Molecular Basis of Mechanotransduction in Endothelial Cells

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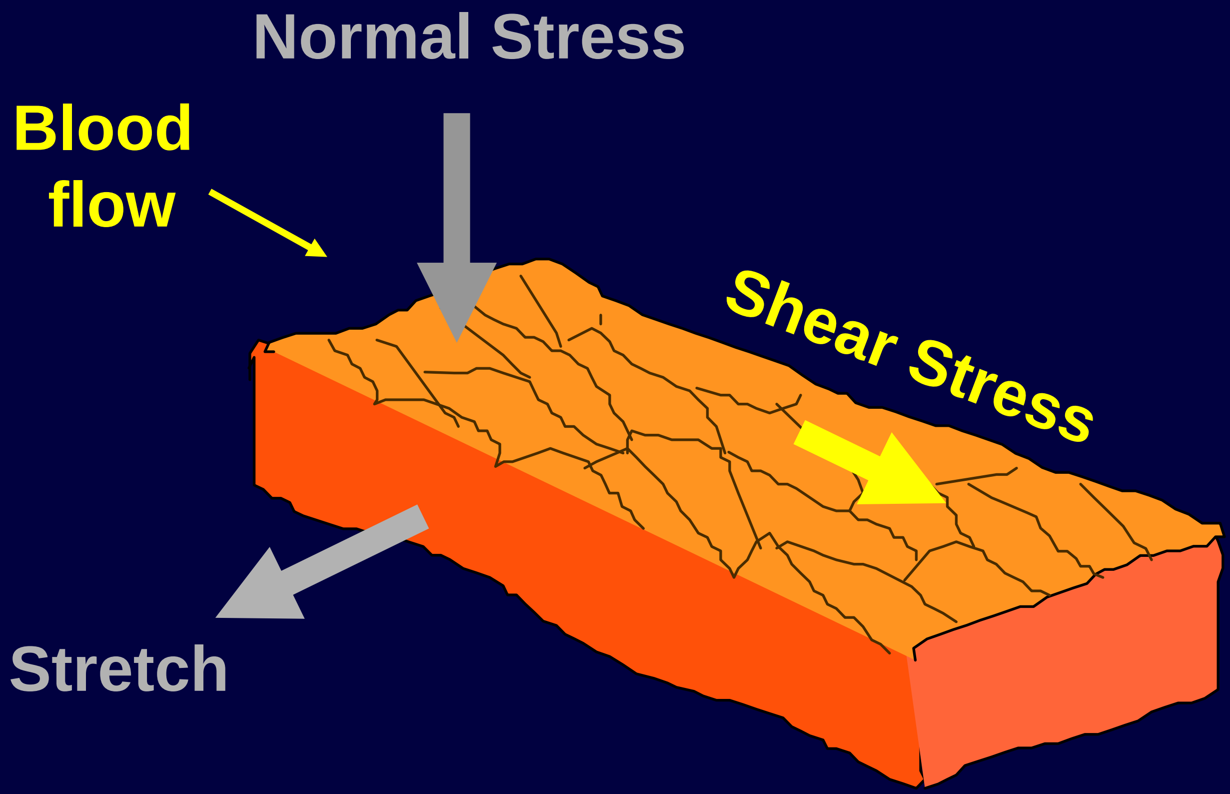
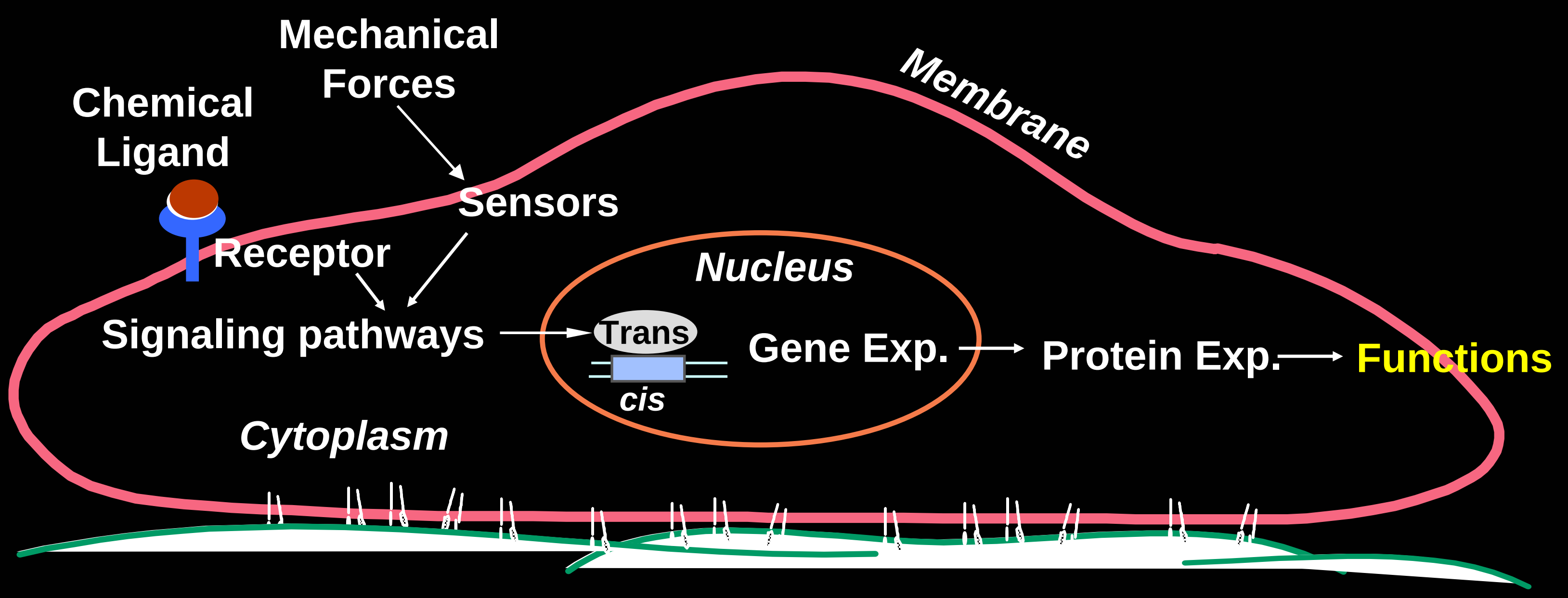
May 16, 2006

Hemodynamic Forces Acting on The Blood Vessel

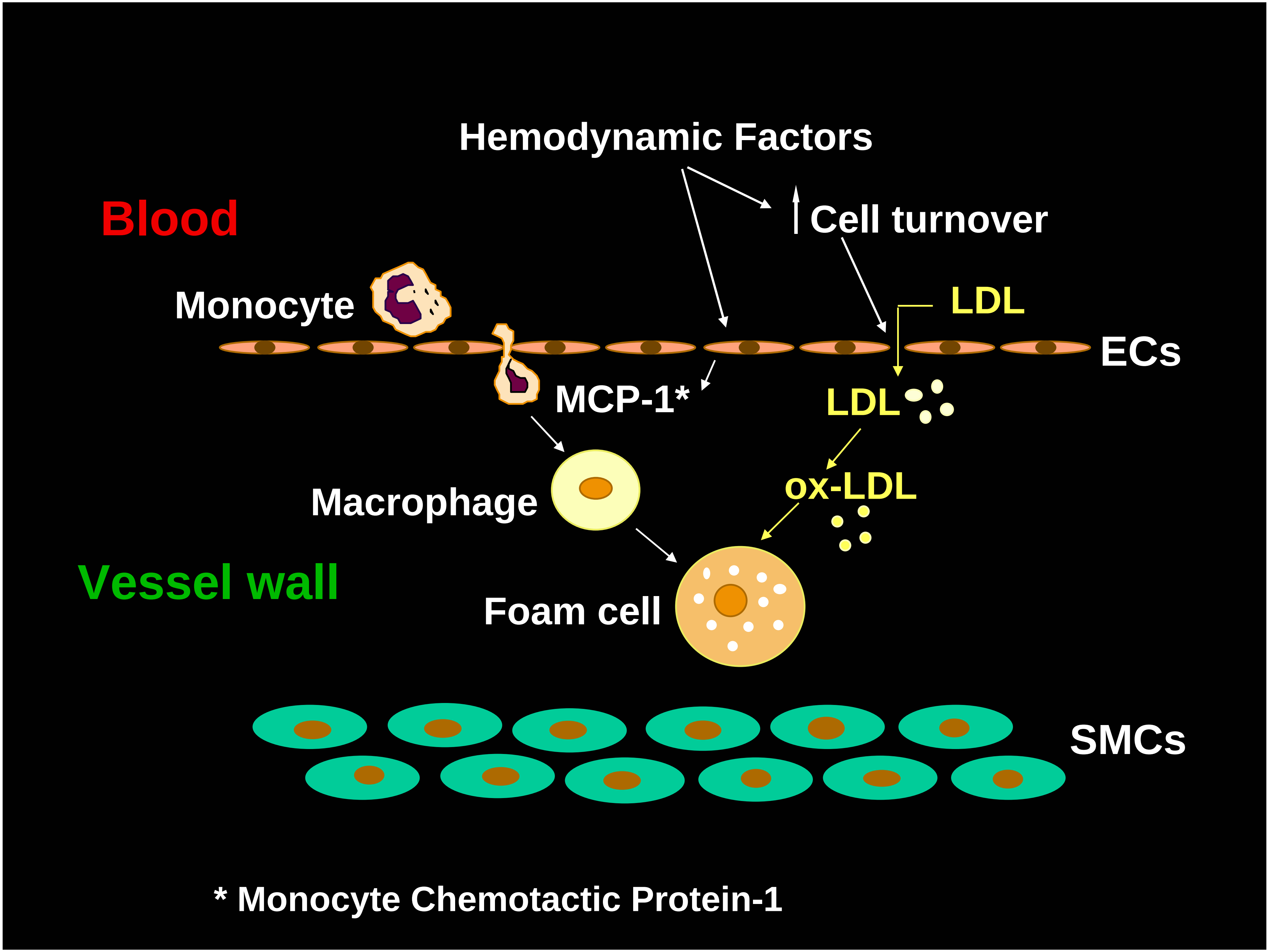


Effects of Physico-chemical Stimuli on Signal Transduction and Gene Expression

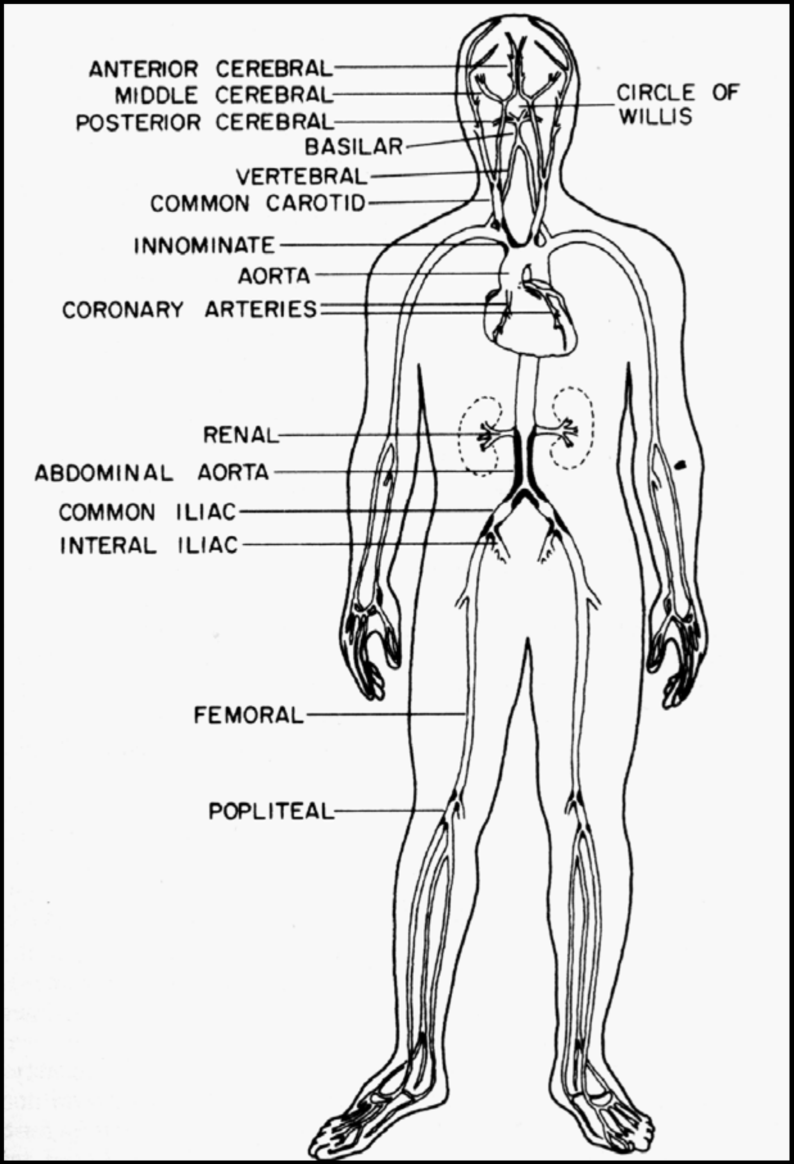


Functions: Secretion, Migration, Remodeling, Proliferation, Apoptosis, etc.

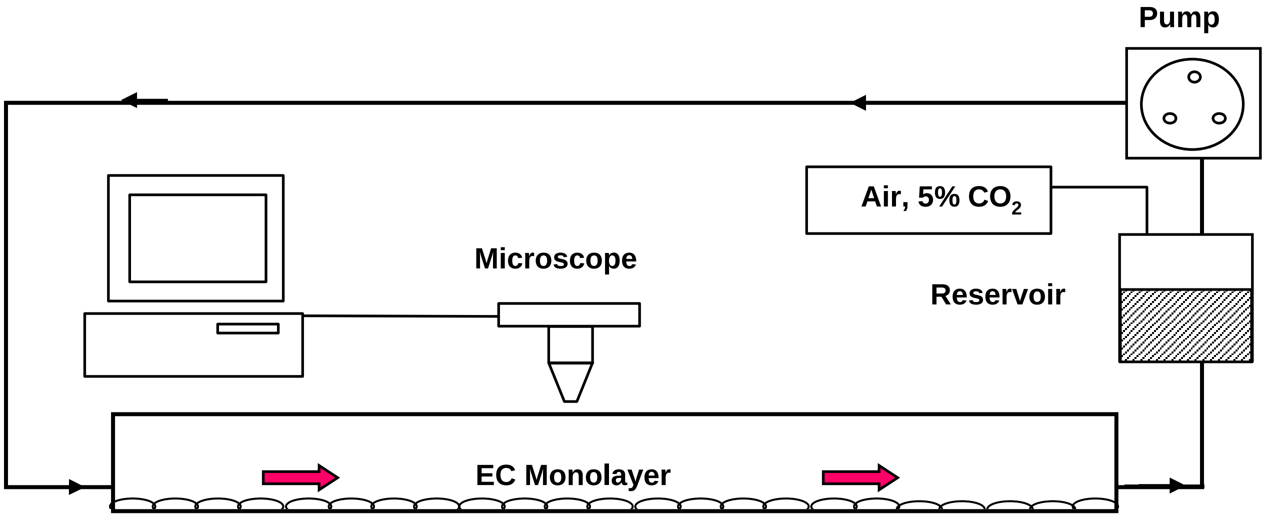
Mechanotransduction is a fundamental homeostatic process in health and disease.

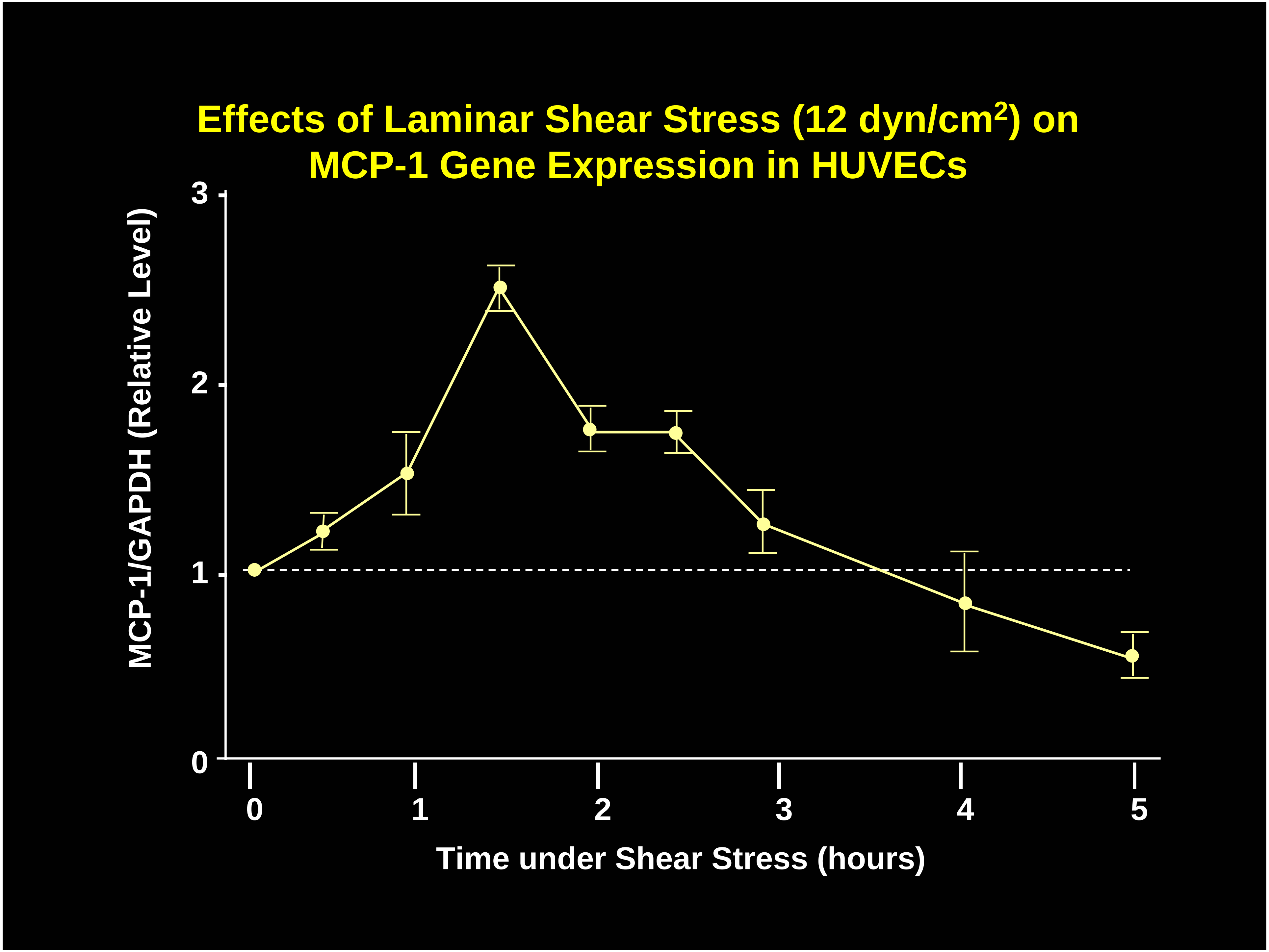

Hemodynamic Factors
Blood
Cell turnover
Monocyte
LDL
ECs
MCP-1*
LDL
Macrophage
ox-LDL
Vessel wall
Foam cell
SMCs
* Monocyte Chemotactic Protein-1

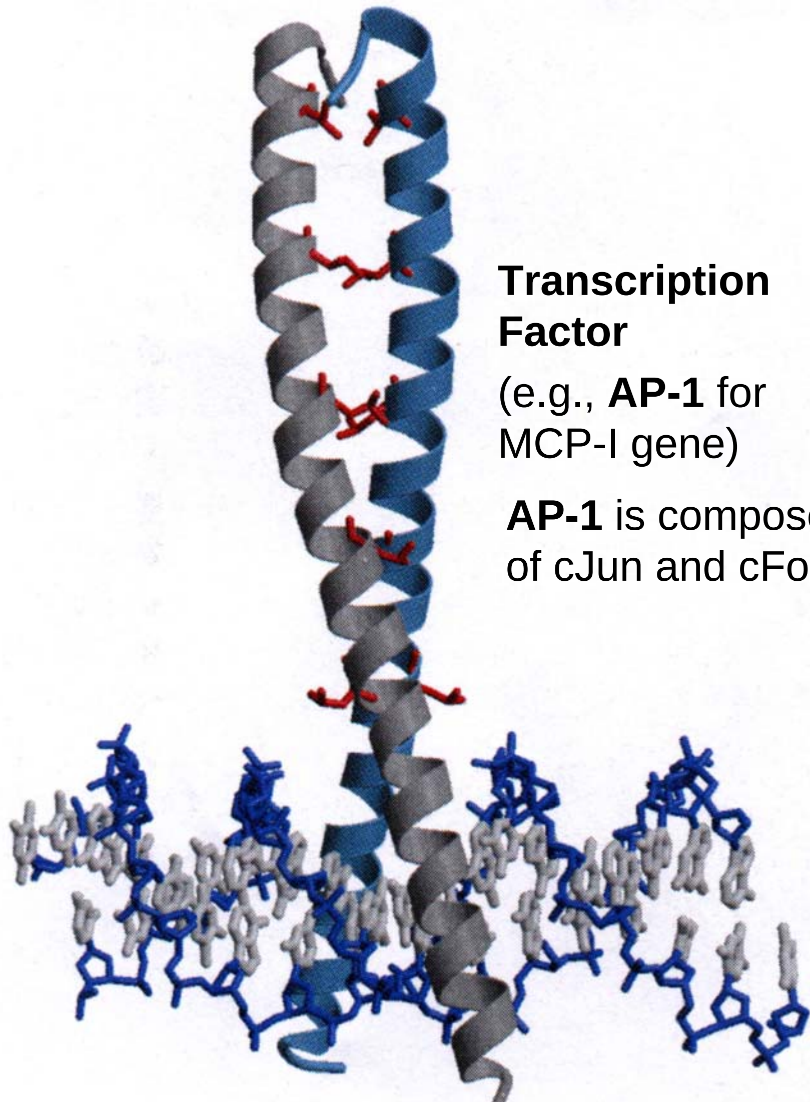
Atherosclerotic Lesions are Preferentially Located at Regions with Complex Flow Patterns



