Invited Review

THE CANNON AWARD LECTURE

Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell

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Chien S. Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell. *Am J Physiol Heart Circ Physiol* 292: H1209–H1224, 2007. First published November 10, 2006; doi:10.1152/ajpheart.01047.2006.—Vascular endothelial cells (ECs) play significant roles in regulating circulatory functions. Mechanical stimuli, including the stretch and shear stress resulting from circulatory pressure and flow, modulate EC functions by activating mechanosensors, signaling pathways, and gene and protein expressions. Mechanical forces with a clear direction (e.g., the pulsatile shear stress and the uniaxial circumferential stretch existing in the straight part of the arterial tree) cause only transient molecular signaling of pro-inflammatory and proliferative pathways, which become downregulated when such directed mechanical forces are sustained. In contrast, mechanical forces without a definitive direction (e.g., disturbed flow and relatively undirected stretch seen at branch points and other regions of complex geometry) cause sustained molecular signaling of pro-inflammatory and proliferative pathways. The EC responses to directed mechanical stimuli involve the remodeling of EC structure to minimize alterations in intracellular stress/strain and elicit adaptive changes in EC signaling in the face of sustained stimuli; these cellular events constitute a feedback control mechanism to maintain vascular homeostasis and are atheroprotective. Such a feedback mechanism does not operate effectively in regions of complex geometry, where the mechanical stimuli do not have clear directions, thus placing these areas at risk for atherogenesis. The mechanotransduction-induced EC adaptive processes in the straight part of the aorta represent a case of the "Wisdom of the Cell," as a part of the more general concept of the "Wisdom of the Body" promulgated by Cannon, to maintain cellular homeostasis in the face of external perturbations.

feedback; gene expression; Rho GTPase; shear stress; signal transduction; strain

ENDOTHELIAL CELLS (ECs), besides being a permeability barrier between the blood and vessel wall, perform many important functions, e.g., cell migration, remodeling, proliferation, apoptosis, and the production, secretion, and metabolism of biochemical substances, as well as the regulation of contractility of vascular smooth muscle cells (SMCs). In addition to their modulations by chemical ligands, ECs respond to mechanical factors, such as fluid shear stress and stretch, which can also be sensed by the EC to modify intracellular signaling, gene expression, and protein expression to result in functional regulations (Fig. 1).

The mechanical stresses (forces per unit area, with a unit of dyn/cm^2) acting on the vessel wall include the normal and circumferential stresses that result from the action of pressure and the shear stress that acts parallel to the luminal surface of the vessel due to flow (Fig. 2). The circumference stress acts along the vessel wall perimeter to cause stretching. The shear stress acts parallel to the cell surface and is a product of fluid viscosity and the velocity gradient between adjacent layers of the flowing fluid. The patterns of these stresses are different between the straight part of the arterial tree versus the branch points and curved regions (10, 16, 49). In the straight part, the shear stress and stretch have well-defined directions, and they can induce feedback mechanisms to minimize the effects of the external stresses imposed by pressure and flow, thus maintaining vascular homeostasis. In contrast, the mechanical stimuli at branch points and curved regions do not have defined directions, and they do not elicit the feedback mechanisms to minimize cellular responses. As a result, the persistence of the external stresses would lead to undesirable signaling responses that are proatherogenic. This presentation summarizes the work done in our laboratory on mechanotransduction in the EC in response to shear stress and stretch. The results, together with the data in the literature, have led to the formulation of the hypothesis that the feedback control of intracellular mechanics and signaling in response to the externally imposed stresses serves to maintain homeostasis at the cellular level, which is required for normal endothelial functions and protection against pathophysiological changes such as atherosclerosis.[1]

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[1] The Walter B. Cannon Award Lecture commemorates the 6th President of the American Physiological Society, Walter Bradford Cannon, who pioneered research addressing the emergency function of the sympathetic nervous system and the key concept of physiological homeostasis. The award is presented to an outstanding scientist who has made lasting and seminal contributions to research in physiology. The 2003 Walter B. Cannon Award Lecture was presented by Dr. Shu Chien at the American Physiological Society 2003 Experimental Biology Meeting.

Fig. 1. Schematic diagram showing that mechanical forces act in a manner similar to chemical ligands to stimulate endothelial cells through the activation of mechanosensors, some of which may be the receptors that respond to ligands. The sensors then activate the signaling pathways, which in turn activate transcription factors (Trans), which bind with the appropriate *cis* elements in the promoter region of the gene to modulate its expression. Such mechanotransduction leads to modulations of protein expression and cellular functions.
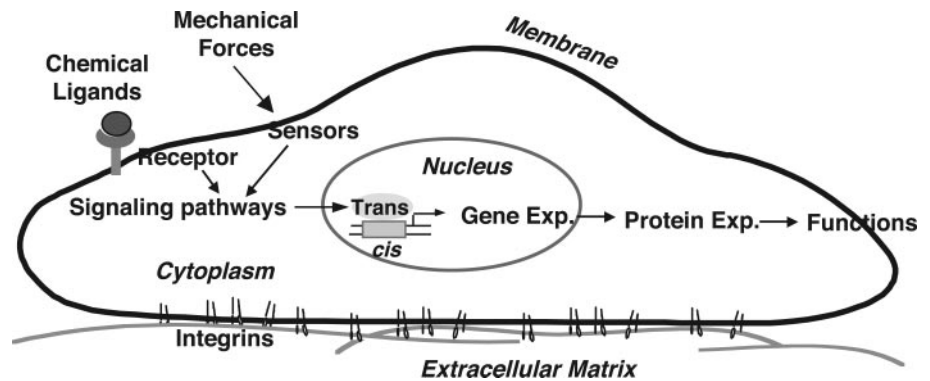
Walter B. Cannon advanced the important concept of homeostasis in his classical book on *The Wisdom of the Body* (6). As indicated in the subtitle, *How the Human Body Reacts to Disturbance and Danger and Maintain the Stability Essential to Life*, Cannon used many examples to illustrate how the body responds (mainly through the autonomic nervous activities) to perturbations of its external environment with the goal of maintaining the constancy of its internal environment, i.e., the physicochemical properties of the extracellular fluid bathing the cells. The current paper presents an extension of this important concept by showing how ECs maintain their intracellular homeostasis in the face of variations of their extracellular microenvironment, especially the mechanical stimuli due to shear stress and stretch. This concept of "The Wisdom of the Cell," with appropriate modifications, can be applied to cells other than ECs and to other forms of physicochemical stimuli besides the mechanical factors discussed here. In fact, such feedback controls at cellular and subcellular levels have been demonstrated in many cellular, molecular, and genetic studies.

MECHANOTRANSDUCTION IN RESPONSE TO SHEAR STRESS

We have studied mechanotransduction in ECs using both in vitro and in vivo approaches. In vitro studies have the advantage that the experimental variables, including the mechanical conditions, can be controlled. To have relevance to physiological and pathophysiological conditions, however, in vivo studies are valuable in determining the applicability of the in vitro findings. The following are summaries of our in vitro and in vivo studies on the response of ECs to shear stress in relation
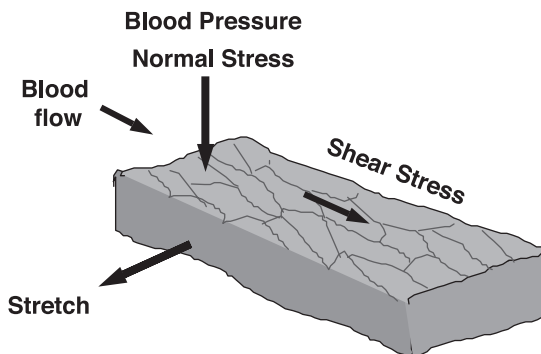

Fig. 2. Schematic diagram showing the generation of shear stress parallel (tangential to the endothelial cell surface) by blood flow and the generations of normal stress (perpendicular to the endothelial cell surface) and circumferential stretch due to the action of pressure. Modified from Chien (11).

to our hypothesis that the feedback control in mechanotransduction serves to maintain vascular homeostasis.

In Vitro Flow Chamber Studies on Mechanotransduction in Response to Shear Stress

Flow chambers have been employed in our laboratory for in vitro studies on the responses of cultured bovine aortic ECs (BAECs), human aortic ECs (HAECs), and human umbilical vein ECs (HUVECs) to shear stress. ECs cultured to confluence on the bottom surface of the rectangular channel are exposed to the flow generated by a pressure difference between the inlet and the outlet of the chamber (Fig. 3*A*). The parallel-plate flow channel created by using a gasket with a rectangular cutout has a uniform channel height along the flow path, and it can be used to study the effects of steady shear at 12 dyn/cm^2 (Fig. 4*A*), "static" control with shear stress at 0.5 dyn/cm^2 (Fig. 4*B*), pulsatile shear at 12 ± 4 dyn/cm^2 (Fig. 4*C*), and reciprocating shear at 0.5 ± 4 dyn/cm^2 (Fig. 4*D*). The steady component 0.5 dyn/cm^2 for the reciprocating shear (Fig. 4*D*) is used to maintain nutrient supply; this low level does not cause shear-induced responses (41, 76). For experiments on the effects of disturbed shear, a step-flow channel (13) is used in which a vertical step expansion of channel height at the entrance is created by using two silicone gaskets, with the top one having a longer longitudinal cutout than the one below (Fig. 3*B*). The disturbed flow pattern beyond the step consists of a region with flow recirculation in a direction opposite to the inflow, a region of flow reattachment (where the wall shear stress is near zero but the shear stress gradient is high), and then transition to a region of forward flow similar to that seen in the channel with a uniform height. Thus the step-flow channel allows the study of the responses of ECs to different shear flow patterns in a single chamber. The different flow patterns shown in Fig. 4 can be applied to shear ECs in the step-flow chamber.

Effects of shear stress on mechanosensing and intracellular signaling. The application of shear stress to ECs can activate a number of mechanosensors (11, 38). These include membrane proteins such as receptor tyrosine kinase (e.g., the vascular endothelial growth factor receptor Flk-1) (8, 77), the integrins (especially $\alpha_v\beta_3$, but also $\alpha_2\beta_1$, $\alpha_5\beta_1$, and $\alpha_6\beta_1$) (28, 45, 59), G proteins and G protein-coupled receptors (36, 38, 51, 71), Ca^{2+} channel (83), and intercellular junction proteins (52, 73). Membrane lipids (5, 22) and membrane glycocalyx (57, 80) may also play a role.

