Secretion of Surfactant Protein C, an Integral Membrane Protein, Requires the N-terminal Propeptide*

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Proteolytic processing of surfactant protein C (SP-C) proprotein in multivesicular bodies of alveolar type II cells results in a 35-residue mature peptide, consisting of a transmembrane domain and a 10-residue extramembrane domain. SP-C mature peptide is stored in lamellar bodies (a lysosomal-like organelle) and secreted with surfactant phospholipids into the alveolar space. This study was designed to identify the peptide domain of SP-C required for sorting and secretion of this integral membrane peptide. Deletion analyses in transiently transfected PC12 cells and isolated mouse type II cells suggested the extramembrane domain of mature SP-C was cytosolic and sufficient for sorting to the regulated secretory pathway. Intratracheal injection of adenovirus encoding SP-C mature peptide resulted in secretion into the alveolar space of wild type mice but not SP-C (−/−) mice. SP-C secretion in null mice was restored by the addition of the N-terminal propeptide. The cytosolic domain, consisting of the N-terminal propeptide and extramembrane domain of mature SP-C peptide, supported secretion of the transmembrane domain of platelet-derived growth factor receptor. Collectively, these studies indicate that the N-terminal propeptide of SP-C is required for intracellular sorting and secretion of SP-C.

Type II epithelial cells synthesize and secrete pulmonary surfactant, a complex mixture of phospholipids and proteins that reduces surface tension along the alveolar air-liquid interface at end respiration. The peptide components of surfactant, in particular surfactant protein B (SP-B)1 and SP-C, are critical for surfactant film formation and function. Newborn infants and mice lacking SP-B have dramatically reduced pulmonary compliance and develop lethal, respiratory distress syndrome shortly after birth (1, 2). Disruption of the SP-B locus results in incomplete processing of pro-SP-C to its mature peptide, leading to deficiency of both SP-C and SP-B. SP-C null mice have normal levels of SP-B and survive with subtle changes in lung function but normal lung structure and surfactant pool sizes.2 The importance of SP-C for normal lung function is inferred from experiments in which intratracheal administration of surfactant containing SP-C as the sole protein component to preterm animals restored lung function to values comparable with animals treated with native surfactant (3–5). Collectively, these results suggest that SP-C and SP-B are functionally interchangeable with respect to biophysical activity.

SP-C is synthesized by the alveolar type II epithelial cell as a 197-amino acid proprotein in which the mature peptide (residues 24–58) is flanked by N-terminal (residues 1–23) and C-terminal (residues 59–197) peptide domains. Unlike SP-B, SP-C is an integral membrane protein, which contains a single membrane-spanning domain located within the mature peptide (6). The topology of the SP-C proprotein in the membrane is not clear, with reports of both type II (7) and type III (8) orientations. SP-C proprotein is detected in endoplasmic reticulum, Golgi, and multivesicular bodies but not in lamellar bodies, the intracellular storage compartment for pulmonary surfactant (8, 9). Processing of the proprotein results in cleavage of the propeptides and generation of the 35-amino acid mature peptide that is detected only in the multivesicular body and lamellar body (8, 10). Colocalization of both proprotein and mature peptide in the multivesicular body strongly suggests that processing of the SP-C precursor to the biologically active peptide occurs within this compartment.

