BIOGRAPHICAL SKETCH

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NAME: Weigert, Roberto

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

POSITION TITLE: Senior Investigator – Laboratory Cellular and Molecular Biology and Director- Intravital microscopy Core, Center for Cancer Research, National Cancer Institute, and Chief, Intracellular Membrane Trafficking Section, Oral and Pharyngeal Cancer Branch, NIDCR, National Institutes of Health

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 Catania, Catania, Italy	M.Sc.	07/1992	Chemistry
Open University of London, London, UK	Ph.D.	03/2000	Life Sciences
Mario Negri Sud Institute, Lanciano, Italy	Post-Doctoral	01/2001	Cell Biology
National Institutes of Health	Post-doctoral	09/2006	Cell Biology

A. Personal Statement

I have worked in the field of membrane remodeling since 1994 and my focus has been understanding the role of mechanical forces in controlling the remodeling of intracellular membranes. The three central questions that drive my research are: 1) how extracellular signals are transduced into mechanical forces by the actomyosin cytoskeleton; 2) how membrane remodeling is coordinated with cellular metabolism, and 3) how membrane remodeling is deregulated in pathological states and in particular during tumor progression, invasion, and metastasis. Eighteen years ago, my lab pioneered intravital subcellular microscopy (ISMic), which enables the direct observation of the dynamics of intracellular processes in live animals. We have complemented ISMic with the development of a series of pharmacological, molecular, and genetic tools that have made possible the investigation of membrane remodeling *in vivo* at a molecular level. Our pioneering work in this field led to major breakthroughs in the understanding of the coordination among membrane dynamics, cytoskeletal remodeling, cell signaling, and mitochondrial metabolism in live animals, thus opening the door to investigate cell biology in mammalian tissues in situ.

B. Positions and Honors

Positions and Employment

2019-present	Director, Intravital Microscopy Core, Center for Cancer Research, NCI, NIH, Bethesda
2015-present	Senior Investigator, Laboratory of Cellular and Molecular Biology, Center for Cancer Research, NCI, NIH, Bethesda
2014-present	Senior Investigator, Chief, Intracellular Membrane Trafficking Section, Oral and Pharyngeal Cancer Branch, NIDCR, NIH, Bethesda, USA
2006-2014	Principal Investigator, Chief, Intracellular Membrane Trafficking Unit, Oral and Pharyngeal Cancer Branch, NIDCR, NIH, Bethesda, USA
2001-2006	Research Fellow, Laboratory of Cell Biology, NHLBI, NIH, Bethesda, USA.
2000-2001	Post-Doctoral Fellow, Dept. of Cell Biology and Oncology, Mario Negri Sud Institute, Italy
1995	Special Volunteer, Cell Biology and Metabolism Branch, NICHD, NIH, Bethesda USA
1994-1996	Pre-Doctoral Fellow, Dept. of Cell Biol. and Oncology, Mario Negri Sud Institute, Italy
1993-1994	Technical Brand Manager, Dept. of Research and Development, Procter & Gamble, Italy

Honors

2023	NCI Director Award's for the development of ISMic and establishing the IVM core
2023	NCI Director Award's implementing a response to a unique disease outbreak
2019	NCI Federal Technical Transfer Award
2019	NCI Research Highlight Award
2016	Chair, Gordon Research Conference on "Lysosomes and Endocytosis"
2015	Chair, Gordon Research Conference on "Salivary Glands and Exocrine Biology"

2013 NHLBI Orloff Science Award
2012 Olympus Bioscape award
2011 Distinguished Research Award - University of South Australia
2007 Chesapeake Society for Microscopy, Award

Other Experience and Professional Memberships

2016 Member of the Italian Society of Cell Biology
Scientific Advisory board of the O'Brien Center for Microscopy University of Indiana, IN
2012-2016 Editor-in-chief, Intravital (Landes Biosciences, Taylor and Frances)
2013 Associate Editor, Frontiers in Cell and Developmental Biology
2001 Member of the American Society of Cell Biology

C. Contributions to Science

My primary interest is unraveling the mechanisms that regulate membrane remodeling during intracellular trafficking and cell motility, with a particular emphasis on the machinery that coordinates cytoskeleton dynamics and membrane remodeling. As a graduate student, my work on the formation of post-Golgi transport carriers, led to the discovery of CtbP/BARS, a molecule controlling membrane fission. During my post-doctoral fellowship, my studies led to the identification of the small GTPase Rab22, as key regulator of the recycling of molecules to the plasma membrane during clathrin-independent endocytosis. As an independent investigator at the National Institutes of Health, I took the challenge of studying membrane remodeling in live multicellular organisms (i.e. rodents) by pioneering ISMic and to extend our interest to pathological states, which include inflammation and tumor progression. Specifically, my lab has focused on the four main areas described below:

