BIOGRAPHICAL SKETCH

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NAME: **Anjon Audhya**

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

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
Brown University, Providence, RI	B.S.	05/1997	Biochemistry
University of California, San Diego, CA (with Scott Emr)	Ph.D.	12/2002	Biomedical Sciences
Ludwig Institute for Cancer Research, La Jolla, CA (with Karen Oegema)	Postdoc	03/2008	Biochemistry and Cell Biology

A. Personal Statement

A major focus of my laboratory is to define the regulatory mechanisms that control vesicle biogenesis and membrane trafficking in the endocytic and secretory pathways of metazoan cells. I have a strong background in the study of membrane transport, including specific training in genetics and biochemistry. We are currently taking advantage of stem cell-based and in vivo rodent models to define the contributions of secretory and endocytic trafficking to normal endocrine function in reproduction and development. In particular, we hypothesize that secretion from uterine epithelial cells supports embryo implantation and deficits in the secretory pathway may underlie conditions, which lead to miscarriage. Additionally, defects in early secretory pathway function lead to ER stress, which has been implicated in many endocrine disease states and defects in normal development in utero. Knowledge obtained from our work can be extended to all of these systems, providing a better picture of how the early secretory pathway influences reproduction and endocrinology, as well as maturation to adulthood and reproductive capacity.

B. Positions and Honors

EMPLOYMENT:

1997-2002	Graduate Student, Biomedical Sciences Program, University of California, San Diego
2003-2008	Helen Hay Whitney Postdoctoral Fellow, Ludwig Institute for Cancer Research, La Jolla, CA
2008-2014	Assistant Professor, Biomolecular Chemistry, University of Wisconsin-Madison
2014-2019	Associate Professor, Biomolecular Chemistry, University of Wisconsin-Madison
2014-current	Director, Molecular and Cellular Pharmacology Graduate Program
2015-current	Director, UW-Madison Optical Imaging Core Facility
2016-current	Affiliate, Department of Neuroscience, University of Wisconsin-Madison
2019-current	Professor, Biomolecular Chemistry, University of Wisconsin-Madison
2019-current	Director, UW-Madison School of Medicine and Public Health Cores Office
2019-current	Associate Dean for Basic Research, School of Medicine and Public Health

HONORS, AWARDS AND OTHER PROFESSIONAL ACTIVITIES:

1997	Inducted into the Sigma Xi Honorary Society
1997	Magna cum Laude, Brown University
1997	Harvey Almy Baker Graduate Fellowship (Brown University)
1998-2002	National Cancer Institute Training Grant Award

2004-2007	Helen Hay Whitney Foundation Postdoctoral Fellowship
2006	DeLill Nasser Award for Professional Development in Genetics
2010	March of Dimes Basil O'Connor Starter Scholar Research Award
2010	Shaw Scientist Award, Greater Milwaukee Foundation
2011	Member, University of Wisconsin Comprehensive Cancer Center
2012	American Cancer Society Research Scholar Award
2012	Member, Institute for Clinical and Translational Research
2013, 2015	Ad hoc Member, NIH, Cell Biology IRG, MBPP Study Section
2013	Ad hoc Member, NIH, Molecular, Cellular, and Developmental Neuroscience IRG, SYN Study Section
2013	Review Editor, *Frontiers in Membrane Traffic*
2013	Member, Stem Cell and Regenerative Medicine Center
2013	Affiliate, Morgridge Institute for Research
2013-2017	Human Frontier Science Program (HFSP) Organization Fellowships Review Panel (Vice-Chair, 2016; Chair, 2017)
2014	Founding Director of the UW-Madison Center for Training in Pharmacology and Drug Development
2015	Ad hoc Member, NIH, Cell Biology IRG, NCSD Study Section
2015	Vilas Associate Award, University of Wisconsin-Madison
2016	Vilas Faculty Early Career Investigator Award
2016-2021	T32 Training Grant (GM008688) for Predoctoral Training in Pharmacology
2016	Faculty Director, Genome Editing and Animal Models (GEAM) core facility
2017	H.I. Romnes Faculty Fellowship, University of Wisconsin-Madison
2017-2023	Standing Member, NIH, Cell Biology IRG, MBPP Study Section
2018	Advanced Scholarship, Tom Wahlig Foundation

