.,* 1997; Nag *et al.,* 1996b). Nag *et al.,* (1996a) indicated that SP-C perturbs the packing of neutral and anionic phospholipid monolayers even when the latter systems are condensed by calcium, indicating that interactions between SP-C and the lipids are predominantly hydrophobic in nature. The dipalmitoleoyl-phosphatidylethanolamine (DPOPE) films at the air/water interface in presence than in absence of SP-C revealed the formation of more uniform and homogeneously packed DPOPE monolayers (Jordanova *et al.,* 2009).

Cholesterol leads to a condensation of the monolayers. An increasing amount of liquid-expanded domains could be visualized in lung surfactant monolayers after addition of either cholesterol or POPE. At surface pressures of 50 mN/m, protrusions were formed which differed in size and shape as a function of cholesterol or POPE content, but only if SP-C was present. Low cholesterol (10 mol %) resulted in an increased number of protrusions, which also grew in size. This is interpreted as a stabilizing effect of cholesterol on bilayers formed underneath the monolayer. Extreme amounts of cholesterol (30 mol %), however, caused an increased monolayer rigidity, thus preventing reversible multilayer formation. In contrast, POPE, as a nonbilayer lipid thought to stabilize the edges of protrusions, lead to more narrow protrusions. The lateral extension of the protrusions is thereby more influenced than their height (Malcharek *et al.,* 2005).

Monolayer-bilayer transformations: Lung surfactant causes the surface tension (γ) in the alveoli to drop to nearly zero on exhalation; in the upper airways γ is ~30 mN/m and constant. Hence, a surface tension gradient exists between alveoli and airways that should lead to surfactant flow out of the alveoli and elimination of the surface tension gradient. The lowering of γ by surfactant requires the exchange of material between the lipid monolayer at the interface and lipid reservoirs under dynamic compression and

expansion of the interface during the breathing cycle. Baoukina *et al.,* (2007) simulated the reversible exchange of material between the monolayer and lipid reservoirs under compression and expansion of the interface using a mixture of DPPC, POPG, cholesterol, and SP-C as a functional analog of mammalian lung surfactant. In these simulation studies, the monolayer collapsed into the water subphase on compression and formed bilayer folds. On monolayer re-expansion, the material was transferred from the folds back to the interface. The simulations indicated that the connectivity of the bilayer aggregates to the monolayer is necessary for the reversibility of the monolayer-bilayer transformation. The simulations also showed that the bilayer aggregates were unstable in the air subphase and stable in the water subphase (Baoukina *et al.,* 2007).

Atomic force microscopy on Langmuir-Blodgett films verified the formation of multilayers in a DPPC, DPPG, cholesterol, and SP-C model system. Multilayer formation was not detected in the absence of SP-C. At the air/water (A/W) interface, the spectra of SP-C revealed that the predominantly helical structure changes its orientation in monolayers versus multilayers. The helix tilt angle changed from approximately 80 degrees in monolayers to a transmembrane orientation in multilayers (Wang *et al.,* 2005). Studies demonstrated that a multilayer structure can be formed in exogenous surfactant even at very low concentrations and indicated that multilayers need to be incorporated into present interpretations of in vitro studies of similar lung surfactant preparations, which are largely based on monolayer models (Follows *et al.,* 2007).

On the basis of the SP-C molecular structure, Li *et al.,*(2007) described that: (1) in an alveolus monolayer, SP-C molecules are surrounded by phosphatidylglycerol (PG). When the monolayer is compressed, SP-C molecules can promote PG molecules to be squeezed out; (2) during compressing of the monolayer, unsaturated-PG molecules form a collapse pit firstly when liquid-expanded state (LE) components achieve the collapse pressure. Then, SP-C's α-helix is attracted by the collapse pit and both α-helix and PG molecules are squeezed out speedily. Finally, the squeezed-out matters can form a lipid-protein aggregation in the subphase. The lipid-protein aggregation, in the centre of which, there is the hydrophobic α-helix section surrounded by PG molecules; (3) during the monolayer expanding, because of the increasing of the monolayer's surface tension, the structure of the lipid-protein aggregation is disturbed and reinserts into the surface of the monolayer rapidly. On the basis of analyzing the energies change of the squeeze-out process, a mathematical model is obtained to calculate the squeezed-out number of DPPG molecules when a SP-C molecule squeezes out in a monolayer. According to the model, it is concluded that SP-C has the capability to promote the squeeze-out and the reinsertion of most of PG component in an alveolus monolayer, the prediction data agree well with the experimental data.

Intrinsic propensity of N-terminal segment to interact with phospholipid bilayers: Since the N-terminal end of SP-C is located at the surface of the membranes and is exposed to the aqueous environment, studies on membranes containing phosphatidylglycerol (PG) suggest that electrostatic lipid-protein interactions induce important effects on the structure and disposition of the N-terminal segment of protein in these membranes. Thus, conformation and interactions of the N-terminal segment of SP-C could be important in regulating the lateral distribution of the protein in surfactant bilayers and monolayers (Plasencia *et al.,* 2001). Polycationic, palmitoylated-cysteine containing N-terminal segment of SP-C is likely the only structural motif the protein projects out of the bilayer in which SP-C is inserted and is therefore a candidate motif to participate in interactions with other bilayers or monolayers. The 13-residue peptides with sequences corresponding to the native N-terminal segment of pulmonary SP-C have been synthesized and their interaction with phospholipid bilayers characterized.

The peptides are soluble in aqueous media but associate spontaneously with bilayers composed of either zwitterionic (phosphatidylcholine) or anionic (phosphatidylglycerol) phospholipids. The peptides show higher affinity for anionic than for zwitterionic membranes. Interaction of the peptides with both zwitterionic and anionic membranes promotes phospholipid vesicle aggregation, and leakage of the aqueous content of the vesicles. The lipid-peptide interaction includes a significant hydrophobic component for both zwitterionic and anionic membranes, although the interaction with phosphatidylglycerol bilayers is also electrostatic in nature. The effects of the SP-C N-terminal peptides on the membrane structure are mediated by perturbations of the packing order and mobility of phospholipid acyl chain segments deep in the bilayer. The peptide expands the pi-A compression isotherms of interfacial phospholipid/peptide films, and perturbs the lipid packing of phospholipid films during compression-driven liquid-expanded to liquid-condensed lateral transitions, as observed by epi-fluorescence microscopy. Plasencia *et al.,* (2004; 2005) suggested that the SP-C N-terminal sequence has intrinsic ability to interact with, insert into, and perturb the structure of zwitterionic and anionic phospholipid films, even in the absence of the palmitic chains attached to this segment in the native protein. SP-C has the ability to facilitate reinsertion of surface active lipid

molecules into the lung interface during respiratory compression-expansion cycling (Plasencia *et al.,* 2004; 2005)

Interactions between lung SP-C and LPS and Susceptibility to infection

Among the different hydrophobic components of mouse surfactant, only SP-C exhibited the capacity to bind to LPS. Unlike SP-A and SP-D, the binding of SP-C to LPS did not require calcium ions (Augusto *et al.,* 2001). The palmitoyl residues of SP-C are not required for the interaction with LPS and that both the hydrophilic and hydrophobic regions of SP-C are required for specific binding of a rough-type LPS. In addition, the terminal phosphate group at the reducing end of the lipid A disaccharide in α configuration and the N-linked fatty acyl chain on the reducing glucosamine of lipid A take part in the interaction (Augusto *et al.,* 2002, 2003a). Since, the N-terminal segment of SP-C was the most likely region responsible for these effects the synthetic analogs of this stretch showed that [SPC(1-13)] binds LPS to the same extent as porcine SP-C. This might help in the design of new derivatives to fight endotoxic shock and pro-inflammatory events (Garcia-Verdugo *et al.,* 2009).

Augusto *et al.,* (2003b) suggested cross talk among SP-C, LPS, and CD14 indicating SP-C as immunological target to a large panel of microorganisms that can enter the airways. Hypoxia-induced mitogenic factor (HIMF), found in inflammatory zone, is important in lung inflammation. The LPS induces intensive HIMF production in mouse lung, but not in heart, liver, spleen or kidney. It appeared that HIMF production, at least partly, contributes to LPS-induced vascular cell adhesion molecule-1 (VCAM-1) upregulation and mononuclear cell sequestration to lung parenchyma, while protecting alveolar type II cells from LPS-resulted decrease in SP-C production and cell death. It indicates that HIMF participates in LPS-induced acute lung injury and inflammation through modulating VCAM-1 and SP-C expression (Tong *et al.,* 2006).

STRUCTURAL BIOLOGY OF SP-C

The three-dimensional structure determined by NMR spectroscopy revealed one continuous 37 A long alpha-helix encompassing residues 9-34 as the only regular structural element. The central 23 A of the helix contains exclusively aliphatic residues with branched side-chains, mainly valines, and exposes an all-hydrophobic regular surface. The size of the entire helix matches the thickness of a fluid dipalmitoylphosphatidylcholine membrane, and the all-hydrophobic part of the helix matches the acyl-chain part of such a bilayer. This supports a transmembrane orientation of SP-C in pulmonary surfactant bilayers. In a phospholipid monolayer, the SP-C helix is tilted, thereby maximizing the interactions with the lipid acyl-chains also in this environment. The palmitoylcysteines of SP-C, which are located in the flexibly disordered N-terminal octapeptide segment, appear to be important both for integrity of the alpha-helical structure and for functional properties (Johansson, 1998).

SP-C α-helix is metastable: The SP-C primary and secondary structures of different species are highly conserved. SP-C is composed of a flexible N-terminal end, where two palmitoyl groups are linked to cysteine residues at positions 5 and 6, and a α-helical C-terminal part (Johansson, 1998; Curstedt *et al.,* 1990). The SP-C helix contains a polyvaline segment (containing 10–12 valine residues out of 16) which is intriguing since valine has the highest β-strand propensity of all residues. This feature likely underlies the tendency of SP-C to unfold and aggregate, since replacement of valine with leucine (which favours helix formation) results in a stable α-helix (Kallberg *et al.,* 2001; Hosia *et al.,* 2002). The N-terminal propeptide of SP-C is required for intracellular sorting and secretion of SP-C (Conkright *et al.,* 2001).

The SP-C consisting of 35 amino acid residues, adopts mainly α helical secondary structure conformation. While α-helix includes residues from 9 through 34; the remaining residues (Augusto *et al.,* 2003a; Dluhy *et al.,* 2003, Weaver *et al.,* 2002; Krüger *et al.,* 1999; 2002) are not assigned any specific secondary structural conformation. However, studies with synthetic peptides of this segment demonstrated the presence of β-turn structure in N-terminal segment (Plascencia *et al.,* 2004). The SP-C α-helix is metastable and can irreversibly transform into β-sheet aggregates, forming amyloid fibrils under pathological conditions, or during incubation in aqueous organic solvents (Wustneck *et al.,* 2003; Gustafsson *et al.,* 2003; Szyperski *et al.,* 1998; Luy *et al.,* 2004; Dluhy *et al.,* 2003). Removal of the SP-C palmitoyl groups accelerates aggregation (Gustafsson *et al.,* 2001). However, the detailed mechanisms underlying SP-C fibril formation are not clear. The length and diameter of the helix are ~39 Å and 12 Å, respectively. Presence of two fatty acyl chains in native SP-C at the Cys^5 and Cys^6 residues are thought to stabilize the protein when present in lipid monolayers and bilayers. The helical nature of fully acylated SP-C at the air-water interface has been confirmed by infrared studies. However, few reports suggest that SP-C helical structure is not the only structure adopted by this molecule (Dluhy *et al.,* 2003, Johansson, 2003). The central 23 A of the helix contains exclusively aliphatic residues with branched side-chains, mainly valines, and exposes an all-hydrophobic regular surface. The size of the entire helix perfectly matches the thickness of a fluid dipalmitoylphosphatidylcholine membrane, and the all-hydrophobic part of the helix matches the acyl-chain part of such a bilayer. This supports a trans membrane

orientation of SP-C in pulmonary surfactant bilayers. In a phospholipid monolayer, the SP-C helix is tilted, thereby maximizing the interactions with the lipid acyl-chains also in this environment. The palmitoyl cysteines of SP-C, which are located in the flexibly disordered N-terminal octapeptide segment, appear to be important both for integrity of the α-helical structure and for functional properties (Johansson, 1998).