In order to promote absorption and spreading of surfactant lipids at the alveolar air-liquid interface, mature SP-C peptide must be secreted by the type II cell. The mature peptide consists of an extremely hydrophobic transmembrane domain and a 10–12-amino acid extramembrane domain that contains palmitoylated cysteines at positions 5 and 6 (residues 28 and 29 of the proprotein) in most species (11–13). The mechanism underlying secretion of this integral membrane peptide is not clear but probably involves two discrete steps. The proprotein is first sorted to the multivesicular body that ultimately fuses with the lamellar body, a lysosome-related organelle. Sorting of integral membrane proteins to lysosomes and secretory granules is dependent upon information encoded in the cytosolic domain of the protein (14, 15), suggesting that either the N-terminal or C-terminal peptide domain of the proprotein plays an important role in sorting SP-C to lamellar bodies. Since SP-C mature peptide is detected only in the lumen of the lamellar body, a second step is required to relocate SP-C from the limiting membrane to the lumen of the multivesicular/ lamellar body. It is likely that the N-terminal or C-terminal peptide domain also facilitates luminal internalization of SP-C. This study was designed to identify the compartment in which
SP-C internalization occurs and the peptide domains required for sorting and secretion of SP-C into the airspace.

MATERIALS AND METHODS

DNA Constructs and Transfection—Full-length human SP-C cDNA was cloned into pcDNA3 (Invitrogen, San Diego, CA). To generate SP-C deletion constructs (Fig. 2A), SP-C fragments were amplified by polymerase chain reaction using specific primers to human SP-C containing 5′ SacI and 3′ SacI cleavage sites. Polymerase chain reaction fragments were subcloned into eGFP vector (Clontech, San Jose, CA) and subjected to bidirectional sequencing to verify the fidelity of the polymerase chain reaction product throughout the SP-C coding sequence and the green fluorescent protein (GFP)/SP-C junction.

PC12 Cell Culture, Metabolic Labeling, and Immunoprecipitation—PC12 cells (a gift from D. Cutler, University College London) were cultured as described previously (16). Cells were grown in T25 flasks until 80% confluent and transiently transfected with 6 μg of plasmid DNA and 60 μl of Fugene 6 (Roche Molecular Biochemicals) according to the manufacturer’s instructions. For 48 h of culture, PC12 cells were labeled with 0.5 mCi of [35S]methionine/cysteine (Amersham Pharmacia Biotech) for 4 h. Cell lysates and media were immunoprecipitated at room temperature. All cells were washed with cold PBS and fixed with cold 4% paraformaldehyde in PBS at 4 °C. After fixation, cells were permeabilized with 0.2% saponin, and stained with antibodies directed against the SP-C N-terminal propeptide (18). SDS-PAGE and autoradiography were performed as previously described (17).

Immunostaining and Confocal Microscopy—1.5 × 10⁶ PC12 cells were plated on laminin/polylysine-coated coverslips. After 24 h of culture, PC12 cells were transiently transfected with 2 μg of plasmid DNA and 20 μl of Fuge6 (Roche Molecular Biochemicals) according to the manufacturer’s instructions. 48 h post-transfection, PC12 cells were fixed with 4% paraformaldehyde in PBS, permeabilized with 0.2% saponin, and stained with antibodies directed against the SP-C N-terminal propeptide (18) and chromogranin A (1:100 (ICN Biomedicals, Costa Mesa, CA) for 1 h at room temperature. Cells were washed and incubated with anti-rabbit Texas Red (1:100) and anti-mouse Cy5 secondary antibodies (1:400) (Jackson Immunoresearch, West Grove, PA) for 1 h at room temperature. After cooling to 4 °C, nonpermeabilized cells were incubated in 4 °C with antibody directed against the SP-C N-terminal (18) or C-terminal peptide domain (9), biotinylated wheat germ agglutinin lectin (1:100) (Vector Laboratories, Burlingame, CA), and TOPRO3 to assess membrane integrity. Cells were washed with cold PBS and fixed with 4% paraformaldehyde in PBS, permeabilized with 0.2% saponin, and double distilled H₂O₃ mounted on slides with Vectashield mounting medium (Vector Laboratories), and sealed with nail polish. Fluorescence was imaged with a Leica confocal microscope and analyzed using Metamorph Imaging Software (Universal Imaging Corp., West Chester, PA).

