Synthetic Processing of Surfactant Protein C by Alevolar Epithelial Cells

THE COOH TERMINUS OF proSP-C IS REQUIRED FOR POST-TRANSLATIONAL TARGETING AND PROTEOLYSIS

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Surfactant protein C (SP-C) is synthesized by alveolar type II cells as a 21-kDa propeptide (proSP-C21) which is proteolytically processed in subcellular compartments distal to the trans-Golgi network to yield a 35-residue mature form. Initial synthetic processing events for SP-C include post-translational cleavages of the COOH terminus of proSP-C21 yielding two intermediates (16 and 6 kDa). To test the role of specific COOH-terminal domains in intracellular targeting and proteolysis of proSP-C21, synthesis and processing of SP-C was evaluated using a lung epithelial cell line (A549) transfected with a eukaryotic expression vector containing either the full-length cDNA for rat SP-C (SP-Cwt) or one of six polymerase chain reaction (PCR)-generated COOH terminally truncated forms (SP-C1-185, SP-C1-175, SP-C1-147, SP-C1-120, SP-C1-72, and SP-C1-59). Using in vitro transcription/translation, each of the seven constructs produced a [35S]-labeled translation product of appropriate length which could be immunoprecipitated by epitope specific proSP-C antisera. Immunoprecipitation of [35S]-labeled A549 cell lysates from SP-Cwt transfectants demonstrated rapid synthesis of [35S]proSP-C21 with processing to SP-C16 and SP-C6 intermediates via cleavages of the COOH-terminal propeptide. Both the intermediates as well as the kinetics of processing in A549 cells were similar to that observed in rat type II cells. In contrast, constructs SP-C1-185, SP-C1-175, SP-C1-147, SP-C1-120, SP-C1-72, and SP-C1-59 were each translated but degraded without evidence of proteolytic processing. Fluorescence immunocytochemistry identified proSP-Cwt in cytoplasmic vesicles of A549 cells while all COOH-terminal deletional mutants were restricted to an endoplasmic reticulum/Golgi compartment identified by co-localization with fluorescein isothiocyanate-concanavalin A. We conclude that SP-Cwt expressed in A549 cells is directed to the ER membrane and routed to the distal secretory pathway where it has been shown to undergo synthetic processing leading to the appearance of SP-C3.7 in alveolar surfactant (3, 7, 14). Nonetheless, proSP-C21 must be translocated across the ER membrane and routed to the distal secretory pathway where it has been shown to undergo synthetic processing leading to the appearance of SP-C3.7 in alveolar surfactant (3, 7, 14).

The alveolar epithelium synthesizes pulmonary surfactant, a surface active lining film consisting of a biochemically complex mixture of lipids and proteins, which serves to reduce surface tension at the alveolar surface, thereby allowing for maintenance of alveolar stability at low lung volumes (end-expiration) (1). Organic extracts of isolated surfactant have been shown to contain two small lipophilic proteins (SP's), SP-B and SP-C, which alone or in combination are sufficient to confer properties of rapid surface adsorption and surface tension lowering to reconstituted mixtures of synthetic phospholipids (2). SP-C, an extremely hydrophobic 3.7-kDa peptide, is the exclusive product of the alveolar type II cell (3–5) and is a component of most clinical surfactant preparations (3, 6, 7).

