BIOGRAPHICAL SKETCH
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NAME:William Tobey Wickner M.D.

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POSITION TITLE: James C. Chilcott '20 Distinguished Professor of Biochemistry

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
Yale University	B.A.	1967	Chemistry
Harvard University Medical School	M.D.	1973	Medicine
Stanford University	Postdoc.	1971-74	Biochemistry

A. **Personal Statement**

I am a devoted, enthusiastic biochemist. My lab's enzymology has always challenged current models in our field, whether testing for co- or post-translational protein translocation earlier in my career or our recent findings-- that tethers are essential for membrane fusion, that fusion requires particular lipid head group and fatty acyl chain properties, that trans-SNARE complex assembly is catalyzed, and that αSNAP (Sec17p) has novel functions at fusion per se-- which are changing central tenets of the current membrane fusion paradigm.

In each phase of my professional life, I've picked a biological problem at an early time in its study, performed biochemical study at the organelle level, purified and reconstituted the relevant proteins and lipids, and studied the process at a chemical level. After starting my own lab, my first project was studying how newly made proteins insert into, and across, the plasma membrane of E. coli (1974-2005). My second project (1988- today) is studying how membranes fuse, using the vacuole (lysosome) of S. cerevisiae. We studied fusion in vivo, developed a rapid quantitative assay for studying fusion with the purified organelle, and now have reconstituted fusion with all-pure and physiological catalysts, the topic of this proposal.

As much as I enjoy working at the bench, and my lab's science, I've equally enjoyed teaching (and learning from) my students and postdocs, who have, in general, done very well. These include Gail Mandel (NAS, HHMI), Ulrich Hartl (NAS, Max Planck Director), Koreaki Ito, Janet Shaw, Lois Weisman, Pamela Silver, Roland Lill, Christian Ungermann, and over 80 other highly successful scientists around the world.

My training was in membrane proteins (EP Kennedy) and enzymology (Kornberg), during which I learned the power of the reductionist approach to complex biology.

1. **Early adventures (during my training) in the enzymology of complex cell biology**
A. Purification of a functional integral membrane enzyme
Dowhan, W., Wickner, W. and Kennedy, E.P. (1974). Purification and properties of phosphatidylserine decarboxylase from Escherichia coli. J. Biol. Chem. **249**, 3079-3084. I purified this enzyme 5,240-fold with a 2% yield from 100lb blocks of E. coli from 1968-1971. I had purified this protein when I left Boston in 1971, and Bill Dowhan did beautiful functional characterization. This was, to my knowledge, the first isolation of a catalytically active integral membrane protein in mixed micellar solution; I learned the hard way that solubility is lost when detergent is removed, years before the fluid mosaic model appeared.
B. DNA replication proteins

Wickner, W., Brutlag, D., Schekman, R. and Kornberg, A. (1972). RNA synthesis initiates <u>in vitro</u> conversion of M13 DNA to its replicative form. Proc. Natl. Acad. Sci. U.S.A. **69**, 965-969. Randy Schekman and I, with others in the Kornberg lab, dissected the complex biology of DNA replication through enzymology.

2. Dissection of protein translocation in E. coli. My group studied protein translocation from 1974 to 2000, starting with *in vivo* studies, progressing to studies with proteins synthesized *in vitro* and translocated into inverted sealed plasma membrane vesicles, then isolating the multisubunit E. coli preprotein translocation complex, a long-sought goal of the field, and characterizing the translocation process at a chemical level.

Brundage, L., Hendrick, J.P., Schiebel, E., Driessen, A.J.M., and Wickner, W. (1990). The purified E. coli integral membrane protein SecY/E is sufficient for reconstitution of SecA-dependent precursor protein translocation. Cell **62**, 649-657. We found how to prepare a detergent extract from which proteoliposomes that would perform preprotein translocation could be reconstituted, then fractionated the proteins in the detergent extract to isolate the active trimeric integral membrane domain of preprotein translocase.

Lill, R., Dowhan, W., and Wickner, W. (1990). The ATPase activity of SecA is regulated by acidic phospholipids, SecY, and the leader and mature domains of precursor proteins. Cell, **60**, 271-281. We found the basic relationships among the preprotein substrates and the parts of the translocation complex. This was our first dissection of a complex membrane event.