Type II Cell Isolation and Transfection—Type II cells were prepared from 6-week-old female C57B/6 mice as described by Corti et al. (19) with the following modifications. A crude cell suspension was prepared by rapid instillation of 3 ml of Dispase into the lung followed immediately by 0.5 ml of 1% low melting temperature agarose, warmed to 45 °C. Single cell suspensions were subsequently isolated as previously described (19) and added to 100-mm culture dishes coated with 42 μl of 20% FBS and 16 μg of 22/23 antibodies (Pharmingen, San Diego, CA) in 6 ml of PBS followed by incubation for 1 h at 37 °C. Plates were gently “panned” to free settled type II cells and pelletted at 130 × g for 7 min at 4 °C. Type II cells were resuspended in culture media (Dulbecco’s modified Eagle’s medium, 25 mM HEPES, 10% fetal bovine serum, and 1% penicillin/streptomycin), plated at a density of 3 × 10⁵ cells/well, and allowed to attach to collagen-coated 22 × 22 coverslips for 4 h. Cells were washed with calcium-free Hanks’ balanced salt buffer (Life Technologies, Inc.) and transfected with a hand-held gene gun (Bio-Rad) at 100 p.s.i. with GFP/SP-C24–58 plasmid-coated 0.6 μm gold particles. Culture medium was replaced, and cells were cultured for 24 h followed by immunostaining with antibody directed against mature SP-C as described above.

Generation of Adenoviral Constructs—The sequence encoding amino acids 24–58 or 1–58 was amplified from cDNA generated by reverse transcriptase-polymerase chain reaction of type II cell RNA isolated from C57B/6 mice using specific primers that included a Kozak consensus sequence in the 5′ primer and a hemagglutinin tag (YPYDVPDYA) in the 3′ primer. The SP-C1–21,000hP2GFP/SP-C24–58HA adenoviral construct was generated by using overlapping primers to include the SP-C cytosolic domain (residues 1–33 of the propeptide), the PDGF transmembrane domain (residues 531–53), and an HA tag. These polymerase chain reaction fragments were cloned into the Adv2 adenoviral shuttle vector (20) and sequenced bidirectionally to confirm the integrity of the reading frame. Recombination and virus production were performed as described previously (20).

Analysis of Lung Tissue and Surfactant from Adenovirus-infected Mice—Purified adenoviral particles were intratracheally injected into wild type Swiss Black and SP-C (−/−) mice (Stephan Glasser, Cincinnati, OH). Mice were anesthetized and injected with 2 × 10⁹ plaque-forming units of adenovirus/mouse in Hanks’ balanced salt buffer containing 12 mM EGTA (21) in a final volume of 100 μl. Three or four days after infection, lung tissue was fixed with 2% paraformaldehyde and 0.5% glutaraldehyde, and the fixed, cryoprotected frozen tissue was processed for immunogold labeling with HA antiserum (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) as described previously (9). Injected mice were lavaged with saline (5 × 1 ml), and surfactant was isolated by centrifugation at 18,000 × g for 30 min at 4 °C. Bronchoalveolar lavage from three injected mice was pooled, and large and small aggregate fractions were isolated on a 0.8 M sucrose gradient as described previously (22). Lamellar bodies were isolated from pooled lungs of three injected mice as previously described by Osanai et al. (23). Equal amounts of protein, determined by bicinechonic acid protein assay (24), from large and small aggregate fractions were analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting with an antibody directed against the HA tag (Santa Cruz Biotechnology).

RESULTS

This study was designed to identify the peptide domain(s) that directs sorting and secretion of SP-C. Because isolated type II cells are very difficult to transfec, initial sorting studies were performed in transiently transfected PC12 cells. PC12 cells were transfected with wild type SP-C1–197 and labeled with [35S]cysteine/methionine, and cell lysates and media were immunoprecipitated with antibodies directed against the SP-C propeptide (18). SDS-PAGE and autoradiography were performed as previously described (17).

