
BIOGRAPHICAL SKETCH

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NAME: Kirchhausen, Tomas

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POSITION TITLE: Professor of Cell Biology and Professor of Pediatrics, Harvard Medical School, Senior Investigator, Boston Children's Hospital; Springer Family Chair, Boston Children's Hospital

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Universidad Peruana Cayetano Heredia, Peru	B.S.	04/72	Biology
Instituto Venezolano de Investigaciones Cientificas, Venezuela	M.S.	06/75	Biophysics
Universidad Peruana Cayetano Heredia, Peru	M.S.	06/75	Biophysics
Instituto Venezolano de Investigaciones Cientificas, Venezuela	Ph.D.	06/77	Biophysics and Physiology
Harvard University	M.A. (HON)	12/99	Cell Biology

A. Personal Statement

Record of successful and productive research projects in structural biology, cell biology, chemical genetics, live cell and single-molecule fluorescence microscopy imaging. My expertise and experience have prepared me to advise predoctoral and postdoctoral students. Leadership record to successfully carry out the longest lasting international collaborative educational and research program at Harvard Medical School.

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
2016-2017 Visiting Scientist at Janelia Research Campus, HHMI
2016- Honorary Professor in the school of Biomedical Sciences, Hong Kong

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 Chair, Boston Children's Hospital
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

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 clathrin coats*

The clathrin pathway is the principal route by which ligands such as protein growth factors and hormones enter cells; it is critical for reuptake of membrane at synapses, and it is a mode of entry usurped by many viral pathogens. We defined the structure, interactions, and assembly mechanisms of clathrin and many of its associated proteins, through studies extending over three decades (Kirchhausen et al., 2014). The clathrin heavy-chain sequence -- at the time, the longest derived by cDNA cloning (Kirchhausen et al., 1987a) -- and a similar analysis of the light chains (Kirchhausen et al., 1987b) led us ultimately to the crystal structure of a large N-terminal fragment (Haar et al., 1998) and to the determination of the first membrane coat, a complete molecular model of a clathrin coat from electron cryomicroscopy (cryoEM) (Fotin et al., 2004b).

Fotin, A., Cheng, Y., Sliz, P., Grigorieff, N., Harrison, S. C., Kirchhausen, T., and Walz, T. (2004b). Molecular model for a complete clathrin lattice from electron cryomicroscopy. *Nature* 432, 573–579.

Haar, ter, E., Musacchio, A., Harrison, S. C., and Kirchhausen, T. (1998). Atomic structure of clathrin: a beta propeller terminal domain joins an alpha zigzag linker. *Cell* 95, 563–573.

Kirchhausen, T., Harrison, S. C., Chow, E. P., Mattaliano, R. J., Ramachandran, K. L., Smart, J., and Brosius, J. (1987a). Clathrin heavy chain: molecular cloning and complete primary structure. *Proc Natl Acad Sci USA* 84, 8805–8809.

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. (1987b). Clathrin light chains LCA and LCB are similar, polymorphic, and share repeated heptad motifs. *Science* 236, 320–324.

Kirchhausen, T., Owen, D., and Harrison, S. C. (2014). Molecular structure, function, and dynamics of clathrin-mediated membrane traffic. *Cold Spring Harb Perspect Biol* 6. PMID: 2478982. PMC3996469.

2. *Mechanism of uncoating*

The structure from cryoEM of a clathrin coat with a bound fragment of auxilin suggested a site for Hsc70 interaction (Fotin et al., 2004a). We determined the structure of a coat with bound auxilin and Hsc70, after carefully determining conditions under which the former directed specific recruitment of the latter (Xing et al., 2010). We showed that Hsc70 binding is accompanied by a distortion of the clathrin lattice and proposed a mechanism for uncoating in which Hsc70:ATP, recruited by auxilin, captures a transient distortion that exposes its site on clathrin; ATP hydrolysis tightens its grip, locking in the distortion and destabilizing the coat. Our subsequent in vitro single-molecule studies of uncoating supported and extended this model, which shows how a purely local set of molecular interactions, consistent with the known mechanism for Hsc70-mediated stabilization of unfolded proteins, can generate a delocalized remodeling of a very large assembly (Böcking et al., 2011).

