Identification of LBM180, a Lamellar Body Limiting Membrane Protein of Alveolar Type II Cells, as the ABC Transporter Protein ABCA3*

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Lamellar bodies are the specialized secretory organelles of alveolar type II (ATII) epithelial cells through which the cell packages pulmonary surfactant and regulates its secretion. Surfactant within lamellar bodies is densely packed as circular arrays of lipid membranes and appears to be the product of several trafficking and biosynthetic processes. To elucidate these processes, we reported previously on the generation of a monoclonal antibody (3C9) that recognizes a unique protein of the lamellar body membrane of 180 kDa, which we named LBM180. We report that mass spectrometry of the protein precipitated by this antibody generated a partial sequence that is identical to the ATP-binding cassette protein, ABCA3. Homology analysis of partial sequences suggests that this protein is highly conserved among species. The ABCA3 gene transcript was found in cell lines of human lung origin, in ATII cells of human, rat, and mouse, as well as different tissues of rat, but the highest expression of ABCA3 was observed in ATII cells. Expression of this transcript was at its maximum prior to birth, and hormonal induction of ABCA3 transcript was observed in human fetal lung at the same time as other surfactant protein transcripts were induced, suggesting that ABCA3 is developmentally regulated. Molecular and biochemical studies show that ABCA3 is targeted to vesicle membranes and is found in the limiting membrane of lamellar bodies. Because ABCA3 is a member of a subfamily of ABC transporters that are predominantly known to be involved in the regulation of lipid transport and membrane trafficking, we speculate that this protein may play a key role in lipid organization during the formation of lamellar bodies.

Pulmonary surfactant is a complex mixture of phospholipids and proteins that functions to prevent atelectasis by reducing alveolar surface tension at low lung volumes (1, 2). Surfactant consists predominantly of phospholipids, synthesized by alveolar type II (ATII) cells, and several unique proteins. Surfactant protein (SP)-A, SP-B, SP-C, and SP-D are synthesized by ATII cells, but with the exception of SP-C, they are also produced by other cells (3, 4). The alveolar surfactant pool size appears to be controlled primarily by ATII cells that regulate both secretion and re-uptake from the alveolar space. Prior to secretion, surfactant lipids along with surfactant proteins are stored in lamellar bodies as densely packed lamellae and are secreted into the alveolar lumen by regulated exocytosis (5).

The average ATII cell normally contains 150 ± 30 lamellar bodies (6) with an in vivo basal secretion rate of ~15 lamellar bodies per h in rat lung (7). ATII cells also endocytose surfactant from the alveolar space, some of which is recycled to lamellar bodies (8–12) and the remainder is degraded (9). Because of its central role in lung surfactant turnover, we have focused on the assembly of this organelle and the proteins that distinguish it from other secretory organelles. Lamellar bodies appear to be at the intersection of several membrane trafficking and vesicle sorting pathways. Surfactant lipids and proteins are targeted to lamellar bodies from both the secretary and endocytic pathways. SP-B and SP-C are delivered to lamellar bodies from the endoplasmic reticulum via the secretary pathway (13, 14), whereas SP-A is secreted to the airspace and subsequently internalized and trafficked to lamellar bodies via clathrin-coated pits through early and late endosomes (12, 15). Newly synthesized lipids, such as phosphatidylcholine, are also delivered to the lamellar body from the endoplasmic reticulum via the secretory pathway (16–18). Surfactant lipids are internalized and delivered to lamellar bodies by two pathways, one clathrin-dependent and another clathrin-independent but actin-dependent (19).

Proteins essential for the formation of lamellar bodies have not been completely defined. SP-B appears to be one of the key proteins necessary for the proper organization of lamellar bodies. Newborns with hereditary SP-B deficiency have poorly formed lamellar bodies and abnormal surfactant (20, 21). Similar abnormalities have been demonstrated in SP-B-deficient mice (22). However, other contributing factors must also exist because loss of lamellar bodies or poorly formed lamellar bodies

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1 The abbreviations used are: ATII, alveolar type II; ABC, ATP binding cassette; MDR, multidrug-resistant; SP, surfactant protein; RT, reverse transcription; Dex, dexamethasone; BMX, isobutylmethylxanthine; EGFP, enhanced green fluorescent protein; PC, phosphatidylcholine; mAb, monoclonal antibody.
have been observed in abnormalities associated with refractory neonatal respiratory failure and congenital alveolar proteinosis where SP-B appears normal (23, 24).

