Increased lung inflation induces gene expression after pneumonectomy

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Gilbert, Kirk A., and D. Eugene Rannels. Increased lung inflation induces gene expression after pneumonectomy. Am. J. Physiol. 275 (Lung Cell. Mol. Physiol. 19): L21–L29, 1998.—Rapid hyperplastic growth of the remaining lung is initiated by partial pneumonectomy in many mammalian species. The response restores normal tissue structure and function. Although physiological control of compensatory lung growth is documented, little is known about the molecular mechanisms that underlie the process. The aim of this study was to investigate the role of mechanical signals in the induction of immediate-early gene (IEG) expression after pneumonectomy. Expression of c-fos and junB increased nine- and fourfold, respectively, in the right lung within 30 min after left pneumonectomy in rats. In contrast, changes in expression of c-jun and c-myc were not observed. When isolated lungs were subjected to elevated airway pressures in vitro, expression of c-fos and junB was induced in a time- and dose-dependent manner similar to that observed in vivo. Similarly, in vitro lung perfusion induced c-fos and junB expression in the absence of increasing lung inflation. These results support the premise that rapid changes in IEG expression after pneumonectomy are initiated by mechanical signaling in the remaining lung. Elevated IEG expression may contribute to initialization of compensatory lung growth.

lungs growth; lung perfusion; mechanochemical signals; immediate-early genes; protooncogenes

IN A WIDE VARIETY of mammalian species, partial pneumonectomy induces rapid, hyperplastic compensatory growth of the remaining lung lobes. In rat, the completed response results in restoration of total lung mass and volume (6, 28); DNA, RNA, and protein (28); collagen content (8, 16); cell number (34); and alveoli number (19, 27) to values normal in both lungs of age-matched control animals. Current literature suggests the response requires that lung growth is ongoing at the time of surgery, and, accordingly, clinical observations indicate that a similar compensatory response occurs in infants and young children (20).

Cellular and subcellular mechanisms that signal and regulate compensatory lung growth remain poorly understood. Studies in animals or with isolated lung tissue or cells have led to proposals that several pathways may play a role in the physiological control of the growth response (12). These pathways include 1) postoperative changes in inflation of or blood flow to the remaining lung lobes and 2) release of local or circulating growth-promoting soluble mediators such as hormones or growth factors.

In a number of model systems, lung growth proceeds to fill space available within the thorax (see Ref. 12 for a review). After pneumonectomy and surgical resolution of the associated pneumothorax, transmural pressure gradients inflate the remaining lung into the space vacated by the resected lobe (13). This acute increase in inflation of the remaining lobes during the immediate postoperative interval may play a key role in signaling accelerated tissue growth (see Ref. 12 for a review). Accelerated growth proceeds until normal total lung mass is restored. The later events are consistent with observations that the response to pneumonectomy is absent if the resected tissue is replaced with inert material, thereby preventing increased inflation of the remaining lobes. Compensatory lung growth has been blocked in this manner by plombage with wax (5, 8), Gelfoam sponge (11), or an inflatable balloon (23).

Resolution of mechanisms that account for the regulation of postpneumonectomy lung growth at the cell and molecular levels requires identification and localization of specific markers of the early phases of the response. Specific and rapid changes in expression of some cellular immediate-early genes (IEGs) are associated with the early stages of growth in the liver after partial hepatectomy (32, 35). IEGs are a class of cellular genes, the products of which serve important functions in cell proliferation and differentiation. Products of several IEGs, including the protooncogenes c-fos and the jun family, are nuclear protein transcription factors that form heterodimers and bind to specific DNA elements containing activator protein-1 consensus sequences. The resulting action of these gene products is to activate transcription of target genes containing the activator protein-1 regulatory element. Similarly, the c-myc protooncogene encodes a nuclear phosphoprotein that acts as a transcription factor when complexed as a heterodimer with partner protein Max (4). The level of c-myc expression appears to regulate cell cycle progression in many cell types (7, 14). As has been the case in liver growth after partial hepatectomy, identification of pneumonectomy-induced changes in IEG expression may provide useful information concerning the regulatory mechanisms that underlie compensatory lung growth.

