

Mechanical Distention Modulates Alveolar Epithelial Cell Phenotypic Expression by Transcriptional Regulation

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The development of a normal pulmonary alveolar epithelium, essential for gas exchange, is critical for the successful adaptation to extrauterine life. From observations of natural and experimental developmental abnormalities, it has been hypothesized that mechanical factors may play a role in regulating differentiation of the pulmonary alveolar epithelium. To test this hypothesis directly, we have investigated the *in vitro* effects of mechanical distention on the expression of specific markers for the type I and type II cell phenotypes. Fetal rat lung (18-d) explants were mechanically distended in culture for 18 h. Mechanical distention caused an increase in RTI 40 messenger RNA (mRNA), a marker of the type I cell phenotype, of 10.6 times ($n = 3$, $P < 0.05$) that of undistended controls. In contrast, mechanical distention resulted in a decrease in mRNA content of two markers of the type II cell phenotype, surfactant protein (SP)-B and SP-C. SP-B was reduced to $10 \pm 9\%$ ($n = 3$, $P < 0.005$) and of SP-C to $12 \pm 7\%$ ($n = 3$, $P < 0.0001$) of undistended controls. Mechanical distention had no effect on content of mRNA for SP-A or 18S ribosomal RNA. Examined by nuclear run-on assays, mechanical distention caused changes in transcriptional rates of RTI 40, SP-B, and SP-C. These data show that mechanical distention stimulates expression of a type I cell marker and inhibits expression of markers for the type II phenotype; these effects occur at least in part at the transcriptional level. These studies support the hypothesis that mechanical distention of fetal lung tissue stimulates expression of the type I cell phenotype and inhibits expression of the type II phenotype. **Gutierrez, J. A., R. Ertsey, L. M. Scavo, E. Collins, and L. G. Dobbs. 1999. Mechanical distention modulates alveolar epithelial cell phenotypic expression by transcriptional regulation. *Am. J. Respir. Cell Mol. Biol.* 21:223–229.**

The pulmonary alveolar epithelium is comprised of two morphologically distinct types of cells, type I and type II cells, both of which are essential for normal mammalian lung function and successful adaptation to extrauterine life. Anatomically, the two cell types are strikingly different. Type I cells are extremely large, very thin squamous cells. Type II cells are much smaller cuboidal cells characterized by morphologically distinct lamellar bodies that contain surfactant proteins and lipids. Type I cells are thought to play an important role in lung function largely because they cover 95% of the alveolar surface, providing both the tight barrier and the short diffusion pathway be-

tween the air and blood components that are essential for efficient gas exchange (1). Type II cells, which cover the remaining alveolar surface, are known to synthesize, secrete (2), and recycle (3, 4) surfactant components, which are responsible for lowering surface tension at the alveolar air-liquid interface, preventing alveolar collapse. Although the establishment of a normal alveolar epithelium is critical for gas exchange, little is currently known about the factors that control the developmental expression of the differentiated phenotype of type I and type II cells.

The two major aspects of fetal lung development that have been studied in detail are lung growth and maturation of the surfactant system. Since the initial report by Liggins in 1969 (5) showing that treatment with glucocorticoids stimulates maturation of the fetal lung, numerous studies have demonstrated that maturation of the surfactant system is controlled principally by endocrine factors (6–8). In contrast, physical forces appear to be the major determinants of fetal lung growth (9–12). Despite this body of information regarding lung growth and the surfactant system, the factors involved in regulating phenotypic expression *in utero* remain largely undefined. On the basis of experiments of nature and studies *in vivo*, it has been suggested that mechanical factors may be important in

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Abbreviations: complementary DNA, cDNA; messenger RNA, mRNA; ribosomal RNA, rRNA; reverse transcriptase/polymerase chain reaction, RT-PCR; standard deviation, SD; sodium dodecyl sulfate, SDS; surfactant protein, SP; saline sodium citrate, SSC.

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modulating alveolar epithelial phenotypic expression during development. The results of congenital diaphragmatic hernia, which causes underdistention of the developing lung *in utero* are pulmonary hypoplasia and surfactant deficiency (13). Overdistention of the lung, as seen in congenital laryngeal atresia, results in large lungs with an increased number of alveoli and a more mature architecture than expected for gestational age (14). Experiments with fetal model systems in which the fetal lung is distended by tracheal ligation or occlusion suggest that mechanical effects may be involved in determining alveolar epithelial phenotypic expression during development (15). There is little information about how mechanical factors affect alveolar epithelial phenotypic expression *in vitro*. Our results suggest that mechanical factors may be potent regulators of alveolar epithelial phenotypic expression.

