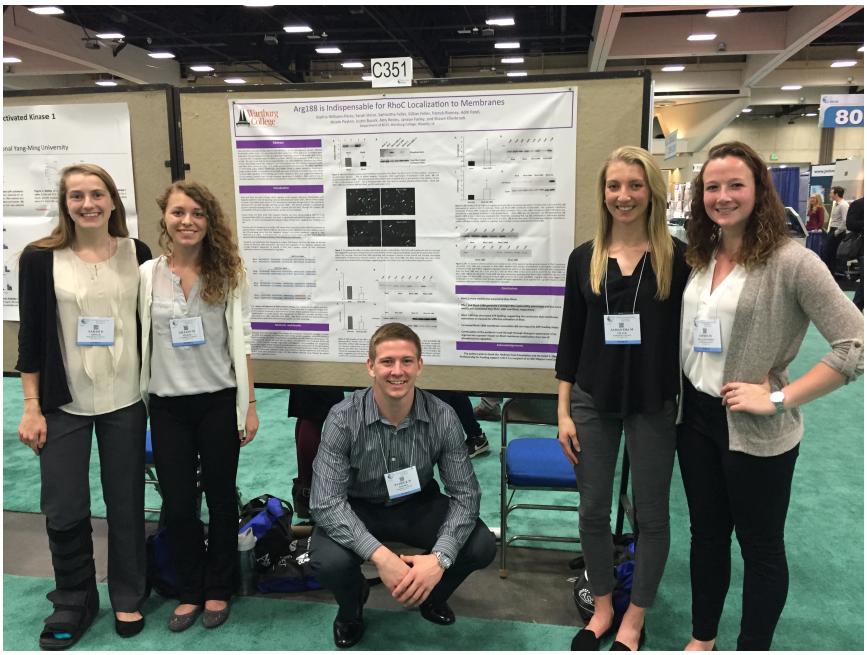
Student Research Presentations

Undergraduate biochemical research at Wartburg College has resulted in a number of student presentations at national and regional meetings. Aside from gaining presentation experience, attendance at scientific meeting offers an opportunity for biochemistry students to travel and attend talks across many scientific disciplines. Recent student presentations include:

Annual Meeting of the American Society for Biochemistry and Molecular Biology, San Diego, CA, (2016).



(LtoR) Sarah Shirar, Gillian Feller, Patrick Rooney, Sam Feller, Sophia Williams-Perez

Arg188 is Indispensable for RhoC Membrane Localization

Sophia Williams-Perez, Sarah Shirar, Gillian Feller, Samantha Feller, Patrick Rooney, Shawn Ellerbroek

RhoA and RhoC GTPases are 92% identical and possess unique cellular functions. Phosphorylation of Ser188 has widely been reported to inhibit RhoA activity. RhoC retains a canonical PKA recognition sequence (KRR) at positions 185-187, but possesses Arg188 in place of Ser188. We report here that transiently expressed RhoC was more membrane associated than RhoA. Further, RhoC-R188S was a PKA substrate *in vitro* and exhibited less GTP loading compared to wild-type RhoC when expressed in cells. RhoC-R188S was less membrane associated than RhoC regardless of GTP-binding status (Q63L). Surprisingly, RhoC-R188A shared a similar membrane fractionation profile as RhoC-R188S. In accordance, RhoA-S188R was more membrane associated than either RhoA or RhoA-S188A regardless of GTP-binding status (Q63L). Altogether, these data suggest that expansion of the polybasic carboxyl tract through divergent appearance of Arg188 contributes more to increased RhoC membrane stabilization than loss of phospho-regulation.

Annual Meeting of the American Society for Biochemistry and Molecular Biology, Washington, DC, (2013).

