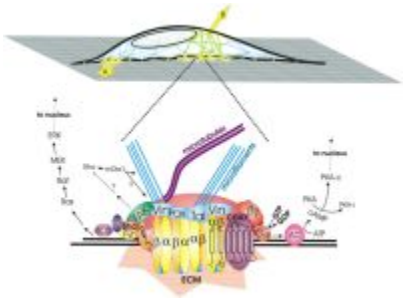
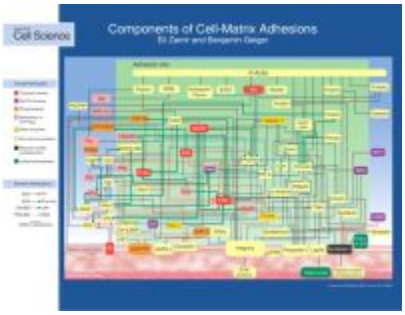
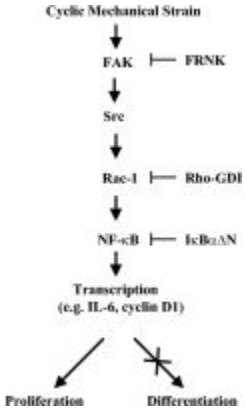
Focal Adhesions

Focal Adhesions

Most papers tend to focus more on elucidating the pathway rather than looking for cis regulatory elements in the stress-upregulated promoters. Papers of interest/ ones that need attention are marked in the section "Important/ Of Interest". Figures of pathways are included for interest in case we want to find binding sequences for specific TFs in our synthetic promoters.







Important/ Of Interest

Giant list of upregulated genes in primary fibroblasts in response to mech strain

Cyclic stretch response elements in Fibroblast Procollagen 1(I), possibly TGFB dependent

Mechanical Stretch Up-Regulates the Human Oxytocin Receptor in Primary Human Uterine Myocytes

Could be an interesting paper but need access

Role of Protein--Protein Interaction in Mechanosensation

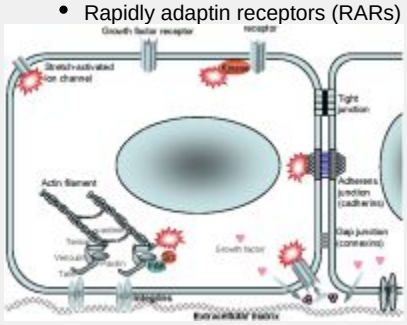
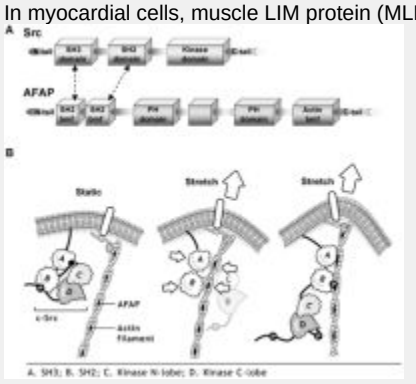
- Examples of mechanosensors in various organisms
  - Touch receptors in C. elegans
  - bristle receptors in Drosophila
  - hair cells of inner ear
  - brain sodium channel 1: essential for mechanosensory complex in skin for detection of light touch
  - plycystins: cell membrane proteins, mediate mechanosensation in primary cilium of kidney cells
  - Rapidly adaptin receptors (RARs) in lungs, associated with voltage-gated ion channels
- 
  Mechanosensors on the cytoplasma membrane. Stretch-activated ion channel, receptors for growth factors and other ligands, ECM-integrin-cytoskeletal complex are commonly accepted as mechanosensors on the cytoplasm membrane. Intercellular cell-cell adherens junctions and gap junctions are also involved in mechanical force-induced signaling.
- Stretch-activated ion channels
  - Can be cell-type specific
  - See Paper
- ECM-Integrin-Cytoskeleton Complex
  - Integrin attaches cytoskeleton to ECM
  - See Paper
- Growth Factor Receptors
  - In fetal rat lung cells, PDGF and its receptors were also shown to be involved in stretch-induced cell proliferation, but it was considered through an autocrine or paracrine mechanism
  - compressive stress shrinks the lateral intercellular space surrounding airway epithelial cells, increasing local ligand concentrations, which triggers cellular signaling via autocrine binding of epidermal growth factor family ligands to the epidermal growth factor receptor in airway epithelial cells
- Intercellular Mechanical Signaling
  - intercellular calcium propagation is mediated through gap junction proteins, connexins
  - forces applied to adherens junctions in fibroblasts activate stretch-sensitive calcium-permeable channels and increase actin polymerization; these effects appear to be mediated via N-cadherins
- Intracellular Protein-Protein Reactions
  - In myocardial cells, muscle LIM protein (MLP) in the Z-disc complex has been proposed as a stretch sensor
  - 