Rectangular Flow Chamber to Study the Effects of Laminar Flows on EC Monolayer




Effects of Laminar Shear Stress (12 dyn/cm2) on
MCP-1 Gene Expression in HUVECs
MCP-1/GAPDH (Relative Level)
3
2
1
0
0
1
2
3
4
5
Time under Shear Stress (hours)



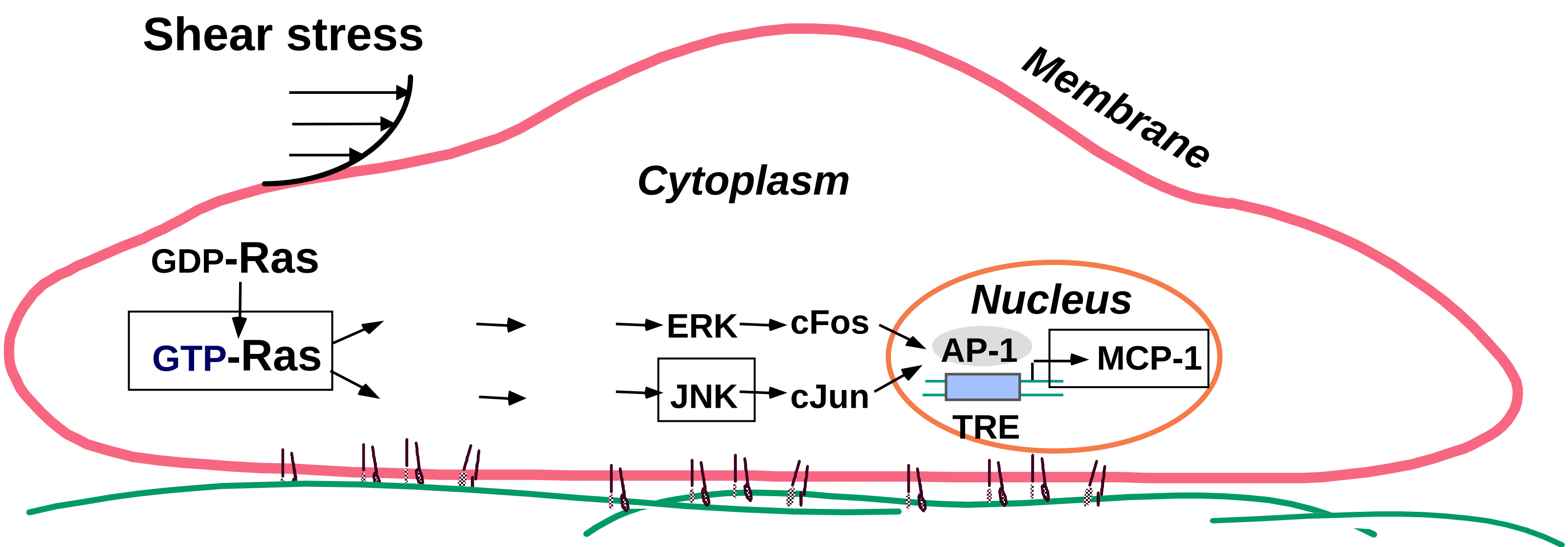
Transcription Factor

(e.g., **AP-1** for MCP-I gene)

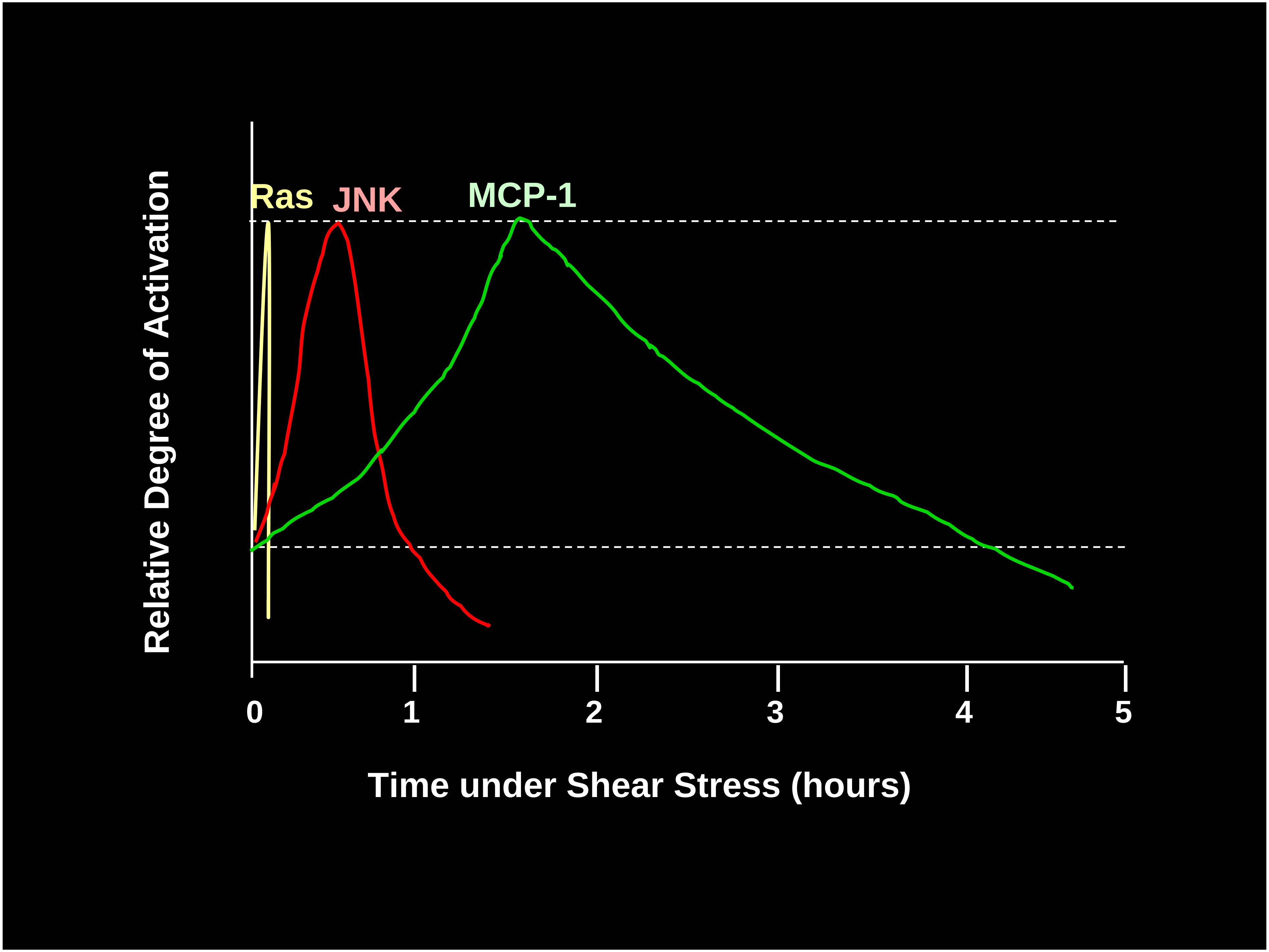
AP-1 is composed of cJun and cFos

Cis element in the promoter region of a gene (e.g., **TRE** for MCP-I gene)

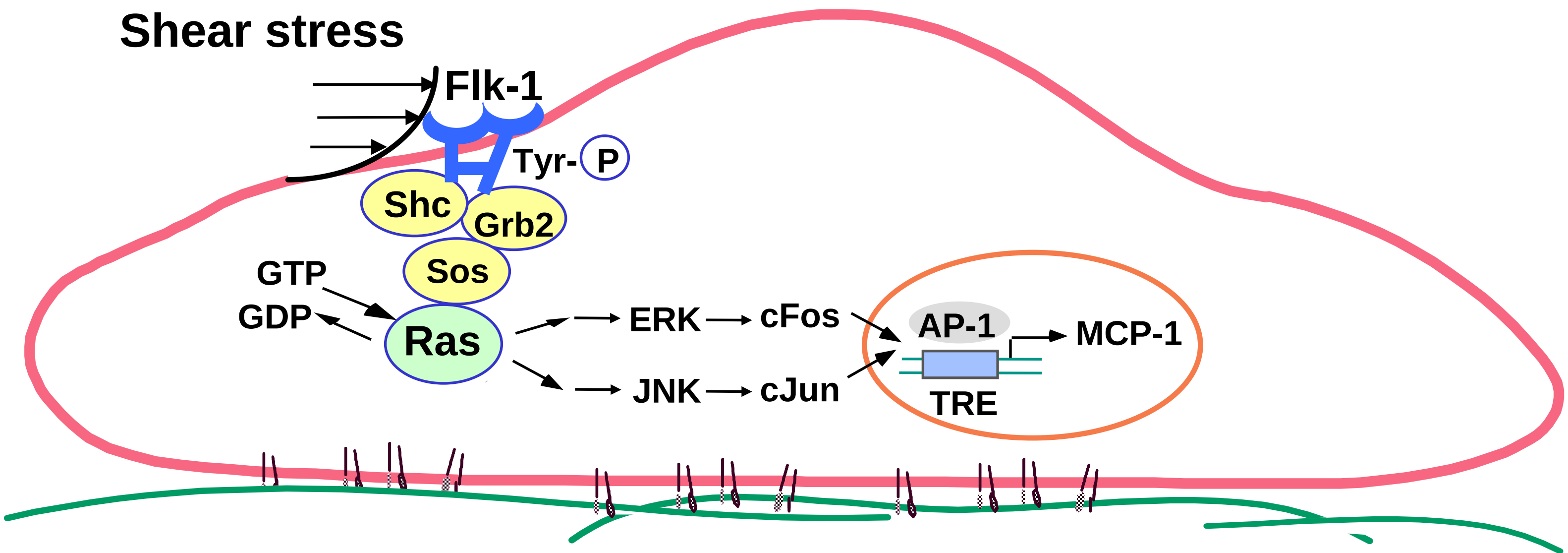
Roles of Ras and MAP Kinases in Shear-Induced Signal Transduction and Gene Expression



Phosphorylation cascade.

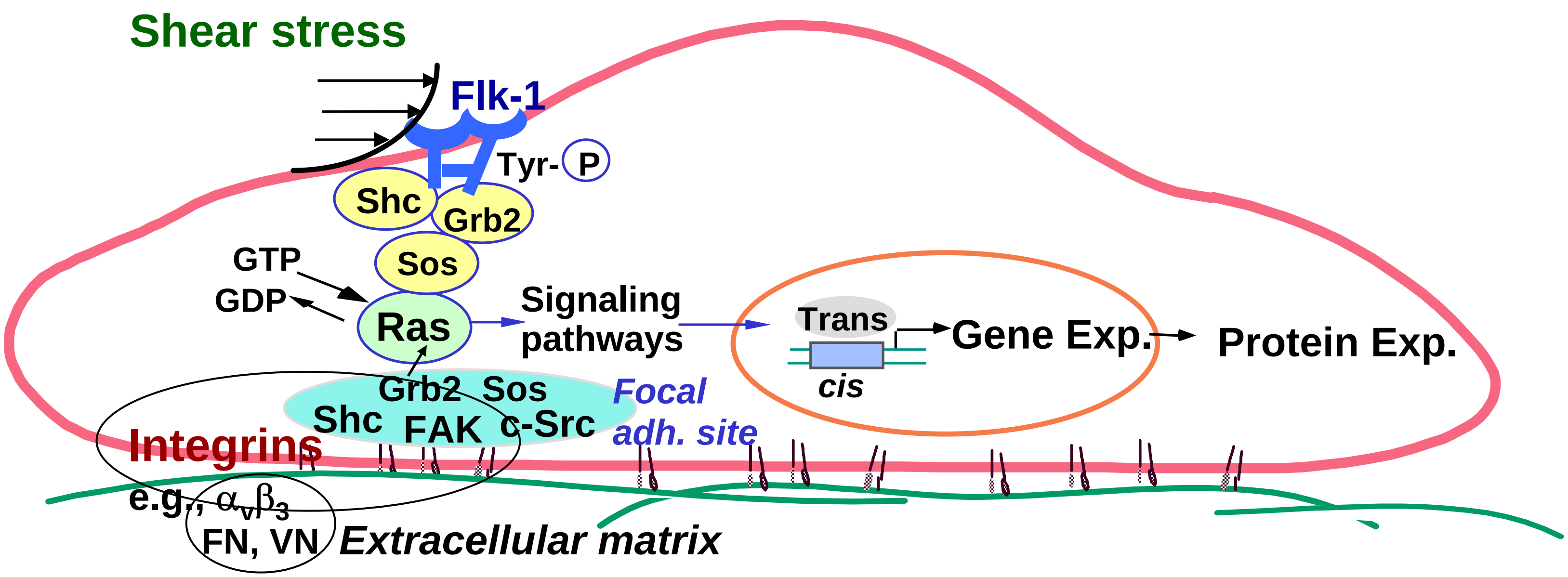

Ras
JNK
MCP-1
Relative Degree of Activation
0
1
2
3
4
5
Time under Shear Stress (hours)

Roles of Adapter Molecules Shc, Grb2 and Sos in Shear-Induced Signal Transduction and Gene Expression



Receptor Tyrosine Kinases (RTKs), e.g., the Vascular Endothelial Growth Factor (VEGF) Receptor Flk-1, can mediate the shear-induction of signaling.

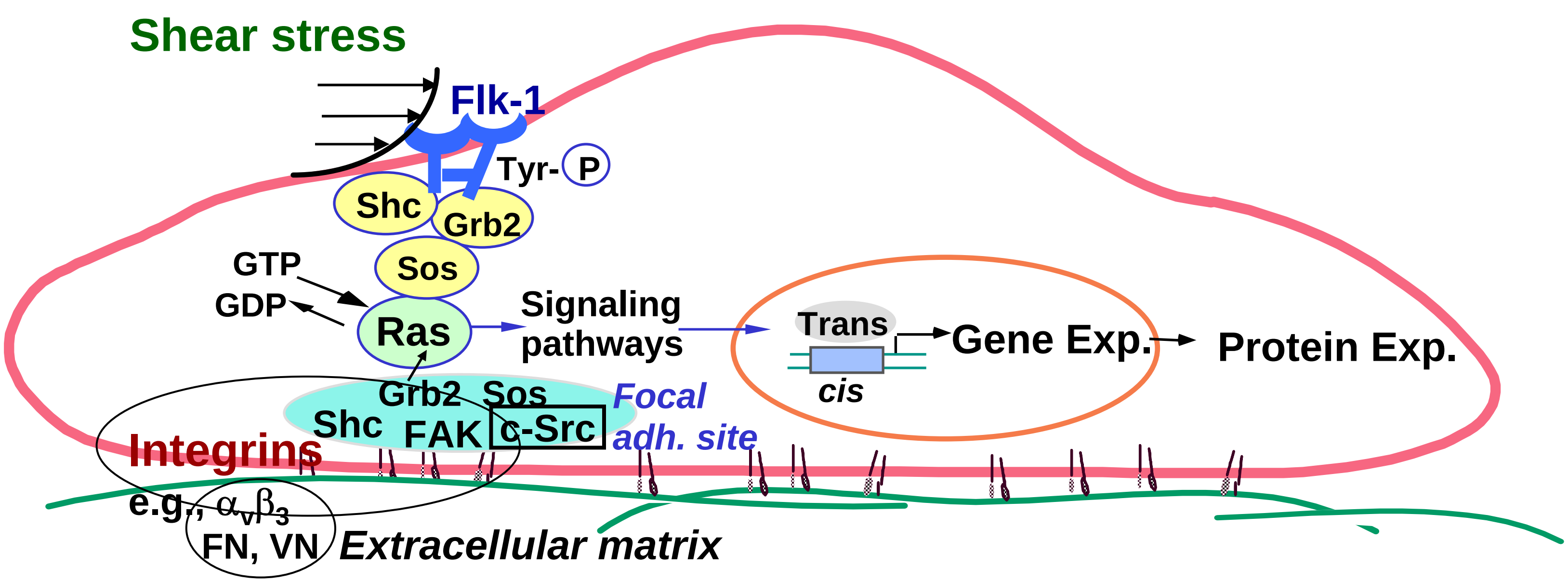
Mediation of Shear-induced Signal Transduction by *Integrins* and Focal Adhesion Proteins



$\alpha_v\beta_3$ integrin interacts specifically with fibronectin and vitronectin, but not laminin or collagen.

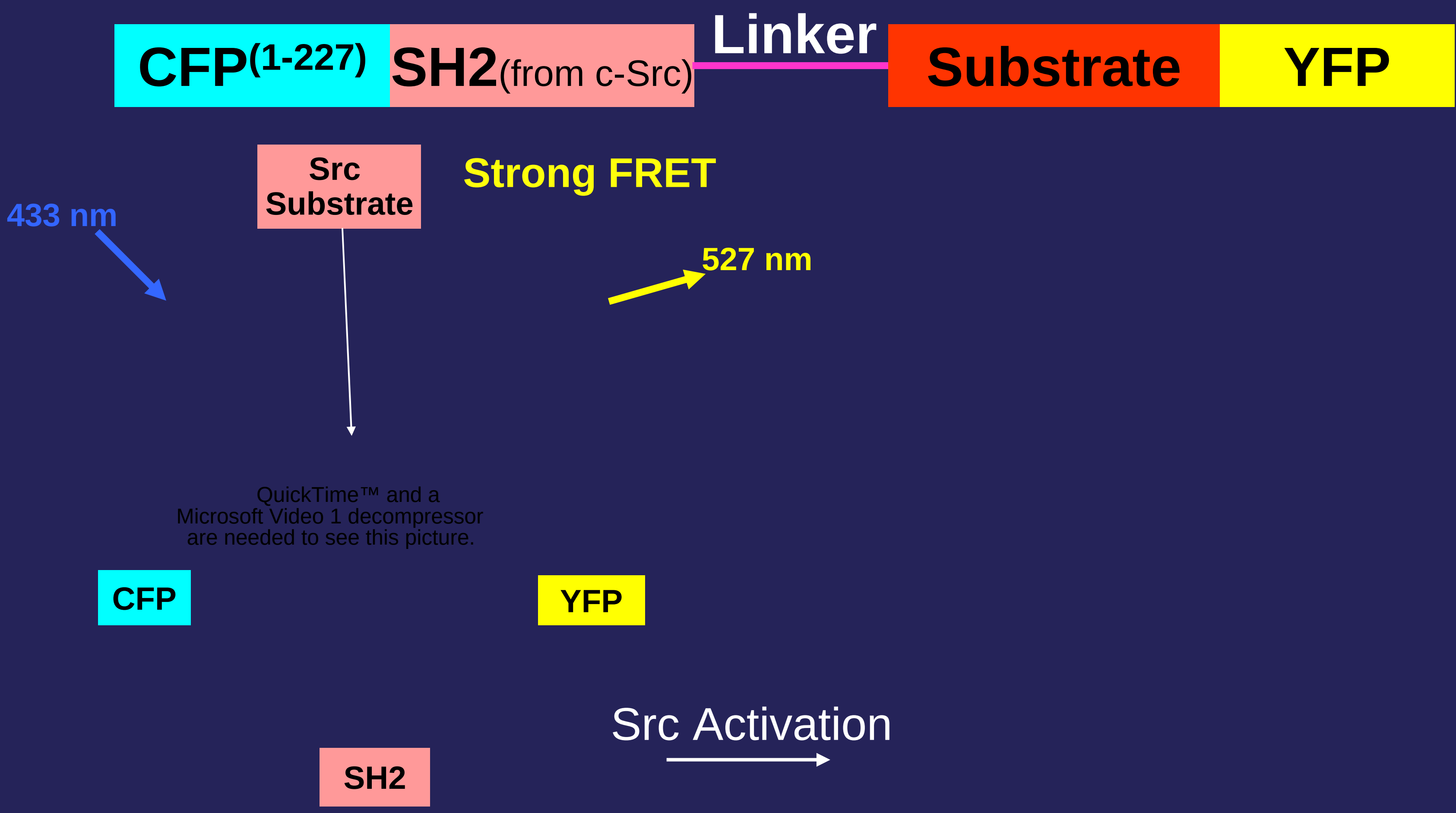
Shear-induced integrin activation leads to its association with FA proteins.

