BIOGRAPHICAL SKETCH

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NAME: David M. Miller

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

POSITION TITLE: Professor

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
University of Southern Mississippi (Hattiesburg MS)	B.S.	1973	Biology
Rice University (Houston TX)	Ph.D.	1981	Biochemistry
Baylor College of Medicine (Houston TX)	Postdoc	1983	Muscle assembly Mentor: *H.F. Epstein, MD*
MRC-Laboratory of Molecular Biology (Cambridge UK)	Postdoc	1984	Myogenesis Mentor: *S. Brenner, MD*

A. PERSONAL STATEMENT

The Miller lab uses the model organism *C. elegans* to investigate neural development and function. Research topics include mechanisms of synaptic specificity, neuronal remodeling and dendrite morphogenesis. The goal of this work is to identify molecular pathways that control these events and to establish the cell biological mechanism of each process. The PI has acquired extensive experience with key approaches used in these studies including *C. elegans* genetics, high-resolution light microscopy, genomic analysis and protein biochemistry. Of particular significance is our leadership in the development of cell-specific expression profiling methods for *C. elegans* **(Spencer et al. 2011, Spencer et al., 2014, Taylor et al., 2019)**, and their use for the identification of transcription factor targets that regulate synaptic specificity **(Von Stetina et al., 2007),** circuit remodeling (**Petersen et al., 2011)** and dendrite morphogenesis (**Smith et al., 2013)**. The PI is actively involved in graduate education in the classroom as well as serving on 85 PhD and MS committees and mentoring a total of 19 graduate students and 11 postdoctoral fellows in the Miller lab. Graduate students from the Miller lab are now either in academic positions (e.g., Jennifer Wolf, Assoc. Prof., Carlton College; Sarah Petersen, Assist. Prof., Kenyon College; Laurie Earls, Assist. Prof., Tulane University; Cody Smith, Assist. Prof., University of Notre Dame; Mallory Hacker, Assist. Prof., Vanderbilt University) or have joined biotech or consulting firms (e.g., Kim Lickteig, Takeda Pharmaceuticals; Rebecca Fox, Phosphorous; Rachel Skelton, Leica Biosystems; Siwei He, Boston Consulting Group). Former Miller lab postdocs are pursuing a variety of careers (e.g., Maureen McDonnell, JD, Regulatory Affairs, Beckman Coulter; Judsen Schneider, Nashville Biosciences; Joseph Watson, Rho). The PI has maintained an ongoing role in the Fisk-Vanderbilt Masters-PhD Bridge program by serving on the committees of eleven Fisk Students. Five of these students, Erica Tross, Corey Roach, Kai Bracey, Jennifer Quinde and Destane Garrett have been accepted into PhD programs at Vanderbilt. In addition, the PI has hosted student interns for the Vanderbilt Summer Research Program (Amanda Mitchell, 2017) and MSTP Summer Research Program (Isaiah Swan) and is currently mentoring a Vanderbilt Academic Pathways postdoctoral fellow (Jamie Stern).

WC Spencer*, G Zeller*, JD Watson, SR Henz, **KL Watkins**, **RD McWhirter**, **SC Petersen**, VT Sreedharan, C Widmer, J Jo, V Reinke, L Petrella, S Strome, **S Von Stetina**, M Katz, S Shaham, G Raetsch, **DM Miller, III** (2011). A spatial and temporal map of *C. elegans* gene expression. *Genome Research 21: 325-341.* *Equal contributions. PMID: 21177967.

WC Spencer, R McWhirter, T Miller, P Strasbourger, O Thompson, LW Hillier, RH Waterston, **DM Miller, III** (2014) Isolation of specific neurons from *C. elegans* larvae for gene expression profiling. PLOS ONE 9, e112102. PMCID: PMC4221280.

SC Petersen, JD Watson, JE Richmond, M Sarov, WW Walthall, **DM Miller, III** (2011). A transcriptional program promotes remodeling of GABAergic synapses in *Caenorhabditis elegans*. *J. Neuroscience 31, 15362–15375*. PMCID: PMC3229156.

***CJ Smith, *T O'Brien,** M Chatzigeorgiou, **WC Spencer**, **E Feingold-Link**, SJ Husson, S Hori, S Mitani, A Gottschalk, WR Schafer, **DM Miller, III** (2013). Sensory neuron fates are distinguished by a transcriptional switch that regulates dendrite branch stabilization. *Neuron* 79, 266-280. PMID: 23889932. *These authors contributed equally.