In an effort to understand the formation and organization of lamellar bodies, we began the process of identifying proteins that are uniquely expressed in this organelle. In our previous report (25), we described the generation of a panel of 30 antibodies against the limiting membrane of lamellar bodies isolated from rat ATII cells. One of these antibodies, monoclonal antibody (mAb) 3C9, labels lamellar bodies of ATII cells with high specificity. Further characterization revealed that the antigen is an integral membrane protein with a molecular mass of 180 kDa, which we named LBM180. The present study identifies LBM180 as ABCA3, a member of ATP-binding cassette (ABC) transporter family, and characterizes temporal, spatial, and regulated expression.

ABC transporters represent the largest family of transmembrane proteins, and its members have been found in every organism examined so far (26). These proteins bind ATP and use the energy of its hydrolysis to drive the transport of various substrates across cell membranes (27–29). The human ABC proteins are classified into subfamilies that include the ABCA subfamily of lipid transporters, multidrug resistance/transmembrane-associated proteins, adrenoleukodystrophy proteins, and the cystic fibrosis transmembrane conductance regulator (30). They transport a wide variety of substrates including lipids, ions, amino acids, peptides, sugars, vitamins, steroid hormones, and toxic compounds (30). Their distinguishing features are two nucleotide binding domains with conserved Walker A and B motifs, both involved in ATP binding (present in many other ATP-utilizing proteins), one conserved sequence diagnostic to the ABC unit called the “ABC signature” located between the Walker A and B sequences, and 12 membrane-spanning helices (31). Substrate specificity appears to be determined by the transmembrane domains, whereas coupling of the two nucleotide binding domains provides the energy required for transport.

ABCA3 was originally cloned from a human medullary thyroid carcinoma cell line, and in humans, its message is expressed most highly in lung (32, 33). Recently, Yamano et al. (34) cloned ABCA3 from human lung and showed, by immunohistochemical analyses, that ABCA3 is an ATII cell lamellar body membrane protein. They also demonstrated that ABCA3 protein had a molecular mass of 150 kDa in human lung but when expressed in HEK 293 cells, the protein had a molecular mass of 180 kDa. ABCA3 is a member of the ABCA subfamily of transporters and shares high homology with ABCA1, ABCC1, and ABCG1. ABCA3 of Alveolar Type II Cells

Experimental Procedures

DNA Constructs—The human full-length ABCA3 cDNA was prepared by amplifying three overlapping –2 kb segments of cDNA by reverse transcription (RT)-PCR method according to the manufacturer’s recommendations (Advantage, CLONTECH, Palo Alto, CA). Total RNA of cells and tissues was transcribed by enzymatic reverse transcription followed by PCR amplification. The three segments chosen had unique restriction sites in their overlapping regions, namely BamHI, DraIII, and BsmI, and each were ligated into a TOPO-TA vector (pCR 2.1-TOPO, Invitrogen, Carlsbad, CA). Following amplification of the plasmids in competent bacterial cells (Invitrogen), the three segments were cut with the aforementioned restriction enzymes and were ligated to each other. The primers (primer nucleotide numbering is based on the data base, from the National Center for Biotechnology (NCBI), data accession number NM001089, of the human ABCA3) generated for these segments are as follows: forward primers, 5′-GAC-CACCTACTTCTTAGCAGCACTGGGCG-3′, corresponding to nucleotides –47 to –18 from the START (ATG) codon; 5′-GGGCGATCTGGGATCTTTCC-3′, corresponding to nucleotides 2681–2681; and 5′-GAGGCGGCGGTGTATTGAGTGC-3′, corresponding to nucleotides 3962–3962; reverse primers, 5′-GTAGGTCGGAGGGACGGACGAGCTGATGGTCTGC-3′, corresponding to nucleotides 1311–1343; 5′-CTGGCACAGCGAGGAACTG-3′, corresponding to nucleotides 4061–4078; and 5′-CGGAGATCCGGGGCTTACCTGAGTACG-3′, corresponding to nucleotides 5841–5873. The 310-bp ABCA3 cDNAs of cells and tissues were prepared by RT-PCR using the following primers: forward primer, 5′-GCGAATGGGCTTTTCCCCTGCCAAGGG-3′, 3′-GGGCCATCTGG-3′, corresponding to nucleotides 874–906; and reverse primer, 5′-CCGGAATGATCAGGAGGCACTTGCC-3′ (complement to bases corresponding to the peptide SPDQPEG-YYR, corresponding to nucleotides 1151–1183. EGFPA/ABCA3 cDNA fusion constructs were generated by ligating the full-length hABCA3 cDNA into a pEGFP-N1 amino-terminal protein fusion vector (CLON-TECH) after removing the STOP (TAG) codon at the internal BglII restriction site located near the STOP site of ABCA3. All resultant plasmids were transformed into competent bacterial cells (Invitrogen) for amplification.

