BIOGRAPHICAL SKETCH

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NAME: William Edward Miller

eRA COMMONS USER NAME (credential, e.g., agency login): millerwe

POSITION TITLE: Associate Professor of Molecular Genetics

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
The Pennsylvania State University University Park, Pennsylvania	B.S.	05/1990	Biology-Genetics
The University of North Carolina Chapel Hill, North Carolina	Ph.D.	11/1997	Virology
Duke University Medical Center Durham, North Carolina	Post-Doc	09/2002	Biochemistry/ Pharmacology

A. Personal Statement

My laboratory is interested in the mechanisms by which microbial pathogens manipulate host cell signal transduction pathways. In particular, we are using the cytomegaloviruses and *Bordetella pertussis* as model systems to examine how pathogens alter G-protein coupled receptor (GPCR) signaling pathways. GPCRs are the largest family of cell surface receptor proteins and play primary roles in physiological processes ranging from regulation of cardiovascular tone to direction of cellular migration to salivary secretion. Given the large number of physiologically relevant signaling pathways regulated by GPCRs, it is not surprising that viruses and bacteria encode proteins in their genomes that directly regulate signaling via this system. The cytomegaloviruses encode actual GPCR homologs in their genomes that signal via the Gαq class of G-proteins. We are currently examining the biochemical properties and downstream signaling activities associated with the human cytomegalovirus (HCMV) US28 and murine cytomegalovirus (MCMV) M33 GPCRs. We are using a variety of molecular and biochemical techniques to investigate the roles that these proteins play in latency, dissemination, cellular migration, etc. We are especially excited about recent progress that we have made on the development of primary human salisphere systems that we believe will have widespread applicability for the study of vGPCRs and other CMV gene products involved in replication in epithelial-based tissues. Using genetics-based approaches, we have constructed numerous viral mutants with targeted mutations in the MCMV M33 and HCMV US28 GPCR and are studying their mechanisms of action *in vivo*.

I am uniquely positioned to study the molecular and physiological properties of the cytomegalovirus GPCRs having been formally trained as both a herpesvirologist and a GPCR biochemist. I earned my graduate degree with Nancy Raab-Traub at UNC-Chapel Hill studying the Epstein-Barr virus latent membrane proteins (LMP1 and LMP2) and completed post-doctoral training with Robert Lefkowitz studying the regulation of GPCR signaling pathways. I have published extensively in both areas and have been selected to present our work at national meetings including the Herpesvirus and Cytomegalovirus Workshops. Since I started my laboratory in 2002 we have devoted the majority of our time integrating virological techniques with molecular/cellular techniques to study the cytomegalovirus GPCRs. We have generated important reagents, garnered a better understanding of

how these proteins function in vitro and are poised to continue to make fundamental discoveries regarding the *in vivo* properties of these interesting and important proteins.

B. Positions and Honors

Positions and Employment

1991-1998 Graduate Student, Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC (Nancy Raab-Traub, Thesis Advisor)
1998-2002 Post-doctoral Fellow, Howard Hughes Medical Institute, Duke University Medical Center, Durham, NC (Robert J. Lefkowitz, Post-Doctoral Advisor)
2002-2009 Assistant Professor, Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati College of Medicine, Cincinnati, OH
2009-Present Associate Professor, Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati College of Medicine, Cincinnati, OH

Other Experience and Professional Memberships

2001-Present Member, American Society for Biochemistry and Molecular Biology
2005 NIH Study Section: NIH IDM-G90 (S) Virology Special Emphasis Panel
2007-Present Member, American Society for Pharmacology and Experimental Therapeutics
2007-Present Member, American Society for Microbiology
2008 NSF Study Section: Molecular Signaling (Ad Hoc)
2008-2011 AHA Study Section: Molecular Signaling I
2014-Present Editorial Board Member, Journal of Biological Chemistry
2015-Present NIH Study Section: NIH IMM-R12 (B) Study Section
2015 NIH Study Section: NIH CF10 Study Section
2016 NIH Study Section: NIH IMM-R90 (B) Study Section

Honors and Awards

1988-1990 C.D. Prutzman Undergraduate Scholarship, The Pennsylvania State University
1997 Lineberger award for excellence in research, The University of North Carolina at Chapel Hill
2004 Basil O'Connor Starter Scholar, March of Dimes
2016 Fellows of the Graduate School Inductee, The University of Cincinnati