The focus of the present study was to detect and quantify early changes in pulmonary gene expression after pneumonectomy in vivo and to establish whether these changes could be due to increased inflation of the remaining lung. Expression of mRNAs encoding c-fos, c-jun, junB, and c-myc was quantified in the right lung immediately after left pneumonectomy in the rat. In vivo, pneumonectomy initiated rapid and selective changes in gene expression. Increased inflation of isolated lungs in vitro produced similar dose- and time-dependent changes in IEG expression. Together, these results support the premise that rapid changes in the expression of growth-regulatory genes are trig-
determined after pneumonectomy by postoperative increases in lung inflation. Thus changes in lung inflation appear to initiate early events in the postpneumonectomy growth response.

METHODS

Surgical Procedures

In vivo pneumonectomy experiments. Adult male Sprague-Dawley rats (250 g body weight) were used in this study. Under chloral hydrate anesthesia (7.2%, 300 mg/kg), the animals were positioned on their backs; the chest was shaved and cleaned. A 5-cm incision was made in the skin of the left thorax, which was then pulled laterally to expose the muscles of the anterior chest wall. A small incision was made through the muscles of the fourth intercostal space into the left thoracic cavity. An open silk ligature was placed over the incision, and the single lobe of the left lung was gently pulled through the ligature, tied at the hilus, and resected. Mediastinal structures were allowed to retract into the chest cavity. The ribs and chest wall muscles were approximated with polyester sutures, and residual air was removed from the thoracic cavity. After the skin was closed with stainless steel wound clips, the animal was placed on its left side under a mild heat source and allowed to recover. Sham operations were performed in the same manner except that the left lung was not pulled from the thoracic cavity. Total time of the operation was <5 min; survival from the surgical procedures was >95%. Unoperated control animals were administered anesthesia only.

At specific times after left pneumonectomy, the animals were reanesthetized with chloral hydrate as above and killed by exsanguination via the abdominal aorta. The lungs were quickly removed, trimmed free of large airways and other extraneous tissues, rinsed in ice-cold PBS, blotted on filter paper, and frozen between aluminum blocks cooled to the temperature of liquid nitrogen. Tissue samples were subsequently pooled, powdered at the temperature of liquid nitrogen, and stored at −70°C.

In vitro ventilation studies. Adult male Sprague-Dawley rats were anesthetized as described in In vivo pneumonectomy experiments. After the chest was shaved, the animals were placed on their backs in a temperature-controlled (37°C) Plexiglas chamber (Liver Perfusion Apparatus, Vanderbilt University Instrument Shop, Nashville, TN). A tracheostomy was performed, and a stainless steel cannula was inserted into the trachea and tied in place. Mechanical ventilation was established with a solenoid-operated pressure-relief ventilator (model SAR-2D, Analytical Specialists, St. Louis, MO). A warmed, humidified O₂-N₂-CO₂ (20:75:5) gas mixture was delivered at an airway pressure (Pₐw) of 15 cm H₂O, with a positive end-expiratory pressure of 2 cm H₂O, and a rate of 70 breaths/min until any remaining surgical procedure was completed.

The effect of altered lung inflation on gene expression was determined with one of three experimental protocols: protocol 1, mechanical ventilation at different Pₐw values combined with stable exogenous perfusion of the pulmonary vasculature; protocol 2, mechanical ventilation alone at different Pₐw values with the thoracic cavity surgically opened to remove constraints on lung inflation imposed by the thoracic cage; and protocol 3, mechanical ventilation alone at different Pₐw values with the thoracic cage intact. These protocols are detailed in Protocol 1: Mechanical Ventilation Combined With Exogenous Lung Perfusion and Protocols 2 and 3: Mechanical Ventilation Alone With Thorax Open or Closed.

Protocol 1: Mechanical Ventilation Combined With Exogenous Lung Perfusion

The pulmonary circulation was cannulated through the heart for perfusion of the lungs as previously described (29, 37). Both lungs were perfused (pulmonary perfusion [arterial] pressure (Pₐ) set at 20 cm H₂O) and ventilated (Pₐw 15 or 20 cm H₂O) for 30–60 min. Briefly, the surgical procedures were as follows. After tracheal intubation, the abdomen was opened, and the diaphragm was trimmed from the ribs to open the thoracic cavity. The chest was further opened anteriorly along the sternum, and the ribs were reflected laterally using hemostats as retractors. Care was taken to avoid contact with the lungs at any time during the procedure.