    Figure 2. Schematic model of protein--protein interaction through specific binding as a mechanism for intracellular mechanosensation. (A) Proteins are built by functional modules. Protein tyrosine kinase c-Src and actin-filament associated protein (AFAP) are used as examples to illustrate the specific binding and interaction between proteins. Bmf: binding motifs. (B) Physical force transmitted via the cytoskeleton may increase the contact between AFAP and c-Src. The high-affinity Src binding sites on AFAP may competitively bind to c-Src, and lead to its activation by SH2 or SH3 domain displacement.
  - c-Src (protein tyrK) activation by stretch, pressure overload, shear stress, common response to external mech stimuli
  - adaptor protein AFAP links signaling molecules to actin filaments, and it is also involved in the actin-filament organization and Src-related intracellular signal transduction
  - kinase activity of c-Src at low basal level maintained by 1)binding of SH3 domain to linker between the SH2 domain and the kinase domain; 2) binding of its SH2 domain to the phsophorylated tyrosine residue 527 in its C-terminal tail

Up-regulation of COX2 Expression by Uni-axial Cyclic Stretch in Human Lung Fibroblast Cells

I looked up the COX2 gene online but couldn't find the promoter sequence.

- Cell line: Human lung fibroblast cell line (TIG-1)
- Mech Stimulation: uni-axial cyclic stretch
- Measured mRNA levels
- Application of Gd3+, which blocks stretch-activated channels, and removal of extracellular Ca2+, inhibited COX2 mRNA levels
- Similar Paper Showed that NF-kB is involved. Same cell line, similar results for Gd3+. In response to uni-axial cyclic stretch, NF-kB was found to be translocated into the nucleus. The NF-kB was first detectable 2 min after the onset of stretch and then peaked at 4 min and returned to the basal level within 10 min. However, NF-kB response may be cell type dependent as other papers using different cell lines showed that cyclic stretching did not lead to any response in NF-kB.
- Possibly of interest: Inhibition of mechanical strain-induced fetal rat lung cell proliferation by gadolinium, a stretch-activated channel blocker.

Review: MS at cell-matrix and cell-cell contacts

- Mechanotransduction at mesenchymal cells relies primarily on cell-matrix contacts, whereas that in epithelial cells makes use of both cell-matrix and cell-cell contacts. All cells may be exposed to shear and hydrostatic stresses from surrounding fluid.

Review of Methods

- Early experiments: spatially uniform mechanical environments, measured averaged response of cells to these stimuli.
    - To control the degree of cell spreading, cells were cultured on substrates coated with different densities of ECM proteins, denser--> better spread.
    - To control the rigidity of the substrate, cells were cultured on thin flexible sheets of polymer, or in three-dimensional gels of ECM (usually collagen) that were cross-linked for different times. \
    - To control the ability of a cell to generate contractile forces, cells were cultured in the presence of agents that destabilized or enhanced cytoskeletal filaments throughout the entire cell.
- New Techniques (see paper for more details)
    - Application of localized forces to specific areas of cell membrane
    - Measurement of forces exerted by cells
    - controls where cells can exert force with subcellular resolution

Mechanotransduction at Cell-Matrix Contacts

- Focal Adhesions (FA)
    - All adherent cells bind to the ECM through integrins---transmembrane receptor.
    - The binding of integrins to the ECM causes them to cluster and leads to the recruitment of a battery of cytoplasmic signaling and structural proteins to form FAs at the site of integrin clustering. Numerous structural proteins (e.g., vinculin, talin, -actinin, and paxillin) act as scaffolding proteins that strengthen cell adhesion by anchoring FAs to the actin cytoskeleton
    - four different cell-matrix adhesions (FAs, focal complexes, fibrillar adhesions, and three-dimensional matrix adhesions)
    - mechanical forces have a direct role in the formation of FAs. application of force causes FAs to increase in size, to stabilize, and to strengthen their coupling to the cell (presumably through the actin cytoskeleton). Externally applied mechanical forces can replace the activation of Rho-associated kinase (ROCK), a downstream effector of Rho that regulates cell contractility, but cannot replace the function of mDia1, an effector of Rho that allows actin to polymerize
    - Many signaling proteins (e.g., src, FAK, Ras) localize within cell-matrix adhesions; growth factor receptors themselves are thought to localize to FAs.
    - Control progression through cell cycle: inhibition of Rho greatly reduces the formation of FAs. Second, cell spreading increases the total area of FAs quantity in a tension-dependent manner. Third, the activation of FAK reduces the activation of Rho.

