Intercellular Ca^{2+} signaling in alveolar epithelial cells through gap junctions and by extracellular ATP

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Inter- and extracellular-mediated changes in intracellular Ca^{2+} concentration ([Ca^{2+}]) can ensure coordinated tissue function in the lung. Cultured rat alveolar epithelial cells (AECs) have been shown to respond to secretagogues with increases in [Ca^{2+}], and have been shown to be gap junctionally coupled. However, communication of [Ca^{2+}] changes in AECs is not well defined. Monolayers of AECs were mechanically perturbed and monitored for [Ca^{2+}] changes. Perturbation of AECs was administered by a glass probe to either mechanically stimulate or mechanically wound individual cells. Both approaches induced a change in [Ca^{2+}], in the stimulated cell that was propagated to neighboring cells (Ca^{2+} waves). A connexin mimicetic peptide shown to uncouple gap junctions eliminated Ca^{2+} waves in mechanically stimulated cells but had no effect on mechanically wounded cells. In contrast, apyrase, an enzyme that effectively removes ATP from the extracellular milieu, had no effect on mechanically wounded cells through gap junctions and by extracellular ATP. Am J Physiol Lung Cell Mol Physiol 280: L221–L228, 2001.— Inter- and extracellular-mediated changes in intracellular Ca^{2+} concentration ([Ca^{2+}]) can ensure coordinated tissue function in the lung. Cultured rat alveolar epithelial cells (AECs) have been shown to respond to secretagogues with increases in [Ca^{2+}], and have been shown to be gap junctionally coupled. However, communication of [Ca^{2+}] changes in AECs is not well defined. Monolayers of AECs were mechanically perturbed and monitored for [Ca^{2+}] changes. Perturbation of AECs was administered by a glass probe to either mechanically stimulate or mechanically wound individual cells. Both approaches induced a change in [Ca^{2+}], in the stimulated cell that was propagated to neighboring cells (Ca^{2+} waves). A connexin mimicetic peptide shown to uncouple gap junctions eliminated Ca^{2+} waves in mechanically stimulated cells but had no effect on mechanically wounded cells. In contrast, apyrase, an enzyme that effectively removes ATP from the extracellular milieu, had no effect on mechanically stimulated cells but severely restricted mechanically wounded Ca^{2+} wave propagation. We conclude that AECs have the ability to communicate coordinated Ca^{2+} changes using both gap junctions and extracellular ATP.

Calcium; cell communication; connexins; adenosine 5’-triphosphate

PULMONARY ALVEOLAR EPITHELIAL CELLS (AECs) line the lung and provide an impermeable barrier against the external environment. The primary constituents of the alveolar epithelium are alveolar type I (ATI) and alveolar type II (ATII) cells. ATI cells are thought to provide a pathway for gas exchange, whereas ATII cells have been shown to be more physiologically diverse. ATII cells provide critical surfactant (e.g., Ref. 54) and extracellular matrix (ECM) protein (e.g., Ref. 19) secretions, participate in H_{2}O and ion transport to regulate fluid composition of the lung (40), and play a crucial role in response to injury, disease, and infection within the lung (16, 40, 42). The ability of ATII cells to act as progenitors of ATI cells (reviewed in Ref. 52) is vitally important for the lung response to injury. After acute alveolar epithelial injury, ATII cells can migrate into a denuded area of lung epithelium, differentiate to an ATI phenotype to restore the epithelial layer, and prevent fibrosis (20, 56). A similar transition occurs in primary cultures of ATII cells after several days in culture on a collagen matrix where ATII cells can lose specific cell markers and take on a more flattened, or ATI type, morphology (15). Dysfunction of AECs in vivo, such as seen after severe cases of acute respiratory distress syndrome, infection, or injury, can have serious effects on lung physiology, including reduced O_{2} transfer, surfactant release, or fibrosis. Although intercellular communication via gap junctions or extracellular signaling may provide for such coordination, there is little known concerning the specific cellular mechanisms that allow for AECs to coordinate epithelial function in the lung.

