BIOGRAPHICAL SKETCH

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NAME: Mark L. Grimes

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

POSITION TITLE: Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Kalamazoo College, Kalamazoo, Michigan	B.A.	06/1978	Chemistry & Biology
University of Oregon, Eugene, Oregon	Ph.D.	03/1986	Chemistry & Molecular Biology (Advisor: Ed Herbert)
University of Oregon, Eugene, Oregon	Postdoctoral	07/1987	Yeast secretion
University of California, San Francisco	Postdoctoral	05/1991	Sorting in the secretory pathway

A. Personal Statement

This multi-investigator application arises from our discovery of a method to produce neural crest-derived craniofacial cartilage from human stem cells (1). We initiated the project to derive neural crest stem cells (NCSCs) from human embryonic stem cells (hESCs) because of an interest in how cells differentiate. We initially wanted to test the hypothesis that NCSC differentiation employs similar mechanisms that we elucidated in neuroblastoma cells (2, 3). The study of cell signaling mechanisms in cancer is highly relevant to mechanisms that drive cell differentiation. Cancer arises by various mechanisms as cells break out of their normal differentiated state in multicellular organisms, and neuroblastoma begins with an early failure in neural crest differentiation during development. To study cell signaling mechanisms, we have developed computational approaches to analyze large data sets of proteins and protein post-translational modifications (3-6) Our techniques employ machine learning to help recognize patterns from statistical relationships in data, and combine that information with protein-protein interactions to visualize data structure as networks at different levels (3, 5, 6). Recently, we have added another level to these network models of data structure that elucidates interactions among cell signaling pathways (4). We are now able to examine differentiation using single nucleotide RNA sequencing to complement these approaches (manuscript submitted). We have also used cell biological approaches to study cell signaling and membrane traffic in a study that directly addresses mechanisms of cell differentiation (2). We fractionated neuroblastoma cells to examine the location of signaling proteins in different membranes and organelles and learned that the scaffold protein, PAG1, which was known to control SRC-family kinase (SFK) activity in lipid rafts, was one of the most highly phosphorylated proteins in neuroblastoma endosomes (3). This led to discovery of a novel cell signaling mechanism that distinguishes receptor tyrosine kinase (RTK) promotion of neuronal differentiation vs. proliferation: PAG1 influences SFK sequestration in multivesicular bodies and is required for differentiation but not proliferation (2). In addition, we transplanted neuroblastoma cells into developing chick embryos and showed that neuroblastoma cells were multipotent, capable of migrating and differentiating into many cell types expected of normal neural crest cells (3). These studies demonstrate that we are capable of mechanistic analysis of chondrocyte differentiation from neural crest. We will use this information to design of conditions for seeding cells into biological scaffolds for three-dimensional printing to provide the correct microenvironment for chondrocytes to build craniofacial cartilage. Note that these studies also demonstrate the ability to work together in teams from different institutions.

1. Foltz, L.E., Levy, T., Possemato, A., and Grimes, M.L. (2021). Craniofacial cartilage organoids from human embryonic stem cells via a neural crest cell intermediate. bioRxiv. 10.1101/2021.05.31.446459.

2. Foltz, L., Palacios-Moreno, J., Mayfield, M., Kinch, S., Dillon, J., Syrenne, J., Levy, T., and Grimes, M. (2020). PAG1 directs SRC-family kinase intracellular localization to mediate receptor tyrosine kinase-induced differentiation. Mol Biol Cell 31, 2269-2282. 10.1091/mbc.E20-02-0135.

3. Palacios-Moreno, J., Foltz, L., Guo, A., Stokes, M.P., Kuehn, E.D., George, L., Comb, M., and Grimes, M.L. (2015). Neuroblastoma tyrosine kinase signaling networks involve FYN and LYN in endosomes and lipid rafts. PLoS Comput Biol 11, e1004130. 10.1371/journal.pcbi.1004130.

4. Ross, K.E., Zhang, G., Akcora, C., Lin, Y., Fang, B., Koomen, J., Haura, E.B., and Grimes, M. (2023). Network models of protein phosphorylation, acetylation, and ubiquitination connect metabolic and cell signaling pathways in lung cancer. PLoS Comput Biol 19, e1010690. 10.1371/journal.pcbi.1010690.