A Rectangular Flow Chamber
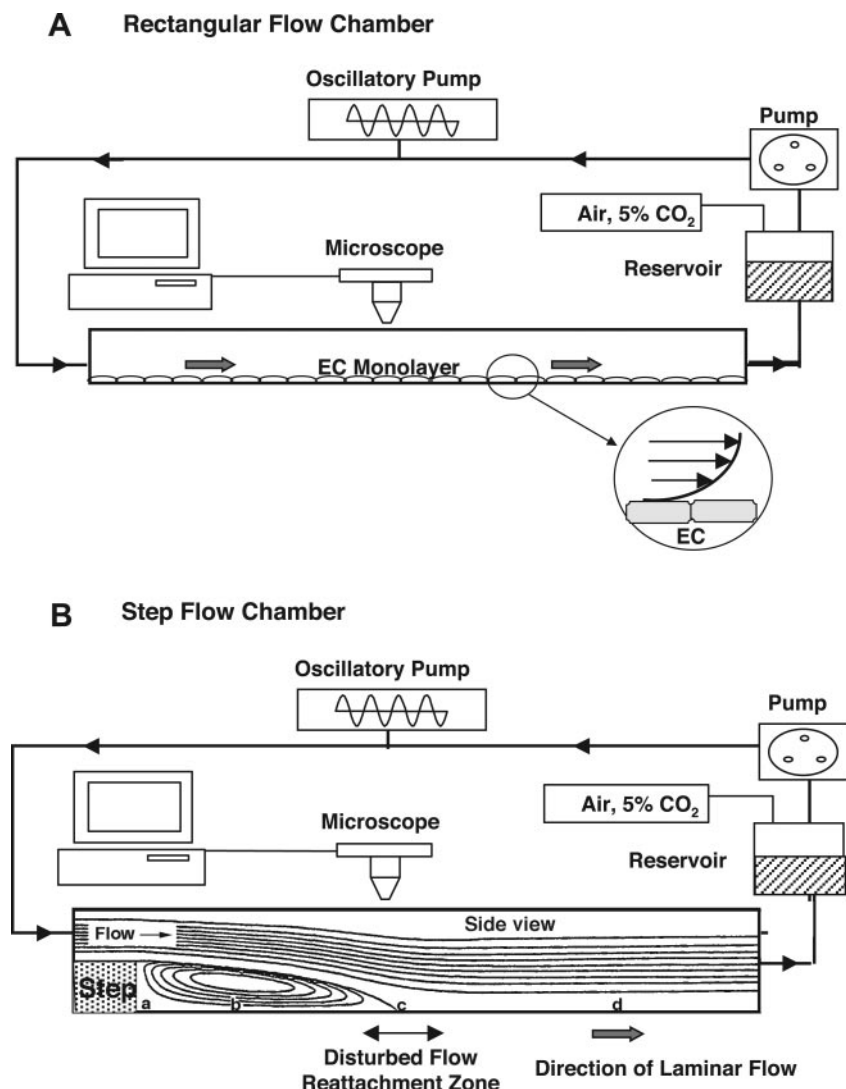
B Step Flow Chamber

Fig. 3. Side views of rectangular flow chamber (*A*) and step-flow chamber (*B*). In both bases, the shear flow can be either steady without oscillations or superimposed with sinusoidal oscillation by using the oscillatory pump. The endothelial cell (EC) monolayer is shown only in *A*; *inset* in *A* shows an enlarged view of two ECs and the velocity gradient due to the applied shear stress. In *B*, the flow direction in *zone b* is opposite to the incoming flow and to that in *zone d*, where forward flow is reestablished. *Zone c* is the reattachment zone where the flow fluctuates between the forward and backward directions, with a low shear stress but large shear stress gradient; this is the area of disturbed flow.

The mechanosensing, mediated through adaptor molecules (e.g., Shc, Grb2, and Sos), triggers a cascade of signaling pathways, and consequently modulates the expression of a number of genes, e.g., genes concerned with proliferation or growth arrest, inflammation or anti-inflammation, and many others. The changes in gene and protein expressions, in turn, regulate the functional behavior of ECs in health and disease (39). The mechanotransduction processes depend on the mode of shearing. Studies on cultured ECs have shown that shear stress with a significant forward direction (whether steady shear stress without oscillation, Fig. 4*A*, or pulsatile shear stress in which an oscillatory component is superimposed on a steady shearing, Fig. 4*C*) generate comparable effects. In contrast, shearing without a significant forward direction, e.g., reciprocating flow with little net forward component (Fig. 4*D*) or disturbed flow generated near the reattachment zone in a step-flow channel (Fig. 3*B*) (13, 25, 45, 65), yields similar results that tend to be opposite to shearing with a significant forward direction. The effects of different modes of shearing are discussed below.

Effects of shear stress on EC expression of monocyte chemotactic protein-1. The expression of the monocyte chemotactic protein-1 (MCP-1) gene is modulated by the Ras-mitogen-acti-

vated protein kinases (MAPKs) pathway. The activation of MAPKs entails the phosphorylation of a series of serine-threonine protein kinases (Fig. 5), with Ras serving as an upstream molecule and ERK, JNK, and p38 as three key downstream molecules. The application of a steady shear stress (e.g., 12 dyn/cm^2) to ECs causes Ras to become bound with GTP instead of GDP, and this is followed sequentially by the activation of MAPKs (29, 39, 67) and the MCP-1 expression (62). These responses are transient in nature; sustained laminar shear stress causes the deactivation/downregulation of Ras (a few seconds), MAPKs (on the order of an hour), and MCP-1 gene expression (63), which decreases to below the preshear level by 5 h (40, 62) (Fig. 6). Therefore, whereas laminar shear stress has a short-term effect of upregulation of MCP-1, it has a long-term effect of downregulation when its application is sustained, which is analogous to the condition encountered in the straight part of the arterial tree. Thus ECs in static culture respond to the applied shear flow by a transient MCP-1 activation, which then vanishes when the cells adapt to the long-term shear stress. The induction of MCP-1 expression by oxidative stress has been shown to be attenuated by pulsatile shear stress and augmented by reciprocating shear stress (25). The functional consequence of this downregulation in response
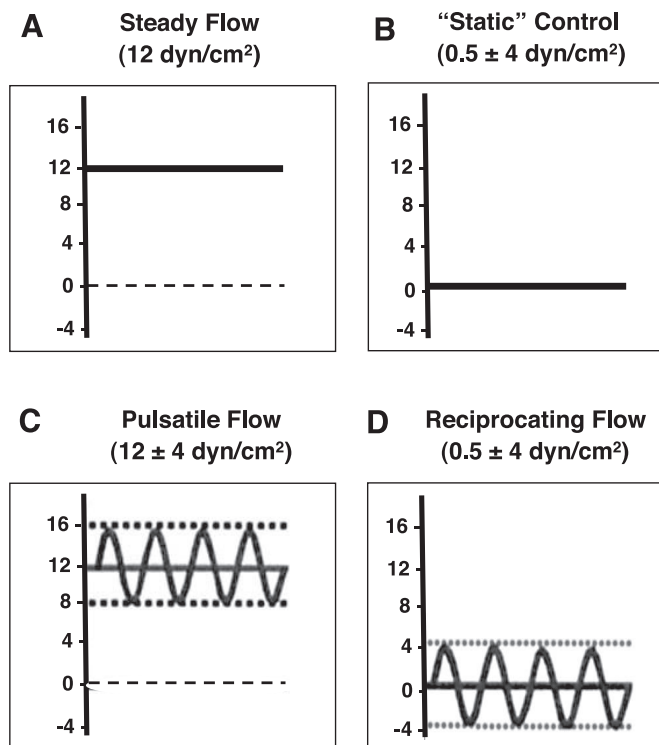
A **Steady Flow**
(12 dyn/cm²)

B **"Static" Control**
(0.5 ± 4 dyn/cm²)

C **Pulsatile Flow**
(12 ± 4 dyn/cm²)

D **Reciprocating Flow**
(0.5 ± 4 dyn/cm²)

Fig. 4. Differential flow patterns used to study EC responses to shearing in the flow chambers. *A*: steady flow with a shear stress of 12 dyn/cm² without oscillation. The dashed line shows the zero shear stress for comparison. *B*: "static" control with a very low shear stress of 0.5 dyn/cm² without oscillation, for the delivery of nutrient. *C*: pulsatile flow with a sinusoidal oscillatory shear stress of 12 ± 4 dyn/cm². *D*: reciprocating shear stress with a very low mean shear stress of 0.05 dyn/cm² and a sinusoidal oscillation of ±4 dyn/cm².

to sustained shearing is a suppression of monocyte attraction into the vessel wall and is thus atheroprotective.

Effects of shear stress on EC KLF-2 expression and EC survival. Krüppel-like factor-2 (KLF-2) is a member of the KLF family zinc finger-containing transcription factors (2, 58). It is abundantly expressed in ECs (37) and is beneficial to EC survival (76). We have contrasted the effects of pulsatile shear stress (12 ± 4 dyn/cm² at 1 Hz), which has a significant forward direction, with those of reciprocating shear stress (0.5 ± 4 dyn/cm² at 1 Hz), which has a minimal forward direction, on the expression of KLF-2 in human umbilical vein ECs (76). The mRNA level of KLF2 increases significantly after exposure to both pulsatile and reciprocating shear stresses at 1 h (Fig. 7). With continued reciprocating shearing, however, KLF2 gene expression decreases below the basal level at 4 h and remains low throughout the 24 h of shearing. In contrast, pulsatile shear stress results in a sustained upregulation of KLF2 for as long as 24 h under continuous shearing (Fig. 7). There is no significant change in KLF-2 expression in static control over the 24-h experiment.

Blockade of KLF-2 by transfecting small interfering RNAs (siRNAs) into ECs causes a significant decrease in KLF2 gene expression. The siRNA knockdown of KLF2 gene does not affect the HUVEC viability under basal condition, but it significantly decreases the HUVEC viability after exposure to oxidized LDL. These results indicate that 24-h reciprocating shear stress, which lacks a significant forward component, inhibits KLF2 expression and decreases the ability of ECs to survive against oxidative stress. Twenty-four-hour pulsatile shear stress, which has a significant forward component, results in continued expression of KLF2 and is beneficial to EC survival.

