**From the Archive**

**BFA sends proteins back**

Observing a step backward brought Jon Yewdell a big step forward in understanding protein trafficking pathways in the cell. As a newly minted assistant professor at the Wistar Institute, Yewdell was examining where in the cell the influenza A virus (IAV) hemagglutinin (HA) trimerized. Using monoclonal antibodies (mAbs) specific for monomers or trimers, he had found by 1985 that monomers localized to the endoplasmic reticulum (ER), whereas trimers were first detected in the Golgi complex without a trace of ER staining (Yewdell et al., 1988). And there the trimers might have stayed except for a chance conversation.

Hearing Yewdell’s seminar at Case Western Reserve University, Alan Tartakoff urged him to read Akira Takatsuki’s paper showing that the drug Brefeldin A (BFA) blocked transport from the ER (Misumi et al., 1986). Yewdell wrote to Takatsuki, who generously sent a glassine envelope with a few milligrams of powder.

Recruited to the National Institutes of Health in 1987, Yewdell forgot about BFA until the envelope tumbled out of a lab notebook while he was unpacking. He treated IAV-infected cells with BFA, and observed for the first time ER staining with trimer-specific mAbs.

“I pretty much knew that I had discovered retrograde transport,” says Yewdell, he eventually performed an intricate pulse-chase experiment in which he first blocked HA egress from the ER with BFA and then removed the BFA in the presence of cycloheximide to prevent new HA synthesis. Now, the trimers completely cleared the ER and moved to the Golgi on their way to the cell surface. When Yewdell added back BFA during the chase, HA trimers magically reappeared in the ER.

“I would have never learned about BFA, except for the search for BFA’s target also really boosted our understanding of the coatomer and Arf1 proteins,” involved in retrograde transport. Arf1 is a key regulator of Golgi architecture, and it turns out that BFA stabilizes an Arf1 exchange factor, GBF1, on Golgi membranes (Niu et al., 2005). This inactivates Arf1, leading to exaggerated retrograde trafficking and the Golgi–ER fusion seen with BFA occur via tubules (Sciaky et al., 1997).

Simultaneously, Jennifer Lippincott-Schwartz, working with Richard Klausner, came to similar conclusions about BFA while tracking T cell receptors (Lippincott-Schwartz et al., 1989). “It was totally exciting when we saw the redistribution of Golgi back into ER,” she says. “It was the first example of organelle disassembly.” She says the two papers ignited the fields of retrograde transport and Golgi biogenesis. Lippincott-Schwartz and colleagues later demonstrated that both normal retrograde trafficking and the Golgi–ER fusion seen with BFA occur via tubules (Sciaky et al., 1997).

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**Jon Yewdell uses Brefeldin A to detect retrograde trafficking.**

Shifting infected cells to 32°C released the protein. Using this system, they showed that BFA induced the retrograde transport of Golgi enzymes that could now modify oligosaccharides of the ER-retained glycoprotein (Doms et al., 1989). They concluded that BFA caused both resident Golgi proteins and those in transport to be shifted to the ER. Whether the mechanism was by an exaggerated recycling pathway or an artificial fusion of the two organelles, the authors left open to further experimentation.

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**Suggested Reading**

- Doms, R.W., et al. 1989. *J. Cell Biol.* 109:61–72.
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