BIOGRAPHICAL SKETCH

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NAME: Troy Stevens

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POSITION TITLE: Lenoire Locke Professor and Chair, Physiology and Cell Biology
Director, Center for Lung Biology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Chadron State College, Chadron, NE	B.S.	1986	Health Education
Colorado State University, Ft. Collins, CO	M.S.	1988	Exercise Physiology
Colorado State University, Ft. Collins, CO	Ph.D.	1991	Physiology
University of Colorado-HSC, Denver, CO	Postdoc.	1994	Respiratory Physiology

A. Personal Statement

I have a long-standing interest in mechanisms pertaining to endothelial cell heterogeneity, particularly in the lung. Our work focuses on molecular mechanisms that account for unique endothelial cell behaviors in pulmonary artery, capillary and vein endothelium. Chief among these interests is a systematic study of: barrier function, neo-angiogenesis, vasoreactivity, and site-specific host-pathogen interactions, not only pertaining to how microorganisms interact with endothelium along the vascular axis, but how toxins access intracellular compartments and uniquely modify the behavior of pulmonary artery, capillary and vein endothelium. A principal goal of these studies is to understand how vascular disease manifests in discrete vascular locations and resolve novel molecular signatures that can be exploited to target therapy to the appropriate vascular site. Examples of our work in this area are shown below.

1. ***Stevens*** **T**, Rosenberg R, Aird WC, Quertermous T, Johnson FL, Garcia JGN, Hebbel RP, Tuder RM, and Garfinkel S. NHLBI Workshop Report: endothelial cell phenotypes in heart, lung and blood diseases. Am. J. Physiol. - Cell Physiol., 281: C1422-C1433, 2001.
2. Gebb S and ***Stevens*** **T**. On lung endothelial cell heterogeneity. Microvasc. Res., 68: 1-12, 2004.
3. Ochoa CD*, Wu S*, and ***Stevens*** T. New developments in lung endothelial heterogeneity: von Willebrand factor, P-selectin and the Weibel-Palade Body. Semin. Thromb. Hemost., 36: 301-308, 2010. PMCID: PMC2917989
4. Ochoa CD* and ***Stevens*** **T**. Studies on the cell biology of interendothelial cell gaps. Am. J. Physiol. - Lung Cell. Mol. Physiol., 302: L275-L286, 2012. PMCID: PMC3289273

B. Positions, Honors and Professional Service

Positions:

1994-1996 Senior Instructor (1994-95) and Assistant Professor (1995-96), Department of Anesthesiology, University of Colorado Health Sciences Center, Denver CO.

1996-2014 Assistant Professor (1996-1999); Associate Professor (1999-2003); Professor (2003-2014), Department of Pharmacology, University of South Alabama College of Medicine, Mobile AL

2002-present Director, Center for Lung Biology, University of South Alabama College of Medicine, Mobile, AL

2008-present Professor (joint), Department of Internal Medicine, University of South Alabama College of Medicine, Mobile, AL

2014-present Lenoire Locke Professor and Chair, Department of Physiology and Cell Biology, University of South Alabama College of Medicine, Mobile AL

Honors and Professional Service (selected examples):

1995-1998	Parker B. Francis Fellowship Award for Research in Pulmonary Medicine.
1996	Giles Filley Memorial Award for Research in Respiratory Physiology and Medicine.
1999-present	Member, Editorial Board (1999-present); Associate Editor (2006-2012), *American Journal of Physiology: Lung Cellular and Molecular Physiology*.
2001	US Delegate for US-Russia Symposium on Basic Research in Cardiovascular and Pulmonary Diseases, Moscow, Russia.
2002	US Delegate for US-Italy Symposium on Pathophysiology and Therapeutic Approaches for Vascular Remodeling, Atlanta, GA, USA.
2003-2007	Associate Editor of "*Microvascular Research"* for Elsevier Science.
2003-2007	Member, Respiratory Integrative Biology and Translational Research Study Section, NIH
2008-present	Ad hoc Reviewer, Respiratory Integrative Biology and Translational Research Study Section, and various PPG Special Emphasis Panels, NIH
2014-present	Pulmonary Circulation Assembly Chair, American Thoracic Society

C. Contribution to Science (* denotes active and former students)

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/pubmed?term=troy+stevens&cmd=DetailsSearch&log$=activity