The full-length rat SP-C mRNA (0.9 kilobases) is produced by splicing of multiple exons and yields a primary translation product 194 amino acids in length (proSP-C21) (8). In vitro translation of lung RNA produces SP-C primary translation products of M, 21,000–22,000 (8). Similar sized products have been detected in freshly isolated rat type II cells (9, 10) and produced in cultured Chinese hamster ovary cells transfected with a human SP-C cDNA (11). The predominant form of SP-C isolated from extracellular surfactant ("mature SP-C") is a 35-amino acid monomer which also contains I–2 covalently linked palmitic acid residues (12, 13). Mature SP-C17 is contained within the larger precursor proprotein, encompassing residues 24–58 of the proSP-C21 sequence. Unlike other surfactant-associated proteins, the NH2 terminus of the primary translation product does not contain a classic "signal" sequence and there are no sites for asparagine-linked glycosylation (3, 7, 14). Nonetheless, proSP-C21 must be translocated across the ER membrane and routed to the distal secretory pathway where it has been shown to undergo synthetic processing leading to production of the 3.7-kDa alveolar form (9, 10, 15–17). The processing events triggered by delivery of proSP-C21 from the ER include post-translational addition of covalent palmitic acid residues and intracellular proteolysis involving cleavage of 23 residues of NH2- and 136 residues of COOH-terminal flanking domains of the precursor molecule (3, 7, 14).

Using several different in vitro models, the processing events which lead to the appearance of SP-C3.7 in alveolar surfactant have been partially characterized (9–11, 15–17). In both a perfused rat lung preparation and freshly isolated rat type II cells, we have demonstrated processing of proSP-C21 through

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1 The abbreviations used are: SP-B, pulmonary surfactant protein B (9 kDa); SP-C, pulmonary surfactant protein C (3.7 kDa); Tricine, N-(2-hydroxyethyl)-1,1-bis(hydroxyethyl)ethylglycine; PAGE, polyacrylamide gel electrophoresis; FITC, fluorescein isothiocyanate; ConA, concanavalin A; PCR, polymerase chain reaction; ER, endoplasmic reticulum.
16- and 6-kDa intermediate forms (proSP-C₁₀₆, proSP-C₉₁₇) (9, 15, 16). A similar processing pattern has been confirmed by others utilizing pulse-chase analysis of type II cells and immunoprecipitation with different polyclonal antisera (10). The proteolysis of proSP-C₂₁ can be blocked either by the use of brefeldin A (9, 16) or by low temperature incubation (20 °C) (10), indicating that intracellular processing of proSP-C is occurring in subcellular compartments located distal to the trans-Golg. SP-C₃₋₇ has also been recovered from the isolated lamellar body, a phospholipid storage organelle found within type II cells (18, 19), which indicates that all proteolysis of proSP-C₂₁ and proSP-C intermediates occurs intracellularly prior to secretion of the mature peptide into the alveolus. Furthermore, the use of inhibitors of organellar acidification has further elucidated that these intracellular proteolytic events are taking place within acidic subcellular compartments of the exocytic pathway (15).

Despite what is known about the major cleavage events, their localization, and cellular factors important for the regulation of SP-C synthetic processing, the role of specific peptide domains contained within the proSP-C sequence in the direction of its post-translational processing and/or of its subcellular targeting has not been forthcoming due to limitations imposed by previous experimental models. Additional insights have been hampered because primary alveolar type II cells in culture are phenotypically unstable and not easily transfected (20, 21), and a relevant experimental lung epithelial cell line capable of demonstrating synthesis, targeting, and post-translational proteolysis of transfected proSP-C cDNA constructs has not been characterized.

The present study was undertaken to identify peptide domains that facilitate intracellular transport and processing of proSP-C. Initially, synthetic processing of wild type SP-C was defined using the A549 lung epithelial cell line transfected with a eukaryotic expression vector containing a full-length rat SP-C cDNA under the control of a strong viral promoter. Results obtained using this system show that the patterns of expression and processing of recombinant SP-C were similar to that observed for endogenous SP-C in native rat type II cells and demonstrate the feasibility of this model for use in studies aimed at evaluating functional domains contained within the proSP-C primary sequence. COOH terminally truncated forms of proSP-C were generated using PCR-based mutagenesis. Transfection of these mutant constructs into A549 cells demonstrated that deletion of as little as 10 amino acids from the COOH terminus of the proSP-C molecule causes mistargeting of the translated protein resulting in disruption of post-translational proteolytic events showing that an intact COOH-terminal peptide of proSP-C is necessary for proper post-translational processing.