To collect venous return, a stainless steel cannula was inserted through the mitral valve and tied in place in the right atrium after an intracardial injection of heparin (10.0 mg/kg in 0.15 M NaCl). To provide arterial flow, a fluid-filled cannula was inserted through the right ventricle and tied in place in the pulmonary artery. Pₐ was set at 20 cm H₂O and remained stable throughout the experiment. The perfusate was Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 4.5% bovine serum albumin and 5.6 mM glucose and was equilibrated with a humidified mixture of O₂-N₂-CO₂ (20:75:5).

Perfusate (100 ml) was placed in a rotating drum, continually oxygenated with the aforementioned gas mixture, and humidified to prevent evaporation. This reservoir supplied the arterial cannula via a peristaltic pump (Gilson Minipuls 2). Venous outflow was returned to the drum. Before recirculation of the perfusate, ~30 ml of buffer were collected to wash the lungs free of blood.

Protocols 2 and 3: Mechanical Ventilation Alone With Thorax Open or Closed

The animals were ventilated at different Pₐw values for 30 or 60 min with the endogenous circulation intact, that is, without the intervention of exogenous perfusion of the pulmonary vasculature. One group of animals was ventilated with the chest opened surgically (protocol 2). The surgery was similar to that described in Protocol 1: Mechanical Ventilation Combined With Exogenous Lung Perfusion except that the pulmonary circulation was not cannulated. A second group of animals was ventilated without further surgical intervention (protocol 3).

RNA Isolation

Total RNA was isolated from whole lung tissue with TRIzol (Molecular Research Center, Cincinnati, OH) according to the protocol of the manufacturer. Samples were resuspended in formamide (Formazol, Molecular Research Center). RNA concentration was determined by measuring absorbance at 260 and 280 nm. All RNA samples were stored at −70°C.

Northern Blot Analysis

RNA samples (20 µg) were denatured and size fractionated on 1.2% agarose gels containing 0.4 M formaldehyde. Even loading of RNA samples was monitored initially by the addition of ethidium bromide to the RNA samples before the gel was loaded, followed by subsequent examination of the fluorescence pattern at the completion of electrophoresis. Fractionated RNA was transferred to nylon membranes (MSI, Westboro, MA) with downward capillary blotting. 32p-labeled probes used for hybridization were the full-length 2.1-kb cDNA specific for rat c-fos and a 1.9-kb cDNA fragment derived from mouse junB (American Type Culture Collection...
no. 63025); a 2.6-kb cDNA fragment derived from mouse c-jun (American Type Culture Collection Jac.1 clone); and a 40-mer oligonucleotide specific for rat c-myc (Oncogene Science, Uniondale, NY). Control probes included a 40-mer oligonucleotide specific for rat glyceraldehyde-3-phosphate dehydrogenase (Oncogene Science) and/or a 2-kb cDNA fragment of the rat homologue to prokaryotic elongation factor Tu (21). All cDNA fragments were labeled with $[^{32}P]dCTP$ by random priming with hexanucleotide primers (Multi-Prime DNA Labeling System, Amersham Life Science, Arlington Heights, IL). The 40-mer oligonucleotides were 5'-end labeled with $[^{32}P]ATP$ with T4 polynucleotide kinase (Promega, Madison, WI).

Hybridization experiments were performed in a rolling incubator at 65°C overnight with a prehybridization and hybridization sodium phosphate-based solution. This solution consisted of 7% SDS, 0.5 M Na$_2$HPO$_4$, 1% nonfat dry milk, and 1 mM EDTA. Washes were performed in 2× saline-sodium phosphate-EDTA (0.3 M NaCl, 20 mM NaH$_2$PO$_4$, pH 7.4, and 20 mM EDTA, pH 7.4) containing 0.5% SDS at 65°C for 30 min. For autoradiography, the blots were exposed to Fuji RX film, with intensifying screens, at 270°C.

Statistics

When multiple samples were analyzed, data (means ± SE) were subjected to two-tailed t-test analysis; differences were considered significant at the P < 0.05 level.