1. ***Lung endothelium is heterogeneous in structure and function.*** The structure and function of endothelium along the pulmonary arterial-capillary-venous axis varies significantly. We have studied the vascular segment-specific endothelial structure-function relationship in an attempt to understand how cellular origin and tissue microenvironment informs acquisition of phenotypic specification. We have developed molecular approaches that discriminate pulmonary artery, capillary and vein endothelial cells in vivo, and have used these approaches to isolate and culture pure populations of cells from each segment. A marked "macro-heterogeneity" is retained in cell culture, even with passaging, enabling rigorous evaluation of molecular cues that are responsible for segment-restricted cell function. Comprehensive molecular profiling [mRNA, RNAseq, epigenetic (methylation), glycomic, proteomic] has revealed signatures that define endothelium within a given vascular segment. This body of work has contributed to the following new physiological principles: (1) Capillary endothelium forms a highly restrictive barrier when compared with endothelium in arterial and venous segments; (2) Activation of store operated calcium entry channels induces cell retraction that increases endothelial permeability, especially in extra-alveolar segments; (3) Increased permeability in extra-alveolar segments causes perivascular cuffing, which decreases lung compliance in the absence of alveolar flooding; (4) Activation of mechanosensitive and thermosensitive calcium channels causes alveolar flooding, without formation of perivascular cuffs; (5) Activation of α_{1G} T type calcium channels in lung capillary endothelium promotes P-selectin surface expression important for leukocyte trafficking, but does not increase permeability; (6) Capillary endothelial cells are enriched with replication competent progenitor cells that are responsible for rapid neo-angiogenesis and vascular repair following injury; and, (7) A transition in endothelial phenotype – where pulmonary artery adjoins capillary endothelium - exists in small precapillary arterioles, at a nidus for occlusive arteriopathy in pulmonary arterial hypertension. Examples include:

1a. Wu S*, Haynes J, Taylor JT, Obiako BO, Stubbs JR, Li M, and ***Stevens* T**. $C_{av}3.1$ (α_{1G}) T-type calcium channels mediate vaso-occlusion of sickled erythrocytes in lung microcirculation. Circ. Res., 93: 346-353, 2003. (see accompanying editorial by Anthony Varghese and Edward Kenneth Weir).

1b. King JAC, Hamil T, Creighton J*, Wu S*, Bhat P, McDonald F, and ***Stevens* T**. Structural and functional characteristics of lung macro- and microvascular endothelial cell phenotypes. Microvasc. Res., 67: 139-151, 2004.

1c. Wu S*, Cioffi EA, Alvarez D*, Sayner SL*, Chen H, Cioffi DL*, King JAC, Creighton JR*, Townsley M, Goodman SR, and ***Stevens* T**. Essential role of a calcium selective, store operated current (I_{SOC}) in endothelial permeability: Determinants of the vascular leak site. Circ. Res., 96: 856-863, 2005.

1d. Wu S*, Zhou C, King JAC, and ***Stevens* T**. A unique pulmonary microvascular endothelial cell niche revealed by Weibel-Palade bodies and *Griffonia simplicifolia*. Pulm. Circ., 4: 110-115, 2014. PMCID: PMC4070765

2. ***Resolving the molecular anatomy of calcium channels that increase endothelial permeability.*** Studies prior to 1990 revealed that neurohumoral inflammatory mediators increase cytosolic calcium in endothelium. The calcium signal stimulates actomyosin interaction, increases centripetal tension, promotes gap formation, and increases paracellular permeability. However, prior to 1990, not a single endothelial cell calcium channel was known. We have contributed to understanding the molecular basis of endothelial cyclic nucleotide gated, α_{1G} T type, and canonical transient receptor potential and Orai1 store operated calcium entry channels. Most of our work has focused on gating, stoichiometry, and function of store operated calcium entry.