Effects of shear stress on cell proliferation. Using the DNA microarray approach, we have investigated gene expression
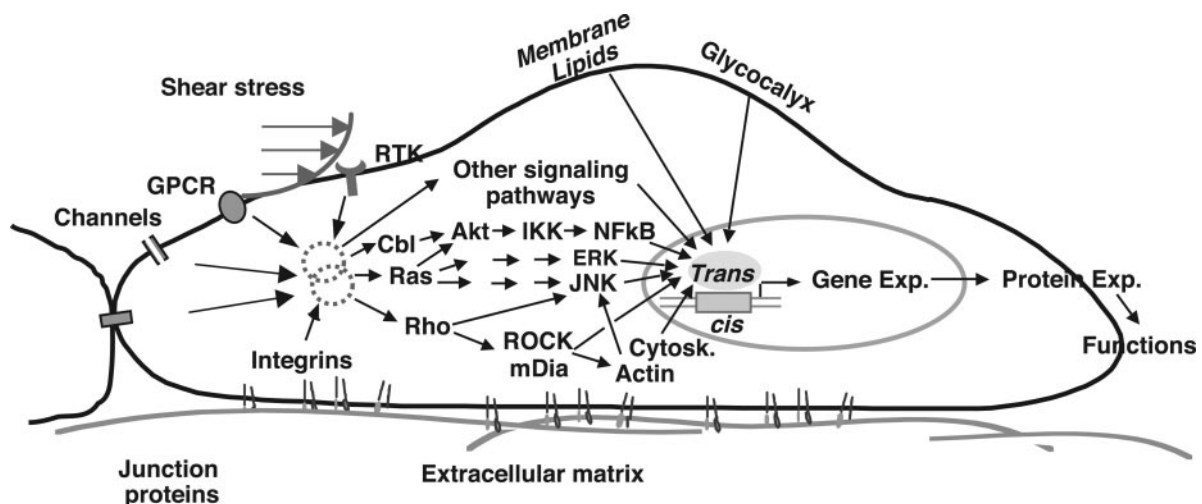

Fig. 5. Schematic diagram showing the action of shear stress on potential sensors in endothelial cells, including receptor tyrosine kinases (RTK), G protein-coupled receptor (GPCR), ion channels, junction proteins, and integrins, as well as membrane lipids and glycocalyx. These mechanosensors act through adaptor molecules (represented by the two dotted circles) to activate upstream signaling molecules such as Ras, which then activates the mitogen-activated protein (MAP) kinase pathways, including ERK and JNK, and then the transcription factors (e.g., activator protein-1, AP-1) for gene expression (e.g., monocyte chemotactic protein-1, MCP-1). Arrows to the left of ERK and JNK are used to represent the phosphorylation cascade that involves the sequential phosphorylation of protein kinases one after the other. Various mechanotransduction pathways are used to modulate the expression of different genes. Also shown are the small GTPase Rho and its downstream molecules Rho kinase (ROCK) and mDia, which are stimulatory to actin. It is also possible for mechanical signals to be perceived by the cytoskeleton, including actin, to modulate gene expression. Shown are the interactions (direct or indirect) between JNK and actin. There are many signaling pathways that are not shown in this figure. This diagram illustrates that mechanotransduction involves the interplay among many mechanosensors, signaling molecules, and genes, all of which form complex networks to modulate EC structure and function.
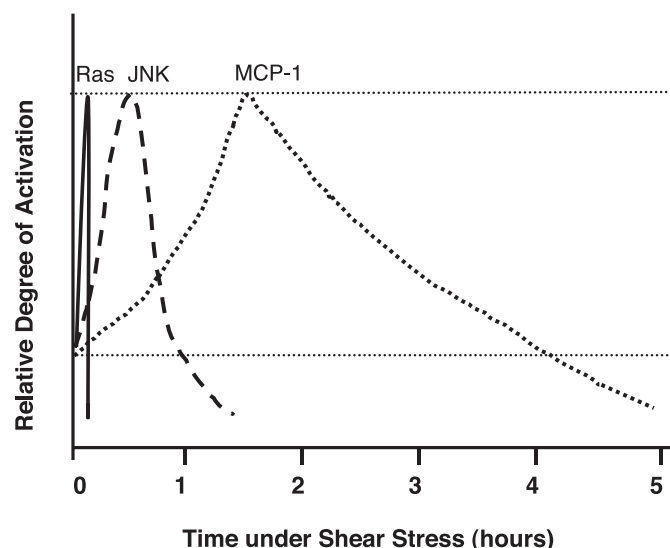
Fig. 6. Time courses of the sequential activations of Ras, JNK, and MCP-1 followed by their downregulations. The activation of Ras reaches a peak in <1 min, followed by an increase in the kinase activity of JNK with a peak at 30 min. The induction of the MCP-1 gene occurs later with a peak at 90 min. After prolonged shearing, the activities of the signaling molecules and the MCP-1 gene expression fall below those in the static controls. Lower horizontal line represents the static control level; upper horizontal line represents the peak values for the three parameters measured. Reprinted from *Progress of Biophysics and Molecular Biology*, vol. 83, Chien, "Molecular and mechanical bases of focal lipid accumulation in arterial wall," pages 131–151, 2003 with permission from Elsevier (10), based on the data in Refs. 40 and 63.

profiles in cultured human aortic ECs in response to 1–24 h of steady shear stress at 12 dyn/cm^2 (7, 86). After being sheared for 24 h, several genes related to EC proliferation and inflammation are downregulated, suggesting that sustained shearing with a forward component keeps ECs in a relatively nonproliferative and noninflammatory state compared with that of static cells. In contrast, genes involved in EC survival and angiogenesis (e.g., Tie2 and Flk-1) and vascular remodeling (e.g., matrix metalloproteinase-1) are significantly upregulated by the 24-h shear stress. Such gene expression profile in shear-adapted ECs would be relevant to the native ECs in the straight part of the aorta in vivo.

On the basis of the finding that relatively long-term shearing with a significant forward component tends to keep ECs in a low-proliferative state, Lin et al. (41) have studied the mechanism underlying the inhibition of EC growth by steady shear stress. The tumor suppressor protein p53 is found to increase in BAECs subjected to 24 h of shear stress at 3 dyn/cm^2 or higher. This shear-induced increase in p53 is attributable to its stabilization following its phosphorylation by JNK. Twenty-four-hour steady shear stress also increases the expression of the growth arrest proteins GADD45 (growth arrest and DNA damage inducible protein 45) and p21^{cip1}, and decreases the phosphorylation of the retinoblastoma gene product, thus leading to cell cycle arrest, which has also been reported by Akimoto et al. (1). This inhibition of EC proliferation by steady shear stress may serve an atheroprotective function in the straight part of the arterial tree, which is constantly subjected to high levels of shearing with a forward direction.

We have assessed the effects of disturbed flow on vascular EC proliferation by determining the incorporation of a labeled nucleotide 5-bromo-2-deoxyuridine (BrdU) into cultured ECs in the step-flow channel (Fig. 8). Under static condition, BrdU incorporation is low and randomly distributed throughout the channel. After 24 h of laminar shear at 12 dyn/cm^2, BrdU incorporation is markedly enhanced in the reattachment area and its vicinity but is much lower in the downstream laminar flow region (10). The same distribution pattern is seen for the activation of signaling molecules for proliferation such as ERK. The increase in BrdU incorporation induced in the area of disturbed flow can be blocked by the ERK inhibitor PD98059. These results indicate that the flow pattern in the reattachment area at branch points stimulates cell proliferation via ERK activation. In contrast, the region with significantly forward flow has a low cell proliferation rate due to the upregulation of growth-arrest genes.

Effects of shear stress on EC lipid metabolism. We have investigated the effects of different flow patterns on the activity of sterol regulatory element binding protein 1 (SREBP1) in ECs and the mechanotransduction mechanism involved (45). In response to sterol depletion, SREBPs are activated to increase the expressions of genes encoding for LDL receptor, cholesterol synthase, and fatty acid synthase, thus restoring the intracellular sterol level (4). The application of laminar shear stress (12 dyn/cm^2) causes a transient activation of SREBP1 and the ensuing translocation of its transcription factor domain into the nucleus; this shear effect is independent of the sterol level. Blockade of β_1-integrin with AIIB2 blocking-type MAb or disruption of actin cytoskeleton with cytochalasin D inhibits the shear-activation of SREBP1, indicating that integrins and the actin cytoskeleton play significant roles in the modulation of EC lipid metabolism in response to shear stress. Studies using the step-flow channel indicate that, in contrast to the transient activation of SREBP1 in ECs under steady flow, disturbed flow causes a sustained activation of SREBP1 (Fig. 9), which would lead to transcriptional activation of EC genes encoding for LDL receptor, 3-hydroxy-3-methylglutaryl (HMG) CoA synthase, and fatty acid synthase, all of which tend to impair lipid homeostasis in ECs.
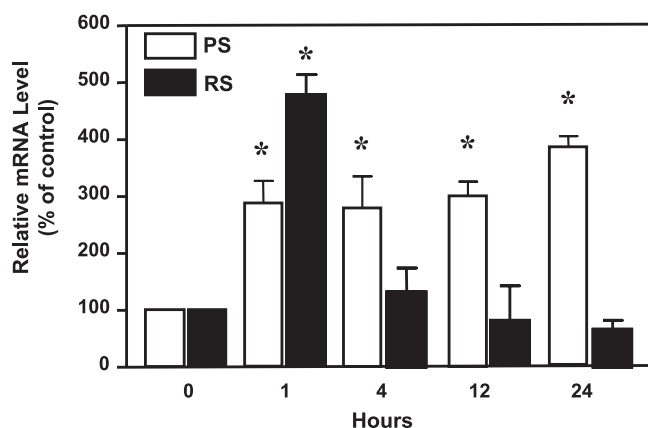

Fig. 7. Flow pattern-specific regulation of Krüppel-like factor-2 (KLF2) gene expression in ECs. Confluent ECs are subjected to pulsatile shear (PS) at 12 ± 4 dyn/cm^2, reciprocating shear (RS) at 0.5 ± 4 dyn/cm^2, or low steady flow (0.5 dyn/cm^2 as "static" control) for 24 h. The mRNA levels of KLF2 obtained 0, 1, 4, 12, and 24 h after PS and RS are normalized by that for static control. *$P < 0.05$. PS induces a sustained expression of the KLF2, whereas RS causes a transient induction with an ensuing repression. Modified from Wang et al. (76).
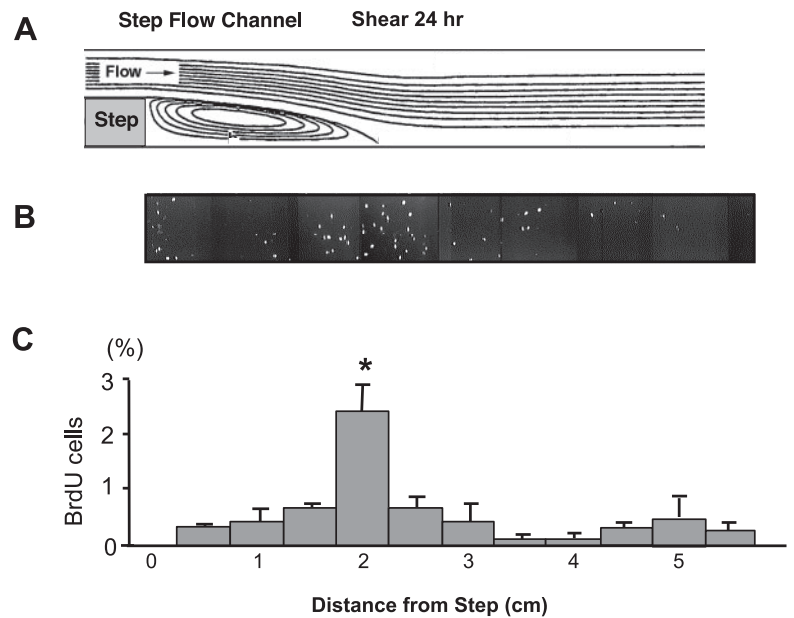
A