RESULTS

Changes in Gene Expression After Pneumonectomy

Initial experiments were designed to establish the postpneumonectomy time course of change in the pulmonary expression of the cellular IEGs c-fos, junB, c-jun, and c-myc. Differential expression of some, but not all, IEGs was evident during the early postoperative interval. This is shown both qualitatively and quantitatively in Figs. 1 and 2, respectively. Within 15 min after surgery, the earliest time point examined, pneumonectomy increased expression of mRNA for c-fos and junB (Figs. 1 and 2) compared with that in lungs of sham-operated or unoperated control animals. Peak expression of the c-fos message was elevated five- to sevenfold above that in control lungs 30 min after pneumonectomy and returned to basal values after 2 h (Fig. 2A). Similarly, junB message levels peaked 30 min after pneumonectomy (Fig. 2A). In contrast, both c-jun and c-myc remained essentially unchanged throughout the early postpneumonectomy interval (Figs. 1 and 2B). When the level of gene expression was low, accuracy of detection of mRNA expression was limited, primarily for c-myc. Thus, in some experiments, poly(A)$^+$ RNA was used in Northern analyses. Generally, the changes in gene expression were similar when the analysis was based on either poly(A)$^+$ or total RNA.

Additional experiments were performed 30 min after pneumonectomy to characterize the consistency of changes in IEG expression among individual animals.

![Fig 1. Altered gene expression after pneumonectomy (P). Representative autoradiographs depict early time course of immediate-early gene expression. Northern blot analysis was performed with 5 µg of poly(A)$^+$ RNA pooled from 4 animals/time point to quantify expression of c-fos, junB, c-jun, and c-myc mRNAs. Each blot was normalized against control gene expression with either glyceraldehyde-3-phosphate dehydrogenase (G$_3$PDH) or rat homologue to elongation factor Tu (EFTu) (21). Unoperated (U) and sham-operated (S) animals served as controls.](http://ajplung.physiology.org/)
In one group of rats, the lungs were perfused with PBS before resection and subsequent RNA isolation. This control was included for two reasons: to determine whether perfusion procedures alone altered IEG expression and to eliminate the possibility that increased IEG mRNA expression could, in part, reflect a contribution of blood components. Autoradiographs in Fig. 3A show qualitatively that c-fos expression was elevated 30 min postsurgery in all animals subjected to left pneumonectomy independent of whether the pulmonary vasculature was washed with PBS. Quantitatively, c-fos expression was elevated above that in sham-operated and unoperated control animals in both washed and unwashed postpneumonectomy tissue samples (Fig. 3B).

On the basis of these results, additional experiments were performed 30 min after surgery; c-fos, junB, c-jun, and c-myc message levels from pneumonectomy, sham-operated, and unoperated groups were quantified and compared to confirm statistical significance of the apparent changes in gene expression. Left pneumonectomy consistently increased c-fos and junB expression in all animals (Fig. 4A). Within 30 min, a ninefold increase in c-fos mRNA was observed, with a fourfold increase for junB (Fig. 4B). These results confirm changes in expression observed in pooled tissue samples (Fig. 2). Similarly, as in pooled samples, both c-myc and c-jun mRNA remained low after pneumonectomy compared with both sham-operated and unoperated control animals (Fig. 4).

Changes in Gene Expression In Vitro

To investigate the premise that postoperative changes in lung inflation cause induction of IEG expression in vivo, isolated rat lungs were subjected to increased inflation in vitro (37). Initial experiments combined mechanical ventilation with exogenous perfusion according to protocol 1 (see METHODS). When the lungs were both mechanically ventilated (Paw 20 cmH2O) and perfused in vitro (Pa 20 cmH2O), expression of both c-fos and junB was elevated (Fig. 5). In apneic lungs (Paw 0 cmH2O), where the ventilator was disconnected but the lungs were continually perfused, expression of c-fos and junB was also elevated independent of increased Paw.
Expression of c-fos was greatest in experiments of 30-min duration (Fig. 6), whereas junB expression was greater after 60 min (Fig. 6), suggesting differing time courses of induction. Lung dry-to-wet weight ratios determined for each individual animal were not statistically different among groups (data not shown), indicating that pulmonary edema did not contribute to changes in gene expression.

Control animals were again included in these experiments to determine whether the intubation and washout procedures accounted for changes in c-fos or junB expression. These animals were intubated and perfused in the same manner as the experimental groups (Paw, 15 cmH2O, 70 breaths/min, Pa, 20 cmH2O) to clear the pulmonary circulation. As shown in Fig. 6 (Control), these procedures did not alter the expression of c-fos or junB. The above experiments from protocol 1 thus revealed that continuous exogenous perfusion of the pulmonary vasculature for 30–60 min caused maximal induction of c-fos and junB expression such that any further change in expression caused by altered inflation was not evident.

