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## Regulation of surfactant secretion in alveolar type II cells

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**Andreeva AV, Kutuzov MA, Voyno-Yasenetskaya TA.** Regulation of surfactant secretion in alveolar type II cells. *Am J Physiol Lung Cell Mol Physiol* 293: L259–L271, 2007. First published May 11, 2007; doi:10.1152/ajplung.00112.2007.—Molecular mechanisms of surfactant delivery to the air/liquid interface in the lung, which is crucial to lower the surface tension, have been studied for more than two decades. Lung surfactant is synthesized in the alveolar type II cells. Its delivery to the cell surface is preceded by surfactant component synthesis, packaging into specialized organelles termed lamellar bodies, delivery to the apical plasma membrane and fusion. Secreted surfactant undergoes reuptake, intracellular processing, and finally resecretion of recycled material. This review focuses on the mechanisms of delivery of surfactant components to and their secretion from lamellar bodies. Lamellar bodies-independent secretion is also considered. Signal transduction pathways involved in regulation of these processes are discussed as well as disorders associated with their malfunction.

lung surfactant; lamellar bodies; multivesicular bodies

THE LUNG ALVEOLAR SYSTEM is the largest surface of our body that is exposed to the environment, covering up to ~120 m<sup>2</sup> (128). Alveolar surface is formed by two types of epithelial cells, pneumocytes I and pneumocytes II, or alveolar cells types I and II, respectively (Fig. 1). Most of the alveolar surface (~95%) is covered by type I cells. These are flattened cells, which are unable to divide, contain just a few organelles, and serve as a thin barrier between blood and the air. A role of type I cells in cation transport has recently been proposed (80). They may also function as signaling partners of type II cells. More than 600 genes have been found to be differentially expressed in type I and type II cells (60). Another cell type present in alveoli are macrophages, which remove particles and microorganisms coming with the air (128).

A number of functions are known for type II cells (recently reviewed in Ref. 97). They participate in clearance and in repair by proliferating and migrating to damage areas. Type II cells are able to divide and to differentiate into type I cells. They participate in defense responses by expressing various receptors (in particular, Toll-like receptors). Type II cells are involved in the lung cytokine/chemokine network by secreting and responding to an array of cytokines and chemokines (74, 139, 145, 164). They regulate transmigration of monocytes across epithelial layer and possibly participate in T cell activation. However, the main (and the most extensively studied) function of type II cells is secretion of the lung surfactant. Recently, a pure preparation of type II cells was obtained from human embryonic stem cells (151).

Type II cells possess specialized organelles of 0.1–2.4 μm in diameter, termed lamellar bodies (LB) (128). Each type II cell contains 120–180 LBs (170), which consist of tightly packed concentric membrane lamellae with little intralamellar space,

surrounded by the outer (limiting) membrane (128) (see Fig. 2). Whether this is their true in vivo morphology has been a matter of some debate (21, 168). Because of their predominantly lipid composition, structure of LBs is easily modified by conventional preparation methods for transmission electron microscopy, and their ultrastructural characterization represents a considerable challenge. In particular, an “in vivo cryotechnique” has recently revealed that LBs may contain no interspaces and few lamellar structures (168).

Lung surfactant forms a lipid monolayer at the interface of liquid and air and reduces surface tension along the alveolar epithelium. In addition, surfactant may also play a role in protection against oxidants and against infection (128). Lung surfactant consists of glycerophospholipids [~80%, with dipalmitoyl phosphatidylcholine (DPPC) as predominant glycerophospholipid], cholesterol (~10%), and proteins [~10%, with surfactant proteins (SP)-A, SP-B, SP-C, and SP-D as predominant components]. Fatty acid composition of phospholipids may be subject to regulation, for example, by hyperoxic conditions (49), or differ in fetal, neonatal, and adult type II cells (116). Virtually all surfactant phospholipids originate from exocytosed LBs, as evidenced by the similarity of the phospholipid composition of LBs isolated from type II cells and that of whole lung surfactant from bronchoalveolar lavage (83).

This review aims to provide an update on biogenesis and mechanisms that regulate LB secretion. A separate important issue not covered here is regulation of synthesis of surfactant components, in particular during lung maturation, which is known to be regulated by a number of hormones and other factors (123, 137) and can eventually influence the efficiency of surfactant secretion.

### SURFACTANT LIPID SECRETION

A massive secretion of surfactant that occurs at birth with the first breath requires prior massive synthesis of surfactant

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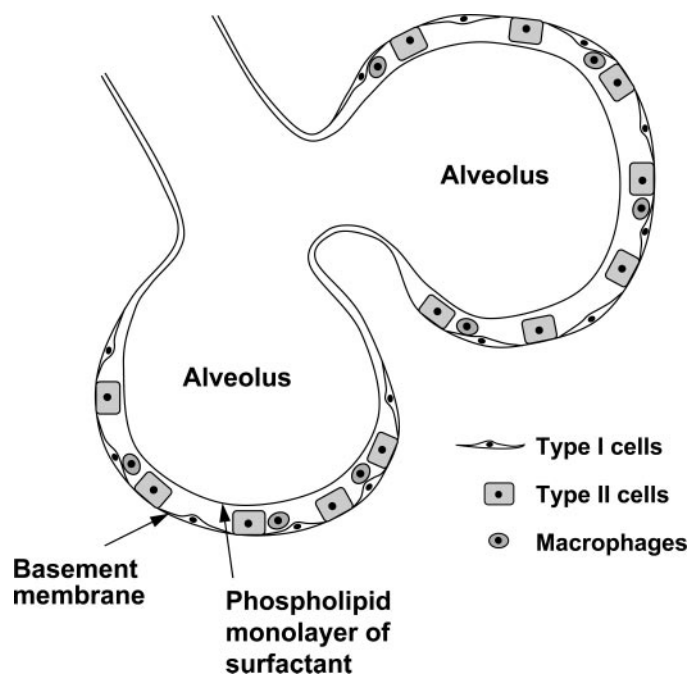


Fig. 1. Cell types present in alveoli. [Adapted from Mulugeta and Beers (97).]

components by type II cells. After birth, considerable proportion of surfactant is continuously recycled, and the demand for de novo synthesis is much lower. Most of the cholesterol of the surfactant is thought to be derived from serum lipoproteins (63), whereas phospholipids are synthesized by type II cells.

Fetal type II cell precursors accumulate considerable amounts of glycogen, which has been suggested to serve as carbon source for lipid synthesis (20, 29). This glycogen pool, however, does not include endoplasmic reticulum (ER) or Golgi apparatus (GA), where lipids are typically synthesized (120). Yet, two key enzymes of phosphatidylcholine (PC) synthesis have been detected in the glycogen pool as well as the presence of phosphorus-rich domains and LBs of varying sizes, including small, probably immature, LBs (120). Conversely, glycogen granules are detectable in some of the LBs (136). These observations strongly suggest that the glycogen pool is the place of synthesis of surfactant phospholipids and LB assembly (120).

It is commonly believed that after birth, surfactant phospholipids are synthesized in the ER (121). Whatever the site of their synthesis, lipid components of the surfactant first have to be transported to the LBs and then translocated across the LB limiting membrane.

#### Trafficking of Lipids from the ER to the LBs

The details of the pathway responsible for lipid delivery to the LBs are not clear. Transport of PC from the ER to the LBs appears to bypass the GA, since disruption of the GA by brefeldin A in cultured type II alveolar cells completely blocked protein secretion, but had no effect on PC transport to the LBs (109). On the other hand, an early work, which employed a pulse chase approach using [ $^3$ H]choline and electron microscopy, suggested GA involvement in PC transport (37). However, these data do not allow determining whether GA-localized PC is then transported to the LBs or elsewhere.

It cannot be excluded that both GA-dependent and GA-independent pathways exist, but the latter is upregulated when GA is disrupted by brefeldin A (109).

The carrier responsible for PC delivery to the LBs has not been identified. A PC transfer protein is expressed in the lung, which is highly specific for PC; however, its function could not be confirmed in vivo, since mice lacking this protein still have normal LBs and normal surfactant composition (143). It has been suggested that although the PC transfer protein may be the physiological PC carrier to the LBs, other nonspecific or novel transfer proteins may compensate for its loss in knockout mice (156). Autophagy has also been suggested as a possible mechanism of phospholipid delivery into LBs (69, 87, 154).

The mechanisms responsible for lipid packaging into LBs appear to be selective toward the length of acyl chains rather than the extent of their saturation, since unsaturated PC forms may constitute significant proportion of the surfactant under some circumstances, in particular in fetal or neonatal type II cells (116).

#### Translocation of Lipids Across LB Limiting Membrane

Lipid translocation across the membrane is facilitated by transporters of the ATP-binding cassette (ABC) family (45), and in particular, the ABCA subfamily (81). Ample evidence indicates that ABCA transporters are involved in the translocation of lipids in the LBs.

Disruption of the gene for ABCA1 transporter in mice was found to lead to multiple morphological defects in the lung and to a respiratory distress with rapid, shallow breathing (11). In particular, the authors observed type II cell hyperplasia and greatly enlarged LBs in some of these cells, yet most LBs were morphologically normal. ABCA1 has been suggested to mediate basolateral, rather than apical, lipid transfer, and to function as a modulator of the overall surfactant lipid pool size (4, 173).

The best documented evidence for a role of an ABC transporter in surfactant secretion concerns the ABCA3 isoform, which is highly expressed in the lung. ABCA3 can be detected exclusively in type II cells, where it is located mostly at the limiting membrane of the LBs (167). ABCA3 mutations result in the lack of mature LBs (50, 132). Similarly, downregulation of ABCA3 by small interfering RNA in differentiated type II cells results in immature and distorted LBs (36). Notably, expression of ABCA3 in HEK-293 cells (i.e., non-lung cells) is able to induce formation of LB-like structures (36, 99).

ABCA3 mutations result in a decreased PC content in the surfactant and in an increased surface tension, as determined by the analysis of bronchoalveolar lavage fluid (56). ABCA3 mutations can also lead to abnormal processing and routing of SP-B and SP-C (23). A recent study suggested that ABCA3 mutations may cause surfactant deficiency by two separate mechanisms: 1) due to abnormal intracellular localization of the transporter in the ER and 2) due to impaired ATP binding and/or ATP hydrolysis in normally localized ABCA3 (95). A number of human cases of respiratory deficiency of different severity due to ABCA3 mutations have been reported (see GENETIC DISORDERS ASSOCIATED WITH SURFACTANT SECRETION).

Subcellular localization of ABCA5 in alveolar type II cells overlaps with that of ABCA3; however, its deletion does not result in detectable abnormalities in the lung (85), suggesting that even if ABCA5 may play a role in LB biogenesis, its

absence can be compensated by other mechanisms (such as ABCA3).

In contrast to ABCA3, deletion of another ABC transporter, ABCG1, which is located either at the plasma membrane or at perinuclear membranes, leads to LB accumulation both in type II cells and in extracellular spaces, and to a severalfold increase in surfactant levels (9). The precise mechanism by which ABCG1 affects surfactant metabolism is unknown.

### SURFACTANT PROTEIN SECRETION

Whereas secretion of phospholipids is mediated solely by LBs, secretion of proteins is more complex. Small hydrophobic polypeptides SP-B and SP-C are localized inside LBs and cosecreted with LB contents. Both of them are probably involved in enrichment of the surfactant in DPPC by squeezing out non-DPPC components during film formation and compression (129). In contrast, two other components of surfactant, large hydrophilic proteins SP-A and SP-D, are secreted in an LB-independent manner (Fig. 2).

### LB-Dependent Secretion

Two of the surfactant proteins, SP-B and SP-C, share a common route towards LBs. LBs contain late endosomal and lysosomal markers (such as lysosomal enzymes acid phosphatase, cathepsins C and H, and membrane proteins LAMP-1, LAMP-2, and CD208) (75, 153). Similar to lysosomes, LBs also have an acidic pH (150). Despite their lysosomal features, LBs are not degradative but secretory organelles. In postnatal type II cells, LBs are thought to form upon redistribution of phospholipid membranes within late endosomes termed multivesicular bodies (MVBs) (156) (Fig. 2B). Intermediate stages of such fusion are termed composite bodies (CBs) (156). LB biogenesis per se is a complex issue, detailed coverage of which is beyond the scope of this review. In addition to SP-B and SP-C, LB biogenesis is affected by multiple factors.