Step Flow Channel   Shear 24 hr

B

Fig. 8. EC proliferation rate is elevated in regions of disturbed flow. *A*: side view of the step-flow channel. The top view of the bovine aortic EC (BAEC) monolayer in *B* shows 5-bromo-2-deoxyuridine (BrdU) incorporation in one experiment, with the positions aligned with the channel length in *A*. *C*: BrdU incorporation into BAECs in four experiments; bars are means ± SE. *Significant difference in BrdU incorporation ($P < 0.01$) in the region of disturbed flow near the reattachment point. Not shown in the bar graph is an increase in BrdU incorporation immediately next to the step.

C

Effects of shear stress on cytoskeleton organization and cell morphology. In ECs exposed to sustained shear stress with a clear direction, triple staining of actin, tubulin, and vimentin shows that the cytoskeletal fibers undergo remodeling to become oriented with the direction of shear flow (20), with a consequent alignment of the cell with the shear flow direction (Fig. 10). The effects of sustained shear stress on stress fiber and cell alignments are accompanied by the thickening of the stress fibers, a decrease of peak cell height, and an increase of cell mechanical stiffness (19, 23, 48, 50, 54, 55). Such cytoskeletal remodeling is not seen under disturbed flow, where the cytoskeletal fibers and the cell show random orientation similar to that seen in the static condition. BAECs subjected to reciprocating shear stress without a net direction also have a polygonal morphology similar to that of static controls, and they have a partial loss of peripheral bands of actin (24, 69).

Simultaneous exposure to both directional shear stress and uniaxial stretch can have synergistic effects in enhancing stress fiber size and alignment in ECs (85).

In Vivo Studies on Mechanotransduction in Response to Shear Stress

In vivo, the flow pattern in the straight part of the arterial tree is pulsatile with a marked forward flow, whereas that at the branch points has a much lesser forward component and is similar to the reciprocating shearing in the reattachment zone in the step-flow channel (31, 68).
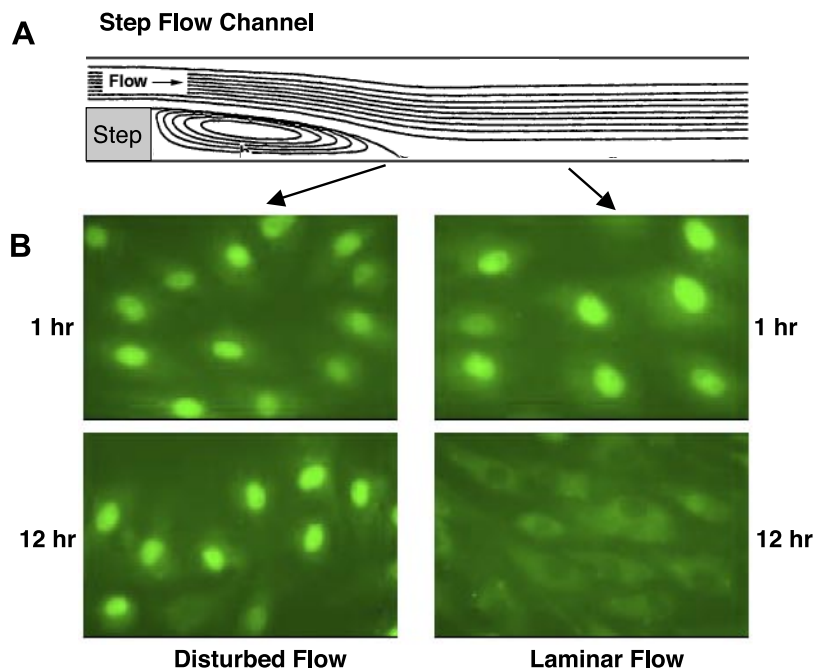
A   Step Flow Channel

Fig. 9. Activation of sterol regulatory element binding protein 1 (SREBP1) is transient with laminar flow but sustained with disturbed flow. *A*: side view of a step-flow channel in which confluent cultured BAECs are subjected to different flow patterns. After being sheared for 1 or 12 h, the cells were fixed and immunostained for SREBP1. Whereas disturbed flow induced a sustained activation of SREBP1, as indicated by its translocation into the nuclei (*B, lower left*), laminar flow activated SREBP1 in a transient manner, as evidenced by the lack of nuclear staining of SREBP1 at 12 h (*B, lower right*). Modified from Lui et al. (45).

B

A

Static Condition
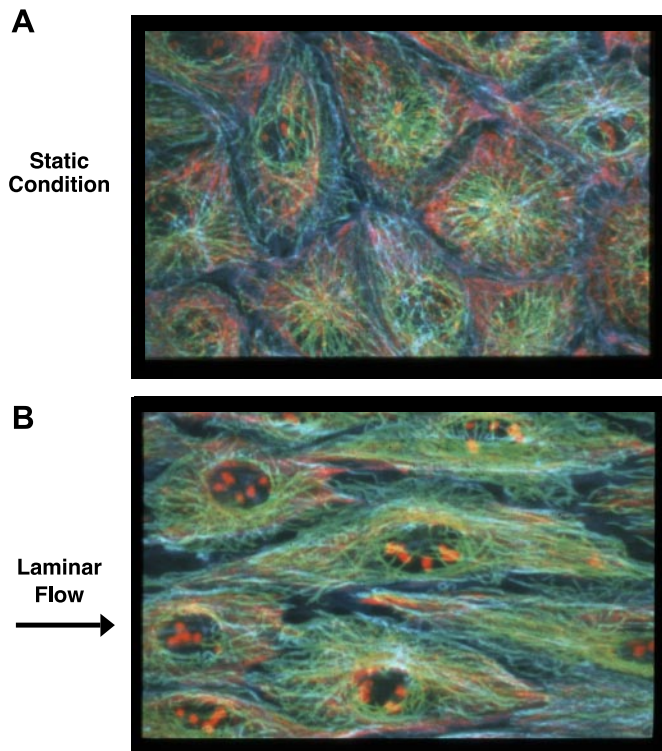
B

Laminar Flow →

Fig. 10. Effect of laminar shear flow on cytoskeleton organization and cell orientation. The cytoskeleton elements are triple stained for actin with fluorescein isothiocyanate-conjugated phalloidin (pseudocolor blue); microtubules with monoclonal anti-β-tubulin (green), and intermediate filaments with polyclonal anti-vimentin (red). Photomicrographs were taken under static condition (*top*) and 24 h after laminar shear flow at 12 dyn/cm^2 (*bottom*). Cytoskeletal elements following disturbed or reciprocating shear flow are similar to those seen under static condition. Based on the work by Galbraith, Skalak, and Chien (20).

Effects of local shear stress pattern on KLF2 and MCP-1 expression in ECs in vivo. To examine whether the flow-pattern-specific regulation of KLF2 expression found in cultured ECs in vitro also exists in the native circulation in vivo, we have examined the branch areas between the abdominal aorta and the celiac artery of normal rats by immunostaining with an anti-KLF2 antibody and histological sections (Fig. 11). In the abdominal aorta, KLF2 expression is continuous throughout the luminal lining (Fig. 11, *region 3*). In the celiac artery, substantial differences in KLF2 expression are observed between its medial and lateral aspects. The expression level of KLF2 on the medial flow divider apex (Fig. 11, *region 2*), where the shear stress is high and more laminar, is much higher than that of the lateral aspects (Fig. 11, *region 1*), where disturbed flow is more likely to occur.

To further define the effect of a change in flow pattern from forward to disturbed on KLF2 expression in vivo, we have used a stenosis model in which the rat abdominal aorta was constricted by using a U-shaped titanium clip. Immunohistochemical examination of the aorta 4 wk later shows high expression of KLF2 in ECs at regions of forward pulsatile flow but virtually no expression in ECs at the poststenotic site, where the flow pattern is disturbed (76). These results, together with our observation in rat artery bifurcation (Fig. 11), indicate that KLF2 expression can be modulated by different flow patterns in vivo in a manner similar to that observed in vitro (Fig. 7).

Dai et al. (18) have shown by microarray studies that exposure of cultured ECs to a waveform simulating the wall shear stresses of distal internal carotid artery (atheroresistant) results in the upregulation of KLF2 compared with that simulating the carotid sinus (atherosusceptible). These results on whole vessels are also in concert with our in vivo and in vitro findings.

Examination of MCP1 protein expression by immunocytochemistry has shown a preferential expression near the intercostal artery orifices compared with the straight part of the aorta (10); mapping of monocyte distribution in the aorta also shows that there is a preferential localization at branch points (46). The expression patterns of KLF2 and MCP1 at branch points versus straight vessel in vivo are similar to what was observed in response to reciprocating-disturbed flow versus pulsating-steady flow in vitro. These in vivo findings suggest that local flow patterns can affect EC functions through differential regulations of the atherogenic versus atheroprotective gene products.

