BIOGRAPHICAL SKETCH

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NAME	POSITION TITLE
Joshua A. Lees	Postdoctoral Associate
eRA COMMONS USER NAME JOSHUALEES	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Houghton College (Houghton, NY)	B.S.	1999-2003	Biology
Princeton University (Princeton, NJ)	Ph.D.	2004-2010	Molecular Biology
Yale University (New Haven, CT)	Post-doctoral	2013-pres.	Structural Biology

A. Personal Statement

I am interested in the cellular machinery that regulates eukaryotic organellar membrane lipid composition, both through the activity of lipid transfer proteins that localize to membrane contact sites and through the activity of lipid kinases and phosphatases responsible for the interconversion of phosphoinositide species that shape the identity and function of organelle membranes. I approach these questions primarily using the tools of structural biology and biochemistry. As a structural biologist, I am trained in the use of both X-ray crystallography and cryoEM, with a productive track record of publications making use of both techniques. I am also highly experienced in protein purification and lipid biochemistry, including the *in vitro* analysis of protein-lipid interactions and reconstitution of protein-mediated lipid transfer between membranes.

B. Positions

2004-2010 Ph.D. student, Princeton University (laboratory of F.M. Hughson)
2010-2013 Assistant Professor of Biology, Ave Maria University
2013-present Postdoctoral Associate in Cell Biology, Yale University (laboratory of K.M. Reinisch)

C. Selected peer-reviewed publications (Selected from 5 peer-reviewed publications)

1. Lees, Joshua A, Calvin K Yip, Tom Walz, and Frederick M Hughson. (2010) **Molecular organization of the COG vesicle tethering complex**. *Nat Struct Mol Biol* 17(11):1292-1297.

2. Lees, Joshua A, Mirko Messa, Elizabeth Sun, Heather Wheeler, Federico Torta, Markus Wenk, Pietro DeCamilli, and Karin M Reinisch. (2017) **Lipid transport by TMEM24 at ER-plasma membrane contacts regulates pulsatile insulin secretion**. *Science* 355(6326):eeah6171.

3. Lees, Joshua A, Yixiao Zhang, Michael Oh, Curtis M. Schauder, Xiaoling Yu, Jeremy Baskin, Kerry Dobbs, Luigi D. Notarangelo, Pietro De Camilli, Tom Walz, Karin M Reinisch. (2017) **Architecture of the PI4KIIIα lipid kinase complex**. *Proc Natl Acad Sci USA* 114(52):13720-13725.

4. Kumar, Nikit, Marianna Leonzino, William Hancock-Cerutti, Florian A. Horenkamp, PeiQi Li, Joshua A. Lees, Heather Wheeler, Karin M. Reinisch, Pietro De Camilli. (2018) **VPS13A and VPS13C are lipid transport proteins differentially localized at ER contact sites.** *J Cell Biol* 217(10):3625-3639.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
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NAME: Karin M. Reinisch

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

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
Harvard-Radcliffe, Cambridge, MA	B.A.	05/1989	Chemistry
Harvard University, Cambridge, MA	Ph.D.	05/1995	Chemistry (biochemistry/structural biology)
Harvard University, Cambridge, MA	N.A.	07/2001	Molecular & Cell Biology