SP-B and SP-C are transported from the ER via GA and trans-Golgi network to the MVBs, then to CBs, and finally to the LBs, and then secreted together with lipid components of the surfactant. SP-B is not only transported along the secretory pathway, but it plays an active role in LB biogenesis: fusion of

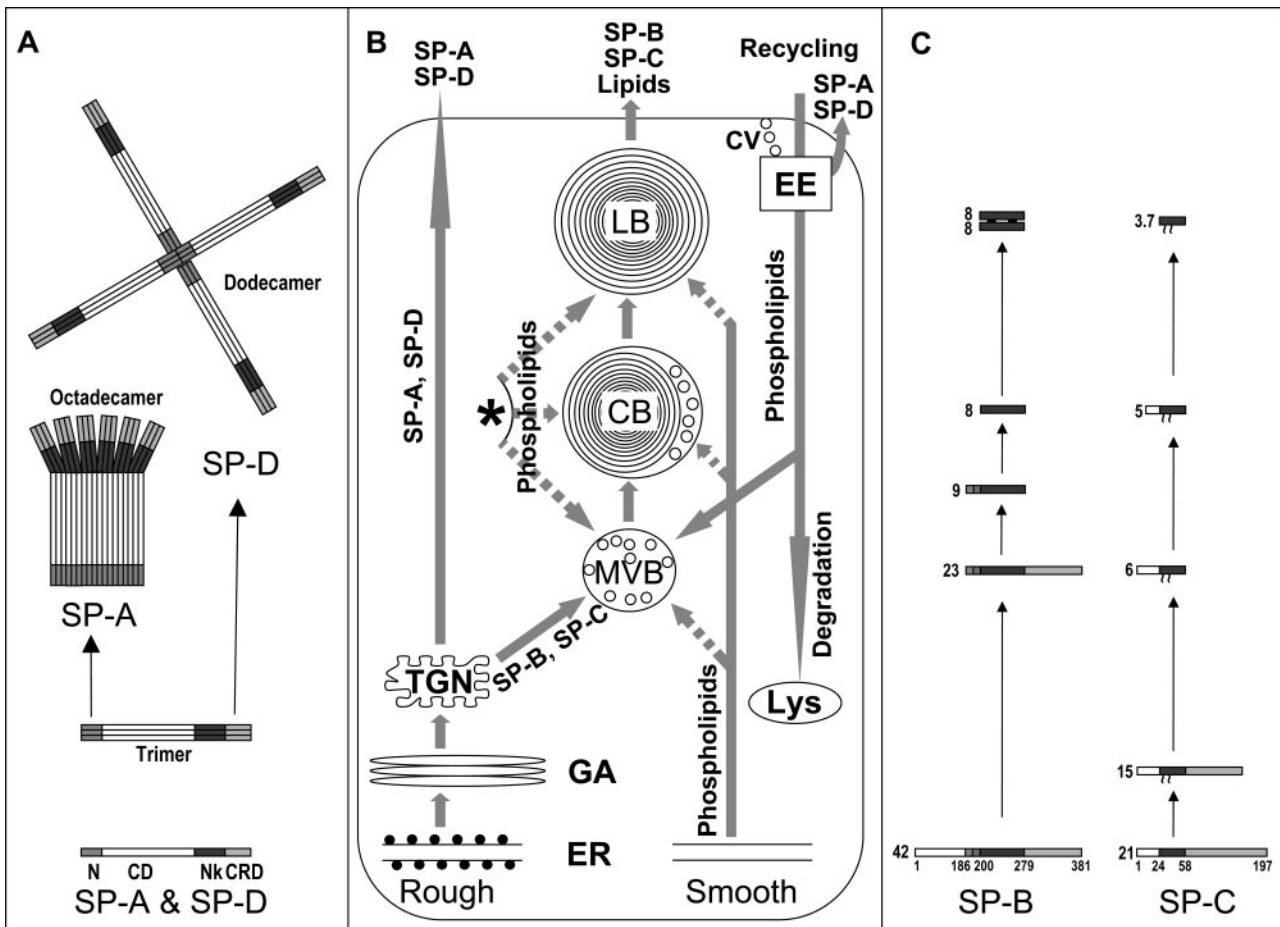


Fig. 2. Processing and secretion of surfactant components. *A*: domain structure and assembly of surfactant protein (SP)-A and SP-D. SP-A/SP-D domain organization: N, NH<sub>2</sub>-terminal domain; CD, collagenous domain; Nk, neck domain; CRD, carbohydrate recognition domain. *B*: lamellar body (LB)-independent (SP-A and SP-D) and LB-dependent (SP-B, SP-C, and phospholipids) secretion. Recycling of surfactant components via clathrin-coated vesicles (CV) is also shown, followed by either degradation or recycling to multivesicular bodies (MVB) and eventually resecretion. ER, endoplasmic reticulum; GA, Golgi apparatus; TGN, trans-Golgi network; EE, early endosomes; Lys, lysosomes; CB, composite bodies. \*Phospholipids synthesized in the cytoplasm, e.g., in the glycogen pool (see main text). *C*: domain structure of SP-B and SP-C preproteins and their processing. Processing products are shown at the level of their presumed locations in the organelles of secretory pathways shown (middle). [Adapted from Brasch et al. (21) and Mulugeta and Beers (97).]

internal vesicles of the MVBs into characteristic lamellar membranes of the LBs is thought to be promoted by SP-B. Indeed, fusogenic properties of SP-B have been demonstrated in vitro (118). In vivo, targeted disruption of SP-B results in the presence of MVBs but not LBs in type II cells (39, 136). Although the contents of these immature LBs (MVBs) are secreted, a functional surfactant film fails to form, eventually leading to death both in mice (136) and in humans (105). A specific role of SP-B in LB biogenesis is also illustrated by a recent finding that PC insufficiency in mice lacking choline cytidyltransferase- $\alpha$  (a rate-limiting enzyme for PC biosynthesis) is accompanied by a strong decrease in the levels of surfactant proteins; an exception is SP-B, which is elevated (140), likely as a compensatory mechanism.

SP-B is synthesized as a preproprotein of 381 amino acids (Fig. 2C). Processing of the SP-B precursor occurs between the GA and the MVBs (21, 97) (Fig. 2C) and requires an aspartic protease napsin A and a cysteine protease cathepsin H (22, 65, 142). Mature SP-B associates with luminal vesicles in MVBs and CBs, promoting their fusion into lamellae of mature LBs (70). As mature SP-B (residues 201 to 279) is hydrophobic, its trafficking is facilitated by the hydrophilic propeptide (residues 24 to 200) (155).

SP-C is a type II transmembrane protein, expressed exclusively by type II cells (155). Unlike SP-B, SP-C is not involved in LB biogenesis, since SP-C null mice have normal LBs (58). However, persistent inflammation and progressive structural alteration of alveoli have been observed in mice and in humans lacking SP-C (103, 160).

SP-C is synthesized as a proprotein of 197 amino acids (Fig. 2C), consisting of a short NH<sub>2</sub>-terminal cytosolic tail containing a motif for targeting to secretory organelles, a transmembrane domain, and a COOH-terminal luminal domain (14, 97, 160). SP-C precursor is included in the invaginations of the outer MVB membrane and relocates into the lumen as intraluminal vesicles are formed. Sorting of SP-C to MVB and its internalization require the cytosolic domain, although the molecular machinery involved in its recognition has not been identified (41). Mature SP-C consists of a part of the cytosolic domain (containing the 2 palmitoylation sites and LPS binding site) and the transmembrane domain; additional hydrophobicity is conferred by palmitoylation at two cysteines (Fig. 2C) (14, 97). Following SP-B-mediated incorporation of luminal vesicles into lamellae, SP-C is cosecreted with surfactant phospholipids.

SP-C facilitates lipid movement between sheets of membrane and vesicles and functions along with SP-B to promote surfactant film formation (14, 97, 160). However, the mechanisms of action of the two proteins may be different, since SP-C is a transmembrane polypeptide, whereas SP-B is located on the membrane surface (97). SP-C also stimulates reuptake of surfactant phospholipids by type II cells and may play a role in surfactant recycling.

#### *LB-Independent Secretion*

SP-A and SP-D belong to the collectin subgroup of the C-type lectin superfamily and have similar domain structure (Fig. 2A). They consist of a short NH<sub>2</sub>-terminal segment, followed by a collagen-like domain, a neck domain, and a carbohydrate recognition domain (43). Both SP-A and SP-D

form homotrimers, which assemble in their turn into higher-order structures different for SP-A and SP-D (Fig. 2A) (86). SP-A forms a bouquet-like octadecamer consisting of six trimers (147). SP-D assembles into a dodecamer, consisting of four trimers. Two Cys residues in the NH<sub>2</sub>-terminal domain are essential for stabilizing this structure (25).

In addition to the primary role of pulmonary collectins in pathogen clearance and inflammatory responses (Refs. 165 and 166 and references therein), SP-A is involved in formation of tubular myelin (a lattice-like structure representing an intermediate between secreted LB contents and the lipid monolayer at the liquid/air interface) (117) and in preservation of surfactant properties (40). SP-A was also reported to inhibit surfactant secretion (48, 119) and to stimulate uptake of phospholipids by type II cells (10, 141). Deletion of SP-D in mice results in accumulation of phospholipids in tissues and in surfactant and in increased numbers of activated macrophages, leading to inflammation and emphysema (158, 169). SP-D was found to affect the physical structure of phospholipid aggregates in surfactant and their uptake and catabolism by type II cells (78). Increased expression of SP-A could not correct the characteristic phenotype of SP-D-deficient mice (171). Expression of SP-D with deleted collagen-like domain in SP-D-deficient mice did correct deficiencies in innate immune responses but failed to correct abnormalities in macrophage activation and surfactant lipid homeostasis (84).

Using radioactive labeling and brefeldin A treatment, SP-A was found to be transported to the GA, glycosylated, and then constitutively secreted and reuptaken into the LB (110, 111). A number of studies concluded that secretion of SP-A and SP-D is constitutive and LB independent (reviewed in Ref. 121) (Fig. 2B). Some authors, however, were able to detect stimulation of SP-A secretion by phorbol ester treatment (47). The presence of SP-A and SP-D in the LBs has also been a matter of some controversy (see Refs. 53, 121, 127 and references therein). This may reflect the presence of some proportion of recycled SP-A/SP-D in the LBs (see below) and possibly also variations between different species (53). The possibility of considerable interspecies differences is illustrated by the findings that rat type II cells contain and secrete considerable amounts of lysozyme, whereas in human lungs, lysozyme could be detected in serous submucosal glands but not in type II cells (134).

#### **RECYCLING**

A considerable proportion (25%-95%) of surfactant is recycled, i.e., it can be reinternalized into the LBs and resecreted again (128). Thus LBs are at an intersection of secretory and endocytic pathways (66, 101, 128).

It has long been recognized that SP-A promotes reuptake of phospholipids and that type II cells expose a high-affinity SP-A receptor on their surface (123). Such a receptor was recently convincingly identified as p63/ERGIC63, a reversibly palmitoylated type II transmembrane protein, initially identified as a resident of the ER-GA intermediate compartment (62). Several earlier studies (see Ref. 62 and references therein) have detected other candidate SP-A receptors. Whether there is more than one receptor for SP-A recycling remains to be demonstrated. Although SP-B, SP-C, and SP-D are also recycled, there is no evidence for specific receptors for these proteins (71, 123).