Mediation of Shear-induced Signal Transduction by *Integrins* and Focal Adhesion Proteins

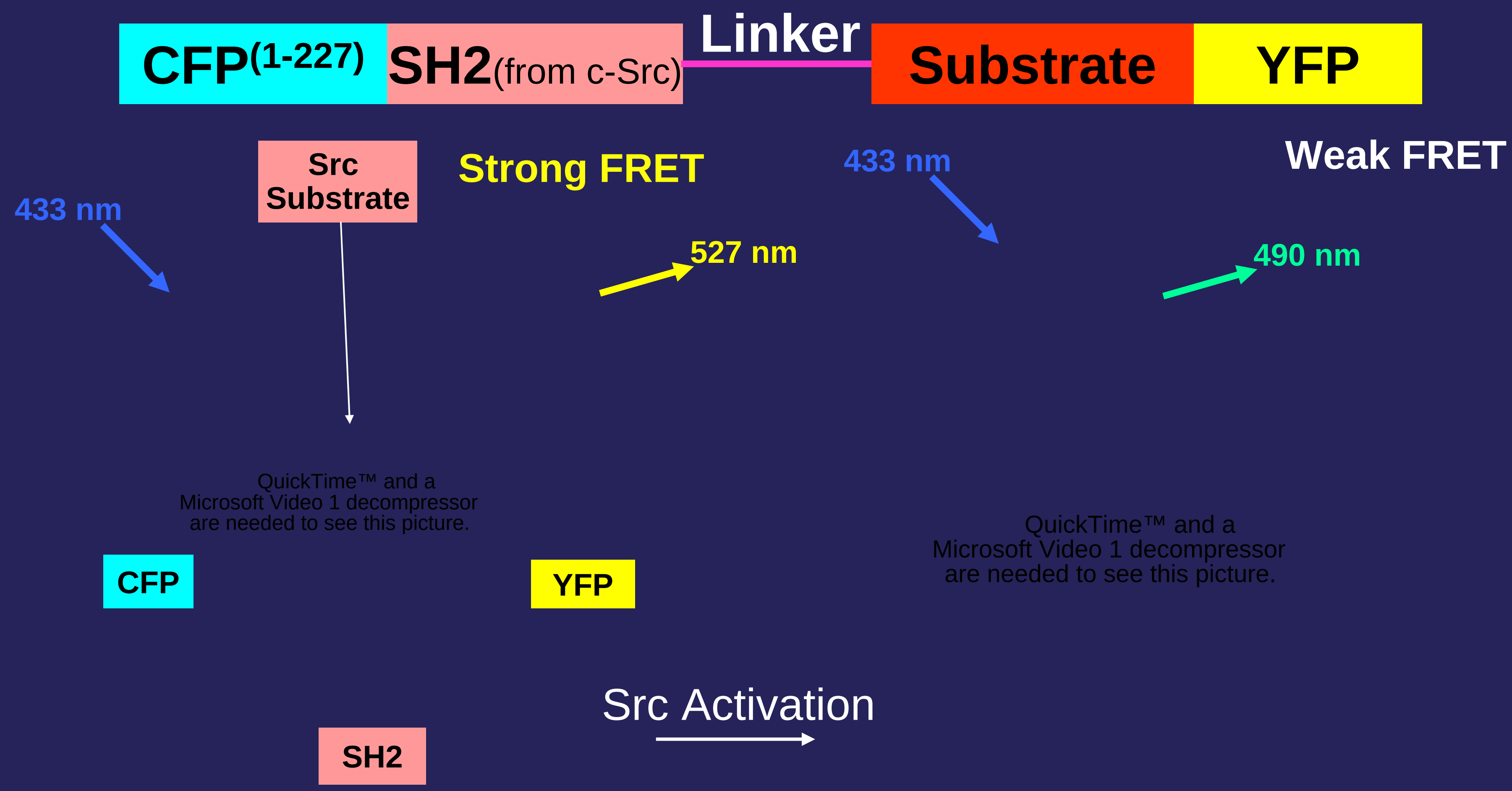


Study of temporal and spatial characteristics of Src activation by Fluorescence Resonance Energy Transfer (FRET)

Design Strategy for a Novel Reporter for Src Activation by Fluorescence Resonance Energy Transfer (FRET)

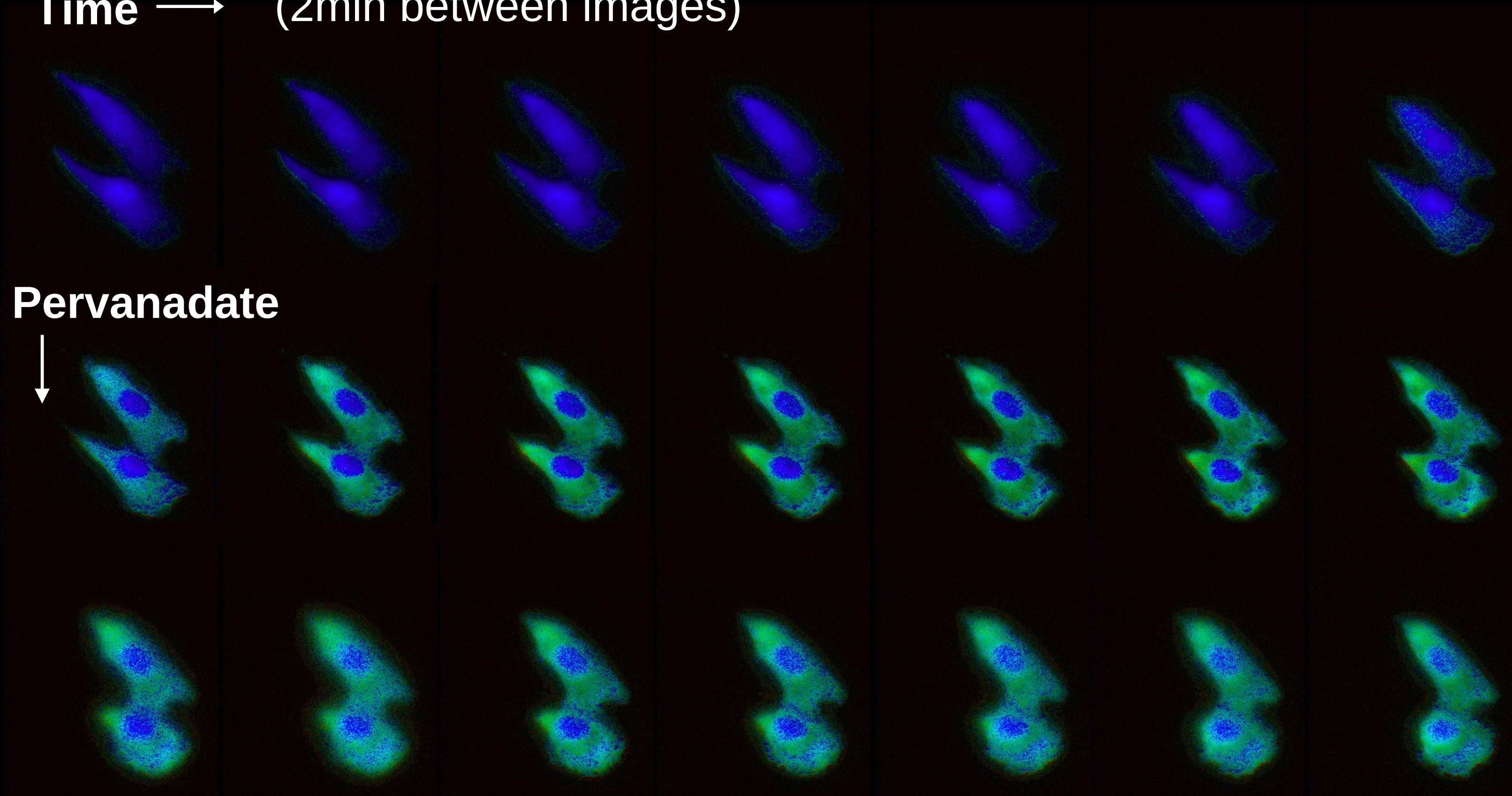


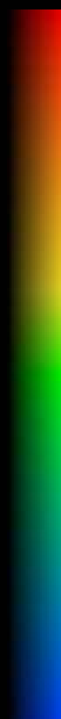
Design Strategy for a Novel Reporter for Src Activation by Fluorescence Resonance Energy Transfer (FRET)



FRET Response of Src Reporter Induced by Pervanadate
(A phosphatase inhibitor that increases phosphorylation)

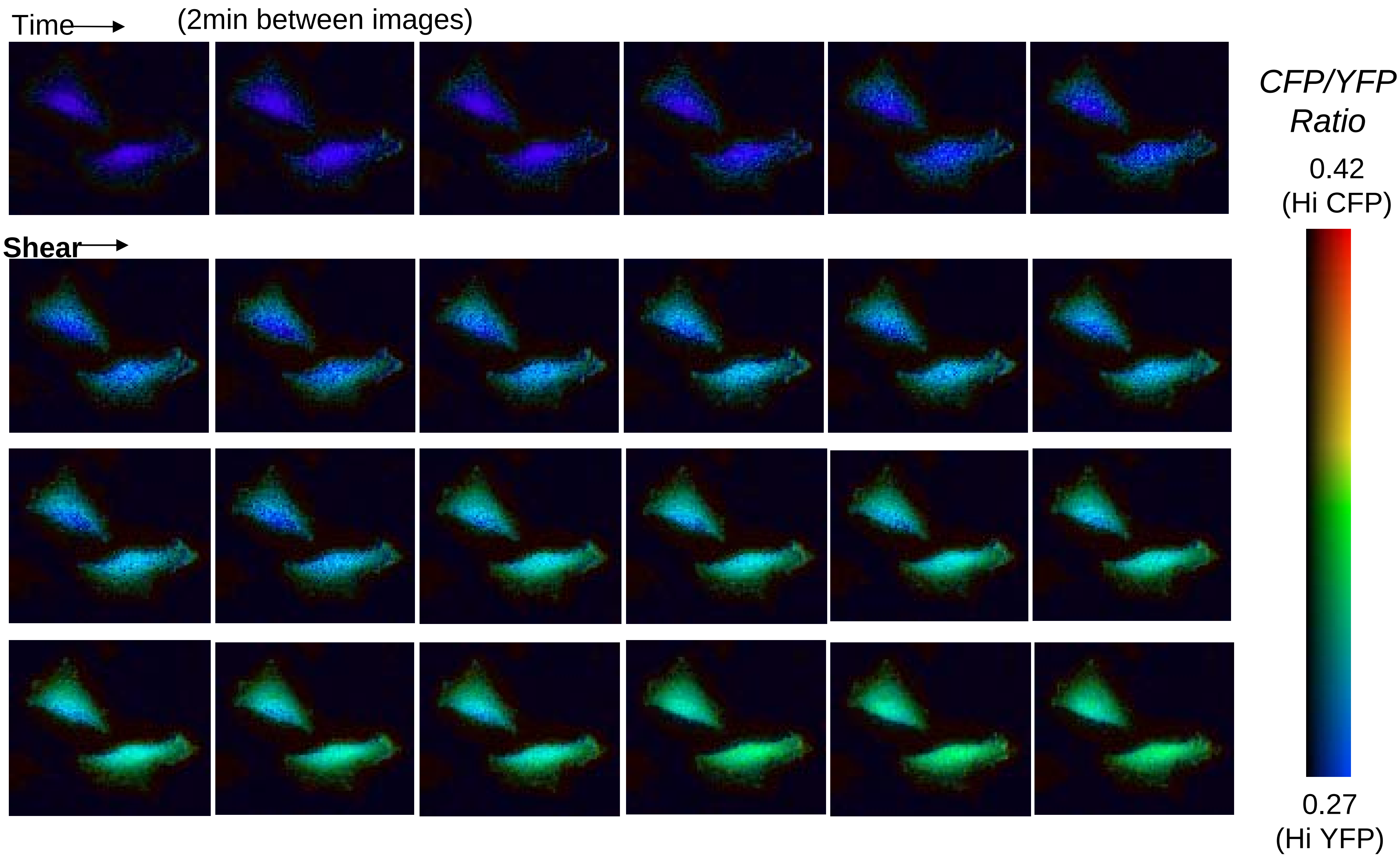
Src Activation: **YFP ⟶ CFP**





The results indicate that Src activity is increased by pervanadate.

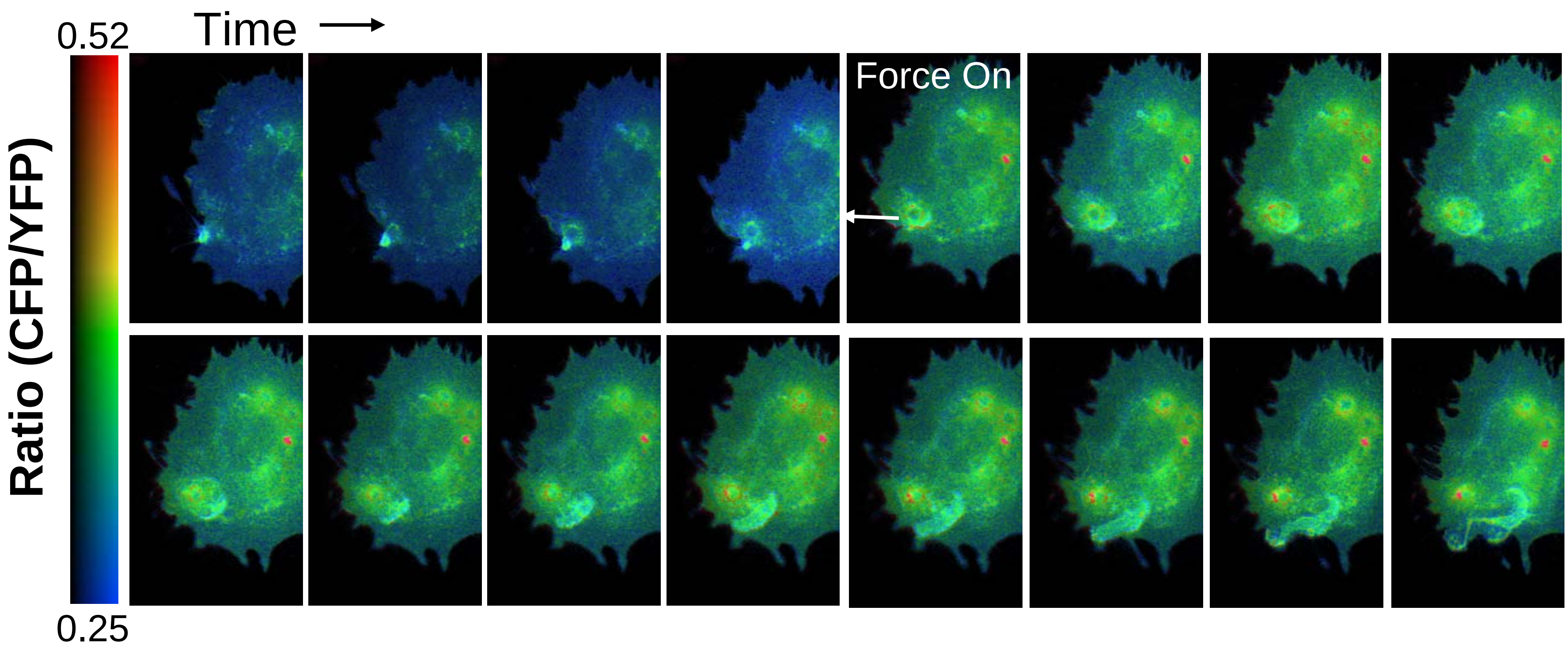
FRET Response of Src Reporter Is Induced by Shear Stress



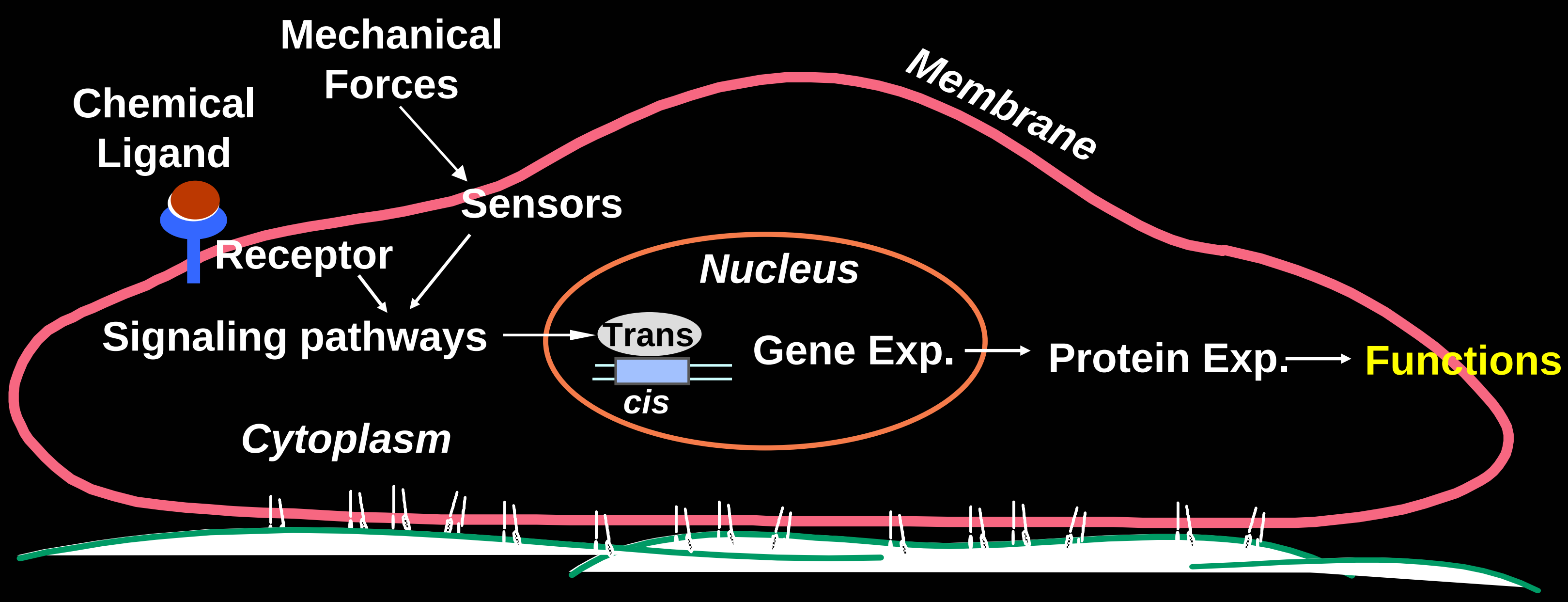
The results indicate that Src activity is increased by shear.