Materials and Methods

Explant Culture and Mechanical Distention

Fetal rat lungs were obtained at 18 d of gestation from pregnant Sprague-Dawley rats (Charles River, Hollister, CA) under protocols approved by the Committee on Animal Research at the University of California San Francisco. The lung parenchyma was minced into 1-mm³ pieces and cultured as explants on circular silicone membrane dishes coated with fibronectin in serum-free Waymouth's MB 752/1 medium supplemented with penicillin 100 U/ml, streptomycin 100 U/ml, and amphotericin B 2.5 µg/ml (all from Cell Culture Facility, University of California San Francisco). Cultures were incubated in 95% air/5% CO₂ on a rocker platform as previously described (16) for 4 h. Preliminary studies showed that by 4 h of culture on fibronectin-coated membranes, explants were attached firmly enough that stretching of the membranes did not cause the detachment of explants. In contrast, when we stretched explants before 4 h, they became detached from the surface of the membrane. After 4 h, membranes were placed in stretching devices that provided space for two groups of three membranes per device. Each group of three membranes could be stretched independently, allowing three stretched membranes to be compared with three unstretched membranes under equivalent conditions in the same stretching device. Membranes were placed onto a porous base overlying a fluid-filled chamber. Membranes were held in place by an acrylic top plate with round borings forming the wells. One-half of the membranes in each chamber were distended by applying hydrostatic pressure beneath the membranes. The amount of distention was controlled by the volume of fluid added. Changes in two-dimensional membrane surface area had been previously correlated with volume of fluid added to the same system (17). In this device, each group of three membranes could be manipulated separately, allowing three stretched membranes to be compared with three unstretched membranes under otherwise equivalent conditions in the same stretching device, as previously described (17, 18). Membranes were maintained in the distended state for 18 h. Maintenance of distention was assessed by evaluating the devices for leaks as well as the amount of fluid removal required to return the membranes to their relaxed state. The vol-

ume of fluid within the device remained constant; there were no leaks. After the 18-h experimental period, tissue culture media were removed and explants were harvested and processed as described later.

Preparation of RNA; Reverse Transcriptase/Polymerase Chain Reaction; Southern Blotting; and Hybridization

Total cellular RNA was extracted and isolated using RNA-STAT (Tel-Test, Friendswood, TX). The amount of total recovered RNA varied less than 20% among samples and between groups. In preliminary experiments, we found that Northern blot analysis was not sensitive enough to detect several of the genes of interest. We therefore elected to measure messenger RNA (mRNA) content by reverse transcriptase/polymerase chain reaction (RT-PCR)/Southern blotting. To control for quantitation of mRNA, the concentration of each sample was adjusted to 0.125 µg RNA/µl, 0.5 µg of RNA was loaded onto a 1% agarose gel, and the RNA was fractionated by electrophoresis. Ethidium bromide staining showed that lanes contained bands of comparable intensity for 18S and 28S RNA and documented the integrity of the RNA. RNA was then transferred to Nytran filters and filters were probed with α-³²P-labeled sheep (partial) 18S complementary DNA (cDNA) probe that had identical sequence to rat 18S RNA. Radiolabeled bands were quantitated by phosphorimage analysis. Once integrity and quantitation of RNA was assured, equal aliquots of 2 µl (0.250 µg) of RNA were then subjected to RT-PCR for RTI 40, surfactant protein (SP)-A, SP-B, and SP-C as well as 18S RNA, using reverse transcriptase-avian myeloblastosis virus (RT-AMV) (Boehringer Mannheim, Indianapolis, IN) in the presence of 10× PCR buffer (Perkin-Elmer, Branchburg, NJ), all four deoxyribonucleotides, and Taq polymerase (Perkin-Elmer). The following specific oligonucleotide PCR primer pairs (Biomolecular Resource Center, San Francisco, CA) were used to amplify the genes of interest: RTI 40, 5'-GCC-ATC GGT GCG CTA GAA GAT GAT CTT-3' (identical to bases 53-80), 5'-GTG ATC GTG GTC GGA GGT TCC TGA GGT-3' (complementary to bases 201-257); SP-A, 5'-TTT CCA GCT TAC CTG GAT GAG G-3' (identical to bases 13-25), 5'-GGA GTC TGG TCT TCA ATC ATG C-3' (complementary to bases 301-323); SP-B, 5'-AAT GAC CTG TGC CAA GAG TGT G-3' (identical to bases 196-218), 5'-AGG ACC AGC TTG TTC AGC AGA G-3' (complementary to bases 509-531); and SP-C, 5'-GTG GTT GTG GTG GTA GTC CTT G-3' (identical to bases 127-149), 5'-TAG CAG TAG GTT CCT GGA GCA GCT G-3' (complementary to bases 380-402). The oligonucleotides for amplification of 18S ribosomal RNA (rRNA) were obtained from Ambion (Austin, TX). The number of amplification cycles was determined by evaluating samples subjected to serial amplifications to determine the linear range for each target cDNA and then choosing a cycle number within the linear range (5 for 18S, 12 for RTI 40, 18 for SP-A and SP-B, and 30 for SP-C). PCR products were separated by electrophoresis through 2% agarose gels, stained with ethidium bromide, and visualized with ultraviolet light. The cDNA was then transferred to Nytran filters (Schleicher and Schuell, Keene, NH) by capil-