### EXPERIMENTAL PROCEDURES

#### Materials

Trans-[³⁵S]-label (70% L-methionine 15% L-cysteine; 1100 mCi/ml as methionine) was purchased from ICN/Flow, Inc., Irvine, CA. Protein A-agarose was obtained from Bethesda Research Labs, Gaithersburg, MD. FITC-concanavalin A was obtained from Sigma. Except where noted, other reagents were electrophoretic grade and were purchased from Bio-Rad or Sigma.

#### ProSP-C Antisera

Monospecific polyclonal rat proSP-C antisera were produced from synthetic peptide immunogens and have been previously characterized (16, 17). Anti-NPROSP-C (Met¹⁵⁶-Glu¹⁷²), anti-hCPRSP-C (His²²⁹-Ser³¹²), and anti-CTERMS-P-C (Ser³⁴⁶-Ser³⁶⁶) each recognize spatially distinct regions of the linearized proSP-C molecule but do not recognize mature SP-C.

#### Cell Lines

A549 Cells—The lung epithelial cell line A549 (22) utilized in all transfection studies were originally obtained through the American Type Culture Collection (Rockville, MD) and made available as a gift of Dr. S. I. Feinstein. A549 cells were grown at 37 °C, 5% CO₂ in minimal essential medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100 µg/ml streptomycin.

Type II Cells—Type II pneumocytes were isolated using elastase digestion of lungs from adult Sprague-Dawley rats (age 2–3 months) by the method of Dobbs et al. (23). The preparation obtained after panning on IgG coated plates (i.e. fresh type II cells) contained approximately 80–85% type II cells.

#### SP-C cDNA Expression Constructs

The expression vector chosen as the backbone for transfection of epithelial cells in culture is the pcDNA3 eukaryotic expression plasmid (Invitrogen, Inc., San Diego, CA) which contains the human cytomegalovirus early promoter and enhancer region, bovine growth hormone polyadenylation sequence, β-lactamase and neomycin resistance genes, as well as T7 and SP6 promoters for sense/antisense in vitro transcription.

All procedures involving oligonucleotide and cDNA manipulations were performed essentially as described by Ausbel et al. (24). The wild type rat SP-C (Met¹⁵⁶-Ile²⁰⁴) and six mutant construct inserts containing progressively larger truncations of the proSP-C COOH terminus are schematically illustrated in Fig. 1.

#### Wild Type SP-C

A full-length rat SP-C cDNA (816 base pairs) insert was prepared by EcoRI digestion of a previously characterized prokaryotic SP-C expression vector, PGM4Z-SP-C (8+8). Purified SP-C insert was ligated into pcDNA3 polylinker at the 5’ end of each reverse primer (3'-1'-ATTGTTGAGCTCCC-5').
are listed in Table I. Amplification reactions containing 0.2 μM primers, 1.25 μM dNTP mixture, 1.5 μM MgCl₂, 10 ng of template, and 2.5 units of Taq polymerase (Perkin-Elmer, Inc., Foster City, CA) consisted of 30 cycles of: denaturation at 95 °C for 30 s, primer annealing at 50–55 °C for 30 s, and primer extension at 72 °C for 15 s. After the last cycle, the mixture was incubated at 72 °C for 7 min. Purified inserts were ligated into pcDNA3 sequentially digested with KpnI and XhoI.

Automated DNA sequencing in both directions performed at the Core Facility in the Department of Genetics at the University of Pennsylvania detected no nucleotide mutations in the coding region of SP-C wt or any deletional constructs. For SP-C185, a single deviation from the published rat SP-C cDNA (8) sequence occurred at nucleotides 612–613 located within the 3′-untranslated region (GC for CG).

The open reading frame of each construct was characterized by production of 35S-labeled protein using sequential in vitro transcription/translation of SP-C cDNAs with Trans35S-label and the TNT T7 reticulocyte lysate system (Promega, Inc., Madison, WI) as described by the manufacturer.

