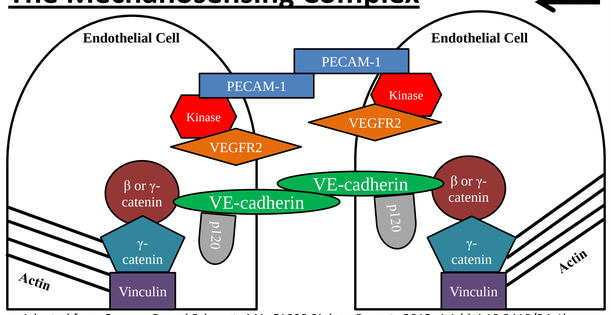
Cofilin regulation of actin realignment is essential for vascular endothelial barrier integrity during shear stress

Joshua B. Slee and Linda J. Lowe-Krentz
Lehigh University, Department of Biological Sciences, Bethlehem, PA 18015

Abstract

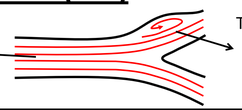
Fluid shear stress (FSS) induces vascular endothelial cell and actin microfilament realignment in the direction of FSS in vitro and in vivo, yet the molecular mechanisms underlying this process are not completely understood. At least one mechanosensing mechanism has been identified involving a complex of PECAM-1, VE-cadherin, and VEGFR2. While PECAM-1 and VEGFR2 are reported to be responsible for downstream signaling, VE-cadherin functions as an endothelial cell-specific component of adherens junctions which is essential for maintaining endothelial barrier integrity. The cytoplasmic domain of VE-cadherin is known to associate with p120-, β-, γ-, and α-catenin to mediate connections to the actin cytoskeleton. Regulation of actin microfilament turnover depends in part upon the Actin Depolymerizing Factor (ADF) family of proteins, of which cofilin is a prominent player. We have determined that FSS induces p-cofilin accumulation in nuclei of vascular endothelial cells and decreased p-cofilin in the cytoplasm. Two cofilin mutants (S3A and S3D) both disrupt multiple stages of the actin realignment process indicating that continued modulation of cofilin phosphorylation is important for actin realignment. Vascular endothelial cells expressing either of the cofilin mutants were utilized to study the role of actin realignment during FSS in maintaining endothelial barrier integrity. Inhibition of dynamic changes in cofilin phosphorylation through cofilin mutants decreased barrier integrity as determined by immunofluorescent staining for VE-cadherin and β-catenin. In similar experiments, inhibition of stress kinases, JNK and p38, also interfered with actin realignment and maintenance of barrier structure. These results identify the importance of actin realignment in maintaining the endothelial barrier during FSS and the necessity of cofilin phospho-regulation in control of the actin realignment.

The Mechanosensing Complex

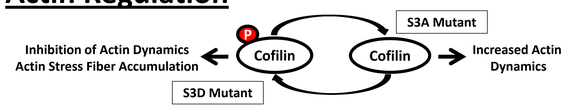


Adapted from Conway D. and Schwartz MA. F1000 Biology Reports 2012, 4:1 (doi:10.3410/B4-1) with established links to actin added.

Fluid Shear Stress (FSS)

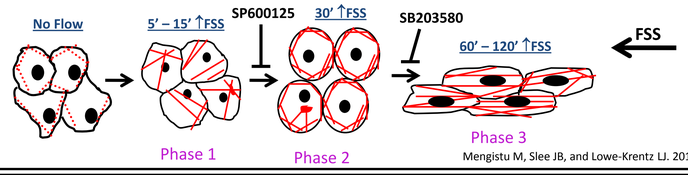


Actin Regulation



FSS-Induced Realignment

- *In vivo* and *in vitro* evidence indicates that FSS causes endothelial cell and actin microfilament realignment in the direction of flow.