B. Positions and Honors

Positions

1968-71	Harvard Medical School, Department of Biochemistry (predoctoral research supervisor: Dr. Eugene P. Kennedy)
1971-74	Stanford University Medical School, Department of Biochemistry (postdoctoral research supervisor: Dr. Arthur Kornberg)
1975-76	Senior Research Fellow, Stanford University Medical School Department of Biochemistry
1976-93	Assistant, Associate, and Full Professor, University of California, Los Angeles Department of Biological Chemistry and Molecular Biology Institute
1993-00	Chairman, Department of Biochemistry, Dartmouth Medical School
1993-	James C. Chilcott '20 Distinguished Professor of Biochemistry

Honors

1966, 67	Phi Beta Kappa, Magna Cum Laude
1971-73	Graub Fellow of Cystic Fibrosis Foundation
1974-76	Mellon Fellow
1979	American Cancer Society Faculty Research Award
1982-83	Guggenheim Fellow
1987	Co-chairman, Membrane Molecular Biology Gordon Conference
1987-91	NIH Cell Biology Study Section
1988-98	NIH Merit Award
1989-91	Chairman, NIH Cell Biol. Study Section
1991-95	Editorial board, Annual Reviews of Biochemistry
1995	Fellow of American Society for Microbiology
1996	National Academy of Sciences Member
2000	European Molecular Biology Organization Associate (foreign) Member
2003	American Academy of Arts and Sciences Member
2008	Bavarian Academy of Sciences, corresponding member
2009	Bonhoeffer Lecturer, University Gottingen
2012	Sigman Lecturer, UCLA; Van Deenen Medalist, Utrecht; Gruber Lecturer, Groningen

C. Contributions to Science. Training students and fellows has been as important to me as the science itself. Protein translocation work is reviewed in Section A above. Here are our contributions to the field of membrane fusion, from ~1988 to the present.

1. Fusion of vacuoles; studying complex biology with a simple colorimetric assay. This work began as an investigation of organelle inheritance by a brilliant postdoc (Lois Weisman). When we realized that isolated vacuoles would fuse *in vitro*, my lab turned its attention to the mechanism of fusion, while Weisman pursued organelle inheritance very successfully in her own lab.

Weisman, L.S. and Wickner, W. (1988). Intervacuole exchange in the yeast zygote defines a new pathway in organelle communication. Science, **241**, 589-591. Lois discovered that vacuole inheritance is spatially and cell-cycle controlled, culminating in fusion of vesicles in the new cell.

Weisman, L.S., Emr, S.D., and Wickner, W.T. (1990). Mutants of Saccharomyces cerevisiae which Block Intervacuole Vesicular Traffic and Vacuole Division and Segregation. Proc. Natl. Acad. Sci. USA, **87**, 1076-1080. The start of *vac* (vacuole inheritance) mutants, which continued in the Weisman lab.

Conradt, B., Shaw, J., Vida, T., Emr, S.D., and Wickner, W. (1992). *In vitro* reactions of vacuole inheritance. J. Cell Biol. **119**, 1469-1479. Vacuoles incubated with ATP and cytosol are seen to fuse.

Haas, A., Conradt, B., and Wickner, W. (1994). G-protein ligands inhibit *in vitro* reactions of vacuole inheritance. J. Cell Biol. **126**, 87-97. The development of a simple, rapid, quantitative assay which measures the complex biology of membrane fusion.

2. Identification of the major proteins of vacuole fusion; Rab, SNAREs, Sec17/18, HOPS. We had leads of which proteins were likely important for fusion from the genetics of vacuole structure (Wada, Emr, Stevens). We evaluated each candidate protein by applying our colorimetric fusion assay to vacuoles isolated from mutant strains and by studying the effects of antibodies and other ligands.

Haas, A., Scheglmann, D., Lazar, T., Gallwitz, D., and Wickner, W. (1995) The GTPase Ypt7p of *Saccharomyces cerevisiae* is required on both partner vacuoles for the homotypic fusion step of vacuole inheritance. EMBO J. **14**, 5258-5270. An active, organelle specific Rab is shown to be essential for fusion in vitro.

Nichols, B.J., Ungermann, C., Pelham, H.R.B., Wickner, W.T., and Haas, A. (1997) Homotypic vacuolar fusion mediated by t- and v-SNAREs. Nature **387**, 199-202. An early demonstration that SNAREs on complementary organelles function to mediate fusion.