Positive charges influence structure and function of SP-C: Reports suggest that the charges on SP-C are located near the bilayer surface. The possibility that the head group response is sensitive to the degree of clustering of surface charge has been suggested and that the torsion angle about the Cα-Cβ bond might be sensitive to steric interactions between the lipid head group and the protein (Morrow *et al.,* 1993). Creuwels *et al.,* (1995a) studied natural porcine SP-C and modified porcine SP-C (SP-Cm) in which the positive charges had been blocked by phenylglyoxal. Circular dichroism experiments showed that SP-Cm had an increased content of α-helix. Natural SP-C, but not SP-Cm, catalyzed insertion of phospholipids into a monolayer at the air water interface. This reduced insertion was due to a strong reduction of binding of phospholipid vesicles to the monolayer. The insertion catalyzed by the natural porcine SP-C was decreased by an increased pH of the subphase. In contrast to natural SP-C, SP-Cm induced lipid mixing between phospholipid vesicles. It was suggested that the positively charged residues of SP-C are important for the binding of phospholipid vesicles to the monolayer, a process that precedes the insertion of phospholipids into the monolayer (Creuwels *et al.,* 1995a; Qanbar and Possmayer, 1995).

Juxtamembrane lysine and arginine residues of SP-C precursor influence palmitoylation: Acylation of SP-C alters structural and physical properties of SP-C (Creuwels *et al.,* 1993), a step crucial for its interactions with phospholipids over the full spectrum of adsorption and surface behavior of lung surfactant (Wang *et al.,* 1996). In bovine lung SP-C Cys^4 and Cys^5 are acylated with palmitoyl chains. In humans ProSP-C is palmitoylated on Cys^5 and Cys^6 before mature SP-C is formed by several proteolytic steps. The observation that substitution by alanines of the amino acids localized between the cysteines and the transmembrane domain had no effect on palmitoylation, suggested that the palmitoylation of proSP-C depends not on specific sequence motifs, but more on the probability that the cysteine is in the vicinity of the membrane surface. This is probably determined not only by the number of amino acids between the cysteines and the transmembrane domain, but also by the hydrophobic interaction of the N-terminus with the membrane. This may also be the case for the palmitoylation of other transmembrane proteins (ten Brinke *et al.,* 2002a; b).

Substitution of juxtamembrane basic residues lysine and arginine by uncharged glutamines led to a large decrease in palmitoylation level of proSP-C. Reports indicate that the two basic juxtamembrane residues influence palmitoylation of proSP-C by preventing the transport of proSP-C out of the ER, implying that proSP-C becomes palmitoylated normally in a compartment distal to the ER (ten Brinke *et al.,* 2001). Double substitution mutation of these juxtaposed residues from positive to neutral charged species resulted in complete reversal of trans membrane orientation of pro-SP-C and total abrogation of post-translational processing. Since, mutation of a single residue resulted in mixed orientation, it seems that juxtamembrane positively charged residues play crucial role in establishing membrane topology and their influence on the trafficking and processing of pro-SP-C (Mulugeta and Beers, 2003)

The lipid-associated α-helix form of deacylated SP-C (dSP-C) is retained only at the surface at low surface pressures and dissociates from the membrane at higher surface pressures. Although SP-C palmitoylation has little effect on its ability to enhance lipid adsorption and surface tension reduction, it greatly enhances lipid re-spreading and film stability and is therefore important for surfactant function. From a non- or dipalmitoylated SP-C analogue ($SP\text{-}C^{Leu}$), in which all helical Val residues were replaced with Leu and Cys-5 and Cys-6 were replaced with Ser, it appeared that the covalently linked palmitoyl groups of the SP-C analogue were important for the mechanical stability of the lipid film, for the capacity to incorporate material from the reservoir into the surface active film upon area expansion, and for the low film compressibility of dynamically cycled films (Gustafsson *et al.,* 2000). Plasencia *et al.,* (2008) proposed that palmitoylation may be important to promote and facilitate association of SP-C and SP-C-containing membranes with ordered lipid structures such as those potentially existing in highly compressed states of the interfacial surfactant film. However, Schürch *et al.,* (2010) found that in the naturally derived surfactant lipid mixtures, SP-B promoted film formation and reextension to lower surface tensions than SP-C. SP-B in particular played a vital role in sustaining film stability at the most compressed states, whereas SP-C produced no stabilization. It illustrates the crucial need to include SP-B or an efficient SP-B analog for optimal function (Schürch *et al.,* 2010).

Role of palmitoylation in α-helical length SP-C: The influence of palmitoylation on full length SP-Cs as well as truncated variants with the N-terminal residues 1-17 and 1-13, respectively revealed a fine-tuned balance

between the influence of the palmitoyl chains and α-helical length. Native SP-C added to DPPC/DPPG monolayers induced the formation of the surface confined reservoir independent of its palmitoylation degree. The influence of palmitoylation increased when α-helical length was considerably reduced to 17 or even 13 amino acid residues. In these truncated SP-C peptides palmitoyl chains increased monolayer stability and anchored the peptides in lipid film. However, no multilayer formation was observed at all for all shortened peptides. The α-helix of SP-C seems to be a pre-requisite for the formation of extended three-dimensional structures and obviously has to be able to span a lipid bilayer. Palmitoylation obviously mediates interactions between lipids and/or peptides not only within a protein/lipid film but also between neighbouring layers and induces a stacking of bilayers (Na Nakorn *et al.,* 2007). Gonzalez-Horta *et al.,* (2008) suggested that SP-C-attached acyl chains could be important for coupling of lipid and protein motions in surfactant bilayers and monolayers, especially in the context of ordered phospholipid structures such as those potentially formed during exhalation, when stabilization of the respiratory surface by surfactant is the most crucial.

Structure and C-terminal dimerization: The solution structure of a recombinant mutant (rSP-C (FFI)) of hSP-C in a mixture of chloroform and methanol by NMR spectroscopy showed a helix from Phe^5 to the C-terminal Leu^{34} and is thus longer by two residues than the helix of porcine SP-C (pSP-C), which is reported to start at Val^7 in the same solvent. Two sets of resonances at C-terminus of the peptide were explained by low-order oligomerization, probably dimerization of rSP-C (FFI) in its α-helical form. The dimerization may be induced by hydrogen bonding of the C-terminal carboxylic groups or by conserved C-terminal heptapeptide segment with a motif similar to GxxxG dimerization motif of glycophorin A (Kairys *et al.,* 2004a). Computational docking of two SP-C helices revealed a dimer with a helix-helix interface that strikingly resembles that of glycophorin A and is mediated by an AxxxG motif similar to the experimentally determined GxxxG pattern of glycophorin A. It is likely that mature SP-C adopts such a dimeric structure in the lamellar bilayer systems found in surfactant. Dimerization has been shown in previous studies to have a role in sorting and trafficking of SP-C and may also be important to the surfactant function of this protein (Kairys *et al.,* 2004b).

The α-helix form of SP-C is metastable, and under certain circumstances may transform from an α-helix to a β-strand conformation that resembles amyloid fibrils (Fig. 3A and Fig. 3B). This transformation is accelerated when the protein is in its deacylated form (dSP-C). Palmitoyl groups reduce unfolding into a fibrillogenic intermediate and that no partly helical intermediates exist and that the unfolding is highly cooperative (Gustafsson *et al.,* 2001). Results on lipid-protein monomolecular films showed that the fibril β-form of dSP-C is not surface-associated at the air-water interface. The dSP-C transforms from a α-helical to a β-type amyloid fibril structure via a pH-dependent mechanism. In solution at low pH, dSP-C is α-helical in nature, but converts to an amyloid fibril structure composed of short β-strands or β-hairpins at neutral pH. The α-helix structure of dSP-C is fully recovered from amyloid β-structure when the pH is once again lowered. Thus, pH is one of the mechanisms that cause the pathogenesis of pulmonary alveolar proteinosis (Dluhy *et al.,* 2003).

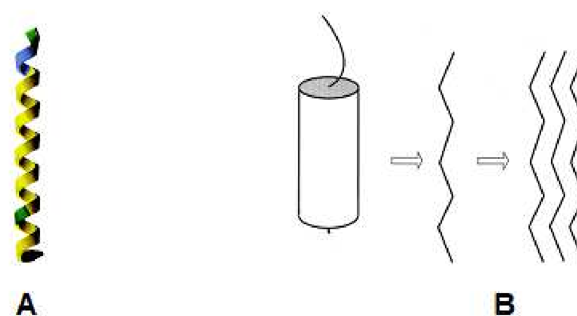

Fig. 3. A. The SP-C α-helix. Polypeptide backbone fold of the helical part of SP-C encompassing residues 9–34. The N-terminal end is located at the top. The amphiphilic nature of the helix is highlighted. Basic residues (Lys, Arg) are colored blue, non-polar residues (Val, Ile, Leu, Ala, Met) are yellow and remaining residues are in green (Johansson, 1998). **B. Structural transitions of SP-C.** Schematic presentation of possible structural transitions of SP-C in solution, deduced from analysis of the native peptide and synthetic analogs. First, native SP-C (left) where residues 9–34 are α-helical (symbolized by the barrel) and the remaining residues are flexibly disordered can probably over time convert into an extended non-helical conformation (middle), which eventually can form an aggregate of several SP-C molecules in extended conformation (right). Second, organic chemical synthesis of SP-C results in peptides with extended structure (middle) which are more likely to aggregate than to form helical peptides (with permission from Johansson, 1998).

GENETIC PHENOTYPES OF *SFTPC* AND SP-C DEFICIENCY DISEASES

***SFTPC* and Interstitial lung diseases:** The interstitial lung diseases are a heterogeneous group of rare disorders of largely unknown etiology. There is an overlap in both the clinical and histopathologic features of these disorders. SP-C spans the phospholipid bilayer of pulmonary surfactant and contributes to maintenance of alveolar expansion at end expiration (Wert *et al.,* 2009; Schürch *et al.,* 2010). Dominantly expressed, rare, exonic mutations in *SFTPC* cause respiratory dysfunction of varying severity and age which is thought to result from aggregation of misfolded or misrouted proSP-C peptides that exceed

the capacity of cell stress response pathways to maintain cellular homeostasis (Nogee *et al.,* 2002; Thomas *et al.,* 2002; Hamvas *et al.,* 2007; Cameron *et al.,* 2005).

