Editorial

Multiple Signaling Pathways in Flow-Mediated Endothelial Mechanotransduction

PYK-ing the Right Location

Peter F. Davies

The endothelial monolayer is a signal transduction interface for blood-borne mechanical as well as chemical stimuli. The structural deformation arising from a mechanical stimulus, such as a change of hemodynamic shear stress, is conceptually different from the binding of a hormone or other agonist to its specific receptor, yet both elicit important endothelial signaling responses. Mechanical and chemical signaling appear to use common as well as unique pathways downstream of the initial stimulus. For example, signaling pathways arising from membrane deformation and hormone-receptor coupling may appear to converge through activation of phospholipases[1] or nuclear factor κB transcription factor complex[2] but later diverge at the level of DNA binding[3] (Figure 1). However, simple interpretations of mechanotransduction are confounded by intracellular and pericellular force transfer, principally by the cytoskeleton,[4,5] that results in the generation of transduction pathways at multiple sites throughout the cell, ie, *decentralized* mechanotransduction.[6,7] Thus, although flow-induced shear stress acts at the luminal surface of the endothelial monolayer, the forces are transmitted to cell junctions, nuclear structures, basal adhesion sites, and organelles that are structurally interconnected. This spatial organization of intracellular signaling may result in the stimulation of multiple parallel, convergent, and/or divergent mechanotransduction pathways. The mechanism of transduction of a purely mechanical signal to second messenger pathways such as IP_3-driven intracellular calcium mobilization[8] or mitogen-activated protein kinases (MAPK) activation[9] is unknown. Major efforts are therefore underway to understand endothelial mechanotransduction in terms of the initial stimulus, the generation of second messengers, the activation of transcription factors, and altered gene and protein expression that lead to structural and functional consequences. Recently, the spatial elements of mechanotransduction are receiving attention, in part because of recognition of structural continuity in the distribution of cellular strain,[5] and an appreciation of the 3-dimensional dynamics of signaling pathways. A new study reported in this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*[10] identifies for the first time a role for the spatially versatile protein-rich tyrosine kinase, PYK2, in shear stress mechanotransduction.

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PYK2 is spatially interesting because it has homology to focal adhesion kinase (FAK) localized to adhesion plaques at the basal side of the cell. However, PYK2 is localized to the cytosol[11] with versatility to relocalize to the focal adhesion sites,[12] the perinuclear region,[13] or the nucleus.[13] In nonendothelial cells, it has been shown to be downstream to G-protein–coupled receptors and able to link G-protein activation to nuclear factor κB activation through phosphoinositide 3-kinase, the serine/threonine kinase *akt*, and IκB kinase.[14] PYK2 is phosphorylated in response to a variety of agonists and hormones, ion channel activation, and in endothelium it associates with Crk-associated substrate p130 (Cas), which is tyrosine-phosphorylated through a calcium-dependent c-Src activation initiated by flow.[15] FAK and PYK2 share their association with Cas through SH domains.[10] Using anti-oxidants, Tai et al[10] demonstrated that tyrosine phosphorylation of PYK2 in endothelial cells by shear stress was dependent on the generation of reactive oxygen species (ROS). The obligatory role of intracellular calcium mobilization for PYK2 phosphorylation was shown, mediated through phospholipase C (PLC) activation and IP_3, confirming PYK2 as a calcium-dependent kinase. While stimulation of PLC activity by shear stress has been shown to lead to protein kinase C (PKC) activation, inhibition of PKC directly had no effect on flow-mediated PYK2 phosphorylation suggesting a PLC-calcium-PYK2 pathway. The correlation of calcium dependence, flow-activation through ROS, and shared SH domain led Tai et al[10] to postulate that tyrosine phosphorylation of Cas was dependent on PYK2 activation. To test this hypothesis more directly, endothelial cells were transfected with a kinase-inactive PYK2, the overexpression of which blocked flow/ROS–mediated phosphorylation of both PYK2 and Cas. Transfection with a kinase-inactive Src did not inhibit PYK2 phosphorylation suggesting that Src-dependent Cas phosphorylation occurred downstream of PYK2. Although transfection with a kinase-inactive c-Src had no effect on PYK2 phosphorylation by flow, other unknown Src family kinases appear to regulate PYK2 activation as demonstrated by the effectiveness of the global Src family kinase inhibitor PP2 to inhibit flow-mediated PYK2 activation. Both Cas and PYK2 phosphorylation were inhibited by depletion of ROS by the antioxidant *N*-acetylcysteine and

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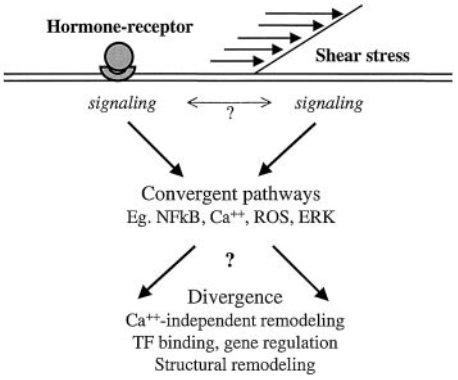

Figure 1. Mechanical and hormone-receptor signaling may interact and share common intracellular pathways. Divergent outcomes suggest parallel rather than simultaneous use of common second messengers. The spatial relationships that may influence nonlinear signaling are unknown.

conversely were stimulated by superoxide-generating agents. Overall, the study links together several elements in mechanical signaling: flow activation of ROS (mechanism unknown, possibly through G-protein activation[11]), PLC-mediated intracellular calcium mobilization, and Src family kinase (but not c-Src)-mediated PYK2 phosphorylation which appears obligatory for p130 Cas phosphorylation. A summary of these findings is outlined in Figure 2.

