Fluid Shear Stress Modulation of Gene Expression in Endothelial Cells

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The vascular endothelium, lining the blood vessel wall, is constantly exposed to wall shear stresses generated by flowing blood. Gene regulation, critical for endothelial cell function, depends on complex interactions at the promoter level and utilizes overlapping signal transduction cascades to activate the expression of genes involved in many biological processes.

Life is surrounded by stress! More correctly, cells are constantly exposed to different stress stimuli. These may be acute or chronic in nature and may take the form of both chemical and physical stimuli. Changes in environment, injury, or disease may place an organism under stress. Evolution has provided many different means by which organisms can adapt to changes in stress, and the stress response is a well-conserved homeostatic reaction that applies equally to prokaryotic and eukaryotic organisms. This reaction may include a diverse spectrum of responses ranging from the induction of heat shock response proteins resulting from an acute change in environmental temperature to the activation of signaling pathways in response to chemicals, such as phorbol ester activation of protein kinase C.

What is fluid shear stress?

The pulsatile flow of blood throughout the branched network of the vasculature generates biomechanical forces that act on blood vessels to modulate their function and activity. Such hemodynamic forces include hydrostatic pressures, cyclic strains, stretch, and both laminar and turbulent (nonlaminar) frictional wall shear stresses. Shear stress is defined as force per unit area and may be considered as pressure, frictional shear at the cell surface, and tensile or compensatory forces acting to counter an externally applied force. All cells in the body receive some form of force, whether they are endothelial cells located in the blood vessels, epithelial cells located in the respiratory tract, or osteoclasts and osteoblasts found in bone. The rate at which the axial velocity rises from the vessel wall toward the lumen is called the shear rate (dv/dr). This velocity gradient causes a shear stress (τ) on the endothelium that is parallel to the blood flow and proportional to the blood viscosity such that viscosity (μ) is defined as $\tau = \mu \cdot (dv/dr)$. To simulate flow over endothelial cells grown in culture, one of the most popular experimental devices is a parallel plate flow chamber, which allows predictable and highly uniform laminar fluid shear stresses to be generated by simple use of a peristaltic pump (5). Endothelial cells cultured on a glass coverslip or slide flaskette are enclosed within the flow chamber, and the reproducible variation in flow rate of tissue culture medium over a given surface area of confluent cells allows for flowing cells in multiple chambers set in series. With the use of such a closed sterile system in which tissue culture medium is constantly recirculated, gassed with CO_2, and held at a constant 37°C, it is possible to maintain cells under flow conditions for several days. Wtih the peristaltic pump capacity, the separation between the endothelial cell monolayer and the roof of the flow chamber, and the internal diameter of capillary tubing, shear stresses of between 1 and 80 dyn/cm^2 are readily achievable. For a laminar flow system, wall shear stress (τ) may be defined as $3 \cdot \mu \cdot Q/2a^2b$, where μ is the viscosity of blood at 37°C (in poise), Q is the volumetric flow rate (in ml/s), a is the half-channel height (in cm), and b is the channel width (in cm).

"...biomechanical forces that act on blood vessels...."

Blood flow and the development of atherosclerosis: an atheroprotective role for shear stress?

Evidence from both animal models of atherosclerosis and human subjects demonstrates that thickening of the intima and plaque localization occur predominantly in regions of the vasculature characterized by low-wall shear stress and complex flow patterns that fluctuate in direction

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(3). In atherosclerosis, plaque lesions are generally found at sites of arterial curvature and bifurcation. Numerous studies in humans have confirmed these observations, leading to the opinion that low and/or fluctuating shear stress is conducive to atherogenesis. Thus one may envision both lesion-prone and lesion-protected areas within the vascular tree. These findings in vivo are supported by a number of in vitro studies that demonstrate that shear stress may protect the endothelium from apoptosis, reduce the potential of the endothelium to be activated by proinflammatory cytokines, inhibit smooth muscle cell proliferation, and inhibit the expression and activity of angiotensin-converting enzyme. The follow-up hypothesis from this is that genes with activity that may be either negatively or positively modulated by shear stress may confer an atheroprotective phenotype on the endothelium. Consequently, many laboratories including our own have exploited endothelial cells grown in culture, subjecting them to flow and developing differential gene expression strategies for discovering new genes that respond to changes in shear stress. It is now becoming clear that both endothelial cells and vascular smooth muscle cells can sense and respond to their localized hemodynamic environment and that the resulting response may be important for the pathogenesis of vascular diseases.