5. Grimes, M., Hall, B., Foltz, L., Levy, T., Rikova, K., Gaiser, J., Cook, W., Smirnova, E., Wheeler, T., Clark, N.R., et al. (2018). Integration of protein phosphorylation, acetylation, and methylation data sets to outline lung cancer signaling networks. Science signaling 11, eaaq1087. 10.1126/scisignal.aaq1087 PMID - 29789295.

6. Grimes, M.L., Lee, W.J., van der Maaten, L., and Shannon, P. (2013). Wrangling phosphoproteomic data to elucidate cancer signaling pathways. PLoS One 8, e52884. 10.1371/journal.pone.0052884.

Underpinning these studies are several papers that demonstrate our innovative approaches to studying the compartmentalization of signal transduction. We developed high-resolution organelle fractionation methods based on mass and density to resolve endosomes from other organelles, and different signaling endosomes from one another (7-10). We showed that the nerve growth factor (NGF) receptors, TrkA (NTRK1) and $p75^{NTR}$, (NGFR) partition into membrane rafts by different mechanisms, and that the fraction of TrkA that associates with lipid rafts is internalized but does not directly form signaling endosomes (11). Rather, by attracting microtubules to lipid rafts, TrkA may mediate other processes such as axon guidance. We resolved two independent NGF-regulated signaling particles with an estimated size of 60–75 S, one containing ERK1 and MEK1 and the other containing B-Raf, which we hypothesize control the temporal and spatial regulation of kinase activity inside cells (12).

7. Lin, D.C., Quevedo, C., Brewer, N.E., Bell, A., Testa, J.R., Grimes, M.L., Miller, F.D., and Kaplan, D.R. (2006). APPL1 associates with TrkA and GIPC1 and is required for nerve growth factor-mediated signal transduction. Mol Cell Biol 26, 8928-8941. 10.1128/MCB.00228-06.

8. McCaffrey, G., Welker, J., Scott, J., der Salm, L., and Grimes, M.L. (2009). High-resolution fractionation of signaling endosomes containing different receptors. Traffic 10, 938-950. 10.1111/j.1600-0854.2009.00909.x.

9. Xin, X., Gfeller, D., Cheng, J., Tonikian, R., Sun, L., Guo, A., Lopez, L., Pavlenco, A., Akintobi, A., Zhang, Y., et al. (2013). SH3 interactome conserves general function over specific form. Mol Syst Biol 9, 652. 10.1038/msb.2013.9.

10. Caliva, M.J., Yang, W.S., Young-Robbins, S., Zhou, M., Yoon, H., Matter, M.L., Grimes, M.L., Conrads, T., and Ramos, J.W. (2021). Proteomics analysis identifies PEA-15 as an endosomal phosphoprotein that regulates alpha 5 beta 1 integrin endocytosis. Scientific Reports 11. ARTN 19830 10.1038/s41598-021-99348-z.

11. Pryor, S., McCaffrey, G., Young, L.R., and Grimes, M.L. (2012). NGF causes TrkA to specifically attract microtubules to lipid rafts. PLoS One 7, e35163. 10.1371/journal.pone.0035163.

12. Maccormick, M., Moderscheim, T., Salm, L.W.M.v.d., Moore, A., Pryor, S.C., McCaffrey, G., and Grimes, M.L. (2005). Distinct signalling particles containing ERK/MEK and B-Raf in PC12 cells. The Biochemical journal 387, 155 164. 10.1042/bj20040272 PMID - 15500439.

Analyzing mass spectrometry data on a large scale required acquiring new skills. With help from collaborators from the fields of pattern recognition and computational bioinformatics, we developed new, effective methods to cluster proteins based on statistical relationships obtained from sparse data (3-6). In addition, the papers below are collaborative contributions to the computational and systems biology fields. The combination of sophisticated data analysis techniques and high-resolution cell fractionation methods puts my laboratory in a

unique position to make valuable contributions towards understanding signaling networks that control cell fate decisions.

13. Fernandez, N.F., Gundersen, G.W., Rahman, A., Grimes, M.L., Rikova, K., Hornbeck, P., and Ma'ayan, A. (2017). Clustergrammer, a web-based heatmap visualization and analysis tool for high-dimensional biological data. Sci Data 4, 170151. 10.1038/sdata.2017.151.