On the basis of these observations, only ventilatory parameters were altered in subsequent experiments. According to protocols 2 and 3, the endogenous circulation remained intact in 30-min experiments with preset levels of Paw. Results from protocol 2 (chest opened surgically) revealed that both c-fos and junB gene expression were elevated significantly in a linear, dose-dependent manner when the lungs were ventilated at increasing Paw values with the thorax opened surgically (Figs. 7 and 8).

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In contrast to the twofold increase in c-fos gene expression in open-chest preparations, increasing P_{aw} from 15 to 20 cmH2O had no significant effect on c-fos expression when the thoracic cage remained intact (data not shown). Similarly, increasing positive end-expiratory pressure from 2 to 10 cmH2O while maintaining a P_{aw} of 20 cmH2O had no additional effect on the expression of c-fos or junB (Fig. 8). These results suggest a relationship between maximum inflation pressure and the induction of IEGs.

**DISCUSSION**

Although the general characteristics of compensatory lung growth after pneumonectomy have been described in detail, mechanisms that regulate the response at the cellular and molecular levels have not been characterized. Additionally, pathways involved in the induction of IEG transcription have not been completely defined. Nevertheless, understanding the underlying mechanisms of stimulus-transcription coupling of IEG expression after pneumonectomy is essential to define the early events in compensatory lung growth. Most IEGs function by coupling stimuli received at the cell surface to regulation of target gene expression and ultimately to changes in cell phenotype. The present results show that pneumonectomy induces a consistent, rapid, and selective increase in the expression of growth-associated IEGs in the residual lung lobes. Furthermore, the data demonstrate that IEG expression is progressively increased in vitro with increasing lung inflation, thus reproducing results observed in vivo. These observations provide strong evidence in support of a role for postoperative increases in lung inflation as initiating signals for compensatory growth of the tissue.

Mechanical stimuli such as increased inflation or blood flow in the remaining lung likely contribute to the regulation of compensatory lung growth (26). Inhibition of compensatory growth by plombage (5, 8, 11, 23) provides indirect evidence that postoperative changes in lung inflation play a role in regulation of the response. These observations also are consistent with the induction of c-fos and junB expression by elevated P_{aw} in open-chest (Figs. 7 and 8) but not in closed-chest rats. Removal of inflation constraints imposed by the thoracic cage allows a greater expansion of the lung, thereby introducing increased mechanical forces that may cause altered gene expression. Consistent with this premise, Berg et al. (3) recently demonstrated that elevated lung inflation in open-chest rabbits causes induction of mRNAs coding for extracellular matrix components, basic fibroblast growth factor, and transforming growth factor-β1.

Left pneumonectomy changes the distribution of pulmonary blood flow in that the entire cardiac output is diverted to the right lung. Although the resulting 50% increase in flow is accommodated by the remaining vasculature (36), it is likely that transitions in pulmo-
nary vascular pressure occur immediately after lung resection, thus providing an additional source of mechanical signaling in the remaining lung. Associated changes in shear stress may contribute to the induction of gene expression. Khachigian et al. (15) identified a 6-bp core sequence in the promoter regions of several genes that are regulated by alterations in fluid shear stress. This sequence, termed the shear-stress response element, is a putative transcription factor binding site. The shear-stress response element core sequence is present in the 5' region of c-fos, c-jun, and junB but not of c-myc, suggesting that transcription of some IEGs may be modulated by shear stress. In the present study, in vitro lung perfusion over 30–60 min at 20 cmH2O P^aw resulted in maximal expression of both c-fos and junB such that a further increase in expression could not be elicited by increased inflation. Similarly, Xu et al. (38) demonstrated elevated c-fos and c-jun expression when rat arteries were subjected to acute elevations in blood pressure. Thus acute changes in pulmonary blood volume, perfusion pressure, and/or blood flow to the remaining lung after pneumonectomy may induce IEG expression and contribute to compensatory lung growth.