"...the normal response of coronary arteries to increased flow is vasodilation."

How do endothelial cells detect fluid shear stress?

The endothelium in blood vessels is uniquely located at the interface between the blood and the vessel wall. Essentially, the endothelium provides a nonthrombogenic surface and a permeability barrier. Together with other cells in the vessel wall, it is capable of modulating blood flow and responding to a range of insults. In 1984, and extended in 1993, Russell Ross (10) and colleagues proposed that atherosclerosis may develop as a result of localized endothelial dysfunction brought on by numerous external stimuli, including, for example, mechanical stress, viruses or bacteria, hypertension, and free radical attack generated from components of cigarette smoke. All of these components may singly or in concert trigger the vascular endothelium to respond abnormally. Blood flow regulates the internal diameter of arteries both acutely, by relaxation and contraction of smooth muscle cells, and chronically, by vascular remodeling of cellular and extracellular components. For example, the normal response of coronary arteries to increased flow is vasodilation. The mechanical stimulus to this endothelium-dependent vasodilation is shear stress. Increasing the vessel diameter counteracts the effects of increased flow and reduces shear stress. This compensatory mechanism does not occur in atherosclerotic segments of the coronary artery (14). Consequently, increased flow rates will cause the intima to thicken, since the vessel attempts to reestablish a normal wall shear stress and eventually the artery may maintain a thickened intima. Thus, in diseased arteries, which have lost their capacity for adaptive change to shear stress, the endothelium will be rapidly and transiently exposed to changes in fluid shear stress. These rapid changes, at least in the context of endothelial cell culture, may have profound effects on changes in cellular gene expression (as discussed below).

When endothelial cells are subjected to fluid shear stress or mechanical stretching, a diverse set of responses is triggered. The nature of these responses encompasses all aspects of cellular biochemistry, ranging from electrophysiological modulation of membrane proteins and ion channels to activation of transcription factors and concomitant transcriptional activation of their cognate target genes or to cellular reorganization and changes in shape. Some responses occur within seconds, whereas others require many hours before a measurable adaptive change to this stimulus becomes apparent (Fig. 1). Many studies have suggested that endothelial cells sense alterations in shear stress much the same as any anchorage-dependent cell may sense this stimulus. The sensing apparatus is likely to be a combination of cytoskeletal elements and release and/or activation of biochemical signals at the mechanotransducer sites. For example, epithelial-like HeLa cells are readily responsive when subjected to shear stress in a laminar flow chamber, yet these cells do not experience fluid shear stress in vivo. It seems plausible that, whatever the cell type, the ability to recognize laminar shear stress in the local environment is brought about by a general set of shear stress "receptors" that may sense other physical stimuli such as pressure. With the use of a differential display approach, a number of genes have been identified with a steady-state messenger RNA level that is increased by laminar fluid flow. For example, manganese superoxide dismutase, cyclooxygenase-2, endothelial nitric oxide synthase, intercellular adhesion molecule-1 (ICAM-1, Ref. 12), and the proteins termed "mothers against decapentaplegism" (MAD), isoforms 6 and 7, were upregulated by laminar fluid shear stress (13).

