Structural Requirements for Targeting of Surfactant Protein B (SP-B) to Secretory Granules in Vitro and in Vivo*

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Human surfactant protein B (SP-B) is synthesized by type II cells as a 381-residue preproprotein which is proteolytically processed to a 79-residue mature peptide and targeted to lamellar bodies for secretion. To identify secretory granule targeting determinants, constructs encoding the SP-B preproprotein (SP-B), COOH-terminally deleted SP-B (SP-B<sub>C</sub>), the NH<sub>2</sub>-terminal propeptide (SP-B<sub>N</sub>), and a chimeric molecule consisting of albumin and the mature peptide (ALB/SP-B<sub>M</sub>) were transfected into AtT-20 and PC12 cells. Pulse-chase studies demonstrated that 10–30% of SP-B and SP-B<sub>C</sub> remained in cells in an endoglycosidase H-resistant form. Secretion of stored SP-B was stimulated by forskolin/12-O-tetradecanoylphorbol-13-acetate and intracellular SP-B was localized to secretory granules by immunoelectron microscopy. In contrast, SP-B<sub>N</sub> and ALB/SP-B<sub>M</sub> were constitutively secreted and not detected in secretory granules. Specific processing of SP-B was not detected in either AtT-20 or PC12 cells. Expression of SP-B<sub>C</sub> in transgenic mice resulted in secretion of fully processed mature SP-B, indicating correct processing and targeting of this construct in vivo. We conclude that 1) SP-B processing occurs in a cell-specific manner, 2) the proproprotein contains secretory granule targeting determinants that are not cell-specific, 3) the NH<sub>2</sub>-terminal propeptide and the mature peptide are required for targeting SP-B to lamellar body, and 4) the COOH-terminal propeptide is not required for processing or sorting of SP-B.

 Proteins reach their extracellular destination by one of two distinct secretory pathways: the constitutive secretory pathway, common to all mammalian cells, is the default pathway in which proteins are rapidly released from the cell by exocytosis; the regulated secretory pathway, present in certain cell types such as neuronal, endocrine, and exocrine cells, is characterized by storage of selected proteins in secretory granules which are released in response to appropriate external stimuli (1, 2). The coexistence of constitutive and regulated secretory pathways within the same cell implies that segregation of proteins must occur, a process which is believed to take place in the trans-Golgi network (3–6). Previous studies have identified ways within the same cell implies that segregation of proteins are released in response to appropriate external stimuli (1, 2).

The coexistence of constitutive and regulated secretory pathways; up to 85% of surfactant lipids are recycled to the lamellar body and to determine if these targeting epitopes are recognized by the sorting machinery of both endocrine and neuronal cells.

Human SP-B is synthesized by the alveolar type II epithelial cell as a preproprotein of 381 amino acids. Within the proprotein the 79-residue mature peptide is flanked by propeptides of 177 and 102 amino acids at the NH<sub>2</sub>- and COOH termini, respectively. The propeptides are removed by endoproteolytic cleavage in the multivesicular body, prior to incorporation of the mature peptide into the lamellar body, for storage with the phospholipid components of surfactant (18). Using domain-specific deletion mutants we demonstrate that the NH<sub>2</sub>-terminal propeptide and the mature peptide are necessary and sufficient for sorting of SP-B to dense core granules in AtT-20 and PC12 cells; we further demonstrate that these peptide domains

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1 The abbreviations used are: SP-B, surfactant protein B; SP-B<sub>C</sub>, COOH-terminally deleted SP-B; SP-B<sub>N</sub>, NH<sub>2</sub>-terminal propeptide of SP-B; ALB, albumin; ALB/SP-B<sub>M</sub>, albumin and mature SP-B peptide; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; TPA, 12-O-tetradecanoylphorbol-13-acetate; BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbent assay; kb, kilobase; bp, base pair; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.
are sufficient to mediate appropriate targeting and processing of SP-B in type II cells in vivo.

MATERIALS AND METHODS

Cell Culture—Both ATII-20/D16v-F2 cells and PC12 cells were obtained from the American Type Culture Collection (Rockville, MD). ATII-20 cells were grown in DMEM (Sigma) supplemented with 10% fetal bovine serum (FBS) (Sigma), 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc.) in an atmosphere of 15% CO2 at 37°C. PC12 cells were grown in DMEM supplemented with 10% horse serum (Sigma) and 5% FBS, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin in an atmosphere of 12.5% CO2 at 37°C. CHO cells were maintained as described previously (19).

