BIOGRAPHICAL SKETCH

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NAME: Hite, Richard

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

POSITION TITLE: Assistant Member

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)	END DATE MM/YYYY	FIELD OF STUDY
Emory University, Atlanta, GA	BS	05/2003	Biology and Mathematics
Harvard University, Boston, MA	PHD	12/2010	Cell and Developmental Biology
Rockefeller University, New York, NY	Postdoctoral Fellow	08/2016	Neurobiology and Biophysics

A. Personal Statement

During my Ph.D. training with Thomas Walz at Harvard Medical School, I elucidated principles for how annular lipid interact with membrane proteins. These interactions were analyzed by determining high-resolution structures of the lens water channel Aquaporin-0 embedded in lipid bilayers with different lipid compositions. Following my Ph.D., I joined the lab of Roderick MacKinnon for my postdoctoral studies on the gating mechanisms of voltage- and ligand-gated potassium channels. Using single-particle cryo-EM and electrophysiological approaches, I determined the mechanisms by which anionic phospholipids bias the gating of voltage-gated potassium channels, the gating mechanism of the Na^+-activated potassium channel, Slo2.2, and the gating mechanisms of the voltage- and Ca^{2+}-activated BK potassium channel.

As an independent investigator, my lab research has primarily focused on the roles of intracellular ion transport in signaling and organellar homeostasis. We combine high-resolution cryo-EM structural studies with biochemical, biophysical and electrophysiological approaches to elucidate the molecular mechanisms that underlie intracellular ion transport. Insights from these mechanistic approaches are then applied to cell biological studies to better understand the role of intracellular ion transport in human health and disease.

Recently, we have established a second major focus on genome integrity. Working with the Dirk Remus lab, which specializes in the *in vitro* reconstitution of the eukaryotic replication machinery, we are applying single-particle cryo-EM approaches to elucidate the mechanisms by which DNA damage is recognized in order to initiate checkpoint cascades and ensure genome integrity.

Recent publications that highlight my group's expertise in the structural and functional characterization of intracellular transport proteins include the (1) the first full-length high-resolution structures of an IP_3R, (2) the first structure of a mammalian CLC transporter, and (3) the first structure of the lysosomal potassium channel TMEM175, and (4) the first structural characterization of the yeast 9-1-1 checkpoint clamp loader in complex with the Rad24-RFC checkpoint clamp loader and a 5' ss/dsDNA substrate,

1. Schrecker M, Korobenko J, Hite RK. Cryo-EM structure of the lysosomal chloride-proton exchanger CLC-7 in complex with OSTM1. Elife. 2020 Aug 4;9PubMed PMID: 32749217; PubMed Central PMCID: PMC7440919.
2. Oh S, Paknejad N, Hite RK. Gating and selectivity mechanisms for the lysosomal K^+ channel TMEM175. Elife. 2020 Mar 31;9PubMed PMID: 32228865; PubMed Central PMCID: PMC7141809.
3. Paknejad N, Hite RK. Structural basis for the regulation of inositol trisphosphate receptors by Ca^{2+} and IP_3. Nat Struct Mol Biol. 2018 Aug;25(8):660-668. PubMed PMID: 30013099; PubMed Central PMCID: PMC6082148.

4. Castaneda JC*, Schrecker M*; Remus D[#]; Hite RK[#]. Mechanisms of loading and release of the 9-1-1 checkpoint clamp. bioRxiv, 2021. https://doi.org/10.1101/2021.09.13.460164 * These authors contributed equally to this work. # Corresponding author

B. Positions and Honors

Positions and Employment

2003 - 2004	Research Technician, New York University, New York, NY
2004 - 2010	Graduate Student, Harvard University, Boston, MA
2011 - 2016	Postdoctoral Fellow, Rockefeller University, New York, NY
2016 -	Assistant Member, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY

Other Experience and Professional Memberships

2020 -	Member, Biophysical Society

Honors

2004 - 2005	National Collegiate Athletic Association Postgraduate Scholarship, National Collegiate Athletic Association
2012 - 2015	Howard Hughes Medical Institute Fellow of the Helen Hay Whitney Foundation, Helen Hay Whitney Foundation
2016	Tri-Institutional Breakout Prize for Junior Investigators, Tri-Institutions of Memorial Sloan Kettering, Rockefeller University and Weill Cornell Medical
2016 - 2021	Josie Robertson Investigator Program, Memorial Sloan Kettering Cancer Center
2018 - 2021	Searle Scholar