Numerous reports have implicated F-actin microfilaments as the principal force transmission structure. When endothelial cells are

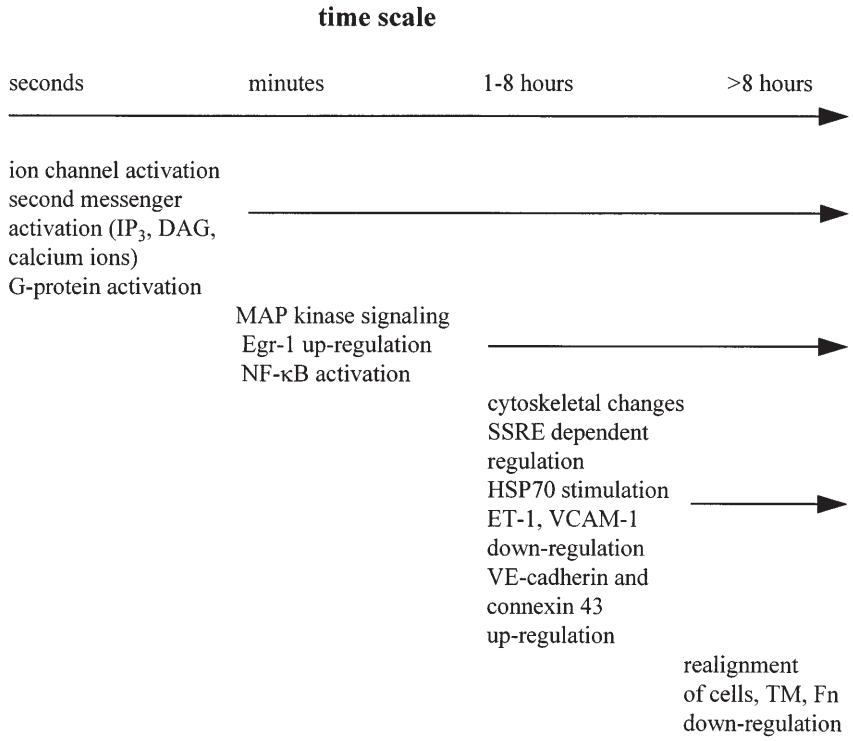

FIGURE 1. Biochemical responses to fluid shear stress. Shear stress activates many biochemical processes. Some occur in seconds: second messengers, inositol 1,4,5 trisphosphate (IP_3), and diacylglycerol (DAG); some in minutes: mitogen-activated protein (MAP) kinase signaling; some in hours: shear stress response element (SSRE) regulation, heat shock protein (HSP) 70 stimulation, endothelin-1 (ET-1), vascular cell adhesion molecule-1 (VCAM-1) downregulation, focal adhesion alignment and kinase phosphorylation, and thrombomodulin (TM) and fibronectin (Fn) downregulation.

exposed to flow, morphological changes in cytoskeletal architecture and cellular realignment occur in the direction of flow. All of these physical changes are inhibited by drugs that interfere with microfilament turnover. Members of the integrin family of adhesion molecules have been extensively studied as sites of cytoskeleton anchorage to the plasma membrane. Linkage of integrins to F-actin on the cytoplasmic side of the plasma membrane occurs via talin, vinculin, and α-actinin. On the cell surface, integrins bind to the extracellular matrix, thus forming a bridge of protein-protein interactions between the filamentous cytoskeleton and the outside of the cell. Integrin-mediated signaling events are important in shear stress-mediated signal transduction on the basis of the observation that shear stress stimulated focal adhesion kinase tyrosine phosphorylation and activation of β_1-integrins in human umbilical endothelial cells (2). Endothelial cell shape is regulated by integrin extracellular matrix attachments that dictate the tensegrity of the cell. When a cell is exposed to fluid shear stress, the tensegrity transiently changes and allows the cell to adapt to this stimulus, rebalancing the force throughout the cytoskeleton. It now seems likely that integrins function not only as mechanotransmitters but also as mechanotransducers.