SP-A and phospholipids are initially transported together to early endosomes (a Rab5 and EEA1-positive compartment) via clathrin-coated vesicles (163) (Fig. 2B). Internalization of SP-A and lipids is fast (<5 min) (163) and depends not only on clathrin but also on actin polymerization (79). Since it is not completely abolished by inhibitors of clathrin and actin, possible involvement of additional, yet unidentified pathway(s) has been suggested (79). Moreover, inhibition of clathrin-mediated endocytosis by phenylarsine oxide has no effect on the reuptake of a 180-kDa LB marker (12) [later identified as ABCA3 (98)], indicating that some LB components may be recycled by a clathrin-independent pathway.

Upon internalization, SP-A is recycled rapidly to the cell surface via Rab4-associated and calmodulin-sensitive pathway (163). Some proportion of SP-A is directed for degradation, rather than for recycling, by a process that requires actin cytoskeleton (79). Internalized lipids are transported to the LBs by a process sensitive to bafilomycin A<sub>1</sub> (an inhibitor of V-ATPase) and requiring calmodulin (163). The mechanisms that determine sorting of SP-A and lipids for recycling to the LBs as opposed to degradation are not understood. It appears that alveolar macrophages (that specifically express lysosomal phospholipase A<sub>2</sub>) rather than type II cells are involved in phospholipid degradation (1, 73).

## REGULATION OF LB SECRETION

### Stimuli *In Vitro* and *In Vivo*

Regulated secretion of surfactant may be initiated by chemical or physical stimuli. The primary physiological stimulus is thought to be direct mechanical stretching of the type II cells that results from breathing (123, 162). One deep breath appears to be sufficient to induce surfactant secretion (102). This effect can also be replicated with cultured type II cells (54, 162). Mechanical stretching of type II cells triggers an increase in cytoplasmic Ca<sup>2+</sup> levels, which is required for the cell response (7, 54). Mechanical contraction is also required to maintain expression of surfactant protein components (64).

Many agonists and second messengers can stimulate secretion in type II cells (121). These stimuli activate one or more of the following three protein kinases: protein kinase A (PKA), protein kinase C (PKC), or Ca<sup>2+</sup>/calmodulin-dependent protein kinase (CaMK) (Fig. 3). Activation of PKC is the most potent way to stimulate surfactant secretion (approximately fivefold), whereas stimuli that activate PKA or CaMK increase secretion by two- to threefold. Simultaneous activation of different pathways results in an up to 12- to 15-fold stimulation of secretion (53). Elevation of intracellular Ca<sup>2+</sup> not only activates CaMK but also acts directly to stimulate secretion by acting via annexins (see below).

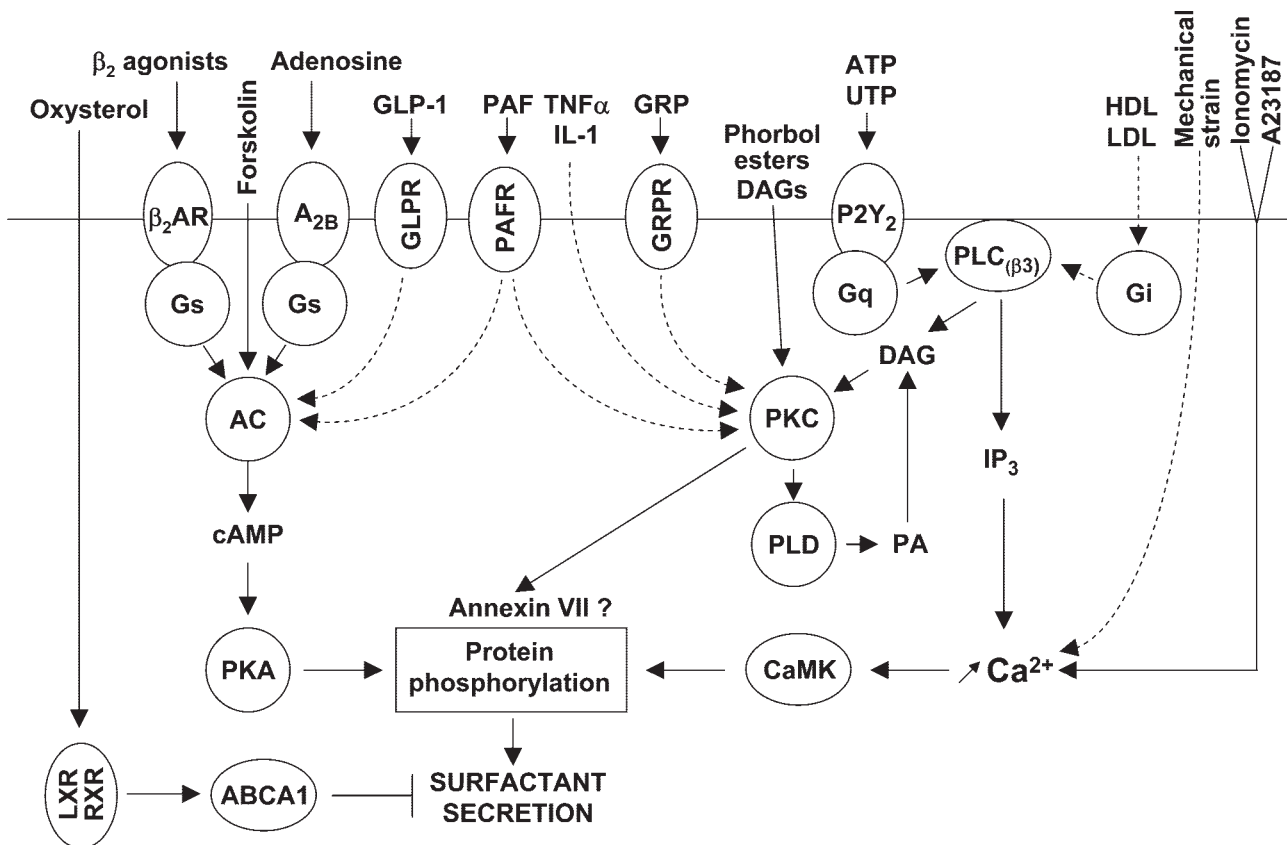


Fig. 3. Signaling pathways involved in regulation of surfactant secretion. The major pathways dependent on the 3 kinases, PKA, PKC, and CaMK, as well as action of several pharmacological stimulators, are shown. [Adapted from Rooney (121).] Additional suggested signaling pathways are shown (see references in text and in Table 1). Dashed lines indicate pathways with unknown intermediates and/or unconfirmed physiological roles. GLP-1, glucagon-like peptide-1; PAF, platelet-activating factor; GRP, gastrin-releasing peptide; DAG, diacylglycerol; LXR, liver X receptor; RXR, retinoic acid receptor.

Labor is known to enhance both synthesis of surfactant lipids and surfactant secretion (122), and adrenergic stimulation of surfactant secretion is probably important during labor and birth (94). Whether it remains physiologically relevant postnatally is less clear. Cholinergic stimulation is well documented in lower vertebrates and in hibernating mammals and is thought to play a role at low body temperatures, whereas type II cells of homeothermic mammals (including humans) are not responsive to cholinergic agonists (108). Yet, cholinergic agonists still may affect surfactant secretion indirectly, via activation of the type II cell  $\beta$ -adrenergic receptors in response to catecholamines released from the adrenal medulla (121).

Although the presence of purinergic receptors in type II cells and the ability of ATP to stimulate surfactant exocytosis are well documented [e.g., (152)], the physiological relevance of purinergic stimulation has long been obscure. A recent study suggests the existence of a paracrine pathway whereby ATP is produced by type I cells in response to mechanical stimulation, and then stimulates surfactant secretion by type II cells, enhancing their response to mechanostimulation (112).

It should be noted that the rate of surfactant secretion appears to be coupled to the rate of its reuptake, as evidenced by enhanced reuptake of ABCA3 (12, 98) by type II cells stimulated by a variety of secretagogues (12, 126).

Conflicting evidence exists concerning the effects of irradiation on the basal and regulated secretion in type II cells, which is of considerable clinical importance due to potential side effects of radiotherapy of thoracic cancer (discussed in Ref. 161).

### Receptors and Downstream Pathways

Signaling pathways triggered by three receptors, which ultimately lead to activation of PKA, PKC, and CaMK and stimulation of secretion (Fig. 3), have been characterized in detail. These are briefly described below. More details, in particular, concerning isoforms of the proteins involved and various agonists that have been used to study these pathways, can be found in recent reviews (46, 121).

**$\beta_2$ -adrenergic receptor.**  $\beta_2$ -adrenergic receptor involvement is suggested by pharmacological evidence (51, 52).  $\beta_2$ -receptor is coupled to a heterotrimeric G protein  $G_s$ , which stimulates adenylyl cyclases II and IV (114), leading to elevation of cAMP levels and activation of PKA. As for  $\beta_1$ -receptor, its involvement is controversial (see Ref. 59).  $\beta_3$ -receptor is undetectable in the lung (121). PKA substrates in this pathway have not been identified. PKA has been suggested to be required for agonist-induced disassembly of filamentous actin (135), which appears to facilitate secretion (see ROLE OF CYTOSKELETON IN LB SECRETION).

**Adenosine  $A_{2B}$  receptor.** Adenosine  $A_{2B}$  receptor involvement (as opposed to  $A_1$ ,  $A_{2A}$ , and  $A_3$  adenosine receptors, all of which are expressed in type II cells) is suggested by pharmacological analysis (121).  $A_{2B}$  receptor, like  $\beta_2$ -receptor, is coupled to  $G_s$ , and initiates a similar signaling pathway resulting in cAMP production and stimulation of PKA. An involvement of another unidentified adenosine receptor, also coupled to cAMP production, has been suggested (121).

**Purinergic  $P2Y_2$  receptor.** Purinergic  $P2Y_2$  receptor involvement (as opposed to  $P2X$  receptor, which is an ion channel and not a G protein-coupled receptor) is suggested by

extensive pharmacological data (see Ref. 121 and references therein).  $P2Y_2$  receptor is coupled to a heterotrimeric G protein  $G_q$ , which stimulates phospholipase C (PLC)- $\beta_3$ . PLC- $\beta_3$  hydrolyzes phosphatidylinositol biphosphate into diacylglycerol (DAG) and inositol trisphosphate ( $IP_3$ ), thus providing bifurcation of the pathway towards PKC activation and elevation of cytoplasmic  $Ca^{2+}$ , respectively (Fig. 3).

DAG directly stimulates PKC, which phosphorylates and activates phospholipase D (PLD), thus initiating a positive feedback loop: PLD hydrolyzes PC and produces phosphatidic acid, which is dephosphorylated by phosphatidate phosphatase and converted into DAG, which further stimulates PKC (Fig. 3). Inhibition of PLD was found to interfere with the late stages of the LB-plasma membrane fusion response in type II cells (55).

$IP_3$  produced by PLC- $\beta_3$  promotes release of free  $Ca^{2+}$  from intracellular stores, which is thought to activate CaMK. The involvement of CaMK II is supported by the observations that its specific inhibitor, KN-62, completely abolishes surfactant secretion induced by a  $Ca^{2+}$  ionophore A-23187 (90, 146). The role of CaMK in transducing signals elicited by  $Ca^{2+}$  elevation as a result of purinergic stimulation or other physiological stimuli (besides lipopolysaccharide, see below) remains to be demonstrated.

Events downstream of PKC are unknown. Notably, direct stimulation of PKC by phorbol esters is inefficient when intracellular  $Ca^{2+}$  is chelated (55). This would mean that either signaling downstream of PKC involves PKC-induced  $Ca^{2+}$  elevation or that increased PKC signaling is permissively dependent on the  $Ca^{2+}$  levels. The first possibility can be excluded, since no increase in free  $Ca^{2+}$  is detectable upon direct activation of PKC by phorbol esters (55, 149). It has therefore been proposed that the role of PKC activation is to sensitize the exocytotic machinery for  $Ca^{2+}$  (46). PKC substrates in this pathway have not been identified. Phorbol ester-induced translocation of PKC to the LBs has been reported (125), suggesting that it might phosphorylate proteins located at or near LBs. One likely candidate may be annexin VII (synexin), which has been implicated in  $Ca^{2+}$ -dependent LB fusion with the plasma membrane (see Ref. 31 and references therein). Binding of annexin VII to the membranes is enhanced upon phorbol ester treatment in rat type II cells (32). At least in chromaffin cells, phosphorylation of annexin VII by PKC has been demonstrated and found to be strongly dependent on  $Ca^{2+}$  levels (28). Moreover, phosphorylation by PKC considerably potentiated the ability of annexin VII to fuse phospholipid vesicles and lowered the half-maximal concentration of  $Ca^{2+}$  required for fusion (28), observations that are reminiscent of the findings by Frick et al. (55) (see above). It would be important to determine whether similar regulation exists in alveolar type II cells.