Immunostaining with antibody directed against the C-terminal peptide, consistent with an exocytotic sorting of the SP-C proprotein to the regulated secretory pathway. Internalized wild type SP-C1–197 was sorted to the regulated secretory pathway, PC12 cells were transfected with SP-C1–197 and stained with antibodies directed against the SP-C proprotein and chromogranin A, a marker for dense core granules. Wild type SP-C1–197 proprotein colocalized with chromogranin A consistent with sorting to the regulated secretory pathway (Fig. 1B, upper panel). These results, coupled with previous reports that SP-C is an integral membrane protein (7, 25), suggested that the proprotein was located in the limiting membrane of the dense core granule and that exocytosis would lead to localization of SP-C on the cell surface. To test this hypothesis, PC12 cells were transiently transfected with wild type SP-C1–197 and stained with an antibody directed against the N-terminal or C-terminal peptide domains and wheat germ agglutinin lectin to define the cell surface. In nonpermeabilized cells, SP-C immunoreactivity was detected with antibody directed against the C-terminal peptide, consistent with an extracellular location for this peptide domain (Fig. 1B). The N-terminal propeptide detected only after cell permeabilization, confirming that this peptide domain is located in the cytoplasm (Fig. 1B). Collectively, these data suggest that SP-C is a type II integral membrane protein and that sorting to the regulated secretory pathway occurs independently of proprotein processing.

To determine the minimal peptide sequence required for sorting of the SP-C proprotein to the regulated secretory pathway, a series of deletion constructs (Fig. 2A) were cloned in frame with GFP, transfected into PC12 cells, and analyzed for colocalization with chromogranin A. All deletion constructs, including a construct consisting of only the mature SP-C peptide (SP-C24–58), colocalized with chromogranin A (Fig. 2B); further, SP-C was sorted to the regulated secretory pathway irrespective of the position of GFP at the N- or C terminus of the mature SP-C peptide (data not shown). SP-C24–58 contains...
two candidate-sorting motifs, a dileucine motif at positions 31 and 32 (residues 54 and 55 of the proprotein) and two palmitoylated cysteines at positions 5 and 6 (residues 28 and 29 of the proprotein). The sorting activity of the dileucine motif was tested by deleting the last 5 residues of the mature peptide (SP-C24–53); the sorting activity of the palmitoylated cysteines was tested by mutating both cysteine residues to serine or alanine (SP-CCCSS and SP-C CCAA) in the context of the proprotein (Fig. 2A). The proteins encoded by all of these constructs colocalized with chromogranin A following transfection of PC12 cells, indicating that neither motif is involved in SP-C sorting (data not shown). We conclude from these experiments that the N- and C-terminal peptide domains of SP-C are not required for sorting to the regulated secretory pathway of PC12 cells and that a novel sorting motif is located in the 10-residue extramembrane, cytosolic domain of the mature peptide (residues 24 and 33 of the proprotein).

The results in PC12 cells predicted that the mature SP-C peptide (SP-C24–58) would be sorted to lamellar bodies in type II cells. This hypothesis was tested by transfecting freshly isolated mouse type II cells with a construct encoding the mature SP-C peptide (GFP/SP-C24–58). The plasmid was precipitated onto gold particles and propelled into type II cells with a hand-held gene gun (Bio-Rad). Following 24 h of culture, GFP fluorescence was detected in vesicular structures that also stained positively for the mature SP-B peptide (Fig. 3). Since SP-B mature peptide is only detected in multivesicular bodies and lamellar bodies of type II cells (26), these results confirm that the mature SP-C peptide is sorted to the secretory pathway in the absence of the flanking N- and C-terminal peptide domains.

