From the Archive

Lost in translation: the signal hypothesis

It was cell biology's version of the ship in the bottle. How do proteins a cell intends to secrete end up in the endoplasmic reticulum? Winkling out the details of the translocation mechanism that spirits these proteins into the ER required more than 20 years and earned Günter Blobel of Rockefeller University the 1999 Nobel Prize in Physiology or Medicine.

Another Rockefeller laureate, George Palade, had demonstrated that ribosomes free in the cytoplasm manufactured non-secreted proteins, whereas ribosomes stuck to the ER made proteins for export. Cell biologists searched in vain for distinctions between free and attached ribosomes that might explain their contrasting behavior. A new assistant professor at Rockefeller and Palade's protege, Blobel suspected that the difference must lie in the proteins themselves. He and colleague David Sabatini conjectured that secretory proteins might carry a short segment near the NH_2 terminus (Blobel and Sabatini, 1971). Once this sequence protruded from the ribosome during translation, a "binding factor" would hook onto the protein and guide it and the ribosome to the ER membrane. Continued translation would then thread the elongating protein into the ER's interior. "It was a beautiful idea," says Blobel. It was also, he admits, "pure speculation."

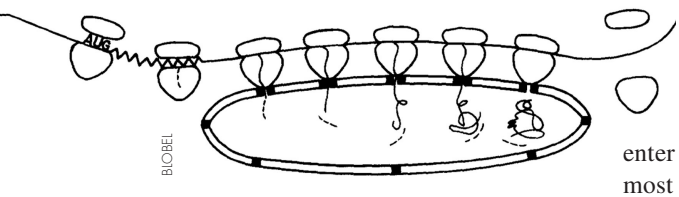
But it didn't take long for evidence of a "signal sequence" to start accruing. The cell-free translation system concocted by Philip Leder and colleagues (Swan et al., 1972) churned out an antibody light chain that was 6 to 8 amino acids longer than the normal secreted version in the body. Tonegawa and Baldi (1973) and Schechter (1973) obtained similar results.

Unaware of Blobel and Sabatini's hypothesis, Cesar Milstein of Cambridge University proposed a similar idea based on his team's cell-free system. It also pumped out an overweight light chain, but when the researchers checked the output of microsomes (ER fragments), they found only the normal-sized protein (Milstein et al., 1972). Milstein speculated that the extra amino acids help direct the growing protein to the ER.

Despite this suggestive data, detractors argued that the protein's extra heft was an artifact of in vitro translation or isolation errors, Blobel recalls. To answer their complaints, he crafted a protein-synthesizing system with help from post-doc Bernhard Dobberstein (now at the University of Heidelberg). Using detergent, they dislodged ribosomes from rough microsomes, and then slipped the particles—which carried unfinished light chains—into a solution that allowed protein making to resume. Because the researchers also added a compound that blocks new translation, the ribosomes could only complete chains they had started.

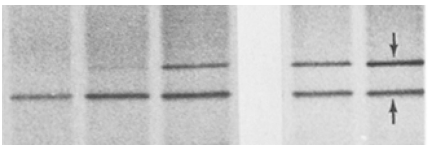
At first, only the smaller, processed chain appeared (Blobel and Dobberstein, 1975a). These proteins came from ribosomes that were well into translation when they parted from microsomes, the researchers concluded, and the chains they held had already undergone pruning to remove the signal sequence. After a few minutes, however, the synthesis mixture started producing longer chains as well. The bulkier proteins emerged from ribosomes that had just started translating when isolated from microsomes. At the time, they bore stubby chains that hadn't yet shed their signal sequence. When translation restarted, these short chains didn't lose the sequence—evidence that the processing enzyme that removes the signal is part of the ER membrane.

In another key experiment, Blobel and Dobberstein let rough microsomes—which carry ribosomes and some associated mRNA—produce proteins. The scientists detected only the shorter version. Adding the protein-dissolving enzymes trypsin and chymotrypsin (which rarely enter the microsomes) did not digest most of the chains, confirming that the trimmed protein ends up tucked away within the microsomes, as the signal hypothesis predicted.



The signal hypothesis in 1975, with the signal peptide as a dotted line.

The next goal, Blobel recalls, was to build the "translation-translocation" mechanism from scratch, using isolated mRNA, small and large ribosome units, and microsomes. But the work stalled. No matter what animal the microsomes came from, they always stifled translation in the cell-free system. After numerous setbacks, Blobel was "prepared for failure" when he tried microsomes from dog pancreas. Instead, in December of 1974, the procedure finally worked.



Ribosomes severed from microsomes make first a smaller, processed protein (left) and later a longer form with signal sequence intact (upper band on right).

The pair quickly showed (Blobel and Dobberstein, 1975b) that this combination produced mostly the short form of the light chain. If primed with the right mRNA, the system would also make globin, a non-secreted protein. Unlike the processed light chain, globin fell victim to the protein-dissolving enzymes, indicating that it didn't slip into the microsomes. Moreover, if complete, oversized light chains were added after the microsomes, they didn't lose the signal sequence, verifying that the removal of the segment occurs during translation, not afterwards. That their Rube Goldberg concoction of mouse RNA, rabbit ribosomes, and dog ER actually synthesized proteins demonstrated something else, Blobel says. "[It] had the virtue of showing that this is a universal system." JCB

