JCB Journal of Cell Biology

IN MEMORIAM

Linton Mark Traub (1962–2020)

Meir Aridor[1] and David J. Owen[2]

Mensch (Yiddish: מענטש, mentsh): "a person of integrity and honor." This sums up Linton Traub in one word—the scientist and the individual. As a scientist, he was a gifted experimentalist as well as someone with a deep and broad theoretical understanding of both his own field of vesicle trafficking, to which he made a number of major contributions, and of cell biology in its widest sense.

Linton was born in London within the sound of Bow Bells in 1962 and was therefore, by its traditional definition, a cockney. He was educated in South Africa, where he studied microbiology and immunology at the University of the Witwatersrand in Johannesburg. He then moved to Israel to do his graduate work with Ronit Sagi-Eisenberg at the Weizmann Institute, whose group studied signal transduction with a membrane traffic twist, asking how a stimulus couples to secretion in mast cells. Linton specifically focused on the role of GTP-binding proteins. This interest led him to St. Louis for his postdoctoral studies with Stuart Kornfeld at Washington University.

At Washington University, Linton's initial focus on GTPases led to a seminal discovery of the role of Arf1 in recruiting the clathrin adaptor complex AP-1 to the TGN (Traub et al., 1993). The work helped to establish the prevailing model of small Arf/SAR1 family GTPases as the initiators of coat-mediated biosynthetic secretion. Together with Ernst Ungewickell, Linton went beyond the GTPase priming event, delving into the multiple protein–protein interactions that nucleate AP-1 and clathrin at the TGN (Traub et al., 1995). He became fascinated with how the arrival of coat protein complexes is spatially and temporally orchestrated at bud sites to sort cargo, form, and release a vesicle.

In his laboratory, initially in St. Louis and later at the University of Pittsburgh School of Medicine, where Linton was appointed as an assistant professor in 2000, he began the fascinating journey of deciphering the network of interactions that pioneers and orchestrates clathrin assembly with cargo selection and vesicle formation at the plasma membrane. While GTPase activation initiates coat assembly at the ER and Golgi, the assembly of the AP-2 adaptor complex and clathrin at the plasma membrane apparently lacked such a priming event. AP-2 is both conformationally activated and stabilized at the plasma membrane by binding PIP2, cargo, and clathrin and can play a key role in driving clathrin-mediated endocytosis (CME). Linton used, and to a large extent initiated the use of, protein structure in membrane trafficking, first with Daved (Fremont) and later with David (Owen). Linton's elegant cell biology and biochemistry/structure analyses were often enhanced by John Heuser's beautiful quick-freeze/deep-etch EM. Linton initially focused on the appendage domains, which are the interaction hubs of AP-2 (Edeling et al., 2006; Traub et al., 1999), mapping functional interactions between clathrin, the AP-2 appendages, and a myriad of cargo selective adaptors that he termed clathrin-associated sorting proteins (CLASPs). A meticulous deciphering of how the binding of short linear amino acid motifs and conformation-dependent interactions with both AP-2 and clathrin began to explain the roles and hierarchy of CLASPs and regulators in orchestrating coat assembly and cargo recruitment (Drake et al., 2000; Drake and Traub, 2001; Jha et al., 2004; Miele et al., 2004; Mishra et al., 2004; Thieman et al., 2009). Using combinations of multiple low affinity motifs, CLASPS capture both AP-2 and clathrin and link them with cargo and PIP2 to trigger coat assembly. The usage of linear low affinity motifs in regulating coat assembly is now an accepted generality (Ma and Goldberg, 2016).

Linton Mark Traub at the bench during his graduate studies at the Weizmann Institute in Israel (A) and more recently in Pittsburgh, PA (B). Panel A image courtesy of Andre Levitan, and panel B image courtesy of the Traub family.

The seminal discoveries of receptor-mediated low density lipoprotein (LDL) uptake by Goldstein and Brown always inspired Linton. He studied the CLASPs that mediate the internalization of LDL and LDL-like receptors (Mishra et al., 2005; Mishra et al., 2002a; Mishra et al., 2002b) and used model

[1]Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Cambridge Institute for Medical Research, University of Cambridge, Cambridge, UK.