**Transfection**

SP-C constructs were transiently transfected into A549 cells using calcium phosphate precipitation (0.18 ml of 0.25 M CaCl₂ was added dropwise to 0.18 ml of plasmid DNA dissolved in 2 × HEPES-buffered saline (50 mM HEPES, 280 mM NaCl, 1.5 mM NaPO₄, pH 7.1)) (24).

**Immunocytochemistry**

Immunocytochemical localization of expressed proSP-C proteins was performed on transfected epithelial cell lines fixed and stained as described previously (17). ProSP-C staining was visualized using primary anti-NPROSP-C (1:200) and secondary goat anti-rabbit IgG (25). Fluorescent images were captured using a 12-bit CCD camera and processed using IMAGE 1 software (Universal Imaging Corporation, West Chester, PA).

**Metabolic Labeling and Immunoprecipitation**

Sixty hours following the introduction of plasmid DNA, transiently transfected cell line monolayers (80–90% confluence) equilibrated in serum-free Dulbecco's modified essential medium-Cys/Met were labeled for 30 min with 100 μCi/ml Trans35S-label, then chased in Met/Cys replete minimal essential medium for up to 4 h. Labeled cells were harvested by scraping and pelleted by centrifugation at 130 × g for 10 min. Fresh type II cells were metabolically labeled with Trans35S-label in serum-free, Met/Cys-free Dulbecco's modified Eagle's medium, using suspension cultures (3–5 × 10⁶ cells/ml) as described previously (9).

All radiolabeled cell pellets were solubilized in buffer containing 150 mM NaCl, 50 mM Tris-HCl, pH 7.40, 1 mM phenylmethylsulfonyl fluoride, 1% (v/v) Triton X-100, 5 mM EDTA, and 5 μg/ml each of aprotinin, leupeptin, and pepstatin and immunoprecipitated using proSP-C antisera as previously published (9, 15). Captured proteins were separated by SDS-PAGE and visualized by autoradiography as described below.

**Analytical Methods**

**Polyacrylamide Gel Electrophoresis and Immunoblotting**—One-dimensional SDS-PAGE was performed in 16.5% polyacrylamide gels using a Tris-Tricine buffer system (26) as modified in our laboratory for surfactant proteins (9, 15, 16). Immunoblotting of transferred samples was done using proSP-C antisera and bands were visualized by enhanced chemiluminescence using the ECL kit (Amersham, Inc.).

**Protein Determination**—Total protein was quantified by the Bradford (27) method using bovine immunoglobulin as standard.

**RESULTS**

**pcDNA3-rSP-C Constructs**—Each pcDNA-SP-C construct (Fig. 1) generated in vitro translation products which were immunoprecipitated by epitope-specific proSP-C antisera (Fig. 2). SDS-PAGE of a TNT rabbit reticulocyte lysate reaction containing full-length or truncated plasmid DNA identified 35S-labeled bands of predicted molecular weight which were not seen in reactions omitting plasmid DNA (not shown). The three proSP-C antisera specifically recognized the appropriate in vitro translation product in a pattern restricted by epitope specificity. Similar patterns were obtained using translation products from SP-C1–147, SP-C1–120, and SP-C1–59 (not shown).