Changes in intracellular Ca^{2+} concentration ([Ca^{2+}]) are a common theme in initiating cellular processes in a variety of cells (3, 4), including those of the lung epithelium (for reviews, see Refs. 11, 37, 41, 48). Despite the growing abundance of in vitro modeled Ca^{2+} events involving ATII cells, there is little known concerning if and how coordination of Ca^{2+} signaling occurs to aid in the tissue response in vitro or in vivo. Because of the ability to spatiotemporally control the initiation of cellular signaling, micropipette perturbation of cultured cells has emerged as an attractive technique to induce coordinated changes in [Ca^{2+}], for the study of intercellular communication (reviewed in Ref. 48). Two major pathways for Ca^{2+} communication have been proposed for mechanically induced Ca^{2+} waves, gap junctional intercellular communication (GJIC) and the release of extracellular signaling molecules, most commonly ATP; these pathways need not be mutually exclusive (12, 14, 21, 30, 51). Recent reports (1, 25, 28, 31, 33) support a role for gap junctions in cell-cell signaling in AECs. However, these reports elucidate considerable differences in the expression of gap junction proteins (connexins) in AEC cultures. The mixed results from these studies and the aforementioned potential for ATP-dependent signaling empha-
size that the mechanisms of communication in AECs may not be straightforward, and current studies to assess the significance of the varied gap junction expression and their function in AECs are inadequate to model intercellular signaling in AECs.

In this study, connexin expression patterns in 7-day cultured AECs were identified by immunocytochemistry. Additionally, localized mechanical force was used on single cells to initiate two distinct types of intercellular Ca2+ signaling (Ca2+ waves) in these cultures. When single cells are mechanically perturbed without compromising the plasma membrane (mechanical stimulation), Ca2+ waves are propagated through gap junctions. However, if mechanical perturbation results in the release of cytosolic contents (mechanical wound ing), Ca2+ waves are primarily propagated via extracellular diffusion of ATP. These redundant pathways for intercellular Ca2+ communication and the varied effects of Ca2+ signaling suggest an important role for Ca2+ waves in the alveolar epithelium.

MATERIALS AND METHODS

Chemicals. Hank’s balanced salt solution (HBSS), Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium in a 1:1 ratio, bovine serum albumin (BSA), trypsin inhibitor, penicillin, streptomycin, and amphotericin were purchased from Gibco BRL (Life Technologies, Grand Island, NY). Fura 2 and fura 2-acetoxymethyl ester (fura 2-AM) were purchased from Calbiochem (La Jolla, CA). Elastase was purchased from Worthington Biochemical. Gap 27 peptide derived from rat cDNA of connexin (Cx) 43 to include amino acids 204–214 (SRPTEKTIFII) from the second extracellular loop was purchased from Genosys (Cambridge, UK) or Severn Biotech (Worcester, UK). Antibodies were raised against the conserved peptide sequences from individual connexins and have been shown to react to connexins from several mammalian species. Antibodies generated to Cx26 were raised in rabbit against a human peptide sequence. Antibodies to Cx32 and Cx43 were raised in rabbit against rat peptide sequences. Antibodies to Cx40 (rabbit anti-mouse) and Cx46 (rabbit anti-rat) were purchased from Al pha Diagnostics International (San Antonio, TX). Secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA) or Sigma (St. Louis, MO) and were of the highest grade available.

Cell cultures. Primary ATII cells were harvested by modified methods previously described (6). Male Sprague-Dawley rats weighing 250–300 g were killed by peritoneal pentobarbital sodium injection. The lungs were initially washed with solution I (136 mM NaCl, 2.2 mM Na2HPO4, 5.3 mM KCl, 5.6 mM glucose and 10 mM HEPES, pH 7.4), incubated for 20 min with antibody dilution solution [0.25% gelatin, 3.0% BSA, 0.05% Tween 20, and 0.2% Na2EDTA in Tris-buffered saline (150 mM NaCl and 15 mM Tris, pH 7.4)], and incubated with the appropriate primary connexin antibody diluted 1:100 and applied for 1 h at 37°C. The coverslips were washed with PBS and antibody dilution solution, and incubated in cold incubation buffer containing 1:250 anti-rabbit IgG FITC conjugate for 1 h at 37°C. The coverslips were thoroughly washed with PBS and deionized water, mounted, and viewed on a Leica TSD-4D confocal laser scanning microscope.