lary action. Filters were then probed with the corresponding full-length cDNAs for rat RTI 40, SP-A, SP-B, SP-C, and 18S. (The cDNAs for rat SP-A, -B, and -C were a kind gift of Dr. John Shannon [University of Colorado, Denver, CO].) All cDNA inserts were excised intact from their vectors with an appropriate restriction enzyme (Boehringer Mannheim), purified by electrophoresis through an agarose gel, then labeled with [α - 32 P]-deoxycytidine triphosphate (NEN Research Products, Boston, MA) by random-primer second-strand synthesis using Random Primer Labeling Kit (GIBCO BRL, Gaithersburg, MD). Unincorporated nucleotides were removed using a Nuc-Trap Probe Purification column (Stratagene, La Jolla, CA). Filters were prehybridized for 10 min in QuikHyb hybridization solution (Stratagene) at 68°C. Hybridization was performed in 10 ml of QuikHyb solution containing 1.25×10^6 dpm/ml for 18 h at 68°C. Hybridized filters were washed three times with a solution of $2 \times$ saline sodium citrate (SSC)/0.1% sodium dodecyl sulfate (SDS) at 25°C for 15 min and once with a solution of $0.1 \times$ SSC/0.1% SDS at 60°C for 30 min. Autoradiography was performed using Hyperfilm (Amersham, CEA AB, Uppsala, Sweden) before quantifying radiolabeled bands by volume integration of pixels measured by phosphorimage analysis (Molecular Dynamics, Sunnyvale, CA).

Isolation of Nuclei and Run-On Transcription Assay

Explants were removed from membranes by scraping membranes in phosphate-buffered saline (PBS) and then pelleted by centrifugation at $500 \times g$ at 4°C for 10 min. Nuclei were prepared from explants by Dounce homogenization in a buffer consisting of 0.2 M tricine, 0.02 M CaCl₂, 0.01 M MgCl₂, 0.25 M sucrose, and 2 mM dithiothreitol (DTT). Nuclei were pelleted by centrifugation at 1,500 rpm at 4°C. The pellet was homogenized in the same buffer and centrifuged. Nuclei were resuspended in a nuclear storage buffer of 50 mM Tris-HCl, 40% glycerol, 5 mM MgCl₂, and 0.1 mM ethylenediaminetetraacetic acid and stored in aliquots of 2.5×10^7 nuclei/100 μ l of nuclei storage buffer. All chemicals were purchased from Sigma (St. Louis, MO).