C. Contribution to Science

1. As a graduate student in Nancy Raab-Traub's laboratory at the University of North Carolina, my work examined the role that the Epstein-Barr Virus oncogene LMP1 played in regulating signal transduction in epithelial cells. Understanding LMP1 signaling in epithelial cells is crucial to understand how the virus contributes to the genesis of nasopharyngeal carcinoma. This work led to the discovery that LMP1 directly caused an increase in epidermal growth factor receptor (EGFR) expression thus driving proliferation of the epithelial cells. We also investigated this phenomenon from a mechanistic standpoint and demonstrated that the upregulation of EGFR expression involved the domain within LMP1 that would eventually be termed CTAR1. In collaboration with George Mosialos and Elliott Kieff we were able to show that this EGFR upregulation involved the engagement of LMP1 with the newly discovered TRAF proteins. Our work, demonstrated that LMP1 expression in epithelial cells resulted in signaling through TRAF proteins, upregulation of EGFR expression and increased cellular proliferation and viability. Thus, our studies provided definitive evidence that LMP1 oncogenic activity and signaling through TRAFs was effective in not only lymphocytes, but also in epithelial cells. Representative publications include:
    a. **W.E. Miller**, H.S. Earp, and N. Raab-Traub. The Epstein-Barr Virus Latent Membrane Protein 1 (LMP1) Induces Expression of the Epidermal Growth Factor Receptor. 1995 *Journal of Virology*, **69**:4390-4398. PMCID: PMC189180

b. **W.E. Miller**, G. Mosialos, E. Kieff, and N. Raab-Traub. Epstein-Barr Virus LMP1 Induction of the Epidermal Growth Factor Receptor is Mediated Through a TRAF Signaling Pathway Distinct From NF-κB Activation. 1997 *Journal of Virology,* **71**:586-594. PMCID: PMC191088
c. **W.E. Miller**, J.L. Cheshire, Baldwin Jr., A.S. and N. Raab-Traub. The NPC derived C15 LMP1 protein confers enhanced activation of NF-κB and induction of the EGFR in epithelial cells. 1998 *Oncogene*, **16**:1869-1877. PMID: 9583684 DOI: 10.1038/sj.onc.1201696
d. **W.E. Miller**, J.L. Cheshire and N. Raab-Traub. Interaction of the Tumor necrosis factor receptor signaling proteins with the latent membrane protein 1 PXQXT motif is essential for induction of epidermal growth factor receptor expression. 1998 *Molecular and Cellular Biology*, **18**:2835-2844. PMCID: PMC110662

2. Based on my interests in plasma membrane proteins and their impact on signal transduction, I pursued post-doctoral work in the laboratory of Robert Lefkowitz at Duke University. In this work I was part of a team that was exploring the novel concept that G-protein coupled receptors signal via the adapter protein βarrestin in addition to signaling via traditional G-proteins. This paradigm shifting work is now central to much of the ongoing work in the GPCR field and has led to emerging concepts such as biased agonism. My work, in particular, made use of yeast-two hybrid screening to identify βarrestin interacting proteins and decipher how these interactions regulate GPCR signaling pathways. Our studies identified numerous βarrestin interacting proteins that continue to be the focus of several laboratories working in the field of GPCR signaling and βarrestins. Representative publications include:
    a. **W.E. Miller**, S. Maudsley, S. Ahn, K. Khan, L. Luttrell, and R. Lefkowitz. Beta-arrestin1 interacts with the catalytic domain of the tyrosine kinase c-Src. 2000 *Journal of Biological Chemistry,* **275**:11312-11319. PMID: 10753943
    b. **W.E. Miller**, P.H. McDonald, S.F. Cai, M.E. Field, R.J. Davis, and R. Lefkowitz. Identification of a motif in the carboxy terminus of β-arrestin2 responsible for activation of JNK3. 2001 *Journal of Biological Chemistry,* **276**:27770-27777. PMID: 11356842 DOI: 10.1074/jbc.M102264200
    c. W. Chen, L. Hu, M. Semenov, S. Yanagawa, A. Kikuchi, R. Lefkowitz, and **W.E. Miller**. Beta-arrestin1 modulates LEF transcriptional activity through interaction with phosphorylated Dishevelled proteins. 2001 *Proc. Natl. Acad. Sci,* **98**:14889-14894. PMID: 11742073 PMCID: PMC64954
    d. **W.E. Miller***, D.A. Houtz, C.D. Nelson, P.E. Kolattakudy, and R.J. Lefkowitz. GRK phosphorylation and beta-arrestin binding regulate the constitutive signaling activity of the human cytomegalovirus US28 GPCR. 2003 *Journal of Biological Chemistry,* **278**:21663-21671. ***Corresponding author. PMID: 12668664 DOI: 10.1074/jbc.M303219200