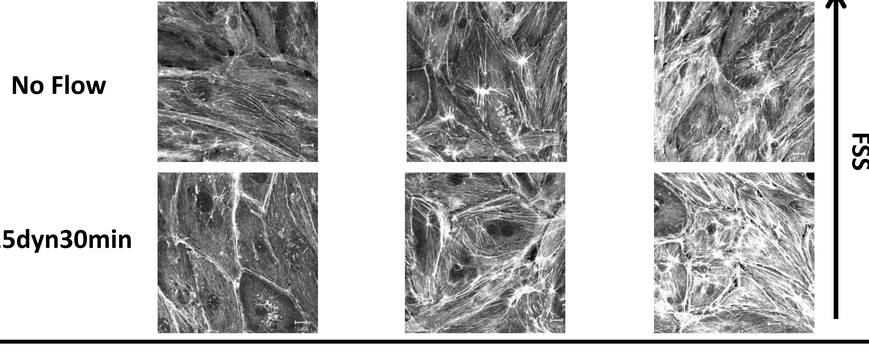
Mengistu M, Slee JB, and Lowe-Krentz LJ. 2012

Methods

- BAOECs obtained from Cell Applications were grown on glass cover slips, coated with bovine collagen type I.
- Shear media was propagated over the BAOECs at 15dynes/cm^2 using a REGLO Digital continuous flow pump (ISMATEC) attached to a POC mini chamber (Hemogenix).
- In experiments using stress kinase inhibitors (SP600125 and SB203580), cells were incubated with 10 μM of inhibitor in shear media for 1 hr prior to FSS exposure.
- BAOECs were electroporated with 20μg/ml of cofilin construct (S3A – serine-3-alanine or S3D – serine-3-aspartic acid) and GFP-vinculin as a fluorescent control using the Bio-Rad Gene Pulser XCell System (160V, 15 ms pulse length, 2 mm cuvette). BAOECs were plated on 100 mm plates two days prior to electroporation, electroporated on day three and re-plated onto 100 mm dishes. Once confluent, the 100 mm plates were split onto cover slips and allowed to reach confluency (approx. 18 hrs). Once confluent, these cells were exposed to FSS.
- Cells were methanol fixed/permeabilized and stained for VE-cadherin and β-catenin or 2% formaldehyde & 0.2% Triton fixed/permeabilized and stained for actin stress fibers with TRITC-phalloidin.
- Cells were imaged using a Zeiss© LSM 510 Meta confocal microscope with a 63X oil-immersion lens at room temperature.