To determine if SP-C mature peptide could be sorted to lamellar bodies in vivo, an adenoviral construct encoding SP-C mature peptide with a hemagglutinin tag (SP-C24–58HA) was generated for intratracheal injection into adult mice. The subcellular distribution of SP-C24–58HA in infected mice was ana-
lyzed by immunogold labeling of fixed, cryoprotected frozen lung sections. Gold particles were detected throughout the regulated secretory pathway including the endoplasmic reticulum, Golgi, multivesicular bodies, and lamellar bodies, but not in the nucleus, mitochondria, or plasma membrane (Fig. 4, B and C). Within multivesicular bodies, HA labeling was detected on the limiting membrane and the inner vesicles (Fig. 4B) similar to the distribution of SP-C proprote in wild type (data not shown) and SP-B null mice (Fig. 4A). Localization of SP-C on the inner vesicles of multivesicular bodies suggested that SP-C24–58HA could be secreted.

To determine if the cytosolic domain of the mature SP-C peptide (residues 1–10) was sufficient to direct secretion of SP-C, mice were intratracheally injected with SP-C24–58HA adenovirus. Four days postinfection, mice were lavaged, and the surfactant pellet was isolated. SP-C24–58HA was detected in the surfactant pellet of all seven infected mice, consistent with secretion of the peptide into the airway (Fig. 5A). Fractionation of BAL into large and small aggregates revealed that SP-C24–58HA co-sedimented with wild type mature SP-B in the surface-active, large aggregate fraction (Fig. 5B). Immunohistochemical analyses of the lungs from infected mice detected HA staining in epithelial cells and in the alveolar spaces without any evidence of cytotoxicity; further, when mice were infected with adenovirus encoding luciferase, reporter activity was not detected in BAL (data not shown). These data indicate that SP-C mature peptide is secreted into the alveolar space and associates with the large aggregate surfactant fraction.

To determine if SP-C24–58HA could be secreted in the absence of endogenous SP-C, SP-C knockout mice were intratracheally injected with SP-C24–58HA adenovirus. Four days after infection, mice were lavaged, and large and small aggregate fractions were isolated. SP-C24–58HA was detected in the large aggregate fraction of wild type but not SP-C null mice (Fig. 6A). To determine if SP-C24–58HA was sorted to lamellar bodies in the absence of endogenous SP-C, lamellar bodies from lung homogenates were isolated from infected wild type and SP-C knockout mice. SP-C24–58HA was detected in lamellar body fractions of wild type mice but not SP-C (−/−) (Fig. 6B). We conclude from these experiments that, in the absence of endogenous SP-C, the cytosolic domain of the mature peptide is not sufficient to sort SP-C to the regulated secretory pathway in type II cells.

To test the hypothesis that the N-terminal propeptide of SP-C could direct sorting and secretion of SP-C24–58HA in SP-C (−/−) mice, an adenovirus encoding the SP-C N-terminal propeptide and mature peptide (SP-C1–58HA) was generated. Three days after infection, SP-C (−/−) mice were lavaged, and the large aggregate fraction was isolated. Western analysis
detected SP-C1–58HA in the large aggregate surfactant fraction that also contained mature SP-B peptide (Fig. 7). SP-C1–58HA comigrated with SP-C24–58HA in wild type mice, consistent with cleavage of the N-terminal propeptide. The results of these experiments indicate that the N-terminal propeptide is required for secretion of SP-C.

To determine if the cytosolic domain of SP-C could direct sorting and secretion of a heterologous transmembrane domain, an adeno viral construct consisting of the SP-C cytosolic domain (residues 1–33), the platelet-derived growth factor receptor (PDGFR) transmembrane domain (residues 531–553), and the 9-amino acid HA tag (SPC1–33PDGFR531–553HA) was generated. Three days after infection, SP-C (−/−) mice were lavaged, and the large aggregate fraction isolated; in addition, lamellar bodies were extracted from lung tissue. SPC1–33PDGFR531–553HA was detected in both large aggregate and lamellar body fractions (Fig. 8); however, SPC1–33PDGFR531–553HA did not comigrate with SP-C1–58HA, suggesting that the N-terminal propeptide was not cleaved from the chimeric protein. These results demonstrate that the cytosolic domain of the SP-C proprotein can direct the sorting and secretion of a heterologous transmembrane domain but is not sufficient to direct processing of the propeptide.