From cell surface to nucleus: signal transduction cascades

The stimulus derived from fluid shear stress is transduced to the nucleus to regulate gene expression via a distinct set of mitogen-activated protein (MAP) kinase signal transduction cascades; these include the extracellular signal-related kinases (ERKs), the c-*jun* NH_2-terminal kinases (JNKs), and p38 kinase. This is illustrated in Fig. 2 for two sets of target genes regulated by early growth response element-1 (Egr-1) and activator protein-1 (AP-1) transcription factors. On the basis of experiments subjecting endothelial cells to flow in vitro, both the Ras-MEKK (MAP kinase kinase kinase)-JNK and ERK1/2 MAP kinase pathways have been implicated (6). These data, derived from in vitro observations, are highly significant insofar that both JNK and ERK MAP kinase pathways are activated in vivo in models of acute hypertension (15) and may suggest that small changes in pressure or shear stress can influence the adaptive response of the vessel wall. Both ERK1/2 and JNK signaling pathways are transiently activated by shear stress levels as low as 1 or 0.5 dyn/cm^2. Research from our own laboratory has "reverse dissected" the signal transduction pathway from a shear stress-activated gene. Tissue factor, an initiator of the blood

"Linkage of integrins to F-actin on the cytoplasmic side of the plasma membrane. . . ."

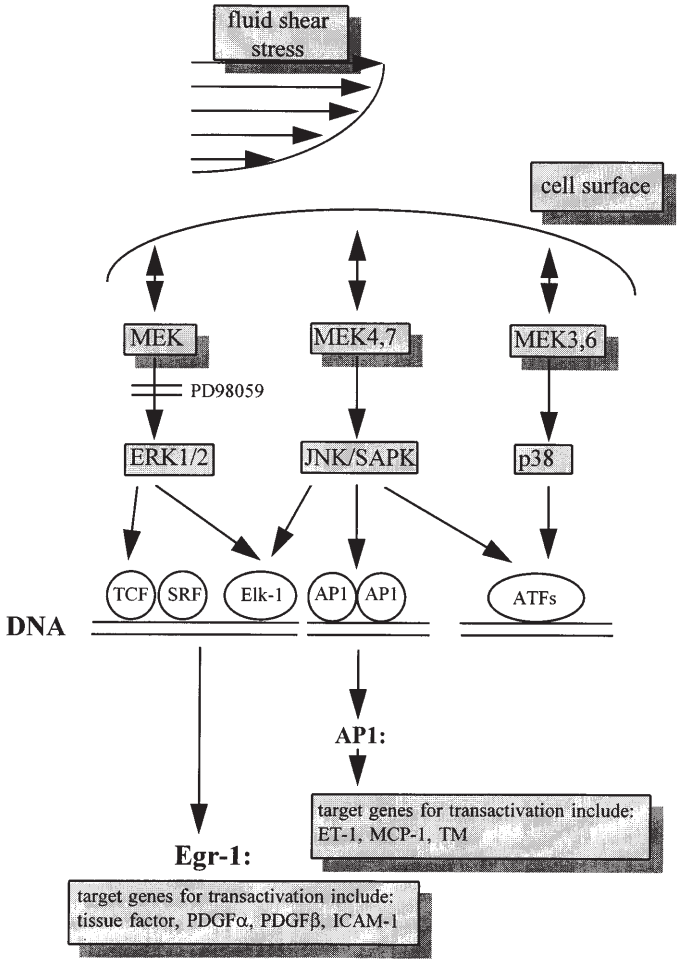

FIGURE 2. Signal transduction cascades and transcriptional modulation of target genes by fluid shear stress. Biomechanical stimulus of fluid shear stress is transduced to the nucleus via extracellular signal-related kinase (ERK) 1/2, c-Jun NH_2-terminal/stress-activated protein kinase (JNK/SAPK), and p38 mitogen-activated protein (MAP) kinase cascades. Transcription factors ternary complex factor (TCF), serum response factor (SRF), Elk-1, and the ATF family are phosphorylated by ERK1/2, and activator protein-1 (AP-1) is phosphorylated by JNK, which is necessary for DNA binding to target genes. PD-98059 blocks MAP kinase kinase-1 (MEK1) phosphorylation of ERK1/2. ET-1, endothelin-1; MCP-1, monocyte chemoattractant protein-1; TM, thrombomodulin; PDGF, platelet-derived growth factor; ICAM, intracellular adhesion molecule.

