BIOGRAPHICAL SKETCH

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NAME: Kirchhausen, Tomas

eRA COMMONS USER NAME: kirchhausen

POSITION TITLE: Professor of Cell Biology and Springer Family Professor of Pediatrics, Harvard Medical School and Senior Investigator, Program in Cellular and Molecular Medicine, Boston Children's Hospital

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	Completion Date	FIELD OF STUDY
Universidad Peruana Cayetano Heredia, Peru	B.S.	04/1972	Biology
Instituto Venezolano de Investigaciones Cientificas, Venezuela	M.S.	06/1975	Biophysics
Universidad Peruana Cayetano Heredia, Peru	M.S.	06/1975	Biophysics
Instituto Venezolano de Investigaciones Cientificas, Venezuela	Ph.D.	06/1977	Biophysics and Physiology
Harvard University	M.A. (HON)	12/1999	Cell Biology

A. Personal Statement

Successful and productive research projects in structural biology, cell biology, chemical genetics, live cell and single-molecule fluorescence microscopy imaging, have enabled strong track record in advising pre-doctoral and post-doctoral trainees. Experience in teaching and organizing international, graduate-level courses in different countries. Principal participant since 1997 in biennial membrane-biology EMBO course (Cargese). Chair/co-chair of two Gordon Research Conferences. Leader (and co-founder) for 7 years of the Harvard-Portugal program, the longest lasting international collaborative educational and research program at HMS.

B. Positions and Honors

Positions and Employment

1980- Tutor in Biochemical Sciences, Harvard University
1981-1982 Postdoctoral, Harvard University, Department of Biochemistry and Molecular Biology
1982-1985 Research Associate in Biochemistry, Harvard University
1984-1985 Assistant Head Tutor, Biochemical Sciences, Harvard University
1986-1991 Assistant Professor of Anatomy and Cellular Biology, Harvard Medical School
1991-1993 Associate Professor of Anatomy and Cellular Biology, Harvard Medical School
1992-1999 Investigator, The Center for Blood Research, Inc.
1993-1999 Associate Professor, Department of Cell Biology, Harvard Medical School
1999- Professor, Department of Cell Biology, Harvard Medical School
1999-2012 Senior Investigator, Immune Disease Institute, (formerly CBR Inst. for Biomedical Research)
2006-2012 Chair IT Committee, Immune Disease Institute.
2007-2012 Executive Faculty Committee, Immune Disease Institute.
2009-2015 HMS Director, Harvard-Portugal Program in Translational Research and Medical Education
2012- Senior Investigator, Program in Cellular and Molecular Medicine (PCMM) at Boston Children's Hospital
2012- Professor, Department of Pediatrics, Harvard Medical School
2013- Springer Family Professor of Pediatrics, Harvard Medical School
2016- Visiting Scientist at Janelia Research Campus, HHMI

Honors

1982-1984	Research Fellow, Charles A. King Trust
1986-1991	Established Investigator Award, American Heart Association
2002-	Honorary Professor, Universidad Peruana Cayetano Heredia, Peru
2008-	AAAS Fellow
2012	John Cebra Endowed Lecture, Dynamics and Endocytosis. Marine Biological Laboratory, Woods Hole
2013-	Springer Family Professor of Pediatrics, Harvard Medical School
2013-	Doctor Honoris Causa, Universidad Ricardo Palma, Peru
2014-	Associated Member EMBO
2014-	SMB Jose Laguna Lecture for Outstanding Basic Research in Dynamics of Endocytosis
2015-	Netherlands Society for Biochemistry and Molecular Biology: Speaker of the year
2015	EMBO Keynote Lecture, Systems Biology of Infection
2016-	Academia Nacional de Ciencias (Peru), Académico Correspondiente
2016-	Honorary Professor, University of Hong Kong
2017	EMBO Keynote Lecture, FEBS – EMBO Advance Lecture Course in Molecular Architecture, Dynamics and Function of Biomembranes
2017-	Honorary Member, Associazione di Biologia Cellulare e del Differenziamento, Italy
2018	EMBO Global Lecturer, India
2020	IUBMB Jubilee Lecture, Lanzen Peru 2020

Other Experience and Professional Memberships

1996-1999	Ad-hoc member of NIH Study Section, CB-1
1999-2003	Member, NIH Study Section, CDF-2
2001-	Member, Advisory Committee, Imaging Facility, Dept. of Cell Biology, Harvard Med. School
2004-2012	Ad-hoc member of NIH Study Sections CD-4 and ZRG1