A possibility of a complex interplay between  $Ca^{2+}$  and G protein-coupled signaling is suggested by observations that GTP $\gamma$ S (nonhydrolyzable GTP analog that permanently activates G proteins) differentially regulates secretion in permeabilized type II cells depending on the  $Ca^{2+}$  levels (113).

In addition to activation of the receptors discussed above, several other naturally occurring agonists have been found to affect surfactant secretion in cultured type II cells (Fig. 3; Table 1), but physiological relevance of this regulation still has to be demonstrated.

Table 1. Natural agonists reported to stimulate secretion in alveolar type II endothelial cells

Agonist	Receptor	Pathway	Reference No.
Adenosine	A <sub>2B</sub>	PKA	Reviewed in 121
ATP	P2Y <sub>2</sub>	PKC	Reviewed in 121
Glucagon-like peptide-1	GLP-1 receptor?	PKA	15, 26, 146
Gastrin-releasing peptide	GRP-R	PKC	8
IL-1	Unknown	PKC	16
LPS	Unknown	Ca <sup>2+</sup> , CaMKII	17, 18
LDL and HDL	Unknown; G <sub>i</sub> -coupled	PKC	115, 148
Oxysterol	LXR/RXR	ABCA1	4
TNF- $\alpha$	Unknown	PKC	16
Platelet-activating factor	Unknown	PKA and PKC	17
UTP	P2Y <sub>2</sub>	PKC	Reviewed in 121

$\beta_2$ -agonists are not listed, see Fig. 3. GLP-1, glucagon-like peptide-1; GRP, gastrin-releasing peptide; LXR, liver X receptor; RXR, retinoic acid receptor.

**Glucagon-like peptide-1.** Glucagon-like peptide-1 (GLP-1) is produced by intestinal L cells as well as in the brain. GLP-1 receptor expression in type II cells has been detected by in situ hybridization (26). In cultured rat (15) and human (146) type II cells, GLP-1 induces cAMP synthesis, activates PKA, and stimulates surfactant secretion.

**Gastrin-releasing peptide.** Gastrin-releasing peptide (GRP), a peptide acting as a growth factor and neuroregulator, was found to stimulate slow surfactant secretion by a PKC-dependent mechanism (8). Since GRP is able to stimulate surfactant phospholipid synthesis (44), it may be difficult to distinguish between its effect on synthesis as opposed to secretion.

**Platelet-activating factor.** Alveolar macrophages (see Fig. 1) can release platelet-activating factor (PAF) following exposure to LPS (34). PAF was found to strongly stimulate surfactant secretion by a mechanism that involves both cAMP and DAG production and activation of PKA and PKC, respectively (17). Which of the known PAF receptors is (are) involved in this effect has not been determined.

**LPS.** LPS can also stimulate surfactant secretion by acting directly on type II cells, although this effect is weaker than that of PAF (17). LPS acts through a different mechanism that is not dependent on PKA and PKC but involves elevation of cytoplasmic Ca<sup>2+</sup> and activation of CaMK II (18).

**TNF- $\alpha$  and IL-1.** TNF- $\alpha$  and IL-1 were reported to stimulate PC secretion via PKC activation in a Ca<sup>2+</sup>-independent manner (16). Notably, TNF- $\alpha$  also decreases PC synthesis, which results in surfactant deficiency and lung injury (30).

**Low- and high-density lipoproteins.** Low- and high-density lipoproteins (LDL and HDL) have been found to stimulate surfactant secretion (115, 148) by a pathway that involves a heterotrimeric G protein G<sub>i</sub>, an increase in phosphoinositide hydrolysis and in cytosolic Ca<sup>2+</sup>, and PKC activation.

Oxysterol, a product of LDL cholesterol oxidation, binds a nuclear steroid receptor [liver X receptor (LXR)], which exists in an obligate complex with 9-*cis* retinoic acid receptor (RXR). The receptor complex binds to LXR/RXR response elements within target genes and modulates their transcription. Oxysterol was found to reduce PC secretion by transcriptionally activating ABCA1 transporter, which promotes basolateral transport of PC (see above), thus reducing its availability for apical surfactant secretion (4). In addition, two mechanisms of inhibition of PC biosynthesis by oxysterols have been described. One involves cleavage of choline cytidyltransferase by calpain (172), and the other one relies on inhibition of its activity by ERK-dependent phosphorylation (5).

G<sub>i</sub> activation and increase in cytosolic Ca<sup>2+</sup> may also be involved in downstream signaling from purinergic P2Y<sub>14</sub> receptor activated by UDP-glucose, which stimulates IL-8 secretion in A549 cells (which are thought to resemble type II cells) (96). The same group has recently implicated several serotonin receptors in secretion of a number of cytokines in A549 cells as well as type II cells (13). It would be interesting to test whether these pathways may stimulate surfactant secretion as well.

### Ca<sup>2+</sup> Signaling

Ca<sup>2+</sup>-induced fusion is a general feature of regulated exocytosis (27), and LB fusion with the plasma membrane in alveolar type II cells is no exception. Ample evidence indicates the importance of Ca<sup>2+</sup> as a second messenger in LB secretion. A study using intact alveoli suggested that the Ca<sup>2+</sup> signal originates in type I cells and then propagates to type II cells via gap junctions (7). The authors found that synchronous Ca<sup>2+</sup> oscillations were induced by lung expansion in all alveolar cells and that the rate of exocytosis correlated with the frequency of the oscillations. Furthermore, a recent work has revealed that an interalveolar Ca<sup>2+</sup> signaling mediated by gap junctions exists in rat lungs (77). The relative role of type I and type II cells as primary mechanosensors remains controversial, as yet another group has demonstrated that cultured type II cells are able to respond to stretching without any contacts with type I cells (54). Interestingly, cell-cell contacts are still indispensable in this case, since lone cells did not exhibit Ca<sup>2+</sup> signals (54). The authors also concluded that strain of type II cells initially stimulates Ca<sup>2+</sup> entry, which is a prerequisite for Ca<sup>2+</sup> release from intracellular stores. The sites of Ca<sup>2+</sup> storage inside type II cells and possible routes of Ca<sup>2+</sup> entry/release have been covered in detail in a recent review (46) and will not be discussed here.

Fusion machinery in type II cells is unusually sensitive to Ca<sup>2+</sup> concentration, with 90% saturation being achieved at 320 nM Ca<sup>2+</sup> (67). Elevation of free Ca<sup>2+</sup> serves as a trigger for fusion, but the presence of high Ca<sup>2+</sup> concentrations per se does not appear to be necessary, since LB fusion still continues after the Ca<sup>2+</sup> levels have returned to the resting state (see discussion in Ref. 46). Free Ca<sup>2+</sup> regulates not only fusion, but also the expansion of fusion pores, the size of which appears to be a limiting factor for the release of the LB contents by diffusion (68).

What the  $\text{Ca}^{2+}$  targets are in this process is far from clear. Early works provided evidence for the involvement of calmodulin (61, 149), which is consistent with high affinity of the hypothetical  $\text{Ca}^{2+}$  sensor and also with the involvement of CaMK (see above). Calmodulin was indeed detected on LB surface (72). Synaptotagmin, which functions as a  $\text{Ca}^{2+}$  sensor in a number of vesicle fusion systems (6), has not been detected in type II cells.

Other  $\text{Ca}^{2+}$  sensors likely involved in surfactant secretion are annexins (33, 92). In addition to annexin VII discussed above, annexin II tetramer (but not monomer or annexins I, III, IV, V, and VI) was found to drive LB fusion with the plasma membrane (35). Importantly, arachidonic acid reduces  $\text{Ca}^{2+}$  levels required for annexin II action to physiologically relevant values (35). Inhibition of cytosolic phospholipase  $\text{A}_2$  or DAG lipase reduces PC secretion by type II cells stimulated by a variety of secretagogues (91), indicating that formation of arachidonic acid indeed plays a physiological role in LB secretion. Annexin II was found to translocate from cytoplasm to the plasma membrane upon stimulation with a phorbol ester (89), consistent with its function downstream of PKC. It was also reported to destabilize cortical actin (135), thus facilitating secretion (see also ROLE OF CYTOSKELETON IN LB SECRETION). Sensitivity of annexin VII to  $\text{Ca}^{2+}$  may also be modulated by arachidonic acid (130) as well as by its PKC-dependent phosphorylation, as discussed above.

Components of canonical vesicle fusion machinery have been identified in type II cells. These include SNARE proteins synaptobrevin-2 (VAMP-2), syntaxin-1, SNAP-23 and SNAP-25 (2, 3, 174), NSF and  $\alpha$ -SNAP, proteins that function to disassemble SNARE complexes (3), and rab3D and cysteine string protein, two putative regulators of SNARE-mediated exocytosis, both of which are associated with LBs (144). The cellular levels of  $\alpha$ -SNAP appear to be rate limiting for fusion (3). Interestingly, rab3D is present on only ~25% of the LBs, indicating their probable functional heterogeneity (144). Since rab3D-positive LBs tended to be located closer to the apical plasma membrane, the authors suggested that these represent a subpopulation of "mature" LBs ready for secretion. Lipid rafts have been suggested to serve as platforms for LB secretion (38). Annexin II colocalizes with syntaxin-2 and SNAP-23 [which are required for surfactant secretion (2)] at lipid rafts.

#### ROLE OF CYTOSKELETON IN LB SECRETION

Two opposite roles of cytoskeletal elements have been traditionally envisaged in respect to vesicle secretion: a "prosecretory" role that consists of promoting secretory vesicle movement toward plasma membrane, and "antiselectory" action whereby cytoskeletal elements (in particular cortex actin) form a physical barrier that hampers vesicle movement.

In the case of LBs, an active microtubule-based mechanism that delivers them to the plasma membrane was suggested by early observations that the microtubule-disrupting agent colchicine inhibits surfactant secretion induced by stretch and by agonists (24, 42). An antiselectory role of F-actin in type II cells is indicated by observations that botulinum C-2 toxin-induced decay of F-actin leads to an increase in surfactant secretion (124) and that the actin microfilament stabilizer jasplakinolide inhibits secretion stimulated by a variety of agonists (135). Moreover, transient decrease in F-actin content

following type II cell stimulation by agonists that induce surfactant secretion was reported (19, 124, 135). In contrast, tubulin polymerization was reported under similar conditions with a similar time course (24). These observations suggest that coordinated changes in cytoskeleton may occur under physiological conditions during LB secretion.

Another line of evidence that cytoskeleton remodeling is involved in LB secretion comes from observations that calpain is activated during regulated exocytosis in type II cells, and this activation is required for secretion (88, 176). One of the substrates cleaved by calpain is spectrin (175), a protein that links cytoskeletal elements to the membrane.

Early works suggested that LBs, including those in the process of exocytosis, are surrounded by actin-like material (140a). These findings have recently been confirmed using F-actin staining with fluorescent phalloidin (144). Yet, only ~10% of the LBs had an actin coat in the latter study. The majority of these were Rab3D-negative (as mentioned above, Rab3D is found on ~25% of LBs), yet a very small proportion contained both Rab3D and actin, leading the authors to speculate that these may represent a transition state between rab3D-labeled LBs and actin-coated LBs. Notably, a myosin ATPase inhibitor reportedly causes a dramatic increase in the number of rab3D-positive LBs, whereas a combination of this inhibitor and latrunculin A (an F-actin-disrupting agent) results in an increase in LBs that are positive for both actin and rab3D (144). These findings indicate that Rab3D is probably a regulatory protein that links LBs with the actomyosin system. Actin was also suggested to play a regulatory role in controlling the diameter of the fusion pore (133).