Run-on transcription assays were performed as previously described (18). Briefly, 2.5×10^7 nuclei were resuspended in reaction buffer containing 10 mM Tris-HCl, 5 mM MgCl₂, and 300 mM KCl; 10 mM adenosine 5' triphosphate, 10 mM cytidine 5' triphosphate, and 10 mM guanosine 5' triphosphate (Boehringer Mannheim); 10 mM DTT (Promega, Madison, WI); and 10 U ribonuclease (RNase) inhibitor (GIBCO BRL) and 200 μ Ci of [α - 32 P]uridine triphosphate (UTP) (NEN) and incubated at 37°C for 20 min. The nuclei were then successively digested with RNase-free deoxyribonuclease I and proteinase K. Unincorporated nucleotides were removed using a Centricon 100 concentrator (Amicon, Beverly, MA).

Equal amounts of radioactive elongated RNA (1 to 3×10^6 dpm) were hybridized to 5 μ g of linearized denatured plasmids containing cDNA inserts for RTI 40, SP-A, SP-B, SP-C, or control plasmid DNA with no insert, that had been spotted onto Nytran filters (Schleicher and Schuell) using a vacuum slot blotter. The filters were hybridized at 42°C for 4 d in a hybridization solution of $10 \times$ Denhardt's

solution, 50% formamide, $5 \times$ SSC, 50 mM *N*-2-hydroxyethylpiperazine-*N*-ethane sulfonic acid, 0.05% SDS (all from Sigma), 50 μ g yeast transfer RNA/ml (GIBCO BRL), and 50 μ g salmon sperm DNA/ml (Stratagene). Hybridized filters were washed under high-stringency conditions. Autoradiography and phosphorimage analysis were performed as previously described.

Immunocytochemistry

Explants were fixed in place for 2 h at room temperature in 4% freshly prepared paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) and then were cryoprotected overnight at 4°C in 4% paraformaldehyde and 0.1 M phosphate buffer in 30% sucrose. The samples were embedded in Tissue-Tek optimal cutting temperature compound (Miles, Elkhart, IN) and were frozen and stored in liquid N₂. Cryostat sections 1 to 2 μ m thick (Reichert-Jung Cryocut 1800) were mounted onto Fisher Superfrost Plus slides, dried, and ringed with rubber cement to form wells to contain solutions.

Samples were prepared for immunofluorescence by incubation with RTII, a monoclonal antibody specific for the apical surfaces of rat type II cells (19), and RTI 40, a monoclonal antibody specific in lung to the apical surface of rat type I cells (20). The sections were rinsed in buffer (0.1% bovine serum albumin and 0.3% triton in PBS), blocked with 10% rabbit serum in buffer, and incubated for 1 h at room temperature with RTI 40 in blocking solution. The tissue was rinsed again before a 1-h incubation with rabbit antimouse immunoglobulin (Ig)G₁ conjugated to fluorescein isothiocyanate (Zymed Laboratories, South San Francisco, CA). The tissue was blocked with rabbit serum as previously described and incubated overnight at 4°C with RTII 70 diluted 1:100 in blocking solution. After rinsing again, the sections were reacted with rabbit antimouse IgG₃ conjugated to biotin (Zymed Laboratories), followed by Neutralite avidin-Texas red (Molecular Probes, Eugene, OR). The sections were mounted with Prolong mounting media (Molecular Probes), examined, and photographed using phase and fluorescent optics on a Leitz Orthoplan microscope.

Statistical Analysis

Results are expressed as the percent of change from undistended controls, means \pm standard deviation (SD) of three experiments, each with a different preparation of explants. Distended samples were compared with undistended controls by Student's *t* test. *P* < 0.05 was considered statistically significant.

Results

Distention of Explants Causes an Increase in the RTI 40 mRNA

Specific mRNAs were quantitated by Southern blot and phosphorimage analysis of RT-PCR products. Mechanical distention caused an increase in RTI 40 mRNA of 10.6 times (*n* = 3 from three experiments using 3 litters per experiment, *P* < 0.05) that of undistended controls (Figure

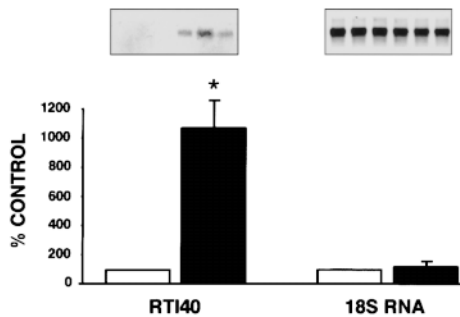


Figure 1. Mechanical distention increases mRNA content of RTI 40. Southern blots were performed from RNA obtained from unstretched control and stretched explants and subjected to RT-PCR for RTI 40 and 18S RNA as described in MATERIALS AND METHODS. Autoradiograms were obtained before quantifying radioactivity by volume integration of pixel values as measured by phosphorimage analysis. In each upper panel, the first three lanes represent triplicate control samples and the next three lanes represent triplicate stretch samples. Results are expressed as the percent of change from unstretched controls, means \pm SD of three experiments, each with a different preparation of explants. * $P < 0.05$.