3. When I started my own laboratory I chose to meld the expertise that I had acquired as a graduate student with that acquired as a post-doc and began investigating the GPCRs encoded by the human cytomegalovirus. We have focused on examining the signaling of receptors like US28 in the context of infection and have made real strides in our understanding of how these proteins work in clinically relevant cells advancing the field from earlier studies done primarily in transfected cells. Representative publications include:
    a. M.P. Stropes, O.D. Schneider, W.A. Zagorski, J.L.C. Miller and **W.E. Miller.** The carboxy-terminal tail of human cytomegalovirus (HCMV) US28 regulates both chemokine-independent and chemokine-dependent signaling in HCMV-infected cells. *2009 Journal of Virology*, **83**:10016-10027. PMID: 19605482 PMCID: PMC2748033
    b. **W.E. Miller***, W.A. Zagorski, J.D. Brenneman, D. Avery, J.L. Miller, and C.M. O'Connor. US28 is a potent activator of phospholipase C during HCMV infection of clinically relevant target cells. *2012 PLoS ONE **7(11)**: e50524.*
    ***Corresponding author. PMID: 23209769 PMCID: PMC3510093
    c. S. Wu and **W.E. Miller**. The Human Cytomegalovirus Lytic Cycle Is Induced by Vitamin D in Peripheral Blood Monocytes and in the THP-1 Monocytic Cell Line. *2015 Virology,***483**:83-95. PMID: 25965798 PMCID: PMC4516672
    d. H. Low, H.L. Cui, N. Mukhamedova, B.P. McSharry, S. Avdic, Y. Lui, Y. Fu, P. Meikle, M. Blomberg, K. Polyzos, **W.E. Miller**, P. Religa, M. Bukrinsky, C. Soderberg-Naucler, B.

Slobedman, D. Sviridov. Cytomegalovirus Restructures Lipid Rafts via a US28/CDC42 Mediated Pathway Enhancing Cholesterol Efflux from Host Cells. *2016 Cell Reports,* **16**:186-200. PMID: 27320924 PMCID: PMC5389417

e. S. Wu and **W.E. Miller.** The HCMV US28 vGPCR induces potent Gαq/PLC-β signaling in monocytes leading to increased adhesion to endothelial cells. *2016 Virology,* **497**:233-243. PMID: 27497185 PMCID: PMC5026607

4. In addition to our work on the human cytomegalovirus GPCRs, I felt that it would be necessary to incorporate studies with the murine cytomegalovirus GPCRs in order to begin to probe how these proteins affected pathogenesis in animal models. The cytomegalovirus GPCRs are dispensable for replication in tissue culture systems and therefore the inclusion of animal models in our experimental approach would be key to understanding the function of these proteins. Our work has used recombinant viruses to demonstrate that the MCMV M33 GPCR signals via G-proteins to facilitate cytomegalovirus growth within the salivary gland itself. Representative publications include:
    a. J.D. Sherrill and **W.E. Miller**. G protein-coupled receptor (GPCR) kinase 2 regulates constitutive Gq/11 signaling from the mouse cytomegalovirus GPCR M33. 2006 *Journal of Biological Chemistry,* **52**:39796-39805. PMID: 17088245 PMCID: PMC2767100
    b. J.D. Sherrill, M.P. Stropes, O.D. Schneider, D.A. Koch, F.M. Bittencourt, J.L.C. Miller, and **W.E. Miller.** Activation of intracellular signaling pathways by the MCMV GPCR M33 occurs via PLC-β/PKC dependent and independent mechanisms. *2009 Journal of Virology,* **83**:8141-8152. PMID: 19494016 PMCID: PMC2715766
    c. C.M. O'Connor and **W.E. Miller.** Methods to study the function of cytomegalovirus GPCRs. *2014 Methods in Molecular Biology: Human Cytomegalovirus Methods and Protocols,***119**:133-164. PMID: 24639223 DOI: 10.1007/978-1-62703-788-4_10
    d. F.M. Bittencourt, S. Wu, J. Bridges and **W.E. Miller**. The M33 GPCR encoded by MCMV is dispensable for hematogenous dissemination but is required for growth within the salivary gland. *2014 Journal of Virology,* **88**:11811-1182. PMID: 25100846 PMCID: PMC4178735