Several other organs exhibit compensatory growth in response to partial resection. The most thoroughly studied is that of compensatory liver growth after partial hepatectomy. Like compensatory lung growth, compensatory liver growth is hyperplastic and proceeds until normal liver mass is restored. Several investigators have examined liver IEG expression after partial hepatectomy, demonstrating elevated levels of c-fos and c-myc transcripts (25, 32, 35) as well as of c-jun and junB (25). Increased IEG expression after hepatectomy is thought to prime hepatocytes for entry into the cell cycle and subsequent replication, thereby providing initial steps necessary for restoration of liver mass. The mechanism of cellular IEG induction after partial hepatectomy, however, remains unknown. As in the present study, c-fos expression is elevated within 15 min after partial hepatectomy, but unlike the lung (7, 24), the c-myc message is also increased between 30 min and 2 h postoperative (35). Additional work confirmed similar changes in c-myc after partial hepatectomy, although elevated c-myc expression was also observed during inflammation (33).

Compensatory growth also occurs in the kidney after unilateral nephrectomy, although it is primarily a hypertrophic response. Cell growth occurs in the absence of proliferation or differentiation; quiescent cells undergo hypertrophy without entering the cell cycle or undergoing phenotypic change (10). Consequently, IEG expression during compensatory renal hypertrophy differs from systems in which hyperplasia predominates. Although it has been reported that c-myc, c-H-ras, and c-fos remain unchanged after unilateral nephrectomy (2), the earliest time point examined was 2 h postoperative, well past the time of peak c-fos expression in other models. IEG expression clearly differs, however, between renal hypertrophy and induced renal hyperplasia (18), suggesting that compensatory renal hypertrophy involves a unique pathway of cell growth.

Fig. 7. Lung c-fos and junB expression in open-chest animals ventilated at different levels of lung inflation. A: Northernblots of lung total RNA (20 µg) from open-chest animals subjected to 30 min of ventilation in situ at 15 or 20 cmH2O P^aw. Unoperated animals were used as controls. EFTu was control probe. B: normalized c-fos and junB expression. Data are means ± SE.

Fig. 8. Dose-response expression of c-fos and junB in open-chest animals with increasing P^aw. Northern blot analysis of normalized lung c-fos and junB expression in lungs ventilated for 30 min at P^aw levels ranging from 15 to 25 cmH2O positive inspiratory pressure (PIP) and 2 cmH2O positive end-expiratory pressure (PEEP). Inset, linear dose-response relationship for both c-fos (r = 0.973; □) and junB (r = 1.000; ●) expression over a range of P^aw levels. Data from each experimental group were derived from 4 animals and are means ± SE. Slopes of lines differ by a factor of 4. Lung dry-to-wet weight ratios were not statistically different among groups (data not shown).
that does not follow the same course of events observed in other models of hyperplastic compensatory growth. Several studies have demonstrated increased IEG expression in response to increased mechanical forces administered at the tissue level. In in vitro models of cardiac hypertrophy, expression of c-fos and c-jun is elevated three- to fourfold when isolated, beating rat hearts are subjected to elevated systolic wall stress (31). Fos protein is confined mainly to cardiac myocytes, with little immunostaining in fibroblast or vascular cell populations (31). Compensatory hypertrophy of heart muscle in response to an elevated workload is accompanied by increased expression of several IEGs (see Ref. 39 for a review). A study (17) using cultured cardiac myocytes suggested that IEG expression may be elicited by mechanical signals at the cell surface, thereby initiating signal transduction pathways that lead to gene transcription. Strain in cardiac myocytes plated on deformable substrates also induces transient c-fos expression (17) and causes activation of numerous second messenger pathways that ultimately result in c-fos induction (30). Similar elevations in c-fos mRNA expression, as well as expression of other IEGs, in response to mechanical strain have been reported in glomerular mesangial cell cultures (1), vascular smooth muscle cell cultures (22), and gastric myenteric neurons (9).

In summary, partial pneumonectomy in the rat results in the induction of some IEGs during the early postoperative interval. Similar changes in IEG mRNA levels are produced independently in vitro by increased lung inflation and exogenous lung perfusion. These data suggest that expression of IEG products may be increased by mechanical signaling mechanisms that are initiated by lung resection. This selective elevation in gene expression may be a prerequisite event for the initiation of compensatory growth. Identification of downstream target genes that may be regulated by the transient, pneumonectomy-induced expression of c-fos and junB remains to be established. The present results encourage further investigation of the role that IEG products may play in the response to pneumonectomy.

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