Mayer, A., Wickner, W., and Haas, A. (1996). Sec18p (NSF)-driven release of Sec17p (α-SNAP) can precede docking and fusion of yeast vacuoles. Cell **85**, 83-94. Sec18p (NSF) was thought at the time to be the ATP-driven engine for the final step of bilayer mixing; here it's shown that Sec18p:ATP only acts early in the fusion reaction.

Seals, D., Eitzen, G., Margolis, N., Wickner, W., and Price, A. (2000) A Ypt/Rab effector complex containing the Sec1 homolog Vps33p is required for homotypic vacuole fusion. Proc. Natl. Acad. Sci. USA **97**, 9402-9407. Six of the proteins encoded by Wada's *VAM* genes (and the corresponding *VPS* genes) are together as 1 large complex, which we named HOPS.

3. Interdependence of lipids and proteins in forming fusion-competent microdomains. The large size of purified vacuoles, and the ability to create strains with GFP-tagged fusion proteins, allowed us to discover a special fusion microdomain.

Wang, L., Seeley, S., Wickner, W. and Merz, A. (2002) Vacuole fusion at a ring of vertex docking sites leaves membrane fragments within the organelle. Cell **108**, 357-369. The Rab, its effector (HOPS), and SNAREs become highly enriched at a ring-shaped microdomain which surrounds the two apposed membranes of docked vacuoles prior to fusion.

Wang, L., Merz, A., Collins, K., and Wickner, W. (2003) Hierarchy of protein assembly at the vertex ring domain for yeast vacuole docking and fusion. J. Cell Biol. **160**, 365-374. This study showed the interdependence of proteins for their assembly into the ring-shaped fusion microdomain.

Fratti, R., Jun, Y., Merz, A.J., Margolis, N., and Wickner, W. (2004) Interdependent assembly of specific "regulatory" lipids and membrane fusion proteins into the vertex ring domain of docked vacuoles. J. Cell Biol. **167**, 1087-1098. Using lipid ligands as inhibitors and as fluorescent probes, we discovered the specific enrichment of certain lipids that are essential for fusion into the same ring-shaped microdomain as the fusion proteins. Strikingly, the lipids depend on the proteins for this spatial enrichment, and the proteins depend on these lipids too. This may be the first documentation of protein and lipid interdependence for membrane microdomain formation.

Zick, M., Stroupe, C., Orr, A., Douville, D., and Wickner, W. (2014) Membranes linked by trans-SNARE complexes require lipids prone to non-bilayer structure for progression to fusion. eLife. doi: 10.7554/eLife.01879. While the paradigm of membrane fusion has heretofore focused on the proteins, we showed here that lipids with headgroups that are less likely to fit bilayers are essential for fusion even after trans-SNARE complexes have formed. Of these, DAG and ergosterol were shown by Fratti (2004) to be enriched at the vertex ring.

4. Reconstitution of fusion with all purified components. Until a rapid and efficient fusion can be reconstituted with all purified proteins and lipids, and at their physiological concentrations, one can't know if a major catalytic element is missing. We have now achieved this goal (Zick and Wickner, in preparation). The steps which led there are in the papers below:

Stroupe, C., Collins, K.M., Fratti, R.A., and Wickner, W.T. (2006) Purification of active HOPS complex reveals its affinities for phosphoinositides and the SNARE Vam7p. EMBO J. **25**, 1579-1589. The first isolation of an active large tethering complex, "HOPS", allowing the direct demonstration of its lipid and SNARE affinities.

Mima, J., Hickey, C., Xu, H., Jun, Y, and Wickner, W. (2008) Reconstituted membrane fusion requires regulatory lipids, SNAREs and synergistic SNARE chaperones. EMBO J. **27**, 2031-2042. With active HOPS in hand, reconstituted proteoliposomes bearing SNAREs could be shown to fuse. HOPS was shown to act synergistically with the SNARE disassembly chaperones Sec17p/Sec18p to promote fusion.

Stroupe, C., Hickey, C.M., Mima, J., Burfeind, A.S. and Wickner, W. (2009) Minimal membrane docking requirements revealed by reconstitution of Rab GTPase-dependent membrane fusion from purified components. Proc. Natl. Acad. Sci. USA **106**, 17,626-17,633. This paper describes the purification of a prenylated Rab-family GTPase that could be reconstituted into SNARE-bearing proteoliposomes and, with HOPS, Sec17p, and Sec18p, catalyze rapid and efficient fusion.