A. Development of intravital subcellular microscopy (ISMic). We have pioneered a powerful approach to study the dynamics of subcellular organelles in live rodents *in situ*. Our work has shown that various processes *in vivo* differ significantly from what reported in *in vitro* or *ex vivo* model systems (e.g. cell cultures or explanted organs) both in term of modality and regulation. Our findings have opened the door to the possibility of studying cell biology under true physiological conditions.

1. Masedunskas, A. and **Weigert, R**. Intravital two-photon microscopy for studying the uptake and trafficking of fluorescently-conjugated molecules in live rodents (2008) Traffic 9(10): 1801-10

2. Sramkova M., Masedunskas A., Parente L., Molinolo A., and **Weigert R**. Expression of plasmid DNA in the salivary gland epithelium: novel approaches to study dynamic cellular processes in live animals (2009) Am. J Physiol Cell Physiol Dec 297(6):C1347-57

3. **Weigert R**, Porat-Shliom N, and Amorpnphimoltham, P. Imaging Cell Biology in Live Animals: Ready for Primetime (2013) J. Cell Biol. 201(7):969-979

4. **Weigert R**, Imaging the dynamics of endocytosis in live mammalian tissues. (2014) Cold Spring Harbor Perspective Biol 6(4)

5. Ebrahim S, and **Weigert R.** Intravital microscopy in mammalian multicellular organisms. (2019) Curr Opin Cell Biol. 59:97-103. doi: 10.1016/j.ceb.2019.03.015

B. Membrane remodeling during invasion and metastasis in head and neck cancer. We applied our innovative approach to study the mechanisms of invasion and metastasis during head and neck cancer. Specifically, we have investigated tumor progression in animal models and discovered novel regulators of this process, including the small GTPase Rab25. We showed that this molecule, that is downregulated in head and neck cancer patients, controls the metastatic process by regulating endosomal recycling and the actin cytoskeleton at the plasma membrane.

1. Patel V, Marsh CA, Dorsam RT, Masedunskas A, Amornphimoltham P, Nathan CO, Singh B, **Weigert R**, Molinolo AA, and Gutkind JS. Decreased Lymphangiogenesis and Lymph Node Metastasis by mTOR Inhibition in Head and Neck Cancer (2011) Cancer Research 71(22):7103-12

2. Amornphimoltham P, Rechache K, Thompson J, Masedunskas A, Leelahavanichkul K, Patel V, Molinolo A, Gutkind JS, and **Weigert R**. Rab25 regulates invasion and metastasis in head and neck cancer (2013) Clin. Can. Res. 19(6):1375-88

3. Amornphimoltham P, Thompson J, Melis N, **Weigert R**. Non-invasive intravital imaging of head and neck squamous cell carcinomas in live mice (2017) Methods. 128:3-11. doi:10.1016/j.ymeth.2017.07.026

4. Chrisafis G, Wang T, Moissoglu K, Gasparski AN, Ng Y, **Weigert R**, Lockett SJ and Mili S. Collective cancer cell invasion requires RNA accumulation at the invasive front (2020) Proc. Natl. Acad. Jour. (in press)

C. Mechanisms of membrane remodeling during cell motility. By using ISMic we have unraveled a novel regulation of the actomyosin complex in neutrophils during their response to inflammatory stimuli. We

discovered a novel pathway that regulates this process and is activated by the secondary chemoattractant Leukotriene B4 (LTB4). We found that: 1) LTB4 is released by the neutrophils into the vasculature by means of large extracellular vesicles (EVs); 2) EVs adhere to the vascular endothelium and release LTB4, which activates the BLT1 receptor on the surface of the neutrophils in a paracrine/autocrine fashion, 3) BLT1 signaling activates the actin-based motor protein myosin IIA (NMIIA), which in turn controls neutrophil adhesion by promoting the recycling of the adhesion molecule through the Beta2-integrin (Itgb2), and neutrophil extravasation through its contractile activity.