Correspondence to Meir Aridor: aridor@pitt.edu; David Owen: djo30@cam.ac.uk.

organisms that provided unique physiological perspectives. Using mosquitoes, Linton would even insert his hand through a sleeve in a cage to provide them with a blood meal since this enhances the internalization of yolk precursors and directs the maturation of developmentally repressed previtellogenic egg chambers. He also had to master fly genetics, as flies exhibit developmentally staged uptake of yolk precursors during ovarian follicle development (Jha et al., 2012; Mishra et al., 2008). His work defined how phosphotyrosine binding domain (PTB)–containing CLASPs such as ARH or DAB2 in mammals, trephin in mosquitoes, or CED6 in flies recognize the internalization motifs (FxNPxY/A) of LDL receptor superfamily members while binding PIP2 and connecting with the AP-2 appendages and clathrin to mediate receptor internalization.

Although a stochastic mechanism for coat assembly was emerging, the rules that govern the temporal arrival and spatial organization of CLASPs within the bud remained undefined. In collaborative work with Beverly Wendland (Johns Hopkins at that time), Linton began to focus on a potential new class of CLASPs, the BAR and mu homology domain–containing muniscins: Syp1 in yeast and FCHO1/2 in mammals (Reider et al., 2009; Umasankar et al., 2012). FCHO1/2 together with the EH proteins EPS15/R are two of the earliest arriving proteins at the clathrin bud sites. Linton's team demonstrated how EPS15/R present multivalent AP-2 binding motifs that engage AP-2, yet also bind the mu homology domain of FCHO1/2 proteins. EPS15/R direct the formation of this transient tripartite complex that links AP-2 with FCHO proteins. FCHO1/2 interact with AP-2 to promote an open conformation for cargo and PIP2 binding. AP-2 activation is followed by the displacement of EPS15/R and FCHO1/2, positioning the two pioneering proteins at the edges of the bud during vesicle formation (Ma et al., 2016; Traub, 2019; Umasankar et al., 2014). In Linton's recent single-author paper, completed while he was battling disease, he demonstrated how dismantling the assembly of these pioneers blunts CME, uncovering the elusive priming event that he set out to find in a truly deserving scientific victory (Traub, 2019).

We met Linton at different stages in his career. One friendship (with Meir Aridor) began in Israel 33 years ago during our graduate studies. We shared one desk in the laboratory and many interests and dreams. Our friendship continued while collaborating across the country during our postdoctoral training (Traub et al., 1996) and then, reuniting in Pittsburgh, having side-by-side offices and laboratories for the past 20 years. I was truly the luckiest person to be able to walk in every morning and meet Linton most often at his bench, carrying out an experiment. We would always discuss emerging questions. Linton's wit, dry humor, incredible scientific insights, advice, endless knowledge, enthusiasm, and yes, also criticism will always stay with me. Every small discovery was exciting; every new find would be properly contextualized and celebrated. Linton's friendship with David Owen began in the late 1990s at a Gordon Research Conference. They discovered they were direct competitors, but in true Linton fashion, rather than displaying any animosity as he could so easily have done to an unheard of upstart from a different field, Linton explained, helped, and advised him, and they became not only collaborators but firm friends for the next 20+ years.

Linton was diagnosed with multiple myeloma in 2017 and endured various treatments as he fought to stay with his beloved wife and daughter; he was above all else a loving family man. Many of us know him as an all-knowing, dedicated, generous, experimental scientist and great mentor, but there was yet another side to him. He was a knowledgeable art lover; his house and office were amazing galleries of pieces of work in all media bought from local and up-and-coming artists. He himself was an artistic craftsman in the true spirit of the Morris and Stickley–inspired Arts and Crafts movement, which he admired. He specialized in ceramics and especially glazes—a love he shared with his daughter. He was an avid reader who exchanged books with many of his colleagues and collaborators and a wordsmith to boot; but he was also a "petrol head," delighting in the smell, speed, and roaring sound of vintage racing cars, as well as on other occasions the smell and roaring taste of vintage cheese. A man of many facets but above all a husband, father, and brother.