Mehanotransduction at Cell-Cell Contacts

- Types of cell-cel adhesions:
    - Ajs (Adherens Junctions): cadherins
    - Tight junctions: occludins
    - Gap junctions: connexins
    - Some blood borne cells: integrins
- AJ = most important for transmitting mech signal directly to actin cytoskeleton
    - Very similar to Fas
    - Cadherin-based adhesions can sustain large forces: E-cadherin >100nN to separate
    - Important components: vinculin (imp in Fas, unknown for AJ)
    - B-catenin link cadherin to actin, phosphorylation -> nucleus, interact with TCF/LEF Tfs to reg expression of cyclin D, c-myc -> cell cycle

Fibroblasts in Mechanically Stressed Collagen Lattices Assume a “Synthetic” Phenotype

*Fibroblasts grown in stretched lattices compared with relaxed lattices

- (Pretranslational) Induced: alpha1,2,aand3 colalgen, al three alpha-chains of type VI colalgen, fibronectin, beta-actin. Elastin slightly incuded. MMP-1 strongly repressed.
- Comparative promoter analysis: core sequence of GAGACC, same as PDGF

- Used: Fibroblasts (primary)
- 3D collagen lattice
- 0.23 Hz (force transducer: KG 7A with Bridge-Amplifier DUBAM 7C, Sci Instruments)
- Mech strain device (FX-3000, Flexcell International Corp): radial and circumferential cellular strain on monolayer

Table I

Selection of genes with known function induced in dermal fibroblasts grown in mechanically stressed lattices analyzed by cDNA microchip analysis
Differential expression1-aTranscriptGenBank™ accession numbers| A. Cell-cycle regulators and proliferation-associated genes | **Fold increase >6 are bolded**

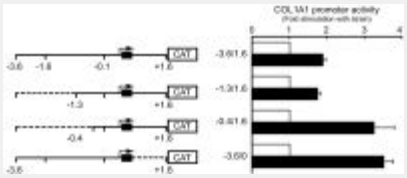
4.6	Proliferation-associated protein 2G4	U59435
3.3	Human minichromosome maintenance (*MCM2*)	AA251518
3.0	Human RAS-related nuclear protein (RAN)	AA305368
2.9	RAN-binding protein 1	AI334312
2.9	Cyclin D3	AI356287
2.6	Proliferation-associated gene A (peroxiredoxin 1)	AA315886
B. Intracellular modulators/ion channels/signal transducers		
6.3	**Human leukemia virus receptor 1**	L20859
4.5	Tax interaction protein 1 (*TIP1*)	AA292422
4.3	Chloride intracellular channel	N27723
3.7	Annexin I	AA348570
3.6	Calmodulin 1 (phosphorylase kinase)	AI205289
3.4	Rho GTPase activating protein 1	AA443506
3.1	Calbindin 2	AI199351
3.0	Myosin, light polypeptide 1 (skeletal fast)	M312211
3.0	Myristoylated alanine-rich PKC substrate	AA931788
2.3	Shc-transforming protein	X68148
2.3	Annexin IV	D78152
C. DNA synthesis/modification/transcription and nucleoside metabolism		
3.4	DNA (cytosine-5)-methyltransferase 1	AA296506
3.3	AHNAK nuclear protein (desmoyokin)	M80899
3.1	DNA-directed RNA polymerase II	N85585
3.0	Replication protein A2	AA130737
3.0	Nucleoside phosphorylase	H64313
D. Transcription factors and DNA-binding proteins		
7.6	**Inhibitor of DNA-binding 1 (*Id1*)**	S78825
6.8	**Inhibitor of DNA-binding 3 (*Id3*)**	AL021154
3.4	Homeobox protein B2	X16665
3.3	Muscle segment homeo box 1 (*MSX1*)	AA464197
2.9	Forkhead-related activator 4 (*FREAC-4*)	U59831
E. Receptors and cell surface proteins		
15.9	**Podocalyxin-like protein (PCLP)**	N80294
9.9	**FGF-inducible protein 14 (*Fn14*)**	AI492143
4.0	Endoglin	AA565269