C. Contributions to Science

1. Regulation of protein secretion and its implications in cancer and other diseases. The directed movement of proteins and membranes between different cellular locations is a fundamental process required for the proper functioning of all eukaryotic cells. Many diseases including cancer, diabetes, immune dysfunction, and neurodegenerative disorders such as hereditary spastic paraplegias can be caused by intracellular protein transport defects. My lab has discovered an essential role for Trk-fused gene (TFG; also called SPG57) in COPII-mediated protein secretion from the endoplasmic reticulum (ER). Our work over the past several years has led to a revised view of how the early secretory pathway is organized in metazoan cells, highlighting a function for TFG in clustering COPII-coated transport carriers near their site of formation to facilitate their subsequent fusion with neighboring ER-Golgi intermediate compartments (ERGIC) upon uncoating, thereby promoting interorganellar cargo transport. In several cases, chromosomal translocation events fuse the amino-terminal portion of *TFG* to other genes, resulting in oncogenic chimeras. Our findings indicate that TFG fusion proteins interact with native TFG at the ER/ERGIC interface, which modulates secretory flow to potentially enhance cell transformation and oncogenesis. In addition, we recently discovered that mutations in *TFG* cause complicated forms of hereditary spastic paraplegia (HSP), a neurodegenerative disorder that severely limits motor control of the lower limbs. Our structural studies indicate that the mutations impair normal assembly of TFG complexes. We are now establishing tractable rodent and stem cell-based models for HSP, and we plan to develop a facile platform for identifying pharmaceutical agents to combat disease.

• Witte, K., Schuh, A.L., Hegermann, J., Sarkeshik, A., Mayers, J.R., Schwarze, K., Yates, J.R., Eimer, S., and **Audhya, A.** (2011) Mechanisms by which TFG functions in protein secretion and oncogenesis. *Nat. Cell Biol.* 13: 550-558. PMCID: PMC3311221.

• Beetz, C., Johnson, A., Schuh, A., Thakur, S., Varga, R., Fothergill, T., Hertel, N., Bomba-Warczak, E., Thiele, H., Nurnberg, G., Altmuller, J., Saxena, R., Chapman, E.R., Dent, E.W., Nurnberg, P., and **Audhya, A.** (2013) Inhibition of TFG function causes hereditary axon degeneration by impairing ER structure. *Proc. Natl. Acad. Sci. USA*. 110: 5091-5096. PMCID: PMC3612678.

• Hanna, M.G., Block, S., Frankel, E.B., Hou, F., Johnson, A., Yuan, L., Knight, G., Moresco, J.J., Yates, J.R., Ashton, R., Schekman, R., Tong, Y., and **Audhya, A.** (2017) TFG facilitates outer coat disassembly on COPII transport carriers to promote tethering and fusion with ER-Golgi intermediate compartments. *Proc. Natl. Acad. Sci. USA.* 114: E7707-E7716. PMCID: PMC5604033.

• Slosarek, E.L., Schuh, A.L., Pustova, I., Johnson, A., Bird, J., Johnson, M., Frankel, E.B., Bhattacharya, N., Hanna, M.G., Burke, J.E., Ruhl, D.A., Quinney, K., Block, S., Peotter, J.L., Chapman, E.R., Sheets, M.D., Butcher, S.E., Stagg, S.M., and **Audhya, A.** (2018) Pathogenic TFG mutations underlying hereditary spastic paraplegia impair secretory protein trafficking and axon fasciculation. *Cell Rep*. 24: 2248-2260. PMCID: PMC6152936.