1). These findings support the hypothesis that mechanical distention favors expression of the type I phenotype.

Distention of Explants Causes a Decrease in the mRNA Content of SP-B and SP-C

In contrast to the effects of mechanical distention on RTI 40 mRNA, mechanical distention caused a reduction in the expression of mRNA for two of the markers of the type II phenotype. Phosphorimage analysis of Southern blots of mRNA subjected to 24 cycles of amplification by RT-PCR for SP-B showed that mechanical distention resulted in a decrease in mRNA content for SP-B to $9.5 \pm 9.4\%$ ($n = 3$, $P < 0.005$) of control (undistended) explants cultured in parallel chambers of the same device (Figure 2). Similar analysis of RT-PCR products after 30 cycles of amplification for SP-C revealed a decrease in mRNA content for SP-C to $12.6 \pm 6.8\%$ ($n = 3$, $P < 0.0001$) of undistended control explants (Figure 2).

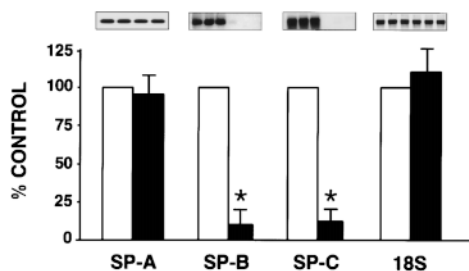


Figure 2. Effect of mechanical distention on the markers of the type II cell phenotype. Southern blot analysis of RNA obtained from unstretched control and stretched explants and subjected to RT-PCR for SP-A, -B, and -C, and 18S RNA as described in MATERIALS AND METHODS. In each upper panel, the first two or three lanes represent control samples and the next two or three lanes represent stretch samples. Results are expressed as the percent change from unstretched controls, means \pm SD of three experiments, each with a different preparation of explants. * $P < 0.005$.

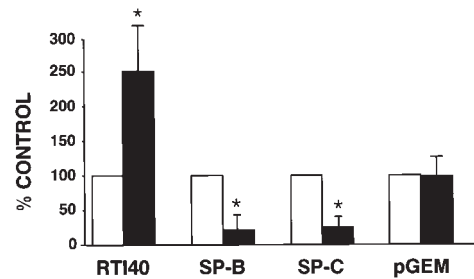


Figure 3. Nuclear run-on transcription. Nuclear run-on transcription assays were performed on nuclei prepared from explants maintained in the distended state for 18 h and from control unstretched explants cultured in the same device. Freshly prepared nuclei were incubated with [α - 32 P]UTP. Radiolabeled RNA was isolated and hybridized to denatured linearized cDNAs for RTI 40, SP-B, and SP-C. Denatured linearized plasmid without an insert (pGEM) was used as a negative control. Filters were subjected to autoradiography before radioactivity was quantitated by volume integration of pixel density by phosphorimaging. One autoradiogram representative of three experiments is shown above the columns, which express the data (means \pm SD) from the three experiments, each with a different preparation of explants. * $P < 0.05$.

Distention Does Not Affect the mRNA Content of SP-A and 18S rRNA in Fetal Lung Explants

A third marker of the type II phenotype, SP-A, was unaffected by mechanical distention. The content of SP-A mRNA after 24 cycles of RT-PCR was not significantly different when RNA from explants from distended membranes was used, in comparison with RNA from explants from control membranes (Figure 2).

Aliquots of RNA were also amplified for 18S rRNA. Mechanical distention did not result in a significant change in the mRNA content of 18S rRNA.