Antiserum—Rabbit antisera 28031, generated against mature SP-B peptide, was used to detect bovine alveolar lavage, was used to detect SP-B preproprotein, SP-B<sub>MC</sub>, and mature SP-B (19). Rabbit antisera 55522, generated against purified recombinant full-length SP-B preproprotein, was used to detect SP-B preproprotein, SP-B<sub>B</sub>, and SP-B<sub>N</sub> (19). Anti-human albumin antibody was purchased from Calbiochem.

Adenoviral Infection—Construction of a replication-deficient vector Av1 containing full-length human SP-B cDNA has been described previously (20). freshly isolated type II cells (21) or ATII-20 cells were infected with Av1-SP-B at a multiplicity of infection of 50 in DMEM medium containing 2% FBS, 50 units/ml penicillin, and 50 μg/ml streptomycin at 37°C, 5% CO2 for 90 min. After infection, plates were incubated for an additional 48 h before pulse-chase studies were initiated (22).

DNA Constructs and Transfection—All procedures involving oligonucleotide and cDNA manipulations were performed essentially as described by Sambrook et al. (23). To generate SP-B constructs (Fig. 1), DNA fragments encoding SP-B, SP-B<sub>C</sub>, and SP-B<sub>N</sub>, were subcloned into the mammalian expression vector pcDNA3 (Invitrogen, San Diego, CA) as described previously (19). To generate the chimeric construct ALB/SP-B<sub>C</sub>, the sequence encoding the mature SP-B peptide was amplified by polymerase chain reaction (PCR) using modified primers to introduce a Bsu36I site at the 5' end and two stop codons followed by a Xhol site at the 3' end; this PCR fragment was ligated to the Bsu36I site at the 3' end of the coding sequence of the human albumin cDNA and the resulting product subcloned into the EcoRI/Xhol sites of pcDNA3 (Fig. 1). As a control, full-length human albumin cDNA was also subcloned into pcDNA3 (ALB). The expression constructs were characterized by DNA sequencing and in vitro transcription/translation. SP-B, SP-B<sub>C</sub>, and SP-B<sub>N</sub> expression plasmids were stably transfected into ATII-20 and PC12 cells using the standard calcium phosphate precipitation procedure (23). Transfected cells were selected using 0.5 mg/ml of G418 (Life Technologies, Inc.) for ATII-20 cells and 0.8 mg/ml of G418 for PC12 cells. For each construct, 40–60 clones were isolated, and stable clones were maintained in 0.2 mg/ml of G418. For cells transfected with SP-B<sub>C</sub> and SP-B<sub>N</sub>, constructs, clones were screened by immunoblot analyses with antisera 55522 as described previously (19). Constructs ALB and ALB/SP-B<sub>C</sub> were transiently transfected into CHO cells and PC12 cells by calcium phosphate precipitation and analyzed by metabolic labeling and pulse-chase experiments 48 h after transfection (19, 22).

Pulse-chase Labeling and Immunoprecipitation—Pulse-chase studies of type II cells and ATII-20 cells infected with adenovirus containing the full-length human SP-B cDNA were carried out as described previously (22). Briefly, 48 h after infection, cells were labeled for 15 min with 1 μCi/ml [35S]Met/Cys (DuPont), washed, and chased in medium containing 1.5 mg/ml methionine, 2.4 mg/ml cysteine, and 10% dialyzed FBS (Sigma). The media and cells were collected at the indicated time points. Immunoprecipitation, endoglycosidase H digestion, and SDS-PAGE/autoradiography were performed as described previously (19). Quantitation of proteins immunoprecipitated from cells and media was performed by phosphoimage analysis of dried gels.

Secretagogue-stimulated Secretion—Two T25 flasks (Falcon Labware) of cells were labeled for 18 h with [35S]Met (specific activity = 1,000 Ci/mmol, Amersham Corp.). Cells were washed, chased for 30 min, and then stimulated with 10 μM forskolin and 100 μM TPAA for an additional 3 h or with 55 mM KCl for 30 min. Medium was collected at the end of each chase period, and cells were collected after the last chase period and analyzed by immunoprecipitation as described (19).