14. Shannon, P.T., Grimes, M., Kutlu, B., Bot, J.J., and Galas, D.J. (2013). RCytoscape: tools for exploratory network analysis. BMC bioinformatics 14, 217. 10.1186/1471-2105-14-217 PMID - 23837656.

B. Positions and Honors

Positions and Employment

1986 - 1987	Postdoctoral Fellow (Advisor: Tom Stevens) Chemistry Department, University of Oregon, Eugene, OR
1987 - 1991	Postdoctoral Fellow (Advisor: Regis B. Kelly) Department of Biochemistry and Biophysics, University of California, San Francisco, CA
1991 - 1992	Postdoctoral Fellow (Advisor: William C. Mobley) Department of Neurology, University of California, San Francisco, CA
1992 - 1994	Assistant Research Cell Biologist Department of Neurology, University of California, San Francisco, CA
1994 - 2001	Senior Lecturer Massey University, Palmerston North, New Zealand
2001 - 2001	Visiting Scientist Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD
2002 - 2018	Associate Professor Division of Biological Sciences, University of Montana, Missoula, MT
2018 - present	Professor Division of Biological Sciences, University of Montana, Missoula, MT

Honors

1979	Graduate Teaching Fellow, Chemistry Department, University of Oregon
1980	NIH Molecular Biology Predoctoral Training Grant GM 07759, Institute of Molecular Biology, University of Oregon
1986	American Heart Association Research Fellow, American Heart Association, Oregon Affiliate, Inc.
1987	NIH Neurobiology Postdoctoral Training Grant NS 07067-10, Department of Physiology, University of California, San Francisco
1988	National Research Service Award NS 08387-01, National Institute of Neurological and Communicative Disorders and Stroke
1991	Athena Neurosciences Special Fellowship, Athena Neurosciences, South San Francisco, CA
1992	NARSAD Young Investigator Award, National Alliance for Research on Schizophrenia and Depression, Great Neck, NY
2007	National Academies Education Fellow in the Life Sciences, National Academy of Sciences
2011	National Academies Education Mentor in the Life Sciences, National Academy of Sciences
2018-21	Scientific Teaching Host Leader and Mentor, Summer Institutes for Scientific Teaching, National Science Foundation, Howard Hughes Medical Institute, Yale University Center for Scientific Teaching.

C. Contributions to Science

1. In my graduate work with Dr. Ed Herbert, I cloned chromogranin A, a major component of neuroendocrine dense core secretory granules. An interest in secretion led me to Dr. Regis Kelly and work in which sorting of regulated and constitutive secretory granules was reconstituted *in vitro.* Interest in nerve growth factor signaling mechanisms led me to Dr. William Mobley, in whose laboratory an organelle fractionation approach was employed to define signaling endosomes containing activated TrkA/NTRK1, the NGF receptor tyrosine kinase. This work is has been highly cited, and is still cited in recent reviews.

a. Iacangelo, A., Affolter, H.U., Eiden, L.E., Herbert, E., and Grimes, M. (1986). Bovine chromogranin A sequence and distribution of its messenger RNA in endocrine tissues. Nature 323, 82-86. 10.1038/323082a0.

b. Grimes, M., and Kelly, R.B. (1992). Intermediates in the constitutive and regulated secretory pathways released in vitro from semi-intact cells. The Journal of Cell Biology 117, 539 549.

c. Grimes, M.L., Zhou, J., Beattie, E.C., Yuen, E.C., Hall, D.E., Valletta, J.S., Topp, K.S., Lavail, J.H., Bunnett, N.W., and Mobley, W.C. (1996). Endocytosis of Activated TrkA: Evidence that Nerve Growth Factor Induces Formation of Signaling Endosomes. The Journal of Neuroscience 16, 7950-7964.

d. Grimes, M.L., Beattie, E., and Mobley, W.C. (1997). A signaling organelle containing the nerve growth factor-activated receptor tyrosine kinase, TrkA. Proc Natl Acad Sci U S A 94, 9909-9914. 10.1073/pnas.94.18.9909.

e. Beattie, E.C., Zhou, J., Grimes, M.L., Bunnett, N.W., Howe, C.L., and Mobley, W.C. (1996). A signaling endosome hypothesis to explain NGF actions: potential implications for neurodegeneration. Cold Spring Harb Sym 61, 389 406.

f. Zhou, J., Valletta, J.S., Grimes, M.L., and Mobley, W.C. (1995). Multiple levels for regulation of TrkA in PC12 cells by nerve growth factor. Journal of Neurochemistry 65, 1146 1156.