Antibody Generation—Two peptides from putative antigenic regions of the deduced amino acid sequence of human ABCA3 were synthesized and used to immunize rabbits. These regions of the protein have no homology to any other proteins in the NCBI data base and no homology to any known rabbit sequence. The peptides prepared were CQEKERRLKEYM (ABCA3 luminal loop) and CGKPRAVAGKE (ABCA3 cytosolic domain) producing antibody 1 and antibody 2, respectively.

Immunoblot Analysis—Protein samples were separated with SDS-PAGE under reducing conditions. Samples were visualized in sample buffer (125 mM Tris/HCl, 0.32 mM sucrose, 2% (w/v) SDS, 65 mM dithiothreitol, and 0.001% bromphenol blue, pH 6.8) at room temperature. The separated proteins were transferred electrophoretically onto nitrocellulose membranes (BA83, 0.32-μm pore size; Schleicher & Schuell) overnight at 20 mA in transfer buffer (12.5 mM Tris, pH 8.3, 96 mM glycine, 0.1% SDS, 15% (v/v) methanol, pH 8.0). Protein-binding sites were blocked by TBS containing 2–5% non-fat dry milk for 60 min at room temperature. The membrane was then incubated with primary antibody in TBS/milk solution for 2 h at room temperature. After three 15-min washes with TBS, the nitrocellulose was incubated for another 60 min at room temperature in TBS/milk solution containing horseradish peroxidase-conjugated anti-mouse-, rat-, or rabbit-IgG (1:2000 normal mouse/milk dilution). Blots were visualized by enhanced chemiluminescence (ECL System, Amersham Biosciences).

Northern Blot Analysis—Total RNA was prepared from cells and tissues using RNeasy Mini Kit (Qiagen, Valencia, CA) and separated by formaldehyde-agarose gel electrophoresis and transferred to nitrocellulose membrane (BA83, 0.32-μm pore size; Schleicher & Schuell). Blots were prehybridized in 50 mM sodium phosphate, pH 6.5, 5 × SSC, 0.1% SDS, formamide, 10% dextran sulfate, and 100 μg/ml salmon sperm DNA for 4 h at 42 °C. Hybridization was carried out overnight in the same buffer containing 10′-10′′ cm2/ml of a 32P-labeled 310-bp ABCA3 cDNA fragment. The filters were washed twice for 15 min in 1 × SSC, 0.1% SDS at room temperature and then twice for 20 min in 0.2 × SSC, 0.1% SDS at 55 °C, and the filters were exposed to a PhosphorImaging screen for 24–72 h. The intensities of signals on the autoradiogram were quantified on a PhosphorImager using the Quantity One computer software.
software (Bio-Rad). To correct for RNA loading, the obtained signals were normalized with the densitometer quantified ethidium bromide-stained 28 S or 18 S bands.