Throughout his career, Linton has been known for his impeccable intellectual integrity and his truly unwavering scientific focus. As one of his colleagues remarked, Linton was "old school." He performed brilliant science and was rigorous, honest, and unassuming but with the right dose of skepticism and a view of the wider picture. He was driven by a quest for knowledge and shied away from accolades. He was a popular speaker at conferences, where he could always be relied upon to not only give an excellent talk, often presenting unpublished data, but to interact with all the attendees. It was not just primary research that Linton published; he wrote many commentaries, book chapters, and reviews, often engaging other leading researchers in membrane trafficking. Valued for his fairness and encyclopedic knowledge of the field, he sat on numerous NIH review panels and reviewed a staggering number of papers for many journals.

While many will remember Linton for all the fantastic science he performed, those who knew him best—his friends and most especially his family—will keenly miss him for the humane, cultured, generous, considerate, and good person he was.

Rest in peace BIG man.

Acknowledgments

We thank Drs. Marijn Ford, Alexander Sorkin, and Sandra Schmid for comments and suggestions. We thank Jill and Simone Traub for their help, comments, suggestions, and advice.

References

Drake, M.T., and L.M. Traub. 2001. Interaction of two structurally distinct sequence types with the clathrin terminal domain beta-propeller. *J. Biol. Chem.* 276:28700–28709. https://doi.org/10.1074/jbc.M104226200

Drake, M.T., M.A. Downs, and L.M. Traub. 2000. Epsin binds to clathrin by associating directly with the clathrin-terminal domain. Evidence for cooperative binding through two discrete sites. *J. Biol. Chem.* 275: 6479–6489. https://doi.org/10.1074/jbc.275.9.6479

Edeling, M.A., S.K. Mishra, P.A. Keyel, A.L. Steinhauser, B.M. Collins, R. Roth, J.E. Heuser, D.J. Owen, and L.M. Traub. 2006. Molecular switches involving the AP-2 beta2 appendage regulate endocytic cargo selection and clathrin coat assembly. *Dev. Cell.* 10:329–342. https://doi.org/10.1016/j.devcel.2006.01.016

Jha, A., N.R. Agostinelli, S.K. Mishra, P.A. Keyel, M.J. Hawryluk, and L.M. Traub. 2004. A novel AP-2 adaptor interaction motif initially identified

in the long-splice isoform of synaptojanin 1, SJ170. *J. Biol. Chem.* 279: 2281–2290. https://doi.org/10.1074/jbc.M305644200

Jha, A., S.C. Watkins, and L.M. Traub. 2012. The apoptotic engulfment protein Ced-6 participates in clathrin-mediated yolk uptake in Drosophila egg chambers. *Mol. Biol. Cell.* 23:1742–1764. https://doi.org/tlsb

Ma, W., and J. Goldberg. 2016. TANGO1/cTAGE5 receptor as a polyvalent template for assembly of large COPII coats. *Proc. Natl. Acad. Sci. USA.* 113: 10061–10066. https://doi.org/10.1073/pnas.1605916113

Ma, L., P.K. Umasankar, A.G. Wrobel, A. Lymar, A.J. McCoy, S.S. Holkar, A. Jha, T. Pradhan-Sundd, S.C. Watkins, D.J. Owen, and L.M. Traub. 2016. Transient Fcho1/2·Eps15/R·AP-2 Nanoclusters Prime the AP-2 Clathrin Adaptor for Cargo Binding. *Dev. Cell.* 37:428–443. https://doi.org/10.1016/j.devcel.2016.05.003

Miele, A.E., P.J. Watson, P.R. Evans, L.M. Traub, and D.J. Owen. 2004. Two distinct interaction motifs in amphiphysin bind two independent sites on the clathrin terminal domain beta-propeller. *Nat. Struct. Mol. Biol.* 11: 242–248. https://doi.org/10.1038/nsmb736

Mishra, S.K., P.A. Keyel, M.J. Hawryluk, N.R. Agostinelli, S.C. Watkins, and L.M. Traub. 2002a. Disabled-2 exhibits the properties of a cargo-selective endocytic clathrin adaptor. *EMBO J.* 21:4915–4926. https://doi.org/10.1093/emboj/cdf487