Nuclear Run-On Transcription Assays

To determine whether the effects of mechanical distention on the content of specific mRNAs were due to transcriptional or post-transcriptional changes, we performed nuclear run-on assays. The results are shown in Figure 3. Mechanical distention caused an increase in the transcription of RTI 40 of 2.5 ± 0.8 ($n = 3$, $P < 0.05$) times undistended controls. In contrast, there was a marked decrease in the transcription of SP-B and SP-C. Transcription of SP-B was decreased to $21 \pm 9\%$ ($n = 3$, $P < 0.05$) of that of control unstretched samples, and transcription of SP-C was decreased to $23 \pm 6\%$ ($n = 3$, $P < 0.005$) of that of controls. There were no observed differences in SP-A transcription between nuclei obtained from explants subjected to distention and nuclei obtained from control explants (data not shown). Together, these data show that the changes in mRNA content of RTI 40, SP-B, and SP-C may be due in part to changes at the transcriptional level.

Immunocytochemistry

We did not detect consistent differences in the morphologic appearance of stretched and control explants as examined by light microscopy (Figure 4). We then examined RTI 40 (a marker of the type I cell phenotype) and RTII

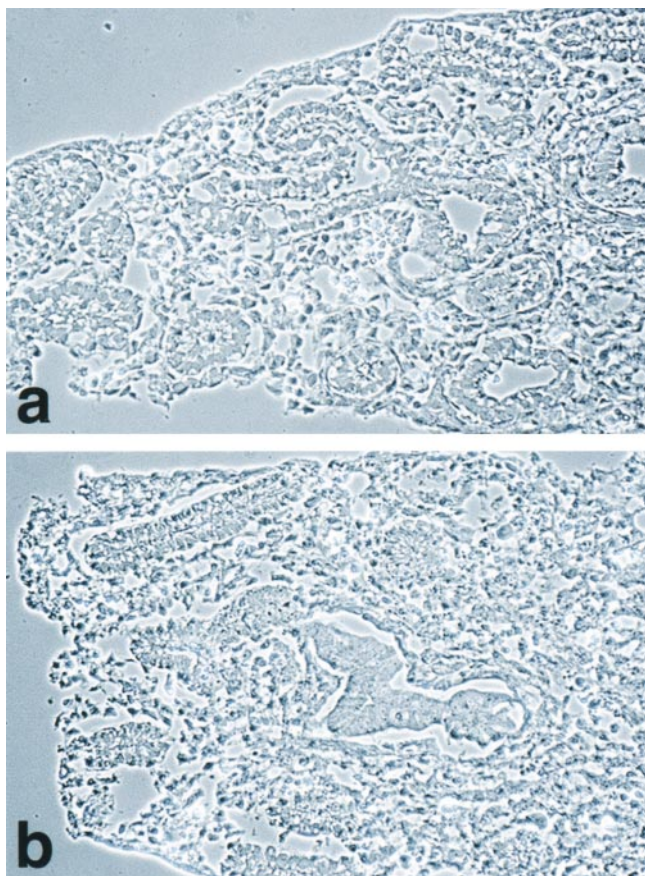


Figure 4. Cultured fetal lung explants. Control (*a*) and stretched (*b*) fetal lung explant tissue. There were no consistent morphologic differences between control and stretched explants (original magnification: $\times 135$).