Böcking, T., Aguet, F., Harrison, S. C., and Kirchhausen, T. (2011). Single-molecule analysis of a molecular disassemblase reveals the mechanism of Hsc70-driven clathrin uncoating. *Nat Struct Mol Biol* 18, 295–301. PMC3056279.

Fotin, A., Cheng, Y., Grigorieff, N., Walz, T., Harrison, S. C., and Kirchhausen, T. (2004a). Structure of an auxilin-bound clathrin coat and its implications for the mechanism of uncoating. *Nature* 432, 649–653.

Xing, Y., Böcking, T., Wolf, M., Grigorieff, N., Kirchhausen, T., and Harrison, S. C. (2010). Structure of clathrin coat with bound Hsc70 and auxilin: mechanism of Hsc70-facilitated disassembly. *Embo J* 29, 655–665. PMC2830701.

3. Mechanisms of coated-vesicle assembly in living cells

We built on the biochemical and structural discoveries we had made during the first twenty-five years of our work on clathrin-mediated endocytosis to analyze the mechanisms of coated-vesicle assembly in living cells, using emerging technologies in fluorescence microscopy and live-cell imaging. We showed that coated pits nucleate at the plasma membrane, grow by steady addition of clathrin triskelions, and finish assembly in a time determined in part by the size of the cargo; a substantial proportion of initiated coated pits abort before completion. Hsc70-mediated uncoating follows promptly upon dynamin-induced membrane scission of completed coated pits; we showed that the timing of this event is determined by arrival of auxilin, the clathrin specific, J-domain co-chaperone for Hsc70 (Massol et al., 2006). We went on, when our TIRF microscopy technology had reached the level of single-molecule counting, to show that coated pits initiate at the plasma membrane by coordinated arrival of clathrin and the AP2 adaptor complex, the latter recruited by interaction with PI-4,5-P2 (Cocucci et al., 2012) and that a single dynamin rung rather than spirals mediate coated pit neck scission (Cocucci et al., 2014). Accessory proteins are then essential for sustained growth. We further showed that clathrin assembly ordinarily provides the principal driving force for membrane invagination but that high membrane tension (Boulant et al., 2011) or very elongated cargo (Cureton et al., 2010) imposes an additional requirement for actin polymerization.

Boulant, S., Kural, C., Zeeh, J.-C., Ubelmann, F., and Kirchhausen, T. (2011). Actin dynamics counteract membrane tension during clathrin-mediated endocytosis. *Nat Cell Biol* 13, 1124–1131. PMID: PMC3167020.

Cocucci, E., Aguet, F., Boulant, S., and Kirchhausen, T. (2012). The first five seconds in the life of a clathrin-coated pit. *Cell* 150, 495–507. PMID: PMC3413093.

Cocucci, E., Gaudin, R., and Kirchhausen, T. (2014). Dynamin recruitment and membrane scission at the neck of a clathrin-coated pit. *Mol Biol Cell*. PMC4230619.

Cureton, D. K., Massol, R. H., Whelan, S. P. J., and Kirchhausen, T. (2010). The length of vesicular stomatitis virus particles dictates a need for actin assembly during clathrin-dependent endocytosis. *PLoS Pathog* 6, e1001127. PMC2947997.

Massol, R. H., Boll, W., Griffin, A. M., and Kirchhausen, T. (2006). A burst of auxilin recruitment determines the onset of clathrin-coated vesicle uncoating. *Proc Natl Acad Sci USA* 103, 10265–10270.