**Expression of Wild Type ProSP-C Protein by Transfected A549 Cells**—A549 cells transfected with pcDNA3-SP-Cwt cells stained with anti-NPROSP-C consistently demonstrated expression of proSP-C within cytoplasmic vesicles (Fig. 3A). The specificity of the immunohistochemical staining was confirmed by the substitution of preimmune serum for primary anti-NPROSP-C (Fig. 3B). Control experiments using an antisense construct, pcDNA3-SP-C (–), transfected under identical con-
The relationship between SP-C21 and SP-C16 can be seen. The A549 cells transfected with pcDNA3-SP-Cwt showed consistent intracellular targeting—immunofluorescence micrographs of SP-Cwt (Fig. 4) demonstrated synthesis and proteolytic processing of 35S-proSP-C21 by type II cells (Fig. 4A). Using anti-NPROSP-C (which recognizes all major proSP-C forms), 35S-proSP-C21 appeared by completion of the pulse (30 min) followed by a time-dependent appearance of a 16-kDa intermediate and low molecular weight proSP-C forms during the chase. 35S-Lysates from SP-Cwt transfected A549 cells immunoprecipitated under identical conditions yielded the same intermediates (Fig. 4B). Immunoprecipitation of SP-Cwt transfected A549 cells with anti-CTERMSP-C demonstrated that initial processing is due to cleavage of COOH-terminal regions (Fig. 4C) as had been previously shown for rat type II cells (16). Immunoprecipitation of the media failed to detect the presence of proSP-C21 or smaller intermediates at chase periods of up to 4 h (not shown).

The kinetic of early proSP-C processing by A549 cells was nearly identical to that of rat type II cells (Fig. 5). In both models, at the conclusion of the pulse period, almost 95% of the total counts were in SP-C21. A quantitative precursor-product relationship between SP-C21 and SP-C16 can be seen. The appearance of low molecular weight forms immediately followed the appearance of SP-C16.

COOH-terminal SP-C Deletional Mutants Are Translated but Not Proteolytically Processed—A549 cells transiently transfected with SP-C1–185, SP-C1–175, SP-C1–147, or SP-C1–72 each produced a 35S-labeled primary translation product of predicted molecular weight (Fig. 6). However, compared with the SP-Cwt (Fig. 4B), there were no lower molecular weight forms indicative of proteolytic processing. ProSP-C forms were not detected in the media (not shown).

Deletion of the COOH Terminus of ProSP-C21 Interrupts Intracellular Targeting—Immunofluorescence micrographs of A549 cells transfected with pcDNA3-SP-Cwt showed consistent expression of proSP-C within cytoplasmic vesicles (Fig. 7A). Expression of pcDNA3-SP-C1–175 was markedly different with proSP-C staining manifested as a juxtanuclear, reticular pattern with no local accumulation in cytoplasmic vesicles (Fig. 7B). This pattern was repeated with SP-C1–72 (Fig. 7C) and SP-C1–185 (Fig. 7D).

To further localize mutant proSP-C, double label staining was performed combining anti-NPROSP-C and Texas Red anti-IgG for proSP-C with FITC-ConA to delineate ER/Golgi compartments (25) (Fig. 8). The specificity of the probes and fluorescence filters was verified by control experiments using preparations of a mixed population of freshly isolated type II cells and lung macrophages (A and A'). Under these conditions, both macrophages ("m") and type II cells ("2") showed ConA staining but proSP-C fluorescence was restricted to the type II cell population. At equivalent exposures, samples stained with proSP-C (Texas red) alone failed to show significant cross-talk in the FITC emission channel (not shown). The expression of the SP-Cwt was found predominantly in cytoplasmic vesicles (Fig. 8B) distinct from ConA staining (Fig. 8B') while localization of the SP-C1–185 mutant protein (Fig. 8C) was restricted to the ConA-labeled compartment (Fig. 8C').

DISCUSSION

The primary sequence of the 3.7-kDa alveolar form of SP-C contains a transmembrane spanning α-helical region and is one of the most hydrophobic amino acid domains known (3). The complete synthesis of SP-C by the type II lung epithelium requires that a series of discrete processing steps occur within the secretory pathway: 1) translation, folding, and translocation of apoprotein across the ER membrane; 2) post-translational addition of covalent palmitic acid; 3) sorting and exit of the lipoprotein from the Golgi followed by cleavage of flanking domains; 4) assembly of mature SP-C with surfactant phospholipid and other proteins in the lamellar body prior to secre-
tion into the alveolus (9, 10, 15–17). Previous studies from our laboratory have shown that early events in the synthetic processing of SP-C involve cleavages of COOH-terminal flanking domains of the propeptide which can only occur after its successful export from the Golgi. We now show that deletion of as little as 10 amino acids from the distal COOH terminus results in retention of proSP-C protein in ER/Golgi compartments of transfected A549 cells. Truncated mutants are translated but not proteolytically cleaved providing evidence that the C terminus is required for sorting of proSP-C to distal compartments for processing.