In familial cases or in children of consanguineous parents, genetic diagnosis is used as a tool to identify the underlying etiology of ILD. The knowledge of gene variants and associated phenotypes is crucial to identify ILD patients in clinical practice (Nogee 2006; Hartl and Griese, 2005). Mutations in genes causing lung malformations include those in the sonic hedgehog, fibroblast growth factor and thyroid transcription factor-1 pathways. Mutations in genes, regulating surfactant homeostasis, necessary for reduction of surface tension in the alveoli, cause lethal respiratory distress at birth or ILD in childhood. Inherited disorders of the surfactant system that affects neonatal respiratory adaptation at birth include hereditary SP-B deficiency and the ABCA3 transporter besides mutations in SP-C. A variety of mutations in SP-C gene (*SFTPC*) have been identified in individuals with family histories of lung disease that include various forms of interstitial pneumonia and fibrosis (Hardie *et al.,* 2009; 2010, Wert *et al.,* 2009). More than 40 distinct mutations in the *SFTPC* gene have been identified. Most sporadic mutations in *SFTPC* occur on the paternal allele, but somatic recombination may be an important basis of mutation in *SFTPC* (McBee *et al.,* 2008). In contrast to *SFTPB*, disease alleles at *SFTPC* do not share a common haplotype background. ILD in both children and adults, linked to mutations in lung-*SFTPC* missense mutation, isoleucine to threonine at codon 73 in human SP- C [hSP-C(I73T)] accounts for ~30% of all described *SFTPC* mutations. The I73T is the most frequent *SFTPC* mutation associated with diffuse lung disease. Unlike the BRICHOS misfolding *SFTPC* mutants, expression of hSP-C(I73T) induces lung remodeling and alveolar lipoproteinosis without a substantial ER stress response or ER-mediated intrinsic apoptosis. In contrast to its wild-type counterpart that is directly routed to lysosomal-like organelles for processing, SP-C(I73T) is misdirected to the plasma membrane and subsequently internalized to the endocytic pathway via early endosomes, leading to the accumulation of abnormally processed proSP-C isoforms (Beers *et al.,* 2011). Functionally, cells expressing hSP-C(I73T) demonstrate both impaired uptake and degradation of surfactant phospholipid, thus providing a molecular mechanism for the observed lipid accumulation in patients expressing hSP-C(I73T) through the disruption of normal phospholipid recycling. These results provide evidence for a cellular mechanism for conformational protein-associated diseases and suggest a paradigm for mistargeted proteins involved in the disruption of the endosomal/lysosomal sorting machinery. The I73T mutation leads also to impaird processing of proSP-C in alveolar type II cells, alters their stress tolerance and surfactant lipid composition, and activates cells of the immune system (Woischnik *et al.,* 2010). In infants with severe respiratory insufficiency, the lung biopsy showed combined histological patterns of nonspecific interstitial pneumonia and pulmonary alveolar proteinosis in association with an intra-alveolar accumulation of SP-A, precursors of SP-B, mature SP-B, aberrantly processed proSP-C, as well as mono- and dimeric SP-C. Genomic DNA detected a de novo heterozygous missense mutation of the *SFTPC* gene (g.1286T>C) resulting in a I73T mutation in the C-terminal propeptide. The I73T mutation in SP- C gene is marked by a distinct trafficking, processing, palmitoylation, and secretion of the mutant and wild-type SP-C (Brasch *et al.,* 2004) and may cause chronic inflammation of the lung or progressive pulmonary fibrosis (Galetskiy *et al.,* 2008; Somaschini *et al.,* 2007). Guillot *et al.,* (2009) screened 121 children for I73T, a mutation that was seen in ten unrelated patients, in six inherited cases, and in four it appeared de novo. Of the 111 patients without the I73T mutation, eight (seven unrelated) subjects showed to carry a novel mutant allele of *SFTPC*. These seven mutations were located in the BRICHOS domain except the p.Val39Ala (V39A) mutation, which was present in SP-C mature peptide.

The finding of heterozygosity for ABCA3 mutations in severely affected infants with *SFTPC* I73T, and independent inheritance from disease-free parents supports that ABCA3 acts as a modifier gene for the phenotype associated with an *SFTPC* mutation (Bullard and Nogee, 2007). A man with usual interstitial pneumonia (age at onset 58 yr) and his two daughters (aged 39 and 43 yr) had the I73T *SFTPC* mutation. In addition, the younger daughter had a transversion encoding an Asp123Asn (D123N) substitution in ABCA3. It seemed that ABCA3 variants could affect disease pathogenesis (Crossno *et al.,* 2010). In ethnically diverse population of newborns with and without RDS the frequencies of 121ins2 in SP-B, E292V in ABCA3, and I73T in SP-C are rare (<0.4%) (Garmany *et al.,* 2008). However, E292V was present in 3.8% of newborns with RDS, a 10-fold greater prevalence than in the Missouri cohort. Two dominant-negative mutations of proSP-C-encoding gene have been reported in families with vertically-inherited ILD (Nogee *et al.,* 2002; Thomas et al. 2002). In a cohort of 34 sporadic or familial cases with unexplained respiratory distress (URD) in which SP-B deficiency related to *SFTPB* mutation had been ruled out, one patient with complete SP-C deficiency had no detectable mutation of *SFTPC*. Of the 10 patients with abnormal pro-SP-C processing, BAL fluid showed two distinct heterozygous *SFTPC* missense mutations. The first, g.1286T > C (p.I73T), was de novo and resulted in progressive respiratory failure with intra-alveolar storage of a granular, protein- and lipid-rich, periodic acid Schiff (PAS)-positive material (pulmonary alveolar proteinosis-PAP), and ILD. The

second, g.2125G > A (p.R167Q), was found in two PAP patients from the endogamous white settler population in which URD had an unexpectedly high prevalence. This mutation was suggested to be due to environmental exposures or modifier genes to play a role in the phenotype (Tredano *et al.,* 2004).

The association of pulmonary fibrosis (PF) with rare inherited disorders and the variable susceptibility of inbred mouse strains to this disease indicate that PF is determined by genetic factors. SP-C and telomerase are susceptibility candidate genes for the development of PF. Recent evidence indicates that mutations in genes of these two different biologic pathways lead to the common phenotype of familial pulmonary fibrosis (FPF) and sporadic IPF (Steele and Brown, 2007; Fernandez *et al.,* 2012). Δ-Linkage analysis and candidate gene approaches have identified four genes that cause the inherited form of IPF, familial interstitial pneumonia (FIP). These four genes encode two surfactant proteins, surfactant protein C (encoded by *SFTPC*) and surfactant protein A2 (*SFTPA2*), and two components of the telomerase complex, telomerase reverse transcriptase (TERT) and the RNA component of telomerase (TERC) (Kottmann *et al.,* 2009; Kropski*et al.,* 2013). Mutations in the genes encoding the lung SF-C and A2 cause increased ER stress in type II alveolar epithelial cells. Mutations in genes encoding telomerase (TERT and TERC) cause IPF through shortening of telomere lengths and probable exhaustion of lung stem cells. All of the mutations are individually rare, but, collectively, TERT mutations are the most common genetic defect found in FPF. The roles of two unrelated biologic pathways-alveolar epithelial ER stress and telomerase dysfunction can elucidate the pathogenesis of IPF. Subsequent studies confirmed that ER stress markers are highly expressed in alveolar epithelium in IPF and FIP (Tanjore *et al.,* 2012; van Moorsel *et al.,* 2010; Mechri *et al.,* 2010).

Familial cases of interstitial pneumonia (FIP) have been reported to be linked to mutations of SP-C gene. Two missense mutations in exon 4 (N138T) and exon 5 (N186S) were identified in SP-C gene from 11 cases with FIP in Japanese population. Each exonic mutation consisted of DNA polymorphism. In particular, the N186S substitution of exon 5 in SP-C gene was shown in FIP or sporadic IP patients with a higher frequency. While pathophysiological mechanisms are yet to be elucidated, the N186S missense variant may have potential susceptibility in the development of IP (Setoguchi *et al.,* 2006). Familial clustering of adult idiopathic interstitial pneumonias (IIP) suggests that genetic factors might play an important role in disease development. Mutations in *SFTPC* are a frequent cause of FPF in adult patients. Non-classifiable radiological patterns with cystic changes and histopathological patterns of usual interstitial pneumonia are characteristics of adult *SFTPC* mutation carriers (van Moorsel *et al.,* 2010). Stevens *et al.,* (2005) described a spontaneous heterozygous mutation resulting in a substitution of lysine for glutamic acid at position 66 (E66K) of the proximal hSP-C COOH flanking propeptide. Lung histology of the patient (E66K) revealed nonspecific interstitial pneumonia (Stevens *et al.,* 2005). In a Spanish family two infants showed progressive neonatal respiratory failure associated with radiological and pathological alterations compatible with ILD. *SFTPC* mutations in general population of 760 individuals from Copenhagen indicated 18 genetic variants, of which 5 were novel. Of the five novel mutations, two A53T and Y106X were situated in highly conserved areas of the *SFTPC* gene. Genotyping identified 36 individuals heterozygous for A53T and 3 individuals heterozygous for Y106X (Baekvad-Hansen *et al.,* 2010).

In another study, 35 adult patients with sporadic idiopathic interstitial pneumonia 25 suffered from IPF and 10 patients from nonspecific interstitial pneumonia. Only two frequent nonsynonymous variants, T138N and S186N, were detected. These mutations in the gene encoding SP-C are not common in sporadic cases of IPF and nonspecific interstitial pneumonia, suggesting that the mutated gene does not play an important role in the pathogenesis of these forms of idiopathic interstitial pneumonia (Markart *et al.,* 2007; van Moorsel *et al.,* 2010). The aetiology of pathogenesis of both idiopathic pulmonary fibrosis (IPF) and familial idiopathic pulmonary fibrosis (FIPF) remains unclear. A consistent genetic basis has not yet been demonstrated in all cases. Other factors, including variable gene expression, co-carriage of other modifying genes and environmental stimuli, particularly cigarette smoke, significantly contribute to disease expression. Using one pulmonary fibrosis kindred, a mutation in the gene encoding SP-C was identified as the cause of pulmonary fibrosis in a family. Subsequently, another individual with IPF was identified with a different mutation in SP-C. These SP-C mutations highlight the importance of the alveolar epithelium in disease pathogenesis (Lawson and Loyd, 2006).

The genetic mutations in *SFTPC* with familial forms of pulmonary fibrosis, including one large family in which a number of family members were diagnosed with usual interstitial pneumonitis (UIP), have been reported with pathological correlate to IPF. Ten SNPs in the *SFTPC* sequence were found in IPF patients and not in controls. Only one of these created an exonic change resulting in a change in amino acid sequence. In this case, a T to C substitution resulted in a change in amino acid 73 of the precursor protein (I73T). Of the remaining polymorphisms, one was in 5' UTR, two were exonic without predicted amino acid sequence

changes, and six were intronic. One intronic mutation suggested a potential enhancement of a splicing site (Lawson *et al.,* 2004). This study indicated that *SFTPC* mutations do not contribute to the pathogenesis of IPF in majority of sporadic cases. In bleomycin-induced lung fibrosis in *SFTPC*$^{-/-}$ mice there was greater lung neutrophil influx after intratracheal bleomycin and increased mortality. SP-C seemed to function to limit lung inflammation, inhibit collagen accumulation, and restore normal lung structure after bleomycin. Susceptibility to IPF probably involves a combination of polymorphisms related to epithelial cell injury and abnormal wound healing (Grutters and du Bois, 2005). Soraisham *et al.,*(2006) reported a term newborn with an unusual lung disease due to mutation in *SFTPC* gene. Observations suggest that individuals with this particular mutation in SP-C gene might be at increased risk of ILD of variety of types (Chibbar *et al.,* 2004). Hamvas *et al.,* (2004) analysed lung tissue at the time of transplantation from a 14-mo-old infant with progressive ILD. An in-frame 9-bp deletion spanning codons 91-93 in Exon 3 of the SP-C gene was present on one allele; neither parent carried this deletion. A dominant negative effect on surfactant protein metabolism and function results from aggregation of misfolded pro-SP-C and subsequent cell injury and inflammation.

Mutations in *SFTPC* gene with ILD patients result in misfolding, ER retention, and degradation of the SP-C proprotein. Transient expression of ERdj4 and ERdj5 in X-box binding protein 1$^{-/-}$ mouse embryonic fibroblasts substantially restored rapid degradation of mutant SP-C proprotein, whereas transfection of HPD mutants failed to rescue SP-C ER-associated protein degradation. ERdj4 and ERdj5 promote turnover of misfolded SP-C and this action is dependent on their ability to stimulate BiP ATPase activity (Dong *et al.,* 2008). Furthermore, two common non-synonymous variants that encode an asparagine for threonine substitution at codon 138 (p.T138N, rs4715) and an asparagine for serine substitution at codon 186 (p.S186N, rs1124) have been associated with RDS among premature infants <34 weeks gestation (Lahti *et al.,* 2004). However, no comprehensive sequence analyses of the contribution of rare variants in *SFTPC* to RDS have been performed (Wambach *et al.,* 2010). The g.1728 G --> A mutation causes misfolding of the SP-C proprotein with subsequent induction of the unfolded protein response; the ER-associated degradation pathways ultimately resulting in disrupted lung morphogenesis (Bridges *et al.,* 2003).