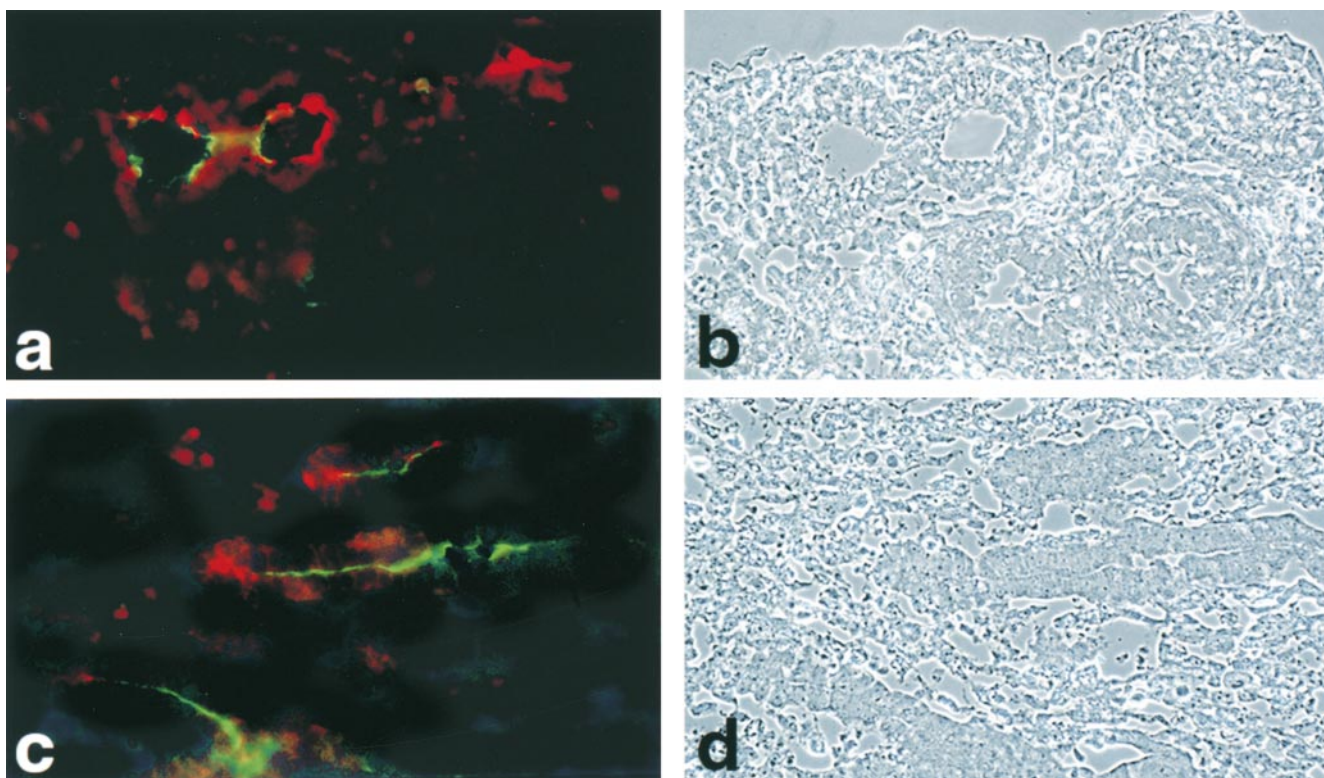


Figure 5. Cultured fetal lung explants. Control (*a* and *b*) and stretched (*c* and *d*) fetal lung explant tissue with double-label immunofluorescence against RTI 40 (*green*) and RTII 70 (*red*). Stretched tissue has a preponderance of RTI 40 over RTII 70. Erythrocytes are weakly autofluorescent (original magnification: $\times 350$).

70 (a marker of the type II cell phenotype) by immunocytochemistry. Stretched tissue exhibited a preponderance of staining for RTI 40 over RTII 70. In contrast, control tissue demonstrated a preponderance of staining for RTII 70 over RTI 40 (Figure 5).

Discussion

Successful adaptation to extrauterine life is dependent upon the development of a normal alveolar epithelium composed of type I cells, which provide both the tight barrier and short diffusion pathway between the air and vasculature that are essential for efficient gas exchange; and type II cells, which synthesize and secrete (2) surfactant, which is responsible for lowering surface tension at the air-liquid interface, preventing alveolar collapse. Although maturation of the surfactant system (5, 6) and growth of the lung (9, 21) have been studied extensively, the factors regulating expression of pulmonary alveolar epithelial phenotype during development have not been defined. On the basis of the results of studies with fetal model systems *in vivo* (10, 12, 15), it has been hypothesized that mechanical factors are important in regulating alveolar epithelial differentiation. From studies performed on fetal sheep lungs *in utero*, in 1977 Alcorn and colleagues (15) concluded that maintaining the fetal lung in an overdistended state by tracheal ligation qualitatively favored the expression of the type I phenotype while inhibiting the expression of the type II phenotype; underdistention of fetal lung *in utero* by chronic tracheal drainage appeared to have the opposite effect. Similar effects were seen with bronchial ligation or drainage (10). However, these initial reports did not provide quantitative data regarding numbers of type I and type II cells or biochemical data supporting these more subjective conclusions. Recent studies have shown that fetal lung distention caused by tracheal obstruction or ligation resulted in a decrease in the number of type II cells and a decrease in mRNA for SP-A, SP-B (22), and SP-C (23), findings consistent with Alcorn and associates' initial hypothesis.