**DISCUSSION**

SP-C is an integral membrane protein that is sorted to the lamellar body and subsequently secreted with surfactant lipids into the alveolar space. The current study was undertaken to identify the peptide domain(s) involved in the sorting of SP-C to the regulated secretory pathway in type II cells and secretion of this integral membrane protein. In the presence of endogenous SP-C, the mature peptide was sorted to the regulated secretory pathway and was secreted into the airspace. However, in the absence of endogenous SP-C, the SP-C N-terminal propeptide was required for secretion of the mature peptide. The importance of the cytosolic domain for secretion of SP-C was confirmed by demonstrating that residues 1–33 of the proprotein were sufficient to direct secretion of a truncated PDGFR receptor.

Integral membrane proteins are sorted to specific subcellular compartments based on information encoded in the cytosolic domain of the protein (14, 15). Previous studies have identified the SP-C proprotein as a type III integral membrane protein (8), in which the C-terminal propeptide is located in the cytoplasm, or a type II membrane integral membrane protein (7), in which the N-terminal propeptide is cytosolic. The results of the current study in PC12 cells suggest a type II membrane orientation consistent with the results reported by Keller et al. (7). This conclusion is also consistent with the presence of a puta-
tive stop-transfer sequence (amino acids 34 and 35 of the proprotein) and the palmitoylation of adjacent cysteine residues (amino acids 28 and 29 of the proprotein), motifs that frequently occur on the cytosolic side of the membrane (27, 28). Further evidence comes from immunogold labeling studies that detected the N-terminal propeptide inside the internal vesicles of multivesicular bodies, consistent with a type II orientation (8, 9). Taken together, these results suggest that the cytosolic domain of SP-C is composed of the 23-amino acid N-terminal propeptide and the first 10 amino acids of the mature peptide.

Consistent with a cytosolic location for the N-terminal propeptide, deletion of the entire 139-amino acid C-terminal propeptide of the SP-C proprotein did not perturb sorting to the regulated secretory pathway in PC12 cells. Moreover, removal of the last 5 amino acids of the mature peptide (residues 54–58 of the proprotein), which contains a dileucine motif at positions 54 and 55, did not affect SP-C sorting. SP-C was also sorted following the deletion of the 23-amino acid N-terminal propeptide, suggesting that the 10-amino acid cytosolic domain of the mature peptide (residues 24–33 of the proprotein) is sufficient to direct SP-C to the regulated secretory pathway. This outcome is difficult to reconcile with results of previous studies suggesting that the C-terminal propeptide of the proprotein was critical for intracellular trafficking of SP-C (25, 29). It is possible that selected mutations/deletions within the C-terminal peptide resulted in misfolding and degradation of SP-C in a cytosolic compartment; for example, in previous studies, a potential intramolecular sulfhydryl bridge was disrupted by deletion of cysteine 189 and/or cysteine 121, residues that are strictly conserved in the SP-C proprotein of all seven species analyzed to date (25, 29, 30). Interpretation of the latter studies is further complicated by expression of SP-C deletion constructs in cell types that lack a typical regulated secretory pathway. PC12 cells used in this study have a well-characterized regulated secretory pathway and have been used extensively for sorting and secretion studies (31–35). We have previously used PC12 cells to identify a sorting determinant in the SP-B proprotein and confirmed the identity of the sorting signal in transgenic mice (16, 17). In the current study, the results of experiments in PC12 cells were confirmed by transfecting isolated type II cells and by intratracheal injection of wild type mice with adenovirus encoding the mature peptide. In both cases, SP-C mature peptide (SP-C1–58HA) was detected in lamellar bodies, consistent with sorting of the mature peptide to this compartment in the absence of the N- and C-terminal peptide domains.