Effects of local shear stress pattern on EC turnover and macromolecular permeability in vivo. At the branch points and curved regions of the arterial tree, which have a predilection for atherosclerosis, blood flow is unsteady and the shear stress shows marked spatial and temporal variations (21). These findings have led to the hypothesis that complex flow patterns cause an accelerated EC turnover (including cell mitosis and death), such that the resulting leaky junctions between the ECs undergoing turnover cause an increase in the permeability of large molecules (e.g., LDL) across the endothelial layer (79).
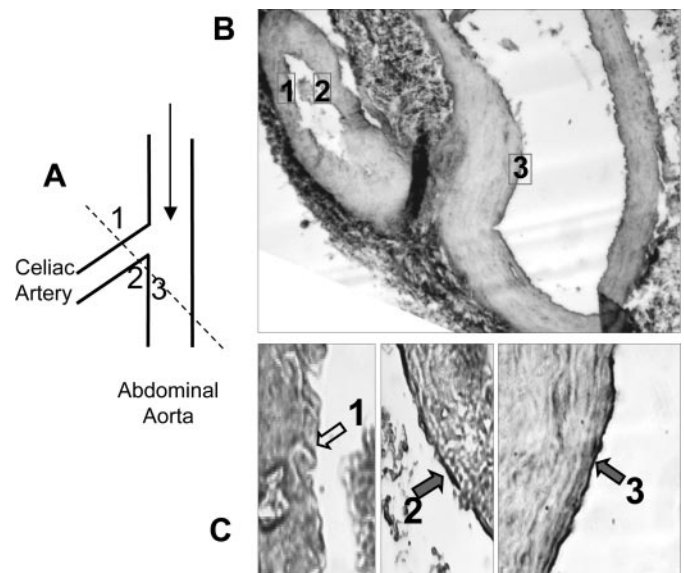

Fig. 11. KLF2 expression in ECs at abdominal-celiac branch point. *A*: drawing to show the branch point of celiac artery (with lateral region marked *1* and medial region *2*) off the abdominal aorta (*3*). Broken line shows the section through which immunohistochemical examinations of KLF2 expression were made in *B* and *C*. *B*: cross section of the branch area showing *regions 1, 2,* and *3* in *A*. *C*: three pictures showing enlarged views of *regions 1, 2,* and *3* in *B*. The expression of KLF2 was high and continuous on ECs of the abdominal aorta (labeled *3*) and the medial aspect (labeled *2*) of the celiac branch, but virtually absent on the lateral aspect (labeled *1*) of the branch. These results are representative of three independent experiments. In *C*, the short filled arrows (in *2* and *3*) indicate the positive staining for KLF2 protein, and the open arrow (in *1*) shows the absence of KLF2 protein in the endothelium. Modified from Wang et al. (76).

Our experimental studies have provided evidence that EC mitosis (12, 44) and death (42) are associated with the leakage of macromolecules such as LDL and albumin on an individual cell basis. Studies performed in a number of laboratories, including our own, have shown that these events of accelerated EC turnover occur primarily in areas with disturbed blood flow, e.g., arterial branch points (15, 43, 60, 72). Electron microscopic studies have identified the widening of the intercellular junctions around ECs that are undergoing mitosis or dying and the leakage of the macromolecular tracer horseradish peroxidase (9, 27).

The potential role of hemodynamic factors and EC turnover in the focal nature of lipid infiltration has been studied in the rabbit thoracic aorta (10). The distribution of shear flow patterns has been inferred from the nuclear orientation and shape index, and the results suggest that flow pattern is rather complex near the small branches, similar to that reported for large branches (3, 31). Mitotic cells and macromolecular leaky spots are distributed primarily around the branch orifices, and their distribution patterns are similar to those of lipid accumulation in cholesterol-fed rabbits (61). These results indicate that the complex flow patterns with little forward component enhance EC turnover and macromolecular permeability to lead to focal lipid accumulation.

Effects of shear stress on cytoskeleton organization in vivo. In the straight part of the aorta in several animal species that have been studied, stress fibers in ECs have prominent orientation parallel to the direction of blood flow (35, 81, 82). In contrast, there is little orientation of ECs or their stress fibers at branch sites lateral to the flow dividers, where flow patterns are more disturbed with no clear forward direction. It has been suggested that the prominent stress fibers with clear orientation at regions exposed to high levels of directional flows may help the ECs to withstand hemodynamic stress and maintain vascular integrity (17, 47, 82).

Perturbations of blood flow by partial obstruction of rabbit abdominal aorta (34) or common carotid arteries (74) have shown that the experimental decrease in shear stress immediately downstream to the obstruction causes a reduction in EC elongation to become polygonal in shape and a disappearance of the oriented stress fibers. By contrast, regions of elevated shear stress, e.g., in the narrowed segment, show an increase in the orientation of stress fibers, as well as EC elongation (74). An increase in shear stress in the canine common carotid artery following the creation of an arteriovenous shunt with the external jugular vein causes an increase in EC stress fibers (47). These results indicate that the in vitro finding of orientation of stress fibers parallel to directional shear stress also occurs in vivo.

EC Response to Shear Stress as a Feedback Control System

Sustained pulsatile or steady shear flow with a large net forward component downregulates the proatherogenic genes such as MCP-1, which would cause monocyte recruitment, and SREBP, which would cause lipid synthesis and accumulation. Such shear flows also upregulate the atheroprotective genes such as the growth arrest GADD45, which reduce cell proliferation and turnover, and KLF2, which improves cell survival. Thus the effects of shear flow with a net forward component, which is seen in the straight part of the aorta, are atheropro-

Table 1. *Comparison of flow patterns, cellular events, and atherogenecity between straight part of the aorta and its branch points*

	Straight Part	Branch Points
Flow pattern	Laminar	Disturbed
Net forward flow	Large	Small
Stress fiber alignment	Along long axis	Not aligned
Monocyte adhesion	Low	High
EC turnover & LDL permeability	Low	High
Genes to raise intracellular lipids	Downregulated	Upregulated
Inflammatory genes	Downregulated	Upregulated
Effects on atherogenesis	Antiatherogenic	Atherogenic

tective (Table 1). In contrast, in the branch regions of the arterial tree, where flow does not have a net forward direction, MCP1 and SREBP expressions are sustained, while growth arrest molecules and KLF2 are downregulated, and hence these areas are prone to atherogenesis. Many of the effects of the complex flow patterns in the branch points can be reproduced by subjecting cultured ECs to disturbed or reciprocating flow with very little forward direction.

In ECs exposed to sustained shear stress with a clear direction, the cytoskeletal fibers undergo remodeling to become aligned with the direction of shear flow. As a result, this would minimize the intracellular stress/strain despite the continued presence of the externally applied shear stress.[2] Besides cytoskeletal remodeling, the adaptive regulation of cell signaling and functions in response to sustained shearing may involve changes in the morphology of cell surface (19, 50) and/or cell stiffness (23, 48, 54, 55), and the consequent modulation of intracellular stress would alter the effects of the externally imposed shear stress. Thus, besides actin stress fibers, other aspects of EC morphometric or biomechanical responses may play an important or even initiating role in the adaptive response of ECs to shearing. Such mechanical adaptation involves alterations in the functional activities of signaling molecules. This is exemplified by the adaptive changes in JNK, ERK, and KLF2 activities and the consequent reductions in MCP-1 level, proliferation, and inflammatory responses for ECs adapted to prolonged laminar shear stress. The feedback control of mechanotransduction may involve modulations of posttranslational regulation such as phosphorylation/dephosphorylation, which are controlled by the check and balance between protein kinases and phosphatases.

MECHANOTRANSDUCTION IN RESPONSE TO CYCLIC STRETCH

The pulsatile nature of blood pressure creates mechanical stimuli to vascular ECs in the form of cyclic stretch. Our studies on stretch deformation of ECs cultured on a deformable substrate provide an in vitro model for the elucidation of molecular and cellular responses to cyclic mechanical stretch.

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[2] The adaptive change in intracellular mechanics in response to the extracellular mechanical forces (shear stress or normal stress) may involve alterations in intracellular stress or strain, or very likely both. Therefore, the term intracellular stress/strain is used here. To simplify the presentation, however, only intracellular stress will be used, with the understanding that the controlled parameter for the feedback system may be intracellular strain as well as intracellular stress.
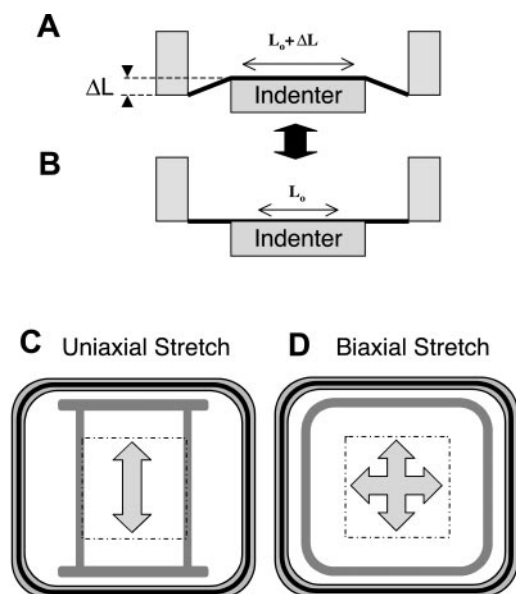
Fig. 12. *A* and *B*: side views of a stretch chamber and indenter to illustrate the principle of cell stretching. *A*: pushing up of the Teflon indenter against a silicone rubber membrane secured to a square frame results in the stretching of the membrane and the ECs cultured on it with a displacement of ΔL. *B*: downward return of the indenter to its original position. The sinusoidal motion of the indenter between *A* and *B* at a frequency of 1 Hz causes a cyclic stretching of the ECs. *C* and *D*: top views of stretch chambers for unilateral (*C*) and bilateral (*D*) stretches. *C*: use of an I-shaped indenter results in a principal stretch oriented along the long axis of the indenter. The small tension generated in the orthogonal direction is opposed by the tendency for the membrane to compress orthogonal to the principal stretch direction. The extensions at the corners of the indenter increase the uniformity of the strain field over the indenter, resulting in a virtually uniaxial stretch. *D*: use of a square frame results in a biaxial stretch oriented along both directions. Cells were seeded in the central 4×4 cm region of the membrane where strain is uniform. Modified from Kaunas et al. (32).

especially the remodeling of actin stress fibers as an adaptation process to the imposed stretch in minimizing the effects of directional stretch on cellular function. These studies were performed on BAECs isolated from the aorta and cultured in DMEM supplemented with 10% fetal bovine serum.

Effects of Cyclic Stretches on EC Stress Fiber Remodeling

To study the remodeling of actin stress fibers of BAECs in response to cyclic strains, we seeded BAECs on silicone membranes coated with 10 μg/ml of fibronectin and subjected the cells to stretch (32, 66) in a stretch chamber that has been designed to apply either uniaxial stretch, which has a defined direction, or biaxial stretch, which does not (Ref. 33, Fig. 12), at a sinusoidal frequency of 1 Hz and a 10% peak change in length. These two modes of stretch elicit different changes in the orientation of stress fibers and cell signaling (32, 33). The experimental results and the molecular mechanisms involved are summarized below.