Although the SP-C null mouse possesses a non-lethal phenotype, a heterozygous substitution of A for G in the first base of intron 4 of human SP-C gene (c.460+1A>G) has been reported in association with familial ILD and absence of mature protein. This mutation produces a splice deletion of exon 4 (Δ^{Exon4}) resulting in removal of a conserved cysteine in C-terminal flanking propeptide. In an earlier study an identical deletion in rat isoform diverted mutant protein to stable aggregates. It was hypothesized that expression of Δ^{Exon4} mutation would result in disruption of intracellular trafficking of both mutant and wild-type proSP-C. Wang *et al.,* (2003) suggested that c.460+1A>G mutation of human SP-C results in disruption of disulfide-mediated folding encoded by Exon 4 leading to diversion of unprocessed proSP-C to aggresomes. The heterotypic oligomerization of hSP-C (1-197) and hSP-C (Δ^{Exon4}) provides a molecular mechanism for the dominant-negative effect observed in vivo. Though, the SP-C $^{-/-}$ mice were viable at birth and grew normally to adulthood without apparent pulmonary abnormalities, neither SP-C mRNA nor mature SP-C protein was detected in lungs of SP-C $^{-/-}$ mice. The levels of the other surfactant proteins (A, B, D) in alveolar lavage were not different from those in wild-type mice. However, decreased stability of captive bubbles with surfactant from SP-C$^{-/-}$ mice indicated that SP-C plays a role in the stabilization of surfactant at low lung volumes, a condition that may accompany RDS in infants and adults (Glasser *et al.,* 2001). Moreover, bleomycin-induced lung fibrosis in *SFTPC* $^{-/-}$ mice, compared with wild-type (*SFTPC*$^{+/+}$), had greater lung neutrophil influx and increased mortality. In these mice, SP-C functions to limit lung inflammation, inhibits collagen accumulation, and restores normal lung structure after bleomycin (Lawson *et al.,* 2005). Zarbock *et al.,* (2012) investigated the effects of the mutation A116D in the BRICHOS domain of SP-C on cellular homeostasis. This mutation leads to impaired processing of proSP-C in alveolar epithelial cells, alters cell viability and lipid composition, and also activates cells of the immune system. Some of the effects of the mutation on cellular homeostasis can be antagonized by application of pharmaceuticals commonly applied in ILD therapy.

SP-C mutations after viral infections: Patients with mutations in the pulmonary SP-C gene develop ILD and pulmonary exacerbations associated with viral infections including respiratory syncytial virus (RSV). The SP-C-deficient (*SFTPC*$^{-/-}$) mice infected with *P. aeruginosa* showed low survival rate. SP-C is implicated as a modifier of alveolar homeostasis and in innate host defense of the lung (Glasser *et al.,* 2008; 2009; 2013). The SP-C deficiency increases the severity of RSV induced pulmonary inflammation through regulation of TLR3 signaling. Infection with RSV caused more severe interstitial thickening, air space consolidation, and goblet cell hyperplasia in SP-C-deficient mice. Bridges *et al.,* (2006) showed that stably transfected cells adapt to chronic ER stress imposed by constitutive expression of SP-CΔ^{exon4} via an NF-kB-dependent pathway. However, the infection of cells expressing SP-

CΔ^{exon4} with respiratory syncytial virus resulted in significantly enhanced cytotoxicity associated with accumulation of the mutant proprotein, pronounced activation of the unfolded protein response, and cell death. Adaptation to chronic ER stress imposed by misfolded SP-C was associated with increased susceptibility to viral-induced cell death. The wide variability in the age of onset of ILD in patients with *SftpC* mutations may be related to environmental insults that ultimately overwhelm the homeostatic cytoprotective response. In Finnish population, with neonatal RDS, two common amino acid variants Asn138Thr and Asn186Ser have been genotyped in children with asthma and children with severe RSV associated diseases. Single polymorphisms showed no association with the diseases, although SP-C haplotypes were associated with severe RSV associated diseases (Glasser *et al.,* 2013). Furthermore, an inverse haplotype distribution was found between children with asthma and RSV. The results of this study might suggest opposing roles of SP- C in the genetic predisposition for RSV associated diseases vs. asthma (Puthothu *et al.,* 2006).

BRICHOS DOMAIN OF PRO-SP-C

The C-terminal domain of lung surfactant pro-SP-C is involved in folding of the transmembrane segment of pro-SP-C. The C-terminal domain includes a Brichos domain with homologs in cancer- and dementia-associated proteins. Mutations in the Brichos domain cause misfolding of pro-SP-C and hence amyloid fibril formation in ILD. ESI-MS showed that the Brichos domain contains the peptide-binding function of CTC. The Brichos domain was also shown to bind fibril-forming peptides containing aromatic/hydrophobic residues (Fig. 2) (Fitzen *et al.,* 2009). Mutations within BRICHOS domain are associated with a severe disease phenotype. The BRICHOS domain was initially defined from sequence alignments of the Bri protein associated with familial dementia, chondromodulin associated with chondrosarcoma and SP-C precursor (proSP-C) associated with RDS and ILD. Today BRICHOS has been found in 12 protein families. Mutations in the Bri2 and proSP-C genes result in familial dementia and ILD, respectively, and both these conditions are associated with amyloid formation. Amyloid, of great medical relevance, is found in several major incurable diseases, like Alzheimer's and Parkinson's disease and diabetes mellitus. Work on recombinant BRICHOS domains and transfected cells indicate that BRICHOS is a chaperone domain that, during biosynthesis, binds to precursor protein regions with high β-sheet propensities, thereby preventing them from amyloid formation. Regions prone to form β-sheets are present in all BRICHOS-containing precursor proteins and are probably eventually released by proteolytic cleavage, generating different peptides with largely unknown bioactivities. Recombinant BRICHOS domains from Bri2 and pro-SP-C have been found to efficiently prevent SP-C, the amyloid β-peptide associated with Alzheimer's disease, and medin, found in aortic amyloid, from forming amyloid fibrils. The data collected so far on BRICHOS raise several interesting topics for further research: (a) amyloid formation is a potential threat for many more proteins than the ones recognized so far in amyloid diseases; (b) amyloid formation of widely different peptides involves intermediate(s) that are recognized by the BRICHOS domain, suggesting that they have distinct structural similarities; and (c) the BRICHOS domain might be harnessed in therapeutic strategies against amyloid diseases (Willander *et al.,* 2011). A heterozygous mutation, c.298G>A (G100S), in BRICHOS domain of pro-SP-C has been reported in a Japanese kindred with no mutation in ABCA3 gene. In vitro expression of mutant gene revealed expression of several ER stress-related proteins. The mutation increased ER stress and induced apoptotic cell death compared with wt SP-C in alveolar type II cells, supporting the role of this mutation in the pathogenesis of pulmonary fibrosis (Ono *et al.,* 2011).

Transformation from monomeric α-helix to a polymeric *β* aggregate: Under certain solvent and temperature conditions, SP-C is transformed from monomeric α-helix to a polymeric *β* aggregate which resembles amyloid fibrils (Dluhy *et al.,* 2003; Szyperski *et al.,* 1998; Gustafsson *et al.,* 1999) (Fig. 3B). These aggregates have been reported as part of amyloid fibril present during pulmonary alveolar proteinosis (PAP). The deacylation of SP-C has shown an increase in the rate of peptide aggregation and fibril formation because helix destabilization promotes the *β* aggregation (Gustafsson *et al.,* 2001). Studies demonstrate that the amino acid composition of the protein is essential to fibril formation (Ohnishi *et al.,* 2004) as stated for SP-C (Johansson, 2003), although other studies suggest that amyloid fibril formation is a property of the protein backbone (Ohnishi *et al.,* 2004; Guijarro *et al.,* 1998). The SP-C helix is composed primarily of valines, which is unusual since valines are underrepresented in helical conformation and over represented in *β*-strands (Johansson, 2003). Because of this, once the helical structure is lost a refold will rarely be observed. However, studies have shown that the *β*-branched amino acids Val and Ile rank among the best helix promoters in a membrane environment (Zangi *et al.,* 2001). Molecular simulations on the SP-C, comprising several consecutive valine residues indicated destabilization in chloroform and remarkably stable in methanol and water. In particular the polyvalyl part comprising residues Val^{15} to Val^{21} remained intact even at elevated temperature, and did not disrupt the α-helical conformation (Kovacs *et al.,* 1995; Zangi *et al.,* 2001). The α-helix from Asn^{9} to Val^{28} was stable up to a period of 5 ns. On the other hand, the segment comprising residues Val^{28}–Leu^{32} unfolded

in methanol and retained the α-helix structure in water. However, molecular dynamics simulations using the NAMD2 package for systems containing from one to seven SP-C molecules showed that unfolding of the protein occurs at the amino terminal and despite this unfolding, no transition from α-helix to β-strand was observed (Ramírez *et al.,* 2006).

The α-helix with a central poly-valine segment perfectly matches the thickness of a fluid DPPC bilayer. NMR and MS studies show that the poly-valine helix is kinetically stabilized, and that once it unfolds, formation of β-sheet aggregates is significantly faster than refolding. The α-Helix unfolding is accelerated after removal of the palmitoyl groups. Secondary structure prediction of SP-C yields β-strand conformation of the poly-valine part. Several amyloid-forming proteins harbor an α-helix in a polypeptide segment that should form a β-strand according to secondary structure predictions, contain a discordant helix and have been found to form fibrils at pH 7.4. It is proposed that α-helix/β-strand-discordant stretches are associated with amyloid fibril formation (Kallberg *et al.,* 2001; Johansson, 2001; 2004)

BRICHOS: A conserved domain in SP-C: A novel domain (the BRICHOS domain) of approximately 100 amino acids has been identified in several unrelated proteins that are linked to major diseases. BRICHOS domains are encoded in >30 human genes, which are associated with cancer, neuro degeneration, and ILD. Proteins include BRI2 protein, which is related to familial British and Danish dementia; Chondromodulin-I (ChM-I), related to chondrosarcoma; CA11, related to stomach cancer; and SP-C, related to RDS. In several of these, the conserved BRICHOS domain is located in the propeptide region that is removed after proteolytic processing (Fig. 2A). The Brichos domain has been related to the complex post-translational processing of these proteins (Sánchez-Pulido *et al.,* 2002). The BRICHOS domain from lung proSP-C is required for membrane insertion of SP-C and has anti-amyloid activity in vitro. The human proSP-C BRICHOS domain may mediate chaperone activity. Only two Cys residues and one Asp residue are strictly conserved in all BRICHOS domains (Hedlund *et al.,* 2009).

BRICHOS domain mutation causes endoplasmic reticulum stress, proteasome dysfunction, and apoptosis: Mutations in BRICHOS domain have been associated with both degenerative and proliferative diseases in several nonpulmonary organs, although the pathogenic mechanisms are mostly undefined. Several mutations in SP-C mapping to the BRICHOS domain located within the proSP-C have been linked to ILDs. In vitro expression of one of SP-C proteins, the exon 4 deletion mutant (hSP-CΔ^{exon4}), causes ER stress, inhibits proteasome function, and activates caspase3-mediated apoptosis. In addition, results support a model whereby proSP-C BRICHOS mutations induce a dynamic toxic gain-of-function, causing apoptotic cell death both by early ER accumulation leading to an exaggerated unfolded protein response and by enhanced deposition of cellular aggregates associated with proteasome dysfunction. To further elucidate mechanisms and common pathways for cellular dysfunction, various assays were performed by transiently expressing two SP-C BRICHOS domain mutant (BRISPC) proteins (hSP-CΔ^{exon4}), hSP-C(L188Q) and control proteins in lung epithelium-derived A549 and kidney epithelium-derived GFP^{u-1} cell lines. Results from these mutant proteins suggest common cellular responses, including initiation of cell-death signaling pathways (Mulugeta *et al.,* 2005; 2007). Proteasome function is impaired in ILD associated with SP-C mutation mapping to the BRICHOS domain located in the proSP-C protein. Proteasome inhibitors decreased TTF-1/Nkx2.1 DNA binding activity. The ubiquitin proteasome pathway is essential for the maintenance of surfactant protein gene expression and that disruption of this pathway inhibits surfactant protein gene expression via reduced expression of TTF-1 protein (Das and Boggaram, 2007).