"...increases in the levels of shear stress may have profound effects on ... gene expression...."

coagulation protease cascade, is *trans*-activated by shear stress in human endothelial cells via the transcription factor Egr-1 (1). Egr-1 is, in itself, *trans*-activated by shear stress via serum response elements (SREs) in the *egr-1* promoter (11). ERK1/2 phosphorylate Elk1-ternary complex factor transcription factors in response to shear stress, which form a ternary complex with the serum response factor on the SREs in the *egr-1* promoter, a process that can be blocked by a specific inhibitor of MEK1 (MAP kinase kinase) phosphorylation of ERK1/2: PD-98059. The ERK1/2 signaling pathway has previously been implicated in the induction of *egr-1* activity by urea, and data showing that shear stress activation of *egr-1* is mediated via this signaling cascade suggest that diverse physical and chemical stimuli may use one and the same pathway.

Slight increases in the levels of shear stress may have profound effects on the levels of gene expression, at least in vitro; this observation allows one to speculate on the implications this may have in vivo. It is conceivable that continuous "normal" levels of shear stress such as 15–30 dyn/cm^2, may exert a protective effect on the endothelium by desensitizing endothelial cells to further stimuli and that low shear stress may result in endothelial cell sensitization to injury. If this theory is correct, then the implications for vascular biology are profound. One would predict that slight increases in shear stress in areas of the endothelium that have become sensitized (i.e., areas of low shear) would have significant effects on gene expression within this cell population. Given the panoply of growth factors, early response transcription factors, adhesion molecules, and vasoactive substances whose expression is shear stress modulated, a deleterious effect to localized regions of the vessel wall may result. Such clinical conditions, in which shear

TABLE 1. Fluid shear stress modulation of transcription in endothelial cells

Gene	Cell Type	mRNA Response	Transcription Factor Binding Sites
Endothelin-1	HUVEC/BAEC	Decrease	AP-1
VCAM-1	HUVEC	Decrease	AP-1, NF-κB
ACE	RAEC	Decrease	SSRE, AP-1, Egr-1
Tissue factor	BAEC	Increase	Sp1
Tissue factor	HAEC/HUVEC	Increase	Egr-1
TM	HUVEC	Increase	AP-1
PDGF-α	BAEC	Increase	SSRE, Egr-1
PDGF-β	BAEC	Increase	SSRE
ICAM-1	HUVEC	Increase	SSRE, AP-1, NF-κB
TGF-β	BAEC	Increase	SSRE, AP-1, NF-κB
Egr-1	HeLa/BAEC	Increase	SREs
c-*fos*	HUVEC	Increase	SSRE
c-*jun*	HUVEC	Increase	SSRE, AP-1
e-NOS	HUVEC	Increase	SSRE, AP-1, NF-κB
MCP-1	HUVEC	Increase	SSRE, AP-1, NF-κB

Specific gene is described together with the cell type in which gene expression was determined. Cell types are human umbilical vein endothelial cell (HUVEC) and human, bovine, and rabbit aortic endothelial cell (HAEC, BAEC, and RAEC, respectively). Involvement of the shear stress response element (SSRE; Ref. 9) or serum response element (SRE; Ref. 11) is indicated. Activator protein-1 (AP-1), early growth response factor-1 (Egr-1), and nuclear factor-κB (NF-κB) transcription factors are involved in activating transcription in response to fluid shear stress. ACE, angiotensin-converting enzyme; eNOS, endothelial nitric oxide synthase; ICAM-1, intracellular adhesion molecule-1; MCP-1 monocyte chemoattractant protein-1; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; TM, thrombomodulin; VCAM-1, vascular cell adhesion molecule-1.

stress modulation of gene expression undoubtedly plays a role, are found in accelerated atherosclerosis, particularly at arteriovenous junctions after bypass graft surgery and during restenosis after balloon angioplasty of a diseased artery.