In the present study, we examined a model system in fetal rat lung explants subjected to 18 h of tonic mechanical distention. We used RTI 40, a protein that is localized in the lung to the apical plasma membrane of type I cells (20), as a marker for the differentiated type I cell phenotype. The cDNA for RTI 40 is highly homologous to OTS-8 (24, 25), a sequence isolated from transformed mouse osteoblastic cells (26). RTI 40 has also been called gp 38 (27) and T1 α (25). Although the function of the protein encoded by this gene remains unknown, RTI 40 has proven to be a useful marker of the alveolar type I cell phenotype. We used SP-A, -B, and -C as markers of the differentiated type II cell phenotype. We measured mRNA content of these markers, but were unable to quantitate SP-A, -B, and -C protein in our samples. Mechanical distention of fetal rat lung explants for 18 h resulted in an increase in the mRNA content of a marker of the type I phenotype of approximately 10 times that of controls, and a decrease in the mRNA content of two of the markers of the type II phenotype to 9% (SP-B) to 13% (SP-C) of control values. Interestingly, there was no observed effect on mRNA con-

tent of SP-A, another (although less specific than SP-C) marker of the type II phenotype. The apparent coregulation of SP-B and SP-C expression, both different from SP-A expression, has been observed previously. For example, in the developing lung, agents that increase intracellular cyclic adenosine monophosphate concentration cause an increase in SP-A mRNA but have only a modest effect on mRNA content of SP-B and SP-C (28, 29). In human fetal lung tissue *in vitro*, glucocorticoids exert a marked stimulatory effect on the levels of SP-B and SP-C mRNAs (30, 31), while exerting both stimulatory (at low concentrations) and inhibitory (at high concentrations) effects on the levels of SP-A mRNA (32). In the current study, mechanical distention of rat fetal lung organ culture favored the type I phenotype while inhibiting two of the markers of the type II phenotype. These findings are consistent with studies we have previously reported describing the effects of mechanical distention on cultured type II cells from adult rats (18) as well as with other model systems, in which there is inverse coregulation of the expression of the type I and type II phenotypes (20, 33, 34, and unpublished observations [L. G. Dobbs, University of California San Francisco, San Francisco, CA]).

The observed differences in mRNAs appear to be due primarily to changes at the transcriptional level. Mechanical distention of explants resulted in an increase in transcription of mRNA for RTI 40 of 2.5 times control. In contrast, distention resulted in a decrease in transcription of mRNA for SP-B to 21% of control and a decrease for SP-C to 23% of control values by nuclear run-on transcription assays. Although we have not directly measured mRNA stability, the differences in transcriptional rates of RTI 40, SP-B, and SP-C suggest that the observed differences are regulated at least in part by transcriptional events.

Mechanical forces have been shown to be important primarily for growth of various systems during development. During cardiac development, experimental interruption of flow through the aortic arches results in abnormalities of the internal cardiac anatomy as well as hypoplasia of the left heart and aorta (35). Mechanical tension has also been shown to be a potent regulator of axonal growth rate during neuronal development (36, 37). The developing lung undergoes either intermittent, repetitive changes in lung shape due to fetal breathing movements (38), or chronic distention due to the distending force caused by a transpulmonary pressure of 2.5 mm Hg (39). Numerous studies have shown that perturbations in these forces are known to result in abnormalities primarily in lung growth. Mechanical distention *in vivo* results in an increase in lung size as measured by lung weight and lung-to-body weight ratio (9-12), and studies suggest that the increase in size may be due to alterations in expression of insulin-like growth factors I (22) and II (40). Previous *in vitro* studies with mixed fetal rat lung cells have shown that mechanical stretch stimulates cell proliferation and that these changes are mediated by phospholipase C and D as well as protein kinase C (41, 42). The results of our current study demonstrate that mechanical forces also affect epithelial phenotypic expression of fetal lung.

The objective of the present study was to determine the effects of mechanical distention on the phenotypic expres-

sion of markers of pulmonary alveolar cell differentiation. The data presented here demonstrate that mechanical distention favors the expression of the type I cell phenotype while inhibiting the type II cell phenotype *in vitro* in fetal lung organ culture. These findings provide a molecular link between mechanical forces and the regulation of alveolar epithelial phenotypic expression during fetal lung development.

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