

# From the Archive

## Curbside recycling at the synapse

When John Heuser finished medical school at the height of the Vietnam War he was immediately eligible for the draft. Luckily for him, and for cell biology, he fulfilled his military service at the National Institutes of Health (NIH) in the United States Public Health Service. It was there that he brought the concept of “membrane recycling” to light.

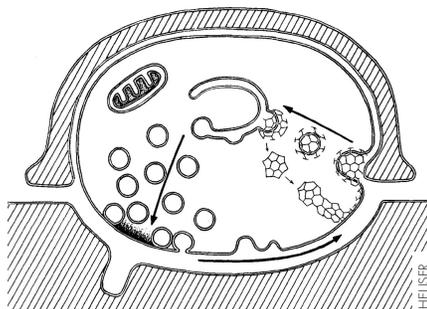
He brought to Thomas Reese’s lab at the NIH a postdoctoral project he had started with Sir Bernard Katz at University College, London—an attempt to capture a picture of neurotransmitter “quanta” being released as Katz had proposed. The only approach available to him at the time, Heuser recalls, “was to stimulate the nerve like hell and throw it into fixative.” But he

**When John Heuser and Thomas Reese visualize neurotransmitter “quanta” being released, they also catch sight of endocytic recycling that forms new synaptic vesicles. Ralph Steinman confirms that significant plasma membrane recycling must also be occurring in other cell types.**

soon realized, “the chances were still almost zero of catching it.” The method, however, gave him images of nerve terminals at the frog neuromuscular junction that had “weird vesicles and membranous cisternae” inside. Heuser,

now at Washington University (St. Louis, Missouri), recalls that other scientists who saw the images said the overstimulated nerves were “disgusting,” “just destroyed,” and “not relevant to anything.”

Heuser had a hunch, however, that the internal structures were not just signs of degradation, but instead were products of endocytosis. Together with Tom Reese, he decided to investigate his idea using horseradish peroxidase (HRP), the endocytic tracer that was just coming into its own as a powerful marker in electron microscopy. They stimulated frog nerve terminals while bathing them in extracellular HRP and found that HRP first appeared in clathrin-coated vesicles that formed from the nerve terminal plasma membrane. These vesicles then coalesced, and the HRP showed up in the internal cisternae (now known to be endosomes). Finally, the HRP ended up in a new population



The original recycling model.

of synaptic vesicles as they reformed (Heuser and Reese, 1973).

This evidence, along with the pair’s meticulous accounting of membrane fluxes between synaptic vesicles, plasma membrane, and cisternae, argued strongly for a rapid recycling of synaptic vesicle membrane via endocytosis. The model figure at the end of the paper headed straight into textbooks and the paper received more than 1,300 citations.

Heuser says he chose the term “recycling” deliberately, both because of the new environmental movement and because it made a critical distinction: “The synaptic vesicle is not like a cola bottle that never loses its integrity when returned to the factory to be filled again. Instead, it melts into the plasma membrane and is completely reformed, like an aluminum beer can.”

And, although the recycling model and paper are widely believed, Heuser is quick to point out another reason for the high number of citations—controversy. A paper published back-to-back with Heuser’s by Bruno Ceccarelli’s group at the University of Milan tipped off the beginning of a still-running debate. That paper also traced the depletion and reformation of synaptic vesicles from the frog neuromuscular junction, but reached an altogether different conclusion: that vesicles reformed directly from the plasma membrane at the site of their release (Ceccarelli et al., 1973).

This laid the groundwork for what would be called the “kiss-and-run” hypothesis: that synaptic vesicles could deliver their cargo by fusing slightly with the membrane and then reform by pinching back off (Fesce et al., 1994). These differ-

ent views led to a decade of competition between the two groups. Heuser says, “We were using the same preparations, and the results were identical, too. But we had opposite interpretations.”