3.1	Bradykinin receptor B2	AA460102
2.4	Syndecan 1	R22500
2.1	Thrombomodulin	R78072
F. Cytokines, growth factors, and chemokines		
7.0	**Cyr61**	AA459762
6.2	**Vascular endothelial growth factor-C (*VEGF-C*)**	H07991
3.2	*ENA-78*	U12709
2.8	Hepatoma-derived growth factor (HDGF)	D16431
2.1	Connective tissue growth factor (CTGF)	AA852384
G. Protease inhibitors		
10.3 Cis and Trans Regulators of PAI Promoter	**Plasminogen activator inhibitor 2 (*PAI-2*)**	H81869
3.8	Protease inhibitor 8 (*PI-8*)	L40377
3.4	Plasminogen activator inhibitor 1 (*PAI-1*)	AA296506
2.3	Tissue inhibitor of metalloproteinase 3 (*TIMP-3*)	AA115348
2.1	Tissue inhibitor of metalloproteinase 1 (*TIMP-1*)	AI199040
H. Extracellular matrix proteins		
2.9	Collagen 1(I)	AA456983
2.1	Tenascin-C	AA134100
I. Cytoskeletal components		
5.1	-Tubulin	AA393046
3.6	-Smooth muscle actin	X13839
3.0	-Centractin	Z14978
2.8	Tropomyosin 1	AA093973
2.7	Microtubule-associated protein	AI124707
2.6	Filamin A (actin-binding protein 280)	AA852906
J. Focal adhesion components		
5.3	Leupaxin	AI289901
3.5	Moesin	F08375
3.3	Integrin-linked kinase	U40282
3.2	Zyxin	X94991
2.7	Vinculin	N87853

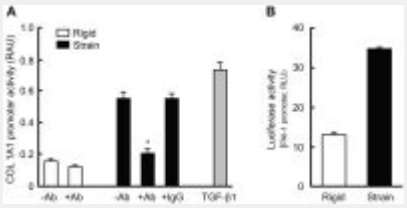
- !F2.medium.gif|thumbnail!** Modulation of TGF -1, -3, and CTGF mRNA levels in stressed and relaxed collagen gels. A, human dermal fibroblasts were cultured in stressed or relaxed () collagen gels for 4, 12, and 20 h. Expression levels of TGF-1, -3, and CTGF mRNA were analyzed by Northern hybridization. In parallel, cells were grown on plastic tissue culture dishes (M) for 20 h.B, methylene blue-stained membranes after transfer, displaying equal loading and 18S rRNA bands.
    - Similar data exist for most other proteins in table

Activation of Fibroblast Procollagen 1(I) Transcription by Mechanical Strain Is Transforming Growth Factor--dependent and Involves Increased Binding of CCAAT-binding Factor (CBF/NF-Y) at the Proximal Promoter

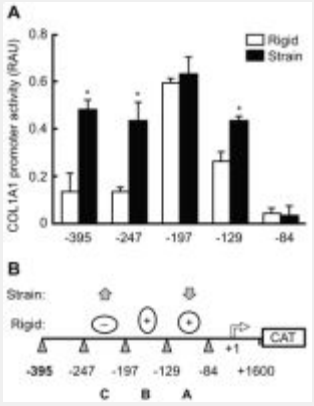
- identify and characterize mechanical strain-responsive elements in the rat procollagen 1(I) (COL1A1) gene
  - increased mechanical load enhances the expression of the COL1A1 gene both in vivo(1) and in isolated fibroblasts in vitro
    - COL1A1 and COL1A2 encode the 1 and 2 chains of type I collagen
    - controlled primarily at the level of transcription and is tightly regulated
  - activity of COL1A1 promoter constructs increased between 2- and 4-fold
  - cardiac fibroblasts
  - continuous cyclic mechanical strain 48 hrs
- Inclusion of a pan-specific TGF- neutralizing antibody inhibited strain-induced COL1A1promoter activation
- Deletion analysis -> two potential strain response regions in proximal promoter, one of which contains an inverted CCAAT-box overlapping a GC-rich element.
  - Both mechanical strain and exogenously added TGF-1 enhanced the binding activity of CCAAT-binding factor, CBF/NF-Y, at this site.
  - Confer strain-responsiveness to an otherwise unresponsive SV40 promoter.
- Stress responsive elements have been shown to bind transcription factors Sp1, NF-I, AP1, Smad proteins
  - Smad: major effector molecules responsible for propagating TGF- signaling following receptor activation
  - CCAAT-binding factor site : electrophoretic mobility shift assays (EMSAs) demonstrated that the ATTGG sequence, within the --147 to --121 fragment, is critical to bind nuclear proteins in the proximal *COL11A1* promoter. We demonstrated that the CCAAT binding factor (CBF/NF-Y) bound to this region using an interference assay with consensus oligonucleotides and a supershift assay with specific antibodies in an EMSA.