Prolonged expression of the BRICHOS mutants, SP-C (Δ^{exon4}) and SP-C (L188Q), destabilizes ER quality-control mechanisms (the UPR), resulting in the induction of ER stress signaling, an inhibition of the ubiquitin/proteasome system, and the activation of apoptotic pathways (Maguire *et al.,* 2012). Based on recent observations that the UPR and ER stress can be linked to the induction of proinflammatory signaling, it is hypothesized that the epithelial cell dysfunction mediated by SP-C BRICHOS mutants would activate proinflammatory signalling pathways. Reports support the role of JNK signaling in mediating SP-C BRICHOS-induced cytokine release, and provide a link between SP-C BRICHOS mutants and proinflammatory cytokine signalling (Maguire *et al.,* 2011).

The ER stress (either induced or due to accumulation of misfolded proteins) is also associated with epithelial-mesenchymal transition (EMT) in alveolar epithelial cells (AECs). Overexpression of SP-C BRICHOS mutant SP-C (Δ^{Exon4}) in A549 cells increased Grp78 and α-SMA and disrupted ZO-1 distribution, and, in primary AECs, SP-C(ΔExon4) induced fibroblastic-like morphology, decreased ZO-1 and E-cadherin and increased α-SMA, mechanistically linking ER stress associated with mutant SP to fibrosis through EMT, suggesting that ER stress induces EMT in AECs, through Src-dependent pathways, a novel role for ER stress in fibroblast accumulation in pulmonary fibrosis (Zhong *et al.,* 2011).

Since the first identification of an interstitial lung disease (ILD)-associated mutation (Nogee *et al.,* 2002) in the proSP-C gene (*SFTPC*), more than 50 SFTPC mutations have been described (Willander *et al.,* 2012). The vast majority of these mutations are located in the linker and BRICHOS domains, with the linker mutation I73T being the most prevalent (Wert *et al.,* 2009).

The proSP-C BRICHOS domain has been suggested to act as a chaperone that targets the SP-C region of proSP-C and prevents its aggregation while assisting its safe membrane insertion as a TM helix (Johansson *et al.,* 2009a,b). Transgenic expression of proSP-C with two different BRICHOS mutations linked to ILD in a mammalian cell line generates Congo red positive inclusions and abundant aggregates of proSP-C, while expression of the I73T mutation only gave rise to low amounts of aggregated proSP-C (Nerelius *et al.,* 2008). In vitro data support the notion that the ILD-associated mutations could give rise to SP-C amyloid formation, but there are no earlier reports of amyloid found in ILD. The crystal structure of the BRICHOS domain of human proSP-C, has been analyzed for protein-peptide interactions and mutations in *SFTPC* mapped for ILD. Observation of amyloid deposits composed of mature SP-C in lung tissue samples from ILD patients with mutations in the BRICHOS domain or in its peptide binding linker region supports the in vivo relevance of the proposed mechanism. The results indicate that ILD mutations interfering with proSP-C BRICHOS activity cause amyloid disease secondary to intramolecular chaperone malfunction (Willander *et al.,* 2012). The mutation A116D in the BRICHOS domain of SP-C leads to impaired processing of proSP-C in alveolar epithelial cells, alters cell viability and lipid composition, and also activates cells of the immune system (Zarbock *et al.,* 2012).

Brichos domain-containing the C-terminal part of proSP-C (CTC) binds to an unfolded poly-val transmembrane segment: Native lung SP-C associates with alveolar surfactant phospholipids via a transmembrane α-helix (Fig. 2B). This helix contains mainly Val, although poly-Val is inefficient in helix formation, and helical SP-C can spontaneously convert to β-sheet aggregates and amyloid-like fibrils. SP-C is cleaved out from a 21-kDa proSP-C, in the alveolar type II cell. The CTC is localized in the endoplasmic reticulum lumen and binds to misfolded (β-strand) SP-C, thereby preventing its aggregation and amyloid fibril formation. Several mutations localized in CTC have been associated with intracellular accumulation of toxic forms of proSP-C, low levels of mature SP-C, and development of interstitial lung disease. CTC contains a approximately 100-residue Brichos domain that is also found in other membrane proteins associated with amyloid formation, dementia, and cancer.

Recombinant CTC binds lipid-associated SP-C, which is in β-strand conformation, and that this interaction results in an increased helical content. In contrast, CTC does not bind α-helical SP-C. The CTC protects the transmembrane part of (pro)SP-C from aggregation into amyloid until it has a folded into an α-helix. Studies suggested that CTproSP-C binds nonhelical SP-C and thereby prevents β-sheet aggregation and that mutations in CTproSP-C can interfere with this function (Johansson *et al.,* 2006). The C-terminal part of proSP-C forms non covalent trimers and supratrimeric oligomers. It contains two intrachain disulfide bridges, and its secondary structure is significantly affected by urea or heat only after disulfide reduction. Reports suggest that the Brichos domain of CTC, with homologs found in proteins associated with amyloid and proliferative disease is several-fold more protected from limited proteolysis than the rest of CTC. The protein exposes hydrophobic surfaces, as determined by fluorescent probe. The exposed hydrophobic surfaces and the structural disordering that result from interactions with phospholipid membranes suggest a mechanism whereby CTC binds to misfolded SP-C in the endoplasmic reticulum membrane (Casals *et al.,* 2008).

Anti-amyloid properties of CTC: The CTC appears to have a more general anti-amyloid effect as demonstrated on TM regions of other proteins. Interactions of CTC with the amyloid A_{β} associated with Alzheimer's disease and medin prevents fibril formation in A_{β} and medin and forms a complex with A_{β} oligomers. The CTC functions as a chaperone that acts preferentially against unfolded TM segments and structural motifs found during amyloid fibril formation, a mechanism that may be exploited in forming a basis for future anti-amyloid therapy. Analysis reveals that CTC binds to all amino acid residues that promote membrane insertion, provided they are in a nonhelical conformation. The CTC is the unique chaperone that may be employed for the development of new diagnostics or anti-amyloid therapies (Nerelius *et al.,* 2009; Johansson *et al.,* 2009a).

CONCLUSIONS AND FUTURE DIRECTIONS

Causes and treatments of pulmonary fibrosis remain significant research problems and as illustrated by IPF, the pathology is complex. The precise pathology likely involves deficiency of critical epithelial proteins that modulate processes involved in epithelial protection and repair including SP-C. Assessing levels of SP-C as a marker or potential therapeutic target may prove useful in future approaches to treating pulmonary fibrosis. SP-C is important component for the function of pulmonary surfactant. Heterozygous mutations in *SFTPC* gene cause sporadic and familial ILD in children and adults. There is obviously an urgent need to define the target mechanism of the treatments

being applied in ILD therapy. Most SP-C mutations cluster within the preprotein's BRICHOS domain and lead to misfolding of the preprotein, aberrant trafficking and processing and result in perinuclear aggregation of the proprotein. The effects of the mutations hSP-CΔ^{exon4} and A116D in the BRICHOS domain of SP-C on cellular homeostasis have to be re-ascertained and the ability of drugs currently used in ILD therapy to counteract the effects have to be evaluated. Studies need more attention on precise pharmacological targets in patients with SP-C deficiency. While the efficacy of surfactant in the prevention and treatment of RDS has been well documented, there are issues to be resolved. Palmitoylation is the key for the functional cooperation of SP-C with SP-B that enables cholesterol-containing surfactant films to reach very low surface tensions under compression. This fact is particularly important in the design of clinical surfactants destined to replacement therapies in diseases as ARDS. It is hoped that further development will allow synthetic surfactant to supersede natural surfactant. Besides, SP-B and SP-C containing surfactant may represent an important development of surfactant. The possible existence of a cooperative action or synergy between the two hydrophobic surfactant proteins SP-B and SP-C to sustain pulmonary surfactant performance is a relevant issue. Since SP-B and SP-C exhibit a truly cooperative behavior in samples containing both proteins simultaneously, there is need to define a threshold for the minimum protein composition required to produce surfactant preparations with optimal surface activity.

REFERENCES

1. Alonso C, Waring A, Zasadzinski JA. (2005): Keeping lung surfactant where it belongs: protein regulation of two-dimensional viscosity. Biophys. J. 89, 266-273.
2. Amrein M, von Nahmen A, Sieber M. (1997): A scanning force- and fluorescence light microscopy study of the structure and function of a model pulmonary surfactant. Eur. Biophys. J. 26, 349-357.
3. Augusto L, Le Blay K, Auger G, Blanot D, Chaby R. (2001): Interaction of bacterial lipopolysaccharide with mouse surfactant protein C inserted into lipid vesicles. Am. J. Physiol. Lung Cell Mol. Physiol. 281, L776-785.
4. Augusto LA, Li J, Synguelakis M, Johansson J, Chaby R. (2002): Structural basis for interactions between lung surfactant protein C and bacterial lipopolysaccharide. J. Biol. Chem. 277, 23484-23492.
5. Augusto LA, Synguelakis M, Espinassous Q, Lepoivre M, Johansson J, Chaby R. (2003a) Cellular antiendotoxin activities of lung surfactant protein C in lipid vesicles. Am. J. Respir. Crit. Care Med. 168, 335-341.
6. Augusto LA, Synguelakis M, Johansson J, Pedron T, Girard R, Chaby R. (2003b): Interaction of pulmonary surfactant protein C with CD14 and lipopolysaccharide. Infect. Immun. 71, 61-67.
7. Baekvad-Hansen M, Nordestgaard BG, Tybjaerg-Hansen A, Dahl M. (2010):Two novel mutations in surfactant protein-C, lung function and obstructive lung disease. Respir Med. 104,418-425
8. Baoukina S, Monticelli L, Amrein M, Tieleman DP. (2007): The molecular mechanism of monolayer-bilayer transformations of lung surfactant from molecular dynamics simulations. Biophys. J. 93, 3775-3782.
9. Beers MF and Fisher AB. (1992): Surfactant protein C: A review of its unique properties and metabolism. Am. J. Physiol. 263, L151-160.
10. Beers MF, Hawkins A, Maguire JA, Kotorashvili A, Zhao M, Newitt JL, Ding W, Russo S, Guttentag S, Gonzales L, Mulugeta S. (2011): A nonaggregating surfactant protein C mutant is misdirected to early endosomes and disrupts phospholipid recycling. Traffic. 12,1196-1210.
11. Beers MF, Kim CY, Dodia C, Fisher AB. (1994): Localization, synthesis, and processing of surfactant protein SP-C in rat lung analyzed by epitope-specific antipeptide antibodies. J. Biol. Chem. 269, 20318-20328
12. Beers MF, Lomax C (1995): Synthesis and processing of hydrophobic surfactant protein C by isolated rat type II cells. Am. J. Physiol. 269, L744-753.
13. Beers MF, Lomax, CA, Russo SJ. (1998): Synthetic processing of surfactant protein C by alevolar epithelial cells. The COOH terminus of proSP-C is required for post-translational targeting and proteolysis. J. Biol. Chem. 273, 15287-15293.
14. Beers MF, Mulugeta S. (2005): Surfactant protein C biosynthesis and its emerging role in conformational lung disease. Annu. Rev. Physiol. 67, 663–696.
15. Bourdos N, Kollmer F, Benninghoven A, Ross M, Sieber M, Galla HJ. (2000): Analysis of lung surfactant model systems with time-of-flight secondary ion mass spectrometry. Biophys. J. 79, 357-369.
16. Brasch F, Griese M, Tredano M, Johnen G, Ochs M, Rieger C, Mulugeta S, Müller KM, Bahuau M, Beers MF. (2004): Interstitial lung disease in a baby with a de novo mutation in the SFTPC gene. Eur Respir J. 24, 30-39.
17. Bridges JP, Wert SE, Nogee LM, Weaver TE. (2003): Expression of a human surfactant protein C mutation associated with interstitial lung disease disrupts lung development in transgenic mice. J Biol Chem. 278,52739-52746.
18. Bridges JP, Xu Y, Na CL, Wong HR, Weaver TE. (2006): Adaptation and increased susceptibility to infection associated with constitutive expression of misfolded SP-C. J. Cell Biol. 172, 395-407.
19. Bullard JE, Nogee LM. (2007): Heterozygosity for ABCA3 mutations modifies the severity of lung disease associated with a surfactant protein C gene (SFTPC) mutation. Pediatr. Res. 62, 176-179.
20. Cameron HS, Somaschini M, Carrera P, Hamvas A, Whitsett JA, Wert SE, Deutsch G, Nogee LM. (2005): A common mutation in the surfactant protein C gene associated with lung disease. J. Pediatr. 146, 370–375.
21. Casals C, Johansson H, Saenz A, Gustafsson M, Alfonso C, Nordling K, Johansson J. (2008): C-terminal, endoplasmic reticulum-lumenal domain of prosurfactant protein C - structural features and membrane interactions. FASEB J. 275, 536-547.