The A549 cell line was chosen as the experimental model because it represents a transformed cell line of type II cell origin (22). Isolated alveolar type II cells are inadequate for use in prolonged transfection studies because when placed in primary culture on most matrices, they have been characterized by marked phenotypic instability which includes a loss of mRNA for all major surfactant proteins (21) and down-regulation of SP-C proprotein expression within 24–48 h of plating (9). It appears that the regulated secretory pathway of alveolar type II cells is also altered by culture. Studies using adenovirus-mediated transfection of SP-B in cultured type II cells have shown that the translated recombinant SP-B proprotein is neither sorted nor proteolytically processed by these primary cultures (20). In contrast, when SP-Cwt was transfected into A549 cells, we observed synthesis and proteolytic processing of

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**Fig. 6.** COOH-terminal SP-C mutants are expressed but not processed. A549 cells at 80% confluence were transfected with the pcDNA3-SP-C1–185, -SP-C1–175, -SP-C1–147, or -SP-C1–72 expression vectors (20 μg/60 mm² dish). 60 h following introduction of plasmid DNA, processing of mutant SP-C was examined by 35S-labeled pulse-chase labeling and immunoprecipitation with anti-NPSP-C as described for Fig. 4. Chase time given in hours is shown at the top. Each autoradiograph, representative of three separate experiments with each construct, was exposed for 30 h except for SP-C1–72 (96 h). Translation of each truncated primary translation product occurs (large arrow) without the appearance of processed forms. Higher molecular weight forms representing oligomer formation are seen in SP-C1–72 as denoted by *.

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**Fig. 7.** Immunocytochemical localization of mutant proSP-C expression in A549 cells. Cells at 80% confluence were transfected with pcDNA3-SP-Cwt, -SP-C1–175, or -SP-C1–72 (20 μg of DNA/60-mm² dish). Sixty hours following introduction of plasmid DNA, cells were fixed and stained with primary anti-NPSP-C “NPSP-C” (1:200) and images acquired by video fluorescence microscopy as described for Fig. 3. N, nucleus.

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**Fig. 8.** Localization of proSP-C and ER/GOLGI compartments. Isolated rat lung cells (panels A and A’) containing fresh type II cells (2) and alveolar macrophage (m) or A549 cells (a) transfected with either pcDNA3-SP-Cwt (panels B and B’) or pcDNA3-SP-C1–185 (panels C and C’), were fixed and stained sequentially with primary anti-NPSP-C “NPSP-C” (1:200), secondary goat anti-rabbit IgG-Texas Red (1:200), and tertiary FITC-ConA (100 μg/ml). Staining patterns were visualized by fluorescence microscopy using Ex 535 nm; Em 615 nm for proSP-C (Texas red) (A, B, and C) and an FITC filter package (OMEGA, Inc.) for ConA (A’, B’, and C’). Arrowheads in A and B showing representative cytoplasmic vesicles staining for proSP-C alone.
3H. Shuman, unpublished data.

with SP-C1–59 (not shown) and have been reported for the in the absence of substantial amounts of flanking propeptide, cells using similar methods.2

have shown that the appearance of SP-C6 in the lysates of type II cells is followed by conversion to mature SP-C3.7 (15), but have not yet, for technical reasons, been able to reliably detect its production in transfected A549 cells using similar methods.2

Prior to transfection, the fidelity of the open reading frame of each mutant SP-C construct and the ability of epitope-specific antisera to recognize translated protein was demonstrated. The pattern of 35S-doublets captured by immunoprecipitation of translated SP-Cwt and truncated mutants (Fig. 2) had been observed by us during in vitro transcription/translation SP-Cwt cDNA in a PGEM-4Z vector and is due to the use of an alternative start site (codon 10) by the TNT reticulocyte lysate kit (16).