Orr, A., Wickner, W., Rusin, S.F., Kettenbach, A.N., and Zick, M. (2015) Yeast vacuolar HOPS, regulated by its kinase, exploits affinities for acidic lipids and Rab:GTP for membrane binding and to catalyze tethering and fusion. Mol. Biol. Cell 26, 305-315. The affinities of HOPS for both acidic lipids and for Ypt7p:GTP combine to give high-affinity binding to the membrane.

5. Chemical studies which revise the paradigm of fusion. The purpose of the above 20 yrs of effort is to harness the power of knowing the relevant components, and being able to add or even remove them at will, to determine fusion mechanism. First came our shocking discovery that the commonly employed assay of fusion could reflect processes other than fusion, necessitating the development of a new, more rigorous fusion assay. In stark contrast to the current paradigm, we found that *trans*-SNAREs alone supported almost no fusion, but that a tether such as HOPS is also required. Furthermore, the bilayer isn't just a neutral sheet; small headgroup (non-bilayer prone) lipids and acidic lipids have vital, specific roles. Most recently (manuscripts in preparation), we have striking findings of novel functions of Sec17p in fusion and of catalysis of SNARE complex assembly by HOPS.

a. A reliable assay of fusion

Zucchi, P. and Zick, M. (2011) Membrane fusion catalyzed by a Rab, SNAREs, and SNARE chaperones is accompanied by enhanced permeability to small molecules and by lysis. Mol Biol. Cell 22, 4635-4646. This paper reports the creation of a reliable assay for fusion (protected lumenal compartment mixing). I left my name off this paper (entirely done in my lab, and wholly supported by GM23377) in recognition of the exceptional contributions of these 2 postdocs.

b. Fusion requires more than trans-SNAREs

Zick, M. and Wickner, W. (2014) A distinct tethering step is vital for vacuole membrane fusion. eLife, doi: 10.7754/eLife.03251. Proteoliposome fusion has commonly been assayed by incorporating 2 fluorescent lipids into one fusion partner and none in the other. Fusion will indeed lead to diminished FRET, ie, dequenching of the donor fluorophore's fluorescence. However, to the suprise of the field, we showed this assay of "dequenching" can measure events other than fusion, and that tethering factors are required for fusion (defined and assayed as protected lumenal content mixing) even when the membranes are linked by trans-SNARE pairs.

c. SNARE complex assembly is proofread and catalyzed

Starai, V.J. and Wickner, W. (2008) Yeast HOPS complex proofreads the SNARE 0-layer and activates it for fusion. Mol. Biol. Cell **19**, 2500-2508. HOPS selectively suppresses vacuolar fusion in the presence of mutant or defective SNAREs.

Zick, M. and Wickner, W. (2013) The tethering complex HOPS catalyzes assembly of the soluble SNARE Vam7p into fusogenic *trans*-SNARE complexes. Mol. Biol. Cell, 24, 3746-3753. The initial evidence that SNARE complex assembly is actually catalyzed by HOPS. Further evidence has come from collaborative studies with the Hughson lab (manuscript in preparation).

d. Sec17p has a novel fusion activity.
Zick, M., Orr, A., Schwartz, M.L., Merz, A.J., and Wickner, W.T. (2015) Sec17 can trigger fusion of trans-SNARE paired membranes without Sec18. Proc. Natl. Acad. Sci. USA, in press. Sec17p is known to support Sec18p's activity of disassembling SNARE complexes, but here we show a surprising second activity associating with trans-SNARE complexes and inserting the Sec17p N-terminal hydrophobic loop into apposed bilayers to trigger membrane fusion.

Complete list of published work:

http://www.ncbi.nlm.nih.gov/pubmed/?term=wickner+w

D. Research Support

My lab has been supported since 1975 by R01 GM23377, now in its 39th year, for which I'm very grateful to NIGMS. The current application is to replace this with MIRA support. The goal of our work is to understand the interplay among proteins (4 SNAREs, Ypt7p, HOPS, Sec17p, and Sec18p) and the vacuolar lipids to mediate rapid and efficient fusion without lysis. The key personnel, Amy Orr and William Wickner, are respectively research associate and PI.