#### GENETIC DISORDERS ASSOCIATED WITH SURFACTANT SECRETION

Since surfactant is indispensable for lung function, failures in its secretion or abnormalities in its composition lead to clinical manifestations of various severities [generally termed respiratory distress syndrome or RDS (128)]. Therefore, identification of mutations underlying these deficiencies should help to develop therapies for lung disorders. On the other hand, identification of mutations underlying lung disorders in humans as well as in animal models sheds new light on biological functions of respective proteins.

Disorders associated with LB biogenesis and/or secretion are linked to either 1) failure to produce LBs or 2) formation of giant LBs due to surfactant overproduction because of insufficiency of its secretion. In addition, lung disorders may be associated with deviations in surfactant composition that do not lead to morphological abnormalities in the LBs [e.g., SP-C deficiency (106)].

##### *Failure To Produce LBs*

Lung disorders known to be due to the failure of LB production originate from deficiencies either in SP-B (39, 50, 105, 107) or in ABCA3 (50, 132). These have been covered in recent reviews (104, 159) and will not be considered here.

##### *Giant LBs*

Giant LBs have been described in patients with Hermansky-Pudlak syndrome (HPS) (100) and in mouse models of both HPS and Chediak-Higashi syndrome (CHS) (57, 93, 138).

These conditions are associated with abnormal biogenesis (HPS) or trafficking (CHS) of lysosomal-related organelles (76).

Eight genes in humans and 15 genes in mice have been linked to HPS. Most of them encode subunits of protein complexes termed BLOCs (biogenesis of lysosome-related organelles complexes). Other genes encode  $\beta_3A$  and  $\delta$ -subunits of the AP-3 adaptor complex,  $\alpha$ -subunit of the Rab geranylgeranyl transferase, a cystine/glutamate exchanger xCT, dysbindin (a protein possibly involved in exocytosis of glutamate in neurons), and pallidin (possible partner of syntaxin-13) (157). A number of combinations of deficiencies in subunits of BLOCs and/or the AP-3 adaptor complex have been described that result in production of giant LBs (57, 66, 93). A controversy exists as to whether a single gene mutation may cause similar phenotype. Whereas Guttentag et al. (66) and Gautam et al. (57) only observed giant LBs in double or triple mutants, Tang et al. (138) described a similar phenotype in mice carrying a single mutation, namely HPS1, a subunit of one of the BLOC complexes. A possible reason for the discrepancy may be the age of mice examined, since the latter study (138) followed phenotypic development in mice aged up to 2 years, whereas the former studies used younger mice.

Human and mouse genes (*CHS1* and *Beige*, respectively) have been cloned, which have mutations that account for CHS and a CHS-like phenotype in mice (131). They encode large (~3,800 amino acids) proteins containing a number of recognizable motifs: ARM motifs (known to mediate membrane association), perilipin domain (mediating association with neutral lipids), WD-40 repeats (which mediate protein-protein interactions), HEAT repeats (thought to play a role in vesicle transport), and BEACH domain of unknown function. The precise role of the CHS1/Beige protein is unknown. Although human CHS patients do not show abnormalities in the LBs, giant LBs have been observed in *Beige* mice (138). The apparent phenotypic discrepancy might be due to the fact that human CHS patients usually die in childhood, whereas *Beige* mice were monitored almost up to the end of their natural lifespan (138).

## SUMMARY

Although several signaling pathways that regulate surfactant secretion have been extensively characterized, a number of aspects remain poorly understood. Although the involvement of the three kinases (PKA, PKC, and CaMK) in regulated surfactant secretion is firmly established, their physiological substrates still need to be identified. Understanding of the interplay between stretch-induced  $Ca^{2+}$  signaling and the pathways leading to kinase activation would considerably improve our understanding of the events leading to surfactant secretion in type II cells. Clarification of the role and regulation of cytoskeleton in LB fusion to the plasma membrane and in recycling of surfactant material, and identification of the molecular mechanisms that couple the rate of recycling to that of secretion, would also be required to build a comprehensive picture of how secretion and recycling of surfactant are regulated.

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## REFERENCES

1. Abe A, Hiraoka M, Wild S, Wilcoxon SE, Paine R 3rd, Shayman JA. Lysosomal phospholipase A<sub>2</sub> is selectively expressed in alveolar macrophages. *J Biol Chem* 279: 42605–42611, 2004.
2. Abonyo BO, Gou D, Wang P, Narasaraaju T, Wang Z, Liu L. Syntaxin 2 and SNAP-23 are required for regulated surfactant secretion. *Biochemistry* 43: 3499–3506, 2004.
3. Abonyo BO, Wang P, Narasaraaju TA, Rowan WH 3rd, McMillan DH, Zimmerman UJ, Liu L. Characterization of alpha-soluble N-ethylmaleimide-sensitive fusion attachment protein in alveolar type II cells: implications in lung surfactant secretion. *Am J Respir Cell Mol Biol* 29: 273–282, 2003.
4. Agassandian M, Mathur SN, Zhou J, Field FJ, Mallampalli RK. Oxysterols trigger ABCA1-mediated basolateral surfactant efflux. *Am J Respir Cell Mol Biol* 31: 227–233, 2004.
5. Agassandian M, Zhou J, Tephly LA, Ryan AJ, Carter AB, Mallampalli RK. Oxysterols inhibit phosphatidylcholine synthesis via ERK docking and phosphorylation of CTP:phosphocholine cytidylyltransferase. *J Biol Chem* 280: 21577–21587, 2005.
6. Andrews NW, Chakrabarti S. There's more to life than neurotransmission: the regulation of exocytosis by synaptotagmin. VII. *Trends Cell Biol* 15: 626–631, 2005.
7. Ashino Y, Ying X, Dobbs LG, Bhattacharya J.  $[Ca^{2+}]_i$  oscillations regulate type II cell exocytosis in the pulmonary alveolus. *Am J Physiol Lung Cell Mol Physiol* 279: L5–L13, 2000.
8. Asokanathan N, Cake MH. Stimulation of surfactant lipid secretion from fetal type II pneumocytes by gastrin-releasing peptide. *Am J Physiol Lung Cell Mol Physiol* 270: L331–L337, 1996.
9. Baldan A, Tarr P, Vales CS, Frank J, Shimotake TK, Hawgood S, Edwards PA. Deletion of the transmembrane transporter ABCG1 results in progressive pulmonary lipidosis. *J Biol Chem* 281: 29401–29410, 2006.
10. Bates SR, Dodia C, Fisher AB. Surfactant protein A regulates uptake of pulmonary surfactant by lung type II cells on microporous membranes. *Am J Physiol Lung Cell Mol Physiol* 267: L753–L760, 1994.
11. Bates SR, Tao JQ, Collins HL, Franccone OL, Rothblat GH. Pulmonary abnormalities due to ABCA1 deficiency in mice. *Am J Physiol Lung Cell Mol Physiol* 289: L980–L989, 2005.
12. Bates SR, Tao JQ, Schaller S, Fisher AB, Shuman H. Lamellar body membrane turnover is stimulated by secretagogues. *Am J Physiol Lung Cell Mol Physiol* 278: L443–L452, 2000.
13. Bayer H, Muller T, Myrtek D, Sorichter S, Ziegenhagen M, Norgauer J, Zissel G, Idzko M. Serotonergic receptors on human airway epithelial cells. *Am J Respir Cell Mol Biol* 36: 85–93, 2007.
14. Beers MF, Mulugeta S. Surfactant protein C biosynthesis and its emerging role in conformational lung disease. *Annu Rev Physiol* 67: 663–696, 2005.
15. Benito E, Blazquez E, Bosch MA. Glucagon-like peptide-1-(7-36) amide increases pulmonary surfactant secretion through a cyclic adenosine 3',5'-monophosphate-dependent protein kinase mechanism in rat type II pneumocytes. *Endocrinology* 139: 2363–2368, 1998.
16. Benito E, Bosch MA. The inflammatory cytokines tumor necrosis factor alpha and interleukin-1beta stimulate phosphatidylcholine secretion in primary cultures of rat type II pneumocytes. *Mol Cell Biochem* 189: 169–176, 1998.
17. Benito E, Bosch MA. Role of platelet-activating factor in phosphatidylcholine secretion in primary cultures of rat type II pneumocytes. *Prostaglandins Other Lipid Mediat* 67: 197–208, 2002.
18. Benito E, Portoles MT, Bosch MA. Involvement of calcium in the stimulation of phosphatidylcholine secretion in primary cultures of rat type II pneumocytes by *Escherichia coli* lipopolysaccharide. *Mol Cell Biochem* 205: 39–44, 2000.
19. Bhandari V, Lu H, Pachter J, Kresch MJ. Actin depolymerization is developmentally regulated in rat type II cells exposed to terbutaline. *Pediatr Res* 41: 166–171, 1997.
20. Bourbon JR, Rieutort M, Engle MJ, Farrell PM. Utilization of glycogen for phospholipid synthesis in fetal rat lung. *Biochim Biophys Acta* 712: 382–389, 1982.
21. Brasch F, Johnen G, Winn-Brasch A, Guttentag SH, Schmiedl A, Kapp N, Suzuki Y, Muller KM, Richter J, Hawgood S, Ochs M.