When transfected into A549 cells, constructs containing deletions of the proSP-C COOH terminus ranging from 10 to 134 amino acids consistently showed synthesis of a primary translation product of predicted length without demonstrable proteolytic processing (Fig. 6). The low level of expression of SP-C1–72 as well as the presence of oligomeric forms suggests that in the absence of substantial amounts of flanking propeptide, mutant protein could be less efficiently translocated into the ER and/or rapidly degraded. Similar findings were obtained with SP-C1–59 (not shown) and have been reported for the translation of a similarly truncated proSP-B construct by transfected Chinese hamster ovary cells (28).

The failure to process truncated proSP-C was associated with an inability of the A549 cells to direct the export of the mutated proteins from early synthetic compartments into cyttoplasmic vesicles (Figs. 7 and 8). Co-localization of SP-C1–185 with ConA indicated that an intact distal COOH terminus (Cys186–Ile194) is necessary for targeting. This data in A549 cells, either the NH2-terminal flanking domain or mature protein to later compartments and suggests another region of proSP-C, either the NH2-terminal flanking domain or mature SP-C itself, serves to enable import into the ER. Previous in vitro studies using deletional human SP-C mutants have provided some indication that the mature SP-C sequence (Phe24–Leu58) could serve this function (31). Since all SP-C constructs in this study contain NH2-terminal and mature SP-C sequences, additional mutagenesis will be needed to further localize a functional signal sequence.

A comparison of proSP-C protein sequences from 4 species indicates a high degree of conserved amino acids in the COOH terminus including the terminal residue Ile194 (Fig. 9). BLAST analysis demonstrates no apparent conserved homo logy with other proteins or conformation to any consensus targeting sequences. However, site-directed mutagenesis of the COOH-terminal Ile of the bitopic lysosome-associated membrane protein, LAMP-2, affects its steady state subcellular distribution (32). This raises the possibility that Ile194 could provide a similar motif for direction of proSP-C to the lamellar body, which contains the lysosomal glycoprotein markers, CD63 (33) and LAMP-1 (36).

Misfolding of the natural conformation of proteins has been associated with aggregation and retention in the early stages of the secretory pathway. The role of intrachain disulfide linkages in propeptide folding is well known. Mutagenesis of residues Cys8 and/or Cys20 in the propeptide NH2 terminus of proopi melanocortin disrupts targeting of proopiomelanocortin to the regulated secretory pathway of Neuro-2a cells (34). For proSP-C, cysteine residues in the COOH terminus (at positions 121 and 186) are conserved across species but Cys186 is deleted during truncation of the distal COOH terminus. Because of the extreme hydrophobicity and membrane avidity of mature SP-C (Phe24–Leu58), the restriction of mutant proSP-C to ConA compartments could be explained by the disruption of propeptide folding and aggregation. Altered post-translational processing secondary to misfolding has been implicated as the mechanism for the absence of SP-B in surfactant observed in cases of congenital SP-B deficiency associated with the R236C mutation (35). While SP-C deficiency has not been reported to date, our data suggests that similar small changes in the distal proSP-C COOH terminus, while not altering mRNA stability, could result in aberrant SP-C processing leading to a deficiency of SP-C3.7.

In summary, like SP-B and many peptide hormones, zymogens, and secreted proteins, SP-C is expressed as a proprotein. Detailed analysis of many of these proteins has failed to identify any conserved amino acid sequences that mediate selective

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2 M. F. Beers, unpublished observations.

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targeting to distal compartments of the exocytic pathway. The results of the present study using A549 lung epithelial cells indicate that the COOH terminus of the SP-C propeptide is critical for its intracellular transport and processing. The transfected A549 cell model will enable further study of other deletional mutants as well as analysis of heterologous chimeric proteins containing the propeptide COOH terminus to further define both the motifs and mechanisms of proSP-C processing.

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