A. Personal Statement

My training is as a structural biologist, and as such my expertise is in protein chemistry, the expression and purification of proteins and macromolecular assemblies, macromolecular crystallography, and biochemistry. I have a long standing interest in how macromolecular machines are assembled and function that began with structural studies of protein-nucleic acid interactions during my graduate studies (1) and continued with the structure determination of the 52 **M**Da reovirus core particle, a massive RNA processing machine, in the laboratory of S. C. Harrison (2). Earliest efforts in my own laboratory continued the theme of my graduate and post-doctoral studies, seeking to understand how proteins interact with and recognize nucleic acids. The strength of Yale's Cell Biology Department in membrane biology prompted me to initiate a new area of research, to unravel the molecular basis for the regulation of membrane trafficking. We made significant contributions in this area, specifically in understanding how small GTPases, which help to determine organelle identity, and tethering complexes, which function in vesicle fusion, are regulated, so that vesicular cargo is delivered to the correct organelle. In the past six years, we have focused on how the lipid composition of different organelles, another determinant of how cargo is delivered to the correct destination, is modulated. In all of our studies, we have collaborated with cell biologists, who can test functionally any hypotheses arising from biochemical and structural studies, and we continue to do so now in exploring membrane lipid homeostasis as proposed here. Our past collaborators have included Peter Novick, Susan Ferro-Novick, Chris Burd, and James Rothman. More recently we have worked closely with my colleague Pietro De Camilli in studying the enzymatic complexes that make and degrade phosphoinositide signaling lipids as well as lipid transport proteins that move specific lipids between compartments, and we are also working with another colleague Thomas Melia in understanding how autophagosomal isolation membranes are formed. Finally, the group is acquiring expertise in cryo-electron microscopy (Cryo-EM) reconstruction to take advantage of the EM "resolution revolution" for our projects.

1. Reinisch KM, Chen L, Verdine GL, Lipscomb WN. The crystal structure of HaeIII methyltransferase convalently complexed to DNA: an extrahelical cytosine and rearranged base pairing. *Cell*. 1995;82:143-53.
2. Reinisch KM, Nibert ML, Harrison SC. Structure of the reovirus core at 3.6 Å resolution. *Nature*. 2000;404:960-7.

B. Positions and Honors

1989-1995 Ph.D. student at Harvard University (laboratory of W.N. Lipscomb)
1995-2001 Post-doctoral Fellow in Molecular and Cellular Biology, Harvard (laboratory of S.C. Harrison)
2001-2007 Assistant Professor,Yale University School of Medicine
2007-2009 Associate Professor, Yale University School of Medicine
2009-2015 Associate Professor with tenure, Yale University School of Medicine
2015- Secondary Appointment in Molecular Biophysics and Biochemistry

2015- Professor, Yale University School of Medicine

Honors

1987 *Phi Beta Kappa*
1989 *Summa cum laude*
2002 Pew Scholars Award
2018- Jean and David W. Wallace endowed chair

C. Contributions to Science

My group investigates the mechanisms underlying membrane biology and trafficking. Our most recent research has centered primarily on the fundamental question of how membrane lipid composition is regulated. Work published in the last five years is indicated in **blue**.

1. Phosphatidylinositol-4-phosphate (PI4P) homeostasis. PI4P is enriched at the Golgi apparatus and at the plasma membrane, where it serves as a determinant of membrane identity, enabling the recruitment for organelle-specific functions of proteins which recognize its headgroup. At the plasma membrane, PI4P also has a critical function in phosphoinositide signaling as a precursor for the signaling molecules phosphatidylinositol-(4,5)-bisphosphate [(PI(4,5)P_2] and phosphatidylinositol-(3,4,5)-trisphosphate. A major effort in the laboratory was directed toward understanding PI4P metabolism, and one of our main projects concerns the PI4KIIIα lipid kinase complex. This complex is conserved in eukaryotes and synthesizes PI4P at the plasma membrane. Our structural studies so far have formed the basis for understanding how the kinase is recruited to the plasma membrane by the scaffolding proteins Efr3, Ypp1/TTC7, and hyccin and suggested how complex assembly may be regulated to modulate kinase activity there (**3**-**5**). We have also obtained insights as to how PI4P levels at the Golgi apparatus are regulated, obtaining a crystal structure of a complex comprising Vps74/GOLPH3 and Sac1. Sac1 is a PI4P phosphatase which resides primarily at the ER, whereas Vps74/GOLPH3 localizes to the Golgi. The structure explains how Sac1 can be recruited to the Golgi by Vps74/GOLPH3 (**6**) to regulate PI4P levels there.

3. Wu X, Chi RJ, Baskin JM, Lucast L, Burd CG, De Camilli P, Reinisch KM. Structural insights into assembly and regulation of the plasma membrane phosphatidyl 4-kinase complex. *Dev Cell*. 2014;28:19-29. PMCID: 4349574

4. [1]Baskin JM, [1]Wu X, Christiano R, Oh MS, Schauder CM, Gazzerro E, Messa M, Baldasarri S, et al et *Reinisch KM, *De Camilli P. The leukodystrophy protein FAM126 (hyycin) regulates PtdIns(4)P synthesis at the plasma membrane. *Nat Cell Bio*. 2016; 18(1): 132-8. PMCID: 4689616. [1]equal contributions. *co-corresponding.