B. POSITIONS AND HONORS

Positions and Employment

1978	Instructor, Department of Biochemistry, Rice University, Houston, TX
1980-1983	Postdoctoral Fellow, Dept of Neurology, Baylor College of Med., Houston, TX (HF Epstein)
1983-84 & S85	Visiting Scientist, MRC Laboratory of Molecular Biology, Cambridge, UK (S. Brenner)
1984-1990	Assistant Professor of Zoology and Genetics, Department of Zoology, North Carolina State University, Raleigh, NC
1990-1994	Assistant Research Professor, Department of Cell Biology, Duke University, Durham, NC
1994-2005	Associate Professor, Department of Cell Biology and Developmental Biology, Vanderbilt University, Nashville, TN
2005-present	Professor, Department of Cell Biology and Developmental Biology, Vanderbilt University, Nashville, TN

Other Experience and Professional Memberships

1983 – 2002	American Society for Cell Biology
1994-	Society for Developmental Biology
1998-	American Association for the Advancement of Science
1999-	Society for Neuroscience
2003-	Genetics Society of America
2005-	Editorial Board: *genesis: the Journal of Genetics and Development*
2004-2005	Ad Hoc reviewer for NIH NIF-7 Study Section
2007	Ad Hoc reviewer for MDCN-K Study Section
2011-2013	NIH Special Emphasis Panel (SEP) reviewer
2015-2019	Member, NIH NST-2 Study Section
2020	Ad Hoc reviewer for SYN Study Section

Honors

1973	Phi Kappa Phi: Outstanding Student in Biochemistry, University of Southern Mississippi
1973-1977	Robert Welch Foundation Fellow, Rice University
1980-1982	Muscular Dystrophy Association Postdoctoral Fellow
1983,1985	Burroughs Wellcome Fund Travel Grant
1984	EMBO Long Term Fellowship
1985	Burroughs Wellcome Fund Travel Grant
2012	Elaine Sanders-Bush Award for Excellence in Teaching, Vanderbilt University
2013	AAAS Fellow
2015	Outstanding Mentor of the Year 2015, Vanderbilt Neuroscience Graduate Program.

C. Contributions to Science

1. Methods for generating transcriptional profiles of specific *C. elegans* cells. The Miller laboratory contributed to the first published description of a primary culture system for *C. elegans* embryonic cells (Christensen et al., 2002). This method has been widely utilized for a range of applications including cell-specific expression profiling, electrophysiology and biochemical analysis (>230 citations). Beginning with this work, we have sustained an ongoing effort to develop innovative approaches to cell-specific profiling and bioinformatic analysis. A series of papers published from the Miller lab demonstrated the utility of FACS for isolating embryonic cells for expression profiling and the application of the mRNA tagging method for cataloging expression in specific larval cells (**Von Stetina et al., 2007, Smith et al., 2010, Smith et al, 2013**). Our paper **(Spencer et al., 2012)** demonstrates the value of this strategy for gene discovery and for prediction of gene regulatory

mechanisms. The Miller lab generated a large database of cell-specific expression profiles that were critically important for the modENCODE effort to map all *C. elegans* transcripts and for comprehensive descriptions of gene expression mechanisms (Gerstein et al., 2014). In a recent paper, we described the first successful use of FACS to isolate larval *C. elegans* neurons for RNA-Seq analysis (SeqCel) (Spencer et al., 2014**).** Culminating in this work, our decade-long effort has validated powerful techniques that can now be used to profile essentially any specific *C. elegans* cell throughout development. This work significantly advanced the prospect of a gene expression map to match the unrivaled single cell resolution of the *C. elegans* body plan. On the basis of these results, NINDS funded a multi-investigator project (see below) involving the Miller lab to achieve this goal by profiling each of the 118 different neurons classes in the adult *C. elegans* nervous system (Hammarlund et al., 2018**).** As a first step toward this objective, the Miller lab has used single cell RNA-Seq to produce gene expression profiles of all known classes of *C. elegans* neurons (Taylor, et al., 2019). In addition, we have made extensive use of this technology to identify the targets of transcriptional pathways that regulate key developmental processes including synaptic specificity (Von Stetina et al., 2007), dendrite morphogenesis (Smith et al., 2013), synaptic remodeling (Petersen et al., 2011), axon regeneration (Byrne et al., 2016), neuronal fate (Lim et al., 2016) and behavior (Oranth et al., 2018; Konietzka et al., 2019). **Miller lab members shown in bold.**

WC Spencer*, G Zeller*, **JD Watson**, SR Henz, **KL Watkins**, **RD McWhirter**, **SC Petersen**, VT Sreedharan, C Widmer, J Jo, V Reinke, L Petrella, S Strome, **S Von Stetina**, M Katz, S Shaham, G Raetsch, **DM Miller, III** (2011). A spatial and temporal map of *C. elegans* gene expression. *Genome Research 21: 325-341.* *Equal contributions. PMID: 21177967.