Heuser and his colleagues substantiated their model with studies showing a correlation between synaptic vesicle exocytosis and quantal transmitter release (Heuser et al., 1979), thus confirming the one vesicle–one quantum theory. By then, Steinman et al. (1976) had shown that in nonneuronal cells so much membrane was coming into the cell (cells pinocytosed their entire cell surface area in ~30 min) that there must be a general recycling flow back to the plasma membrane. This work therefore defined a recycling pathway with the plasma membrane as destination rather than source. Meanwhile, Heuser went on to capture beautiful images of the structural changes that clathrin goes through during receptor-mediated endocytic events (Heuser and Evans, 1980).

For synaptic vesicle exocytosis, the question of kiss-and-run versus full fusion plus recycling is still very much up in the air. Some researchers now believe that both forms of exocytosis occur, but that cells use kiss-and-run when vesicles are in short supply (Wightman and Haynes, 2004). Heuser isn’t so sure. “Kissing and running isn’t an option for membrane compartments as tiny as synaptic vesicles,” he says. By the time these vesicles exocytose they lack any protective coat that could maintain their shape. After fusion, “the surface tension on such small membrane spheres is probably so great that their exocytosis cannot be reversible. I started thinking that after our 1973 paper, and still believe it.” **JCB**

Ceccarelli, B., et al. 1973. *J. Cell Biol.* 57:499–524.

Fesce, R., et al. 1994. *Trends Cell Biol.* 4:1–4.  
Heuser, J.E., and T.S. Reese. 1973. *J. Cell Biol.* 57:315–344.

Heuser, J.E., et al. 1979. *J. Cell Biol.* 81:275–300.

Heuser, J.E. 1980. *J. Cell Biol.* 84:560–583.  
Steinman, R.M., et al. 1976. *J. Cell Biol.* 68:665–687.

Wightman, R.M., and C.L. Haynes. 2004. *Nat. Neurosci.* 7:321–322.

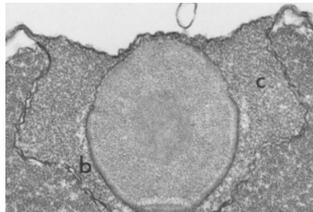
## Actin pushes in bizarre places

Lewis Tilney has always had a gift for looking for biology in strange places. His publishing history, he says, reads like a “Rogue’s gallery” of plants, fungi, parasites, and plenty of unusual invertebrate creatures. “It’s just ridiculous, and nowadays I couldn’t get away with it.” His colleagues say it is part of his genius to look in unusual systems to answer fundamental biology questions, but Tilney shrugs off that idea.

“I have the attention span of a five-year-old,” he says. “It’s always wonderful to start a new project that’s curious and interesting. Then something else attracts my attention and I move on—it’s a lack of commitment on my part.” So it’s no surprise that one of Tilney’s major contributions—proposing actin polymerization as a method of force generation within the cell—came through two landmark papers characterizing unorthodox systems: the acrosomal reaction in both starfish and sea cucumber sperm, and the cell-to-cell motility of the *Listeria monocytogenes* bacterium.

In the late 1960s, cytoplasmic actin that was not bundled into a contractile unit with myosin was just beginning to be recognized. At a lecture at Woods Hole Oceanographic Institute, Tilney heard Jean Dan describe the amazing acrosomal reaction of starfish sperm, which in seven seconds shoots out a process that is 45 times the length of the sperm cell. In Dan’s fuzzy pictures, Tilney could just make out some intracellular filaments inside the process. Could cytoplasmic actin be responsible for this fantastic reaction?

Using a glutaraldehyde and osmium tetroxide “mixed fix” that stabilized actin filaments, Tilney and his colleagues captured stunning pictures of the acrosomal process reaction with a clear bundle of filaments filling the process (Tilney et al., 1973). They identified the major protein in the acrosomal process as actin both by binding myosin in situ and by SDS gel electrophoresis. Since 80% of the actin was a monomer before the reaction and appeared amorphous in the images, Tilney reasoned that the only way to generate the process so quickly would be actin polymerization. “It was pretty obvious it had to be assembled, but I got a lot of heat for this,” he says. “The key question was, how can you push and polymerize at the same time?”