2. Mechanisms that regulate membrane scission during vesicle biogenesis and cytokinesis. Components of the ESCRT (Endosomal Sorting Complex Required for Transport) machinery have been implicated in the formation of multivesicular endosomes (MVEs), essential organelles that facilitate the turnover of integral membrane proteins and maintain cellular homeostasis at least in part by attenuating intracellular signaling mediated by cell surface receptors. MVE biogenesis involves the formation of intralumenal vesicles (ILVs), which bud away from the cytoplasm toward the endosome interior. In topologically similar processes, the ESCRT machinery also participates in membrane abscission during cytokinesis, plasma membrane repair after injury, nuclear envelope resealing after mitosis, and the formation of retroviral particles that bud from the cell surface during infection. My lab discovered the first membrane curvature sensitive component of the ESCRT machinery, which appears to play a key role in targeting ESCRT function during membrane scission events. Specifically, we have shown that a complex composed of ESCRT-II and the ESCRT-III subunit Vps20 binds selectively to membranes of elevated curvature, similar to that found at a vesicle bud neck. Our data further suggest that the ESCRT-II/Vps20 complex is mechanosensitive, binding more tightly to membranes as they become increasingly bent, which may aid in maintaining the spatial distribution of ESCRT-III to promote membrane constriction and scission. Using a combination of cryogenic electron microscopy (cryo-EM), single particle reconstruction, and molecular dynamics simulations, we have determined a pseudo-atomic structural model for the ESCRT-III complex, highlighting a spiral filament architecture composed of a series of repeating globular densities joined by flexible linkers. Our data highlight an assembly mechanism that intrinsically harnesses free energy within spiral arrays, which we speculate is released upon restructuring of the polymer. One of our current efforts is aimed at defining the regulatory components that remodel ESCRT-III filaments to facilitate membrane scission.

• Fyfe, I., Schuh, A.L., Edwardson, J.M., and **Audhya, A.** (2011) Association of the endosomal sorting complex ESCRT-II with the Vps20 subunit of ESCRT-III generates a curvature-sensitive complex capable of nucleating ESCRT-III filaments. *J. Biol. Chem*. 286: 34262-34270. PMCID: PMC3190807.

• Shen, Q., Schuh, A.L. Zheng, Y., Quinney, K., Wang, L., Hanna, M., Mitchell, J.C., Otegui, M.S., Ahlquist, P., Cui, Q., and **Audhya, A.** (2014) Mechanisms governing ESCRT-III spiral filament assembly. *J. Cell Biol*. 206: 763-777. PMCID: PMC4164947.

• Frankel, E.B., Shankar, R., Moresco, J.J., Yates, J.R., Volkmann, N., and **Audhya, A.** (2017) Ist1 regulates ESCRT-III assembly and function during multivesicular endosome biogenesis in Caenorhabditis elegans embryos. *Nat. Commun*. 8: 1439. PMCID: PMC5682282.

• Quinney, K., Frankel, E.B., Shankar, R., Kasberg, W., Luong, P., and Audhya, A. (2019) Growth factor stimulation promotes multivesicular endosome biogenesis by prolonging recruitment of the late-acting ESCRT machinery. *Proc. Natl. Acad. Sci. USA*. 116: 6858-686. PMCID: PMC6452702.

3. Regulatory mechanisms that control membrane composition, structure, and dynamics. Cellular membranes are composed of numerous lipid species that function together to maintain subcellular compartmentalization and recruit downstream effector proteins. In particular, acidic phospholipids, including phosphorylated derivatives of phosphatidylinositol (PIPs) and phosphatidylserine (PS), are ideally suited to bind positively charged peptide sequences within peripheral membrane proteins, often activating these effectors to carry out their specific function(s). We have uncovered key roles for acidic phospholipids in regulating processes as diverse as cell invasion, intercellular adhesion, and intracellular protein trafficking. Our ultimate goal is to understand how specific lipid species function together with membrane binding proteins to orchestrate temporally and spatially regulated membrane remodeling events.

• Green, R., Kao, H.,* **Audhya, A**.,* Arur, S., Mayers, J.R., Fridolfsson, H., Schulman, M., Schloissnig, S., Niessen, S., Laband, K., Wang, S., Starr, D., Hyman, A., Schedl, T., Desai, A., Piano, F., Gunsalus, K.C., and Oegema, K. (2011) A high-resolution *C. elegans* essential gene network based on phenotypic profiling of a complex tissue. *Cell*. 145: 470-482. PMCID: PMC3086541. *Equal contributions

• Morrison, K., Witte, K., Mayers, J.R., Schuh, A.L., and **Audhya, A.** (2012) Role of acidic phospholipids and nucleotides in regulating membrane binding and activity of a calcium-independent phospholipase A_2 isoform. *J. Biol. Chem*. 287: 38824-38834. PMCID: PMC3493924.