5. [1]Lees JA, [1]Zhang Y, Oh MS, Schauder CM et al et *De Camilli P, *Walz T, *Reinisch KM. Architecture of the human PI4KIIIalpha lipid kinase complex. *PNAS* 2017; 114: 13720-13725. PMCID: 5748228. [1]equal contributions.*co-corresponding.

6. [1]Cai Y, [1]Deng Y, Horenkamp F, *Reinisch KM, *Burd CG. Sac1-Vps74 structure reveals a mechanism to terminate phosphoinositide signaling in the Golgi apparatus. *J Cell Biol*. 2014;206(4):485-91. PMCID: 4137058. [1]equal contributions. *co-corresponding.

2. Processes at membrane contact sites. It is becoming clear that membrane lipid composition is modulated at membrane contact sites, where two organelles are apposed closely enough so that non-vesicular lipid exchange between their membranes is possible. We have been at the forefront in exploring the molecular basis for lipid exchange at contact sites. The Extended-Synaptotagmin proteins were previously characterized as tethers that are localized to and maintain ER-plasma membrane contact sites. Our crystal structure of Extended-Synaptotagmin2 (E-Syt2) demonstrated that a protein module of previously unknown function, the SMP domain, within E-Syt2 is a lipid binding module, strongly supporting the idea that lipid exchange at these contact sites is mediated by lipid transfer proteins (**7, 8**). Because SMP-domains are also found at other contact sites (for example, in the ERMES complex at ER-mitochondrial sites) our findings have broad implications for these sites also. We further identified the SMP-domain protein TMEM24 as a transporter of PI, which localizes to ER-plasma membrane contacts reversibly in a calcium dependent manner, elucidating how TMEM24 coordinates calcium and phosphoinositide signaling pathways to control insulin secretion (**9**). To better understand how cholesterol is trafficked between compartments, we determined a structure for the lipid transport module of Ysp2p, a member of a major family of sterol transporters functioning at membrane contact sites in yeast, both in its apo and sterol-bound forms (**10**). In the same study we showed that all metazoan members of this family (GRAMD1a-c) also transfer sterol and likely play a role in sterol dynamics.

7. Schauder CM, Wu X, Saheki Y, Narayanaswamy P, Torta F, Wenk MR, *De Camilli P, *Reinisch KM. Structure of a lipid-bound extended synaptotagmin indicates a role in lipid transfer. *Nature*. 2014; 510: 552-555. PMCID: 4135724. *co-corresponding.

8. Saheki Y, Bian X, Schauder CM, Sawaki Y, Surma MA, Klose, C, Pincet F, Reinisch KM, De Camilli P. Control of plasma membrane lipid homeostasis by the extended synaptotagmins. *Nat. Cell Biol*. 2016; 18:504-515. PMCID: 4848133.

9. [1]Lees JA, [1]Messa M, Sun EW, Wheeler H, Torta F, Wenk MR, *De Camilli P, *Reinisch KM. Lipid transport by TMEM24 at ER-plasma membrane contacts regulates insulin secretion. *Science* 2017; 10.1126/science.aah617. PMCID: 5414417. [1]equal contributions.*co-corresponding.
10. [1]Horenkamp FA, [1]Valverde DP, Nunnari J, Reinisch KM. Molecular basis for sterol transport by StART-like lipid transfer domains. *EMBO J* 2018; 37. 10.15252/embj.201798002. PMCID: 5852651. [1]equal contributions.