Interestingly, SP-C was not detected in lamellar bodies or BAL of SP-C (−/−) mice infected with adenovirus encoding SP-C1–58HA. This outcome suggests that in wild type mice endogenous SP-C associated with transfected mature SP-C peptide to facilitate its trafficking to lamellar bodies; SP-C mature peptide may similarly interact with an endogenous protein(s) in PC12 cells, leading to localization in dense core secretory granules. The sorting deficit in SP-C (−/−) mice was corrected by the addition of the 23-amino acid N-terminal propeptide to SP-C1–58HA. SP-C1–58HA was processed to the mature peptide consistent with trafficking through the multivesicular body, a compartment previously shown to be involved in processing of the SP-C and SP-B proproteins (8, 10, 26); further, the processed peptide was detected in the extracellular, surface-active, large aggregate fraction of surfactant. Collectively, these studies suggest that the N-terminal propeptide is required for efficient sorting of SP-C to the distal secretory pathway in type II cells and that sorting occurs independently of the C-terminal peptide. Furthermore, the cytosolic domain of SP-C was able to direct both the sorting (to lamellar bodies) and secretion of the transmembrane domain of PDGFR. However, SPC1–58PDGF_{531–558}HA was not efficiently processed to a smaller form, suggesting that the SP-C transmembrane domain may contribute to the formation of an optimal cleavage site between the propeptide and mature peptide.

Sorting of SP-C to the distal secretory pathway is necessary but not sufficient for secretion of SP-C into the alveolar space. In transfected PC12 cells, SP-C was detected in the limiting membrane of dense core granules and was transferred to the plasma membrane during exocytosis; however, in type II epithelial cells, SP-C was detected in the lumen of the lamellar body and was secreted with surfactant phospholipids. These observations suggest that the SP-C proprotein is relocated from the limiting membrane of a transport or storage vesicle to the vesicle lumen during transit in the secretory pathway of the type II cell. Ultrastructural analyses detected SP-C proprotein in the limiting membrane and luminal vesicles of multivesicular bodies, suggesting a key role for this compartment in SP-C secretion. Relocation of SP-C within the multivesicular body is probably the result of inward vesiculation of the limiting membrane, similar to the process leading to internalization and subsequent degradation of the epidermal growth factor receptor in lysosomes (36–38). Inward vesiculation of the multivesicular body-limiting membrane is probably dependent on the interaction of specific residues in the cytosolic domain of the SP-C proprotein with unidentified cytoplasmic proteins. Not all proteins on the limiting membrane of the multivesicular body are included in the inner vesicles (39, 40), suggesting that SP-C is selectively sorted to the inner vesicle. The finding that SP-C_{1–58}HA is secreted following infection of SP-C (−/−) mice suggests that residues 1–34 of the proprotein contain one of more motifs that direct sorting to the secretory pathway and internalization of SP-C into the luminal vesicles of the multivesicular bodies. Following internal vesicle formation, the multivesicular body fuses with the lamellar body, resulting in the incorporation of the internal vesicles of the multivesicular body into the internal membranes of the lamellar body and, ultimately, secretion of SP-C with surfactant phospholipids (41, 42).

In summary, the mature SP-C peptide is sorted to the regulated secretory pathway of PC12 cells, isolated mouse type II cells, and adenovirus-infected wild type mice; however, mature SP-C peptide was not sorted to lamellar bodies or secreted into the airway of adenovirus-infected SP-C (−/−) mice. The addition of the SP-C N-terminal propeptide to the mature peptide (SP-C_{1–58}HA) restored sorting, processing, and secretion of SP-C as part of a functional surfactant complex; further, the cytosolic domain of SP-C was sufficient to direct the sorting and secretion of a heterologous transmembrane domain. These studies demonstrate that the N-terminal propeptide of SP-C is critical for the intracellular trafficking and secretion of SP-C in type II cells.

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