• Schuh, A.L., Hanna, M., Quinney, K., Wang, L., Sarkeshik, A., Yates, J.R., and **Audhya, A.** (2015) The VPS-20 Subunit of the Endosomal Sorting Complex ESCRT-III Exhibits an Open Conformation in the Absence of Upstream Activation. *Biochem. J.* 466: 625-637. PMCID: PMC4384658.

• Johnson, A., Bhattacharya, N., Hanna, M., Pennington, J.G., Schuh, A.L., Wang, L., Otegui, M.S., Stagg, S.M., and **Audhya, A.** (2015) TFG clusters COPII-coated transport carriers and promotes early secretory pathway organization. *EMBO J.* 34: 811-827. PMCID: PMC4369316.

4. Regulatory mechanisms that govern clathrin-mediated endocytosis and endocytic recycling. A large number of cell surface molecules undergo internalization in a clathrin-dependent fashion. This process requires multiple endocytic adaptors to recognize largely distinct cargoes in a manner that relies on short signal sequences or post-translational modifications found within substrates. We discovered that the ESCRT-0 complex is recruited to sites of clathrin-mediated endocytosis at the plasma membrane, engaging ubiquitin-modified cargoes prior to their accumulation at the endosome. Although inhibition of ESCRT-0 recruitment to the cell surface does not affect the rate of cargo internalization, transport through the endosomal system slows under these conditions, resulting in a significant delay in cargo degradation. Our findings indicate that preassembly of ubiquitinylated cargoes with the ESCRT-0 complex at the plasma membrane enhances the efficiency of downstream sorting events in the endolysosomal system. In the absence of ubiquitin modification, many integral membrane proteins undergo endocytic recycling, an essential pathway required to maintain cell surface composition and is regulated by several Rab-type GTPases. My lab has helped to uncover new effectors of the endocytic Rab proteins, and we are currently using genetic, biochemical, and fluorescence-based functional assays for organelle remodeling to uncover the roles of these factors during development.

• Shi, A., Chen, C.C., Banerjee, R., Glodowski, D., **Audhya, A.,** Rongo, C., and Grant, B.D. (2010). EHBP-1 functions with RAB-10 during endocytic recycling in *C. elegans*. *Mol. Biol. Cell*. 21: 2930-2943. PMCID: PMC2921114.

• Mayers, J.R., Wang, L., Pramanik, J., Johnson, A., Sarkeshik, A., Wang, Y., Saengsawang, W., Yates, J.R., and **Audhya, A.** (2013) Regulation of ubiquitin-dependent cargo sorting by multiple plasma membrane endocytic adaptor proteins. *Proc. Natl. Acad. Sci. USA.* 110: 11857-11862. PMCID: PMC3718112.

• Wang, L., and **Audhya, A.** (2014) *In vivo* imaging of *C. elegans* endocytosis. *Methods*. 68: 518-528. PMCID: PMC4112158.

• Wang, L., Johnson, A., Hanna, M., and **Audhya, A**. (2016) Eps15 membrane-binding and -bending activity acts redundantly with Fcho1 during clathrin-mediated endocytosis. *Mol. Biol. Cell*. 27: 2675-2687. PMCID: PMC5007088.

5. Mechanisms that regulate post-Golgi cargo sorting and trafficking. Approximately one-third of all translated proteins in human cells are predicted to enter the secretory pathway, many of which must be accurately sorted at the Golgi apparatus to efficiently reach their final destination. Our studies have helped to identify new regulatory mechanisms that govern post-Golgi cargo transport. We have demonstrated a key role for the ESCRT-0 complex in stably associating with ubiquitin-modified cargoes and directing their incorporation into lumenal vesicles within multivesicular endosomes. Additionally, we have participated in the identification of new regulatory factors that direct the trafficking of essential cargoes, including integrin complexes, which mediate cell adhesion, and insulin, which plays critical roles in metabolic regulation. In the future, we plan to determine mechanisms by which these regulatory proteins enable cargo-selective transport toward unique secretory pathways using a combination of biochemical and high-resolution imaging approaches.