**Developmental Study**—Animal protocols were standardized and authorized by the Institutional Animal Care and Use Committee of both the University of Pennsylvania and The Children’s Hospital of Philadelphia (Philadelphia, PA). “Timed-pregnant” Wistar rats (mating day = Gestational Day (GD) 1, term = GD 22) and newborn Wistar rats were used. Pregnant rats were delivered by Caesarian section at GD 17, 19, and 21 or were allowed to deliver naturally. Neonatal rats were designated to be 1-day-old postnatal day (PD) 1. PD 4, 7, 14, and 16-week-old adult rats were subjected to study. All animals were killed by cutting the abdominal aorta after a surgical level (toe pinch) of anesthesia was induced with intraperitoneal injection of pentobarbital (<50 mg/kg).

**Immunofluorescence**—Cells in culture were fixed in 2% paraformaldehyde for 30 min, permeabilized with 0.3% Triton X-100, and washed. Lung tissues were cryosectioned (5–8 μm), and sections adhered to slides. The slides were incubated in NaBH₄ to reduce tissue autofluorescence, permeabilized with Triton X-100, and washed in phosphate-buffered saline (PBS) (137 mM NaCl, 10 mM Na₂HPO₄, 2.7 mM KCl, 1.8 mM KH₂PO₄, pH 7.4). After washing, cells or sections were blocked with 5% normal goat serum, washed with PBS, incubated with primary antibody for 2 h at room temperature, washed, and incubated with fluorescein isothiocyanate (Texas Red)-conjugated secondary IgG for 1 h. Specimens were mounted in Mowiol and examined by fluorescence microscopy.

**Antigen Retrieval**—Antigen retrieval by anti-ABCA3 was carried out as described previously (39). Briefly, cultured cells on glass cover slips were rinsed in serum-free medium, fixed with 4% paraformaldehyde, and washed with PBS. Cells were permeabilized with 0.3% Triton X-100, washed with PBS, and treated with 1% SDS for 5 min before continuing with immunofluorescence described above.

**Microscopy**—Samples were observed with inverted Nikon fluorescence microscopes equipped with either a cooled CCD camera and MetaMorph image analysis software (Universal Imaging, West Chester, PA) or Bio-Rad Micromaniplease 2000 confocal attachment (Bio-Rad).

**Explant Culture**—Human fetal lungs were obtained from 14- to 22-week gestation therapeutic abortions under protocols approved by the Committee on Human Research at The Children’s Hospital of Philadelphia. Fetal lung parenchyma was minced into 1-mm³ pieces and placed in organ culture, as described previously (40). Briefly, tissue pieces were distributed in two parallel strips on 60-mm culture dishes and placed in organ culture, as described previously (40). Briefly, tissue pieces were distributed in two parallel strips on 60-mm culture dishes and placed in organ culture, as described previously (40).

**Isolation of Primary Cultures of ATII Cells from Adult Rats Using Elastase**—ATII cells were isolated using elastase essentially according to the method of Dobbs and Williams (42). The lungs of anesthetized rats were perfused via the pulmonary artery and exsanguinated to the method of Dobbs and Williams (42). The lungs of anesthetized rats were perfused via the pulmonary artery and exsanguinated to the method of Dobbs and Williams (42).

**Immunoprecipitation**—Immunoprecipitation was carried out, as described previously (47), with slight modifications. Lamellar body membrane fraction was resuspended in ice-cold PBS. To reduce nonspecific binding to the lamellar body membrane suspension was incubated with end-over-end rotation for 90 min at 4 °C with 10 μl of preimmune serum (normal goat serum, Jackson ImmunoResearch) followed by 40 μl of protein A-Sepharose (Amersham Biosciences). After centrifugation, the supernatant was transferred into a clean tube and incubated with mAb 3C9 on end-to-end rotation for 90 min at 4 °C. Antibody complex was then precipitated with 50 μl of protein A-Sepharose by incubation for 90 min at 4 °C with end-over-end rotation. The immune complex protein A-Sepharose was collected by centrifugation and washed 4 times with ice-cold lysis buffer (150 mM NaCl, 50 mM Tris-Cl, pH 8.0, 1% Triton X-100, 1% deoxycholate, 0.1% SDS), and the bound proteins were solubilized with 25 μl of SDS-PAGE sample buffer (48) (10% glycerol, 5% β-mercaptoethanol, 2.3% SDS, and 0.062 μl Tris-HCl, pH 6.8). The Sepharose beads were removed by centrifugation, and the proteins were resolved by 10% SDS-PAGE.

