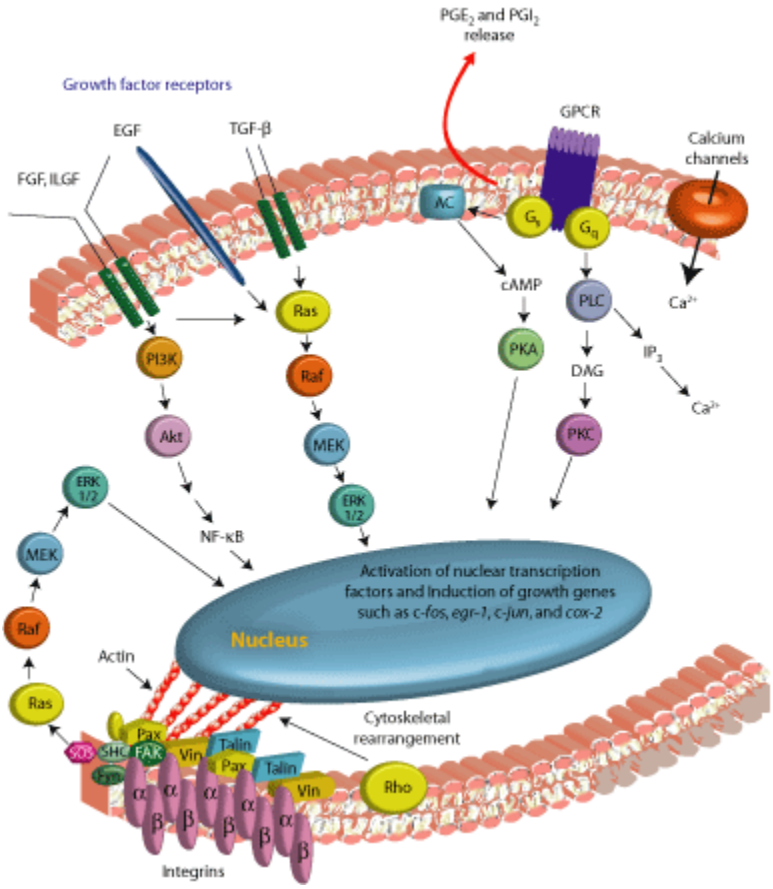
SS responses

Shear Stress Responses



Excellent review detailing mechanosensory responses, particularly in osteoblasts:

- Osteoblasts respond to mechanosensation via intagration of signals from a variety of pathways; many of these appear to be non-cell type specific.
- Animals treated with calcium channel blockers had up to 61% decrease in bone formation factors after mechanical stress.
- The Wnt pathway is also implicated in stress-sensing mechanisms.

h3: Types of Stress

Fluid Shear Stress

- Studies applying FSS of 800 µ demonstrated actin stress fiber formation and induction of mRNA for cox-2 (cyclooxygenase-2) and c-fos within an hour of application .
- c-fos, cox-2, and prostaglandin E2 (PGE2) are induced by growth factors or mechanical stress
- **Interesting Finding:** Oscillating fluid flow on osteoblasts sensed by L-type calcium channels and IP3 induction of intracellular Ca2+; this activated ERK and p38 MAPK, which was followed 24 hours later by an increase in osteopontin (OPN) induction.
  -> very interesting because osteopontin is a marker of bone differentiation; there are more findings connecting in vitro expression of bone differentiation factors with mechanostimulation.

Mechanical strain	Calculated strain	Response measured	Signaling molecules and pathways implicated	Reference
FSS	<800 με	*c-fos, cox-2, actin cytoskeleton,* cAMP, Ca^{2+}	PLC-IP_3, release of intracellular Ca^{2+}, Rho, NF-κB translocation, MAPK	(*12–14, 22, 25, 29*)
FSS	>800 με	Ca^{2+}, cAMP, NO, Cox-2, pERK	IP_3, PKA, adenylate cyclase, MAPK, calcium channels, PGE_2 receptors	(*22, 27, 28, 30, 48*)
PFF or OFF	500–1,300 με	OPN, pERK, PGE_2	IP_3, Ca^{2+} channels and intracellular Ca^{2+}, p38 MAPK	(*11, 31, 32*)
Gravity compression	30–500 με	DNA synthesis, c-*fos*, pERK, *fgf-2*, *cox-2*, and proliferation	PKA, MAPK	(*7, 33-37*)
Four-point bending	3,400–10,000 με	c-*fos*, pERK, and proliferation	pERK-1: IP_3, NO, Ca^{2+} channel; pERK-2: PGI_2, NO	(*39–43, 48*)
Substrate stretch	1,000–10,000 με	c-*fos*, *egr-1*, NF-κB translocation, cAMP, IP_3	Src kinases, MAPK, JNK, FAK, and PKC	(*51–54, 56, 58-60, 64, 90–92*)
Magnetic bead RGB, twisting or pulling, atomic force or vibration	1,000–2,000 με	ET-1	pp60-src, pFAK, IP_3, PLC, PKA, calcium channels, Akt	(*67, 69–71, 73, 89, 93, 94*)