Cyclic mechanical strain activates the ratCOL1A1 promoter. Serum-starved rat cardiac fibroblasts were transfected with four collagen promoter deletion CAT reporter constructs shown schematically on the left. The 1.6 mark in the full-length construct indicates a region around position 1.6 kb, upstream of the transcription start site, in which a major TAE has previously been reported (18). Dashed linesrepresent deleted sequences. Angled arrow indicates the transcription start site. The graph on the right shows fold increase in CAT protein levels in response to 48 h of mechanical strain (black bars) over rigid control cells (white bars) for each construct. CAT protein levels in cell lysates were measured by ELISA as described in “Experimental Procedures.” Transfection efficiency was controlled for by using the Hirt's assay. Data shown are mean ± S.E. (n = 3 wells) from a representative experiment repeated four times. All constructs displayed a significant increase ($p < 0.01$) in promoter activity in response to mechanical strain compared with rigid controls as estimated by the Mann-Whitney U test of data from combined experiments.



Activation of the rat COL1A1promoter is TGF--dependent. A, CAT protein levels were measured in rigid (white bars) and strained (black bars) cells, which had been transfected with the pColCAT-1.3/1.6 construct (lacks the reported TAE at position 1624, Ref. 18), in the presence (+Ab) or absence (Ab) of a pan-specific TGF- neutralizing antibody. The amount of TGF- antibody (75 g/ml) was such that it neutralized 90% of 1 ng/ml TGF-1 added exogenously as determined by the manufacturer. The same amount of IgG control antibody was added as a negative control. TGF-1 (1 ng/ml) was added to some cells as a positive control for TGF- responsiveness of this construct (gray bar). Values presented are the mean relative absorbance units (RAU) ± S.E. from a representative experiment (n = 3 wells).B, conditioned medium from the cells in A was analyzed in a TGF- bioassay based on Mv1Lu stably transfected with a TGF--responsive PAI-1 promoter luciferase reporter (32). Data presented are mean ± S.E. luciferase activity expressed as relative light units (RLU). Medium from strained fibroblasts gave a 2.6-fold increase in luciferase activity in Mv1Lu cells, compared with medium from rigid cells ($p < 0.01$ by Mann-Whitney). These luciferase activities were equivalent to 12versus 30 pg/ml of TGF-1 in medium from rigid and strained fibroblasts, respectively, as extrapolated from a standard curve from mink lung cells treated with exogenous porcine TGF-1.

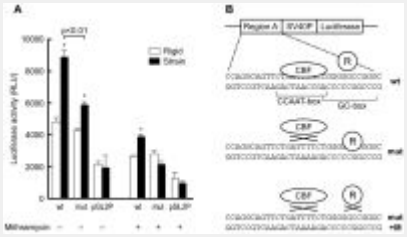


A, promoter activity of deletion constructs of the proximal promoter in rigid (white bars) and mechanically strained (black bars) cells. The number under each set of bars indicates the 5-end position (in base pairs from the transcription start site) of the promoter fragment for that construct. Asterisk denotes a significant increase compared with rigid controls for each construct atp < 0.01 estimated by the Mann-Whitney Utest from combined experiments. B, proposed mechanisms of transactivation of the rat COL1A1 promoter under basal condition and during mechanical strain (arrows). Up arrow indicates lifting of repression in Region C and down arrow indicates increased transactivation in Region A by mechanical strain. Angled arrow indicates the transcription start site.

- The region between position 247 and 197 (Region C) contains a strong repressor element, which under basal conditions counteracts a positive element in the region between 197 and 129 (Region B)
- During mechanical strain the repression in Region C is lifted (indicated by theup-arrow in Fig. 3 B) leading to activation of transcription.
- significant portion of the strain response appears to be mediated via sequences downstream of position 129
- fragment denoted Region A (129 to 84) may be involved in mediating the strain response observed using construct 129. Transcriptional activation of the COL1A1gene by mechanical strain is, therefore, a complex process involving at least two regions (Region A and B/C) in the proximal promoter, which, estimating from the activities of the individual constructs, contribute approximately equally to the response.