**Transfection**—Transfection was performed by the FuGENE 6 method (Roche Molecular Biochemicals) according to the manufacturer’s recommendation. 4 μg of pEGFP-N1-tagged ABCA3-containing plasmid was preincubated with 6 μl of FuGENE 6 in a total volume of 100 μl of serum-free medium at room temperature for 15–30 min. The complex mixture was added dropwise to 60–70% confluent cells in a 35-mm dish containing 3 ml of fresh medium with 10% fetal calf serum. Cells were incubated at 37 °C for various times, and medium was replaced 3 days after start of transfection.

**Statistics**—Experiments were analyzed by one-way analysis of variance. All values were expressed as mean ± S.E. All computations were performed using SigmaStat 2.0 statistical analysis software (Jandel Corp., Chicago, IL).

**RESULTS**

**Purification of the Lamellar Body Specific Protein from Lamellar Body Membranes**—The lamellar body membrane-specific protein LBM180 was identified previously using monoclonal antibody, mAb 3C9 (25). To confirm this finding prior to protein purification, Western blot analysis was repeated on purified lamellar body membrane proteins. Lamellar bodies from rat lungs were purified to near-homogeneity (Fig. 1A), and the membrane fraction was separated from the intra-organelle contents by osmotic shock followed by centrifugation. The proteins from the membrane and content fractions were further separated by SDS-PAGE. Immunoblot with mAb 3C9 revealed a single band at 180 kDa in the membrane fraction and no labeling in the content fraction (Fig. 1B). To isolate the immu-
specific oligonucleotide primers (Fig. 2). A 310-bp PCR product was amplified from mRNA isolated from rat and ATII cells and rat and mouse lung total mRNA. Human adenocarcinoma cell lines A549 and NCI H441 expressed the ABCA3 mRNA as well, although at lower levels, whereas HeLa (a human cervical epithelial cell line) and rat pancreatic βHC9 cells did not. RT-PCR results were confirmed by Northern blot, where A549 and H441 cells had much lower expression of ABCA3 message (not shown) than ATII cells (Fig. 4).

The deduced amino acid sequence of the ABCA3 PCR products had high homology between three species examined (Fig. 3). The human ATII cell sequence is identical to the previously published (32) ABCA3 cDNA sequence obtained from human thyroid medullary carcinoma cells. There is 86% identity and 94% positive homology between murine and human amino acid sequences, whereas the rat is 91% identical and 98% homologous with the mouse sequence.

Expression of ABCA3 Transcripts—Northern blot analysis of mRNA from rat ATII cells and various rat tissues showed a single transcript of 6.5 kb (Fig 4A). For all organs, the highest ABCA3 expression was observed in lung and was consistent with the previously reported level of expression in human tissues (32). Significant expression of mRNA was also clearly detected in stomach, intestine, and kidney with lower levels of expression in thyroid, brain, liver, spleen, heart, testis, and thymus (Fig. 4B). Unlike the human counterpart (32), expression of ABCA3 mRNA was not detected in rat pancreas.

Developmental Expression of ABCA3—Surfactant production is developmentally regulated during gestation and reaches maximal levels prior to birth (49–51). To address the question of whether ABCA3 is induced during lung development, total rat lung RNA was collected on days 17, 19, and 21 of gestation and days 1, 4, 7, 14 and 16-weeks postnatal. Northern blot analyses revealed significant increases of ABCA3 message levels between days 17 and 21 of gestation and then a decrease after birth to a nadir at approximately day 7 postnatal (Fig. 5). By day 14 postnatal, the message had returned to ~75% of the adult level of expression.

Lung maturation and surfactant production can be accelerated by corticosteroid hormones (52–54). To evaluate the hormonal responsiveness of ABCA3, gene array and Northern blot analyses were utilized for the human fetal lung and alveolar type II cells during in vitro differentiation in either the absence (control) or presence of Dex, cAMP, and IBMX (DCI). A Northern blot of fetal lung explants showed increased (~30-fold) expression with DCI treatment, whereas day 1 and control explants had nearly background expression (Fig. 6A). Explants
treated with Dex or cAMP alone also showed increased expression. The increase was synergistic (>35-fold) with combined hormone treatment (Fig. 6B). Similar results were obtained with microarray gene expression analysis of ATII cells harvested from 14- and 17-week old lungs in which DCI greatly increased (44- and 28-fold, respectively) hABCA3 mRNA expression compared with control cells (Table I). Overall, compared with the 159 genes induced >2-fold, the hABCA3 mRNA was the seventh most highly induced gene in differentiating ATII cells.