3. Mechanisms for activating Rab GTPases. Besides the phosphoinositides, a second major determinant of membrane identity is the subset of activated Rab GTPases present at a particular membrane. To better understand how Rabs are activated, we have obtained crystal structures for complexes of Rabs bound to their activating guanine nucleotide exchange factor (GEF). These include the Sec2/Sec4 (**11**), TRAPPI/Ypt1 ((**12**); see also (**13**)), and DENND1B/Rab35 (**14**) complexes, where DENN-domain proteins are the largest GEF family identified to date. Interestingly, although these GEFs all have different folds, our work has helped to show that the mechanisms underlying GTPase activation are similar. In most cases, GEF binding causes restructuring of the Rab switch regions. This removes an aromatic residue that normally stabilizes bound nucleotide from the nucleotide binding pocket, lowering the affinity of the Rab for nucleotide. It also opens the nucleotide binding pocket to solvent, facilitating nucleotide exchange.

11. Dong G, Medkova M, *Novick P, *Reinisch KM. A catalytic coiled coil: structural insights into the activation of the Rab GTPase Sec4p by Sec2p. *Mol Cell*. 2007;25(3):455-62. PMCID: 1847580. *co-corresponding.
12. Cai Y, Chin HF, Lazarova D, Menon S, Fu C, Cai H, et al. et *Ferro-Novick S, *Reinisch KM. The structural basis for activation of the Rab Ypt1p by the TRAPP membrane-tethering complexes. *Cell*. 2008;133(7):1202-13. PMCID: 2465810. *co-corresponding.
13. Tan D, Cai Y, Wang J, Zhang J, Menon S, Chou HT, *Ferro-Novick S, *Reinisch KM, *Walz T. The EM structure of the TRAPPIII complex leads to the identification of a requirement for COPII vesicles on the macroautophagy pathway. *Proc Natl Acad Sci U S A*. 2013;110(48):19432-7. PMCID: 3845172. *co-corresponding.
14. Wu X, Bradley MJ, Cai Y, Kümmel D, De La Cruz EM, Barr FA, Reinisch KM. Insights regarding guanine nucleotide exchange from the structure of a DENN-domain protein complexed with its Rab GTPase substrate. *Proc Natl Acad Sci U S A*. 2011;108(46):18672-7. PMCID: 3219131.

4. Proteins manipulating membranes. Previously, we contributed to understanding the architecture of multi-component tethering complexes in the CATCHR family, which are thought to promote the assembly of the SNARE complex and other membrane fusion machinery. We discovered that CATCHR complex subunits have similar folds in that they are all composed of α-helical bundles assembled into rods (**11**) and demonstrated that the GARP complex, a tether that functions at the Golgi, is a member of the CATCHR family (**12**). We also studied regulation of SNARE assembly at the neuronal synapse, in particular how the protein complexin interacts with assembling SNARE complexes there so as to regulate secretory vesicle fusion (**13**). Our crystal structure of complexin with an incompletely assembled SNARE complex suggested a model for how this protein might inhibit SNARE assembly pending an action potential.

15. Dong G, Hutagalung AH, Fu C, *Novick P, *Reinisch KM. The structures of exocyst subunit Exo70p and the Exo84p C-terminal domains reveal a common motif. *Nat Struct Mol Biol.* 2005;12(12):1094-100. *co-corresponding.
16. Vasan N, Hutagalung A, Novick P, Reinisch KM. Structure of a C-terminal fragment of its Vps53 subunit suggests similarity of Golgi-associated retrograde protein (GARP) complex to a family of tethering complexes. *Proc Natl Acad Sci U S A.* 2010;107(32):14176-81. PMCID: 2922553.
17. Kummel D, Krishnakumar SS, Radoff DT, Li F, Giraudo CG, Pincet F, *Rothman JE, *Reinisch KM. Complexin cross-links prefusion SNAREs into a zigzag array. *Nat Struct Mol Biol.* 2011;18:927-33. PMCID: 3410656. *co-corresponding.

Additionally, we investigated how the bacterium *Legionella pneumophila* subverts autophagy in its host cell in order to replicate. Autophagosome maturation requires the conjugation of Atg8/LC3 to phosphatidylethanolamine in the isolation membrane. Legionella targets its effector protein RavZ to the isolation membrane to irreversibly cleave away Atg8/LC3, thereby stalling autophagy. Our structure and biochemistry studies indicated that RavZ is targeted to the isolation membrane via a phosphatidylinositol-3-phosphate binding domain and further provided first insights as to why RavZ is active only at the isolation membrane and not on soluble Atg8/LC3 (**18**). This paper represents our first collaboration with Tom Melia, with whom we will collaborate further in the next project period.