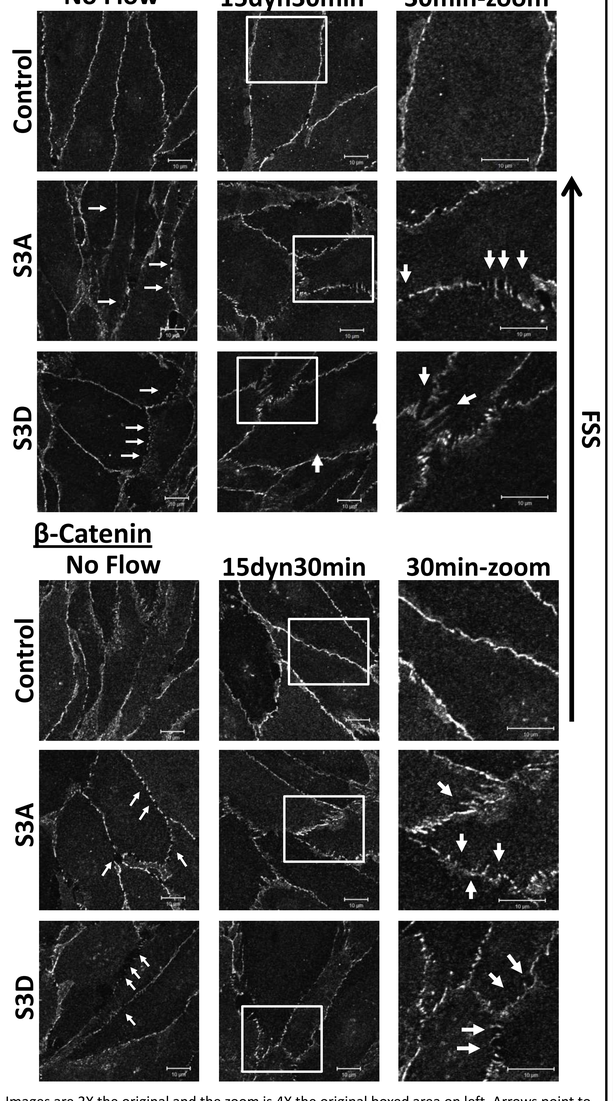
Results: Impaired Realignment

Actin stress fibers stained with TRITC-Phalloidin



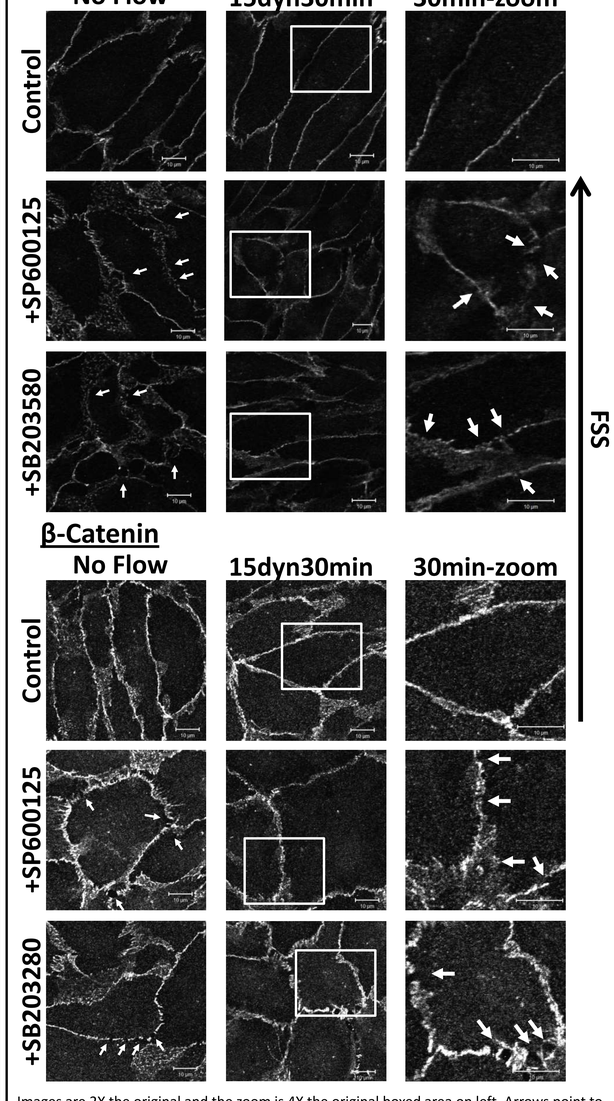
BAOECs expressing GFP-vinculin alone or in combination with S3A or S3D cofilin. GFP-vinculin only cells show normal FSS-induced actin realignment. BAOECs expressing either cofilin mutant exhibit impaired FSS-induced actin realignment; shown for 30 mins here. BAOECs expressing either cofilin mutant fail to elongate in the direction of FSS. Confocal images shown from a single experiment are representative of 8 experiments.

Results: Cofilin Mutants



Images are 2X the original and the zoom is 4X the original boxed area on left. Arrows point to breaks in staining. Images shown from a single experiment representative of 3 experiments.

Results: Stress Kinase Inhibitors



Images are 2X the original and the zoom is 4X the original boxed area on left. Arrows point to breaks in staining. Images shown from a single experiment representative of 2 experiments.

Conclusions

- Cofilin phospho-regulation is necessary for control of actin realignment during shear stress.
- Actin realignment is essential for maintaining/strengthening the endothelial barrier during shear stress.
- Stress kinases (JNK and p38) play a role in maintaining/strengthening the endothelial barrier during shear stress, likely through their established roles in shear stress-induced actin realignment.

Acknowledgements

- Cofilin mutants provided by Theo Rein, PhD. Max Planck Institute of Psychiatry, München, Germany.
- The authors would like to thank Bryan Berger, PhD. (Lehigh Univ.) for use of his lab's electroporation equipment and ongoing collaborations.
- Research in the Lowe-Krentz lab is supported in part by NIH grant HL54269 to LJL-K.
- This work is published in J Cell Biochem. 2012 Oct 11. doi: 10.1002/jcb.24416.