22. Chibbar R, Shih F, Baga M, Torlakovic E, Ramlall K, Skomro R, Cockcroft DW, Lemire EG. (2004): Nonspecific interstitial pneumonia and usual interstitial pneumonia with mutation in surfactant protein C in familial pulmonary fibrosis. Mod Pathol. 17, 973-980.

23. Cirera S, Nygård AB, Jensen HE, Skovgaard K, Boye M, Fredholm M. (2006): Molecular characterization of the porcine surfactant, pulmonary-associated protein C gene. Genomics. 88, 659-668.

24. Conkright JJ, Apsley KS, Martin EP, Ridsdale R, Rice WR, Na CL, Yang B, Weaver TE. (2010): NEDD4-2- Mediated ubiquitination facilitates processing of surfactant protein C. Am. J. Respir. Cell Mol. Biol. 42, 181-189.

25. Conkright JJ, Bridges JP, Na CL, Voorhout WF, Trapnell B, Glasser SW, Weaver TE. (2001): Secretion of surfactant protein C, an integral membrane protein, requires the N-terminal propeptide. J. Biol. Chem. 276, 14658-14664.

26. Creuwels LA, Boer EH, Demel RA, van Golde LM, Haagsman HP. (1995a): Neutralization of the positive charges of surfactant protein C. Effects on structure and function. J. Biol. Chem. 270, 16225-16229.

27. Creuwels LA, Demel RA, van Golde LM, Benson BJ, Haagsman HP. (1993): Effect of acylation on structure and function of surfactant protein C at the air-liquid interface. J. Biol. Chem. 268, 26752-26758.

28. Creuwels LA, Demel RA, van Golde LM, Haagsman HP. (1995b): Characterization of a dimeric canine form of surfactant protein C (SP-C). Biochim. Biophys. Acta. 1254, 326-332.

29. Crossno PF, Polosukhin VV, Blackwell TS, Johnson JE, Markin C, Moore PE, Worrell JA, Stahlman MT, Phillips JA 3rd, Loyd JE, Cogan JD, Lawson WE. (2010): Identification of early interstitial lung disease in an individual with genetic variations in ABCA3 and SFTPC. Chest. 137, 969-973.

30. Curstedt T, Johansson J, Persson P, Eklund A, Robertson B, Lowenadler B, Jornvall H. (1990): Hydrophobic surfactant-associated polypeptides: SP-C is a lipopeptide with two palmitoylated cysteine residues, whereas SP-B lacks covalently linked fatty acyl groups. Proc. Natl. Acad. Sci. USA 87, 2985–2989.

31. Das A, Boggaram V. (2007): Proteasome dysfunction inhibits surfactant protein gene expression in lung epithelial cells: mechanism of inhibition of SP-B gene expression. Am. J. Physiol. Lung Cell. Mol. Physiol. 292, L74-84.

32. Dico AS, Taneva S, Morrow MR, Keough KM. (1997): Effect of calcium on phospholipid interaction with pulmonary surfactant protein C. Biophys. J. 73, 2595-2602.

33. Dluhy RA, Shanmukh S, Leapard JB, Krüger P, Baatz JE. (2003): Deacylated pulmonary surfactant protein SP-C transforms from α-helical to amyloid fibril structure via a pH-dependent mechanism: an infrared structural investigation. Biophys. J. 85, 2417-2429.

34. Dong M, Bridges JP, Apsley K, Xu Y, Weaver TE. (2008): ERdj4 and ERdj5 are required for endoplasmic reticulum-associated protein degradation of misfolded surfactant protein C. Mol. Biol. Cell. 19, 2620-2630.

35. Fernandez BA, Fox G, Bhatia R, Sala E, Noble B, Denic N, Fernandez D, Duguid N, Dohey A, Kamel F, Edwards L, Mahoney K, Stuckless S, Parfrey PS, Woods MO. (2012): A Newfoundland cohort of familial and sporadic idiopathic pulmonary fibrosis patients: clinical and genetic features. Respir Res. 13:64. doi: 10.1186/1465-9921-13-64.

36. Fitzen M, Alvelius G, Nordling K, Jörnvall H, Bergman T, Johansson J. (2009): Peptide-binding specificity of the prosurfactant protein C Brichos domain analyzed by electrospray ionization mass spectrometry. Rapid Commun Mass Spectrom. 23, 3591-3598.

37. Follows D, Tiberg F, Thomas RK, Larsson M. (2007): Multilayers at the surface of solutions of exogenous lung surfactant: direct observation by neutron reflection. Biochim. Biophys. Acta. 1768, 228-235.

38. Galetskiy D, Woischnik M, Ripper J, Griese M, Przybylski M. (2008): Aberrant processing forms of lung surfactant proteins SP-B and SP-C revealed by high-resolution mass spectrometry. Eur. J. Mass Spectrom (Chichester, Eng). 14, 379-390.

39. Garcia-Verdugo I, Garcia de Paco E, Espinassous Q, Gonzalez-Horta A, Synguelakis M, Kanellopoulos J, Rivas L, Chaby R, Perez-Gil J. (2009): Synthetic peptides representing the N-terminal segment of surfactant protein C modulate LPS-stimulated TNF-α production by macrophages. Innate Immun. 15, 53-62.

40. Garmany TH, Wambach JA, Heins HB, Watkins-Torry JM, Wegner DJ, Bennet K, An P, Land G, Saugstad OD, Henderson H, Nogee LM, Cole FS, Hamvas A. (2008): Population and disease-based prevalence of the common mutations associated with surfactant deficiency. Pediatr Res. 63, 645-649.

41. Glasser SW, Burhans MS, Korfhagen TR, Na CL, Sly PD, Ross GF, Ikegami M, Whitsett JA. (2001): Altered stability of pulmonary surfactant in SP-C-deficient mice. Proc. Natl. Acad. Sci. USA. 98, 6366-6371.

42. Glasser SW, Senft AP, Maxfield MD, Ruetschilling TL, Baatz JE, Page' K, Korfhagen KR. (2013): Genetic replacement of surfactant protein-C reduces respiratory syncytial virus induced lung injury. Respir. Res. 14:19 doi:10.1186/1465-9921-14-19.

43. Glasser SW, Korfhagen TR, Perme CM, Pilot-Matias TJ, Kister SE, Whitsett JA. (1988a): Two SP-C genes encoding human pulmonary surfactant proteolipid. J. Biol. Chem. 263, 10326-10331.

44. Glasser SW, Korfhagen TR, Weaver TE, Clark JC, Pilot-Matias T, Meuth J, Fox JL, Whitsett JA. (1988b): cDNA, deduced polypeptide structure and chromosomal assignment of human pulmonary surfactant proteolipid, SPL(pVal). J. Biol. Chem. 263, 9-12.

45. Glasser SW, Senft AP, Whitsett JA, Maxfield MD, Ross GF, Richardson TR, Prows DR, Xu Y, Korfhagen TR. (2008): Macrophage dysfunction and susceptibility to pulmonary Pseudomonas aeruginosa infection in surfactant protein C-deficient mice. J. Immunol. 181, 621-628.

46. Glasser SW, Witt TL, Senft AP, Baatz JE, Folger D, Maxfield MD, Akinbi HT, Newton DA, Prows DR, Korfhagen TR. (2009): Surfactant protein C-deficient mice are susceptible to respiratory syncytial virus infection. Am J Physiol Lung Cell Mol Physiol. 297, L64-72.

47. Gonzalez-Horta A, Andreu D, Morrow MR, Perez-Gil J. (2008): Effects of palmitoylation on dynamics and phospholipid-bilayer-perturbing properties of the N-terminal segment of pulmonary surfactant protein SP-C as shown by ^{2}H-NMR. Biophys. J. 95, 2308-2317.

48. Grutters JC, du Bois RM. (2005): Genetics of fibrosing lung diseases. Eur Respir J. 25, 915-927.

49. Guijarro JI, Sunde M, Jones JA, Campbell ID, Dobson CM. (1998): Amyloid fibril formation by SH3 domain. Proc. Natl. Acad. Sci. USA. 95, 4224–4228.

50. Guillot L, Epaud R, Thouvenin G, Jonard L, Mohsni A, Couderc R, Counil F, de Blic J, Taam RA, Le Bourgeois M, Reix P, Flamein F, Clement A, Feldmann D. (2009): New surfactant protein C gene mutations associated with diffuse lung disease. J Med Genet. 46, 490-494.

51. Gupta A, Gupta GS. (2012a): C-Type Lectins Family In: Animal Lectins: Form, Function and Clinical Applications (Eds. Gupta GS, Gupta A and Gupta RK), pp. 473-500, Springer, Wien.

52. Gupta A, Gupta RK. (2012b): Pulmonary SP-A. In: Animal Lectins: Form, Function and Clinical Applications (Eds. Gupta GS, Gupta A and Gupta RK), pp. 501-526, Springer, Wien.

53. Gupta A. (2012): Pulmonary Collectins in Diagnosis and Prevention of Lung Diseases In: Animal Lectins: Form, Function and Clinical Applications (Eds. Gupta GS, Gupta A and Gupta RK), pp. 955-990, Springer, Wien.

54. Gupta RK, Gupta A. (2012c): Surfactant Protein-D: Forms and Functions. In: Animal Lectins: Form, Function and Clinical Applications (Eds. Gupta GS, Gupta A and Gupta RK), pp. 527-550, Springer, Wien.

55. Gustafsson M, Griffiths WJ, Furusjö E, Johansson J. (2001): The palmitoyl groups of lung surfactant protein C reduce unfolding into a fibrillogenic intermediate. J. Mol. Biol. 310, 937-950.

56. Gustafsson M, Palmblad M, Curstedt T, Johansson J, Schürch S. (2000): Palmitoylation of a pulmonary surfactant protein C analogue affects the surface associated lipid reservoir and film stability. Biochim. Biophys. Acta. 1466, 169-178.

57. Gustafsson M, Thyberg J, Näslund J, Eliasson E, Johansson J. (1999): Amyloid fibril formation by pulmonary surfactant protein C. FEBS Lett. 464, 138-142.

58. Hamvas A, Cole FS, Nogee LM. (2007): Genetic disorders of surfactant proteins. Neonatology 91, 311–317.

59. Hamvas A, Nogee LM, White FV, Schuler P, Hackett BP, Huddleston CB, Mendeloff EN, Hsu FF, Wert SE, Gonzales LW, Beers MF, Ballard PL. (2004): Progressive lung disease and surfactant dysfunction with a deletion in surfactant protein C gene. Am J Respir Cell Mol Biol. 30, 771-776.

60. Hardie WD, Glasser SW, Hagood JS. (2009): Emerging concepts in the pathogenesis of lung fibrosis. Am J Pathol 175, 3-16.

61. Hardie WD, Hagood JS, Dave V, Perl A-K T, Whitsett JA, Korfhagen TR, Glasser S. (2010): Signaling pathways in the epithelial origins of pulmonary fibrosis. Cell Cycle 9, 2769-2776.

62. Hartl D, Griese M. (2005): Interstitial lung disease in children -- genetic background and associated phenotypes. Respir Res. 6, 32.

63. Hedlund J, Johansson J, Persson B. (2009): BRICHOS—A superfamily of multidomain proteins with diverse functions. BMC Res. Notes 2, 180.

64. Hosia W, Johansson J, Griffiths WJ. (2002): Hydrogen/deuterium exchange and aggregation of a polyvaline and a polyleucine alpha-helix investigated by matrix-assisted laser desorption ionization mass spectrometry. Mol. Cell. Proteomics 1, 592–597.

65. Johansson H, Eriksson M, Nordling K, Presto J, Johansson J. (2009a): The Brichos domain of prosurfactant protein C can hold and fold a transmembrane segment. Protein Sci. 18, 1175-1182.