1. Subramanian B, Melis N, Chen D, Wang, W, **Weigert R***, Parent C*. The LTB4-BLT1 axis regulates actomyosin and beta2 Integrin dynamics during neutrophil extravasation (2020) J. Cell Biol 219(10):e201910215

D. Mechanisms and bioenergetics of membrane remodeling during regulated exocytosis. By using intravital microscopy we demonstrated that a novel actomyosin complex composed of F-actin and two isoforms of non-muscle myosin II (NMIIA and NMIIB) is essential for the completion of exocytosis in exocrine glands in different organisms. By introducing super-resolution microscopy in vivo, we recently discovered that this actomyosin complex is arranged in contractile cage-like structures around secretory vesicles, which provide the force necessary to drive protein secretion. This process, which *in vivo* is not energetically favorable, is tightly coupled to mitochondrial dynamics and energy production. Recently, we discovered a novel coupling between mitochondrial energetics and exocytosis, which involves whole-tissue propagating oscillatory mitochondrial NADH waves, traversing through gap junctions.

1. Masedunskas A, Sramkova M, Parente L, Sales KU, Amornphimoltham P, Bugge TH, and **Weigert R**, Role for the acto-myosin complex in regulated exocytosis revealed by intravital microscopy (2011) Proc Natl Acad Sci U S A. 108(33):13552-7

2. Porat-Shliom N, Chen Y, Tora M, Shitara A, Masedunskas A, and **Weigert R**, In vivo tissue-wide synchronization of mitochondrial metabolic oscillations. (2014) Cell Reports 9(2):514-21

3. Tran DT, Masedunskas A, **Weigert R**, Ten-Hagen KG. Arp2/3-mediated F-Actin branching controls regulated exocytosis in vivo (2015), Nature Comm 6:10098

4. Masedunskas A, Chen Y, Stussman R, **Weigert R**, Mather IH, Kinetics of milk lipid droplet (LD) transport, growth and secretion revealed by Intravital imaging: LD release is intermittently stimulated by oxytocin (2017) Mol. Biol. Cell doi: 10.1091/mbc.E16-11-0776.

5. Milberg O, Shitara A, Ebrahim S, Masedunskas A, Tora M, Tran DT, Chen Y, Conti MA, Adelstein RS, Ten Hagen KG, **Weigert R**. Concerted Actions of Distinct Non-Muscle Myosin II Isoforms Drive Intracellular Membrane Remodeling in Live Animals (2017) J. Cell Biol. doi: 10.1083/jcb.201612126

6. Shitara A, Malec L, Ebrahim S, Chen D, Bleck C, Hoffman MP, **Weigert R**. Cdc42 negatively regulates endocytosis during apical membrane maintenance in live animals (2018) Mol Biol Cell. doi: 10.1091/mbc.E18-10-0615.

7. Porat-Shliom N, Harding JH, Malec L, Narayan K, **Weigert R** Mitochondrial populations exhibit differential dynamic responses to increased energy demand during exocytosis *in vivo* (2019) iScience 11:440-449. doi: 10.1016/j.isci.2018.12.036.

8. Ebrahim S, Chen D, Weiss M, Malec L, Ng Y, Rebustini I, Krystofiak E, Hu L, Liu J, Masedunskas A, Hardeman E, Gunning P, Kachar B, **Weigert R.** Dynamic polyhedral actomyosin lattices remodel micron-scale curved membranes during exocytosis in live mice (2019) Nat. Cell Biol. 21(8):933-939

9. Heydecker M, Shitara A, Chen D, Tran D, Masedunskas A, Tora M, Ebrahim S, Appaduray MA, Galeano Niño JL, Bhardwaj A, Narayan K, Hardeman EC, Gunning PW, **Weigert R.** bioRxiv. 2023 Dec 5:2023.12.04.569944. doi: 10.1101/2023.12.04.569944.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1z_L6o6FY-YAo/bibliography/public/

D. Research Support.

1. **Molecular Mechanisms regulating membrane trafficking in salivary glands** 2006-2015
Funding: NIDCR Intramural Support, Role: PI
This project addresses the molecular machineries regulating protein secretion and endocytosis in salivary glands in order to better understand the physiology of this organ and to develop better strategies to cure salivary glands dysfunctions

2. **Molecular Mechanisms regulating membrane remodeling during physiological and pathological conditions**
Funding: NCI-CCR Intramural Support, Role: PI
This project addresses the molecular machineries regulating membrane remodeling during trafficking in live animals and their deregulation during pathological conditions.

E. Diversity Activities

Joined the ICURE program to mentor under-represented minorities. Mentored several women at different stages of their career, including post-bac and post-doctoral fellows, and Tenure-Track investigators.