Biochimica et Biophysica Acta 1831 (2013) 448–458

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbalip

Review

Regulation of lung surfactant phospholipid synthesis and metabolism

Victoria Goss [a], Alan N. Hunt [a,b], Anthony D. Postle [a,b,*]

[a] Southampton Centre for Biomedical Research, NIHR Respiratory Biomedical Research Unit, University Hospitals Southampton, UK
[b] Clinical & Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton UK

ARTICLE INFO

Article history:
Received 21 June 2012
Received in revised form 24 October 2012
Accepted 14 November 2012
Available online 27 November 2012

Keywords:
Pulmonary surfactant
Phospholipid synthesis
Stable isotopes
Mass spectrometry

ABSTRACT

The alveolar type II epithelial (ATII) cell is highly specialised for the synthesis and storage, in intracellular lamellar bodies, of phospholipid destined for secretion as pulmonary surfactant into the alveolus. Regulation of the enzymology of surfactant phospholipid synthesis and metabolism has been extensively characterised at both molecular and functional levels, but understanding of surfactant phospholipid metabolism *in vivo* in either healthy or, especially, diseased lungs is still relatively poorly understood. This review will integrate recent advances in the enzymology of surfactant phospholipid metabolism with metabolic studies *in vivo* in both experimental animals and human subjects. It will highlight developments in the application of stable isotope-labelled precursor substrates and mass spectrometry to probe lung phospholipid metabolism in terms of individual molecular lipid species and identify areas where a more comprehensive metabolic model would have considerable potential for direct application to disease states.

1. Introduction

Lung surfactant is a lipid:protein complex comprising approximately 90% lipid, of which phosphatidylcholine (PC) is the principal component, with four specific surfactant proteins (SP-A, SP-B, SP-C, and SP-D, [135]). Although most surfactant proteins are expressed in a variety of cells (*e.g.* Clara cells of the bronchial epithelium), synthesis and secretion of the functional surfactant complex is uniquely confined to the type II cell (ATII) of the alveolar epithelium. As net exporters of lipids, ATII cells require sensitive homeostatic regulatory mechanisms to both prevent excessive accumulation of stored lipid and simultaneously provide sufficient, readily-mobilised supplies to meet normal alveolar requirements. Genetic, developmental and adverse environmental derangements of these mechanisms lie at the heart of numerous dysfunctional pulmonary surfactant states.

The fundamental pathways for lung surfactant phospholipid synthesis, its packaging into storage lamellar bodies (LB), secretion into the alveolar lining fluid and finally recycling back into the ATII cell are well established. Understanding of these pathways is based on extensive studies in animal and isolated cell culture systems tracking incorporations of radioisotope-labelled lipid precursors. Such studies have provided the basis for current models of lung surfactant lipid metabolism, but have inherent limitations. First, as with all phospholipids, lung surfactant phospholipid is a complex mixture of individual molecular species defined by the combination of fatty acyl moieties esterified at the *sn*-1 and *sn*-2 positions of the glycerophosphate backbone. Studies that, for instance, use osmium tetroxide (OsO_4) to measure total disaturated PC after destructive oxidation of unsaturated lipid species provide no information about this structural complexity. Second, studies using incorporation of radioisotope-labelling precursors track the label and not the molecule and consequently do not allow metabolic analysis in terms of individual molecular species. Third, surfactant lipid metabolism studies using cultured cells lines, whether dissociated primary cells or transformed cell lines, need to be treated with caution. Surfactant lipid metabolism by ATII cells is very dependent on paracrine and nutritional interactions with other cell types in addition to gene expression [81]. Phenotypic characterisation, for instance appearance of LBs and expression of surfactant proteins, is no guarantee of appropriate phospholipid composition and metabolism. Primary cultures of ATII cells maintained on plastic or lamin 5-coated polycarbonate filters rapidly acquire characteristics of type I alveolar epithelial cells and lose their typical saturated PC enrichment, lamellar body