(LtoR) Nicole Peyton, Amy Kobliska, Alexandra Ciota, Sydney Marsh, Aditi Patel, Amy Reicks

Contribution of Residue 188 Identity to RhoA and RhoC Membrane Association

Nicole Peyton, Amy Reicks, Aditi Patel, Shawn Ellerbroek. Biochemistry and Chemistry, Wartburg College, Waverly, IA

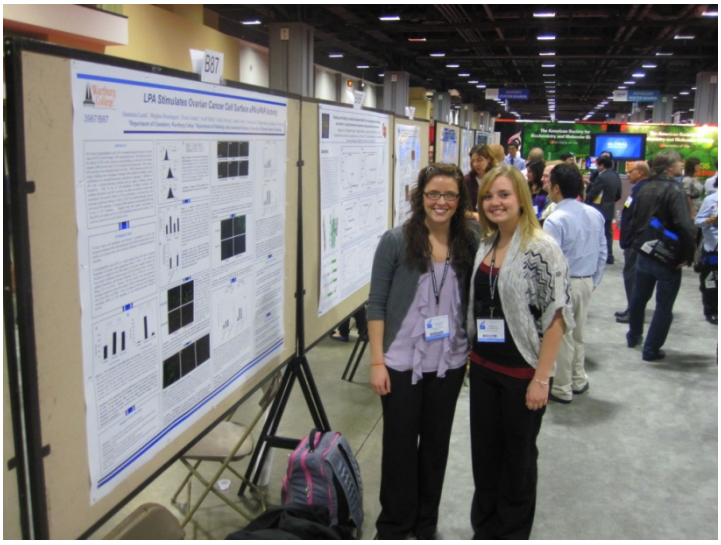
Presence of a negative charge at RhoA residue 188, such as through PKA-catalyzed serine phosphorylation, has been found to destabilize RhoA membrane association. Closely related RhoC retains an adjacent canonical PKA recognition sequence (KRR), but possesses an Arg residue in place of serine at position 188. Therefore, we propose divergence at residue 188 between these two Rho isoforms directly impacts membrane association. We found transiently expressed RhoC more membrane associated than RhoA when expressed in ovarian cancer cells. Using a residue swap approach, RhoA-S188R was found more membrane associated than wild-type RhoA, while RhoC-R188S had similar membrane association as wild-type RhoC. At the same time, RhoC-R188S was more effectively driven from membranes following forskolin treatment than RhoC. Altogether, these data suggest that a positively charged residue at position 188 strengthens RhoC membrane association and provides a mechanism for regulatory divergence.

Lysophosphatidic Acid Regulates uPAR Expression and Localization

Alexandra M Ciota, Amy Kobliska, Sydney Marsh, Shawn M Ellerbroek. Biochemistry and Chemistry, Wartburg College,Waverly, IA

Lysophosphatidic acid (LPA) stimulates in vitro invasion of ovarian epithelial cancer cells through a uPA-dependent process. We therefore analyzed the effects of LPA on the cell surface uPA receptor (uPAR). While both uPAR mRNA and protein expression was consistent with control after 2 hours of LPA stimulation, LPAstrongly stimulated uPAR redistribution into punctate aggregates or to cell junctions, depending on cell line. After 24 hours of stimulation, uPAR mRNA levels increased 4.0±2.3 fold in OVCA429 cells and were modestly impacted in OVCA 433 cells (1.8±0.5 fold). In accordance, cell surface-localized uPA activity increased 4.3±2.5 fold (OVCA 429) or 2.1±0.9 fold (OVCA 433) and was accompanied by increased uPAR immunofluorescence staining.At this same time, mRNA levels of uPA and PAI-1 were not significantly changed. These data provide mechanistic insight into the actions of a lipid that positively correlates with ovarian cancer progression.

Annual Meeting of the American Society for Biochemistry and Molecular Biology, Washington, DC, (2011).



Anastasia Lundt (Biochemistry '12) and Meghan Henningsen (Biochemistry '12)

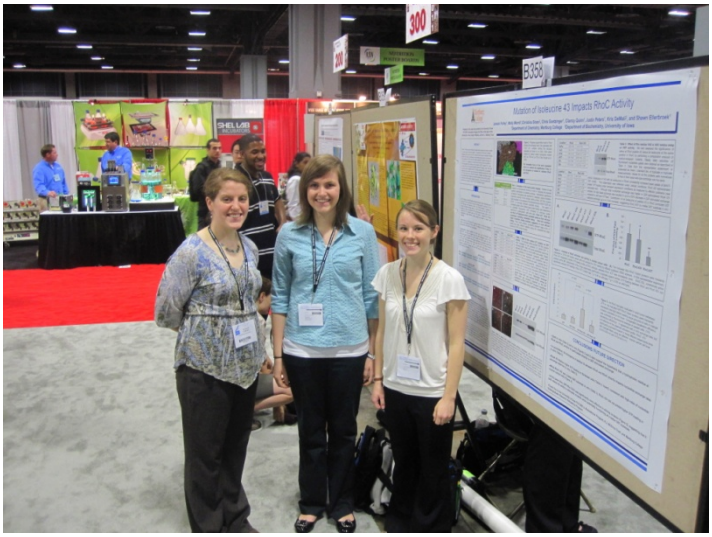
LPA Stimulates Ovarian Cancer Cell Surface uPA/uPAR Activity

