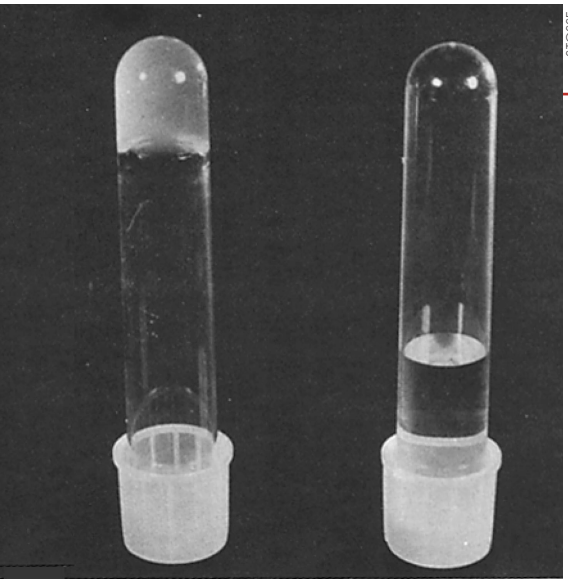

STOSSE

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

Text by Mitch Leslie
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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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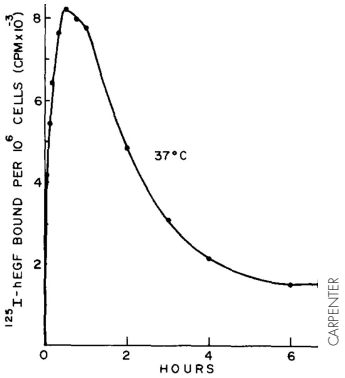
EGF is internalized and degraded

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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