Pulling Fibronectin-coated Beads induced A directional propagation of Src activation

(Color changes indicates increases of Src activity after pulling. Time interval between images= 2min)

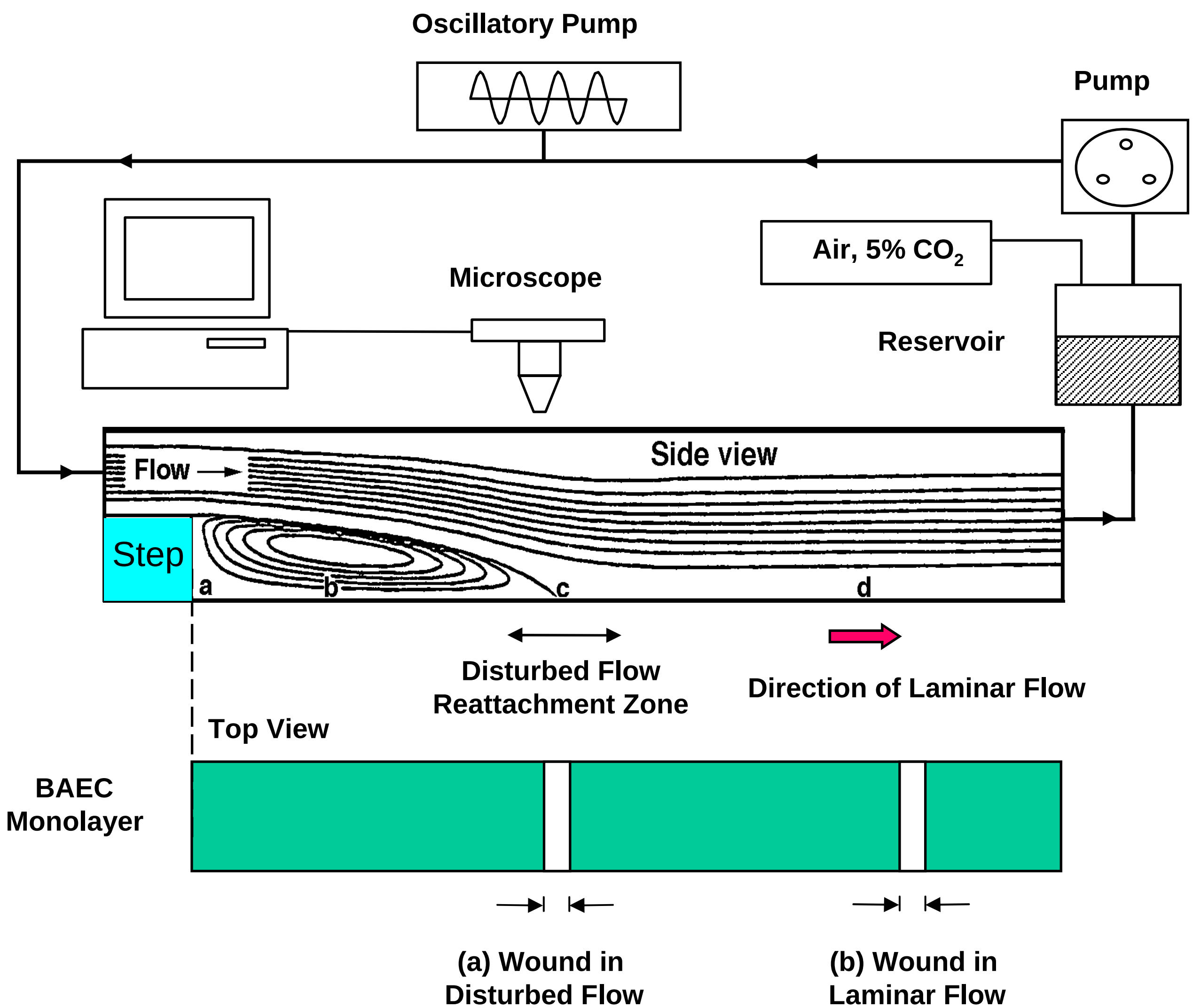


Effects of Physico-chemical Stimuli on Signal Transduction and Gene Expression

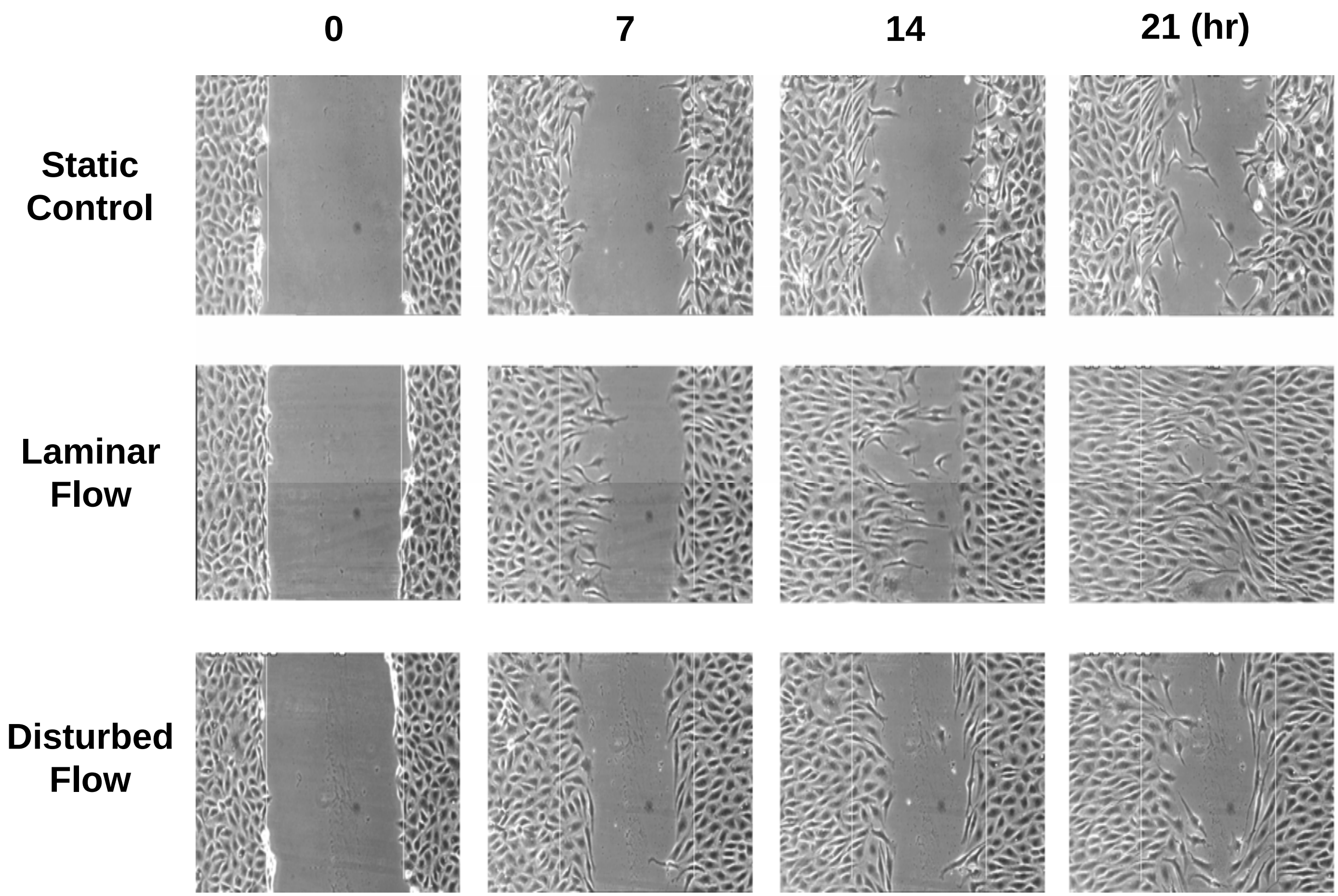


Functions: Secretion, Migration, Remodeling, Proliferation, Apoptosis, etc.

Effects of Laminar and Disturbed Flows on Wound Closure in EC Monolayer

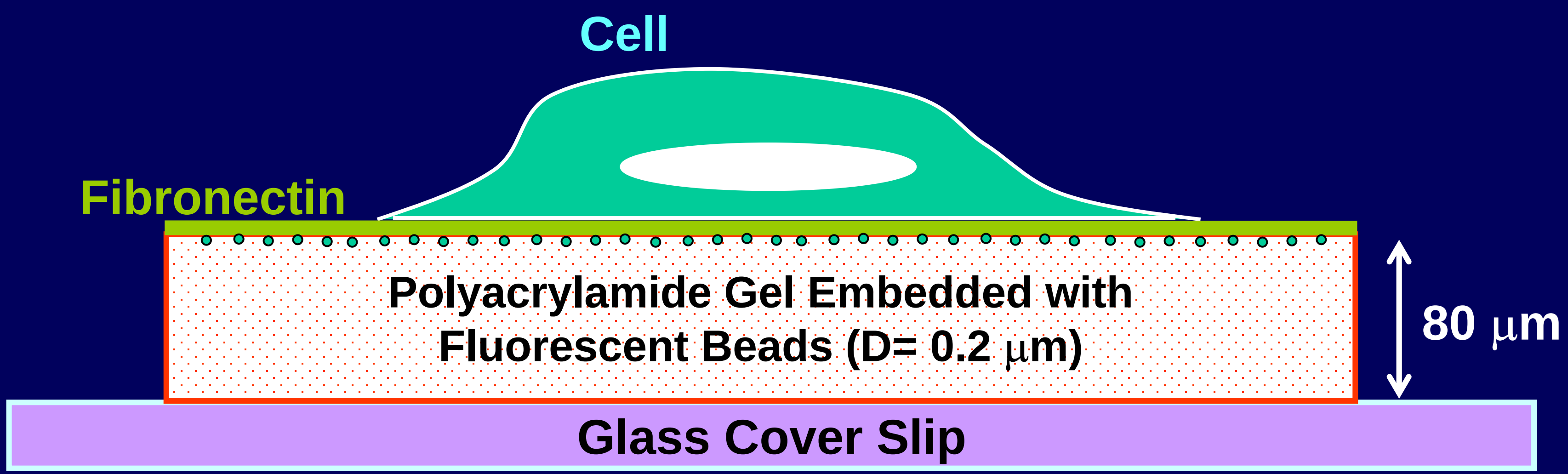


Effects of Flow Endothelial Cell Migration



Laminar flow enhances wound healing, which is much slower under Disturbed flow.

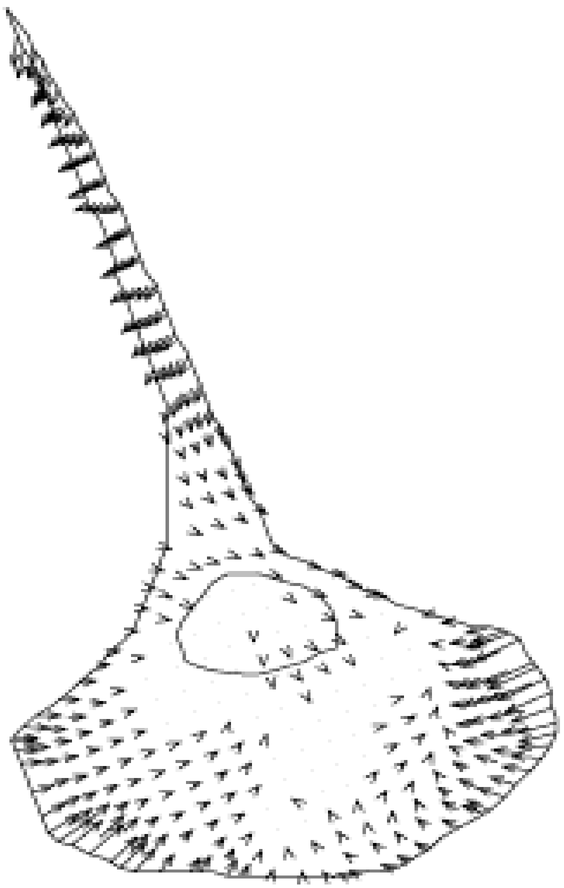
Measurement of Cell Traction Force by Using the Beads-in-Membrane Technique



Side View (not in proportion)

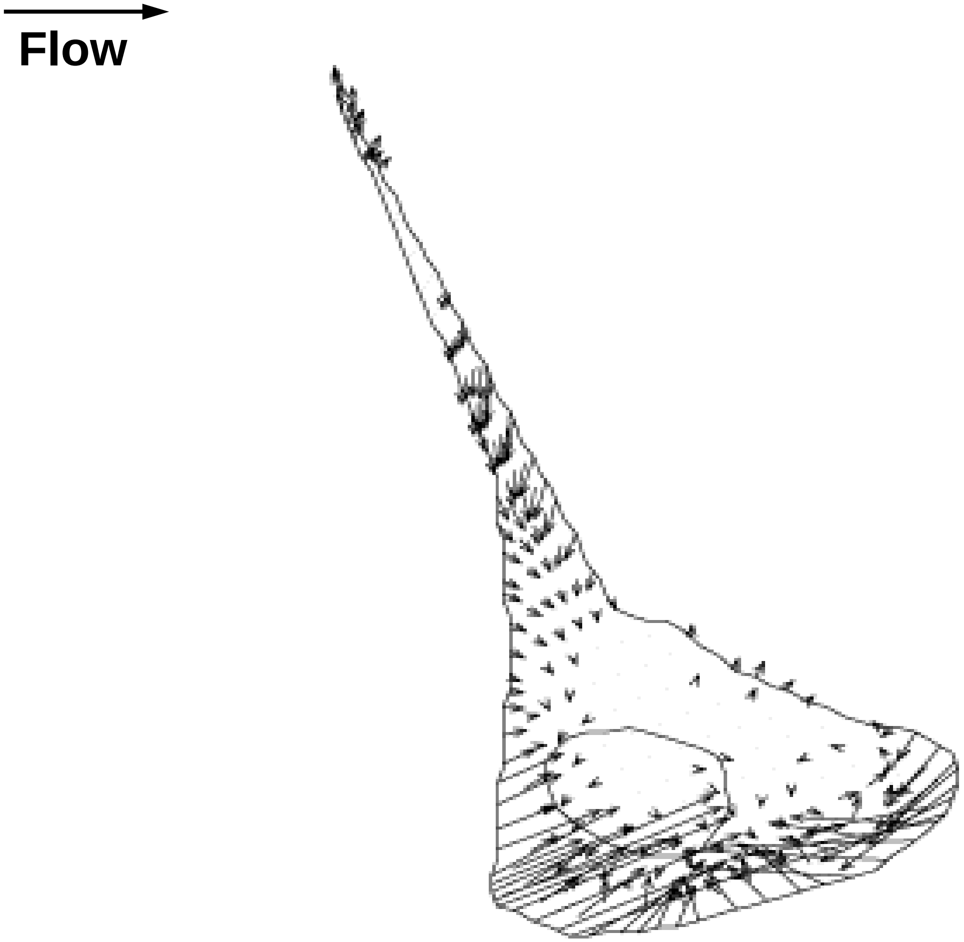
EC Traction on Silicon Membrane Containing Fluorescent Beads

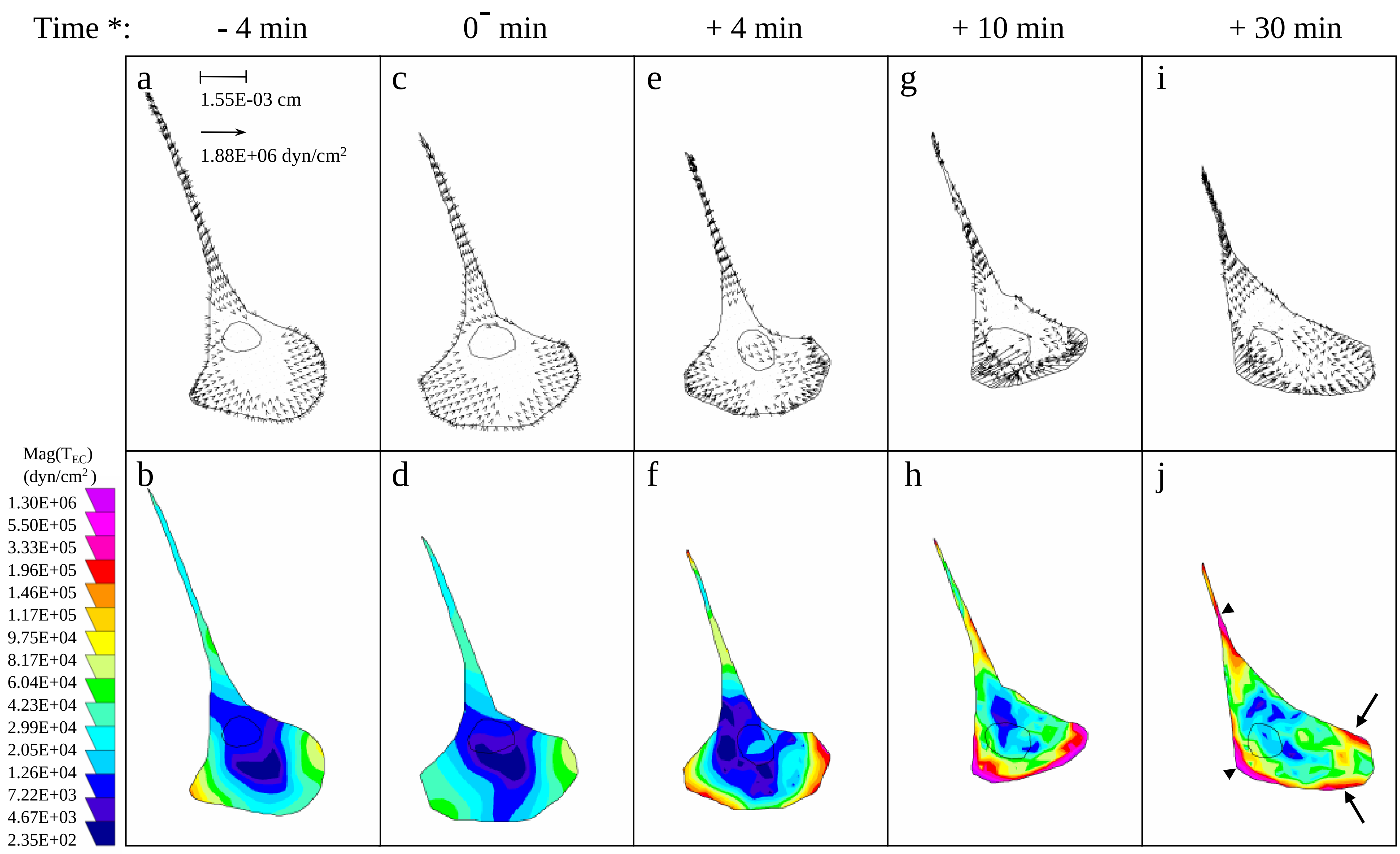
A. No Flow



EC Traction on Silicon Membrane Containing Fluorescent Beads

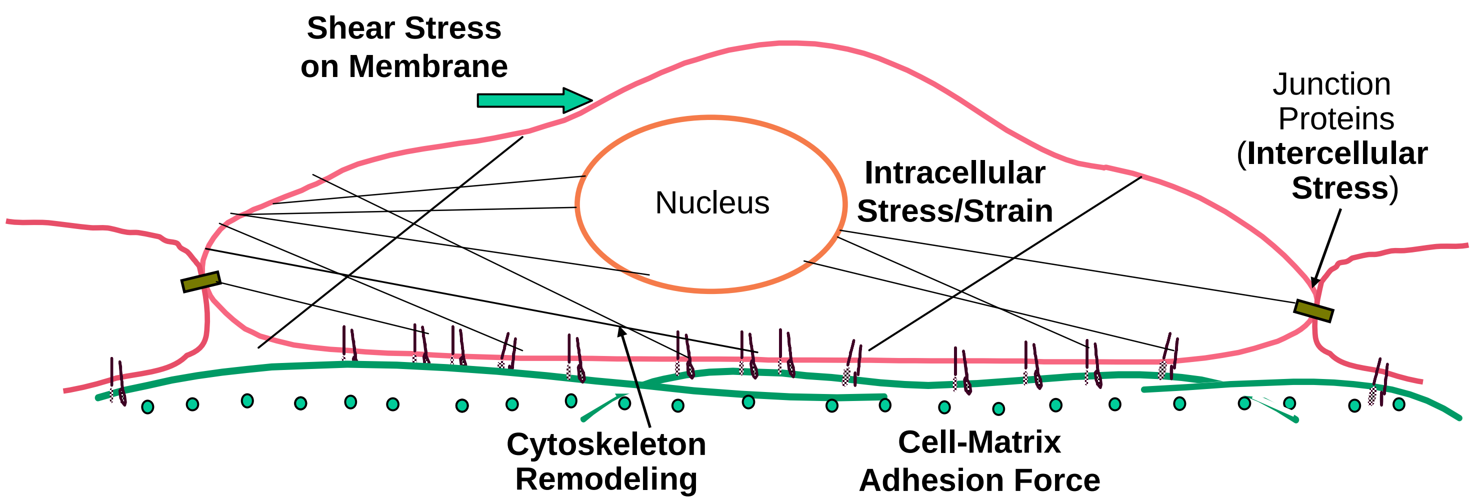
B. 10 min. at 12 dyn/cm²



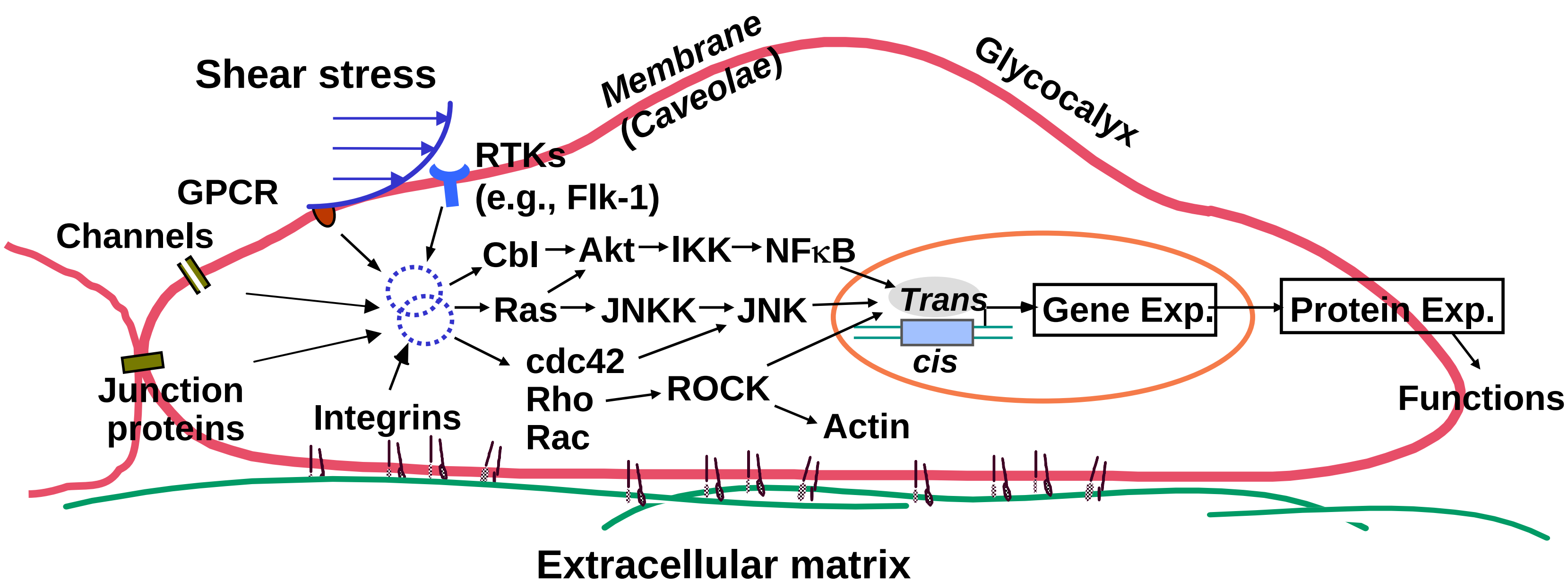


Time *:
- 4 min
0⁻ min
+ 4 min
+ 10 min
+ 30 min
a
c
e
g
i
b
d
f
h
j
1.55E-03 cm
1.88E+06 dyn/cm2
Mag(TEC)
(dyn/cm2)
1.30E+06
5.50E+05
3.33E+05
1.96E+05
1.46E+05
1.17E+05
9.75E+04
8.17E+04
6.04E+04
4.23E+04
2.99E+04
2.05E+04
1.26E+04
7.22E+03
4.67E+03
2.35E+02