Mechanosensory promoters

COX-2

- In vivo, showed COX-2 dependent mechanically induced bone formation.
- Cox-2 transcription can be induced by various pathways implicated in mechanical stress response, e.g. protein kinase A (PKA), Akt, and Wnt
- Rho-mediated formation of stress fibers bound to integrin in focal adhesions may promote the transcriptional activation of cox-2 and c-fos.
- Fluid flow initiates filamentous actin (F-actin) formation and immediate early gene activation through a PLC-IP3--mediated pathway involving IP3-dependent intracellular Ca2+ release (see top figure).

*Intracellular Ca2 release caused nuclear factorka kB (NF-kB) translocation to the nucleus and stimulated cox-2 gene expression during fluid shear.

- FSS stimulation of cox-2 in osteoblastic cells largely dependent on PKA signaling link
- FSS induced expression of cox-2 also partially dependent on ERK pathway link

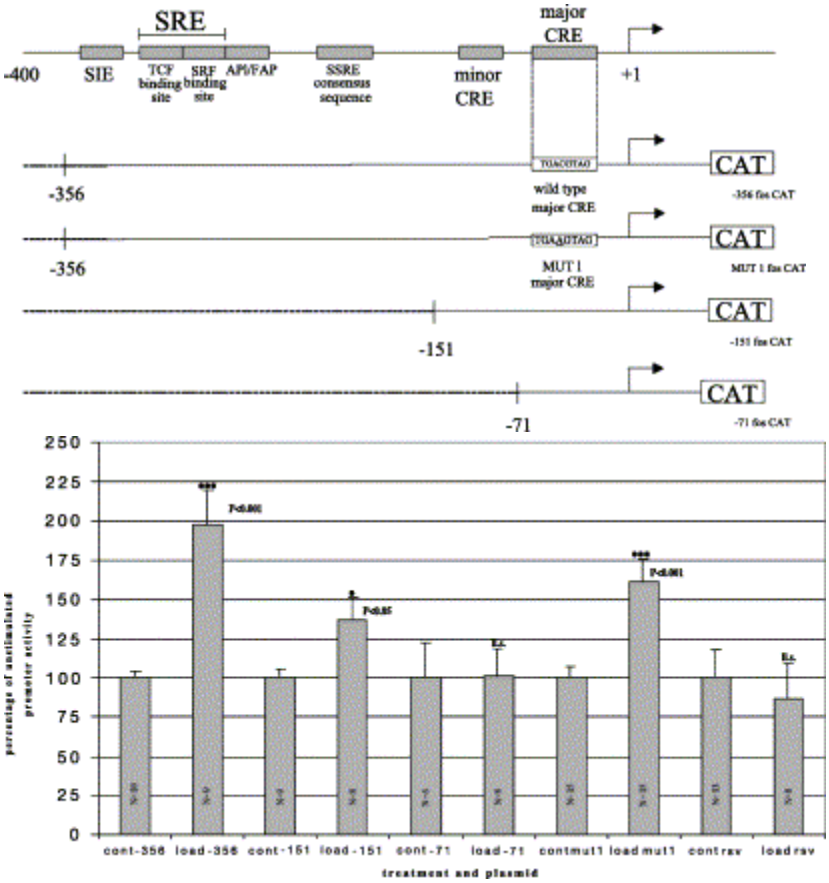
c-fos

- Initially characterized as mechanosensitive in cardiomyocytes. link

c-fos mechanosensitive elements.

- * **VERY INTERESTING!** Paper detailing mechanosensitive elements in c-fos promoter, done in osteoblastic UMR-106 cells. link http://www.sciencedirect.com.libproxy.mit.edu/science?_ob=ArticleURL&_udi=B6T36-47W5GB8-8&_user=501045&_coverDate=02%2F27%2F2003&_fmt=full&_orig=
- Paper looking at elements in the c-fos prom link for c-fos promoter infohttp://carcin.oxfordjournals.org/cgi/reprint/16/3/443.pdf
- Shown that deletion of -356 to -71 resulted in almost complete attenuation of mechanical response.