Anastasia Lundt, Meghan Henningsen, Nicole Gardner, Geoff Miller, Emily Heying, Janean Farley, Yueying Liu, Sharon Stack, and Shawn Ellerbroek

[1]Department of Chemistry, Wartburg College [2]Department of Pathology and Anatomical Sciences, University of Missouri School of Medicine

We found lysophosphatidic acid (LPA) stimulated matrigel invasion of ovarian cancer (OVCA) cells through a uPA-dependent process. We therefore analyzed the effects of LPA on the expression and distribution of uPA and its cell surface receptor, uPAR, at both early (2-4 hr) and late (36 hr) time points. After 2 hours, uPA secretion was stimulated nearly two-fold. uPAR expression and uPA cell surface binding were unchanged, although uPAR underwent dramatic surface redistribution by immunofluorescence (junctional or aggregate). Inhibition of uPA with a function-blocking antibody did not impact E-cadherin junction breakdown. After 36 hrs of LPA-stimulation, E-cadherin junctions still exhibited dissolution and uPA secretion was either non-existent or minor when normalized to the positive effects of LPA on cell density. At the same time, LPA promoted significant binding of uPA to OVCA cell surfaces through a process involving PI3K signaling. In support, uPAR expression was increased following 36 hrs of LPA treatment. Together, these data support involvement uPA/uPAR cell surface activity in LPA-stimulated ovarian cancer invasion. This work was supported by the RJ McElroy Trust.

Annual Meeting of the American Society for Biochemistry and Molecular Biology, Washington, DC, (2011).



Janean Farley (Biochemistry'12), Nicole Peyton (Biochemistry'13), Molly Wernli (Biology '11)

Mutation of isoleucine 43 impacts RhoC activity

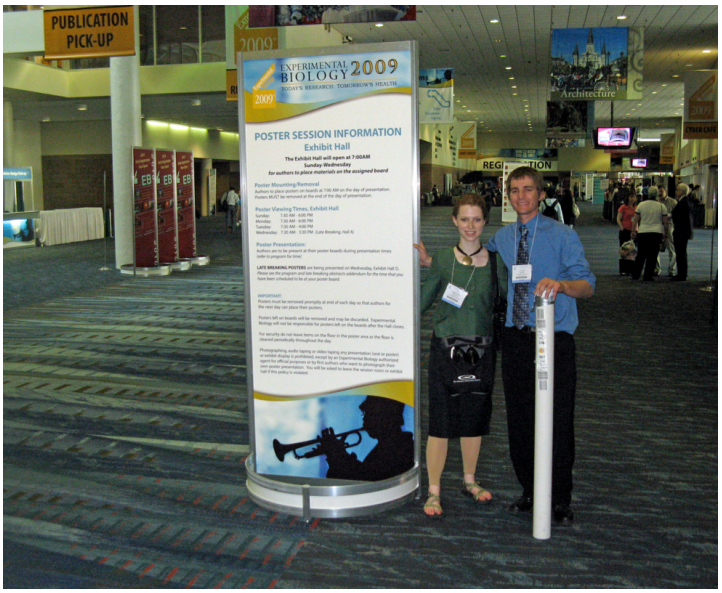
Janean Farley, Molly Wernli, Kris DeMali, and Shawn Ellerbroek