C. Contribution to Science

1. Molecular architecture of clathrin coats and mechanism of uncoating (1981-) [1 2 3 4]

Through studies extending over three decades, we defined the structure and interactions of clathrin and many of its associated proteins and the assembly and uncoating mechanisms for clathrin coats. Highlights: clathrin heavy-chain sequence -- at the time, the longest protein sequence derived by cDNA cloning; high-resolution crystal structures of a large N-terminal fragment of clathrin and its complex with a "clathrin box" peptide, of the core of the AP-1 adaptor complex, and of a complex between Dishevelled and the μ2 subunit of the AP-2 adaptor complex; subnanometer structure of a clathrin coat (22MDa) by electron cryomicroscopy (cryoEM), yielding a complete molecular model for clathrin at 8 Å resolution; cryoEM structure of a clathrin coat with bound auxilin and Hsc70 and subsequent *in vitro* and *in vivo* single-molecule total internal reflection fluorescence (TIRF) microscopy studies of uncoating.

1. Kirchhausen, T., Owen, D. & Harrison, S. C. Molecular structure, function, and dynamics of clathrin-mediated membrane traffic. Cold Spring Harb Perspect Biol 6, a016725 (2014). PMID: 2478982. PMC3996469.
2. Fotin, A. et al. Molecular model for a complete clathrin lattice from electron cryomicroscopy. Nature 432, 573–579 (2004).
3. Xing, Y. et al. Structure of clathrin coat with bound Hsc70 and auxilin: mechanism of Hsc70-facilitated disassembly. EMBO J 29, 655–665 (2010). PMCID: PMC2830701.
4. Böcking, T., Aguet, F., Harrison, S. C. & Kirchhausen, T. Single-molecule analysis of a molecular disassemblase reveals the mechanism of Hsc70-driven clathrin uncoating. 18, 295–301 (2011). PMCID: PMC3056279.

2. Live-cell imaging (2004-) [5 6 7 8]

Building on the biochemical and structural discoveries outlined above, we analyzed mechanisms of coated-vesicle formation in living cells, applying emerging technologies in fluorescence microscopy and live-cell imaging. Our use of spinning disc confocal microscopy led to the following description of molecular events in clathrin-mediated endocytosis: coated pits nucleate at the plasma membrane and grow by steady addition of clathrin triskelions; Hsc70-mediated uncoating follows promptly upon dynamin-induced membrane scission; arrival of auxilin, the clathrin specific, J-domain co-chaperone for Hsc70, determines the timing of this event;

clathrin assembly ordinarily provides the principal driving force for membrane invagination, but at high membrane tension or with very elongated cargo, actin polymerization is also required. When our TIRF microscopy technology had reached the level of single-molecule counting, we showed that coated-pit initiation proceeds by coordinated arrival of clathrin and the AP2 adaptor complex, the latter recruited by interaction with PtdIns (3,4)P2, and that accessory proteins are then essential for sustained growth, and that a single rung of a dynamin tube is sufficient for coated pit neck scission. Our most recent findings that follow our keen and long standing interests on studies focusing on mechanisms of cell host / virus interactions revealed that inhibiting the PIKfyve kinase through two small-molecule related antivirals are potently prevent Zaire ebolavirus and SARS-CoV-2 infection.

5. Ehrlich, M. et al. Endocytosis by random initiation and stabilization of clathrin-coated pits. 118, 591–605 (2004); Massol, R. H., Boll, W., Griffin, A. M. & Kirchhausen, T. A burst of auxilin recruitment determines the onset of clathrin-coated vesicle uncoating. Proc Natl Acad Sci USA 103, 10265–10270 (2006).
6. Cocucci, E., Aguet, F., Boulant, S. & Kirchhausen, T. The first five seconds in the life of a clathrin-coated pit. 150, 495–507 (2012). PMCID: PMC3413093.
7. Cocucci, E., Gaudin, R. & Kirchhausen, T. Dynamin recruitment and membrane scission at the neck of a clathrin-coated pit. Mol Biol Cell 25, 3595–3609 (2014). PMCID: PMC4230619.
8. Kang, Y.-L. et al. Inhibition of PIKfyve kinase prevents infection by Zaire ebolavirus and SARS-CoV-2. Proceedings of the National Academy of Sciences 15, 202007837 (2020). PMC7263545.