Ca2+ imaging. The cells were loaded with fura 2 by a 75-min incubation with 5 µM fura 2-AM in HBSS (1.3 mM CaCl2, 5.0 mM KCl, 0.3 mM KH2PO4, 0.5 mM MgCl2, 0.4 mM MgSO4, 137.9 mM NaCl, 0.3 mM Na2HPO4, and 1% glucose additionally buffered with 25 mM HEPES, pH 7.4). The cells were washed for 20 min in HBSS, mounted on an inverted Olympus IX70 microscope, and observed with a ×40, 1.35-numerical aperture oil-immersion objective. Fura 2 fluorescence was observed after excitation was alternated at 340 and 380 nm by a 75-W xenon lamp linked to a Delta Ram Illuminator (Photon Technologies (PTI)). Images of emitted fluorescence above 510 nm were recorded by an intensified charge-coupled device camera (PTI) and simultaneously displayed on a 21-inch vivitron color monitor. The imaging system was under software control (ImageMaster, PTI) on an IBM clone computer. Calculation of [Ca2+]i, was by published equations (24). A positive response in [Ca2+]i was detected when [Ca2+]i increased over 150 nM, a three- to fourfold increase over resting [Ca2+]i. All cells exhibiting a positive change in [Ca2+]i were counted as communicating cells. Thus a Ca2+ wave of 1 cell represents a [Ca2+]i change only in the stimulated cell.

Mechanical stimulation and wounding. A glass micropipette (tip diameter ~1 μm) under piezoelectric control was positioned with a hydraulic micromanipulator and deflected downward for 150 ms to deform an individual AEC. If the membrane of the deformed cell was not broken by the micropipette, it was considered to be mechanically stimulated; if the membrane of the deformed cell was broken by the micropipette, resulting in dye release, it was considered to be mechanically wounded.

Statistics. Two-sample Student’s t-tests were used in all data comparisons. Results are averages ± SD.

RESULTS

Morphology of cultured ATII cells. ATII cells were isolated as described in MATERIALS AND METHODS. The cells were visually checked for morphology and stained for lamellar bodies, an ATII cell marker, to determine cell type in culture. At isolation, ATII cells comprised 99% of the cells (325 of 325 cells counted from 2 isolations stained positively for lamellar bodies; data
not shown). On day 2 of culture, ATII cells had a typical cuboidal shape ~5–10 μm across, with >98% of the cells staining positive for lamellar bodies (120 of 122 cells counted from 2 isolations; Fig. 1A). On day 7 of culture, the cells were more flattened in shape, were ~20–25 μm across, and displayed a reduced number of lamellar bodies, with 29% of the cells staining positive for the ATII cell marker (100 of 345 cells counted from 2 isolations had at least one lamellar body; Fig. 1B). Because 7-day-old cultures displayed characteristics of both ATII and ATI cells, they are best described as AECs.

Connexin localization in AECs. We used immunocytochemistry and specific antibodies generated to Cx26, Cx32, Cx40, Cx43, and Cx46 to study the expression of connexin proteins in 7-day-old AEC cultures. Immunocytochemistry with antibodies generated to Cx40 (Fig. 2A) and Cx43 (Fig. 2B) resulted in a punctate staining pattern of AECs at the plasma membranes, indicative of these connexins directly participating in cell-cell communication. Staining with antibodies to Cx46 (Fig. 2C) also showed distinct punctate staining. However, this staining was localized to the juxtanuclear region of the AECs. Immunocytochemistry of AECs with antibodies generated to Cx32 (Fig. 2D), and Cx26 (Fig. 2E) resulted in some cellular localization near the juxtanuclear region that was significantly higher than background (Fig. 2F). However, cell surface punctate staining with antibodies to Cx26 and Cx32 was severely reduced compared with results with antibodies generated to Cx40, Cx43, and Cx46.

**AEC response to ATP.** ATP has been used as an effector molecule to raise [Ca²⁺], in lung epithelium and other tissues and has been suggested as an extracellular messenger for coordinated Ca²⁺ communication. To determine whether 7-day-old AECs responded to ATP, we monitored [Ca²⁺], in monolayer cultures of AECs after exposure to 10 μM ATP. Application of ATP resulted in a significant rise in [Ca²⁺] in AECs (Fig. 3, A–D). All cells in the field of view responded uniformly with an increase in [Ca²⁺] by 60 s and a return to baseline levels of [Ca²⁺] by 120 s. To manipulate ATP response, 50 U/ml of apyrase were added to 10 μM ATP immediately before bath application (Fig. 3, E and F). Apyrase effectively eliminated the [Ca²⁺] response to 10 μM ATP in AECs.