• Mayers, J.R., Fyfe, I., Schuh, A.L., Chapman, E.R., Edwardson, J.M., and **Audhya, A.** (2011) ESCRT-0 assembles as a heterotetrameric complex on membranes and binds multiple ubiquitinylated cargoes simultaneously. *J. Biol. Chem*. 286: 9636-9645. PMCID: PMC3058970.

• Wan, J., Zhu, F., Zasadil, L., Yu, J., Wang, L., Johnson, A., Berthier, E., Beebe, D.J., **Audhya, A.**, and Weaver, B.A. (2014) A Golgi localized pool of the mitotic checkpoint component Mad1 controls integrin secretion and cell migration. *Curr. Biol.* 24: 2687-2692. PMCID: PMC4254593.

• Kebede, M., Oler, A., Balloon, A.J., Johnson, A., Rabaglia, M., Stapleton, D., Schueler, K., Floyd, B., Richards, O., Raines, S., Gregg, T., Eliceiri, K., Weisshaar J., Rhodes, C., Thorstenson, C., Keller, M.P., Coon, J., **Audhya, A.**, and Attie, A.D. (2014) Sorcs1 is Required for Normal Dense Core Vesicle Biogenesis in Metabolically Stressed Beta-Cells. *J. Clin. Invest.* 124: 4240-4256. PMCID: PMC4191024.

• Takahashi, H., Mayers, J.R., Wang, L., Edwardson, J.M., and **Audhya, A.** (2015) Hrs and STAM function synergistically to bind ubiquitin-modified cargoes in vitro. *Biophys. J.* 108: 76-84. PMCID: PMC4286613.

Complete List of Published Work in PubMed:
http://www.ncbi.nlm.nih.gov/pubmed/?term=audhya+a+not+nagpure+not+chaudhury

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support:

National Institutes of Health, NIGMS
Grant #1R01GM088151 (Anjon Audhya, PI) 7/1/2010 - 3/31/2020
Molecular Mechanisms that Regulate Lysosomal Protein Transport
The major goals of this project are to determine mechanisms by which the ESCRT machinery recognizes substrates and to define mechanisms that regulate cargo entry into the ESCRT pathway.

National Institutes of Health, NIGMS
Grant #1R01GM110567 (Anjon Audhya, PI) 3/1/2015 - 1/31/2023
Regulatory mechanisms that control vesicle secretion at the endoplasmic reticulum
The major goals of this project are to define the contributions of TFG to the organization of the endoplasmic reticulum (ER)/ERGIC interface.

National Institutes of Health, NIDDK
Grant #1R01DK102948 (Anjon Audhya, co-I) 7/1/2015 - 6/30/2020
Role of Sorcs1 in Diabetes Susceptibility
The major goals of this project are to discover the role of Sorcs1 and Sortilin in insulin degradation, characterize SNPs in human *SORT1* associated with low insulin and hypercholesterolemia in an Amish population, and characterize the SNP in the pro-peptide of Sorcs1.

National Science Foundation
Grant # 1661900 (Anjon Audhya, co-PI) 7/1/2017 - 6/30/2021
Collaborative Research: Multi-scale modeling of membrane fission
The major goals of this project are to develop mathematical theory in ESCRT-III geometry and dynamics on a fixed membrane, nonlocal hydrodynamics of membrane-bound filament motion, and evolution of the fully coupled ESCRT-membrane system.

National Institutes of Health, NIGMS
Grant #1R01GM117473 (Anjon Audhya, co-I) 9/21/2017 - 7/31/2021
Control of COPII vesicle trafficking by intracellular protein glycosylation
The major goals of this project are to dissect the functional impact of O-GlcNAc cycling on COPII vesicle trafficking, define the role of site-specific O-GlcNAcylation of Sec23A and Sec24D in human cells, and determine the contribution of COPII O-GlcNAcylation in vertebrate models of disease.

National Institutes of Health, NIAID
Grant #2R01AI073289 (Anjon Audhya, co-I) 6/1/2018 - 5/31/2023
Biofilm Induced Extracellular Vesicle Pathogenesis
The major goal of this project is to define extracellular vesicle cargo that permits Candida to persist and disseminate from implanted medical devices, with a longer term goal of discovering targets for the development of innovative therapeutic agents.