	SRE	Fos-Ap1 (Fap)	SSRe	
SIE				
SRE				
Fap				
SSRE				



- CRE and AP1 sites in the c-fos promoter: (c-fos on top, CRE on bottom, homologs shown)
- Paper mapping stress response element in c-fos promoter; identifies serum response element as potential mechanosensory element
  http://www.ncbi.nlm.nih.gov/pmc/articles/PMC452298/pdf/emboj00020-0059.pdf
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Heparin-Binding Growth Factor (HB-EGF)

- Identified in cDNA screen of smooth muscle cells for genes upregulated in response to 3, 6, and 12-hour stress respectively; showed highest induction (>10 fold increase in cDNA in response to hydrostatic pressure)
- Thought to be mediated via AP-1 (AP-1 stretch-induced expression of HB-EGF;
  AP-1 binding site at -988 to -947 of promoter in mouse HB-EGF; this could be a potential element for us to recombine in our screen.
- Shown almost complete attenuation of pressure sensitivity in AP-1 site mutant

AP-1 element:

FosB

- Very interesting paper showed induction of FosB in response to Fluid Shear Stress; they attempted to identify oligonucleotide sequences in the promoter by transfecting cells with an oligonucleotide sequence controlling expression of a reporter gene.
  *Identified an element called CRE2/SRE that resulted in 4-fold induction after application of fluid shear stress.
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HB-GAM

- Showed induction in response to mechanical loading (cyclic stretching in flexibottom plate for 0.5 - 1.5 hours); this paper generated deletion constructs to try to identify mechanosensitive elements. link

CCN2

- This paper shows stimulation of transcription of CCN2 in response to approx. 1 hour of mechanosensitive; generated truncated promoter sequences to try to identify mechanosensory elements. AP-1 and NFkB elements in promoter (especially NFkB) identified as probable actuators for stress responsiveness. Between -164 and -122 in CCN2 promoter.

Mechanosensitive Element Sequences

Name	Sequence	Promoters Identified in	Position in promoter	Notes	Link
AP-1	TGACTCA	Mouse HB-EGF promoter	-988 to -974	Showed complete attenuation of mechanosensitivity after mutation of the AP-1 binding site.	
SRE/CRE2	TGGCGAGCTCCTTATATGGCTAATTGCGTCACAGGAA	Mouse FosB	-473 to -401	4-fold induction of reporter gene controlled by CRE2 oligo after fluid shear stress.	link

Screen For Mechanosensitive Promoters

*Employed oligonucleotide arrays to identify stretch-sensitive genes in primary culture human bladder SMC subjected to repetitive mechanical stimulation for 4 h.

- Validated results via RT-PCR (results shown below)
  *

cDNA array for mechanosensitive genes

- Screened chondrocytic cells under hydrostatic pressure for 3, 6, and 12 hours respectively; mRNA transcriptional upregulation was measured via cDNA array.
- Got highest induction for Heparin Binding EGF-like Growth Factor (HB-EGF); we've previously picked out this gene as being potentially sensitive to pressure.
- Observed increased expression of hsp70, hsp40, and hsp27 (heat shock response proteins); common functionality in response to stress.
- Growth arrest and DNA damage-inducible genes (GADD45 and GADD153) also increased under pressure.
- Anti-proliferative proteins tob and p21 also upregulated, whereas ribosomal protein S19, prothymosin alpha and p55CDC (genes associated w/ cell proliferation were down regulated.