**Mechanical stimulation and Ca²⁺ communication in AECs.** To determine whether AECs were capable of communicating [Ca²⁺], changes, [Ca²⁺], was monitored in a field of cells during and after a central cell was briefly stimulated with a glass micropipette. Mechanical stimulation (where the plasma membrane was not damaged by the micropipette) resulted in a Ca²⁺ wave that averaged slightly over 4 cells (n = 17; Figs. 4, A–D, and 5A). All cells returned to baseline [Ca²⁺] within 120 s. The gap junction-inhibiting peptide Gap 27 (5, 13, 53) and the ATPase apyrase were used to determine the pathway of Ca²⁺ communication after mechanical stimulation. After a 30-min incubation with Gap 27, mechanical stimulation resulted in an increase in [Ca²⁺], that was largely restricted to the stimulated cell (n = 7; Figs. 4, E–H, and 5A). This inhibition of communication was a significant reduction from Gap 27-free mechanical stimulation (P <
0.001). A 15-min washout of Gap 27 allowed for a return to normal-sized \( \text{Ca}^{2+} \) waves on mechanical stimulation (Fig. 5A). Similar experiments with apyrase did not significantly reduce \( \text{Ca}^{2+} \) wave propagation in response to mechanical stimulation (\( n = 17; \) Figs. 4, I–L, and 5A).

**Mechanical wounding and \( \text{Ca}^{2+} \) communication in AECs.** To determine whether cell communication between AECs could occur by alternative signaling mechanisms, a glass micropipette was introduced to a single AEC such that the plasma membrane was compromised and the intracellular contents were free to mix with the extracellular milieu, and the \( \left[ \text{Ca}^{2+} \right]_i \) in AEC cultures was measured. After such mechanical wounding, AECs displayed \( \text{Ca}^{2+} \) waves that averaged greater than six cells (\( n = 17; \) Figs. 5B and 6, A–D). As above, Gap 27 and apyrase were used to determine the mechanism of \( \text{Ca}^{2+} \) wave propagation. A 30- to 45-min incubation with Gap 27 did not significantly reduce the size of the mechanical wound-induced \( \text{Ca}^{2+} \) wave (Figs. 5B and 6, E–H). However, when apyrase was applied to the AEC monolayer for 2 min or more, mechanical wound-induced \( \text{Ca}^{2+} \) waves were restricted to the stimulated cell and one or two neighboring cells (\( n = 11; \) Figs. 5B and 6, I–L). This was a significant reduction in wound-induced \( \text{Ca}^{2+} \) waves measured in apyrase-free cultures (\( P < 0.001; \) Fig. 5B). Washout of apyrase for 5 min allowed for a return of mechanical wound-induced \( \text{Ca}^{2+} \) waves to near control values (\( n = 8; \) Fig. 5B).

**DISCUSSION**

Cellular communication pathways are required for coordinated tissue function, including that of the alveolar epithelium. In this report, we show that 7-day-old AECs communicate increases in \( \left[ \text{Ca}^{2+} \right]_i \), in response to local mechanical stimulation via gap junctions. A–L: color maps of \( \left[ \text{Ca}^{2+} \right]_i \) of AECs over time (nos. at bottom right in s) in response to mechanical stimulation of a single cell (arrows). A–D: control conditions. E–H: in the presence of 130 \( \mu \text{M} \) of the gap junction-inhibiting peptide Gap 27. I–L: in the presence of 50 U/ml of apyrase. Time points were picked to emphasize size of \( \text{Ca}^{2+} \) waves. White lines, cell borders. Color bar, approximate \( \left[ \text{Ca}^{2+} \right]_i \). Cell isolations and \( n \) values are listed in Fig. 5. Mechanical stimulation of a single cell resulted in propagated \( \text{Ca}^{2+} \) waves. These mechanical stimulus-induced \( \text{Ca}^{2+} \) waves were restricted by Gap 27 but were not affected by the ATPase apyrase.
cultures of AECs (from purified ATII cells grown on a fibronectin-collagen matrix) express connexin proteins required for GJIC. Through the use of localized mechanical perturbation of a single cell, it is further demonstrated that AECs can coordinate \([Ca^{2+}]_i\) changes among neighboring cells (Ca\(^{2+}\) waves) through two distinct pathways, namely, GJIC and extracellular diffusion of ATP released from the mechanically perturbed cell. The redundancy of pathways for propagation of Ca\(^{2+}\) waves suggests an important function for this type of communication in AECs.