RESULTS

Immunogold Labeling—Postembedding immunolabeling of plastic sections: ATII-20 cells were fixed, dehydrated, and embedded in Eponate 12 (Ted Pella, Inc., Redding, CA) for electron microscopy as described previously (24). Ultrathin (90–120 nm) sections were cut from polymerized blocks and mounted on nickel grids. Sections were etched in 30% sodium metaperiodate, blocked with streptavidin and biotin, and incubated with rabbit antisera 55522 at 1:100 dilution in carbonate/BSA buffer for 1 h (25, 26). Sections were washed three times in buffer, incubated with biotinylated goat anti-rabbit IgG (Cappel-Organon Teknika Co., Durham, NC) 1 h, washed in buffer, and incubated 30 min with streptavidin conjugated with 5-nm gold (gift of Dr. Randall E. Morris, University of Cincinnati, Cincinnati, OH). Grids were rinsed in buffer and distilled water, and counterstained with 2% aqueous uranyl acetate. Sections were observed on a Zeiss EM 912 transmission electron microscope, and photographed. Nontransfected ATII-20 cells were immunolabeled in each experiment as negative controls.

Targeted Expression of SP-B in the Lung Epithelium of Transgenic Mice—A transgene consisting of the 3.7-kb human SP-C promoter (27), 1.7-kb EcoRI fragment encoding the first 279 amino acids of human SP-B preproprotein, and a 400-bp fragment containing the SV40 small t intron and polyadenylation signal was cloned into pUC18. In preparation for injection, the 5.8-kb transgene was excised from pUC18 by NotI/NdeI digestion, isolated by gel electrophoresis, and purified by adsorption to Qiagen resin (Qiagen, Chatsworth, CA). The DNA was extensively dialyzed against 5 mM Tris (pH 7.5) and 0.1 mM EDTA and microinjected into fertilized eggs of the FVB/N mouse strain by the University of Cincinnati Transgenic Animal Core Facility. Founder mice were identified by PCR amplification of DNA isolated from mouse tails using a 5' primer (5'-CGCGAGAACAAACAGGCTTCA-3') specific to human SP-C and a 3' primer (5'-CCAGGGTCCACATCGCTGCT-3') specific to human SP-B to generate a diagnostic 600-bp fragment. 100 ng of genomic DNA isolated from mouse tails was amplified in a 30-cycle PCR with 1 μM of each primer, 100 μM dNTPs, 10 μM Tris (pH 8.3), 50 mM KCl, 1.5 mM MgCl2. PCR results were confirmed by Southern analyses using a 5'- labeled fragment containing the SV40 small t intron. Four transgenic lines were established, and line 9 was selected for further studies based on the elevated level of SP-B protein expression. Expression of mouse and human SP-B mRNA was assessed by S1 nuclease analyses as described previously (20). In order to assess the levels and forms of secreted SP-B, surfactant was isolated from the airways of transgenic mice and nontransgenic littermates by alveolar lavage (28) and analyzed by ELISA and/or immunoblotting using antisera 28031 (19). SP-B was subsequently isolated from alveolar lavage fluid by organic extraction (30), subjected to Tricine SDS-PAGE followed by electrophoretic transfer to polyvinylidene difluoride (31), and analyzed by automated Edman degradation for NH<sub>2</sub>-terminal sequence identification. For analyses of total lung SP-B, lung tissues were harvested at 4–5 weeks of age and homogenized in 10 μM Tris (pH 7.5), 0.25 M sucrose, 1 mM EDTA, 0.1 mM sodium orthovanadate, 2 mM sodium fluoride, and 10 μg/ml pepstatin A, aprotenin, antipain, leupeptin, and chymotrypsin. Samples from transgenic and nontransgenic lung tissue homogenates or alveolar lavage isolates were normalized to protein content (32) prior to ELISA or immunoblotted analyses.