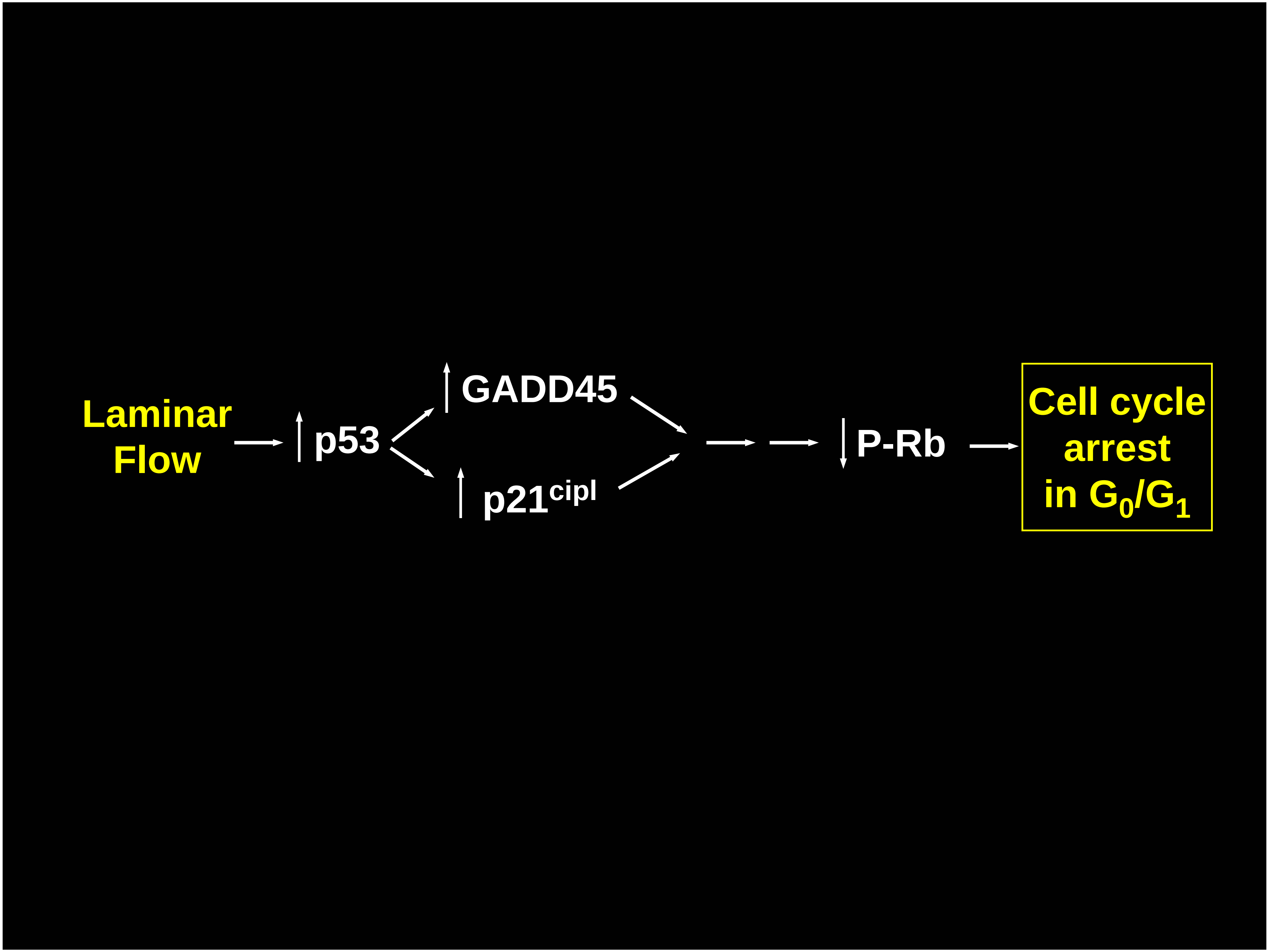
Force Balance in Mechanically Induced Cell Migration



Multi-factorial Mechanotransduction in Shear-sensing, Signal Transduction and Gene Expression

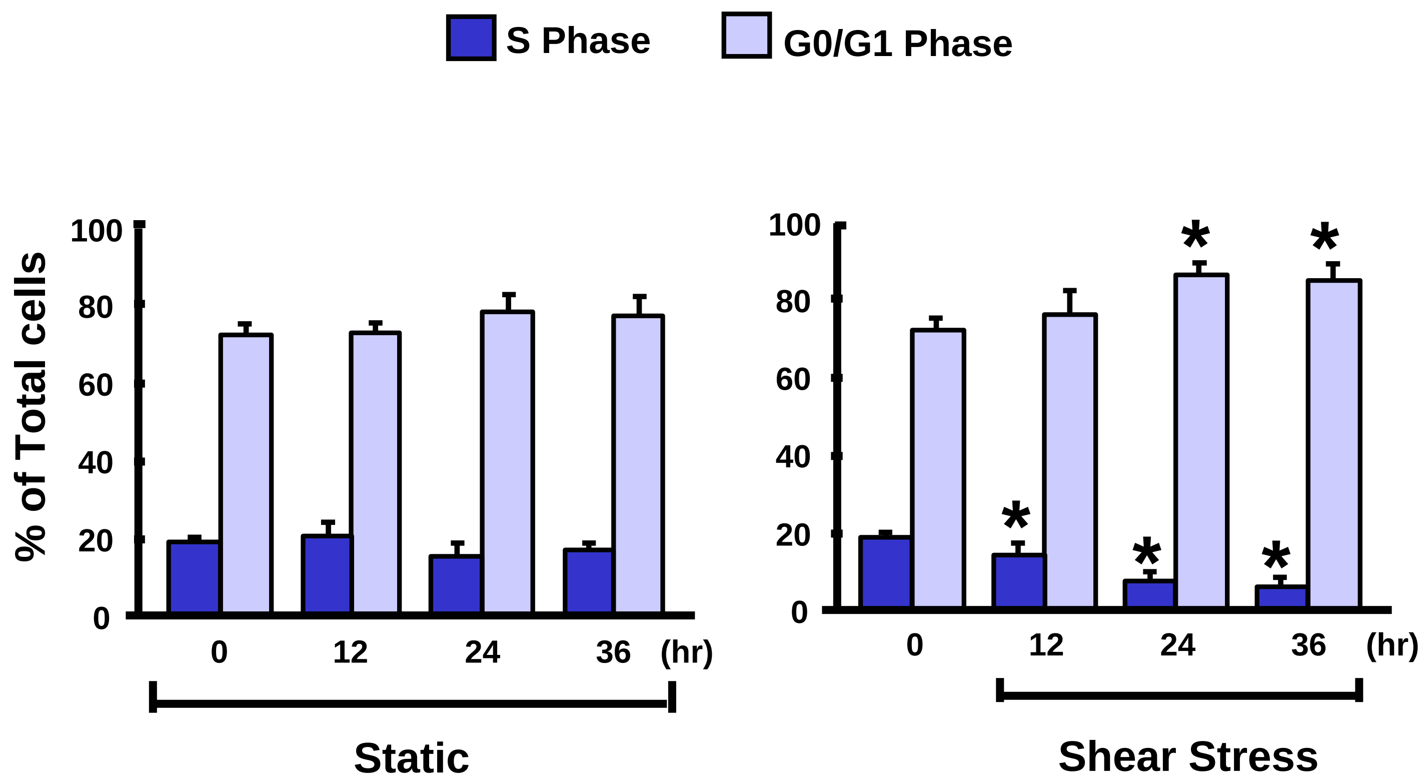


Functions: Secretion, Migration, Remodeling, Proliferation, Apoptosis, etc.

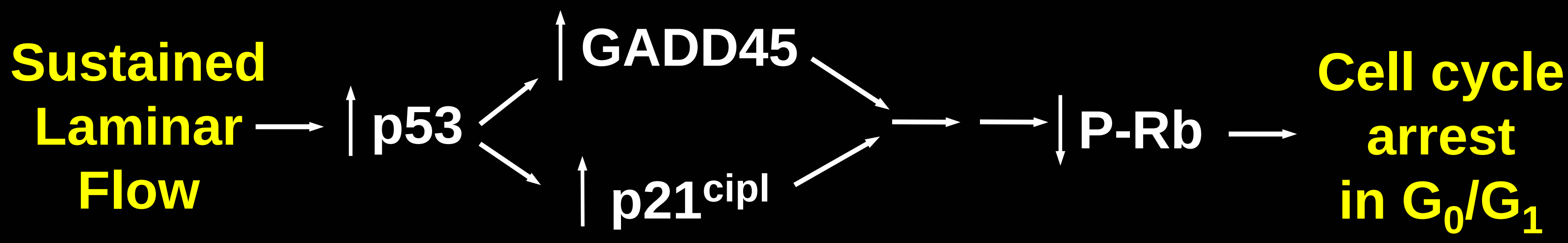


Laminar Flow
p53
GADD45
p21cipl
P-Rb
Cell cycle arrest in G0/G1

Long-term Laminar Shear Stress Causes EC Arrest



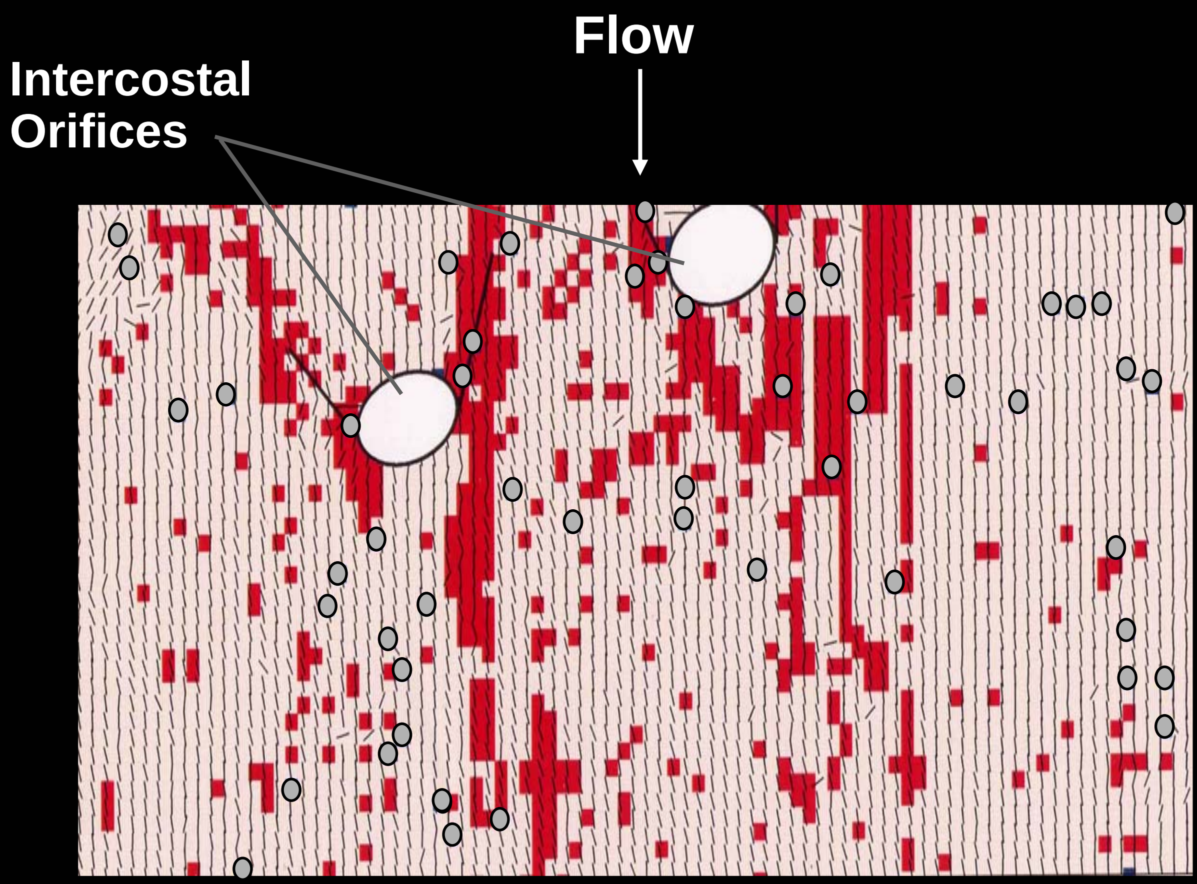
Effects of Flow Patterns on Cell Turnover and Permeability



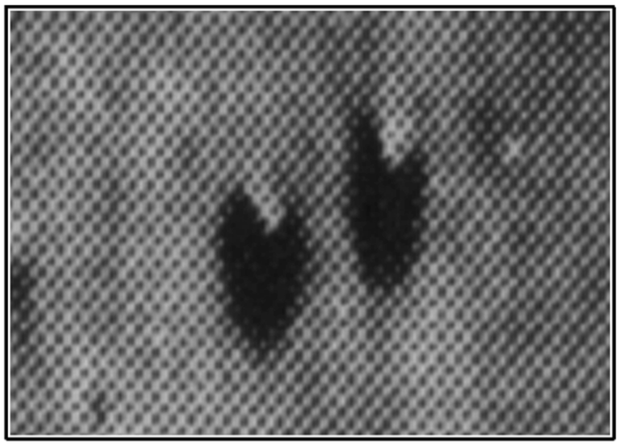
Straight part of the aorta is subjected to sustained laminar shear that leads to cell cycle arrest and hence reduced endothelial permeability.

Branch points have unsteady flow pattern, which accelerates cell turnover and increases permeability.

Endothelial Cell Mitosis (○) and Albumin Leaky Spots (■) In Rabbit Thoracic Aorta

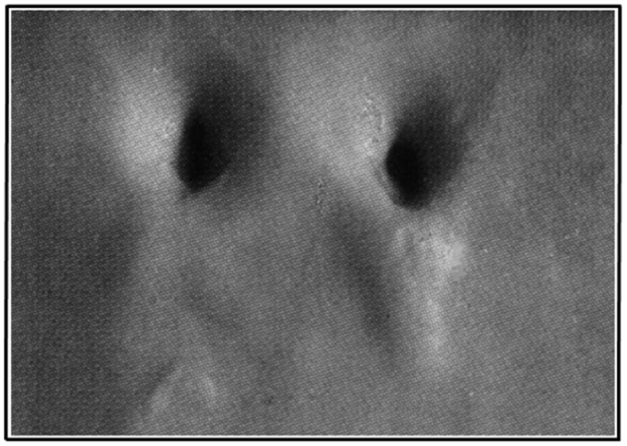


Lipid Accumulation and Atherosclerotic Lesions around Intercostal Orifices



Lipid accumulation (Rabbit Aorta)

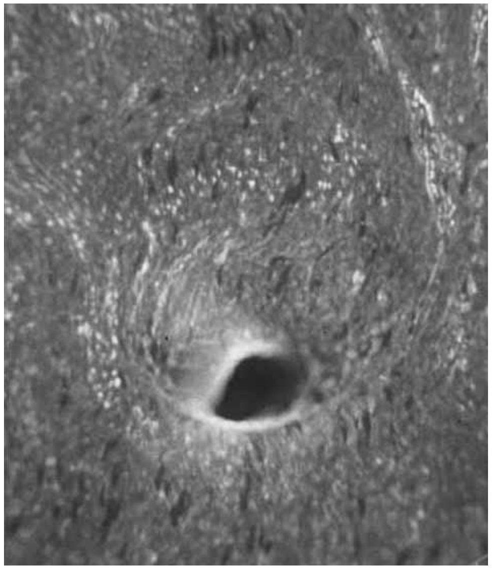
D.C. Schwenke & T.E. Carew Arteriosclerosis 9:895-918, 1989



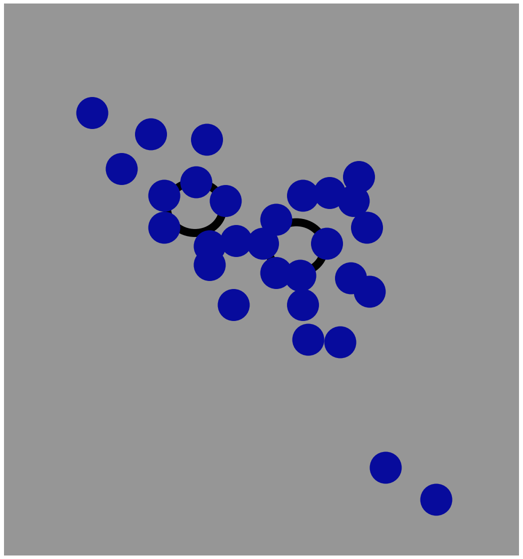
Atherosclerotic lesions (Human Aorta)