The link between ROS and PYK2 phosphorylation is well established. ROS generation by angiotensin II, PDGF, cytokines, and UV radiation as well as peroxide in other cells leads to PYK2 activation.[10] There is circumstantial evidence in other cells that Src family kinase members Yes and/or Fyn may be a missing link in the regulation of PYK2 phosphorylation[15] but flow experiments using endothelial transfectants are lacking at present. Recently, Keogh et al[11] have demonstrated endothelial PYK2 phosphorylation on tyrosines 402, 580, and 881 following stimulation by G-protein–coupled receptor agonists, VEGF, and interleukin-1α, and they have noted its cytosolic distribution and rapid association with structural and adapter proteins, suggesting a kinase with great versatility of location and function.

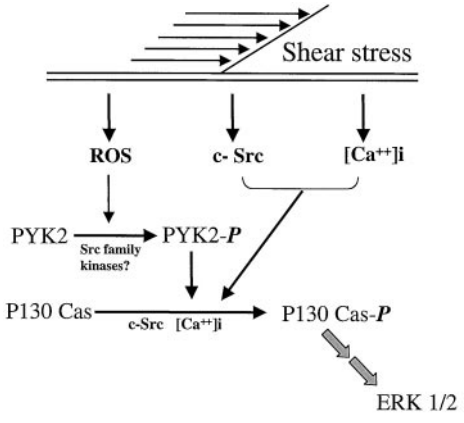

Figure 2. Outline of the involvement of protein-rich tyrosine kinase PYK2 in endothelial mechanotransduction arising from the work of Tai et al.[10]

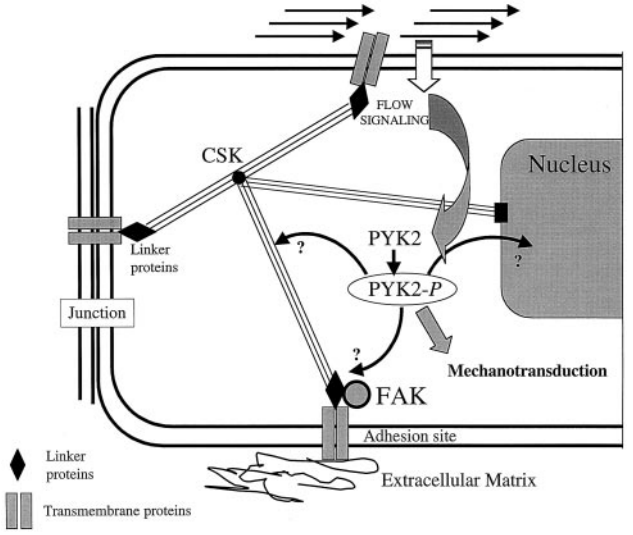

Figure 3. Potential spatial relationships involving PYK2 activation in endothelial mechanotransduction (see text).

If PYK2 plays a significant regulatory role in mechanotransduction, what is known about its intracellular distribution and function (Figure 3)? The protein p130Cas, with which PYK2 appears to associate, is reported to be localized to focal adhesion sites[16] including association with zyxin and LIM proteins[17] and is known to be critical for cell spreading and motility.[16,18] PYK2 can also physically associate with cytoskeleton linker molecules such as paxillin,[11] and these associations are consistent with its reported localization at adhesion sites where its autophosphorylation is regulated by FAK through the FAK focal adhesion-targeting domain.[12] Although phosphorylation of PYK2 by flow was unaffected by treatment with the actin microfilament-destabilizing agent cytochalasin D,[10] suggesting that an intact actin filament network may not be critical for flow-mediated PYK2 phosphorylation, Sawada and Sheetz[19] have recently reported binding of p130 Cas, FAK, and paxillin to actin microfilaments as a consequence of stretch in L929 cells. Paradoxically however, PYK2 is also localized to the perinuclear region in fibroblasts and becomes exclusively nuclear on modification of one of its SH3-binding sites, suggesting a possible direct role in the regulation of transcription.[13]

The spatial structure-function relationships in mechanosignaling are now recognized through the development of local probes and 3-dimensional live cell imaging. In endothelial cells exposed to hemodynamic shear stresses, the spatial considerations associated with mechanotransduction assume great importance, especially because the forces vary greatly over subcellular distances.[6] Thus, there are complex spatiotemporal components of the signaling that need to be taken into account. Now that PYK2 is identified as a player in mechanotransduction, the potential spatial versatility of this important kinase as a component of hemodynamic mechanosignaling is worth further investigation.

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