- Surfactant protein B in type II pneumocytes and intra-alveolar surfactant forms of human lungs. *Am J Respir Cell Mol Biol* 30: 449–458, 2004.
22. **Brasch F, Ochs M, Kahne T, Guttentag S, Schauer-Vukasinovic V, Derrick M, Johnen G, Kapp N, Muller KM, Richter J, Giller T, Hawgood S, Buhling F.** Involvement of napsin A in the C- and N-terminal processing of surfactant protein B in type-II pneumocytes of the human lung. *J Biol Chem* 278: 49006–49014, 2003.
  23. **Brasch F, Schimanski S, Muhlfeld C, Barlage S, Langmann T, Aslanidis C, Boettcher A, Dada A, Schrotten H, Mildenerger E, Prueter E, Ballmann M, Ochs M, Johnen G, Griese M, Schmitz G.** Alteration of the pulmonary surfactant system in full-term infants with hereditary ABCA3 deficiency. *Am J Respir Crit Care Med* 174: 571–580, 2006.
  24. **Brown LA, Pasquale SM, Longmore WJ.** Role of microtubules in surfactant secretion. *J Appl Physiol* 58: 1866–1873, 1985.
  25. **Brown-Augsburger P, Hartshorn K, Chang D, Rust K, Fliszar C, Welgus HG, Crouch EC.** Site-directed mutagenesis of Cys-15 and Cys-20 of pulmonary surfactant protein D. Expression of a trimeric protein with altered anti-viral properties. *J Biol Chem* 271: 13724–13730, 1996.
  26. **Bullock BP, Heller RS, Habener JF.** Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. *Endocrinology* 137: 2968–2978, 1996.
  27. **Burgoyne RD, Morgan A.** Secretory granule exocytosis. *Physiol Rev* 83: 581–632, 2003.
  28. **Caohuy H, Pollard HB.** Activation of annexin 7 by protein kinase C in vitro and in vivo. *J Biol Chem* 276: 12813–12821, 2001.
  29. **Carlson KS, Davies P, Smith BT, Post M.** Temporal linkage of glycogen and saturated phosphatidylcholine in fetal lung type II cells. *Pediatr Res* 22: 79–82, 1987.
  30. **Carroll JL Jr, McCoy DM, McGowan SE, Salome RG, Ryan AJ, Mallampalli RK.** Pulmonary-specific expression of tumor necrosis factor- $\alpha$  alters surfactant lipid metabolism. *Am J Physiol Lung Cell Mol Physiol* 282: L735–L742, 2002.
  31. **Chander A, Naidu DG, Chen XL.** A ten-residue domain (Y11–A20) in the NH<sub>2</sub>-terminus modulates membrane association of annexin A7. *Biochim Biophys Acta* 1761: 775–784, 2006.
  32. **Chander A, Sen N, Naidu DG, Spitzer AR.** Calcium ionophore and phorbol ester increase membrane binding of annexin a7 in alveolar type II cells. *Cell Calcium* 33: 11–17, 2003.
  33. **Chander A, Wu RD.** In vitro fusion of lung lamellar bodies and plasma membrane is augmented by lung synexin. *Biochim Biophys Acta* 1086: 157–166, 1991.
  34. **Chang SW, Feddersen CO, Henson PM, Voelkel NF.** Platelet-activating factor mediates hemodynamic changes and lung injury in endotoxin-treated rats. *J Clin Invest* 79: 1498–1509, 1987.
  35. **Chattoopadhyay S, Sun P, Wang P, Abonyo B, Cross NL, Liu L.** Fusion of lamellar body with plasma membrane is driven by the dual action of annexin II tetramer and arachidonic acid. *J Biol Chem* 278: 39675–39683, 2003.
  36. **Cheong N, Madesh M, Gonzales LW, Zhao M, Yu K, Ballard PL, Shuman H.** Functional and trafficking defects in ATP binding cassette A3 mutants associated with respiratory distress syndrome. *J Biol Chem* 281: 9791–9800, 2006.
  37. **Chevalier G, Collet AJ.** In vivo incorporation of choline-3 H, leucine-3 H and galactose-3 H in alveolar type II pneumocytes in relation to surfactant synthesis. A quantitative radioautographic study in mouse by electron microscopy. *Anat Rec* 174: 289–310, 1972.
  38. **Chintagari NR, Jin N, Wang P, Narasaraaju TA, Chen J, Liu L.** Effect of cholesterol depletion on exocytosis of alveolar type II cells. *Am J Respir Cell Mol Biol* 34: 677–687, 2006.
  39. **Clark JC, Wert SE, Bachurski CJ, Stahlman MT, Stripp BR, Weaver TE, Whitsett JA.** Targeted disruption of the surfactant protein B gene disrupts surfactant homeostasis, causing respiratory failure in newborn mice. *Proc Natl Acad Sci USA* 92: 7794–7798, 1995.
  40. **Cockshutt AM, Weitz J, Possmayer F.** Pulmonary surfactant-associated protein A enhances the surface activity of lipid extract surfactant and reverses inhibition by blood proteins in vitro. *Biochemistry* 29: 8424–8429, 1990.
  41. **Conkright JJ, Bridges JP, Na CL, Voorhout WF, Trapnell B, Glasser SW, Weaver TE.** Secretion of surfactant protein C, an integral membrane protein, requires the N-terminal propeptide. *J Biol Chem* 276: 14658–14664, 2001.
  42. **Corbet AJ, Voelker RM, Murphy FM, Owens ML.** Inhibition by colchicine of phospholipid secretion induced by lung distension. *J Appl Physiol* 65: 1710–1715, 1988.
  43. **Crouch E, Wright JR.** Surfactant proteins A and D and pulmonary host defense. *Annu Rev Physiol* 63: 521–554, 2001.
  44. **Cullen A, Emanuel RL, Torday JS, Asokanathan N, Sikorski KA, Sunday ME.** Bombesin-like peptide and receptors in lung injury models: diverse gene expression, similar function. *Peptides* 21: 1627–1638, 2000.
  45. **Dean M, Hamon Y, Chimini G.** The human ATP-binding cassette (ABC) transporter superfamily. *J Lipid Res* 42: 1007–1017, 2001.
  46. **Dietl P, Haller T.** Exocytosis of lung surfactant: from the secretory vesicle to the air-liquid interface. *Annu Rev Physiol* 67: 595–621, 2005.
  47. **Dobbs LG, Mason RJ, Williams MC, Benson BJ, Sueishi K.** Secretion of surfactant by primary cultures of alveolar type II cells isolated from rats. *Biochim Biophys Acta* 713: 118–127, 1982.
  48. **Dobbs LG, Wright JR, Hawgood S, Gonzalez R, Venstrom K, Nellenbogen J.** Pulmonary surfactant and its components inhibit secretion of phosphatidylcholine from cultured rat alveolar type II cells. *Proc Natl Acad Sci USA* 84: 1010–1014, 1987.
  49. **Dombrowsky H, Tschernig T, Vieten G, Rau GA, Ohler F, Acevedo C, Behrens C, Poets CF, von der Hardt H, Bernhard W.** Molecular and functional changes of pulmonary surfactant in response to hyperoxia. *Pediatr Pulmonol* 41: 1025–1039, 2006.
  50. **Edwards V, Cutz E, Viero S, Moore AM, Noguee L.** Ultrastructure of lamellar bodies in congenital surfactant deficiency. *Ultrastruct Pathol* 29: 503–509, 2005.
  51. **Ewing CK, Duffy DM, Roberts JM.** Characterization of the beta-adrenergic receptor in isolated human fetal lung type II cells. *Pediatr Res* 32: 350–355, 1992.
  52. **Fabisiak JP, Vesell ES, Rannels DE.** Interactions of beta adrenergic antagonists with isolated rat alveolar type II pneumocytes. I. Analysis, characterization and regulation of specific beta adrenergic receptors. *J Pharmacol Exp Ther* 241: 722–727, 1987.
  53. **Fehrenbach H.** Alveolar epithelial type II cell: defender of the alveolus revisited. *Respir Res* 2: 33–46, 2001.
  54. **Frick M, Bertocchi C, Jennings P, Haller T, Mair N, Singer W, Pfaller W, Ritsch-Marte M, Dietl P.** Ca<sup>2+</sup> entry is essential for cell strain-induced lamellar body fusion in isolated rat type II pneumocytes. *Am J Physiol Lung Cell Mol Physiol* 286: L210–L220, 2004.
  55. **Frick M, Eschertzhuber S, Haller T, Mair N, Dietl P.** Secretion in alveolar type II cells at the interface of constitutive and regulated exocytosis. *Am J Respir Cell Mol Biol* 25: 306–315, 2001.
  56. **Garmany TH, Moxley MA, White FV, Dean M, Hull WM, Whitsett JA, Noguee LM, Hamvas A.** Surfactant composition and function in patients with ABCA3 mutations. *Pediatr Res* 59: 801–805, 2006.
  57. **Gautam R, Novak EK, Tan J, Wakamatsu K, Ito S, Swank RT.** Interaction of Hermansky-Pudlak Syndrome genes in the regulation of lysosome-related organelles. *Traffic* 7: 779–792, 2006.
  58. **Glasser SW, Burhans MS, Korfhagen TR, Na CL, Sly PD, Ross GF, Ikegami M, Whitsett JA.** Altered stability of pulmonary surfactant in SP-C-deficient mice. *Proc Natl Acad Sci USA* 98: 6366–6371, 2001.
  59. **Gobran LI, Rooney SA.** Pulmonary surfactant secretion in briefly cultured mouse type II cells. *Am J Physiol Lung Cell Mol Physiol* 286: L331–L336, 2004.
  60. **Gonzalez R, Yang YH, Griffin C, Allen L, Tigue Z, Dobbs L.** Freshly isolated rat alveolar type I cells, type II cells, and cultured type II cells have distinct molecular phenotypes. *Am J Physiol Lung Cell Mol Physiol* 288: L179–L189, 2005.
  61. **Griese M, Gobran LI, Rooney SA.** Signal-transduction mechanisms of ATP-stimulated phosphatidylcholine secretion in rat type II pneumocytes: interactions between ATP and other surfactant secretagogues. *Biochim Biophys Acta* 1167: 85–93, 1993.
  62. **Gupta N, Manevich Y, Kazi AS, Tao JQ, Fisher AB, Bates SR.** Identification and characterization of p63 (CKAP4/ERGIC-63/CLIMP-63), a surfactant protein A binding protein, on type II pneumocytes. *Am J Physiol Lung Cell Mol Physiol* 291: L436–L446, 2006.
  63. **Guthmann F, Harrach-Ruprecht B, Looman AC, Stevens PA, Robenek H, Rustow B.** Interaction of lipoproteins with type II pneumocytes in vitro: morphological studies, uptake kinetics and secretion rate of cholesterol. *Eur J Cell Biol* 74: 197–207, 1997.
  64. **Gutierrez JA, Suzara VV, Dobbs LG.** Continuous mechanical contraction modulates expression of alveolar epithelial cell phenotype. *Am J Respir Cell Mol Biol* 29: 81–87, 2003.