RhoC GTPase has been linked with increased cellular migration and invasion, yet little is known about its molecular regulation. We previously found residue 43 variation between RhoA/B (valine) and RhoC (isoleucine) impacts guanine nucleotide exchange factor (GEF) activity. Specifically, we used a residue swap approach and observed that substitution of RhoC ile43 with a valine increased GEF-catalyzed exchange 1.5-4.0 fold *in vitro*. Substitution of a polar threonine at residue 43 had minor effect on GEF-stimulated RhoC exchange, with the exception of GEFT (reduced 75%). Here, we report RhoC-I43T displayed significantly reduced GTP-loading in cells. RhoC-I43V, on the other hand, displayed similar GTP-loading as wild-type RhoC, yet stimulated significantly more ovarian cancer cell invasion of matrigel than wild-type RhoC, RhoC-I43T, RhoA, RhoA-V43I, or RhoA-V43T enzymes. All together, these findings support the hypothesis that a divergence at residue 43 between RhoA/B and RhoC directly contributes to their molecular regulation and activity. This work was supported with funds from the University of Iowa FUTURE in Biomedicine Program.

\

Annual Meeting of the American Society for Biochemistry and Molecular Biology, New Orleans, LA, (2009).

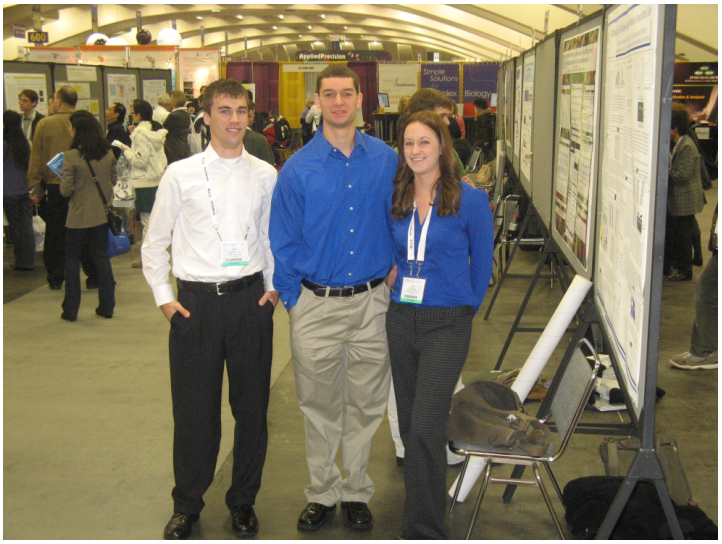
"Rho Residue 43 is a Structural Determinant of GEF Activity and Selectivity"
Chris J. Goetzinger, Christina M. Sloan, Clancy V. Quinn, and Shawn Ellerbroek



Christina Sloan ('10 biochemistry) and Clancy Quinn ('10 biochemistry)

Annual Meeting of the American Society for Cell Biology, San Francisco, CA, (2008).

"LPA Stimulation of Cell Surface-Associated Proteases in Ovarian Cancer Cells"
Kim Chaffin, Kyle Huegel, Tim McKenna, Yueying Liu, Sharon Stack, and Shawn Ellerbroek



Wartburg Students present their biochemical research at the annual meeting of the American Society of Cell Biology held in San Francisco, CA, December 2008. These students are, left to right, Tim McKenna ('09 biology), Kyle Huegel ('09 biochemistry), and Kim Chaffin ('09 biochemistry).

Annual Meeting of the American Society for Biochemistry and Molecular Biology, Washington, D.C. (2007).

"Role of Residue 43 in Activation of RhoA, RhoB, and RhoC" Justin P. Peters and Shawn M. Ellerbroek

"A Novel Monoclonal Antibody Against Phosphorylated RhoA" Julie Schweinfurth, Adi Dubash, Kim Chaffin, and Shawn M. Ellerbroek

Annual Meeting of the Animal Behavior Society, Snowbird, Utah. (2006)

"Behavioral diagnostic methods for the detection of bladder cell carcinoma"
Laura Durant, John Melville, Eric Cassmann, Cynthia Bane, Shawn Ellerbroek, and Roy M. Ventullo.

Regional Meeting of the Society for Neuroscience, Chicago , IL. (2006)

"The sensitivities of biochemical and behavioral diagnostic methods for the detection of bladder cell carcinoma." Laura Durant, Eric Cassmann, Cynthia Bane, Shawn Ellerbroek, and Johnathan Melville.