WC Spencer, R McWhirter, T Miller, P Strasbourger, O Thompson, LW Hillier, RH Waterston, **DM Miller, III** (2014) Isolation of specific neurons from *C. elegans* larvae for gene expression profiling. PLOS ONE 9, e112102. PMCID: PMC4221280.

Hammarlund, M., Hobert, O., **DM Miller, III,** and Sestan, N. (2018). The CeNGEN Project: The Complete Gene Expression Map of an Entire Nervous System. Neuron *99*, 430–433.

Seth R Taylor, Gabriel Santpere, Molly Reilly, Lori Glenwinkel, **Abigail Poff, Rebecca McWhirter**, Chuan Xu, Alexis Weinreb, Manasa Basavaraju, Steven J Cook, Alec Barrett, Alexander Abrams, Berta Vidal, Cyril Cros, Ibnul Rafi, Nenad Sestan, Marc Hammarlund, Oliver Hobert, **David M. Miller, III** (2019) Expression profiling of the mature *C. elegans* nervous system by single-cell RNA-Sequencing. *bioRxiv, August 17, 2019.* doi:https://doi.org/10.1101/737577.

2. <u>Molecular genetic mechanisms that specify synaptic choice</u>. Work in the Miller laboratory has advanced understanding of the genetic mechanisms that regulate wiring specificity in the nervous system. A landmark paper **(Miller et al, 1992)** describes the first report of a transcriptionally-regulated pathway for defining synaptic choice. This work is significant because it demonstrated the explicit role of a genetic program involving the homeodomain transcription factor, UNC-4, in the creation of connections between specific neuron partners. Notably, UNC-4 controls the specificity of both chemical (neurotransmitter) and electrical (gap junctions). **Winnier et al. (1999)** reported the first example of a necessary role of the conserved transcriptional co-repressor protein, Groucho, in motor circuit development and presaged the discovery of a similar function in the vertebrate spinal cord. **Von Stetina et al. (2007)** demonstrated the utility of cell-specific profiling methods for identifying UNC-4-regulated genes and suggested that the homolog of one of these components (CEH-12), the homeodomain transcription factor, HB9, exerts a parallel role in vertebrate motor circuit differentiation. A subsequent paper extended this work to show that *unc-4* antagonizes a canonical Wnt signaling pathway to specify the wild-type pattern of connectivity **(Schneider et al., 2012)**. We have recently determined the molecular basis of this effect by using SeqCel to show that UNC-4 drives expression of a conserved member of protein family of canonical inhibitors of Wnt signaling, the Secreted Frizzled Receptor Protein, SFRP-1. Additional UNC-4 targets revealed by this approach, a GPCR and phosphodiesterase, function in a parallel acting G-protein-dependent pathway (unpublished data). Thus, we are now poised to define, for the first time, the cell biological pathways that drive gap junction specificity in the nervous system.

DM Miller, III, MM Shen, CE Shamu, TR Bürglin, G Ruvkun, **ML Dubois, M Ghee**, **L Wilson**. (1992) *C. elegans unc-4* gene encodes a homeodomain protein that determines the pattern of synaptic input to specific motor neurons. *Nature 355, 841-845.* (142 citations)

***AR Winnier** , ***JY-J Meir**, **JM Ross**, T Ishihara, I Katsura, N Tavernarakis, M Driscoll, M, **DM Miller, III.** (1999) UNC-4/UNC-37-dependent repression of motor neuron-specific genes controls synaptic choice in *Caenorhabditis elegans*. *Genes and Dev 13, 2774-2786*. *Equal contributions.

***SE Von Stetina**, ***RM Fox**, **KL Watkins**, TA Starich, JE Shamu, **DM Miller III** (2007) UNC-4 represses CEH-12/HB9 to specify synaptic inputs to VA motor neurons in *C. elegans.* *Genes Dev* **21**: 332-346. *Equal contributions. PMCID: PMC1785118.

***JD Schneider, *RL Skelton, *SE Von Stetina,** A van Oudenaarden, T Middelkoop, H. Korswagen**, DM Miller, III** (2012). UNC-4 antagonizes Wnt signaling to regulate synaptic choice in the *C. elegans* motor circuit. *Development 139, 2234-2245*. PMCID: PM3357913. *These authors contributed equally.