Gene	Fold of change			Function	GB access
	3 h	6 h	12 h		
Heat shock protein 40	1.8	3.1	0.4	stress response	D49547
Heat shock 27-kDa protein 1	0.9	2.9	1.0	stress response	X54079
Heat shock 70-kDa protein 1	4.5	8.6	4.0	stress response	M11717
Growth arrest and DNA-damage-inducible protein GADD45	2.0	5.7	7.4	stress response/cell cycle control	M60974
Growth arrest and DNA-damage-inducible protein GADD153	1.8	5.0	4.6	stress response/cell cycle control	S40706
tob	1.1	2.0	1.0	cell cycle control	D38305
Cyclin-dependent kinase inhibitor 1A (p21; Cip1; WAF)	1.0	3.2	2.9	cell cycle control	U09579
40S ribosomal protein S19	0.6	1.0	0.8	cell cycle control	M81757
Prothymosin alpha	0.5	1.3	0.9	cell cycle control	M26708
p55CDC (cell division control protein 20)	0.3	1.1	0.0	cell cycle control	U05340
c-jun proto-oncogene	9.9	9.8	6.6	transcription factor	J04111
Endothelial transcription factor GATA-2	4.1	8.9	4.3	transcription factor	M68891
Early growth response protein 1/transcription factor ETR103	1.7	2.7	1.1	transcription factor	M62829
cAMP-dependent transcription factor ATF-4 (TAXREB67)	1.5	2.6	1.8	transcription factor	D90209
fra-1 (fos-related antigen 1)	0.7	1.8	2.6	transcription factor	X16707
Homeobox C1 protein	0.7	1.1	0.4	transcription factor	M16937
Transcription factor AP-2	0.2	0.7	0.7	transcription factor	M36711
DNA-binding protein Id-1	0.6	0.7	0.1	proliferation/differentiation	D13889
DNA-binding protein inhibitor Id-2	1.3	0.9	0.4	proliferation/differentation	M97796
DNA-binding protein inhibitor Id-3	0.8	0.7	0.1	proliferation/differentiation	X69111
NIP3	1.1	0.4	0.2	apoptosis	U15174
Cytoplasmic dynein light chain 1 (HDLC1)	0.4	0.4	0.5	apoptosis/intracellular transport	U32944
Heparin binding EGF-like growth factor	> 10	> 10	> 10	growth factor	M60278
Vascular endothelial growth factor, VEGF	2.5	4.9	1.5	growth factor	M32977
Connective tissue growth factor	0.5	1.4	0.2	growth factor	M92934
Heregulin alpha	0.3	1.1	0.9	growth factor	U02326
EB1 (protein that binds to APC)	0.4	1.1	0.6	microtubular dynamics	U24166
ATP-dependent DNA helicase II (thyroid antigen 70 kDa)	1.1	0.2	0.1	DNA repair	M32865
UV excision repair protein RAD23 (p58/HHR23B)	0.7	0.7	0.5	DNA repair	D21090
Superoxide dismutase	0.6	1.1	0.5	DNA repair/antioxidation	K00065
UV excision repair protein RAD23 (HHR23A)	0.5	0.9	0.7	DNA repair	D21235
DNA-repair protein XRCC1	0.4	0.5	0.5	DNA repair	M36089
Integrin alpha-6	0.7	1.4	0.1	cell adhesion	X53586
Alpha-catenin	0.5	1.1	1.2	cell adhesion	D13866
Cation-independent M-6-P receptor (IGFR-2)	1.4	2.7	1.8	signaling	Y00285
Basic fibroblast growth factor receptor	1.3	2.7	0.6	signaling	M37722

cAMP-dependent protein kinase alpha-catalytic subunit	0.5	0.7	0.4	signaling	X07767
Tyrosine-protein kinase receptor ufo (sky)	0.4	1.2	0.7	signaling	D17517
Tyrosine-protein kinase CAK (EDDR1; TRK E)	0.4	0.7	0.6	signaling	X74979
Activated p21cdc42Hs kinase (ack)	0.3	0.9	0.5	signaling	L13738
Transducin beta-2	0.3	0.9	0.4	signaling	M36429
MAP kinase kinase 3 (MMK3)	0.3	0.6	0.4	signaling	L36719
Transducin beta-1	0.3	0.5	0.4	signaling	M36430
MAP kinase	0.2	0.8	0.3	signaling	L05624
Tyrosine-protein kinase receptor eph-3	0.1	0.3	0.3	signaling	X75208
Prostaglandin E2 receptor EP4 subtype	0.1	0.9	1.2	signaling	L28175
Protein kinase MLK-3	0.0	0.4	0.0	signaling	L32976
Glucose-6-phosphate isomerase	1.2	1.9	0.3	metabolism	K03515
Glutathione S-transferase P	0.5	0.6	0.4	metabolism/apoptosis	X15480
NADH-ubiquinone oxidoreductase B18 subunit (SQM1)	0.4	0.7	0.3	metabolism	M33374
Dioxin-inducible cytochrome *P*450 (CYP1B1)	0.2	0.5	0.2	metabolism/detoxification	U03688