When we began our studies, mechanisms responsible for coupling calcium store depletion with membrane channel activation were unknown, and were hypothesized to be due to either physical coupling, generation of a soluble mediator, or insertion of new channels in the plasma membrane. We first recognized that store operated calcium entry channels functionally interact with protein 4.1, and are tethered to the spectrin membrane skeleton, consistent with the physical coupling model. We learned that the molecular basis of an endogenous endothelial store operated calcium entry channel includes TRPC1 and TRPC4, and an associated Orai1. The TRPC4 carboxy terminus possesses a protein 4.1 binding domain near the channel pore, which binds protein 4.1 and gates the channel. The Orai1 amino terminus also possesses a protein 4.1 binding domain, perhaps as a mechanism of organizing the channel signalplex. Orai1 determines calcium selectivity of the endothelial cell TRPC1/4, as Orai1 seems to select calcium for permeation through the pore, and as such, influences the magnitude of calcium that enters the cell. The resulting TRPC1/4 calcium influx causes endothelial cell barrier disruption. Thus, we define a signalplex, including TRPC1/4-Orai1 and protein 4.1, responsible for the calcium influx that disrupts the endothelial barrier. Examples include:

2a. Brough G, Wu S*****, Cioffi DL*****, Moore TM*, Li M, Dean N, and ***Stevens*** **T**. Contribution of endogenously expressed Trp1 to a calcium-selective, store operated calcium entry pathway. FASEB J., 15: 1727-1738, 2001.

2b. Wu S*, Sangerman J, Li M, Brough GH, Goodman SR, and ***Stevens*** **T**. Essential control of an endothelial I_{SOC} by the spectrin membrane skeleton. J. Cell Biol., 154: 1225-1234, 2001.

2c. Cioffi DL*, Wu S*, Alexeyev M, Goodman SR, Zhu MX, and ***Stevens*** **T**. Activation of the endothelial store operated I_{SOC} calcium channel requires interaction of protein 4.1 with TRPC4. Circ. Res., 97: 1164-1172, 2005.

2d. Cioffi DL*, Wu S*, Chen H, Alexeyev M, St. Croix CM, Pitt BR, Uhlig S, and ***Stevens*** **T**. Orai1 determines calcium selectivity of an endogenous TRPC heterotetramer channel. Circ. Res., 110: 1435-1444, 2012. PMCID: PMC3388001

3. ***Studies on endothelial transmembrane adenylyl cyclase and permeability.*** Neurohumoral inflammatory mediators promote calcium influx through store operated calcium entry channels, which disrupts the endothelial cell barrier. This calcium-dependent barrier disruption is opposed by intracellular cAMP, bringing into question how calcium influences cAMP production. We addressed this question in 1996, at a time when the molecular complexity of adenylyl cyclases was just being realized. We found that endothelium expresses the type 6 calcium inhibited adenylyl cyclase, which provides a physiologically important mechanism for crosstalk between calcium and cAMP signaling. The physiological relevance of this relationship was not fully realized until 2002, when we were able to functionally uncouple calcium from cAMP, and demonstrate that in the absence of AC6 calcium inhibition, inflammatory agonists do not disrupt the endothelial barrier.

Calcium inhibition of AC6 does not necessarily translate into a decrease in whole cell cAMP. We now realize that cAMP is highly compartmentalized within the cell, where membrane-associated cAMP signals do not uniformly distribute throughout the cytosol. Endothelial cells possess soluble adenylyl cyclase isoforms (e.g. AC10 or sAC) that independently contribute to the cell-averaged cAMP concentration. Membrane-localized phosphodiesterase(s) directs the cAMP signal to its relevant targets, while limiting "spillover" into the bulk cytosol. This work has taught us that in endothelium: (1) calcium inhibits membrane adenylyl cyclases to enable cytoskeletal reorganization necessary for barrier disruption; and, (2) cAMP is highly compartmentalized within the cell, to efficiently couple environmental cues with appropriate physiological outcomes. Examples include:

3a. ***Stevens*** **T,** Nakahashi Y, Cornfield DN, McMurtry IF, Cooper DMF, and Rodman D. Calcium inhibitable adenylyl cyclase modulates pulmonary artery endothelial cell cAMP content and barrier function. Proc. Natl. Acad. Sci., 92: 2696-2700, 1996.

3b. Cioffi DL*, Moore TM*, Schaack J, Creighton JR*, Cooper DMF, and ***Stevens* T***. Dominant regulation of inter-endothelial cell gap formation by calcium inhibited type 6 adenylyl cyclase. J. Cell Biol., 157: 1267-1278, 2002.

3c. Creighton JR*, Masada N, Cooper DMF, and ***Stevens* T***. Coordinate regulation of membrane cAMP by calcium inhibited adenylyl cyclase and phosphodiesterase activities. Am. J. Physiol.-Lung Cell. Mol. Physiol., 284: L100-L107, 2003.