M. Texon: Hemodynamic Basis of Atherosclerosis. 1980.

MCP-1 Staining and Subintimal WBC Localization around Intercostal Orifices

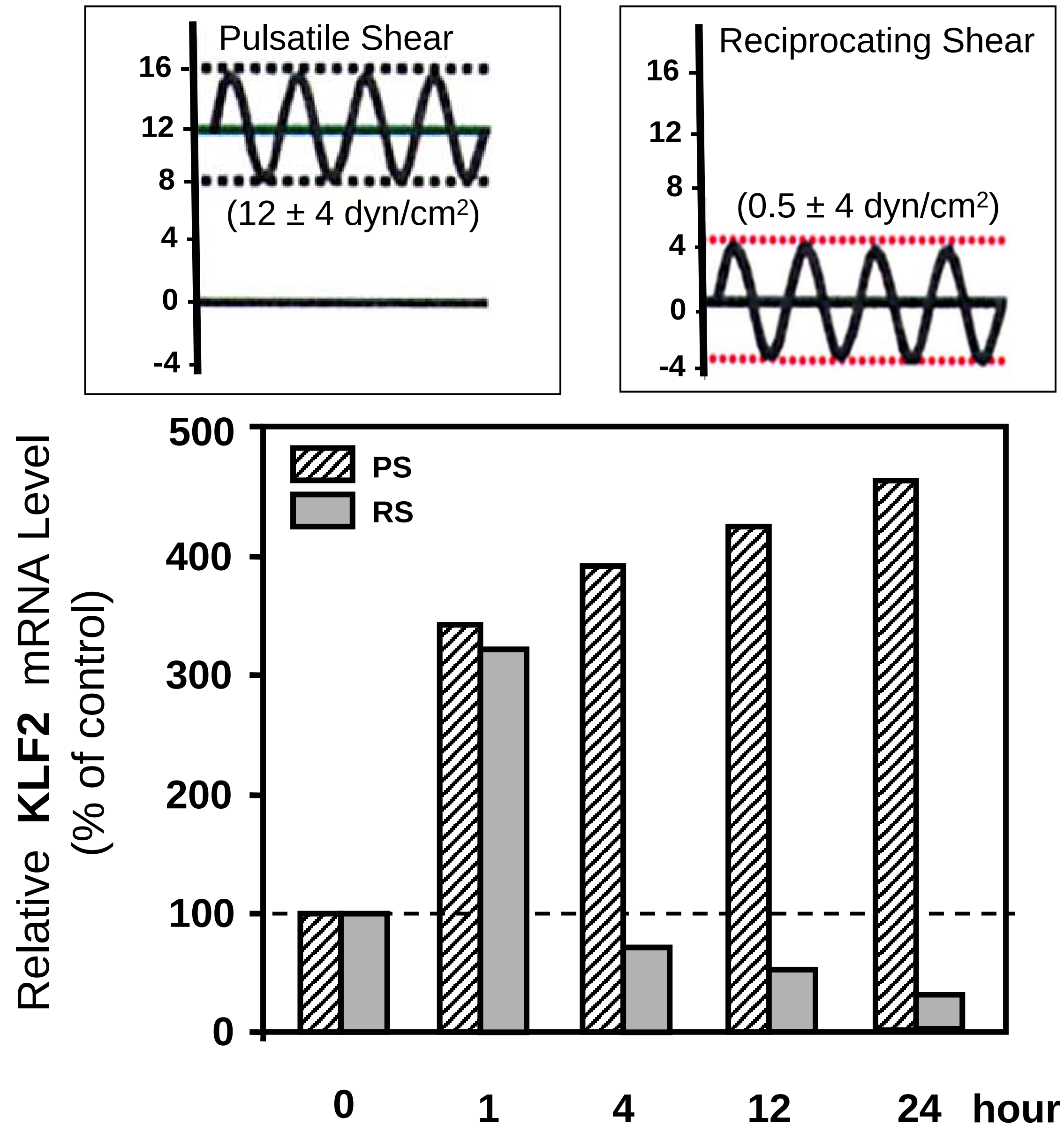


Histochemical Staining of MCP-1 (Rat Aorta)
G. Norwich & S. Chien

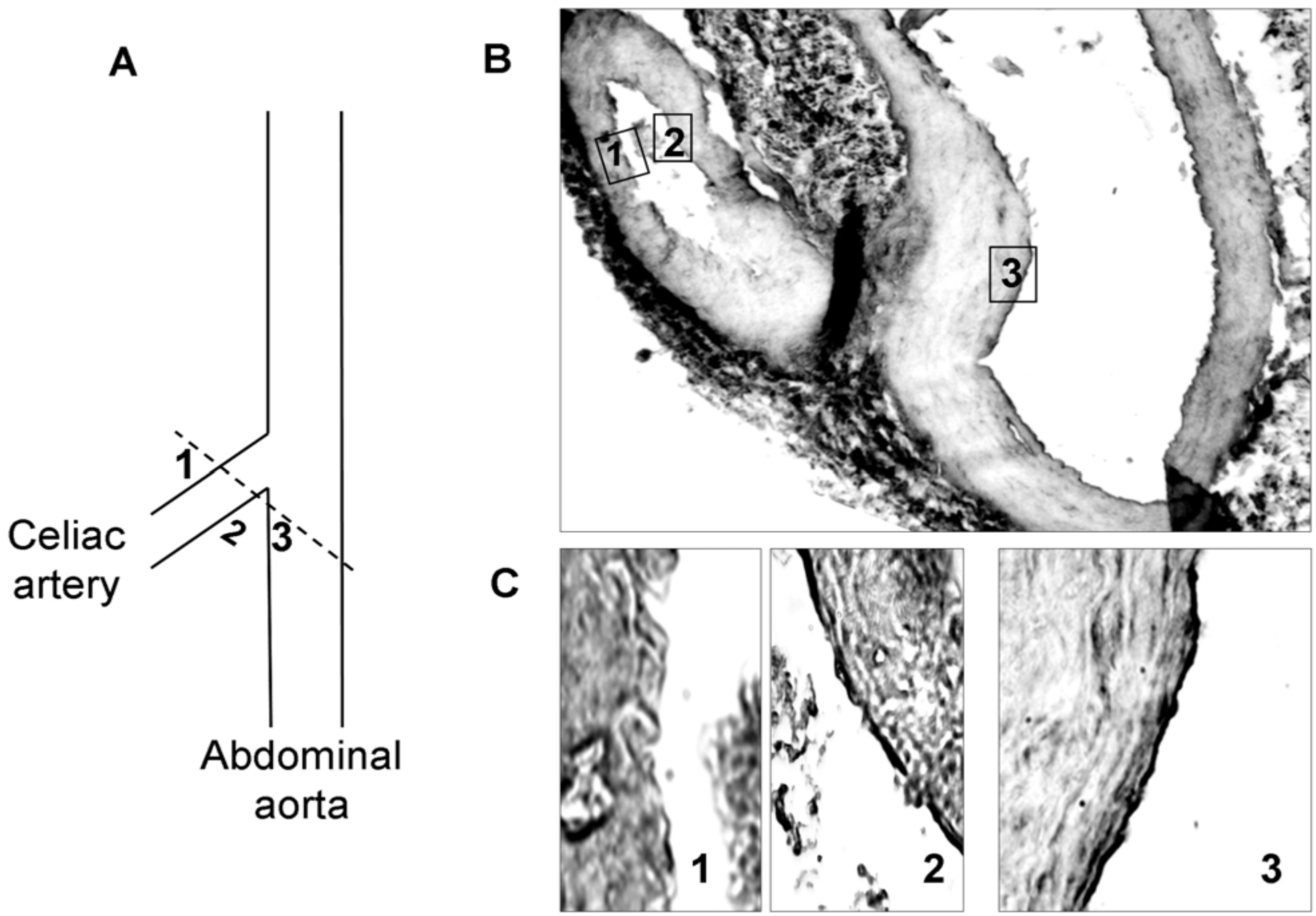


Intimal Distribution of WBC (Rabbit Aorta)
Malinauskas et al., Atherosclerosis 115:145, 1995

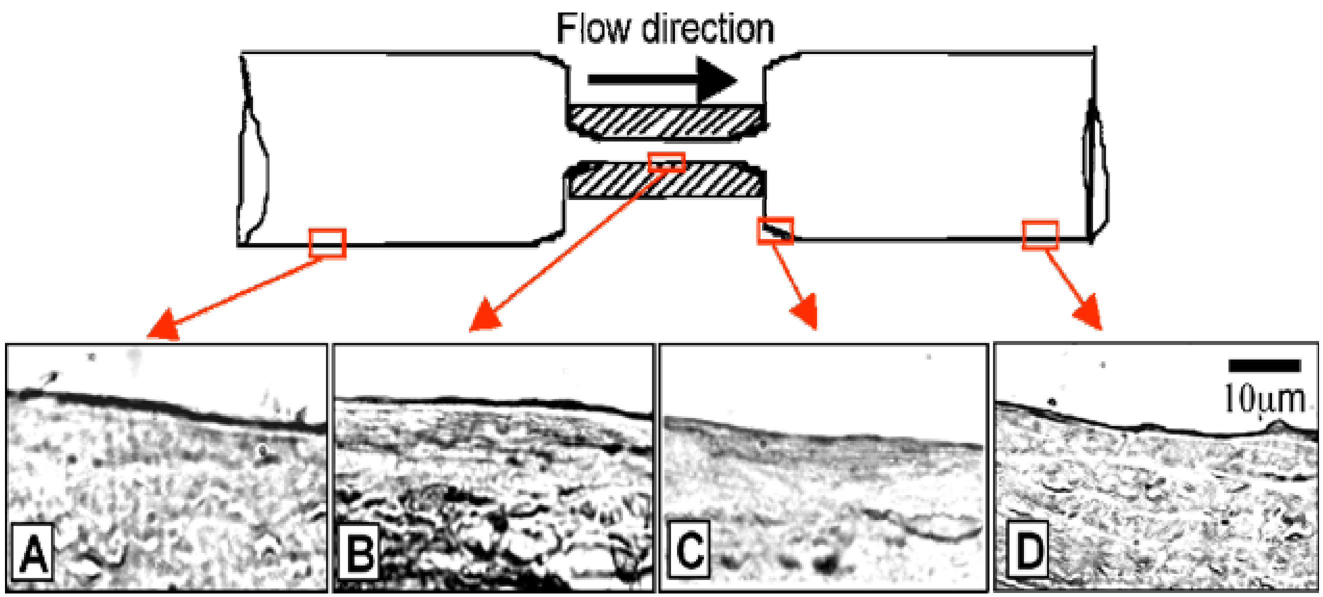
Oscillatory Shears with Different net forward Flows



Differential Regulation of KLF2 at Branch Points in Vivo



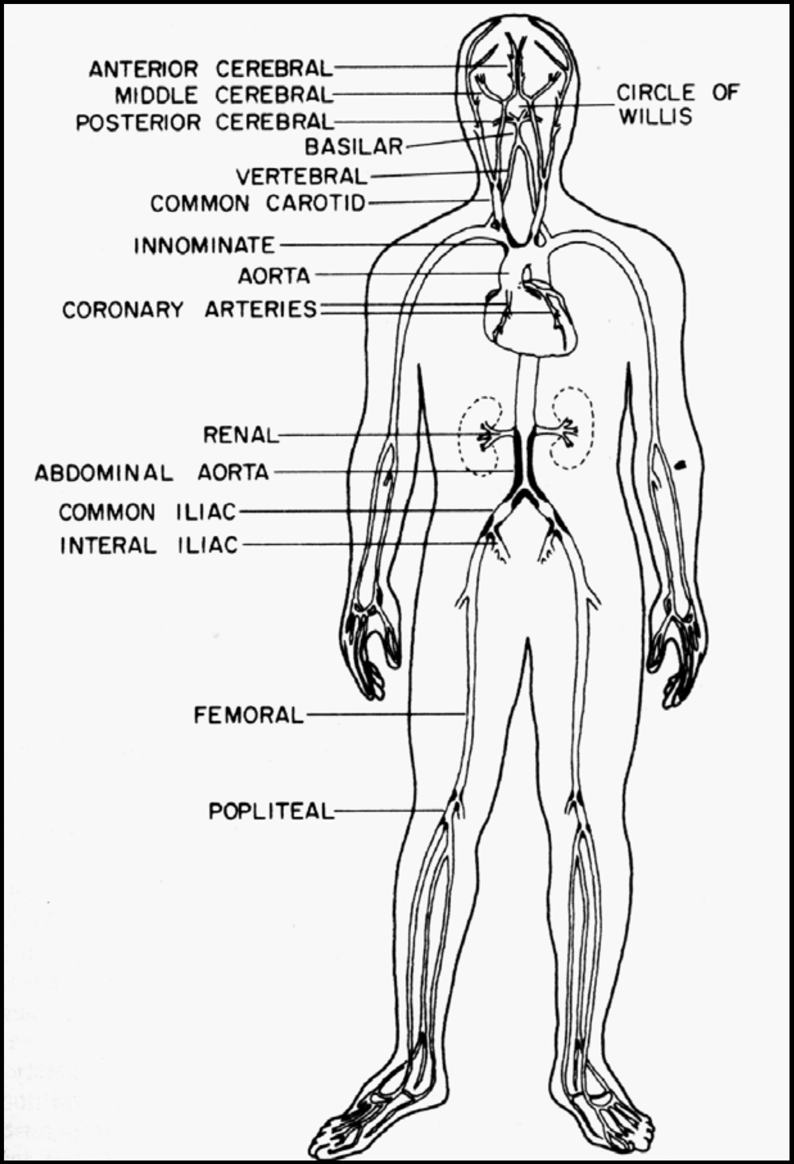
Effects of Local Constriction on KLF2 Expression in Vivo



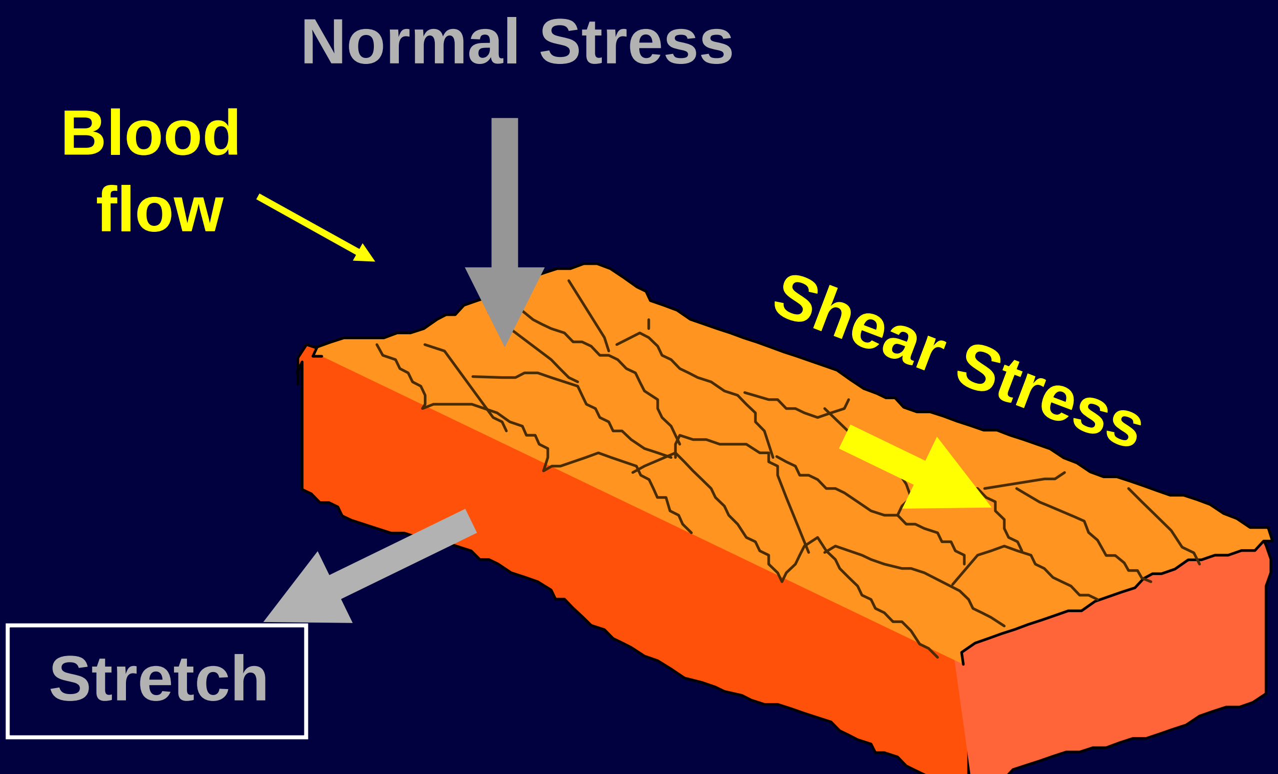
Blood Flow Patterns in Relation to Endothelial Cell Functions and Pathology

	Straight Part	Branch Points
Flow Pattern	Laminar	Disturbed
Monocyte Adhesion	↓	↑
EC turnover & LDL Permeability	↓	↑
Effects on Atherogenesis	Anti-Athero.	Atherogenic

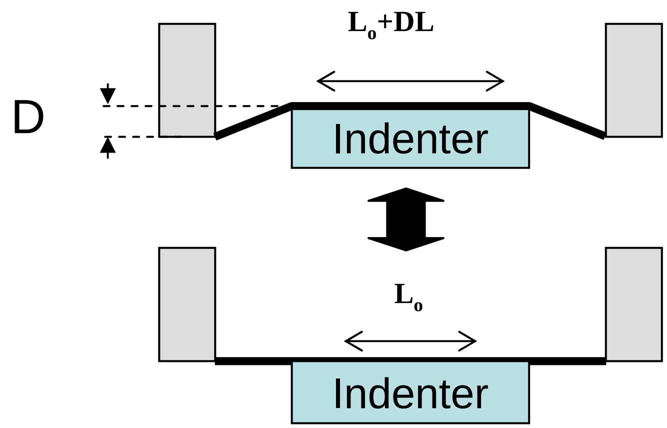
Atherosclerotic Lesions are Preferentially Located at Vessel Bifurcations