Abbreviations: AM, alveolar macrophage; ATII cell, alveolar type II epithelial cell; ABC, ATP binding cassette; BMP, bis(monoacylglycerol)phosphate; CDH, congenital diaphragmatic hernia; CCT, CTP: cholinephosphate cytidylyltransferase; CPT, cholinephosphotransferase; DPPC, dipalmitoyl PC PC16:0/16:0; ESI MS, electrospray ionisation mass spectrometry; GMCSF, granulocyte macrophage colony stimulation factor; INSIG, insulin induced gene; LB, lamellar bodies; C12, laurate; LPCAT1, lysoPC acyltansferase 1; PC16:0/14:0, palmitoylmyristoyolPC; PC16:0/16:1, palmitoylpalmitoleoylPC; PPARγ, peroxisome proliferator activated receptor gamma; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, PE-*N*-methyltransferase; PG, phosphatidylglycerol; PI, phosphatidylinositol; PLA_2, phospholipase A_2; RDS, respiratory distress syndrome; SCAP, SREBP cleavage activating protein gene; SREBP, sterol responsive element binding protein; SP, surfactant protein

* Corresponding author at: Sir Henry Wellcome Laboratories, MP803, Level F, South Block, Southampton General Hospital, Southampton, SO16 6YD, UK. Tel.: +44 2380796161; fax: +44 238087884.
E-mail address: adp@soton.ac.uk (A.D. Postle).

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http://dx.doi.org/10.1016/j.bbalip.2012.11.009

morphology and ability to secrete functional surface active material [35]. ATII cells cultured under different conditions, for instance on transwells at an air:liquid interface [76] or with keratinocyte growth factor [138,139], maintain their phenotypic morphology longer and can secrete functional material in response to agonist stimulation, but it has yet to be determined whether this apparent differentiation state is accompanied by phospholipid synthesis and molecular species composition characteristic of their state *in vivo*. The effect of culture conditions on surfactant phospholipid composition is well illustrated by electrospray ionisation mass spectrometry (ESI-MS) analysis of material secreted from human fetal ATII cells differentiated *in vitro*. Despite the cells having an appropriate lamellar body morphology, expression of surfactant proteins and secreting material which is surface active, the major PC was the monounsaturated palmitoylpalmitoleoylPC species (PC16:0/16:1) and not disaturated dipalmitoylPC (PC16:0/16:0) characteristic of human surfactant *in vivo* [99]. This review will summarise current understanding of the regulation of surfactant phospholipid metabolism and highlight how advance in electrospray ionisation mass spectrometry (ESI-MS) and the use of stable isotope-labelled precursors have provided novel insights into these processes.

2. Normal ATII cell surfactant lipid synthesis and turnover

The established model of ATII synthesis and turnover of lung surfactant envisages a cycle of phospholipid synthesis, trafficking and packaging that is followed by release and reuptake (Fig. 1). Bulk surfactant phospholipid, synthesised at the ER is transported to LBs by mechanisms that may employ specific carrier proteins and/or trafficking through the Golgi. During the processes of synthesis, transport and selection, the PC component becomes increasingly enriched in saturated molecular species by a combination of the acyl remodelling of unsaturated species, selective transport of saturated species or selective exclusion of unsaturated species. LB maturation culminates in regulated actin-dependent fusion with the apical membrane of the ATII cell and the secretion of their contents into the alveolar space. Newly synthesised surfactant then adsorbs to the air–liquid interface where saturated PC contributes to surface activity. It is not clear whether this process involves an unravelling of LB contents or whether there is direct adsorption of LBs without an unravelling step [47]. The cycle is completed by a combination of turnover mechanisms that remove alveolar surfactant from the extracellular space: (i) a proportion is lost by displacement up the bronchial tree and out of the lung, (ii) some is scavenged and taken up by alveolar macrophages for degradation and (iii) the rest is actively internalised by ATII cells, broken down in endosomes and reusable components delivered back to substrate pools.