Synthesis and Secretion of SP-B by Freshly Isolated Type II Cells—Previous studies (33) have established that many aspects of the type II epithelial cell phenotype, including SP-B expression, are rapidly down-regulated during primary cell culture. Therefore, initial experiments were performed to identify an appropriate model system for the study of SP-B sorting and secretion. Freshly isolated rat type II cells were transfected with a mammalian expression vector containing the full-length human SP-B cDNA (Fig. 1). Forty eight hours after
strated rapid secretion of SP-B proprotein, sarcoma virus promoter (20). Pulse-chase experiments demonstrated constitutive secretion of full-length human SP-B cDNA under the control of the Rous sarcoma virus promoter (20). Freshly isolated rat type II epithelial cells were infected with Av1/SP-B, an adenoviral vector containing the full-length human SP-B cDNA. 48 h after infection cells were labeled with [35S]Met/Cys for 15 min and chased for 30, 60, and 120 min, respectively. Both cell lysates and media were immunoprecipitated with antisera 28031 (directed against the mature SP-B peptide). Immunoprecipitated proteins bound to protein G-sepharose were incubated with (+) or without (−) endoglycosidase H and analyzed by SDS-PAGE/autoradiography. Transfected human SP-B proprotein, Mₘ = 42,000, did not accumulate intracellularly but was rapidly secreted in the proprotein form. Mouse SP-B was not detected in uninfected control cells, indicating that endogenous SP-B expression was down-regulated after 48 h of culture (not shown). Protein standards are shown at the right of the panel, in kilodaltons.

Fig. 2. Human SP-B proprotein is constitutively secreted by transfected rat type II epithelial cells in culture. Freshly isolated rat type II cells were infected with Av1/SP-B, an adenoviral vector containing the full-length human SP-B cDNA. 48 h after infection cells were labeled with [35S]Met/Cys for 15 min and chased for 30, 60, and 120 min, respectively. Both cell lysates and media were immunoprecipitated with antisera 28031 (directed against the mature SP-B peptide). Immunoprecipitated proteins bound to protein G-sepharose were incubated with (+) or without (−) endoglycosidase H and analyzed by SDS-PAGE/autoradiography. Transfected human SP-B proprotein, Mₘ = 42,000, did not accumulate intracellularly but was rapidly secreted in the proprotein form. Mouse SP-B was not detected in uninfected control cells, indicating that endogenous SP-B expression was down-regulated after 48 h of culture (not shown). Protein standards are shown at the right of the panel, in kilodaltons.

To determine the COOH-terminal propeptide is required for sorting SP-B to secretory granules, PC12 cells were stably transfected with the construct SP-B₁₋₂₋₃, in which the sequence encoding the COOH-terminal 102 residues of the SP-B proprotein was deleted (Fig. 1). Pulse-chase studies demonstrated that after 24 h of chase approximately 30% of the protein was retained intracellularly in an endoglycosidase H-resistant form (not shown), suggesting that SP-B₁₋₂₋₃ was sorted to secretory granules with a sorting efficiency comparable with that observed for the intact propeptide. Immunogold labeling of ultrathin cryosections demonstrated that gold particles were distributed in the endoplasmic reticulum and Golgi as well as dense core granules (Fig. 4B), confirming transport through the regulated secretory pathway. Further evidence for the sorting of SP-B₁₋₂₋₃ to the regulated secretory pathway was provided by the ability of secretagogues to stimulate secretion of intracellular SP-B; the addition of forskolin/TPA or KCl to the chase medium resulted in a 4- and 2-fold increase in the secretion of intracellular SP-B, respectively, accompanied by a decrease in the intracellular level of SP-B (Fig. 4A). These results indicated that the COOH-terminal propeptide is not required for sorting of SP-B to secretory granules.
Constitutive Secretion of SP-B N in Vitro—To determine if the NH2-terminal propeptide is responsible for the sorting of SP-B, a construct encoding the first 200 residues of the SP-B prepro-protein was generated (Fig. 1) and stably transfected into PC12 cells. The kinetics of SP-B N secretion, as assessed by pulse-chase experiments, demonstrated that the NH2-terminal propeptide was constutively secreted. Secretion kinetics were unaffected by the addition of forskolin/TPA (Fig. 5A). Immunogold labeling was restricted to the endoplasmic reticulum and Golgi and was not detected in dense core granules (Fig. 5B). These data suggest that the NH2-terminal propeptide alone is insufficient to direct SP-B to the secretory granule.

Constitutive Secretion of ALB/SP-BM in Vitro—Previous studies (19) have shown that the mature SP-B peptide is not

**Fig. 3.** SP-B proprotein is sorted to secretory granules in AT20 cells. A, kinetics of SP-B secretion. AT20 cells infected with Av1/SP-B were labeled with [35S]Met/Cys for 15 min and chased for the indicated number of minutes. Cell lysates and media were immunoprecipitated with antiserum 28031, treated with (-) or without (-) endoglycosidase H, and analyzed by SDS-PAGE. Significant amounts of endoglycosidase H-resistant SP-B were detected as early as 30 min of chase, and all intracellular proprotein was endoglycosidase H-resistant at 240 min, consistent with storage in a post-Golgi compartment. Proteolytic processing of SP-B proprotein was not detected. Variable glycosylation of SP-B resulted in detection of multiple proprotein forms following endoglycosidase H digestion. Molecular mass standards are indicated at the right of the panel, in kilodaltons. B, immunolocalization of SP-B proprotein. AT20 cells stably transfected with SP-B (a) and untransfected (control, b) cells were prepared for immunoelectron microscopy with antiserum 55522 as described under "Materials and Methods." Gold particles (arrowheads) were detected in dense core secretory granules of transfected cells but not in control cells. Bars = 0.1 μm.