C. Contributions to Science

1. **Mechanistic basis of loading and release of the 9-1-1 checkpoint clamp.** Incidents of Genomic stress, such as stalled replication forks or DNA double-strand breaks initiate signaling cascades that halt DNA replication and initiate the DNA damage response. To elucidate how 5' ss/dsDNA junctions, which arise DNA double-strand breaks or replication fork stalling, are recognized by the 9-1-1 checkpoint clamp to activate the apical checkpoint kinase, Ataxia telangiectasia and Rad3-related (ATR; Mec1 in yeast), we determined structures of yeast 9-1-1 in complex with the Rad24-RFC checkpoint clamp loader and a 5' ss/dsDNA junction. Structures revealed that the 5' DNA junction is exclusively recognized by Rad24, the defining subunit of the Rad24-RFC checkpoint clamp loader. Moreover, we observed that Rad24 also makes unique contacts with 9-1-1 that together ensure that 9-1-1 is specifically loaded onto 5' ss/dsDNA junctions. Once associated with the DNA junction, Rad24-RFC hydrolyzes ATP to release 9-1-1 and recruit ATR/Mec1. A structure of Rad24-RFC in a post-ATP-hydrolysis state revealed that ATP hydrolysis induces large conformational changes in Rad24-RFC that prevent 9-1-1 and the DNA junction from remaining associated with Rad24-RFC. Thus, our work revealed the mechanisms by which 9-1-1 is specifically loaded onto 5' DNA junctions.

    a. Castaneda JC*, Schrecker M*; Remus D[#]; Hite RK[#]. Mechanisms of loading and release of the 9-1-1 checkpoint clamp. bioRxiv, 2021. https://doi.org/10.1101/2021.09.13.460164 * These authors contributed equally to this work. # Corresponding author

2. **Mechanisms of lysosomal pH regulation.** The lysosome is major cellular recycling center and regulation of lysosomal pH is essential for its function. Dysfunction of several proteins, including the K^+ channel TMEM175 and the chloride-proton exchanger CLC-7, can lead to dysregulation of lysosomal pH and are associated with human disease. TMEM175 is an evolutionarily distinct cation channel that forms the primary potassium permeation pathway in the lysosome and establishes a membrane potential critical for lysosomal function. Mutations in TMEM175 lead to dysregulated lysosomal pH and are associated with the development of Parkinson's Disease. To begin to understand the basis of ion gating, permeation and

selectivity, we determined structures of human TMEM175 in open and closed states in the presence of K^+ at resolutions up to 2.45 Å. In the closed structure, a hydrophobic constriction formed by isoleucine side chains that is too narrow to permit ion permeation is present near the center of the pore. In the open structure, the constriction expands and ordered water molecules are resolved that flank either side. Molecular dynamics simulations reveal that cations can traverse the pore in a partially hydrated state and that relative energetics of dehydration contribute to K^+ selectivity, thereby establishing a novel mechanism for K^+ selectivity by TMEM175. CLC-7 is a member of the CLC family of chloride channel and transporters that forms the primary chloride permeation pathway in lysosomes and the ruffled border of osteoclasts. Its mutation, along with mutations of its obligatory β-subunit, OSTM1, can lead to osteopetrosis, neurodegeneration and other diseases. A comparison of structures of CLC-7 alone and in complex with OSTM1 support a model where the heavily-glycosylated OSTM1 serves as a shield to protect the luminal surface from the degradative environment of the lysosomal lumen. The structures also revealed that CLC-7 contains conserved ATP and phosphatidylinositol lipid binding sites that participate in transporter regulation. Together, our analyses of TMEM175 and CLC-7 provided a mechanistic understanding of the regulation of lysosomal pH by TMEM175 and CLC-7 and OSTM1 and insights into how dysregulation is associated with disease.

a. Schrecker M, Korobenko J, Hite RK. Cryo-EM structure of the lysosomal chloride-proton exchanger CLC-7 in complex with OSTM1. Elife. 2020 Aug 4;9PubMed PMID: 32749217; PubMed Central PMCID: PMC7440919.

b. Oh S, Paknejad N, Hite RK. Gating and selectivity mechanisms for the lysosomal K^+ channel TMEM175. Elife. 2020 Mar 31;9PubMed PMID: 32228865; PubMed Central PMCID: PMC7141809.

c. Oh S*, Zhou W*, Marinelli F*, Lee J, Choi HJ, Kim M, Faraldo-Gómez J#, **Hite RK#**. Differential ion dehydration properties explain selectivity in the non-canonical lysosomal K^+ channel TMEM175. bioRxiv, 2021. * These authors contributed equally to this work. # Corresponding author