18. Horenkamp FA, Kauffman KJ, Kohler LJ, Sherwood RK *et al. et* *Melia TJ, *Reinisch KM. The Legionella Anti-autophagy Effector RavZ Targets the Autophagosome via PI3P- and Curvature-Sensing Motifs. *Dev. Cell* 2015; 34: 569-576. PMCID: 4594837. *co-corresponding.

5. Quality Control. Early efforts in the group continued the theme of my graduate and post-doctoral studies and focused on understanding how proteins interact with and recognize specific nucleic acid sequences. Our studies

of the Ro 60 kDa autoantigen address the question how chaperone proteins can distinguish aberrant non-coding RNAs from properly folded ones. Ro, a major target of the immune response in patients with systemic lupus erythythematosus, is found predominantly in the cytoplasm, complexed with small RNAs called Y RNAs. When the cells are stressed, however, Ro is translocated to the nucleus where it recognizes misfolded non-coding RNAs and targets them for degradation. Our structural and biochemical studies of Ro and Ro/RNA complexes (**19, 20)** suggested that Ro recognizes mainly the shape of misfolded RNAs, rather than any specific sequence, and requires a 3' extension, a feature of many newly transcribed RNAs. As Ro is bound only to improperly folded RNAs in oocyte extracts, Ro likely functions as a scavenger for RNAs that are not recognized by specific binding partners. In addition, we have biochemically and structurally investigated the La protein (**21**), which is involved in tRNA processing and helps certain tRNAs to fold properly, and the heterotrimeric TRAMP complex (**22**), which degrades aberrant non-coding RNAs together with the exosome.

19. Fuchs G, Stein AJ, Fu C, Reinisch KM, Wolin SL. Structural and biochemical basis for misfolded RNA recognition by the Ro autoantigen. *Nat Struct Mol Biol.* 2006;13(11):1002-9.
20. Stein AJ, Fuchs G, Fu C, Wolin SL, Reinisch KM. Structural insights into RNA quality control: the Ro autoantigen binds misfolded RNAs via its central cavity. *Cell.* 2005;121(4):529-39. PMCID: 1769319.
21. Dong G, Chakshusmathi G, Wolin SL, Reinisch KM. Structure of the La motif: a winged helix domain mediates RNA binding via a conserved aromatic patch. *EMBO J.* 2004;23(5):1000-7. PMCID: 380972.
22. Hamill S, *Wolin SL, *Reinisch KM. Structure and function of the polymerase core of TRAMP, a RNA surveillance complex. *Proc Natl Acad Sci U S A.* 2010;107(34):15045-50. PMCID: 2930566. *co-corresponding.

Complete list of published work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/karin.reinisch.1/bibliography/40447932/public/?sort=date&direction=ascending.

D. Research Support
Ongoing:

1R35GM131715-01 (Reinisch-PI)
NIH/NIGMS
Molecular Basis for Membrane Lipid Homeostasis 04/01/2019 – 03/31/2024

This R35 award replaces R01GM080616 and R01GM114068 below.

Completed within the past three years:

5 R01 GM080616-11 (Reinisch-PI) 8/1/2007-03/31/2019
NIH/NIGMS
Lipid transporters and Lipid Homeostasis at Membrane Contact Sites
Our goal is to understand the molecular basis for processes at membrane contact sites.

5 R01 GM114068-03 (Reinisch-PI) 6/1/2015-3/31/2019
NIH/NIGMS
Pathophysiology of Plasma Membrane PI4P Generation
The aim is to elucidate the function and regulation of the PI4KIIIα complex, which is responsible for the bulk of phosphatidylinositol-4-phosphate at the plasma membrane, at the molecular, cellular, and organismal levels.

R03 AG046941 (Reinisch-PI) 5/1/2014-2/29/2016
NIH/NIAID
Cargo Recognition by the Retromer Sorting Complex.
Our goal was to understand the molecular basis for cargo recognition by retromer.