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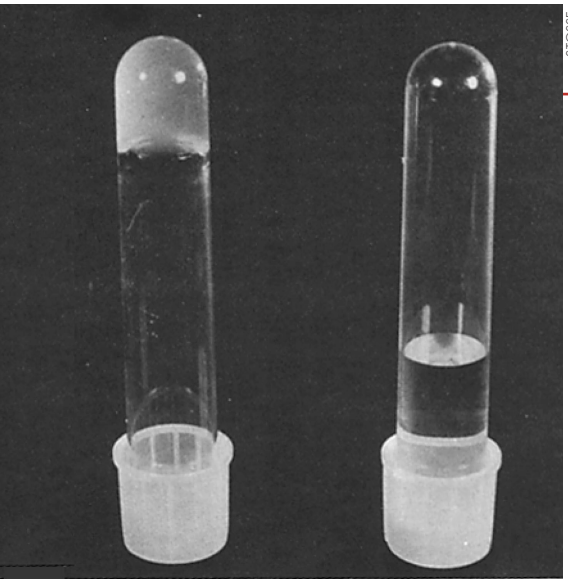

STOSSE

Gelled macrophage supernate sticks to the top of an inverted tube (left).

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Powered by gel

Thomas Stossel was lucky he didn't know how difficult phagocytosis would be to figure out. "If I'd known how complicated it was, I might have gone another way," he says. In the 18th century, some of the first observations with optical microscopes had shown that cells engulf food and slither along by turning part of their cytoplasm into a semi-solid gel, and then liquefying it again. Stossel and his colleague John Hartwig (both at Harvard University) wanted to know what controlled this gel–sol transformation.

At the time, the discovery that nonmuscle cells contained actin and myosin was fresh. But what the pushy proteins accomplished was uncertain—researchers had just discerned that actin helps form the contractile ring that pinches cells in half during division (Schroeder, 1972). Stossel and Hartwig started by nabbing a new molecule they called actin-binding protein—the very first actin-binding protein—that spurred actin fibers in vitro to coalesce into a mesh (Hartwig and Stossel, 1975). This mesh later turned out to provide a substrate for myosin-mediated contraction.

Next, Stossel and Hartwig (1976) reproduced this phenomenon with purified proteins and linked the process with what was happening in vivo during phagocytosis. They showed that extracts of macrophages in the midst of phagocytosis solidified into a gel and did so faster than did those from cells that weren't eating. What's more, cytoplasm from cells that had recently swallowed an oil droplet contained more actin-binding protein than did material from resting cells. A mixture of actin, myosin, and actin-binding protein, but not the duo of actin and myosin alone, would also gel.

The idea that actin molecules can't knit into a gel without help from actin-binding protein was controversial, Stossel recalls. In fact, the preceding paper in the same issue argued the opposite view (Pollard, 1976). Stossel says that it took about 15 years to win over most doubters, and during this time the number of participating molecules swelled. For example, Stossel's lab discovered a protein called gelsolin, which unhooks actin filaments (Yin and Stossel, 1979). Gelsolin and the original actin-binding protein, now called filamin A, are two of the hundreds of molecules that help orchestrate cell movements. JCB

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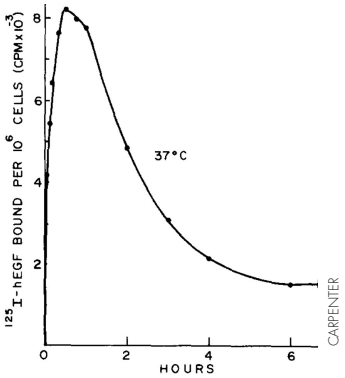
EGF is internalized and degraded

Thirty years ago, cell biologists were convinced that protein hormones and cells had a superficial relationship. Although steroid hormones such as testosterone could squeeze through the cell membrane to deliver commands, their protein counterparts never got beyond receptors on the cell's surface. Graham Carpenter and Stanley Cohen (both then at Vanderbilt University) overturned the conventional wisdom with their study of epidermal growth factor (EGF), a protein hormone that spurs fibroblasts to duplicate their DNA and divide.

When the pair steeped human fibroblasts in EGF tagged with radioactive iodine, they found that the amount of radioactivity affixed to the cell's surface peaked after ~30 to 40 min, and then plummeted (Carpenter and Cohen, 1976). To track the missing radioactivity, Carpenter and Cohen soaked cells in labeled EGF before shifting them to a hormone-free mixture. The hot iodine returned to solution, the researchers discovered, but not as part of EGF. Almost all of it had transformed into monoiodotyrosine and diiodotyrosine, breakdown products of EGF. That finding provided strong circumstantial evidence that after EGF binds to a receptor, cells take in the hormone, chop it up, and eject the fragments.

To bolster that conclusion, the team added antibodies that target EGF to a solution of cells bathed in the hormone. The longer the experiment ran, the fewer antibodies attached to the cells, implying that EGF was vanishing from the plasma membrane. Carpenter and Cohen's results suggested the hormone was ending up in the lysosomes for demolition. When they combined EGF-laden cells with chloroquine, which hinders the organelle's protein-slicing enzymes, the breakdown of EGF slowed. "The key experiment was showing that lysosomal inhibitors prevented degradation [of the hormone]," says Carpenter.

The findings supported the notion that the hormone's receptors are "swallowed" and replaced by fresh proteins—an inference later studies substantiated. The team determined that cells required 10 h to regain their full EGF-binding capacity. But the recovery stagnated if the researchers mixed in molecules that inhibit protein or RNA synthesis. Further work showed that cells absorbed and processed more than just protein hormones. For example, research led by Nobel laureates Michael Brown and Joseph Goldstein demonstrated that cells also engulf low-density lipoproteins and recycle the receptors (Anderson et al., 1976, 1977, 1982). And multiple studies in recent years have emphasized that a lot of signaling occurs even after uptake of receptors into cells. JCB



CARPENTER

Labeled EGF binds to cells but is then taken up and degraded.

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