Mishra, S.K., S.C. Watkins, and L.M. Traub. 2002b. The autosomal recessive hypercholesterolemia (ARH) protein interfaces directly with the clathrin-coat machinery. *Proc. Natl. Acad. Sci. USA.* 99:16099–16104. https://doi.org/10.1073/pnas.252630799

Mishra, S.K., M.J. Hawryluk, T.J. Brett, P.A. Keyel, A.L. Dupin, A. Jha, J.E. Heuser, D.H. Fremont, and L.M. Traub. 2004. Dual engagement regulation of protein interactions with the AP-2 adaptor alpha appendage. *J. Biol. Chem.* 279:46191–46203. https://doi.org/tlsb

Mishra, S.K., P.A. Keyel, M.A. Edeling, A.L. Dupin, D.J. Owen, and L.M. Traub. 2005. Functional dissection of an AP-2 beta2 appendage-binding sequence within the autosomal recessive hypercholesterolemia protein. *J. Biol. Chem.* 280:19270–19280. https://doi.org/tlsb

Mishra, S.K., A. Jha, A.L. Steinhauser, V.A. Kokoza, C.H. Washabaugh, A.S. Raikhel, W.A. Foster, and L.M. Traub. 2008. Internalization of LDL-receptor superfamily yolk-protein receptors during mosquito oogenesis involves transcriptional regulation of PTB-domain adaptors. *J. Cell Sci.* 121:1264–1274. https://doi.org/10.1242/jcs.025833

Reider, A., S.L. Barker, S.K. Mishra, Y.J. Im, L. Maldonado-Báez, J.H. Hurley, L.M. Traub, and B. Wendland. 2009. Syp1 is a conserved endocytic adaptor that contains domains involved in cargo selection and membrane tubulation. *EMBO J.* 28:3103–3116. https://doi.org/10.1038/emboj.2009.248

Thieman, J.R., S.K. Mishra, K. Ling, B. Doray, R.A. Anderson, and L.M. Traub. 2009. Clathrin regulates the association of PIPKIgamma661 with the AP-2 adaptor beta2 appendage. *J. Biol. Chem.* 284:13924–13939. https://doi.org/10.1074/jbc.M901017200

Traub, L.M. 2019. A nanobody-based molecular toolkit provides new mechanistic insight into clathrin-coat initiation. *eLife.* 8:e41768. https://doi.org/10.7554/eLife.41768

Traub, L.M., J.A. Ostrom, and S. Kornfeld. 1993. Biochemical dissection of AP-1 recruitment onto Golgi membranes. *J. Cell Biol.* 123:561–573. https://doi.org/10.1083/jcb.123.3.561

Traub, L.M., S. Kornfeld, and E. Ungewickell. 1995. Different domains of the AP-1 adaptor complex are required for Golgi membrane binding and clathrin recruitment. *J. Biol. Chem.* 270:4933–4942. https://doi.org/10.1074/jbc.270.9.4933

Traub, L.M., S.I. Bannykh, J.E. Rodel, M. Aridor, W.E. Balch, and S. Kornfeld. 1996. AP-2-containing clathrin coats assemble on mature lysosomes. *J. Cell Biol.* 135:1801–1814. https://doi.org/10.1083/jcb.135.6.1801

Traub, L.M., M.A. Downs, J.L. Westrich, and D.H. Fremont. 1999. Crystal structure of the α appendage of AP-2 reveals a recruitment platform for clathrin-coat assembly. *Proc. Natl. Acad. Sci. USA.* 96:8907–8912. https://doi.org/10.1073/pnas.96.16.8907

Umasankar, P.K., S. Sanker, J.R. Thieman, S. Chakraborty, B. Wendland, M. Tsang, and L.M. Traub. 2012. Distinct and separable activities of the endocytic clathrin-coat components Fcho1/2 and AP-2 in developmental patterning. *Nat. Cell Biol.* 14:488–501. https://doi.org/10.1038/ncb2473

Umasankar, P.K., L. Ma, J.R. Thieman, A. Jha, B. Doray, S.C. Watkins, and L.M. Traub. 2014. A clathrin coat assembly role for the muniscin protein central linker revealed by TALEN-mediated gene editing. *eLife.* 3: e04137. https://doi.org/10.7554/eLife.04137