66. Johansson H, Nerelius C, Nordling K, Johansson J. (2009b): Preventing amyloid formation by catching unfolded transmembrane segments. J. Mol. Biol. 389, 227-229.

67. Johansson H, Nordling K, Weaver TE, Johansson J. (2006): The Brichos domain-containing C-terminal part of pro-surfactant protein C binds to an unfolded poly-val transmembrane segment. J. Biol. Chem. 28,: 21032-2139.

68. Johansson J (2003): Molecular determinants for amyloid fibril formation: lessons from lung surfactant protein C. Swiss Med. Wkly. 133 275–282.

69. Johansson J, Szyperski T, Curstedt T, Wüthrich K. (1994): The NMR structure of the pulmonary surfactant-associated polypeptide SP-C in an apolar solvent contains a valyl-rich α-helix. Biochemistry 33, 6015–6023.

70. Johansson J, Weaver TE, Tjernberg LO. (2004): Proteolytic generation and aggregation of peptides from transmembrane regions: lung surfactant protein C and amyloid β-peptide. Cell Mol. Life Sci. 61, 326-335.

71. Johansson J. (1998): Structure and properties of surfactant protein C. Biochim. Biophys. Acta 1408, 161-172.

72. Johansson J. (2001): Membrane properties and amyloid fibril formation of lung surfactant protein C. Biochem. Soc Trans. 29, 601-606.

73. Johnson AL, Braidotti P, Pietra GG, Russo SJ, Kabore A, Wang WJ, Beers MF. (2001): Post-translational processing of surfactant protein-C proprotein: targeting motifs in the NH(2)-terminal flanking domain are cleaved in late compartments. Am J Respir Cell Mol Biol. 24, 253-263.

74. Jordanova A, Georgiev GA, Alexandrov S, Todorov R, Lalchev Z. (2009): Influence of surfactant protein C on the interfacial behavior of phosphatidylethanol- amine monolayers. Eur Biophys. J. 38, 369-379.

75. Kabore AF, Wang WJ, Russo SJ, Beers MF. (2001): Biosynthesis of surfactant protein C: characterization of aggresome formation by EGFP chimeras containing propeptide mutants lacking conserved cysteine residues. J Cell Sci. 114, 293-302.

76. Kairys V, Gilson MK, Luy B. (2004b): Structural model for an AxxxG-mediated dimer of surfactant-associated protein C. Eur. J. Biochem.. 271, 2086-2092.

77. Kalina M, Mason RJ, Shannon JM. (1992): Surfactant protein C is expressed in alveolar type II cells but not in Clara cells of rat lung. Am J Respir Cell Mol Biol. 6, 594-600.

78. Kallberg Y, Gustafsson M, Persson B, Thyberg J, Johansson J. (2001): Prediction of amyloid fibril-forming proteins. J. Biol. Chem. 276, 12945-12950.

79. Keller A, Eistetter HR, Voss T, Schäfer KP. (1991): The pulmonary surfactant protein C (SP-C) precursor is a type II transmembrane protein. Biochem J. 277, 493-499.

80. Kotorashvili A, Russo SJ, Mulugeta S, Guttentag SH, Beers MF. (2009): Anterograde transport of surfactant protein C proprotein to distal processing compartments requires PPDY

mediated association with NEDD4 ubiquitin ligases. J. Biol. Chem. 284, 16667-16678.

81. Kottmann RM, Hogan CM, Phipps RP, Sime PJ. (2009): Determinants of initiation and progression of idiopathic pulmonary fibrosis. Respirology. 14, 917-933.

82. Kovacs H, Mark AE, Johansson J, van Gunsteren WF. (1995): The effect of environment on the stability of an integral membrane helix: molecular dynamics simulations of surfactant protein C in chloroform, methanol and water. J. Mol. Biol. 247, 808-822.

83. Kramer A, Wintergalen A, Sieber M, Galla HJ, Amrein M, Guckenberger R. (2000): Distribution of the surfactant-associated protein C within a lung surfactant model film investigated by near-field optical microscopy. Biophys. J. 78, 458-465.

84. Kropski JA, Lawson WE, Young LR, Blackwell TS. (2013): Genetic studies provide clues on the pathogenesis of idiopathic pulmonary fibrosis. Dis Model Mech. 6, 9-17

85. Krüger P, Baatz JE, Dluhy RA, Lösche M. (2002): Effect of hydrophobic surfactant protein SP-C on binary phospholipid monolayers. Molecular machinery at the air/water interface. Biophys Chem. 99, 209-228.

86. Krüger P, Schalke M, Wang Z, Notter RH, Dluhy RA, Losche M. (1999): Effect of hydrophobic surfactant peptides SP-B and SP-C on binary phospholipids monolayer. Biophys. J. 77, 903–14.

87. Lahti M, Marttila R, Hallman M (2004): Surfactant protein C gene variation in the Finnish population -association with perinatal respiratory disease. Eur J Hum Genet 12, 312–320.

88. Lawson WE, Grant SW, Ambrosini V, Womble KE, Dawson EP, Lane KB, Markin C, Renzoni E, Lympany P, Thomas AQ, Roldan J, Scott TA, Blackwell TS, Phillips JA 3rd, Loyd JE, du Bois RM. (2004): Genetic mutations in surfactant protein C are a rare cause of sporadic cases of IPF. Thorax. 59, 977-980.

89. Lawson WE, Loyd JE. (2006): The genetic approach in pulmonary fibrosis: can it provide clues to this complex disease? Proc Am Thorac Soc. 3, 345-349.

90. Lawson WE, Polosukhin VV, Stathopoulos GT, Zoia O, Han W, et al. (2005): Increased and prolonged pulmonary fibrosis in surfactant protein C-deficient mice following intratracheal bleomycin. Am. J. Pathol. 167: 1267-1277.

91. Li D, Zeng Z, Xue W, Yao Y. (2007): The model of the action mechanism of SP-C in the lung surfactant monolayers. Colloids Surf. B Biointerfaces. 57, 22-28.

92. Li J, Hosia W, Hamvas A, Thyberg J, Jörnvall H, Weaver TE, Johansson J. (2004a): The N-terminal propeptide of lung surfactant protein C is necessary for biosynthesis and prevents unfolding of a metastable α-helix. J. Mol. Biol. 338, 857-862.

93. Li J, Ikegami M, Na CL, Hamvas A, Espinassous Q, Chaby R, Nogee LM, Weaver TE, Johansson J. (2004b): N-terminally extended surfactant protein (SP) C isolated from SP-B-deficient children has reduced surface activity and inhibited lipopolysaccharide binding. Biochemistry 43, 3891-3898.

94. Li J, Liepinsh E, Almlén A, Thyberg J, Curstedt T, Jörnvall H, Johansson J. (2006) Structure and influence on stability and activity of the N-terminal propeptide part of lung surfactant protein C. FEBS J. 273, 926-935.

95. Luy B, Diener A, Hummel RP, Sturm E, Ulrich WR, Griesinger C. (2004): Structure and potential C-terminal dimerization of a recombinant mutant of surfactantassociated protein C in chloroform/methanol. Eur. J. Biochem. 271, 2076–2085.

96. Maguire JA, Mulugeta S, Beers MF. (2011): Endoplasmic reticulum stress induced by surfactant protein C BRICHOS mutants promotes proinflammatory signaling by epithelial cells. Am J Respir Cell Mol Biol. 44, 404-414.

97. Maguire JA, Mulugeta S, Beers MF. (2012): Multiple ways to die: delineation of the unfolded protein response and apoptosis induced by Surfactant Protein C BRICHOS mutants. Int J Biochem Cell Biol. 44,101-112.

98. Malcharek S, Hinz A, Hilterhaus L, Galla HJ. (2005): Multilayer structures in lipid monolayer films containing surfactant protein C: effects of cholesterol and POPE. Biophys. J. 88, 2638-2649.

99. Markart P, Ruppert C, Wygrecka M, Schmidt R, Korfei M, Harbach H, Theruvath I, Pison U, Seeger W, Guenther A, Witt H. (2007): Surfactant protein C mutations in sporadic forms of idiopathic interstitial pneumonias. Eur Respir J. 29, 134-137.

100. McBee AD, Wegner DJ, Carlson CS, Wambach JA, Yang P, Heins *HB*, Saugstad OD, Trusgnich MA, Watkins-Torry J, Nogee LM, Henderson H, Cole FS, Hamvas A. (2008): Recombination as a mechanism for sporadic mutation in the surfactant protein-C gene. Pediatr Pulmonol. 43, 443-450.

101. Mechri M, Epaud R, Emond S, Coulomb A, Jaubert F, Tarrant A, Feldmann D, Flamein F, Clement A, de Blic J, Taam RA, Brunelle F, le Pointe HD. (2010): Surfactant protein C gene (SFTPC) mutation-associated lung disease:high-resolution computed tomography (HRCT) findings and its relation to histological analysis. Pediatr Pulmonol. 45,1021-1029.

102. Mingarro I, Nilsson I, Whitley P, von Heijne G. (2000): Different conformations of nascent polypeptides during translocation across the ER membrane. BMC Cell Biol. 1, 3.

103. Morrow MR, Taneva S, Simatos GA, Allwood LA, Keough KM. (1993): ^{2H}NMR studies of the effect of pulmonary surfactant SP-C on the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine headgroup: a model for transbilayer peptides in surfactant and biological membranes. Biochemistry 32: 11338-11344.

104. Mulugeta S, Beers MF. (2003): Processing of surfactant protein C requires a type II transmembrane topology directed by juxtamembrane positively charged residues. J. Biol. Chem. 278, 47979-47986.

105. Mulugeta S, Beers MF. (2006): Surfactant protein C: its unique properties and emerging immunomodulatory role in the lung. Microbes Infect. 8, 2317–2323.

106. Mulugeta S, Maguire JA, Newitt JL, Russo SJ, Kotorashvili A, Beers MF. (2007): Misfolded BRICHOS SP-C mutant proteins induce apoptosis via caspase-4- and cytochrome c-related mechanisms. Am. J. Physiol. Lung Cell Mol. Physiol. 293, L720-729.

107. Mulugeta S, Nguyen V, Russo SJ, Muniswamy M, Beers MF. (2005): A surfactant protein C precursor protein BRICHOS domain mutation causes endoplasmic reticulum stress, proteasome dysfunction, and caspase 3 activation. Am J Respir Cell Mol Biol. 32, 521-530.

108. Na Nakorn P, Meyer MC, Flach CR, Mendelsohn R, Galla HJ. (2007): Surfactant protein C and lung function: new insights into the role of α-helical length and palmitoylation. Eur. Biophys. J. 36, 477-489.

109. Nag K, Perez-Gil J, Cruz A, Keough KM. (1996a): Fluorescently labeled pulmonary surfactant protein C in spread phospholipid monolayers. Biophys. J. 71, 246-256.

110. Nag K, Perez-Gil J, Cruz A, Rich NH, Keough KM. (1996b): Spontaneous formation of interfacial lipid-protein monolayers during adsorption from vesicles. Biophys. J. 71, 1356-1363.

111. Nerelius C, Gustafsson M, Nordling K, Larsson A, Johansson J. (2009): Anti-amyloid activity of the C-terminal domain of proSP-C against amyloid β-peptide and medin. Biochemistry 48, 3778-3786.

112. Nerelius C, Martin E, Peng S, Gustafsson M, Nordling K, Weaver T, Johansson J. (2008): Mutations linked to interstitial lung disease can abrogate anti-amyloid function of prosurfactant protein C. Biochem. J. 41, 201–209.

113. Nogee LM, Dunbar AE 3rd, Wert S, Askin F, Hamvas A, Whitsett JA. (2002): Mutations in the surfactant protein C gene associated with interstitial lung disease. Chest 121 20S–21S.

114. Nogee LM, Dunbar AE 3rd, Wert SE, Askin F, Hamvas A, Whitsett JA. (2001): A mutation in the surfactant protein C gene associated with familial interstitial lung disease. N. Engl. J. Med. 344, 573–579.