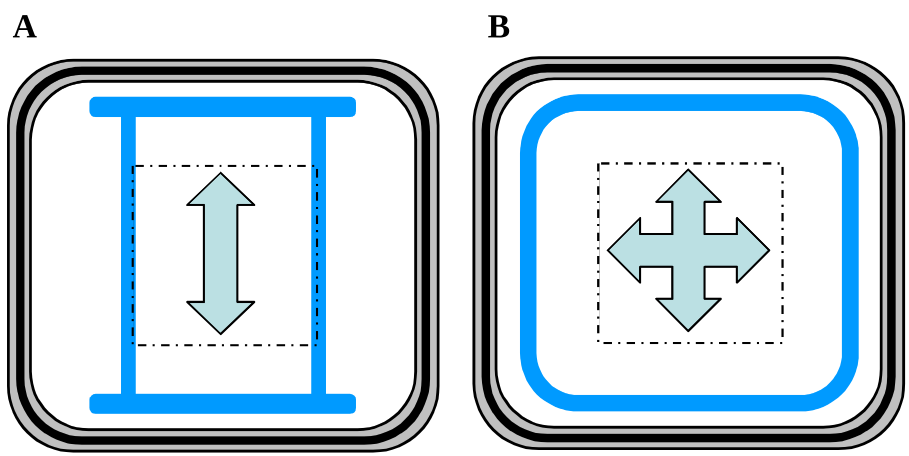


Hemodynamic Forces Acting on The Blood Vessel



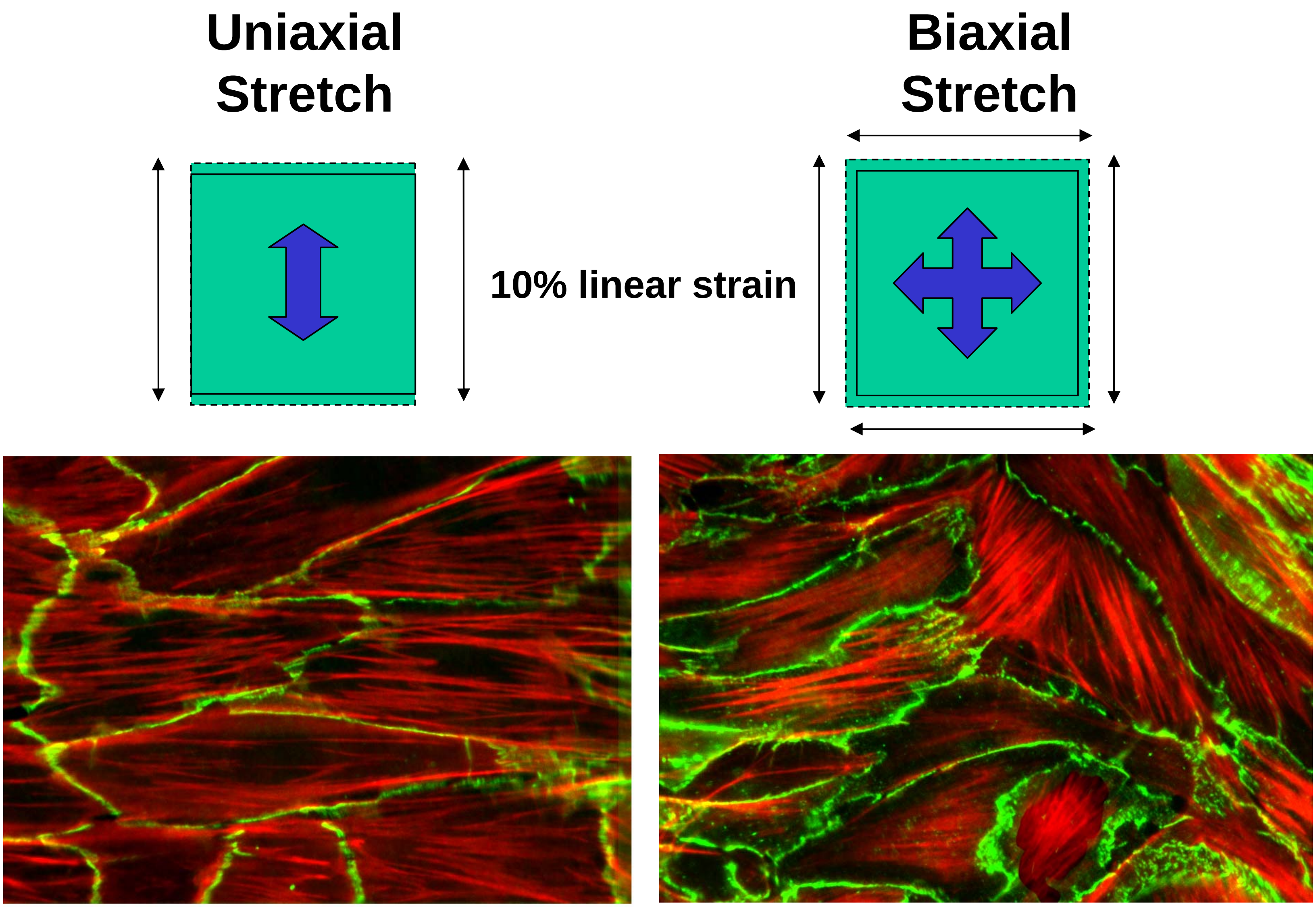
Uniaxial and Biaxial Stretch Devices





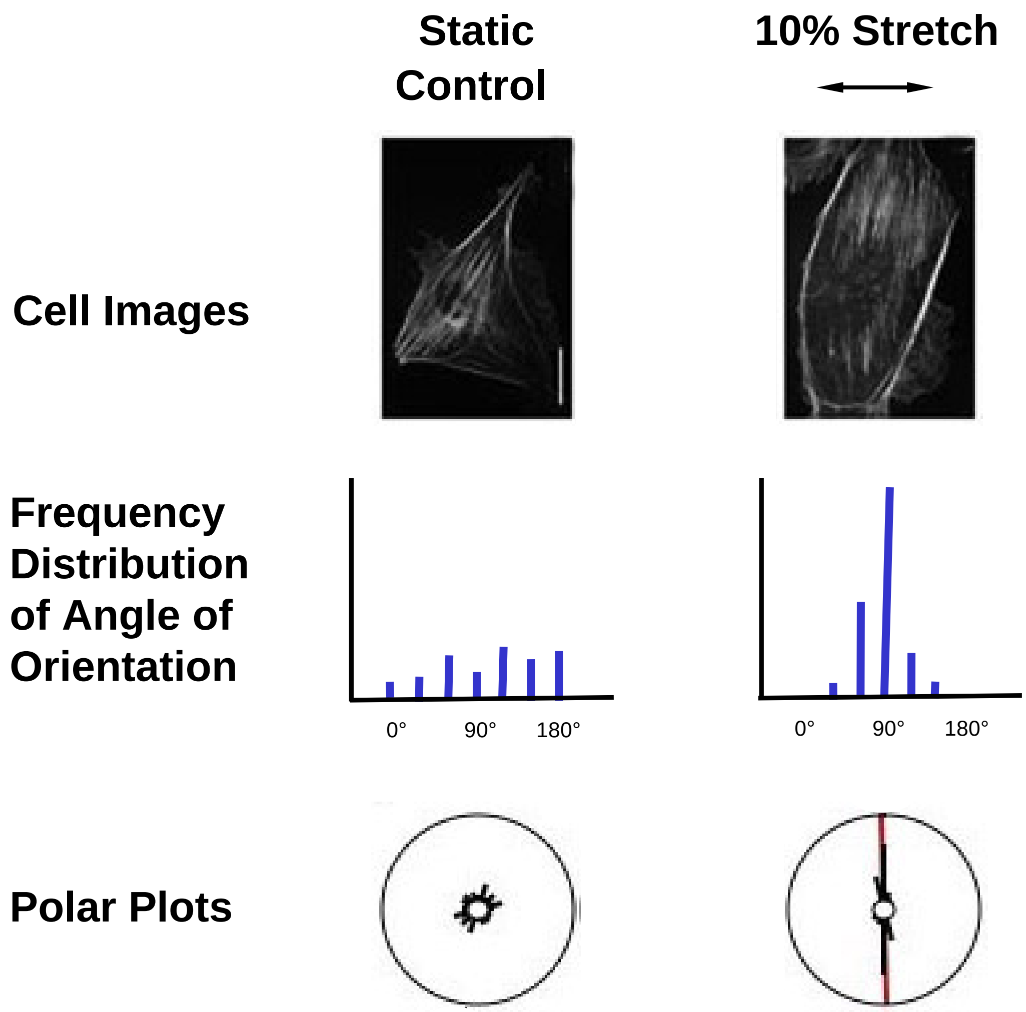
Uniaxial Stretch

Biaxial Stretch

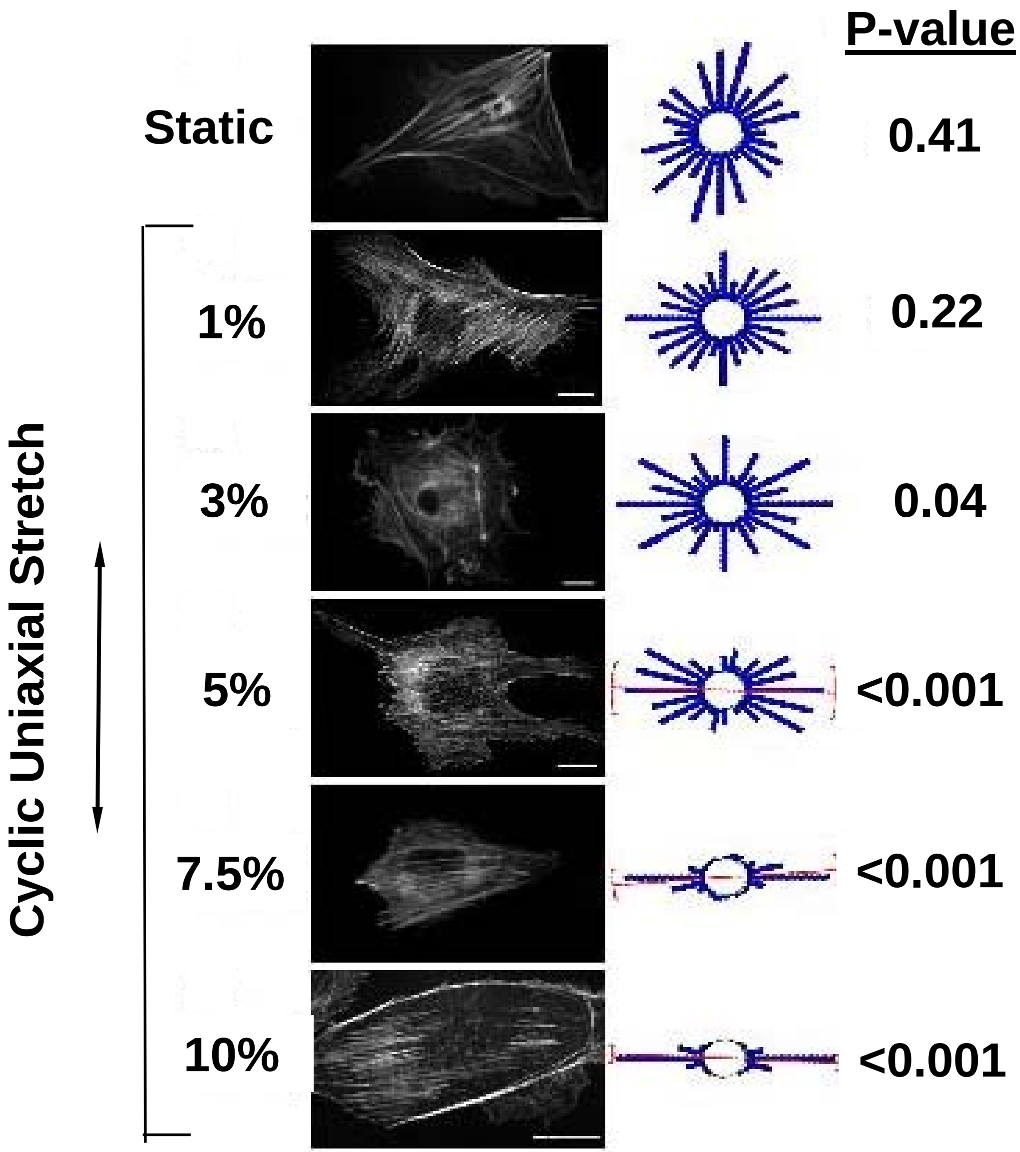


Cell Borders (β-Catenin mAb / Alexa 488 anti-mouse)
F-Actin (Rhodamine Phalloidin)

Effects of Uniaxial Stretch on Stress Fiber Orientation

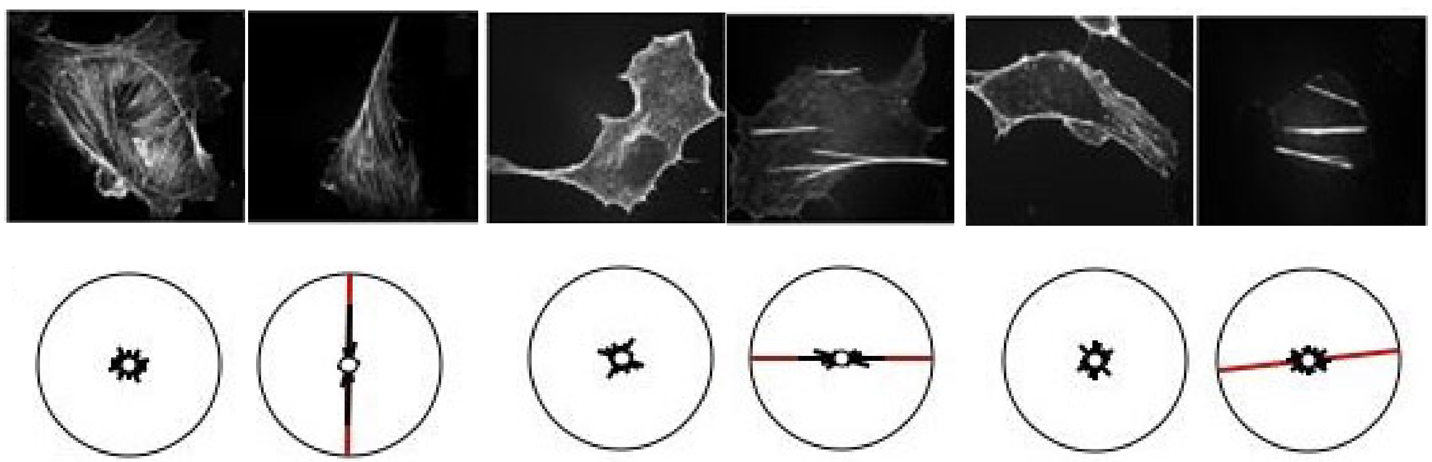


Effects of Uniaxial Stretch Magnitude on Stress Fiber Orientation



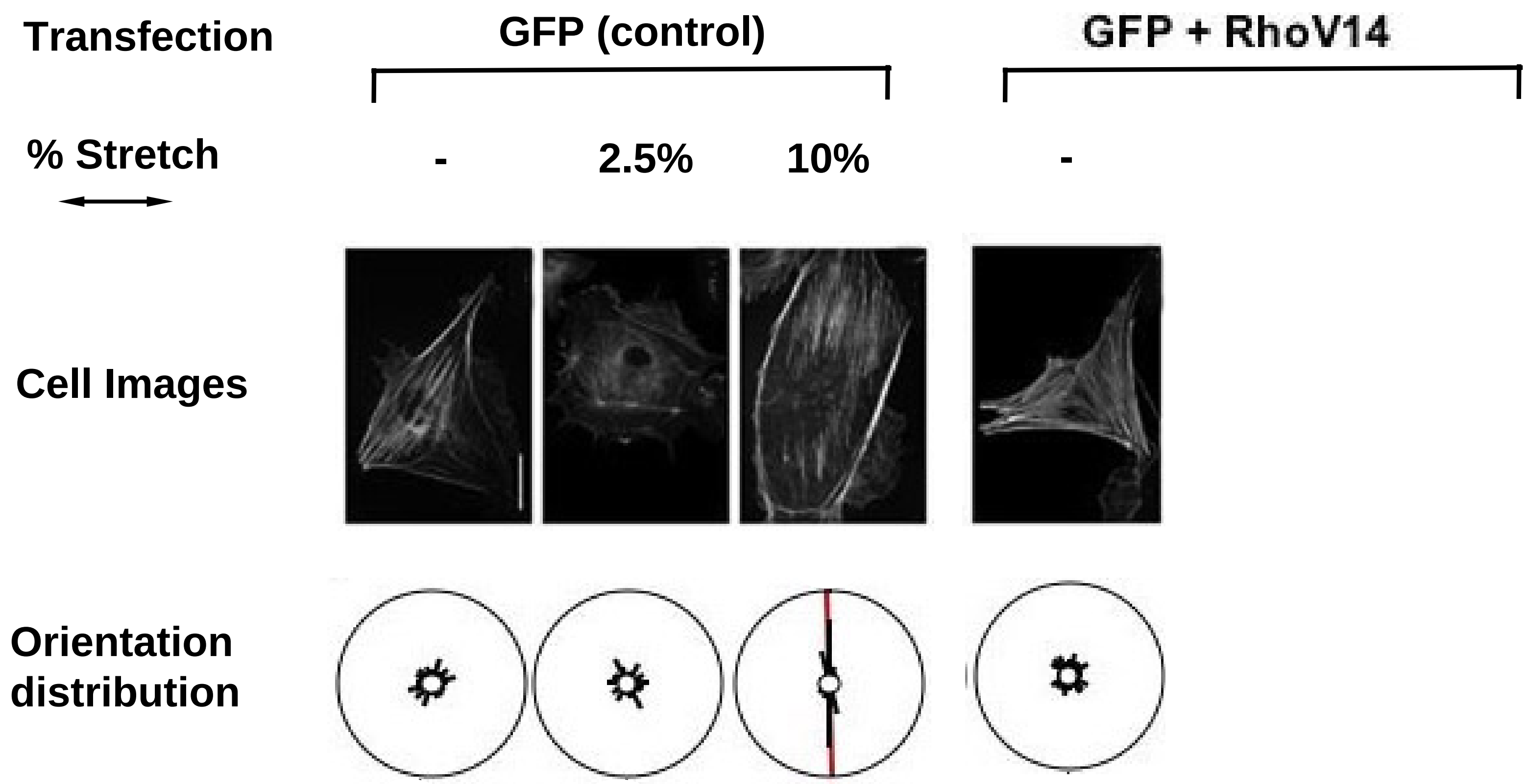
Effects of Inhibition of Downstream Effectors of Rho: Rho Kinase (ROCK) and MDia on Stress Fiber Orientation Induced by 10% Stretch

Transfection	pEF (control)		pEF + Y27632		pEF + F1F2Δ1	
% Stretch ↔	-	10%	-	10%	-	10%
Cell Images						
Orientation distribution						

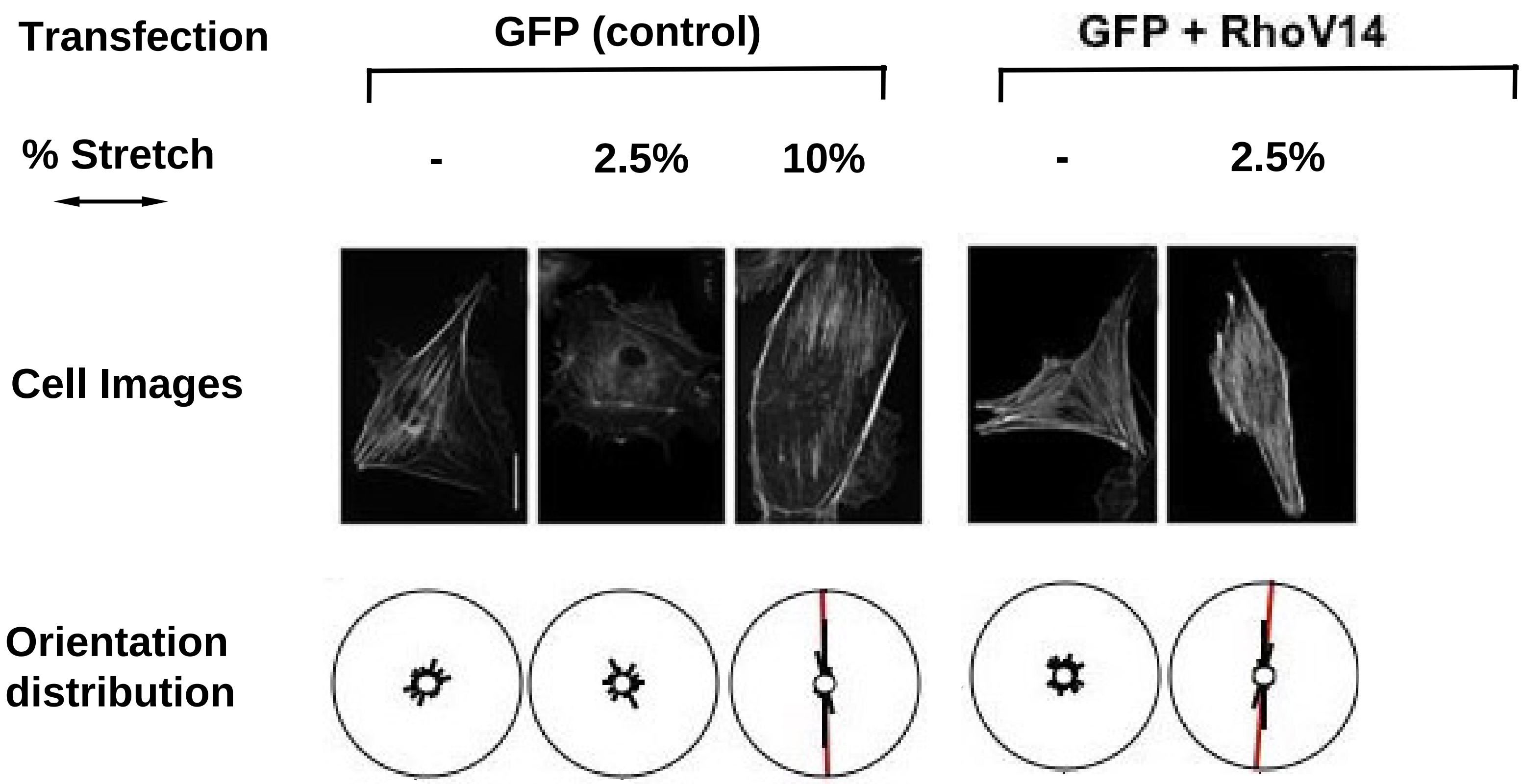


Inhibition of ROCK (Y27632) or MDia (F1F2Δ1) changed the 10% stretch-induced stress fiber orientation from <u>perpendicular</u> to <u>parallel</u>.

Effects of Active Mutant RhoV14 on Stress Fiber Orientation



Effects of Active Mutant RhoV14 on Stress Fiber Orientation



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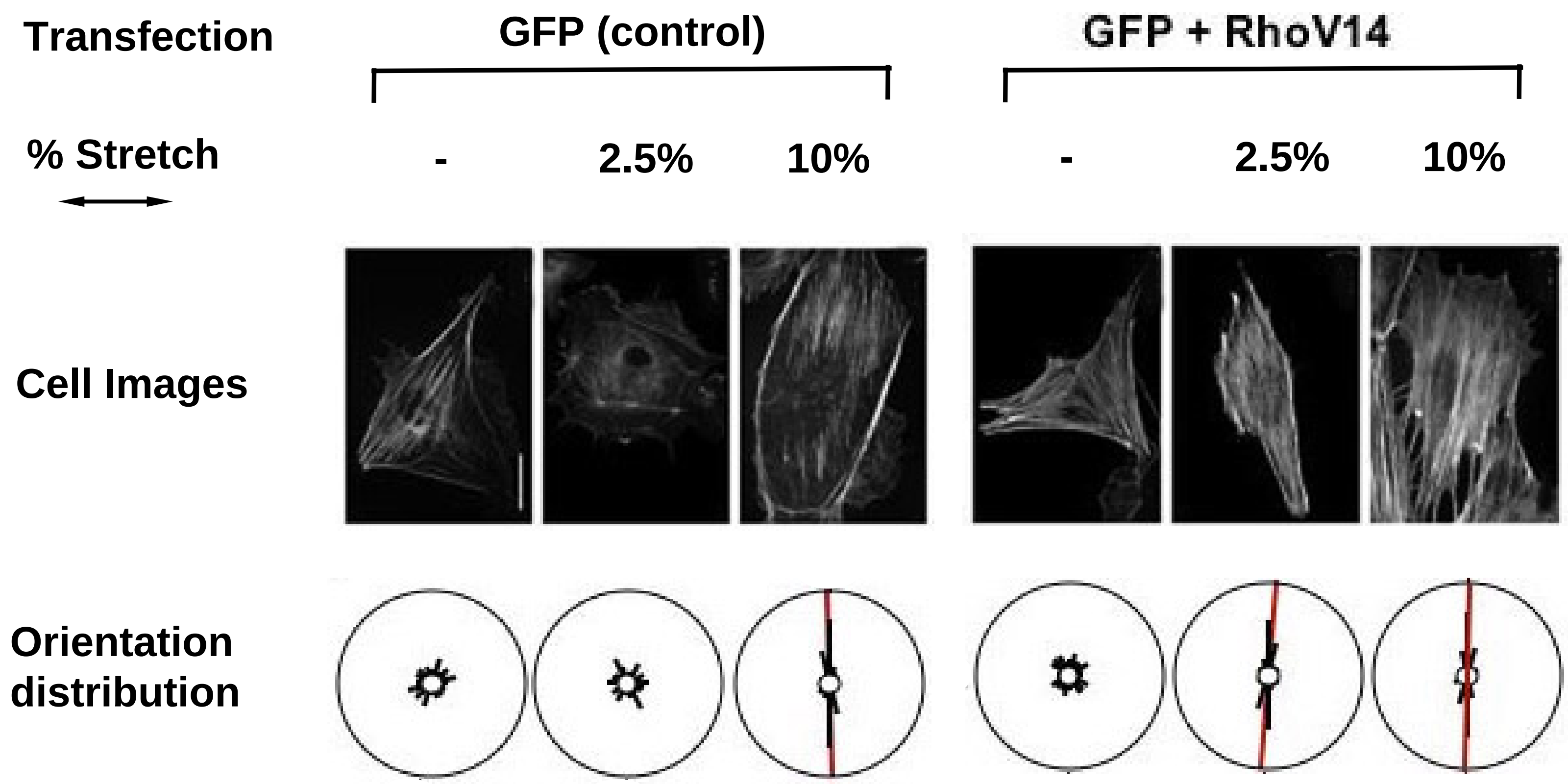
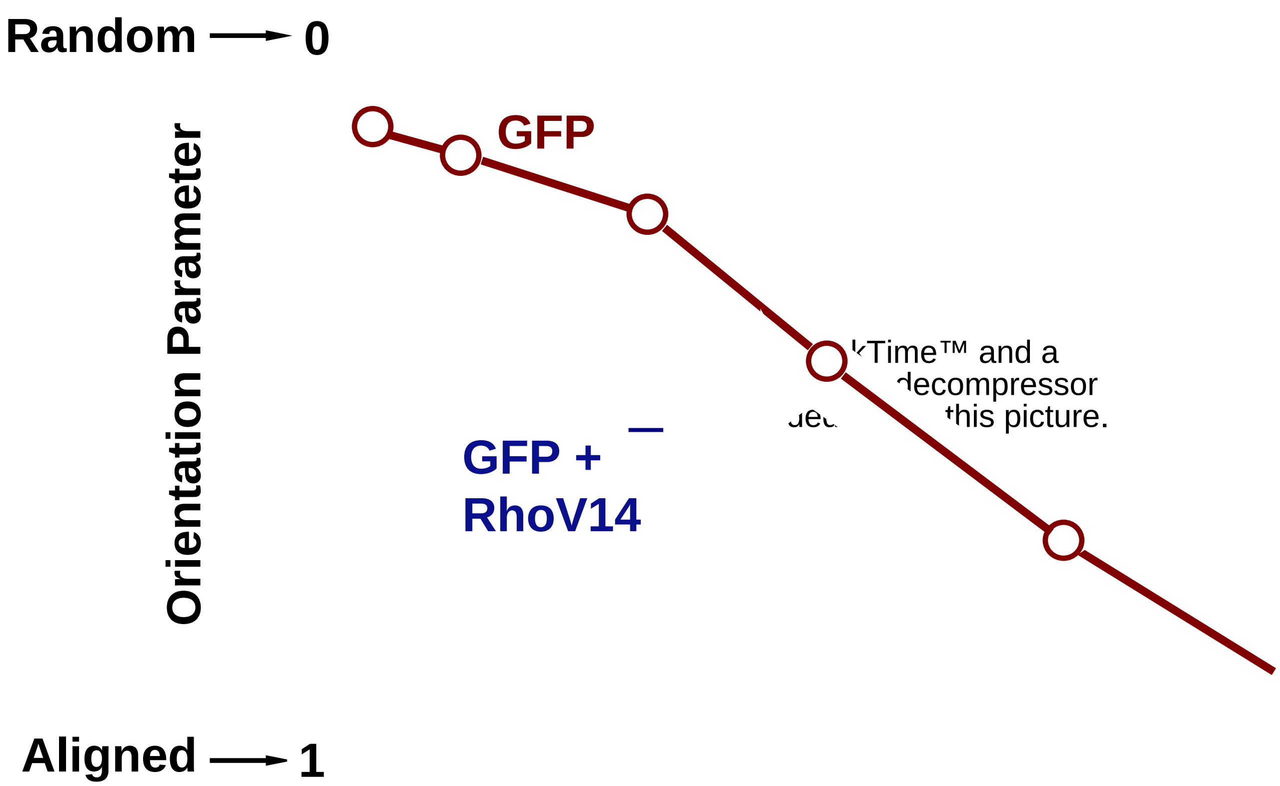