Gap junctions are constructed of connexin proteins, of which ~20 major isoforms are identified in mammals. Expression of connexins and their intracellular trafficking and assembly into gap junctions at cell contact areas is an important indicator that GJIC may occur. In this work, antibodies generated to Cx26, Cx32, Cx40, Cx43, and Cx46 were used to study connexin expression in 7-day-old AECs. It was shown that AECs have punctate expression of Cx40 and Cx43 at the plasma membrane in addition to Cx46 at the juxtanuclear region. Any combination of these connexins could contribute to the GJIC of Ca\(^{2+}\) waves detected after mechanical stimulation (Figs. 4 and 5). Several recent reports (e.g., Refs. 1, 25, 33) have used a variety of techniques to elucidate connexin expression pat-

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**Fig. 5.** Comparison of Ca\(^{2+}\) waves by mechanical stimulation (A) and mechanical wounding (B). Cells responding with an increase in [Ca\(^{2+}\)]\(_i\) after mechanical perturbation are plotted against individual treatments for mechanical stimulation and mechanical wounding. Values are averages ± SD; \(n\), no. of experiments from 13 control, 3 Gap 27 and ATP, and 4 separate cell (apyrase) isolations. Ca\(^{2+}\) waves induced by mechanical stimulation were blocked by the gap junctional inhibitor Gap 27 but not after treatment with apyrase. Ca\(^{2+}\) waves induced by mechanical wounding were not affected by the gap junctional inhibitor Gap 27 but were restricted by the ATPase apyrase. In each case, washout of the inhibitor restored Ca\(^{2+}\) wave propagation. *\(P < 0.001\) by Student’s paired \(t\)-test.

**Fig. 6.** AECs communicate increases in [Ca\(^{2+}\)]\(_i\), in response to local mechanical wounding via extracellular ATP. A–L: color maps of [Ca\(^{2+}\)]\(_i\) of AECs over time (nos. at bottom right in s) in response to mechanical wounding of a single cell (arrows). A–D: control conditions. E–H: in the presence of 130 \(\mu\)M of the gap junction inhibiting peptide Gap 27. I–L: in the presence of 50 U/ml of apyrase. Time points were picked to emphasize size of Ca\(^{2+}\) waves. White lines, cell borders. Cell isolations and \(n\) values are listed in Fig. 5. Color bar, approximate [Ca\(^{2+}\)]\(_i\). Mechanical wounding of a single cell resulted in propagated Ca\(^{2+}\) waves. These mechanical wound-induced Ca\(^{2+}\) waves were not affect by Gap 27 but were restricted by the ATPase apyrase.
terns in rat AECs, mostly focusing on 0- to 4-day-old primary cultures of ATII cells. With Northern and immunoblot analyses in rat ATII cells to detect Cx26, Cx32, Cx43, and Cx45, day 0 isolations consisted of Cx26 and Cx32 expression, whereas Cx43 and Cx45 were not detected (33). By day 3 of culture, however, Cx32 and Cx45 were absent, Cx26 was reduced in expression, and Cx43 was the most commonly expressed connexin. Significantly, by day 6 in culture, the immunoblot analysis of Cx43 showed a reduced protein expression, but the Northern blot analysis reported continued high expression of Cx43 mRNA. In a separate study (1), rat ATII cells were analyzed for 13 different connexins at isolation with RT-PCR; these cells displayed Cx26, Cx30.3, Cx32, Cx37, Cx43, and Cx46 on day 0. In the same study, connexin expression was followed over time with total mRNA assays for the recognized connexins. On day 4 of culture, total mRNA for Cx43 and Cx46 increased and for Cx26 and Cx32 decreased, whereas Cx30.3 and Cx37 remained constant. If the culture medium was changed by reducing the fetal bovine serum supplement and adding keratinocyte growth factor to increase ATII cell morphology, the results discussed above include ATII cell isolations grown on various ECM components, discrepancies in the findings of connexin expression may be linked to the ECM. However, it should be noted that the ECM also likely changes in vivo during wound healing as the cell types that migrate over the ECM can contribute to changes in the ECM. Because it has become increasingly clear that the connexin makeup of gap junctions allows for selectivity of molecule transfer (e.g., Ref. 23), the variation in connexin expression displayed in AEC cultures over time may be an important part of cellular communication in vivo. That is, the expression of connexins during ATII cell differentiation may allow for transfer of select signaling molecules to coordinate distinct physiological processes in the alveolar epithelium. We further speculate that a trigger for the initial changes in [Ca\textsuperscript{2+}], communicated after the pathophysiology and subsequent release of cytosolic constituents from damaged cells in vivo could occur in the absence of gap junctional signaling.