5. Aside from our studies on cytomegalovirus GPCRs we have developed an interest in the mechanisms used by *Bordetella pertussis* toxin (PTx) to regulate G-protein signaling. While the catalytic ADP ribosylation activity of PTx A-subunit had long been known to inhibit signaling via the Gαi proteins, my laboratory has demonstrated that PTx uses a novel mechanism to regulate GPCR signaling pathways. In particular, we discovered that PTx B-subunit binding to the T cell receptor promotes cross-desenstization of chemokine receptors. These studies have shed new light on mechanisms used by *Bordetella pertusis* to interfere with immune cell function by creating an unfavorable environment for migration of immune cells to the site of chemotaxis. Representative publications include:
    a. O.D. Schneider, A. Weiss, and **W.E. Miller**. Pertussis Toxin utilizes proximal components of the T-cell receptor complex to initiate signal transduction events in T-cells. *2007 Infection and Immunity,* **75**:4040-4049. PMID: 17562776 PMCID: PMC1951969
    b. O.D. Schneider, A. Weiss, and **W.E. Miller**. Pertussis Toxin signaling through the T-cell receptor initiates cross-desensitization of the chemokine receptor CXCR4. *2009 Journal of Immunology,* **182**:5730-5739. PMID: 19380820 PMCID: PMC2766007
    c. O.D. Schneider, S.H. Millen, A. Weiss, and **W.E. Miller**. Mechanistic insight into Pertussis Toxin and lectin signaling using T-cells engineered to express a CD8α/CD3ζ chimeric receptor *2012 Biochemistry,***51**:4126-4137. PMID: 22551306 PMCID: PMC3359064
    d. Millen SH, Schneider OD, **Miller WE**, Monaco JJ, Weiss AA. Pertussis toxin B-pentamer mediates intercellular transfer of membrane proteins and lipids. *2013 PLoS ONE* **8(9)** e72885. PMID: 24019885 PMCID: PMC3760862

Complete List of Published Work in MyBibliography (58 publications total):
http://www.ncbi.nlm.nih.gov/sites/myncbi/127cfSc9R-lAN/bibliography/47312807/public/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

1. NIH R56 AIAI121028-01. 8/20/16-7/31/17.
   Mechanisms of vGPCR mediated Cytomegalovirus Growth in the Salivary Gland
   William E. Miller, Ph.D. (Principal Investigator)
   -The major goals are to define the mechanisms by which CMV vGPCRs signal through Gaq/Ga11 proteins leading to altered salivary gland physiology ultimately affecting CMV growth and spread.
   -The current proposal is a revision of the earlier R01 application that was given 1 year of R56 NIH Bridge Funding.

2. NIH R21 AI119415. 5/1/15-4/30/18 (no-cost extension).
   The Role of US28 during HCMV Latency
   Christine M. O'Connor, Ph.D. (Principal Investigator)
   William E. Miller, Ph.D. (Co-Investigator)
   -This grants aims to examine the hypothesis that US28 vGPCR signaling alters hematopoietic progenitor cell differentiation thus regulating the development and maintenance of HCMV latency.
   -The funds from this grant are being evenly split by the O'Connor and Miller laboratories to complete the proposed aims.

3. NIH R01 NHLBI. 4/1/16-3/31/21.
   Role of GPR116 in Alveolar Homeostasis
   James P. Bridges, Ph.D. (Principal Investigator)
   William E. Miller, Ph.D. (Co-Investigator)
   -This grant aims to explore the signaling pathways and mechanisms underlying the function of the GPR116 GPCR in regulating Lung physiology.

Recently Completed Research Support

1. NIH R56 AI095442. 8/1/12-7/31/15 (no-cost extension).
   Role of Cytomegalovirus GPCRs in Pathogenesis in Vivo
   William E. Miller (Principal Investigator)
   -The grant investigated the role of the MCMV M33 GPCR in promoting viral replication in vivo and determined that M33 signaling activity is essential for viral replication within the salivary epithelium itself. The outcomes of these studies are relevant for viral persistence and horizontal viral transmission.