Transcriptional activation of shear stress-responsive genes and the identification of shear stress response elements

As a consequence of the local perturbation in laminar shear stress, a number of genes have been identified whose promoter activity is both positively and negatively regulated by shear stress (Table 1). Several *cis*-acting elements have been implicated in mediating shear modulation of gene expression, and the term shear stress response element (SSRE) was first proposed after the identification of the sequence GAGACC (9). This SSRE was demonstrated to confer shear stress responsiveness on the platelet-derived growth factor-B promoter and has since been located in a number of other promoters that have been shown to be shear stress responsive in endothelial cells . The GAGACC sequence binds the transcription factor nuclear factor κ-B (NF-κB) p50–p65 heterodimers; consistent with this observation, the wild-type human immunodeficiency virus-1 long terminal repeat (LTR), but not an LTR lacking the NF-κB binding site, was also shown to be responsive to shear stress. NF-κB binding activity has itself been shown to respond differentially to fluid shear stress, depending on the flow regime. Human aortic endothelial cells exposed to either low chronic laminar shear or low pulsatile shear conditions were associated with increased and prolonged NF-κB levels, which could be reversed at high shear stress levels (8). This suggests that, at areas of low shear stress associated with atherosclerotic plaque development, elevated and sustained levels of NF-κB may increase levels of adhesion molecules and monocyte chemoattractant protein-1 (MCP-1) and serve as a trigger for monocyte recruitment.

"...sustained levels of NF-κB may increase levels of adhesion molecules...."

Not all SSREs serve to positively regulate gene expression. For example, vascular cell adhesion molecule (VCAM-1) and ICAM-1 are both negatively and positively regulated by increasing shear stress, respectively. Deletion analysis of the VCAM-1 and ICAM-1 promoters isolated both a repressive and active SSRE, which illustrates that SSREs may function not only to differentially modulate the level of gene expression but may function in a sequence-specific context.

Other transcription factors shown to mediate shear stress activation of a promoter are AP-1, Egr-1, and Sp1. AP-1, a heterodimer of c-*jun* and c-*fos* or a homodimer of c-*jun*, is activated by shear stress (4). AP-1 binds to a consensus sequence element termed the 12-*O*-tetra-

canoylphorbol 13-acetate response element, which is found in a number of genes, for example, in MCP-1. MCP-1, as described above, is also upregulated by NF-κB. Thus, at least in the case of MCP-1, two different transcription factors that utilize disparate signal transduction cascades may activate the same promoter in response to fluid shear stress. In contrast, both Egr-1 and Sp1 transcription factors have been shown to regulate the tissue factor promoter by shear stress in human and bovine endothelial cells, respectively (1, 7). This may illustrate that species specificity of transcriptional control is prevalent, which should be taken into account when extrapolating data from animal cells and models.

In this paper, we describe the importance of biomechanical stimuli to the vessel wall, in particular to vascular endothelial cells. We have drawn on many studies that have derived data from experiments conducted in cellular systems in vitro to data taken from animal models and human subjects in vivo. In 1998, we are only just beginning to understand the plethora of transcription factors and signaling cascades that communicate this unique stimulus to modulate the control of gene expression in one specialized cell type: the vascular endothelium. Therapeutic applications of fluid shear stress research include the discovery of gene products whose expression may have atheroprotective properties and understanding how to sensitize the endothelium against acute stimuli, which may have use in bypass graft surgery, in which the endothelium experiences a sudden increase in blood flow and fluid shear stress. Finally, it is possible to construct shear stress-inducible transcription units (SITUs), synthetic promoters that respond to fluid shear stress for gene therapy applications. SITUs may be used to drive the expression of therapeutic genes at areas of increased shear stress, for example at sites of arterial stenosis. Further research can only add to our understanding of the role of shear stress in the maintenance of vascular cell integrity in healthy blood vessels and the role that shear stress contributes to the development and progression of vascular disease.

It is impossible in such a short review to do justice to the many laboratories that have made seminal discoveries in the field of endothelial cell biology. We hope that this review captivates the readers' imagination and enthusiasm and serves as a starting point for more intensive study.

Our laboratory thanks our many friends and colleagues who have offered invaluable criticism and encouragement for our research.

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