65. Guttentag S, Robinson L, Zhang P, Brasch F, Buhling F, Beers M. Cysteine protease activity is required for surfactant protein B processing and lamellar body genesis. *Am J Respir Cell Mol Biol* 28: 69–79, 2003.
66. Guttentag SH, Akhtar A, Tao JQ, Atochina E, Rusiniak ME, Swank RT, Bates SR. Defective surfactant secretion in a mouse model of Hermansky-Pudlak syndrome. *Am J Respir Cell Mol Biol* 33: 14–21, 2005.
67. Haller T, Auktor K, Frick M, Mair N, Dieltl P. Threshold calcium levels for lamellar body exocytosis in type II pneumocytes. *Am J Physiol Lung Cell Mol Physiol* 277: L893–L900, 1999.
68. Haller T, Dieltl P, Pfaller K, Frick M, Mair N, Paulmichl M, Hess MW, Furst J, Maly K. Fusion pore expansion is a slow, discontinuous, and Ca<sup>2+</sup>-dependent process regulating secretion from alveolar type II cells. *J Cell Biol* 155: 279–289, 2001.
69. Hariri M, Millane G, Guimond MP, Guay G, Dennis JW, Nabi IR. Biogenesis of multilamellar bodies via autophagy. *Mol Biol Cell* 11: 255–268, 2000.
70. Hawgood S, Derrick M, Poulain F. Structure and properties of surfactant protein B. *Biochim Biophys Acta* 1408: 150–160, 1998.
71. Herbein JF, Savov J, Wright JR. Binding and uptake of surfactant protein D by freshly isolated rat alveolar type II cells. *Am J Physiol Lung Cell Mol Physiol* 278: L830–L839, 2000.
72. Hill DJ, Wright TC Jr, Andrews ML, Karnovsky MJ. Localization of calmodulin in differentiating pulmonary type II epithelial cells. *Lab Invest* 51: 297–306, 1984.
73. Hiraoka M, Abe A, Lu Y, Yang K, Han X, Gross RW, Shayman JA. Lysosomal phospholipase A<sub>2</sub> and phospholipidosis. *Mol Cell Biol* 26: 6139–6148, 2006.
74. Homer RJ, Zheng T, Chupp G, He S, Zhu Z, Chen Q, Ma B, Hite RD, Gobran LI, Rooney SA, Elias JA. Pulmonary type II cell hypertrophy and pulmonary lipoproteinosis are features of chronic IL-13 exposure. *Am J Physiol Lung Cell Mol Physiol* 283: L52–L59, 2002.
75. Hook GE, Gilmore LB. Hydrolases of pulmonary lysosomes and lamellar bodies. *J Biol Chem* 257: 9211–9220, 1982.
76. Huizing M, Anikster Y, Gahl WA. Hermansky-Pudlak syndrome and Chediak-Higashi syndrome: disorders of vesicle formation and trafficking. *Thromb Haemost* 86: 233–245, 2001.
77. Ichimura H, Parthasarathi K, Lindert J, Bhattacharya J. Lung surfactant secretion by interalveolar Ca<sup>2+</sup> signaling. *Am J Physiol Lung Cell Mol Physiol* 291: L596–L601, 2006.
78. Ikegami M, Na CL, Korfhagen TR, Whitsett JA. Surfactant protein D influences surfactant ultrastructure and uptake by alveolar type II cells. *Am J Physiol Lung Cell Mol Physiol* 288: L552–L561, 2005.
79. Jain D, Dodia C, Fisher AB, Bates SR. Pathways for clearance of surfactant protein A from the lung. *Am J Physiol Lung Cell Mol Physiol* 289: L1011–L1018, 2005.
80. Johnson MD, Bao HF, Helms MN, Chen XJ, Tigue Z, Jain L, Dobbs LG, Eaton DC. Functional ion channels in pulmonary alveolar type I cells support a role for type I cells in lung ion transport. *Proc Natl Acad Sci USA* 103: 4964–4969, 2006.
81. Kaminski WE, Piehler A, Wenzel JJ. ABC A-subfamily transporters: structure, function and disease. *Biochim Biophys Acta* 1762: 510–524, 2006.
82. Kapanci Y, Costabella PM, Cerutti P, Assimakopoulos A. Distribution and function of cytoskeletal proteins in lung cells with particular reference to 'contractile interstitial cells.' *Methods Achiev Exp Pathol* 9: 147–168, 1979.
83. Kikkawa Y, Smith F. Cellular and biochemical aspects of pulmonary surfactant in health and disease. *Lab Invest* 49: 122–139, 1983.
84. Kingma PS, Zhang L, Ikegami M, Hartshorn K, McCormack FX, Whitsett JA. Correction of pulmonary abnormalities in Sftpd<sup>-/-</sup> mice requires the collagenous domain of surfactant protein D. *J Biol Chem* 281: 24496–24505, 2006.
85. Kubo Y, Sekiya S, Ohigashi M, Takenaka C, Tamura K, Nada S, Nishi T, Yamamoto A, Yamaguchi A. ABCA5 resides in lysosomes, and ABCA5 knockout mice develop lysosomal disease-like symptoms. *Mol Cell Biol* 25: 4138–4149, 2005.
86. Kuroki Y, Voelker DR. Pulmonary surfactant proteins. *J Biol Chem* 269: 25943–25946, 1994.
87. Lajoie P, Guay G, Dennis JW, Nabi IR. The lipid composition of autophagic vacuoles regulates expression of multilamellar bodies. *J Cell Sci* 118: 1991–2003, 2005.
88. Li HL, Feinstein SI, Liu L, Zimmerman UJ. An antisense oligodeoxynucleotide to m-calpain mRNA inhibits secretion from alveolar epithelial type II cells. *Cell Signal* 10: 137–142, 1998.
89. Liu L. Calcium-dependent self-association of annexin II: a possible implication in exocytosis. *Cell Signal* 11: 317–324, 1999.
90. Liu L. Inhibition of lung surfactant secretion by KN-62, a specific inhibitor of the calcium- and calmodulin-dependent protein kinase II. *Biochem Mol Biol Int* 45: 823–830, 1998.
91. Liu L. Regulation of lung surfactant secretion by phospholipase A<sub>2</sub>. *J Cell Biochem* 72: 103–110, 1999.
92. Liu L, Wang M, Fisher AB, Zimmerman UJ. Involvement of annexin II in exocytosis of lamellar bodies from alveolar epithelial type II cells. *Am J Physiol Lung Cell Mol Physiol* 270: L668–L676, 1996.
93. Lyerla TA, Rusiniak ME, Borchers M, Jahreis G, Tan J, Ohtake P, Novak EK, Swank RT. Aberrant lung structure, composition, and function in a murine model of Hermansky-Pudlak syndrome. *Am J Physiol Lung Cell Mol Physiol* 285: L643–L653, 2003.
94. Marino PA, Rooney SA. The effect of labor on surfactant secretion in newborn rabbit lung slices. *Biochim Biophys Acta* 664: 389–396, 1981.
95. Matsumura Y, Ban N, Ueda K, Inagaki N. Characterization and classification of ATP-binding cassette transporter ABCA3 mutants in fatal surfactant deficiency. *J Biol Chem* 281: 34503–34514, 2006.
96. Muller T, Bayer H, Myrtek D, Ferrari D, Sorichter S, Ziegenhagen MW, Zissel G, Virchow JC Jr, Luttmann W, Norgauer J, Di Virgilio F, Idzko M. The P2Y<sub>14</sub> receptor of airway epithelial cells: coupling to intracellular Ca<sup>2+</sup> and IL-8 secretion. *Am J Respir Cell Mol Biol* 33: 601–609, 2005.
97. Mulugeta S, Beers MF. Surfactant protein C: its unique properties and emerging immunomodulatory role in the lung. *Microbes Infect* 8: 2317–2323, 2006.
98. Mulugeta S, Gray JM, Notarfrancesco KL, Gonzales LW, Koval M, Feinstein SI, Ballard PL, Fisher AB, Shuman H. Identification of LBM180, a lamellar body limiting membrane protein of alveolar type II cells, as the ABC transporter protein ABCA3. *J Biol Chem* 277: 22147–22155, 2002.
99. Nagata K, Yamamoto A, Ban N, Tanaka AR, Matsuo M, Kioka N, Inagaki N, Ueda K. Human ABCA3, a product of a responsible gene for abca3 for fatal surfactant deficiency in newborns, exhibits unique ATP hydrolysis activity and generates intracellular multilamellar vesicles. *Biochem Biophys Res Commun* 324: 262–268, 2004.
100. Nakatani Y, Nakamura N, Sano J, Inayama Y, Kawano N, Yamanaka S, Miyagi Y, Nagashima Y, Ohbayashi C, Mizushima M, Manabe T, Kuroda M, Yokoi T, Matsubara O. Interstitial pneumonia in Hermansky-Pudlak syndrome: significance of florid foamy swelling/degeneration (giant lamellar body degeneration) of type-2 pneumocytes. *Virchows Arch* 437: 304–313, 2000.
101. Nicholas TE. Pulmonary surfactant: no mere paint on the alveolar wall. *Respirology* 1: 247–257, 1996.
102. Nicholas TE, Power JH, Barr HA. The pulmonary consequences of a deep breath. *Respir Physiol* 49: 315–324, 1982.
103. Noguee LM. Abnormal expression of surfactant protein C and lung disease. *Am J Respir Cell Mol Biol* 26: 641–644, 2002.
104. Noguee LM. Genetic mechanisms of surfactant deficiency. *Biol Neonate* 85: 314–318, 2004.
105. Noguee LM, de Mello DE, Dehner LP, Colten HR. Brief report: deficiency of pulmonary surfactant protein B in congenital alveolar proteinosis. *N Engl J Med* 328: 406–410, 1993.
106. Noguee LM, Dunbar AE 3rd, Wert SE, Askin F, Hamvas A, Whitsett JA. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med* 344: 573–579, 2001.
107. Noguee LM, Wert SE, Proffitt SA, Hull WM, Whitsett JA. Allelic heterogeneity in hereditary surfactant protein B (SP-B) deficiency. *Am J Respir Crit Care Med* 161: 973–981, 2000.
108. Ormond CJ, Orgeig S, Daniels CB, Milsom WK. Thermal acclimation of surfactant secretion and its regulation by adrenergic and cholinergic agonists in type II cells isolated from warm-active and torpid golden-mantled ground squirrels, *Spermophilus lateralis*. *J Exp Biol* 206: 3031–3041, 2003.
109. Osanai K, Mason RJ, Voelker DR. Pulmonary surfactant phosphatidylcholine transport bypasses the brefeldin A sensitive compartment of alveolar type II cells. *Biochim Biophys Acta* 1531: 222–229, 2001.
110. Osanai K, Mason RJ, Voelker DR. Trafficking of newly synthesized surfactant protein A in isolated rat alveolar type II cells. *Am J Respir Cell Mol Biol* 19: 929–935, 1998.