Effects of cyclic stretches on stress fiber orientation. Both uniaxial and biaxial stretches cause an increase in the amount of actin stress fibers in BAECs, but they have different effects on fiber orientation (33). Uniaxial stretch causes an orientation of the stress fibers, over the course of several hours, in a direction perpendicular to that of stretch, whereas biaxial stretch does not result in any specific orientation of the stress fibers (Fig. 13); the difference in orientation of stress fibers in response to the two modes of stretch led to the same difference in orientation of the cells. Such alignment of stress fibers in the direction of the minimal substrate deformation has also been reported by Wang et al. (75). Quantification of the degree of perpendicular orientation of stress fibers as a function of the magnitude of the uniaxial stretch shows that there is no significant orientation following stretches less than 3%; at 3% stretch the perpendicular orientation is marginally significant ($P = 0.04$) (Fig. 14). Further increases in the degree of uniaxial stretch lead to increases in the extent, uniformity, and statistical significance of the perpendicular orientation, which becomes almost totally perpendicular at 10% uniaxial stretch. Such a perpendicular orientation of stress fibers to uniaxial stretch is also seen in arteries in vivo, where the EC stress fibers are aligned along the longitudinal axial direction of the arteries, which are subjected to cyclic circumferential stretch due to the pulsatile pressure. When rat renal arteries are subjected to cyclic stretching along the vessel axis ex vivo, the endothelial stress fibers change their alignment within hours toward the
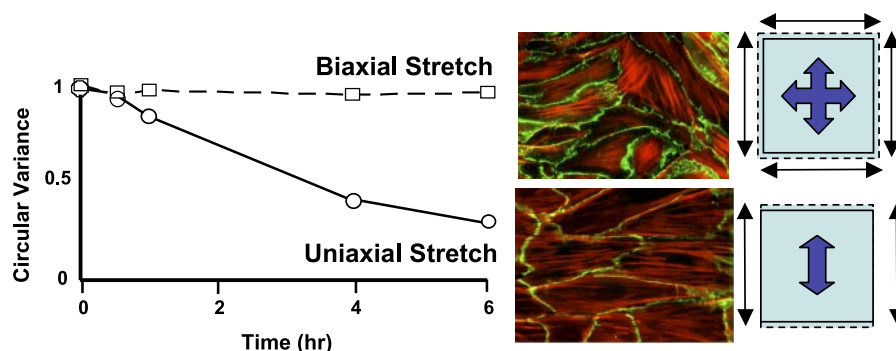
Fig. 13. Differential effects of cyclic biaxial and uniaxial stretches on the orientation of actin stress fibers. Distribution of stress fiber orientation is determined using an intensity gradient algorithm and expressed as circular variance (from 1.0 for random orientation distribution to 0 for perfect alignment) (32). Under static conditions, BAECs are cuboidal and stress fibers are randomly oriented. Over the 6-h period of study, the stress fibers do not show any significant orientation following biaxial stretch but undergo a progressive increase in alignment perpendicular to the direction of stretch. *Insets*: microphotographs of the BAECs double-stained for F-actin and β-catenin to identify stress fibers and cell outlines, respectively. The broad double arrows to the right of the microphotographs indicate biaxial and uniaxial stretches, respectively. Bars = 10 μm. Modified from Kaunas et al. (32).
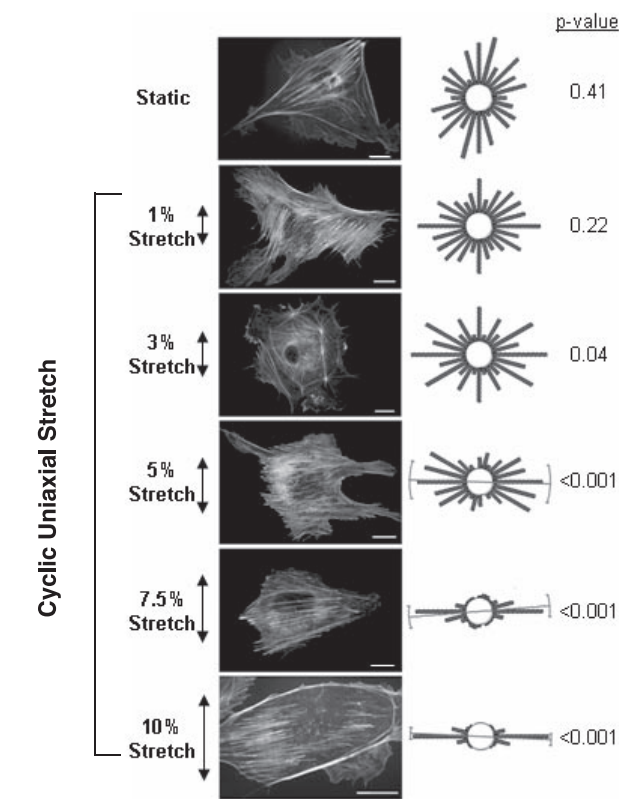
Fig. 14. Effects of the magnitude of cyclic uniaxial stretch on stress fiber orientation. Representative micrographs are shown of BAECs expressing GFP kept as an unstretched control (*A*) or subjected to 1, 3, 5, 7.5, or 10% uniaxial stretch for 6 h. Direction of the stretch was along the long axis of the figure, as indicated by the doubleheaded arrows for each stretch level; 10-μm bars are shown for reference. Polar histograms illustrate the distribution of stress fiber orientations for each condition with the significance of orientation displayed as *P* values to the right of each histogram. In the polar histograms, the mean orientation and 95% confidence interval are shown for the distributions with significant orientation ($P < 0.05$). Based on data from Kaunas et al. (32).

circumferential direction (64), which is again perpendicular to the imposed uniaxial stretch.

Effects of inhibition of Rho GTPase on stretch-induced stress fiber orientation. It is known that activation of the small GTPase Rho causes cell contraction and stress fiber assembly (53). The downstream effectors of Rho are Rho kinase, which regulates the phosphorylation of myosin light chain (14), and mDia, which regulates actin polymerization and focal adhesion turnover through its association with profilin and src-tyrosine-kinase, respectively (56, 78). We have studied the effects of inhibitions of Rho, Rho kinase, and mDia on the stretch-induced stress fiber orientation.

In contrast to the perpendicular orientation seen in control BAECs with normal Rho activities (32), 10% uniaxial stretch results in the formation of stress fibers parallel to the stretch direction following the inhibition of the activities of Rho (with C3 exoenzyme), Rho kinase (with Y27632), or mDia (with its dominant-negative mutant F1F2Δ1) (Fig. 15). These results indicate that the Rho pathways are critical in the stretch-induced perpendicular orientation of the stress fibers in normal cells.

Effects of activation of Rho GTPase on the stretch-induced stress fiber orientation. To further elucidate the interplay between the externally imposed uniaxial stretch and the internally generated Rho-induced actin assembly/disassembly, we have performed experiments on BAECs transfected with RhoV14, an active mutant of Rho, with GFP used for cell identification (32). The cells expressing GFP alone behave in the same manner as the untransfected control, i.e., their stress fibers do not show any orientation when unstretched and only marginally significant orientation following uniaxial stretch at 3%, but they exhibit marked perpendicular orientation following 10% uniaxial stretch (Fig. 14). Co-expression of RhoV14/GFP results in a general increase in stress fiber density, but it does not induce any orientation in the absence of stretch (Fig. 16, static). It is important to note, however, that the stress fibers

Fig. 15. Effects of inhibition of mDia and Rho kinase on the stretch-induced stress fiber orientation in BAECs. Representative micrographs are shown of confluent BAECs transfected with the empty vector pEF as a control (*top*) or treated with pEF plus the negative mutant of mDia (F1F2Δ1) (*middle*), or the Rho kinase inhibitor Y27632 (*bottom*). Cells were either kept as unstretched controls (0%) or subjected to 10% cyclic uniaxial stretch for 6 h. The cells were stained with rhodamine-phalloidin to identify F-actin bundles. Note that 10% stretch leads to perpendicular orientation of the stress fibers in the pEF control but parallel orientation following the inhibition of Rho kinase or mDia. C3 exoenzyme also causes the parallel orientation after 10% uniaxial stretch (data not shown). Modified from Kaunas et al. (32).
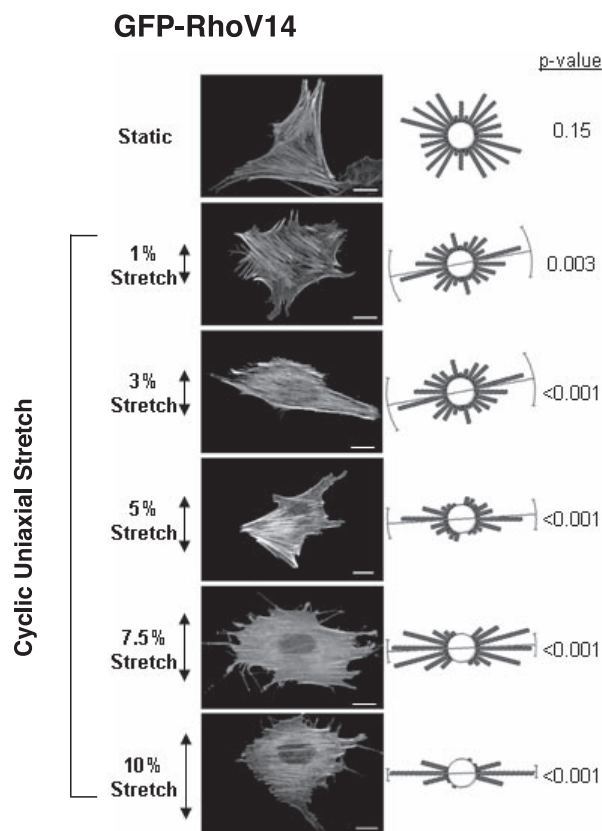
GFP-RhoV14
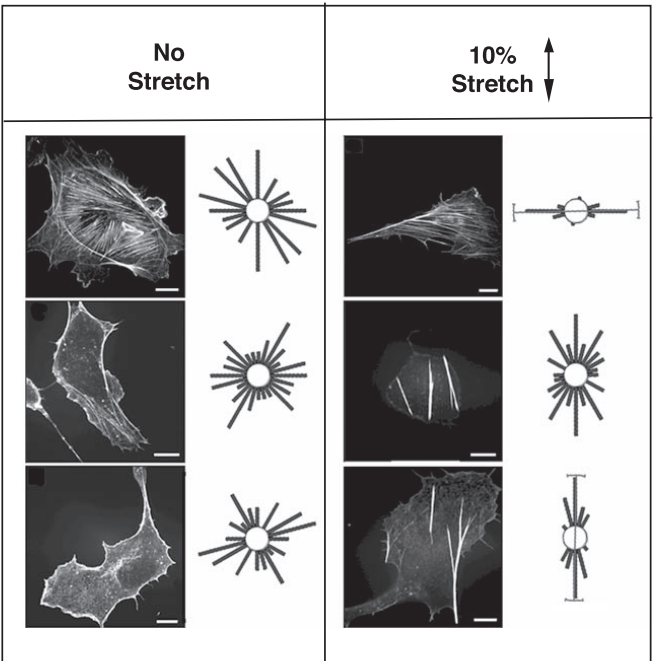
Fig. 16. Cooperative and interactive effects of uniaxial stretch and RhoV14 on stress fiber orientation. Representative micrographs and circular histograms are shown for BAECs coexpressing RhoV14 and GFP. These cells were either kept as an unstretched static control (*top*) or subjected to 1, 3, 5, 7.5, or 10% uniaxial stretch for 6 h. As in the control cells expressing GFP without RhoV14 (see Fig. 14), the direction of the stretch was along the long axis of the figure (doubleheaded arrows), and polar histograms are drawn with the significance of orientation displayed as *P* values; 10-μm bars are shown for reference. Note that following 1% stretch, stress fiber orientation did not occur in the cells expressing only GFP (see Fig. 14) but was prominent in the cells coexpressing RhoV14. Based on data from Kaunas et al. (32).