Localization of mAb 3C9-positive Protein in the Limiting Membrane of Lamellar Bodies of Mouse—To evaluate whether mAb 3C9, previously shown to recognize a lamellar body membrane protein in rat ATII cells (25), also recognizes the mouse...
homologue, lung sections from mouse and rat were used for immunofluorescence studies. Texas Red-conjugated mAb 3C9 labeled both tissues in a similar manner (Fig. 7). The antibody appears to label the limiting membrane of lamellar bodies, as indicated by the ring-like staining surrounding the lamellar bodies (Fig. 7). The antibody immunofluorescence studies. Texas Red-conjugated mAb 3C9 homologue, lung sections from mouse and rat were used for values from two experiments. FAS, fatty-acid synthase.

○ Increase compared with control and day 4 of culture and are individual demonstrate significant increases in ABCA3 expression. Data are fold increase compared with control and day 4 of culture and are individual values from two experiments. FAS, fatty-acid synthase.

**TABLE I**

| Gene          | Increase fold |
|---------------|---------------|
| SP-C          | 133, 143      |
| SP-A          | 97, 119       |
| ABCA3         | 44, 28        |
| SP-B          | 20, 36        |
| Lipoprotein lipase | 12, 6         |
| FAS           | 12, 6         |

Lamellar Body Localization of ABCA3 in ATII Cells—We generated two polyclonal antibodies that recognize ABCA3 using synthetic peptides with amino acid sequences matching putative intracellular (antibody 1) and extracellular regions (antibody 2) of the human ABCA3 protein. Immunofluorescence and immunoblot analysis were performed to determine the localization and expression of ABCA3 by ATII cells using these anti-hABCA3 antibodies. By immunofluorescence, using an antigen retrieval method, anti-hABCA3 antibody 1 labeled membranes of human ATII cell organelles (Fig. 8B, arrows), which was similar to the labeling pattern observed for rat ATII cells with mAb 3C9 (Fig. 8A). Immunoblot analysis revealed that anti-hABCA3 antibody 2 recognized a 180-kDa band in protein samples from a purified lamellar body fraction of human fetal but not rat lung (Fig. 8E). Neither of these ABCA3 antibodies labeled rat protein by immunofluorescence (data not shown).

Post-translational Targeting of hABCA3 in A549 and H441 Cells—The deduced protein structure from the human cDNA sequence and comparisons to other ABC transporters (30) as well as the data provided above indicate that ABCA3 is likely to be a transmembrane protein. To examine the subcellular localization of ABCA3, A549 and H441 cells were transiently transfected with cDNA encoding hABCA3/enhanced green fluorescent protein (EGFP) fusion proteins. The transfected fusion proteins were consistently localized to cytoplasmic vesicles when expressed by either A549 or H441 cells (Fig. 9, A and E). In contrast, cells transfected with the EGFPN1 plasmid alone showed a diffused appearance (Fig. 9, B and F). At higher magnification, ABCA3/EGFP fluorescence in transfected A549 cells had a ring-like appearance (Fig. 9A, inset) suggesting localization of ABCA3/EGFP to vesicle membranes.

DISCUSSION

The lamellar bodies of ATII cells play a central role in surfactant lipid and protein homeostasis in the lung. As a step toward understanding the nature and assembly of lamellar bodies, we identified previously several monoclonal antibodies that react specifically to the limiting membrane of lamellar bodies. One of these antibodies was shown to recognize LMB180, a 180-kDa protein in the limiting membrane of lamellar bodies of rat lung (25). By using immunoprecipitation and tandem array mass spectrometry, we identified LMB180 to be ABCA3, a protein that belongs to a family of transporters that are involved in active transport of various substrates across cell and organelle membranes. By using an antibody raised against a synthetic peptide corresponding to 13 amino acid residues of human ABCA3, ABCA3 was recently shown to be a lamellar body protein of ATII cells (34). Lung tissue had the highest expression of ABCA3 RNA of the dozen rat tissues examined (Fig. 4) similar to that found previously for human tissues (32). Transcripts of ABCA3 were also abundantly expressed in rat stomach, intestine, and kidney suggesting that the function of ABCA3 may not be exclusive to ATII cells.