3. Mechanisms of Synaptic Remodeling: Work from the Miller laboratory offers an unprecedented opportunity to delineate molecular pathways that regulate synaptic plasticity. Neural circuits are actively remodeled during development and in response to injury or disease but the mechanisms that drive these changes are poorly understood. To address this question, we used a novel genetic screen to identify at least 19 proteins with conserved vertebrate homologs that direct the remodeling of GABAergic synapses in *C. elegans* (**Petersen et al., 2011**). Thus, this work holds the promise of exploiting the ready accessibility of a synaptic plasticity program in *C. elegans* to define a pathway that could also drive circuit remodeling in the human brain. Indeed, ongoing work in the Miller lab has shown that one of these proteins, the DEG/ENaC cation channel UNC-8 (**Wang et al, 2013**) is required for presynaptic remodeling in a pathway that depends on neuronal activity. This finding is significant because members of the DEG/ENaC family are known to mediate learning and memory in mammals but the mechanism of this effect is unknown (**Miller-Fleming et al, 2016**). In recent work, we identified an additional effector of synaptic remodeling, an Immunoglobulin domain (Ig) protein OIG-1 that normally prevents the transposition of postsynaptic components to new locations in the GABA circuit. In this case, we show that OIG-1 is down-regulated by a transcriptional cascade to unleash the synaptic remodeling program. This work is important because it has revealed a key effector of a genetic pathway that orchestrates the overall synaptic remodeling program (**He et al., 2015**).

SC Petersen, JD Watson, JE Richmond, M Sarov, WW Walthall, **DM Miller, III** (2011). A transcriptional program promotes remodeling of GABAergic synapses in *Caenorhabditis elegans*. *J. Neuroscience 31, 15362–15375*. PMID: 22031882.

Wang, L Han, C Matthewman, **T Miller**, **DM Miller, III,** L Bianchi (2013) Neurotoxic *unc-8* mutants encode constitutively active DEG/ENaC channels that are blocked by divalent cations. *J. Gen. Phys.* 142, 157-169. PMCID: PMC3727304.

***Siwei He**, *Allison Philbrook, **Rebecca McWhirter**, Christopher V. Gabel, Daniel G. Taub, **Maximillian H. Carter**, **Isabella M. Hanna**, Michael M. Francis, **David M. Miller, III** (2015) Transcriptional control of synaptic remodeling through regulated expression of an immunoglobulin superfamily protein. *Current Biology 25, 2541–2548*. *These authors contributed equally. PMCID: PMC4596794.

***Tyne W. Miller-Fleming, *Sarah C. Petersen**, Laura Manning, Cristina Matthewman, **Megan Gornet, Allison Beers,** Sayaki Hori, Shohei Mitani, Laura Bianchi, Janet Richmond, **David M. Miller, III**. (2016) The DEG/ENaC channel protein UNC-8 drives activity-dependent synapse removal in remodeling GABAergic neurons. *eLIFE 5:e14599*. *These authors contributed equally. PMID: 27403890.

4. Nociceptor morphogenesis and dendrite self-avoidance: Publications from the Miller lab led the field in the use of the *C. elegans* PVD neuron for studies of nociceptor development and function. **CJ Smith et al., (2010)** provided the first comprehensive description of the morphogenesis and gene expression signature of the PVD sensory neuron. This work has contributed significantly to the rapid emergence of the PVD neuron as a useful model for investigations of dendrite morphogenesis and sensory neuron function. A second paper (**Smith et al., 2012**) is important because it provides a new, and unexpected model of dendrite self-avoidance, a widely observed but poorly understood phenomenon. This work showed that a soluble cue, the axon guidance protein, UNC-6/Netrin, mediates self-avoidance in a novel capture and display mechanism involving the canonical axon guidance receptors UNC-40/DCC and UNC-5. We have recently shown that this UNC-6/Netrin-mediated self-avoidance depends on a downstream pathway that stimulates actin assembly and depends on myosin motor activity (**Sundararajan et al., 2019**).The strong conservation of these components argues that this mechanism could be employed for self-avoidance in mammals. Thus, our discovery opens the door for the use of *C. elegans* as a model for rapidly advancing our understanding of the basic cell biology of dendrite self-avoidance. An additional paper (**Smith et al., 2013**) is significant because it describes an elegant transcriptional mechanism that distinguishes the developmental fates of two different classes of mechanosensory neurons. This work uncovered parallel roles for a transcription factor (aryl hydrocarbon receptor/spineless) in the specification of dendrite morphology in nematodes and insects and therefore argues that this transcription factor is similarly employed in mammals. In addition, this study exploits pioneering cell-specific profiling methods from the Miller lab to identify downstream effectors including a member of a conserved class of cell adhesion proteins. This finding is particularly notable because recent work has shown that a surprisingly large number of transcription factors are involved in sensory neuron morphogenesis but few downstream targets are known.