111. **Osanai K, Tsuchihara C, Hatta R, Oikawa T, Tsuchihara K, Iguchi M, Seki T, Takahashi M, Huang J, Toga H.** Pulmonary surfactant transport in alveolar type II cells. *Respirology* 11 Suppl: S70–S73, 2006.
112. **Patel AS, Reigada D, Mitchell CH, Bates SR, Margulies SS, Koval M.** Paracrine stimulation of surfactant secretion by extracellular ATP in response to mechanical deformation. *Am J Physiol Lung Cell Mol Physiol* 289: L489–L496, 2005.
113. **Pian MS, Dobbs LG.** Activation of G proteins may inhibit or stimulate surfactant secretion in rat alveolar type II cells. *Am J Physiol Lung Cell Mol Physiol* 266: L375–L381, 1994.
114. **Pian MS, Dobbs LG.** Evidence for G beta gamma-mediated cross-talk in primary cultures of lung alveolar cells. Pertussis toxin-sensitive production of cAMP. *J Biol Chem* 270: 7427–7430, 1995.
115. **Pian MS, Dobbs LG.** Lipoprotein-stimulated surfactant secretion in alveolar type II cells: mediation by heterotrimeric G proteins. *Am J Physiol Lung Cell Mol Physiol* 273: L634–L639, 1997.
116. **Postle AD, Gonzales LW, Bernhard W, Clark GT, Godinez MH, Godinez RI, Ballard PL.** Lipidomics of cellular and secreted phospholipids from differentiated human fetal type II alveolar epithelial cells. *J Lipid Res* 47: 1322–1331, 2006.
117. **Poulain FR, Allen L, Williams MC, Hamilton RL, Hawgood S.** Effects of surfactant apolipoproteins on liposome structure: implications for tubular myelin formation. *Am J Physiol Lung Cell Mol Physiol* 262: L730–L739, 1992.
118. **Poulain FR, Nir S, Hawgood S.** Kinetics of phospholipid membrane fusion induced by surfactant apoproteins A and B. *Biochim Biophys Acta* 1278: 169–175, 1996.
119. **Rice WR, Ross GF, Singleton FM, Dingle S, Whitsett JA.** Surfactant-associated protein inhibits phospholipid secretion from type II cells. *J Appl Physiol* 63: 692–698, 1987.
120. **Ridsdale R, Post M.** Surfactant lipid synthesis and lamellar body formation in glycogen-laden type II cells. *Am J Physiol Lung Cell Mol Physiol* 287: L743–L751, 2004.
121. **Rooney SA.** Regulation of surfactant secretion. *Comp Biochem Physiol A Mol Integr Physiol* 129: 233–243, 2001.
122. **Rooney SA, Gobran LI, Wai-Lee TS.** Stimulation of surfactant production by oxytocin-induced labor in the rabbit. *J Clin Invest* 60: 754–759, 1977.
123. **Rooney SA, Young SL, Mendelson CR.** Molecular and cellular processing of lung surfactant. *FASEB J* 8: 957–967, 1994.
124. **Rose F, Kurth-Landwehr C, Sibelius U, Reuner KH, Aktories K, Seeger W, Grimminger F.** Role of actin depolymerization in the surfactant secretory response of alveolar epithelial type II cells. *Am J Respir Crit Care Med* 159: 206–212, 1999.
125. **Samuels ER, Harrower T, Kahlon S, Oulton MR, MacDonald J, Siau C, Scott JE.** Calcium-PS-dependent protein kinase C and surfactant protein A in isolated fetal rabbit type II alveolar cells and surfactant-related material. *Pediatr Pulmonol* 27: 95–103, 1999.
126. **Schaller-Bals S, Bates SR, Notarfrancesco K, Tao JQ, Fisher AB, Shuman H.** Surface-expressed lamellar body membrane is recycled to lamellar bodies. *Am J Physiol Lung Cell Mol Physiol* 279: L631–L640, 2000.
127. **Schmiedl A, Ochs M, Muhlfeld C, Johnen G, Brasch F.** Distribution of surfactant proteins in type II pneumocytes of newborn, 14-day old, and adult rats: an immunoelectron microscopic and stereological study. *Histochem Cell Biol* 124: 465–476, 2005.
128. **Schmitz G, Muller G.** Structure and function of lamellar bodies, lipid-protein complexes involved in storage and secretion of cellular lipids. *J Lipid Res* 32: 1539–1570, 1991.
129. **Schurch S, Green FH, Bachofen H.** Formation and structure of surface films: captive bubble surfactometry. *Biochim Biophys Acta* 1408: 180–202, 1998.
130. **Sen N, Spitzer AR, Chander A.** Calcium-dependence of synexin binding may determine aggregation and fusion of lamellar bodies. *Biochem J* 322: 103–109, 1997.
131. **Shiflett SL, Kaplan J, Ward DM.** Chediak-Higashi Syndrome: a rare disorder of lysosomes and lysosome related organelles. *Pigment Cell Res* 15: 251–257, 2002.
132. **Shulenin S, Noguee LM, Annilo T, Wert SE, Whitsett JA, Dean M.** ABCA3 gene mutations in newborns with fatal surfactant deficiency. *N Engl J Med* 350: 1296–1303, 2004.
133. **Singer W, Frick M, Haller T, Bernet S, Ritsch-Marte M, Dietl P.** Mechanical forces impeding exocytotic surfactant release revealed by optical tweezers. *Biophys J* 84: 1344–1351, 2003.
134. **Singh G, Katyal SL, Brown WE, Collins DL, Mason RJ.** Pulmonary lysozyme—a secretory protein of type II pneumocytes in the rat. *Am Rev Respir Dis* 138: 1261–1267, 1988.
135. **Singh TK, Abonyo B, Narasaraaju TA, Liu L.** Reorganization of cytoskeleton during surfactant secretion in lung type II cells: a role of annexin II. *Cell Signal* 16: 63–70, 2004.
136. **Stahlman MT, Gray MP, Falconieri MW, Whitsett JA, Weaver TE.** Lamellar body formation in normal and surfactant protein B-deficient fetal mice. *Lab Invest* 80: 395–403, 2000.
137. **Sullivan LC, Orgeig S, Daniels CB.** The role of extrinsic and intrinsic factors in the evolution of the control of pulmonary surfactant maturation during development in the amniotes. *Physiol Biochem Zool* 76: 281–295, 2003.
138. **Tang X, Yamanaka S, Miyagi Y, Nagashima Y, Nakatani Y.** Lung pathology of pale ear mouse (model of Hermansky-Pudlak syndrome 1) and beige mouse (model of Chediak-Higashi syndrome): severity of giant lamellar body degeneration of type II pneumocytes correlates with interstitial inflammation. *Pathol Int* 55: 137–143, 2005.
139. **Thorley AJ, Ford PA, Giembycz MA, Goldstraw P, Young A, Tetley TD.** Differential regulation of cytokine release and leukocyte migration by lipopolysaccharide-stimulated primary human lung alveolar type II epithelial cells and macrophages. *J Immunol* 178: 463–473, 2007.
140. **Tian Y, Zhou R, Rehg JE, Jackowski S.** Role of phosphocholine cytidylyltransferase alpha in lung development. *Mol Cell Biol* 7: 975–982, 2007.
- 140a. **Tsilibary EC, Williams MC.** Actin in peripheral rat lungs: S1 labeling and structural changes induced by cytochalasin. *J Histochem Cytochem* 31: 1289–1297, 1983.
141. **Tsuzuki A, Kuroki Y, Akino T.** Pulmonary surfactant protein A-mediated uptake of phosphatidylcholine by alveolar type II cells. *Am J Physiol Lung Cell Mol Physiol* 265: L193–L199, 1993.
142. **Ueno T, Linder S, Na CL, Rice WR, Johansson J, Weaver TE.** Processing of pulmonary surfactant protein B by napsin and cathepsin H. *J Biol Chem* 279: 16178–16184, 2004.
143. **van Helvoort A, de Brouwer A, Ottenhoff R, Brouwers JF, Wijnholds J, Beijnen JH, Rijneveld A, van der Poll T, van der Valk MA, Majoor D, Voorhout W, Wirtz KW, Elferink RP, Borst P.** Mice without phosphatidylcholine transfer protein have no defects in the secretion of phosphatidylcholine into bile or into lung airspaces. *Proc Natl Acad Sci USA* 96: 11501–11506, 1999.
144. **van Weeren L, de Graaff AM, Jamieson JD, Batenburg JJ, Valentijn JA.** Rab3D and actin reveal distinct lamellar body subpopulations in alveolar epithelial type II cells. *Am J Respir Cell Mol Biol* 30: 288–295, 2004.
145. **Vanderbilt JN, Mager EM, Allen L, Sawa T, Wiener-Kronish J, Gonzalez R, Dobbs LG.** CXC chemokines and their receptors are expressed in type II cells and upregulated following lung injury. *Am J Respir Cell Mol Biol* 29: 661–668, 2003.
146. **Vara E, Arias-Diaz J, Garcia C, Balibrea JL, Blazquez E.** Glucagon-like peptide-1(7-36) amide stimulates surfactant secretion in human type II pneumocytes. *Am J Respir Crit Care Med* 163: 840–846, 2001.
147. **Voss T, Eistetter H, Schafer KP, Engel J.** Macromolecular organization of natural and recombinant lung surfactant protein SP 28-36. Structural homology with the complement factor C1q. *J Mol Biol* 201: 219–227, 1988.
148. **Voyno-Yasenetskaya TA, Dobbs LG, Erickson SK, Hamilton RL.** Low density lipoprotein- and high density lipoprotein-mediated signal transduction and exocytosis in alveolar type II cells. *Proc Natl Acad Sci USA* 90: 4256–4260, 1993.
149. **Voyno-Yasenetskaya TA, Dobbs LG, Williams MC.** Regulation of ATP-dependent surfactant secretion and activation of second-messenger systems in alveolar type II cells. *Am J Physiol* 261: 105–109, 1991.
150. **Wadsworth SJ, Spitzer AR, Chander A.** Ionic regulation of proton chemical (pH) and electrical gradients in lung lamellar bodies. *Am J Physiol Lung Cell Mol Physiol* 273: L427–L436, 1997.
151. **Wang D, Haviland DL, Burns AR, Zsigmond E, Wetsel RA.** A pure population of lung alveolar epithelial type II cells derived from human embryonic stem cells. *Proc Natl Acad Sci USA* 104: 4449–4454, 2007.
152. **Warburton D, Parton L, Buckley S, Cosico L.** Verapamil: a novel probe of surfactant secretion from rat type II pneumocytes. *J Appl Physiol* 66: 1304–1308, 1989.
153. **Wasano K, Hirakawa Y.** Lamellar bodies of rat alveolar type 2 cells have late endosomal marker proteins on their limiting membranes. *Histochemistry* 102: 329–335, 1994.

154. **Wattiez R, Hermans C, Bernard A, Lesur O, Falmagne P.** Human bronchoalveolar lavage fluid: two-dimensional gel electrophoresis, amino acid microsequencing and identification of major proteins. *Electrophoresis* 20: 1634–1645, 1999.
155. **Weaver TE, Conkright JJ.** Function of surfactant proteins B and C. *Annu Rev Physiol* 63: 555–578, 2001.
156. **Weaver TE, Na CL, Stahlman M.** Biogenesis of lamellar bodies, lysosome-related organelles involved in storage and secretion of pulmonary surfactant. *Semin Cell Dev Biol* 13: 263–270, 2002.
157. **Wei ML.** Hermansky-Pudlak syndrome: a disease of protein trafficking and organelle function. *Pigment Cell Res* 19: 19–42, 2006.
158. **Wert SE, Yoshida M, LeVine AM, Ikegami M, Jones T, Ross GF, Fisher JH, Korfhagen TR, Whitsett JA.** Increased metalloproteinase activity, oxidant production, and emphysema in surfactant protein D gene-inactivated mice. *Proc Natl Acad Sci USA* 97: 5972–5977, 2000.
159. **Whitsett JA.** Genetic disorders of surfactant homeostasis. *Paediatr Respir Rev* 7, Suppl 1: S240–S242, 2006.
160. **Whitsett JA, Weaver TE.** Hydrophobic surfactant proteins in lung function and disease. *N Engl J Med* 347: 2141–2148, 2002.
161. **Willner J, Vordermark D, Schmidt M, Gassel A, Flentje M, Wirtz H.** Secretory activity and cell cycle alteration of alveolar type II cells in the early and late phase after irradiation. *Int J Radiat Oncol Biol Phys* 55: 617–625, 2003.
162. **Wirtz HR, Dobbs LG.** Calcium mobilization and exocytosis after one mechanical stretch of lung epithelial cells. *Science* 250: 1266–1269, 1990.
163. **Wissel H, Lehfeldt A, Klein P, Muller T, Stevens PA.** Endocytosed SP-A and surfactant lipids are sorted to different organelles in rat type II pneumocytes. *Am J Physiol Lung Cell Mol Physiol* 281: L345–L360, 2001.
164. **Witherden IR, Vanden Bon EJ, Goldstraw P, Ratcliffe C, Pastorino U, Tetley TD.** Primary human alveolar type II epithelial cell chemokine release: effects of cigarette smoke and neutrophil elastase. *Am J Respir Cell Mol Biol* 30: 500–509, 2004.
165. **Wright JR.** Immunoregulatory functions of surfactant proteins. *Nat Rev Immunol* 5: 58–68, 2005.
166. **Yamada C, Sano H, Shimizu T, Mitsuzawa H, Nishitani C, Himi T, Kuroki Y.** Surfactant protein A directly interacts with TLR4 and MD-2 and regulates inflammatory cellular response. Importance of supratrimetric oligomerization. *J Biol Chem* 281: 21771–21780, 2006.
167. **Yamano G, Funahashi H, Kawanami O, Zhao LX, Ban N, Uchida Y, Morohoshi T, Ogawa J, Shioda S, Inagaki N.** ABCA3 is a lamellar body membrane protein in human lung alveolar type II cells. *FEBS Lett* 508: 221–225, 2001.
168. **Yang C, Terada N, Ohno N, Fujii Y, Ohno S.** Morphological analysis of lamellar structures in mouse type II pneumocytes by quick-freezing and freeze-drying with osmium tetroxide vapor-fixation. *Med Mol Morphol* 39: 88–96, 2006.
169. **Yoshida M, Korfhagen TR, Whitsett JA.** Surfactant protein D regulates NF-kappa B and matrix metalloproteinase production in alveolar macrophages via oxidant-sensitive pathways. *J Immunol* 166: 7514–7519, 2001.
170. **Young SL, Fram EK, Spain CL, Larson EW.** Development of type II pneumocytes in rat lung. *Am J Physiol Lung Cell Mol Physiol* 260: L113–L122, 1991.
171. **Zhang L, Ikegami M, Korfhagen TR, McCormack FX, Yoshida M, Senior RM, Shipley JM, Shapiro SD, Whitsett JA.** Neither SP-A nor NH<sub>2</sub>-terminal domains of SP-A can substitute for SP-D in regulation of alveolar homeostasis. *Am J Physiol Lung Cell Mol Physiol* 291: L181–L190, 2006.
172. **Zhou J, Ryan AJ, Medh J, Mallampalli RK.** Oxidized lipoproteins inhibit surfactant phosphatidylcholine synthesis via calpain-mediated cleavage of CTP:phosphocholine cytidylyltransferase. *J Biol Chem* 278: 37032–37040, 2003.
173. **Zhou J, You Y, Ryan AJ, Mallampalli RK.** Upregulation of surfactant synthesis triggers ABCA1-mediated basolateral phospholipid efflux. *J Lipid Res* 45: 1758–1767, 2004.
174. **Zimmerman UJ, Malek SK, Liu L, Li HL.** Proteolysis of synaptobrevin, syntaxin, and SNAP-25 in alveolar epithelial type II cells. *IUBMB Life* 48: 453–458, 1999.
175. **Zimmerman UJ, Speicher DW, Fisher AB.** Secretagogue-induced proteolysis of lung spectrin in alveolar epithelial type II cells. *Biochim Biophys Acta* 1137: 127–134, 1992.
176. **Zimmerman UJ, Wang M, Liu L.** Inhibition of secretion from isolated rat alveolar epithelial type II cells by the cell permeant calpain inhibitor II (*N*-acetyl-leucyl-leucyl-methioninal). *Cell Calcium* 18: 1–8, 1995.