3. **Structural characterization of intracellular Ca^{2+} signaling by inositol 3,4,5-trisphosphate receptors.** Cytosolic Ca^{2+} signaling plays critical roles in numerous processes including differentiation, cell death and fertilization. In non-excitable cells, inositol 3,4,5-trisphosphate receptors (IP3R) serve as the primary Ca^{2+} release channels that mediate the release of Ca^{2+} from the endoplasmic reticulum. IP_3Rs are activated by the second messengers IP_3 and nanomolar Ca^{2+} concentrations, but are inhibited by micromolar Ca^{2+} concentrations. Using single particle cryo-EM, we determined high-resolution structures of a human IP_3R in ligand-free closed states, pre-activated IP_3-bound states and Ca^{2+}-bound inhibited states. Comparison of the ligand-free and IP_3-bound structures revealed that conformational changes induced by IP_3 binding at the distal IP_3-binding are propagated to the membrane pore located nearly 100 Å away through rearrangements of several large helical domains. Two Ca^{2+} ions were resolved in the Ca^{2+}-bound structures at subunit interfaces that stabilize the channel in an inhibited conformation in which IP3 binding is uncoupled from the membrane pore. Thus, the structures established a mechanistic basis for Ca^{2+}-dependent inhibition of IP_3Rs and the activation by IP_3.

a. Paknejad N, Hite RK. Structural basis for the regulation of inositol trisphosphate receptors by Ca^{2+} and IP_3. Nat Struct Mol Biol. 2018 Aug;25(8):660-668. PubMed PMID: 30013099; PubMed Central PMCID: PMC6082148.

4. **Structural and functional characterization of gating of voltage- and ligand-gated K^+ channels.** Potassium channels in the plasma membrane establish and regulate the resting membrane potential of excitable and non-excitable cells. In excitable cells, activation of K^+ channels in response to stimuli such as voltage and ligand binding hyperpolarizes the membrane, thereby depressing action potential firing. The Slo family of ligand- and voltage-gated K^+ channels are broadly expressed and play critical roles in smooth muscle tone, neuronal excitation and sperm activation. My postdoctoral studies employed structural and electrophysiological approaches to determine how Slo channels sense and integrate different stimuli to regulate their activity. By comparing structures of Ca^{2+}- and voltage-activated K^+ channel Slo1, we observed that Ca^{2+} and Mg^{2+} bind at inter-domain interfaces to stabilize the pore in an activated state, establishing a molecular basis for the functional coupling of voltage- and ligand-activation that are hallmarks of Slo1 channels. A structural titration performed by collecting cryo-EM data of the Na^+-activated

K^+ channel Slo2.2 channel in the presence of different Na^+ concentrations further allowed us to demonstrate that the Na^+ dependence of conformational change that we resolved in our cryo-EM analysis correlated almost exactly with functional changes in channel activity that we observed in electrophysiological experiments. Together, these studies revealed the molecular basis for gating in Slo channels.

a. Hite RK, MacKinnon R. Structural Titration of Slo2.2, a Na^+-Dependent K^+ Channel. Cell. 2017 Jan 26;168(3):390-399.e11. PubMed PMID: 28111072; PubMed Central PMCID: PMC5382815.

b. Tao X, Hite RK, MacKinnon R. Cryo-EM structure of the open high-conductance Ca^{2+}-activated K^+ channel. Nature. 2017 Jan 5;541(7635):46-51. PubMed PMID: 27974795; PubMed Central PMCID: PMC5500982.

c. Hite RK, Tao X, MacKinnon R. Structural basis for gating the high-conductance Ca^{2+}-activated K^+ channel. Nature. 2017 Jan 5;541(7635):52-57. PubMed PMID: 27974801; PubMed Central PMCID: PMC5513477.

d. Hite RK, Yuan P, Li Z, Hsuing Y, Walz T, MacKinnon R. Cryo-electron microscopy structure of the Slo2.2 Na(+)-activated K(+) channel. Nature. 2015 Nov 12;527(7577):198-203. PubMed PMID: 26436452; PubMed Central PMCID: PMC4886347.

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

Ongoing Research Support

NIH R01 GM132307, National Institutes of General Medical Sciences
Hite, Richard (PI)
04/01/2019-01/31/2024
Molecular characterization of the multi-modal regulation of inositol 1,4,5-trisphosphate receptors
The goal of this study is to determine the mechanisms that underlie the function of inositol 1,4,5-trisphosphate receptors.
Role: PI

Completed Research Support

Searle Scholars Program
Hite, Richard (PI)
07/01/18-06/30/21
Structural Characterization of the Polymodal Regulation of Inositol 1,4,5-trisphosphate Receptor Gating
The goal is this project is to determine the structure of a human Inositol Triphosphate Receptor.
Role: PI