3. Frontier optical-imaging modalities using LLSM (2015-2017) [9] [10] [11] [12]
After initial use at Janelia Research Campus of LLSM to resolve the dorsal from the ventral surfaces of thin lamellipodial protrusions, we built a second generation LLSM with guidance from Betzig and team at Janelia Research Campus; it has been in continuous use since July, 2014. Our published work with this instrument has included: reexamination of the clathrin-coat assembly dynamics over the entire surfaces of 45 cells and ~250,000 AP-2 traces that required MATLAB software development resulting in the generation of 3D cmeAnalysis (clathrin-mediated endocytosis analysis (with Francois Aguet and Gaudenz Danuser) ; full-cell imaging measurements of cell surface area and volume throughout the cell cycle, both for single cells in culture and for cells in the eye of a developing zebrafish embryo; studies of lipid-droplet maturation (with Walter and Farese); studies of assembly mechanisms in the ESCRT pathway in yeast (with David Teis). We provide direct evidence (with Janet Shaw) that the intracellular location of mitochondria shapes subcellular energy gradients. Finally, we developed a new generation of phosphoinositide sensors and showed that a cascade of molecular conversions, made possible by the separation of a clathrin coated vesicle from its parent membrane, can label membrane-traffic intermediates and determine their destinations. In this work, we used LLSM to visualize the sensors across the entire cellular volume, showing that a cascade of molecular signals, two of which are accumulation of PtdIns (3)P or PtdIns(4)P and PtdIns (3,4)P2 during uncoating, may bring about arrival of auxilin and Rab5GTPases, respectively.

9. Kural, C. et al. Dynamics of intracellular clathrin/AP1- and clathrin/AP3-containing carriers. Cell Rep 2, 1111–1119 (2012). PMC4472015.
10. Aguet, F. et al. Membrane dynamics of dividing cells imaged by lattice light-sheet microscopy. Mol Biol Cell (2016). doi:10.1091/mbc.E16-03-0164. PMC5221578.
11. Adell, M. A. Y. et al. Recruitment dynamics of ESCRT-III and Vps4 to endosomes and implications for reverse membrane budding. Elife 6, e31652 (2017). PMC5665648.
12. He, K. et al. Dynamics of phosphoinositide conversion in clathrin-mediated endocytic traffic. Nature 552, 410–414 (2017). PMC6263037.

4. Frontier optical-imaging modalities using AO-LLSM and FIB-SEM (2016-) [13] [14] [15] [16]
Since August 2016, we have collaborated closely with Eric Betzig in developing AO-LLSM and using it to visualize live, multicellular structures (organoids, worms, plants and most extensively, zebrafish embryos). A collaborative study with Sean Megason led to the unexpected discovery of a physical relief valve in the endolymphatic sac, a dead-end epithelial tube connected to the inner ear: Megason. The most recent study is a comprehensive collaboration with Betzig to test AO-LLSM, particularly focused on visualizing developmental processes in zebrafish embryos. Examples include: quantitative tracking of all endocytic coated pits and vesicles in muscle and brain cells (the dynamics are faster in brain, and the coats are smaller); visualization and quantification of migrating lymphocytes and human cancer cells; measurements of cell area and volume and organelle distribution during cell division; tracing of axonal growth in the hindbrain. Through our ongoing collaboration with Betzig and Herald Hess, we contributed to the nanoscale 3D visualization of endosomes

combining low temperature super-resolution fluorescence microscopy of high-pressure frozen cells coupled with focused ion beam scanning electron microscopy (FIB-SEM).

These applications demonstrate the advantage of LLSM and AO-LLSM and how they set a new standard for imaging membrane dynamics in single cells and multicellular assemblies.

13. Liu, T.-L. et al. Observing the cell in its native state: Imaging subcellular dynamics in multicellular organisms. Science 360, eaaq1392 (2018). PMC6040645.
14. Hoffman, D. P. et al. Correlative three-dimensional super-resolution and block-face electron microscopy of whole vitreously frozen cells. Science 367, eaaz5357 (2020). PMC7339343.
15. Swinburne, I. A. et al. Lamellar projections in the endolymphatic sac act as a relief valve to regulate inner ear pressure. Elife 7, 2837 (2018). PMC6008045.
16. Gao, R. et al. Cortical column and whole-brain imaging with molecular contrast and nanoscale resolution. Science 363, eaau8302 (2019). PMC6481610.