4. Chemical genetics. We used the adaptation of small-molecule screening tools for academic settings to introduce "chemical genetics" into the membrane-traffic field. Four compounds we discovered have turned into valuable tools for probing intracellular membrane dynamics. Dynasore, which we found with a screen for inhibitors of its GTPase activity, blocks the membrane scission step (Macia et al., 2006); this molecule and a more soluble derivative (hydroxydynasore) are now standard tools for identifying dynamin-dependent processes. Vacuolin, identified in a phenotypic screen, causes loss of intraluminal vesicle in endosomes (Cerny et al., 2004). BLT-1, identified in a screen for inhibition of cholesterol uptake from HDL, blocks scavenger-receptor mediated cholesterol transfer (Nieland et al., 2002). Secramine was the earliest discovered small-molecule inhibitor of Cdc42 (Pelish et al., 2006).

Cerny, J., Feng, Y., Yu, A., Miyake, K., Borgonovo, B., Klumperman, J., Meldolesi, J., McNeil, P. L., and Kirchhausen, T. (2004). The small chemical vacuolin-1 inhibits Ca(2+)-dependent lysosomal exocytosis but not cell resealing. *EMBO Rep* 5, 883–888.

Macia, E., Ehrlich, M., Massol, R., Boucrot, E., Brunner, C., and Kirchhausen, T. (2006). Dynasore, a cell-permeable inhibitor of dynamin. *Dev. Cell* 10, 839–850.

Nieland, T. J. F., Penman, M., Dori, L., Krieger, M., and Kirchhausen, T. (2002). Discovery of chemical inhibitors of the selective transfer of lipids mediated by the HDL receptor SR-BI. *Proc Natl Acad Sci USA* 99, 15422–15427.

Pelish, H. E., Peterson, J. R., Salvarezza, S. B., Rodriguez-Boulan, E., Chen, J.-L., Stamnes, M., Macia, E., Feng, Y., Shair, M. D., and Kirchhausen, T. (2006). Secramine inhibits Cdc42-dependent functions in cells and Cdc42 activation in vitro. *Nat Chem Biol* 2, 39–46.

5. Organelle Biogenesis

We applied our experience with frontier optical-imaging modalities to examine cellular membrane remodeling processes other than clathrin-coated vesicle traffic. We showed by combining live-cell imaging with EM tomography that during cell division, that the endoplasmic reticulum converts into extended sheets (Lu et al., 2009). We followed reconstitution of the nuclear membrane during late stages of mitosis and showed that nuclear membrane deposition precedes nuclear pore insertion (Lu et al., 2011). We also have studied the regulation of cell-surface area during cell division; constitutive membrane recycling shuts down during early mitosis and reactivates massively at the onset of anaphase. In contrast to the constitutive recycling, the reactivated recycling, like membrane wound healing and synaptic vesicle release, is a Ca²⁺-triggered process (Boucrot and Kirchhausen, 2007).

Boucrot, E., and Kirchhausen, T. (2007). Endosomal recycling controls plasma membrane area during mitosis. *Proc Natl Acad Sci USA* 104, 7939–7944.

Lu, L., Ladinsky, M. S., and Kirchhausen, T. (2009). Cisternal organization of the endoplasmic reticulum during mitosis. *Mol Biol Cell* 20, 3471–3480. PMC2719565.

Lu, L., Ladinsky, M. S., and Kirchhausen, T. (2011). Formation of the postmitotic nuclear envelope from extended ER cisternae precedes nuclear pore assembly. *J Cell Biol* 194, 425–440. PMC3153650.

Complete List of Published Work in MyBibliography:

<http://www.ncbi.nlm.nih.gov/sites/myncbi/tomas.kirchhausen.1/bibliography/40106331/public/?sort=date&direction=ascending>.

Selected peer-reviewed publications (193 in chronological order).

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 Å 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., Goebel, 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.
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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.
39. Sorkin, A., McKinsey, T., Shih, W., Kirchhausen, T. and Carpenter, G. 1995. Stoichiometric interaction of the epidermal growth factor receptor with the clathrin-associated protein complex AP-2. *J Biol Chem*. 270. 2. 619-625.
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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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