Comparison between human, rat, and mouse RT-PCR products showed a highly conserved 82-amino acid deduced sequence that corresponds to the amino-terminal region of ABCA3 (Fig. 3). The 82-amino acid sequence used for comparison represents only

![Figure 6](http://www.jbc.org/content/279/45/22152/F6)

**FIG. 6.** Hormonal induction of ABCA3 message during in vitro differentiation of human fetal explants of lung and isolated cells. Human fetal explants of 14-week gestation lung (A) and 22-week gestation ATII progenitor cells (B) were cultured in the absence (day 1, day 4 control) or presence of Dex/cAMP/IBMX (DCI). Northern blots of total RNA from each culture was hybridized with a hABCA3 cDNA probe. A representative Northern blot demonstrating hybridized bands at 6.5 kb (arrow) from three different lung explants (A) and at least three separate ATII cell experiments (B) are indicated by the arrow, and the corresponding ethidium bromide-stained 28 S bands (arrowhead) are shown. The bands were quantified by PhosphorImaging computer system, and arbitrary units were generated by normalizing the PhosphorImage of each band against its 28 S band. Results are expressed as mean ± S.E. *, p < 0.05 versus control. †, p < 0.01 versus control.


~5% of the total hABCA3 protein. However, this region is not within any of the conserved domains of the ABC transporters, e.g. the Walker ATP-binding motifs. This consideration supports the likelihood of high homology of ABCA3 between species. High homology between mouse and rat amino acid sequences (91% identical and 98% conserved) could explain why mAb 3C9 reacted with mouse lamellar bodies (Fig. 7) even though this antibody was originally generated from purified lamellar body membrane proteins of rat lung (25).

Our study shows that the ABCA3 gene was strongly up-regulated during human and rat fetal lung development. The highest expression of the transcript in the rat lung was observed 1 or 2 days before birth (Fig. 5) at the same time as the induction of the message for surfactant-associated proteins, consistent with involvement of ABCA3 in development of ATII cells and the surfactant system. Expression of ABCA3 mRNA by ATII progenitor cells isolated from human fetal lung was highly induced (>35-fold) by DCI treatment, and ABCA3 protein localizes to newly formed lamellar bodies in these cells. In this cell culture system, however, there is a coordinated induction of lamellar body constituents (SP-B, -C, and phospholipids) and ABCA3 of the limiting membrane, further supporting a role for ABCA3 in lamellar body genesis. These findings are in agreement with earlier studies in other systems that have shown temporal correlations between developmental increases in various enzymes involved in synthesis of fatty acids and surfactant proteins (55–59) and also demonstrated the glucocorticoid-dependent appearance of lamellar bodies and mRNAs of surfactant proteins (60, 61). Numerous other studies have also shown surfactant phospholipid (49, 50, 53, 62) and protein (50, 51, 60, 63) production is developmentally regulated during gestation and reaches maximal levels prior to birth. Endogenous hormones, such as glucocorticoids, appear to be involved in the regulation of this phenomenon (3, 57, 64). The decrease in ABCA3 mRNA content observed at postnatal day 7 (Fig. 5) is compatible with hormonal regulation of expression
because the lowest plasma concentration of total corticosterone is found on days 6–12 in rats (65).

The data presented here and by Yamano et al. (34) place ABCA3 in the lamellar body membrane of ATII cells. The sorting of ABCA3 was mimicked by the ABCA3/EGFP fusion construct, which was sorted to vesicular membranes in A549 cells (Fig. 9). A549 cells were originally derived from a human lung adenocarcinoma, and in early passages they expressed lamellar bodies (66, 67). Although most of the lung epithelial phenotype has been lost in these cells, a fraction of late passage A549 cells still show vesicles with a morphology similar to lamellar bodies which may account for sorting of ABCA3/EGFP in the distinctive ring-like pattern observed (Fig. 9, inset).