***CJ Smith,** ***JD Watson**, **WC Spencer**, **T O'Brien**, B Cha, A Albeg, M Treinin, **DM Miller, III** (2010) Time-lapse imaging

and cell-specific expression profiling reveal dynamic branching and molecular determinants of a multi-dendritic nociceptor in *C. elegans*. *Developmental Biol. 345, 18-33.* PMID: 20537990. *Equal contributions. Cover Art.

CJ Smith, JD Watson, MK Van Hoven, DA Colon-Ramos, **DM Miller, III**. (2012) Netrin (UNC-6) mediates dendritic self-avoidance. *Nature Neuroscience* 15, 731-737. PMID: 22426253. Recommended by Faculty of 1000.

Lakshmi Sundararajan, **Cody J. Smith**, Joseph D. Watson, Bryan A. Millis, Matthew J. Tyska, **David. M. Miller, III** (2019) Actin assembly and non-muscle myosin activity drive dendritic retraction in an UNC-6/Netrin-dependent self-avoidance response*. PLoS Genetics 15, e1008228, PMID:31220078.*

***CJ Smith, *T O'Brien,** M Chatzigeorgiou, **WC Spencer, E Feingold-Link**, SJ Husson, S Hori, S Mitani, A Gottschalk, WR Schafer, **DM Miller, III** (2013). Sensory neuron fates are distinguished by a transcriptional switch that regulates dendrite branch stabilization. *Neuron* 79**,** 266-280. PMID: 23889932. *These authors contributed equally.

Complete List of Published Work in My PubMed Bibliography:
http://www.ncbi.nlm.nih.gov/pubmed/?term=(Miller+DM+AND+gerstein+AND+agarwal+AND+waterston)+OR+(Miller+DM+3rd)+OR+(Miller+AND+unc-4)+OR+(Miller+DM+AND+vanderbilt+AND+elegans)+OR+(Miller-Fleming+UNC-8)+OR+(Oranth+AND+McWhirter+AND+elegans)

D. RESEARCH SUPPORT

ACTIVE

R01 NS100547 Hammarlund, Hobert, Sestan, Miller (Co-PI) 09/25/2017 – 07/31/2022
Discovery and analysis of the C. elegans neuronal gene expression network (CeNGEN).
A multi-investigator project to produce a gene expression profile of each of the 118 classes of neurons in the mature *C. elegans* nervous system.

R01 NS106951 Miller (PI) 02/01/2018 – 01/31/2023
Molecular genetics of synaptic plasticity
Investigates a novel activity-dependent endocytic pathway that drives the removal of the presynaptic apparatus in remodeling GABAergic neurons.

R01 NS113559 Miller (PI) 05/15/2020 – 04/30/2025
Molecular mechanisms for neuron-specific assembly of electrical synapses
cAMP signaling and gap junction trafficking in the placement and specificity of electrical synapses.

R21 NS108505 Paschalis, Miller (Co-PI) 04/01/2019 – 03/31/2021
Identification of the transcriptional targets of three conserved regulatory factors necessary for motor neuron subtype function.
Single cell-RNA-Seq detects differentially regulated genes in specific motor neuron subtypes.

R01 NS118078 Paschalis, Miller (Co-PI) 06/15/2020 – 03/31/2021
Molecular mechanisms of motor neuron terminal identity
Single cell-RNA-Seq reveals genes that maintain motor neuron-specific function.

COMPLETED (last 3 years)

R21 NS100483 Lundquist (Univ. of Kansas, PI) 07/01/2017 – 06/30/2019
The Role of ETR-1/CELF-1, an RNA binding protein, in Neuronal Migration.
Uses RNA-Seq to identify ETR-1/CELF-1 regulated targets in body muscle cells and genetic methods to test for roles in Q neuroblast migration.
Role: Co-PI

R01 NS079611 Miller 06/01/2013 - 05/31/2019
Molecular regulation of dendrite morphogenesis.
Molecular genetic approaches to identify determinants of dendrite branching and self-avoidance.
Role: PI

R56 AG050969 Hammarlund (Yale, PI), 09/30/2016 – 08/31/2018
Mechanisms and Regulation of Neuronal Aging.
Identification of transcriptionally regulated targets that define axonal regenerative capacity in aging neurons.
Role: Co-PI.

R01 NS081259 Miller 06/01/2013-04/30/2018
Molecular determinants of synaptic plasticity
The role of a DEG/ENaC cation channel protein, UNC-8, in an activity-dependent mechanism of synaptic remodeling.
Role: PI