3. The regulated enzymology of PC synthesis by ATII cells

PC synthesis *de novo* in ATII cells occurs predominantly by the CDP:choline (Kennedy) pathway and is catalysed by the sequential activities of choline kinase, CTP:cholinephosphate cytidylyltransferase (CCT) and cholinephosphotransferase (CPT). Comparison of incorporations of radiolabelled methionine and choline *in vivo* shows clearly that the alternative PC synthesis pathway involving sequential N-methylation of phosphatidylethanolamine (PE) catalysed by PE-N-methyltransferase (PEMT) is of minor importance in rat and primate lungs [110], although it may make a significant contribution to lung PC synthesis in the sheep [109]. Considerable evidence indicates that CCT is the major regulatory enzyme of *de novo* PC synthesis in lungs and ATII cells [97,121,123], although CPT can assume a greater regulatory role in certain circumstances. For instance, over-expression of lysoPC acyltansferase 1 (LPCAT1) in epithelial cells, leading to increased PC synthesis from lysoPC, decreased PC synthesis *de novo* by enhancing CPT ubiquitination and degradation [25]. Activity

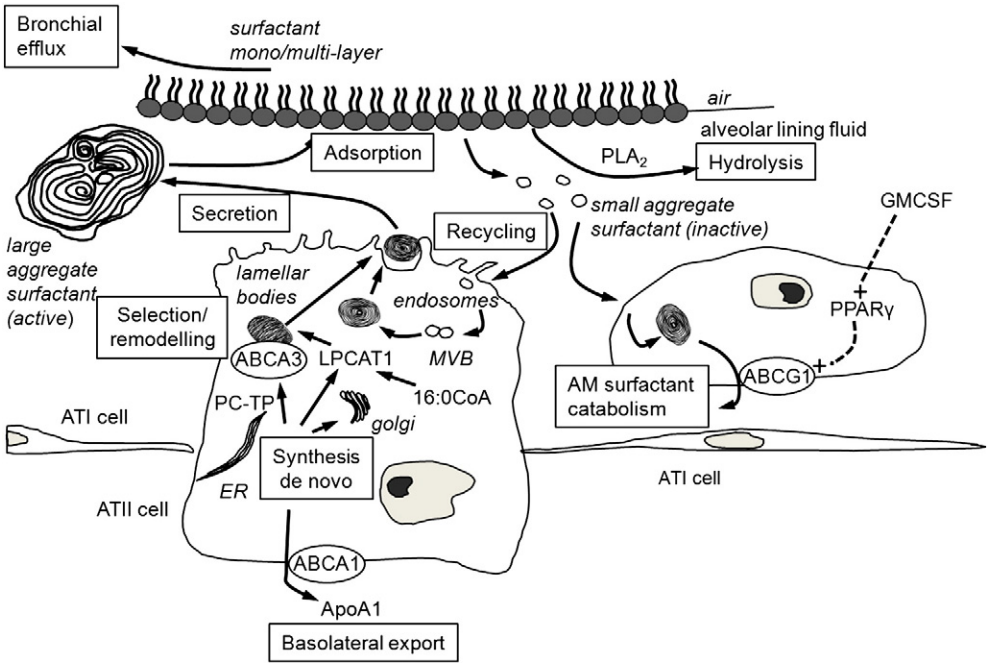

Fig. 1. Integration of surfactant phospholipid metabolism. Surfactant phospholipid is synthesised *de novo* on the endoplasmic reticulum (ER) of the ATII cell. Phospholipid destined for surfactant storage is transported to LBs by some combination of transfer proteins (PC-TP) and Golgi transit. The characteristic phospholipid composition of LBs is determined by ABCA3-mediated LB import and activities of phospholipases and lysoPC acyltransferase (LPCAT1). Basolateral export of unsaturated PC species is mediated by ABCA1, with ApoA1 as the major lipid acceptor in the circulation. Surfactant is secreted as surface active large aggregates when LBs fuse with the ATII cell plasma membrane and then adsorbs rapidly to the air–liquid interface in the alveolus. The high surface pressure of this adsorbed layer drives a proportion of surfactant up the bronchial tree and squeezes out "spent" surfactant back into the alveolar lining fluid as surface inactive small aggregate vesicles. A proportion of these vesicles are recycled through ATII cells by uptake into endosomes and multi-vesicular bodies (MVB) followed by fusion with LBs and eventual re-secretion. Alternatively, surfactant is catabolised by a combination of phospholipase action (PLA_2) and GMCSF-dependent alveolar macrophage (AM) uptake and metabolism.