It has long been postulated that mechanical forces play an important role in both developing (e.g., Ref. 2) and mature (e.g., Refs. 39, 46) lungs in vivo. In vitro, mechanical stretch of ATII cells grown on Silastic membranes has been shown to induce cellular signaling in AECs (e.g., Ref. 55). In this study, we focused mechanical forces onto a single cell and measured the ability of that cell to communicate responses to mechanical force. Mechanical perturbation allows distinct spatiotemporal control to initiate intercellular signaling communication of Ca\textsuperscript{2+} waves through two independent mechanisms dependent on the health status of the stimulated cell. In other in vitro cell systems, including ovine lens cells (14), liver cells (21), osteoblasts (30), and pancreatic cells (9), a duality in mechanisms for mechanically induced Ca\textsuperscript{2+} waves also exists. However, of these systems, only the ovine lens cells were shown to require wounding for ATP-dependent signaling. In a previous study (29) with AECs where [Ca\textsuperscript{2+}], responses to mechanical wounding were measured, wounding was induced by dragging an 18-gauge needle across ATII cell cultures to induce Ca\textsuperscript{2+} waves encompassing hundreds of cells; it was postulated that these Ca\textsuperscript{2+} waves were dependent on an extracellular messenger presumed to be ATP. Our observations with Ca\textsuperscript{2+} waves induced by mechanical wounding of a single cell sensitive to apyrase confirm that nucleotide release from wounded cells can result in a Ca\textsuperscript{2+} wave. GJIC in AECs has also been analyzed with dye transfer (1, 25, 33) and mechanical stimulation-induced Ca\textsuperscript{2+} communication (33). In these studies, traditional gap junctional inhibitors such as 18-glycyrrhetinic acid (25) and octanol (33) could be used to uncouple dye transfer (25, 33) or Ca\textsuperscript{2+} signaling (33) via GJIC in day 2–4 isolated ATII cells. In the present study using Gap 27, a benign, nonpenetrant, and reversible GJIC peptide inhibitor (5, 13, 43, 53), mechanical stimulation-induced Ca\textsuperscript{2+} communication in 7-day-old AECs is reversibly blocked. In contrast to previous results, the present study emphasizes the importance of AEC communication in two ways: 1) the communication of physiologically significant molecules such as the second messenger Ca\textsuperscript{2+} and 2) the redundancy of mechanisms for Ca\textsuperscript{2+} wave propagation to ensure proper communication in AECs.

Ca\textsuperscript{2+} is an important signaling molecule in AECs. AEC [Ca\textsuperscript{2+}], can be raised by changes in pH (22), mechanical stretch (55), insulin (36), endothelin (50), ATP (17, 44), and heavy metal application (34) among other means. Changes in [Ca\textsuperscript{2+}], have distinct physiological effects on AECs. Such changes have been shown to be involved in membrane reorganization (8); secretion of surfactant (27, 44, 45, 47, 49, 55), arachidonic acid metabolites (10), and ECM molecules (18); regulation of channels (35); differentiation of ATII to ATI cells (26); and repair of the epithelial barrier (29). The communication of Ca\textsuperscript{2+} waves either by GJIC (as in mechanical stimulation) or by extracellular signaling molecules (as in mechanical wounding) can provide a mechanism for the coordination of Ca\textsuperscript{2+}-induced physiology (or pathophysiology) in the AECs that line the lung alveolus. How AECs interact in vivo to Ca\textsuperscript{2+} wave communication remains to be studied, but speculation in relation to the above-mentioned effects that [Ca\textsuperscript{2+}], changes have on AECs is intriguing. Coordination of surfactant or other secretions from ATII cells, differentiation from ATII to ATI phenotype, mi-
gration of AECs to cover a denuded epithelial area, or regulation of ion channel activity within the alveolar epithelium are just some of the possible physiological outcomes of Ca\textsuperscript{2+} waves in AECs. In further studies, the use of available mechanisms for coordinated changes in [Ca\textsuperscript{2+}], and subsequent physiological outcomes may be elucidated to better understand the function of the alveolar epithelium in vivo.

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