3d. Creighton JR*, Zhu B, Alexeyev M, and ***Stevens* T***. Spectrin-anchored phosphodiesterase 4D4 restricts cAMP from disrupting microtubules and inducing endothelial gap formation. J. Cell Sci., 121: 110-119, 2008. (see accompanying highlight)

4. *Soluble adenylyl cyclases disrupt the endothelial barrier.* Transmembrane adenylyl cyclases generate an anti-inflammatory, barrier enhancing cAMP signal. However, certain bacteria utilize cyclases as "edema factors." *Pseudomonas aeruginosa* expresses a soluble purine and pyrimidine nucleotidyl cyclase, exoenzyme Y (ExoY), that causes endothelial cell retraction, loss of cell-cell adhesion, and increased permeability. We have discovered this "paradoxical" permeability effect is due to the intracellular location of ExoY (and other soluble cyclases). Unlike transmembrane adenylyl cyclases, ExoY is found in the cytosol where it generates cyclic nucleotides near associated microtubules, leading to their breakdown. Thus, ExoY increases endothelial permeability by causing microtubule disassembly rather than by increasing actin-based contraction.

ExoY is a promiscuous nucleotidyl cyclase, meaning that it synthesizes purine and pyrimidine cyclic nucleotides; through studies with ExoY, we have found that endothelium normally synthesizes cUMP and to a lesser extent cCMP, in addition to cAMP and cGMP. At present, the second messenger function(s) of pyrimidine cyclic nucleotides are unknown and are under investigation. However, these second messengers activate protein kinase A, which leads to phosphorylation of an endothelial cell tau. As a consequence of this "hyperphosphorylation," tau oligomerizes inside the cell and is then released. This oligomerized tau is recovered from the cell supernatant, and from the bronchoalveolar lavage fluid and plasma of patients harboring *Pseudomonas aeruginosa* infections. Examples of this work include:

4a. Sayner SL, Frank DW, King JAC, Chen H, VandeWaa J, and ***Stevens* T**. Paradoxical cAMP-induced lung hyperpermeability revealed by *Pseudomonas aeruginosa* ExoY. Circ. Res., 95: 196-203, 2004.

4b. Sayner SL, Alexeyev M, Dessauer C, and ***Stevens* T**. Soluble adenylyl cyclase reveals the significance of cAMP compartmentation on pulmonary microvascular endothelial cells. Circ. Res., 98: 675-681, 2006. (see accompanying editorial by Rodolphe Fischmeister)

4c. Prasain N, Alexeyev M, Balczon R, and ***Stevens* T**. Soluble adenylyl cyclase-dependent microtubule disassembly reveals a novel mechanism of endothelial cell retraction. Am. J. Physiol., 297: L73-L83, 2009. PMCID: PMC2711814

4d. Ochoa CD, Alexeyev M, Pastukh V, Balczon R, and ***Stevens* T**. *Pseudomonas aeruginosa* exotoxin Y is a promiscuous cyclase that increases endothelial tau phosphorylation and permeability. J. Biol. Chem., 287: 25407-25418, 2012. PMCID: PMC3408204

5. *Lung pathogens induce endothelial production of cytotoxic amyloids with prion characteristics.* We first noticed that *Pseudomonas aeruginosa* ExoY expression in endothelial cells causes cell rounding that results in a chronic loss of cell mobility. We followed this observation by testing the function of surviving cells, after the primary infection. This work revealed that endothelium was releasing factor(s) that impaired their recovery from infection. More specifically, these cells did not proliferate, and they could no longer undergo angiogenesis. We learned that infection, and ExoY in particular, induces endothelial production and release of cytotoxic proteins with amyloid characteristics, including oligomeric tau and amyloid beta (Aβ). These endothelial cell amyloids share characteristics of prion disease, in that they are transmissible among cells and self-replicating. They are heat stable, protease resistant, insoluble in detergents, insensitive to RNase and DNase treatments, and can be resolved in the 50% ammonium sulfate fraction by column chromatography. They can be sedimented by centrifugation with an angular momentum of 1.14×10^{12}. Amyloid cytotoxicity is inactivated using phenol, diethyl pyrocarbonate, and hexafluoro-2-propanol. These findings indicate infection elicits lung endothelial cell production of cytotoxic amyloids that may contribute to end organ dysfunction. In our ongoing work we have tested this principle and found intensive care unit patients with nosocomial pneumonia have amyloids present in the bronchoalveolar lavage fluid, plasma and cerebrospinal fluid. The amyloids present in the cerebrospinal fluid are sufficient to impair long term potentiation in the hippocampus.