Complete List of Published Work:

1. Mateu, L., Kirchhausen, T. and Camejo, G. 1977. A low temperature structural transition in human serum low density lipoproteins. Biochim Biophys Acta. 487. 1. 243-245.
2. Mateu, L., Kirchhausen, T., Padron, R. and Camejo, G. 1977. Small-angle x-ray scattering study of human serum low-density lipoproteins with differential reactivity for an arterial proteoglycan. J Supramol Struct. 7. 3-4. 435-442.
3. Mateu, L., Kirchhausen, T. and Camejo, G. 1978. Small-angle X-ray scattering and differential scanning calorimetry studies on reversibly modified human-serum low density lipoproteins. Biochemistry. 17. 8. 1436-1440.
4. Kirchhausen, T., Untracht, S. H., Fless, G. M. and Scanu, A. M. 1979. Atherogenic diets and neutral-lipid organization in plasma low density lipoproteins. Atherosclerosis. 33. 1. 59-70.
5. Mateu, L. and Kirchhausen, T. 1979. Kinetics of thermal transitions in human serum low density lipoproteins (LDL) and neutral lipids. A dynamic small-angle X-ray scattering study. Acta Cient Venez. 30. 5. 478-483.
6. Kirchhausen, T., Fless, G. and Scanu, A. M. 1980. The structure of plasma low density lipoproteins: experimental facts and interpretations--a minireview. Lipids. 15. 6. 464-467.
7. Kirchhausen, T. and Harrison, S. C. 1981. Protein organization in clathrin trimers. Cell. 23. 3. 755-761.
8. Fless, G. M., Kirchhausen, T., Fischer-Dzoga, K., Wissler, R. W. and Scanu, A. M. 1982. Serum low density lipoproteins with mitogenic effect on cultured aortic smooth muscle cells. Atherosclerosis. 41. 2-3. 171-183.
9. Harrison, S. C. and Kirchhausen, T. 1983. Clathrin, cages, and coated vesicles. Cell. 33. 3. 650-652.
10. Hogle, J., Kirchhausen, T. and Harrison, S. C. 1983. Divalent cation sites in tomato bushy stunt virus. Difference maps at 2-9 A resolution. J Mol Biol. 171. 1. 95-100.
11. Kirchhausen, T., Harrison, S. C., Parham, P. and Brodsky, F. M. 1983. Location and distribution of the light chains in clathrin trimers. Proc Natl Acad Sci U S A. 80. 9. 2481-2485.
12. Leon, V., Kirchhausen, T., Avila, E. M. and Mateu, L. 1983. Thermal effects in human plasma high density lipoproteins (HDL)3: a 13C-FT-NMR study. Acta Cient Venez. 34. 3-4. 209-215.
13. Kirchhausen, T. and Harrison, S. C. 1984. Structural domains of clathrin heavy chains. J Cell Biol. 99. 5. 1725-1734.
14. Heuser, J. and Kirchhausen, T. 1985. Deep-etch views of clathrin assemblies. J Ultrastruct Res. 92. 1-2. 1-27.
15. Kirchhausen, T., Wang, J. C. and Harrison, S. C. 1985. DNA gyrase and its complexes with DNA: direct observation by electron microscopy. Cell. 41. 3. 933-943.
16. Kirchhausen, T., Harrison, S. C. and Heuser, J. 1986. Configuration of clathrin trimers: evidence from electron microscopy. J Ultrastruct Mol Struct Res. 94. 3. 199-208.
17. Kirchhausen, T., Harrison, S. C., Chow, E. P., Mattaliano, R. J., Ramachandran, K. L., Smart, J. and Brosius, J. 1987. Clathrin heavy chain: molecular cloning and complete primary structure. Proc Natl Acad Sci U S A. 84. 24. 8805-8809.
18. Kirchhausen, T., Scarmato, P., Harrison, S. C., Monroe, J. J., Chow, E. P., Mattaliano, R. J., Ramachandran, K. L., Smart, J. E., Ahn, A. H. and Brosius, J. 1987. Clathrin light chains LCA and LCB are similar, polymorphic, and share repeated heptad motifs. Science. 236. 4799. 320-324.
19. Thurieau, C., Brosius, J., Burne, C., Jolles, P., Keen, J. H., Mattaliano, R. J., Chow, E. P., Ramachandran, K. L. and Kirchhausen, T. 1988. Molecular cloning and complete amino acid sequence of AP50, an assembly protein associated with clathrin-coated vesicles. DNA. 7. 10. 663-669.