2. This work fueled further studies on the compartmentalization of signal transduction described above (3, 4). In addition, we have published several papers elucidating mechanisms activated during neural cell apoptosis. We have shown that AKT and CREB are cleaved by caspases during apoptosis, and that populations of cells at different stages of apoptosis can be isolated. In addition, we have contributed to several collaborative studies.

g. François, F., Godinho, M.J., and Grimes, M.L. (2000). CREB is cleaved by caspases during neural cell apoptosis. FEBS Letters 486, 281 284.

h. Francois, F., Godinho, M.J., Dragunow, M., and Grimes, M.L. (2001). A population of PC12 cells that is initiating apoptosis can be rescued by nerve growth factor. Mol Cell Neurosci 18, 347-362. 10.1006/mcne.2001.1035.

i. François, F., and Grimes, M.L. (1999). Phosphorylation-dependent Akt cleavage in neural cell in vitro reconstitution of apoptosis. Journal of Neurochemistry 73, 1773 1776.

j. Grimes, M.L., and Miettinen, H.M. (2003). Receptor tyrosine kinase and G-protein coupled receptor signaling and sorting within endosomes. Journal of Neurochemistry 84, 905 918.

k. Agnihothram, S.S., Dancho, B., Grant, K.W., Grimes, M.L., Lyles, D.S., and Nunberg, J.H. (2009). Assembly of arenavirus envelope glycoprotein GPC in detergent-soluble membrane microdomains. J Virol 83, 9890-9900. 10.1128/JVI.00837-09.

l. Weible, M.W., Ozsarac, N., Grimes, M.L., and Hendry, I.A. (2004). Comparison of nerve terminal events in vivo effecting retrograde transport of vesicles containing neurotrophins or synaptic vesicle components. Journal of Neuroscience Research 75, 771 781. 10.1002/jnr.20021 PMID - 14994338.

m. Blythe, T.J., Grimes, M.L., and Kitson, K.E. (1999). The role of retinoid metabolism by alcohol and aldehyde dehydrogenases in differentiation of cultured neuronal cells. Advances in experimental medicine and biology 463, 199 204.

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

Ongoing Research Support

1R15DE028434-01
Title: Craniofacial cartilage from human stem cells through neural crest stem cells
Total Funds: $424,665
Dates: 7/1/2019-6/30/2023
Role: PI

Completed Research Support

Center for Translational Medicine Pilot Grant
Center for Translational Medicine, University of Montana
P.I.: Mark Grimes
Total Funds: $100,000
Dates: 5/1/2022 - 4/31/2023

University of Montana Foundation
Source: Private donors
Title: Cancer Research Initiative - Neuroblastoma
P.I. Mark Grimes
Total Funds: $16,854
Dates: 8/15/2015-present

Center for Translational Medicine Pilot Grant
Center for Translational Medicine, University of Montana
P.I.: Mark Grimes
Total Funds: $50,000
Dates: 6/1/2019 - 5/31/2020

RFA-HG-14-001 BD2K-LINCS-DCIC External Investigator Award
Title: Elucidation of signaling pathways from post-translational modification data derived from mass spectrometry
P.I.: Mark Grimes
Total Funds: $260,000
Dates: 05/01/2016 – 04/30/2018

RFA-HG-14-001
Title: D2K-LINCS-Perturbation Data Coordination and Integration Center (DCIC) (U54)
P.I.: Avi Malayan, Mt Sinai School of Medicine
co-P.I. Peter Hornbeck, Cell Signaling Technology
Total Funds: $80,625 (sub-award)
Dates: 1/1/15-12/31/17

1R15NS070746-01 Grimes (PI)
National Institutes of Health – NINDS
Title: Receptor Tyrosine Kinase Sorting and Transactivation in Endosomes
Total Funds: $421,525
Dates: 04/01/2010 – 03/31/2013
Role: PI