*Conferring strain-induced transactivation to hereologous promoter (SV40)

- wild-type Region A oligonucleotide (Region A wt) containing the overlapping CCAAT- and GC-boxes used in the EMSAs was cloned into the luciferase reporter gene vector pGL2P



A Region A oligonucleotide containing the overlapping proximal inverted CCAAT-box and downstream GC-box can confer strain-induced transactivation on a heterologous promoter. A, promoter activity of the pGL2P vector with the wild-type Region A oligonucleotide (wt), mutated Region A oligonucleotide (mut), or pGL2P alone in cells after 30 h of rigid (white bars) or strained (black bars) conditions, with and without 50 nm mithramycin (M). Data presented are from one representative experiment (n = 3 wells) of two repeats, which gave similar results. Asterisk denotes a significant increase compared with rigid controls for each construct/condition at p< 0.01 calculated by the Mann-Whitney U test from combined experiments. In the absence of mithramycin, there was a significant reduction in strain response for the mutated construct compared with the wild type at p < 0.01 by analysis of variance (indicated by the line above the two-strain data bars). B, diagram of heterologous promoter construct (Region A oligonucleotide cloned in front of the SV40 promoter without enhancer in pGL2P) and proposed transcription factor binding to the Region A (wt) sequence (top), the mutated Region A (mut) sequence in the absence (middle), or presence (bottom), of mithramycin (M). R represents an uncharacterized transcription factor, likely to be a repressor, previously designated IF2 (40). CBF and the repressor compete for binding at this site, with CBF having higher affinity. Crosses denote inhibition of binding, either to the CCAAT-box by mutations or to the GC-box by mithramycin.

Mechanical Stretch Up-Regulates the Human Oxytocin Receptor in Primary Human Uterine Myocytes

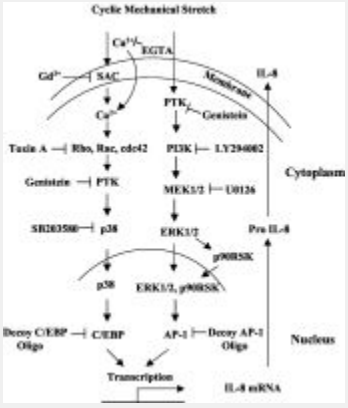
- To investigate the effect of stretch on the transcriptional regulation of OTR, primary NL myocytes were transiently transfected with a construct of the OTR promoter (1.1 kb from the transcription start site) linked to a LUC reporter gene. CMV-Renilla vector was used to control for transfection efficiency. The myocytes were exposed to 16% stretch for 6 h. We found that mechanical stretch resulted in a significant increase in OTR-PROM activity in transiently transfected human primary myocytes control (100 vs. 134% (4%), n = 6, P = 0.001, paired t test)

- EMSAs were performed to identify the presence of specific DNA binding to consensus oligonucleotide probes for C/EBPß, AP-1, and NF-kB and identify any change in DNA binding with stretch. C/EBPß, AP-1, and NF-kB were all detected in the nuclear fraction of myometrial cells in the presence and absence of stretch. Specific DNA binding for AP-1 and C/EBPß (supershifted with specific antibodies to C/EBPß and AP-1 c-jun and c-fos) was significantly increased in cells exposed to stretch, whereas NF-kB binding was not changed by mechanical stretch. Typhoon phosphor imager showed that the DNA binding and supershift for C/EBPß increases by 35% and for AP-1 by 80% after a 16% stretch for 1 h (n = 3, P < 0.05)

Mech stretch activated GFs

Mechanical stretch activates nuclear factor-kappaB, activator protein-1, and mitogen-activated protein kinases in lung parenchyma

Protein Kinase Pathways in Response to Mechanical Stretch of Human Airway Smooth Muscle Cells



I don't have access to this paper

Regulation of extracellular matrix gene expression by mechanical stress

the production of tenascin-C and collagen XII, two ECM proteins typical of tendons and ligaments, is high in fibroblasts attached to a stretched collagen matrix, but suppressed in cells on a relaxed matrix. The response to a change in stretch is rapid and reversible, and is reflected on the mRNA level. Both the tenascin-C and the collagen XII gene promoters contain ‘stretch-responsive’ enhancer regions with similarity to ‘shear stress response elements’ in other genes.