20. Kirchhausen, T., Nathanson, K. L., Matsui, W., Vaisberg, A., Chow, E. P., Burne, C., Keen, J. H. and Davis, A. E. 1989. Structural and functional division into two domains of the large (100- to 115-kDa) chains of the clathrin-associated protein complex AP-2. Proc Natl Acad Sci U S A. 86. 8. 2612-2616.
21. Kirchhausen, T. 1990. Identification of a putative yeast homolog of the mammalian beta chains of the clathrin-associated protein complexes. Mol Cell Biol. 10. 11. 6089-6090.
22. Matsui, W. and Kirchhausen, T. 1990. Stabilization of clathrin coats by the core of the clathrin-associated protein complex AP-2. Biochemistry. 29. 48. 10791-10798.
23. Scarmato, P. and Kirchhausen, T. 1990. Analysis of clathrin light chain-heavy chain interactions using truncated mutants of rat liver light chain LCB3. J Biol Chem. 265. 7. 3661-3668.
24. Tucker, K. L., Nathanson, K. and Kirchhausen, T. 1990. Sequence of the rat alpha c large chain of the clathrin associated protein complex AP-2. Nucleic Acids Res. 18. 17. 5306.
25. Keen, J. H., Beck, K. A., Kirchhausen, T. and Jarrett, T. 1991. Clathrin domains involved in recognition by assembly protein AP-2. J Biol Chem. 266. 12. 7950-7956.
26. Kirchhausen, T., Davis, A. C., Frucht, S., Greco, B. O., Payne, G. S. and Tubb, B. 1991. AP17 and AP19, the mammalian small chains of the clathrin-associated protein complexes show homology to Yap17p, their putative homolog in yeast. J Biol Chem. 266. 17. 11153-11157.
27. Nakayama, Y., Goebl, M., O'Brine Greco, B., Lemmon, S., Pingchang Chow, E. and Kirchhausen, T. 1991. The medium chains of the mammalian clathrin-associated proteins have a homolog in yeast. Eur J Biochem. 202. 2. 569-574.
28. Gallusser, A. and Kirchhausen, T. 1993. The beta 1 and beta 2 subunits of the AP complexes are the clathrin coat assembly components. Embo J. 12. 13. 5237-5244.
29. Kirchhausen, T. 1993. Coated pits and coated vesicles - sorting it all out. Current Opinion in Structural Biology. 3. 182-188.
30. Kirchhausen, T., Staunton, D. E. and Springer, T. A. 1993. Location of the domains of ICAM-1 by immunolabeling and single-molecule electron microscopy. J Leukoc Biol. 53. 3. 342-346.
31. Kirchhausen, T. and Toyoda, T. 1993. Immunoelectron microscopic evidence for the extended conformation of light chains in clathrin trimers. J Biol Chem. 268. 14. 10268-10273.
32. Osborn, L., Vassallo, C., Browning, B. G., Tizard, R., Haskard, D. O., Benjamin, C. D., Dougas, I. and Kirchhausen, T. 1994. Arrangement of domains, and amino acid residues required for binding of vascular cell adhesion molecule-1 to its counter-receptor VLA-4 (alpha 4 beta 1). J Cell Biol. 124. 4. 601-608.
33. Phan, H. L., Finlay, J. A., Chu, D. S., Tan, P. K., Kirchhausen, T. and Payne, G. S. 1994. The Saccharomyces cerevisiae APS1 gene encodes a homolog of the small subunit of the mammalian clathrin AP-1 complex: evidence for functional interaction with clathrin at the Golgi complex. Embo J. 13. 7. 1706-1717.
34. Sogaard, M., Tani, K., Ye, R. R., Geromanos, S., Tempst, P., Kirchhausen, T., Rothman, J. E. and Sollner, T. 1994. A rab protein is required for the assembly of SNARE complexes in the docking of transport vesicles. Cell. 78. 6. 937-948.
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38. Shih, W., Gallusser, A. and Kirchhausen, T. 1995. A clathrin-binding site in the hinge of the beta 2 chain of mammalian AP-2 complexes. J Biol Chem. 270. 52. 31083-31090.
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41. Stepp, J. D., Pellicena-Palle, A., Hamilton, S., Kirchhausen, T. and Lemmon, S. K. 1995. A late Golgi sorting function for Saccharomyces cerevisiae Apm1p, but not for Apm2p, a second yeast clathrin AP medium chain-related protein. Mol Biol Cell. 6. 1. 41-58.

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45. Kirchhausen, T. and Rosen, F. S. 1996. Disease mechanism: unravelling Wiskott-Aldrich syndrome. Curr Biol. 6. 6. 676-678.
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54. Cupers, P., Jadhav, A. P. and Kirchhausen, T. 1998. Assembly of clathrin coats disrupts the association between Eps15 and AP-2 adaptors. J Biol Chem. 273. 4. 1847-1850.
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