in RhoV14/GFP-expressing BAECs show a highly significant perpendicular orientation with a uniaxial stretch of 3%; in fact, such orientation occurs with a uniaxial stretch as small as 1% (Fig. 16). Thus neither 1% stretch in the absence of RhoV14 (Fig. 14, second from *top*) nor RhoV14 in the absence of stretch (Fig. 16, *top*) causes significant orientation of stress fibers, but the combination of RhoV14 and 1% stretch resulted in prominent perpendicular orientation (Fig. 16, second from *top*). These results clearly show the cooperativity of Rho activity and uniaxial stretch in inducing the perpendicular orientation of stress fibers.

Quantitative analysis of the interplay between Rho activity and uniaxial stretch indicates that RhoV14 expression is equivalent to approximately 3% stretch in inducing the perpendicular orientation of stress fibers (32). This effect of RhoV14 in enhancing perpendicular orientation can be blocked by Y27632 or F1F2Δ1, indicating that it is mediated through the Rho pathways involving Rho kinase and mDia.

Possible mechanism of the Rho-mediated perpendicular orientation of stress fibers in response to uniaxial stretch. We propose that stretch-induced activation of Rho pathway leads

to not only an increase in the amount of stress fibers but also an increase in the dynamics of actin assembly/disassembly. The newly assembled actin stress fibers would have a greater propensity to be disassembled when they are aligned in the direction of the uniaxial stretch, whereas those aligned in the perpendicular orientation would have a higher probability to persist. As a result of the greater stability of the stress fibers with a perpendicular orientation than those with a parallel orientation, the net statistical result of this enhanced assembly/disassembly would be for the stress fibers to develop a perpendicular orientation with time. Such an orientation would lead to an adaptive lessening of the net intracellular stress in the face of the constant application of external uniaxial stretch. This adaptive change in stress fiber orientation requires the increase in actin dynamics due to the activation of Rho GTPase and its downstream effectors. Hence, it cannot take place after the inhibition of the Rho pathway, and the stress fibers then align passively, i.e., parallel, with the direction of stretch.

Effects of Cyclic Stretches on JNK Activation and Its Relationship With Stress Fiber Orientation

Effects of cyclic stretches on JNK activity and correlation with stress fiber orientation. The application of 10% uniaxial stretch to BAECs induces a 2.6-fold increase in JNK activity after 30 min (Fig. 17*A*), when the stress fibers have not yet developed an orientation (Fig. 17*B*). But the level of JNK activity then gradually decreases to reach the basal level after 6 h, despite the continued application of stretching, when stress fibers have developed an orientation perpendicular to the direction of stretch (Fig. 13 and 17, *A* and *B*) (33). In contrast to the effects of uniaxial stretch, biaxial stretch leads to sustained activation of JNK at 2.5- to 3.0-fold over 6 h or more of stretching (Fig. 17*A*), because the stress fibers do not show any orientation (Fig. 17*B*) (33). Since uniaxial stretch causes the perpendicular orientation of stress fibers, which would tend to decrease the intracellular stress in the face of the uniaxial stretch, whereas biaxial stretch does not cause any preferred orientation of the stress fibers, we propose that the remodeling of the stress fibers and the consequent modulation of intracellular stress cause the subsidence of JNK response in the face of a continued application of cyclic uniaxial stretch.

Cross plotting the data shows that JNK activation can be correlated with stress fiber orientation (Fig. 17*C*; $R^2 = 0.88$), i.e., a decrease of the level of stretch-induced JNK activation is associated with the development of stress fiber orientation perpendicular to the direction of stretch.

The role of actin remodeling in the stretch-induced JNK activation has been demonstrated by inhibiting actin polymerization with cytochalasin D at 50 nM, a dose that attenuates actin stress fiber formation but does not cause cell rounding. Cytochalasin D changed the response of JNK activation to uniaxial stretch from transient to sustained, thus indicating the important role of the integrity of the actin cytoskeleton for effecting the transient nature of the uniaxial stretch-induced JNK activation (32).

Effects of change in direction of uniaxial stretch on stress fiber orientation and JNK activity. We tested our hypothesis that the stress fiber orientation modulates JNK activation by the following experiment. We first subject BAECs to uniaxial stretch for 6 h, which has led to a perpendicular stress fiber
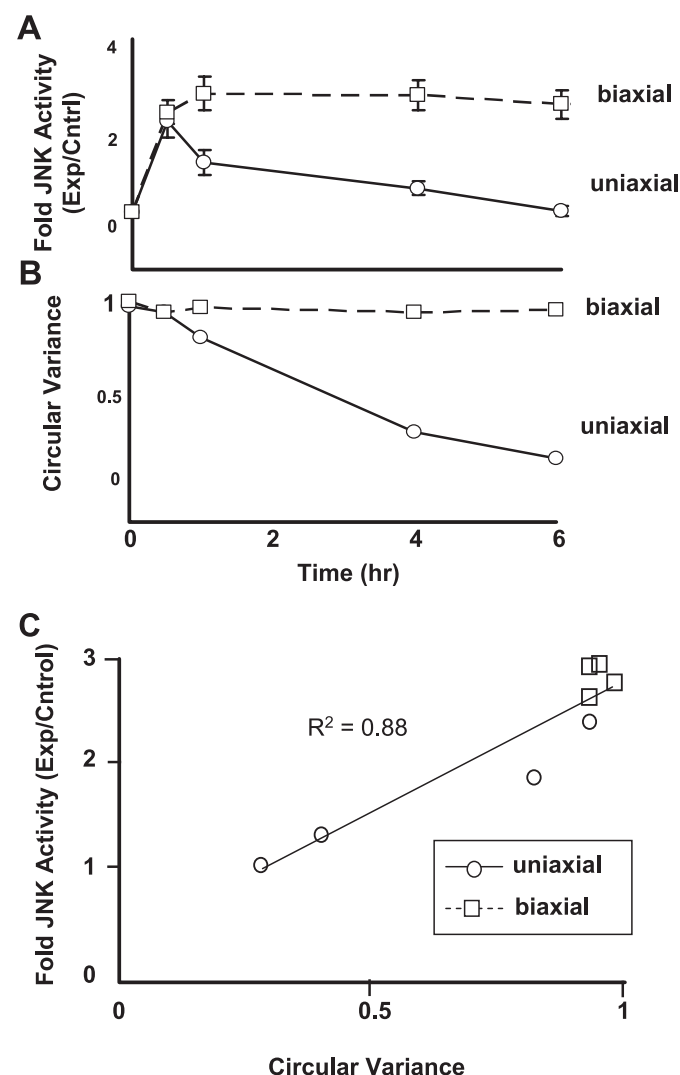
Fig. 17. Correlation of the temporal responses of stress fiber alignment and the degree of JNK activation of confluent BAECs in response to 10% cyclic uniaxial and biaxial stretches at 1 Hz. *A*: time courses of JNK activation (normalization to the unstretched control) following uniaxial and biaxial stretches. The results shown are means $\pm$ SE ($n = 6$). *B*: time course of stress fiber orientation following uniaxial and biaxial stretches (same as Fig. 13, placed here for comparison with *A*). Note that the alignment of the stress fibers toward perpendicular following uniaxial stretch is accompanied by a deactivation of JNK following its transient activation, whereas following biaxial stretch, which does not lead to stress fiber alignment (circular variance $\approx$ 1), JNK activation is sustained. *C*: cross plot of JNK activation and stress fiber alignment. The correlation shows $R^2 = 0.88$. Based on data in Kaunas et al. (33).

Fig. 18. *A*: 90°change in the direction of uniaxial stretch causes disalignment and realignment of stress fibers in the perpendicular direction, which are accompanied by reactivation and deactivation of JNK. BAECs are first exposed to uniaxial stretch for 6 h. At 0.5 h, when the stress fibers are not yet aligned, JNK is activated (*$P < 0.05$ between 0.5 h and 0 h). At 6 h, stress fibers undergo perpendicular alignment (*A,a*) and there is a subsidence of JNK activation (*B,a*). The cells are then subjected to a change in the direction of stretch by 90°; this results in a transient reactivation of JNK (*B,b*; $P < 0.05$ between 6 h + 90 + 0.5 h and 6 h + 180 + 0.5 h) before the realignment of the stress fibers (*A,b*). After 6 h of uniaxial stretch in this new direction, the stress fibers are again oriented perpendicular to the new direction of stretch (*A,c*), and the JNK activity then subsides (*B,c*). Control experiments in which the stretch direction is changed by 180° after 6 h of uniaxial stretch do not cause any significant change in stress fiber alignment, nor the JNK activity (*B*, 4th lane and bar). Based on data in Kaunas et al. (33).