We are examining the nature of these endothelial amyloids, mechanisms leading to their production and release, and their bio-distribution during critical illness. Examples of this work include:

5a. Morrow KA*, Ochoa CD*, Alexeyev M, Balczon R, Frank DW, and ***Stevens* T**. *Pseudomonas aeruginosa* exoenzymes U and Y induce a transmissible endothelial proteinopathy. Am. J. Physiol. Lung Cell. Mol. Physiol., 310: L337-L353, 2016. PMCID: PMC4754902

5b. Balczon R, Morrow KA*, Zhou C, Edmonds B, Alexeyev M, Pittet JF, Wagener BM, Moser SA, Leavesley S, Zha X, Frank DW, and ***Stevens* T**. *Pseudomonas aeruginosa* infection liberates transmissible, cytotoxic prion amyloids. FASEB J., 31: 2785-2796, 2017. PMCID: PMC5471513

5c. Lin MT, Balczon R, Pittet JF, Wagener BM, Moser SA, Morrow KA*, Voth S*, Francis CM*, Leavesley S, Bell J, Alvarez DF*, and ***Stevens* T**. Nosocomial pneumonia elicits an endothelial proteinopathy: Evidence for a source of neurotoxic amyloids in critically ill patients. Am. J. Resp. Crit. Care Med., 198: 1575-1578, 2018. PMCID: PMC6298632

5d. Voth S*, Gwin M*, Francis CM*, Balczon R, Frank DW, Pittet JF, Wagener BM, Moser SA, Alexeyev M, Housley N, Audia JP, Piechocki S, Madera K, Simmons A, Crawford M, and ***Stevens* T**. Virulent *Pseudomonas aeruginosa* infection converts antimicrobial amyloids into cytotoxic prions. FASEB J., 34: 9156-9179, 2020.

D. Research Support

Ongoing Research Support

R37 HL60024 (Stevens) 04/10/1998-06/30/2020
NIH/NHLBI
Store operated calcium entry: lung endothelial permeability
This proposal tests the overall hypothesis that Orai1 interacts with TRPC1/3/4 and establishes the channel's calcium selectivity, which is necessary to increase membrane calcium to concentrations that disrupt the spectrin-actin interaction causing inter-endothelial cell gap formation.
Role: Principal Investigator

P01 HL66299 (Stevens) 12/01/2001-04/30/2023
NIH/NHLBI
Lung endothelial cell phenotypes
This Program Project Grant is founded on the hypothesis that endothelium lining the lung's extra-alveolar and alveolar blood vessels is phenotypically distinct, and that the unique behavior(s) of cells from these different vascular locations is necessary for them to fulfill their site-specific function(s).
Role: Principal Investigator/Project Leader

5R01HL140182-02 (Lin) 04/01/2018-03/31/2022
NIH/NHLBI
Nosocomial pneumonias impair cognitive function
This project takes advantage of vertically integrated approaches, ranging from the use of different bacteria stains and cultured cells, to in vitro brain slice recordings and in vivo animal behavior studies.
Role: Co-Investigator

1 K25 HL136869-01 (Francis) 07/01/2017-06/30/2022
NIH/NHLBI
TRPC4-mediated Calcium Signals Accelerate Vascular Remodeling in Pulmonary Arterial Hypertension
Type: NIH Mentored Quantitative Research Development Award (K25)
Goals: The major goal of this project is to determine the role of interaction between TRPC4-dependent calcium signals and shear stress in driving vascular remodeling in pulmonary arterial hypertension.
Role: Mentor

1R01HL148069-01A1 (Stevens) 07/01/2020-06/30/2024
NIH/NHLBI $385,000
Lung Endothelial Aß in infectious proteinopathy
This project tests the hypothesis that the *P. aeruginosa* type 3 secretion system effector, exoenzyme Y, promotes the production of cytotoxic Aβ, dependent upon γ secretase activating protein.
Role: Principal Investigator