115. Nogee LM. (2006): Genetics of pediatric interstitial lung disease. Curr Opin Pediatr. 18:287-292.

116. Ohnishi S, Takano K (2004): Amyloid fibrils form the viewpoint of protein folding. Cell Mol. Life Sci. 61 511–524.

117. Ono S, Tanaka T, Ishida M, Kinoshita A, Fukuoka J, Takaki M, Sakamoto N, Ishimatsu Y, Kohno S, Hayashi T, et al. (2011): Surfactant protein C G100S mutation causes familial pulmonary fibrosis in Japanese kindred. Eur Respir J. 38:861-869.

118. Pietschmann SM, Pison U (2000): cDNA cloning of ovine pulmonary SP-A, SP-B, and SP-C: isolation of two different sequences for SP-B. Am J Physiol Lung Cell Mol Physiol. 278:L765-78.

119. Plascencia I, Rivas L, Keough KM, Marsh D, Perez-Gil J. (2004): The N-terminal segment of pulmonary surfactant lipopeptide SP-C has intrinsic propensity to interact with and perturb phospholipid bilayers. Biochem. J. 377, 183–193.

120. Plasencia I, Baumgart F, Andreu D, Marsh D, Pérez-Gil J. (2008): Effect of acylation on the interaction of the N-Terminal segment of pulmonary surfactant protein SP-C with phospholipid membranes. Biochim. Biophys. Acta 1778, 1274-1282.

121. Plasencia I, Cruz A, Casals C, Pérez-Gil J. (2001): Superficial disposition of the N-terminal region of the surfactant protein SP-C and the absence of specific SP-B-SP-C interactions in phospholipid bilayers. Biochem. J. 359, 651-659.

122. Plasencia I, Keough KM, Perez-Gil J. (2005): Interaction of the N-terminal segment of pulmonary surfactant protein SP-C with interfacial phospholipid films. Biochim. Biophys. Acta 1713, 118-128.

123. Puthothu B, Krueger M, Heinze J, Forster J, Heinzmann A. (2006): Haplotypes of surfactant protein C are associated with common paediatric lung diseases. Pediatr Allergy Immunol. 17: 572-527.

124. Ramírez E, Santana A, Cruz A, Plasencia I, López GE. (2006): Molecular dynamics of surfactant protein C from single molecule to heptameric aggregates. Biophys. J. 90, 2698–2705.

125. Sánchez-Pulido L, Devos D, Valencia A. (2002): BRICHOS: a conserved domain in proteins associated with dementia, respiratory distress and cancer. Trends Biochem. Sci. 27, 329-332.

126. Schürch D, Ospina OL, Cruz A, Pe´ rez-Gil J. (2010): Combined and independent action of proteins SP-B and SP-C in the surface behavior and mechanical stability of pulmonary surfactant films. Biophys. J. 99, 3290–3299.

127. Setoguchi Y, Ikeda T, Fukuchi Y. (2006): Clinical features and genetic analysis of surfactant protein C in adult-onset familial interstitial pneumonia. Respirology. 11Suppl: S41-45.

128. Solarin KO, Wang WJ, Beers MF. (2001): Synthesis and post-translational processing of surfactant protein C. Pediatr. Pathol. Mol. Med. 20, 471-500.

129. Somaschini M, Nogee LM, Sassi I, Danhaive O, Presi S, Boldrini R, Montrasio C, Ferrari M, Wert SE, Carrera P. (2007): Unexplained neonatal respiratory distress due to congenital surfactant deficiency. J Pediatr. 150, 649-53, 653.e1.

130. Soraisham AS, Tierney AJ, Amin HJ. (2006): Neonatal respiratory failure associated with mutation in the surfactant protein C gene. J Perinatol. 26,67-70.

131. Steele MP, Brown KK. (2007): Genetic predisposition to respiratory diseases: infiltrative lung diseases. Respiration. 74, 601-608.

132. Stevens PA, Pettenazzo A, Brasch F, Mulugeta S, Baritussio A, Ochs M, Morrison L, Russo SJ, Beers MF. (2005): Nonspecific interstitial pneumonia, alveolar proteinosis, and abnormal proprotein trafficking resulting from a spontaneous mutation in the surfactant protein C gene. Pediatr Res. 57, 89-98.

133. Stults JT, Griffin PR, Lesikar DD, Naidu A, Moffat B, Benson BJ. (1991): Lung surfactant protein SP-C from human, bovine, and canine sources contains palmityl cysteine thioester linkages. Am. J. Physiol. 261, L118-125.

134. Szyperski TG, Vandenbussche G, Curstedt T, Ruysschaert JM, Wuthrich K, Johansson J. (1998): Pulmonary surfactant-associated polypeptide C in a mixed organic solvent transforms from a monomeric α-helical state into insoluble β-sheet aggregates. Protein Sci. 7, 2533–2540.

135. Tanjore H, Blackwell TS, Lawson WE. (2012) Emerging evidence for endoplasmic reticulum stress in the pathogenesis of idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 302, L721-9.

136. Ten Brinke A, Batenburg JJ, Gadella BM, Haagsman HP, Vaandrager AB, van Golde LM. (2001): The juxtamembrane lysine and arginine residues of surfactant protein C precursor influence palmitoylation via effects on trafficking. Am. J. Respir. Cell Mol. Biol. 25, 156-163

137. Ten Brinke A, Batenburg JJ, Haagsman HP, van Golde LM, Vaandrager AB. (2002a): Differential effect of brefeldin A on the palmitoylation of surfactant protein C proprotein mutants. Biochem. Biophys. Res. Commun. 290, 532-538.

138. Ten Brinke A, Posthuma G, Batenburg JJ, Haagsman HP, Ridder AN, van Golde LM, Vaandrager AB. (2003): The transmembrane domain of surfactant protein C precursor determines the morphology of the induced membrane compartment in CHO cells. Eur. J. Cell Biol. 82, 285-294.

139. Ten Brinke A, Vaandrager AB, Haagsman HP, Ridder AN, van Golde LM, Batenburg JJ. (2002b): Structural requirements for

palmitoylation of surfactant protein C precursor. Biochem. J. 361, 663-671.

140. Ten Brinke A, van Golde LM, Batenburg JJ. (2002c): Palmitoylation and processing of the lipopeptide surfactant protein C. Biochim. Biophys. Acta. 1583, 253-265.

141. Thomas AQ, Lane K, Phillips J 3rd, Prince M, Markin C, et al. (2002): Heterozygosity for a surfactant protein C gene mutation associated with usual interstitial pneumonitis and cellular nonspecific interstitial pneumonitis in one kindred. Am. J. Respir. Crit. Care Med. 165, 1322–1328.

142. Tong Q, Zheng L, Kang Q, Dodd-O J, Langer J, Li B, Wang D, Li D. (2006): Upregulation of hypoxia-induced mitogenic factor in bacterial lipopolysaccharide-induced acute lung injury. FEBS Lett. 580, 2207-2215.

143. Tredano M, Griese M, Brasch F, Schumacher S, de Blic J, Marque S, Houdayer C, Elion J, Couderc R, Bahuau M (2004): Mutation of SFTPC in infantile pulmonary alveolar proteinosis with or without fibrosing lung disease. Am J Med Genet A. 126A, 18-26.

144. van Moorsel CH, van Oosterhout MF, Barlo NP, de Jong PA, van der Vis JJ, Ruven HJ, van Es HW, van den Bosch JM, Grutters JC. (2010): Surfactant protein C mutations are the basis of a significant portion of adult familial pulmonary fibrosis in a dutch cohort. Am J Respir Crit Care Med. 182, 1419-25.

145. Vandenbussche G, Clercx A, Curstedt T, Johansson J, Jörnvall H, Ruysschaert JM. (1992): Structure and orientation of the surfactant-associated protein C in a lipid bilayer. Eur. J. Biochem. 203, 201-209.

146. von Nahmen A, Post A, Galla HJ, Sieber M. (1997): The phase behavior of lipid monolayers containing pulmonary surfactant protein C studied by fluorescence light microscopy. Eur. Biophys. J. 26, 359-369

147. Vorbroker DK, Profitt SA, Nogee LM, Whitsett JA. (1995a): Aberrant processing of surfactant protein C in hereditary SP-B deficiency. Am. J. Physiol. 268, L647-656.

148. Vorbroker DK, Voorhout WF, Weaver TE, Whitsett JA. (1995b): Posttranslational processing of surfactant protein C in rat type II cells. Am. J. Physiol. 269, L727-733.

149. Wambach JA, Yang P, Wegner DJ, An P, Hackett BP, Cole FS, Hamvas A. (2010): Surfactant protein-C promoter variants associated with neonatal respiratory distress syndrome reduce transcription. Pediatr. Res. 68: 216–220.

150. Wang L, Cai P, Galla HJ, He H, Flach CR, Mendelsohn R. (2005): Monolayer-multilayer transitions in a lung surfactant model: IR reflection-absorption spectroscopy and atomic force microscopy. Eur. Biophys. J. 34, 243-254.

151. Wang WJ, Mulugeta S, Russo SJ, Beers MF. (2003): Deletion of exon 4 from human surfactant protein C results in aggresome formation and generation of a dominant negative. J. Cell Sci. 116, 683-692.

152. Wang Z, Gurel O, Baatz JE, Notter RH. (1996): Acylation of pulmonary surfactant protein-C is required for its optimal surface active interactions with phospholipids. J. Biol. Chem. 271, 19104-19109.

153. Weaver TE and Conkright J. (2001): Function of surfactant proteins B and C. Annu. Rev. Physiol. 63, 555–578.

154. Wert SE, Whitsett JA, Nogee LM. (2009): Genetic disorders of surfactant dysfunction. Pediatr. Dev. Pathol. 12, 253–274.

155. Whitsett JA, Wert SE, Xu Y. (2005): Genetic disorders of surfactant homeostasis. Biol Neonate. 87, 283-287.

156. Willander H, Askarieh G, Landreh M, Westermark P, Nordling K. et al. (2012): High-resolution structure of a BRICHOS domainand its implications for anti-amyloid chaperone activity on lung surfactant protein C. Proc. Natl. Acad. Sci. USA. 109, 2325–2329.

157. Willander H, Hermansson E, and Johansson J, Presto J. (2011): BRICHOS domain associated with lung fibrosis, dementia and cancer--a chaperone that prevents amyloid fibril formation? FEBS J. 278, 3893-3904.

158. Wohlford-Lenane CL, Durham PL, Snyder JM. (1992): Localization of surfactant-associated protein C (SP-C) mRNA in fetal rabbit lung tissue by in situ hybridization. Am J Respir Cell Mol Biol. 6, 225-234.

159. Woischnik M, Sparr C, Kern S, Thurm T, Hector A, Hartl D, Liebisch G, Mulugeta S, Beers MF, Schmitz G, Griese M. (2010): A non-BRICHOS surfactant protein c mutation disrupts epithelial cell function and intercellular signaling. BMC Cell Biol. 20, 11:88.

160. Wustneck N, Wustneck R, Perez-Gil J, Pison U. (2003): Effects of oligomerization and secondary structure on the surface behavior of pulmonary surfactant proteins SP-B and SP-C. Biophys. J. 84, 1940–1949.

161. Zangi R, Kovacs H, Gunsteren WF, Johansson J, Mark AE. (2001): Free energy barrier estimation of unfolding the α-helical surfactant-associated polypeptide C. Proteins 43, 395–402.

162. Zarbock R, Woischnik M, Sparr C, Thurm T, Kern S, Kaltenborn E, Hector A, Hartl D, Liebisch G, Schmitz G, Griese M. (2012): The surfactant protein C mutation A116D alters cellular processing, stress tolerance, surfactant lipid composition, and immune cell activation. BMC Pulm. Med. 12, 15.

163. Zhong Q, Zhou B, Ann DK, Minoo P, Liu Y, Banfalvi A, Krishnaveni MS, Dubourd M, Demaio L, Willis BC, Kim KJ, duBois RM, Crandall ED, Beers MF, Borok Z. (2011): Role of endoplasmic reticulum stress in epithelial-mesenchymal transition of alveolar epithelial cells: effects of misfolded surfactant protein. Am J Respir Cell Mol Biol. 45,498-509.

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