**Fig. 4.** SP-B(1-22) is sorted to secretory granules in PC12 cells. A, effect of secretagogues on SP-B(1-22) secretion. Two flasks of PC12 cells stably transfected with SP-B(1-22) were labeled with [35S]Met for 18 h and chased for two consecutive 3-h periods (chase intervals 1 and 2). At the beginning of the third chase interval, 10 μM forskolin plus 100 nM TPA or 55 mM KCl was added to one of the flasks. Secreted and intracellular SP-B were recovered by immunoprecipitation of media and cell lysates with antiserum 28031, subjected to SDS-PAGE, and quantitated by phosphorimage analyses. SP-B(1-22) secretion was increased 2- and 4-fold in the presence of KCl and forskolin/TPA, respectively. Results shown are representative of three independent pulse-chase experiments. B, immunolocalization of SP-B(1-22). PC12 cells stably transfected with SP-B(1-22) and untransfected (control) PC12 cells were prepared for immunoelectron microscopy with antiserum 55522. Gold particles (arrowheads) were detected in Golgi (a) and dense core granules (b) of transfected cells, but never in control cells. Bars = 0.1 μm.
efficiently translocated into the endoplasmic reticulum in the absence of the flanking propeptides; therefore, to determine if the mature peptide contains sorting determinants, a chimeric construct encoding the first 608 amino acids of human albumin in the mature peptide appears to be essential for SP-B sorting.

Sorting and Secretion of the SP-B<sub>m</sub> peptide. To test this hypothesis, transgenic mouse lines were generated in which human SP-B<sub>m</sub>cDNA was targeted to the 3.7-kb human SP-C promoter (27). Four transgenic lines were established (Fig. 7A); transgenic line 6.1 was selected for these studies because of the elevated level of SP-B expression (Fig. 7B). In situ hybridization with antisense probes specific for human SP-B demonstrated that the expression of SP-B<sub>m</sub>cDNA mRNA was localized exclusively to type II cells of the respiratory epithelium (not shown). Alveolar structure and type II cell ultrastructure, as assessed by electron microscopy, were not affected by overexpression of SP-B (not shown). Because of extensive (78%) homology, mouse and human mature SP-B could not be distinguished by size or immunological methods; therefore, the total amount of SP-B was assessed in transgenic and control lungs by ELISA. In the transgenic lung, secreted and total lung SP-B was increased approximately 2- to 3-fold over control levels, respectively (Fig. 7C); immunoblot analyses revealed that all SP-B recovered from total lung homogenate was present as mature peptide (Fig. 7D). Identification and verification of
appropriate processing of human SP-B was assessed by NH$_2$-terminal propeptide and the mature peptide might be a sorting signal or that both the peptide itself might serve as a sorting signal or that both the

**DISCUSSION**

Analyses of numerous secretory proteins have failed to identify any conserved amino acid sequences that mediate selective targeting to secretory granules of the regulated secretory pathway. However, targeting determinants have been localized to peptide domains, including the propeptides of many secretory proteins (prosomatostatin (34, 35), pro-opiomelanocortin (36, 37), carboxypeptidase E (38), and peptidylglycine $\alpha$-amidating monooxygenase (39)) as well as the biologically active peptide itself (trypsinogen (40) and renin (41)). Based on our previous finding that the NH$_2$-terminal propeptide is essential for intracellular trafficking of the hydrophobic, mature SP-B peptide (19), we postulated that the propeptide might also mediate the sorting of SP-B to the regulated secretory pathway. The observation that only the mature peptide was detected in lamellar bodies (18) raised the additional possibility that the mature peptide itself might serve as a sorting signal or that both the NH$_2$-terminal propeptide and the mature peptide might be required for SP-B sorting.