Actin drives the acrosomal reaction (left to right).

The same puzzle would come up again, 16 years later when Tilney teamed up with Daniel Portnoy. A bacteriologist, Portnoy arrived at the University of Pennsylvania in September of 1988 with a most intriguing observation. He had followed the intracellular bacterium *Listeria* moving from one infected macrophage to another and found that adding the actin inhibitor cytochalasin D had stopped the intercellular infection. So he sought out Tilney and his actin expertise.

Tilney remembers, “Portnoy crashed a department picnic and insisted I look at his damn *Listeria*—I couldn’t even spell *Listeria*—then I took one look, and bam, you’re hooked.” The two “hit it off scientifically,” Portnoy recalls, and the work went

quickly: “I had worked out the tissue culture model of infection and the system was ripe to analyze. Tilney had the right methods to look at actin. We submitted the paper by Christmas.”

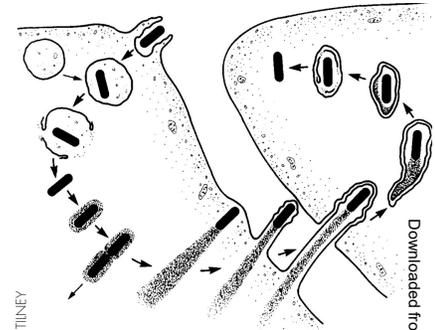
The collaboration demonstrated that *Listeria*, once inside a cell, acquired a “comet tail” of actin. It moved with the comet to the cell surface and into a cell extension that is eventually engulfed by a neighboring cell (Tilney and Portnoy, 1989). Tilney wrote, “thus, this insidious beast has managed to multiply and spread cell-to-cell without leaving the cytoplasm of its host.” (Portnoy had objected to the original wording, which included a description of the beast’s “Machiavellian deviousness.”)

The work led others to discover actin nucleation proteins such as the bacterial ActA protein (Domann et al., 1992; Kocks et al., 1992) and the Arp2/3 complex (Welch et al., 1997). The system could be reconstituted in vitro (Theriot et al., 1994; Loisel et al., 1999), and without myosin, so it clinched the idea that polymerization was generating the motility of the bugs. The force-by-elongation mystery would eventually be answered by a mathematical demonstration that Brownian motion could account for actin elongation pushing a membrane forward (Mogilner and Oster, 1996).

Matt Welch, who purified the actin nucleation complex, says the 1989 *Listeria* paper, “was really the culmination of this whole field of actin polymerization as a mode of motility. You didn’t need myosin to move these bacteria around and people made extremely good use of that system to show that what Tilney was saying in the 1973 [acrosomal process] paper was really true.”

Mark Mooseker, a co-author on that first acrosome paper, says Tilney, still using a 45-yr-old Philips 200 scope, remains one of the best electron microscopists in the field. “He is an absolute hero of mine and his impact is just huge.” Most recently, says Tilney, his studies have “drifted again” to look at a parasitic nematode: “a very curious beast with a sophisticated external gut—it’s both entertaining and disgusting.” We would expect no less. **JCB**

- Domann, E., et al. 1992. *EMBO J.* 11:1981–1990.  
Kocks, C., et al. 1992. *Cell.* 68:521–531.  
Loisel, T.P., et al. 1999. *Nature.* 401:613–616.  
Mogilner, A., and G. Oster. 1996. *Biophys. J.* 71:3030–3045.  
Tilney, L.G., et al. 1973. *J. Cell Biol.* 59:109–126.  
Tilney, L.G., and D.A. Portnoy. 1989. *J. Cell Biol.* 109:1597–1608.  
Theriot, J.A., et al. 1994. *Cell.* 76:505–517.  
Welch, M.D., et al. 1997. *Nature.* 385:265–269.



Actin-powered rocketing by *Listeria* gets it into adjacent cells.