Effect of Active Rho Mutant (RhoV14) on Stretch-Induced Stress Fiber Orientation



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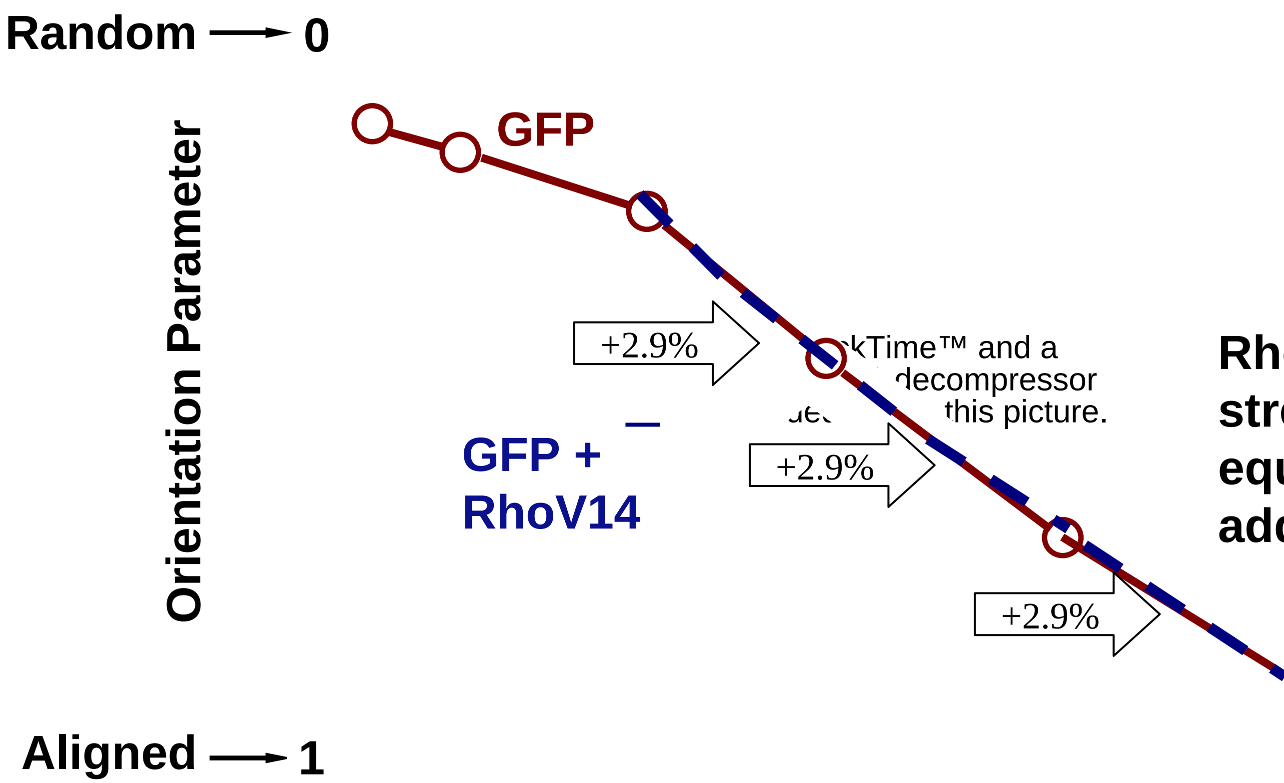
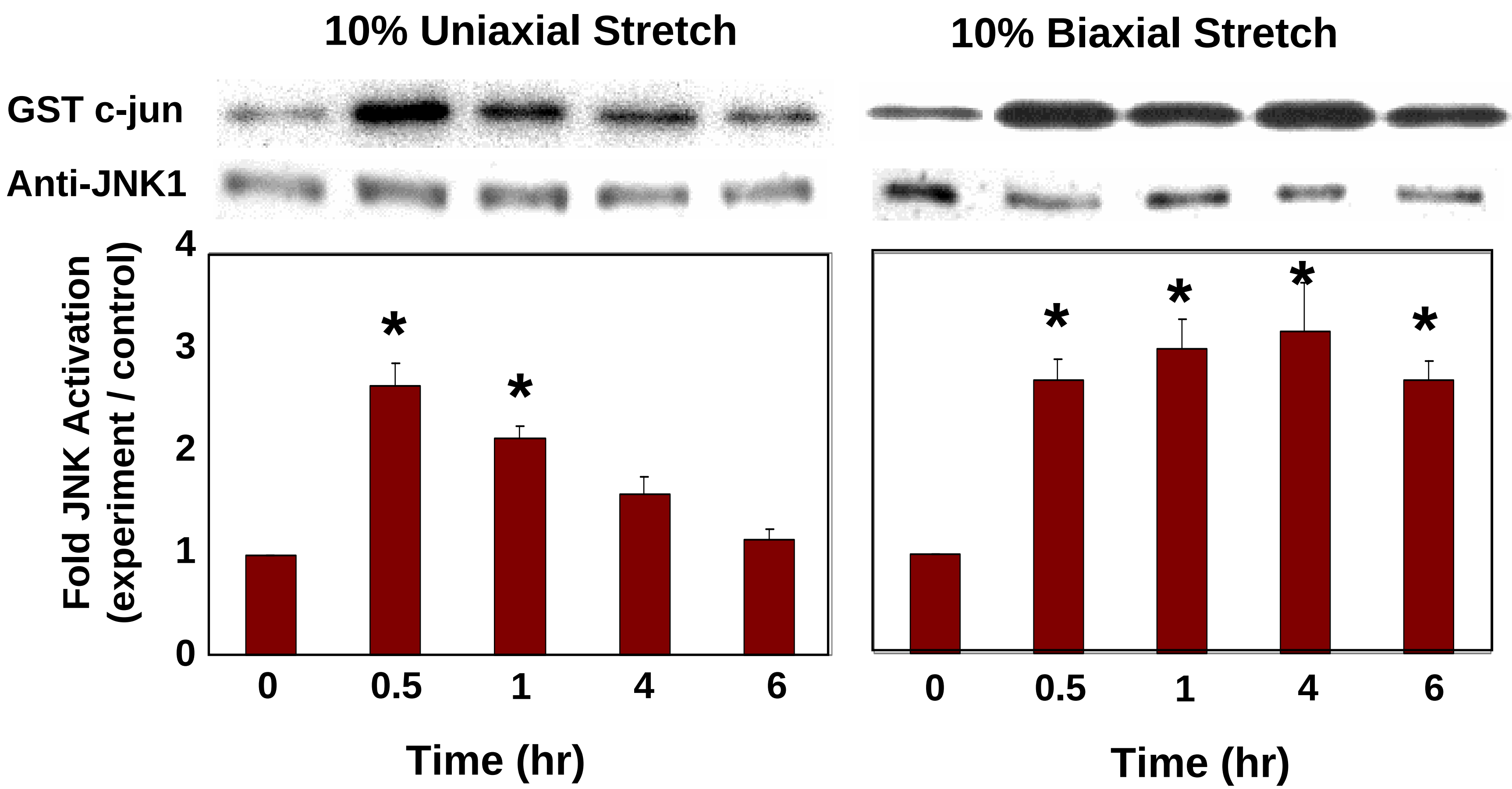


RhoV14 has an effect on stress fiber orientation equivalent to an additional 2.9% stretch.

JNK Activation is Transient in Response to Uniaxial Stretch, but Sustained with Biaxial Stretch



	Uniaxial Stretch	Biaxial Stretch
Cell and Actin Filament Orientation	Perpendicular to Stretch	Random
Time Course of JNK Activation	Transient	Sustained

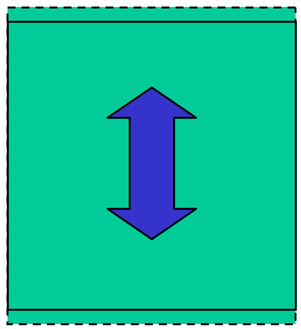
Hypothesis

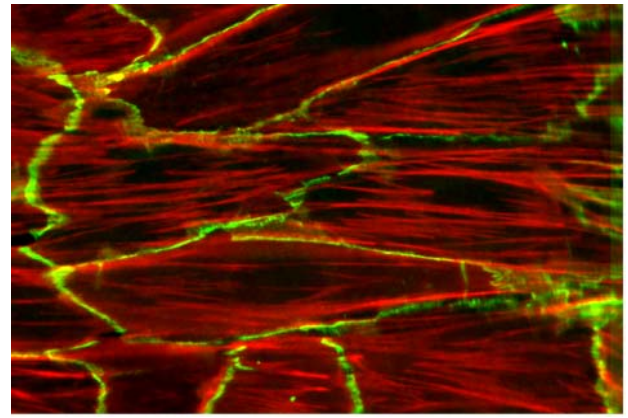
Remodeling in response to uniaxial stretch leads to the subsidence of JNK activation.

Significance

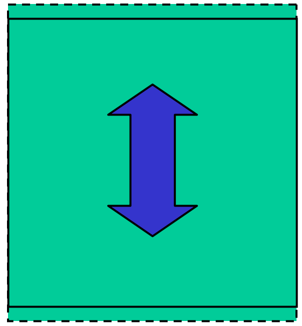
Sustained, but not transient, JNK activation causes apoptosis.

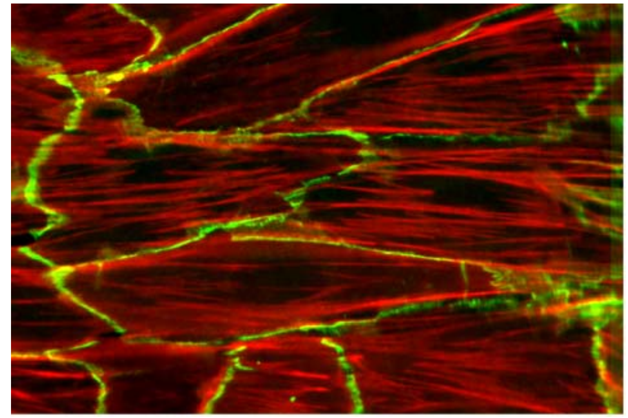
6 hr





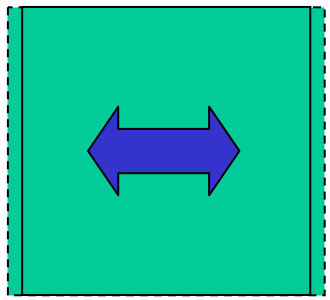
6 hr



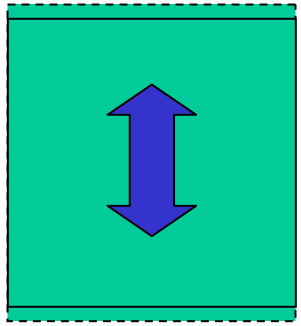


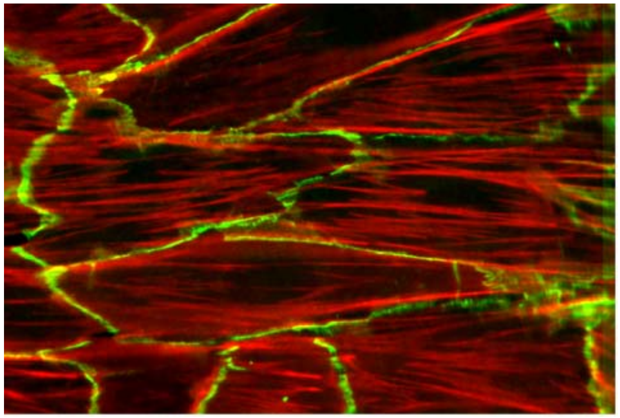
90° turn

0.5 hr



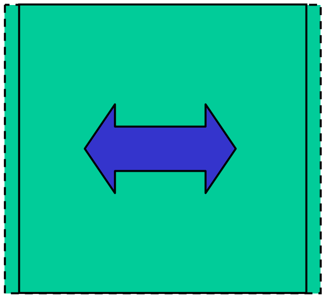
6 hr

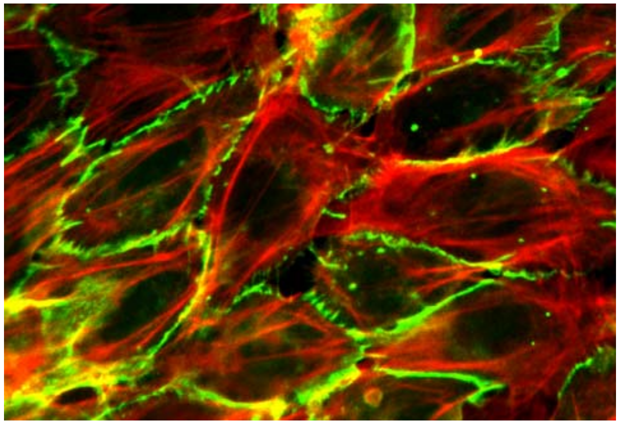


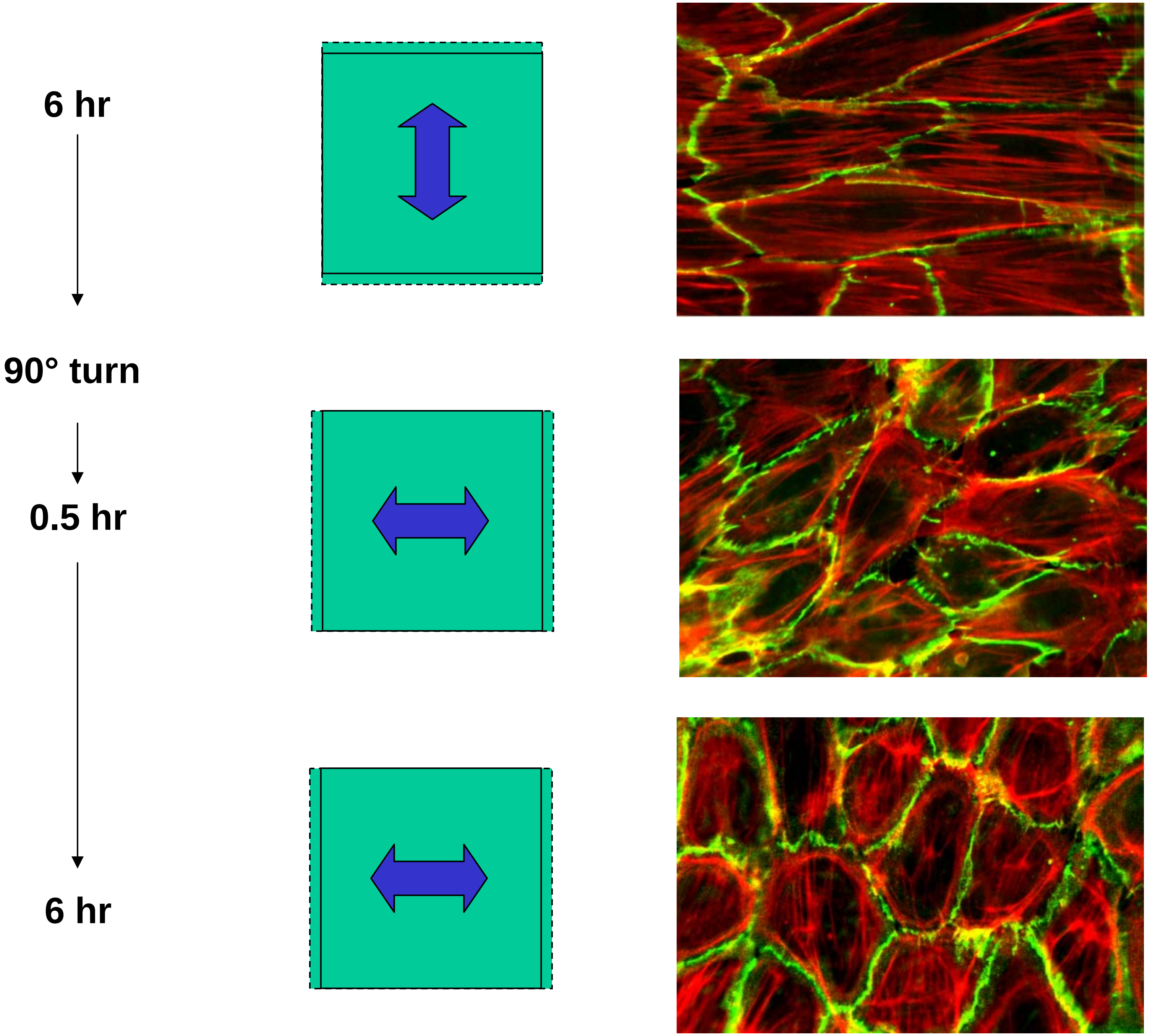


90° turn

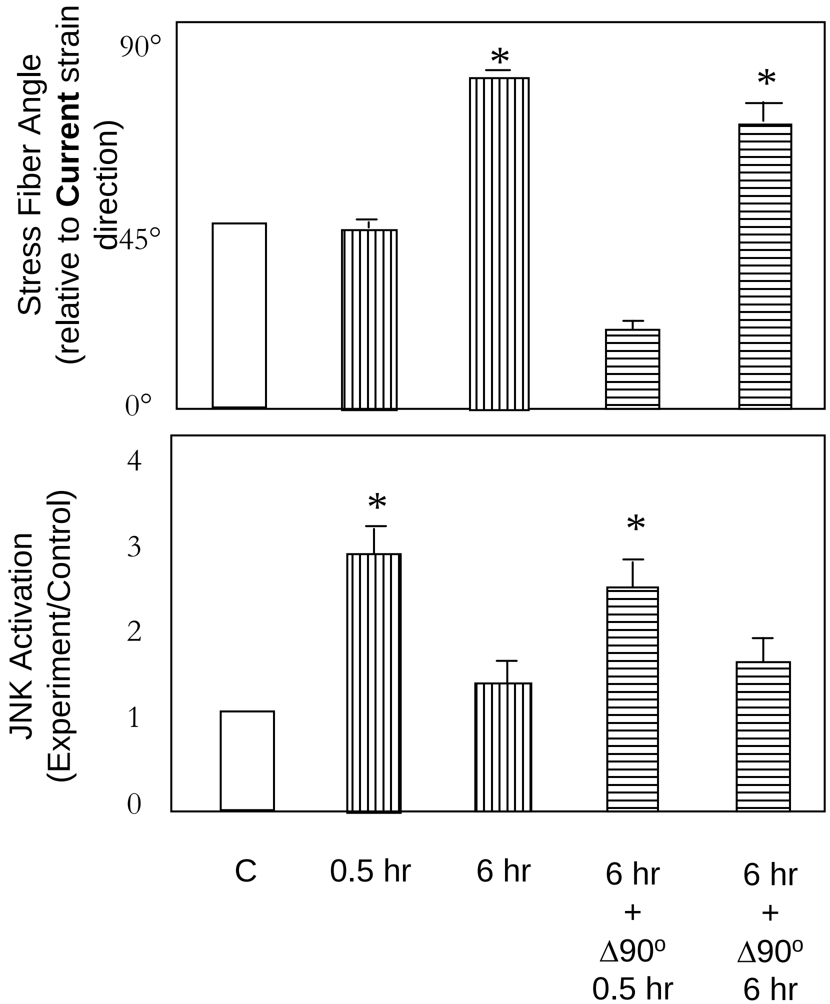
0.5 hr






6 hr
90° turn
0.5 hr
6 hr

Regulation of Stress Fiber Orientation and JNK Activation by 10% Uniaxial Stretch: Effects of Change in Stretch Direction



JNK activation subsided following stress fiber realignment

	Uniaxial Stretch	Equibiaxial Stretch
Cell and Actin Filament Orientation	Perpendicular to Stretch	Random
Time Course of JNK Activation	Transient	Sustained
Apoptosis	Protected	Enhanced

The Rho-mediated stress fiber orientation perpendicular to the direction of stretch represents a mechanism by which cells adapt to mechanical strain that involves molecular and biomechanical responses.

Conclusions:
Importance of Directionality in Mechanotransduction

Laminar flow with a net forward direction is athero-protective, whereas disturbed flow with little net forward direction is athergenic.

Uniaxial stretch with a definitive direction is anti-apoptosis, whereas biaxial stretch without a net direction leads to apoptosis.

The directionality of the mechanical stimuli and the consequent directional remodeling of the cytoskeleton play important roles in the modulation of cell functions in response to mechanotransduction.