alignment (Fig. 18*A,a*) and subsidence of JNK activation (Fig. 18*B,a*), and then change the direction of stretch by 90°. Thus the uniaxial stretch is now applied in a direction parallel to the oriented stress fibers. This change in direction results in a transient reactivation of JNK (Fig. 18*B,b*) when the stress fibers are not yet realigned (Fig. 18*A,b*). After 6 h of uniaxial stretch in this new direction, however, the stress fibers become again oriented perpendicular to the new direction of stretch (Fig. 18*A,c*), and the JNK activity then subsides once again (Fig. 18*B,c*). Control experiments in which the stretch direction is changed by 180° after 6 h of uniaxial stretch do not cause any significant change in stress fiber alignment (which remains
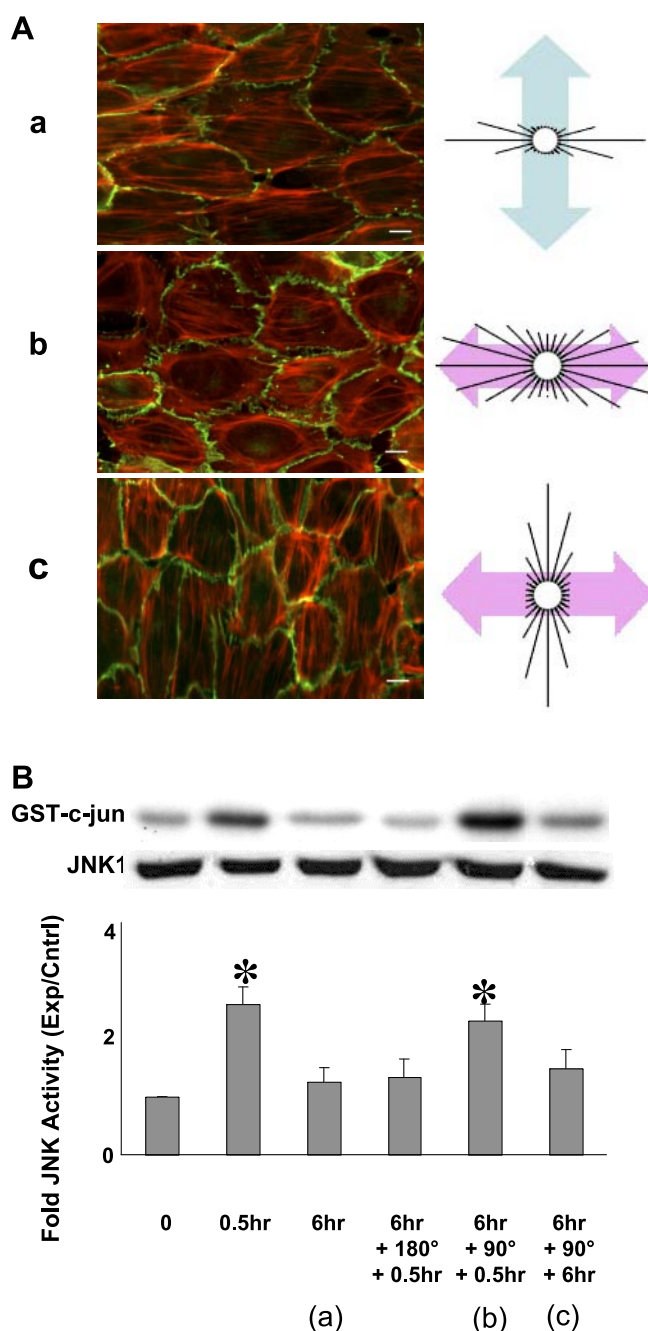
Table 2. *Comparison of flow patterns, cellular events, and atherogenecity between straight part of the aorta and its branch points*

	Straight Part	Branch Points
Primary stretch pattern	Uniaxial stretch	Non-uniaxial stretch
Stretch direction	Circumferential	No definite direction
Cell and actin filament orientation	Perpendicular to stretch	Random
Time course of JNK activation	Transient	Sustained
Apoptosis	Protected	Enhanced

perpendicular to the stretch direction) nor the JNK activity (which remains low at the prestretch level) (Fig. 18*B*, 4th bar).

These results indicate that the directionality of mechanical stretch and the resulting orientation of stress fibers play important roles in the time course of JNK activation in response to mechanical strain. Activation of JNK occurs during the period when the stress fibers are in the process of developing their perpendicular orientation to the direction of uniaxial stretch. Once this remodeling process has fully evolved, JNK activation decreases to control levels. The sustained activation of JNK in response to biaxial stretch can be attributed to the inability of cells to remodel and adapt to the stretch without a defined direction, and hence a high intracellular stress persists to continue to activate JNK. In the case of uniaxial stretch, the feedback mechanism leads to a lessening of intracellular stress and a subsidence of JNK activation. The contrast between unixial and biaxial stretch as found in the experiments described above is summarized in Table 2.

EC Response to Cyclic Uniaxial Stretch as a Feedback Control System

Our results show that ECs subjected to uniaxial stretch orient their stress fibers perpendicular to the direction of stretch. Fibers aligned perpendicular to stretch would bear less tension than when they are aligned parallel to stretch, hence perpendicular orientation of the stress fibers serves to reduce the stretch-induced increase in intracellular mechanical energy generated by uniaxial stretch. These findings and the studies on Rho have led us to postulate a feedback control model. In this model, the direct effect of uniaxial stretch is an increase in intracellular stress that stimulates Rho activity and its downstream signaling targets (Rho kinase and mDia). This activation of the Rho pathway and the consequent increase in the dynamics of actin assembly/disassembly cause the stress fibers to orient perpendicularly to the direction of stretch, thus allowing the cell to adapt to the stretch by minimizing the increase in intracellular stress. This feedback adaptation can modulate cellular signaling events, as exemplified by the JNK activity, and the consequent functional responses such as proliferation and apoptosis. Such a feedback control mechanism serves to maintain the homeostasis of ECs in the straight part of the aorta by reducing proliferation and apoptosis. This is analogous to that discussed above with respect to the feedback regulation of intracellular stress in response to directional shearing. It is interesting to note that both types of mechanical stimuli can increase stress fiber sizes and cause their synergistic alignment in vivo (85).

In contrast, the lack of a clear stretch direction in branch points (70) would not induce the feedback minimization of intracellular stress, and hence JNK activation would be sustained. The differential responses of JNK to uniaxial and biaxial stretches may have significant implications in cellular functions. JNK plays a significant role in cellular regulation of stress responses (reviewed in Ref. 30), and there is evidence that sustained, but not transient, activation of JNK is associated with apoptosis (26). Therefore, cyclic stretch without a clear direction, such as that seen in branch points of the arterial tree, would cause a higher frequency of apoptosis (15) and a greater vulnerability to atherogenesis compared with the straight part of the arterial tree, where the cyclic stretch due to the pulsatile pressure is primarily uniaxial in the circumferential direction.

SUMMARY AND CONCLUSIONS

Hemodynamic forces can modulate the structure and function of ECs in blood vessels. Under normal conditions, these modulating influences allow the vascular wall to adapt to changes in pressure and flow for the optimization of its functional performance.

External mechanical stimuli by shear flow or stretch cause changes in intracellular mechanics and also mechanotransduction. When the externally applied forces have a clear direction (e.g., in the form of pulsatile flow or uniaxial stretch), the directed mechanical stimuli can elicit EC remodeling that would minimize the changes in intracellular stress. Whereas the adaptive minimization of changes in intracellular stress results from molecular signaling due to mechanotransduction, such adaptive responses also modulate the molecular signaling.
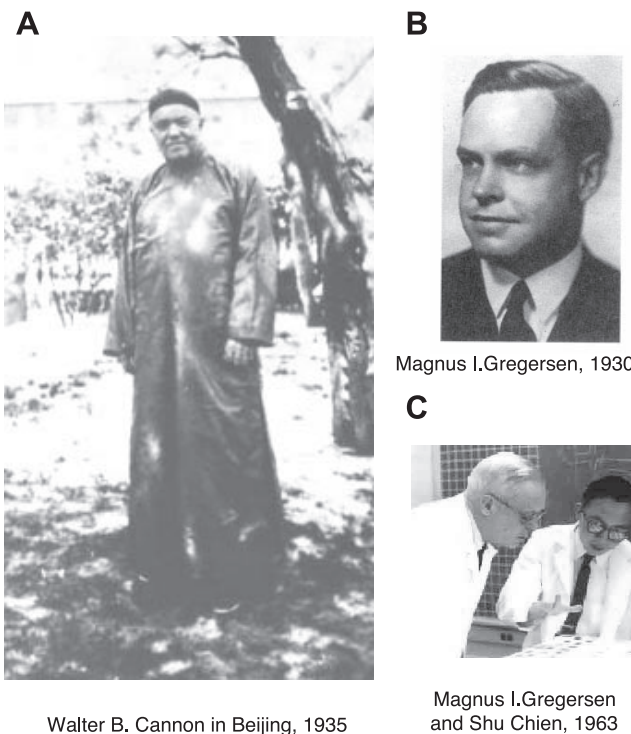

Fig. 19. *A*: Walter B. Cannon in Beijing, 1935. (Reprinted with permission from State University of New York Press.) *B*: Magnus I. Gregersen in Cannon's lab at Harvard University, 1930. *C*: Magnus I. Gregersen and Shu Chien at Columbia University College of Physicians and Surgeons, 1963.

Thus the feedback regulation involves close coupling between mechanics and biology. Such feedback control of cellular homeostasis takes place within the cell, and it represents an example of the wisdom of the cell to regulate its structure and function in the face of external perturbations.

Whereas such feedback regulation works well in the straight part of the arterial tree, where the mechanical forces are directional, it cannot operate effectively in regions with complex geometry such as branch points, where the shear stress and strain are not clearly directional, thus placing these regions at risk for pathophysiological conditions such as atherosclerosis. To achieve the circulatory transport function, it is necessary to have vascular branching and the attendant mechanical consequences. Therefore, to prevent atherogenesis, we need to avoid risk factors such as smoking, obesity, hyperglycemia, and lack of exercise, which superimpose on local hemodynamic factors to cause atherogenesis in the lesion-prone areas. One of the potential beneficial effects of exercise is to change the hemodynamic patterns in these lesion-prone areas by increasing cardiac output and regional blood flows to move the regions of complex flow (e.g., reattachment zone) further downstream. Using computational fluid mechanics methods, Taylor et al. (68) have shown that simulated moderate exercise (but not light exercise) can eliminate the complex flow patterns from several lesion-prone branch points, thus supporting the notion that the elimination of adverse hemodynamic conditions at branch points may be a mechanism by which exercise can promote health. Such considerations indicate that, whereas "The Wisdom of the Cell" by itself can serve to maintain the homeostasis of cells under some conditions, it needs to be aided by other mechanisms that involve "The Wisdom of the Body" beyond the cell, such as the increase in blood flow in exercise. We also need "The Wisdom of the Mind" to make our body do things that are good for our cells and our health, e.g., regular exercise with appropriate intensity and duration, as well as the avoidance of other risk factors such as smoking and poor eating habits.

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