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
employed a pulse chase approach using [3H]choline and elec-
the LBs (109). On the other hand, an early work, which
blocked protein secretion, but had no effect on PC transport to
brefeldin A in cultured type II alveolar cells completely
the LBs are not clear. Transport of PC from the ER to the LBs
be transported to the LBs and then translocated across the LB
their synthesis, lipid components of the surfactant first have to
lipids are synthesized in the ER (121). Whatever the site of
pool is the place of synthesis of surfactant phospholipids and
(136). These observations strongly suggest that the glycogen
versely, glycogen granules are detectable in some of the LBs
sizes, including small, probably immature, LBs (120). Con-
the presence of phosphorus-rich domains and LBs of varying
synthesis have been detected in the glycogen pool as well as
(120). Yet, two key enzymes of phosphatidylcholine (PC)
Golgi apparatus (GA), where lipids are typically synthesized
however, does not include endoplasmic reticulum (ER) or
carbon source for lipid synthesis (20, 29). This glycogen pool,
amounts of glycogen, which has been suggested to serve as
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
components by type II cells. After birth, considerable propor-
tion of surfactant is continuously recycled, and the demand for
de novo synthesis is much lower. Most of the cholesterol of the
surfactant is disrupted be 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 translo-
cation 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 trans-
porter in surfactant secretion concerns the ABCA3 isof orm,
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

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**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, NH₂-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).]**

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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 cytidylyltransferase-α (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 preprotein of 197 amino acids (Fig. 2C), consisting of a short NH2-terminal cysteolic 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 cystolic domain, although the molecular machinery involved in its recognition has not been identified (41). Mature SP-C consists of a part of the cystolic 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 superfamiy and have similar domain structure (Fig. 2A). They consist of a short NH2-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 NH2-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 reuptake 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 A1 (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 A2) 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 culturated type II cells (54, 162). Mechanical stretching of type II cells triggers an increase in cytoplasmic Ca\(^{2+}\) 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\(^{2+}\)/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\(^{2+}\) not only activates CaMK but also acts directly to stimulate secretion by acting via annexins (see below).

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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 β-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).

**β2-adrenergic receptor.** β2-adrenergic receptor involvement is suggested by pharmacological evidence (51, 52). β2-receptor is coupled to a heterotrimeric G protein Gs, which stimulates adenylate cyclases II and IV (114), leading to elevation of cAMP levels and activation of PKA. As for β1-receptor, its involvement is controversial (see Ref. 59). β3-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 A2B receptor.** Adenosine A2B receptor involvement (as opposed to A1, A2A, and A3 adenosine receptors, all of which are expressed in type II cells) is suggested by pharmacological analysis (121). A2B receptor, like β2-receptor, is coupled to Gs, 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 P2Y2 receptor.** Purinergic P2Y2 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). P2Y2 receptor is coupled to a heterotrimeric G protein Gi, which stimulates phospholipase C (PLC-β2). PLC-β2 hydrolyzes phosphatidylinositol bisphosphate into diacylglycerol (DAG) and inositol trisphosphate (IP3), thus providing bifurcation of the pathway towards PKC activation and elevation of cytoplasmic Ca2+, 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).

IP3 produced by PLC-β3 promotes release of free Ca2+ 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 Ca2+ ionophore A-23187 (90, 146). The role of CaMK in transducing signals elicited by Ca2+ 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 Ca2+ is chelated (55). This would mean that either signaling downstream of PKC involves PKC-induced Ca2+ elevation or that increased PKC signaling is permissively dependent on the Ca2+ levels. The first possibility can be excluded, since no increase in free Ca2+ 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 Ca2+ (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 Ca2+-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 Ca2+ levels (28). Moreover, phosphorylation by PKC considerably potentiated the ability of annexin VII to fuse phospholipid vesicles and lowered the half-maximal concentration of Ca2+ 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 Ca2+ and G protein-coupled signaling is suggested by observations that GTPyS (nonhydrolyzable GTP analog that permanently activates G proteins) differentially regulates secretion in permeabilized type II cells depending on the Ca2+ 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

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Receptor</th>
<th>Pathway</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>A&lt;sub&gt;3&lt;/sub&gt;</td>
<td>PKA</td>
<td>Reviewed in 121</td>
</tr>
<tr>
<td>ATP</td>
<td>P2Y&lt;sub&gt;2&lt;/sub&gt;</td>
<td>PKA</td>
<td>15, 26, 146</td>
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<td>Glucagon-like peptide-1</td>
<td>GLP-1 receptor?</td>
<td>PKC</td>
<td>8</td>
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<td>GRP-R</td>
<td>PKC</td>
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<td>PKC</td>
<td>115, 148</td>
</tr>
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<td>4</td>
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<td>17</td>
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<td>P2Y&lt;sub&gt;2&lt;/sub&gt;</td>
<td>PKC</td>
<td>Reviewed in 121</td>
</tr>
</tbody>
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β<sub>2</sub>-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-α and IL-1. TNF-α and IL-1 were reported to stimulate PC secretion via PKC activation in a Ca<sup>2+</sup>-independent manner (16). Notably, TNF-α 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 cytidylyltransferase by calpain (172), and the other one relies on inhibition of its activity by ERK-dependent phosphorylation (5).

GLP-1 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 synchronousCa<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 dispensable 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 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 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 Ca\(^{2+}\) sensor in a number of vesicle fusion systems (6), has not been detected in type II cells.

Other 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 Ca\(^{2+}\) levels required for annexin II action to physiologically relevant values (35). Inhibition of cytosolic phospholipase 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 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 \(\sim 25\%\) 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 “antisecretory” 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 antisecretory 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 \(\sim 10\%\) 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 \(\sim 25\%\) 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 SURFACANT 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 J) 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 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 β1A and δ-subunits of the AP-3 adaptor complex, α-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 phenotype 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 trafficking), and a 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 Ca2+ 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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GRANTS

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