The antibodies against hABCA3 label ATII cell vesicles in a ring-like manner by immunofluorescence (Fig. 8B) (34). Anti-hABCA3 antibodies also recognize either a 180-kDa protein (Fig. 8E, lane 2) or 150-kDa protein, Yamano et al. (34), in Western blots. These data are comparable with the protein characteristics demonstrated by using mAb 3C9 (Figs. 1C, lane 2, and 8A) in rat ATII cells. The difference in apparent molecular weights observed here and by Yamano et al. (34) may be explained by the difference in electrophoresis procedures used in the two studies or by differences in the epitopes recognized by the two antibodies. Because mAb 3C9 and our anti-hABCA3 do not bind to immunoblots after the samples are boiled, our antibody may recognize the full-length ABCA3, with a predicted molecular mass of 191 kDa, whereas the Yamano et al. (34) antibody recognizes a shorter, post-translationally modified protein.

What role does ABCA3 play in the biology of ATII cells? When hABCA3 was originally cloned, it was classified as a member of the ABCA subfamily due to its high homology to ABCA1 (36% identity and 54% conserved at the amino acid level) (32, 33). ABCA1 is critically involved in cellular trafficking of cholesterol and choline phospholipids (68). Tangier disease, characterized by the absence of plasma high density lipoprotein and defective removal of cellular cholesterol and phospholipids, is due to a mutation in ABCA1 (69). The mouse ABCA1 (70) and Caenorhabditis elegans protein, Ced-7 (71) (a homologue of ABCA3 and ABCA1), facilitate apoptotic cell engulfment and dead-cell ingestion, respectively, by a mechanism that involves the transbilayer movement of phospholipids (72). Several other ABC transporters within the ABCA subfamily are also believed to be involved in lipid transport. ABCA4 appears to mediate retinol transport, by flipping modified phosphatidylethanolamine to the outer leaflet of the cell membrane (30, 73). ABCA2, ABCA7, and ABCA8, like ABCA1, are up-regulated in a sterol-dependent mechanism (35, 36).

A number of other ABC transporters, although not in the ABCA subfamily, have been implicated in mediating lipid transport across membranes. ABCB4 (MDR3), a member of the multidrug transporter subfamily, is a specialized translocator required for secretion of phosphatidylcholine (PC) into bile (74). MDR2 (the murine homologue of MDR3) knockout mice are completely unable to secrete PC, which results in damage of the canalicular membrane of hepatocytes and small bile ducts (74). Recently, familial MDR3 deficiency in human was identified and is characterized by intrahepatic cholestasis with high θ-glutamyl transpeptidase, causing severe liver disease (75).

Two members of the ABCG subfamily, ABCG5 and ABCG8, also have been implicated in a genetic form of hyperlipidemia caused by impaired secretion of sterol and related compounds to the intestinal lumen and bile (68). The peroxisomal ABC transporter is an intracellular membrane transporter and has been shown to transport fatty acids and metabolites across peroxisomal membranes (76).

As mentioned earlier, this study and others (34) have localized ABCA3 in lamellar body membranes. We have also reported, from studies using mAb 3C9, that LBM180 is expressed on the plasma membrane of ATII cells and is recycled back to lamellar bodies (77). The site of action of ABCA3 could therefore be either at the cell surface or in membranes of intracellular organelles including lamellar bodies or both.

The most likely possibility for function of this protein is, therefore, that ABCA3 transports lipid across the cell or lamellar body limiting membrane. Specifically, because the lipid content of lamellar bodies is highly enriched in PC, ABCA3 may either transport PC into lamellar bodies or transport other lipids such as phosphatidyleserine, phosphatidylethanolamine, sphingomyelin, or sphingolipids out of lamellar bodies. Either mechanism would have the capacity to enrich the PC content of lamellar bodies.

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Identification of LBM180, a Lamellar Body Limiting Membrane Protein of Alveolar Type II Cells, as the ABC Transporter Protein ABCA3

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