In order to test these hypotheses, primary cultures of type II cells were initially assessed for their ability to sort endogenous and exogenous SP-B to the regulated secretory pathway. The results of these studies indicated that expression of endogenous SP-B was down-regulated in primary culture, as reported previously (33); furthermore, transfected SP-B was neither sorted to lamellar bodies nor proteolytically processed but constitutively secreted in the proprotein form. These findings are consistent with previous reports that incorporation of newly synthesized surfactant phospholipids into lamellar bodies decreases with time in culture (42) and suggest that the regulated secretory pathway of isolated type II epithelial cells is rapidly down-regulated, including both the sorting machinery and the proteins sorted by this machinery.

Since primary cultures of type II cells were not suitable for the study of SP-B sorting, AtT-20 and PC12 cells were selected as alternative models based on the existence of a well characterized regulated secretory pathway in both cell types (43, 44). In contrast to cultured type II cells, stably transfected PC12 and AtT-20 cells were able to sort SP-B to secretory granules. Similar results were obtained following transient transfection of AtT-20 cells with Av1/SP-B, indicating that the outcome of experiments in primary cultures of type II cells was not influenced by nonspecific effects of the adenoviral vector. Taken together, these data support the conclusion that SP-B contains a sorting signal that is recognized by both exocrine and endocrine cells.

Many protein precursors, which are heterologously expressed in AtT-20 and PC12 cells, are appropriately processed to their biologically active peptides in the secretory granule compartment. Proteolytic processing of prohormones occurs frequently after dibasic residues and less frequently after...
monobasic residues (45, 46). The sequences flanking the mature SP-B peptide do not contain basic residues or any other known consensus sequence for endoproteolytic cleavage. In keeping with this observation the SP-B proprotein was not proteolytically processed to the mature peptide in either AT-20 or PC12 cells, suggesting that propeptide cleavage occurs in a type II cell-specific manner. We recently demonstrated that the NH2-terminal propeptide of recombinant human SP-B proprotein could be removed by cathepsin D, a ubiquitous lysosomal enzyme (47). However it is unlikely that cathepsin D is involved in the processing of endogenous SP-B, since cathepsin D knockout mice survive the neonatal period without respiratory complications (48). Processing of the SP-B proprotein to the mature peptide apparently proceeds in the absence of cathepsin D, because complete absence of SP-B mature peptide is neonatal lethal (49). Although the identification of the endoproteases(s) involved in SP-B processing remains unknown, it is clear that propeptide cleavage is not a prerequisite for targeting to the secretory granule compartment in neuroendocrine cells.

The results of the present studies in AtT-20 and PC12 cells indicate that both the NH2-terminal propeptide and the mature peptide are required for sorting of SP-B to secretory granules. The in vivo relevance of this finding is supported by the identification of fully processed, mature SP-B peptide in the airway of transgenic mice expressing the SP-B construct, confirming that the COOH-terminal propeptide is not required for appropriate sorting and processing of the proprotein in vivo. The complete absence of proprotein in the airway of transgenic mice further suggests that the sorting efficiency is much higher in vivo, resulting in sorting of virtually all SP-Bac to the regulated secretory pathway for processing. Finally, the observation that SP-Bac is efficiently processed to the mature peptide in vivo indicates that cleavage of the NH2-terminal propeptide is not dependent on the presence of the COOH-terminal propeptide. Previous studies in freshly isolated type II epithelial cells demonstrated that the temporal sequence of events in proprotein processing involved the initial cleavage of the NH2-terminal propeptide followed by cleavage of the COOH-terminal propeptide (22); the present studies in transgenic mice suggest that the order of propeptide cleavage is not of critical importance for generation of the mature peptide in vivo.

In summary, the targeting of SP-Bac to the regulated secretory pathway in neuroendocrine cells and in transgenic mice is consistent with the conclusion that the targeting of proteins to the lamellar body compartment in type II epithelial cells is dependent on sorting motifs and machinery that are common to both exocrine and endocrine cells; in the case of SP-B, the sorting motif is comprised of both the NH2-terminal propeptide and the mature peptide. In contrast to the lack of specificity in SP-B sorting, proteolytic processing of the proprotein occurs in a type II cell-specific manner. Processing and sorting of the SP-B proprotein are independent events, neither of which requires the 102-amino acid COOH-terminal propeptide. Studies are currently underway to define the function of the COOH-terminal propeptide by breeding the SP-Bac transgenic mice into the knockout background.

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