

and indirect. Cell biologists have attempted to find them by measuring an aspect of how individual cells enlarge—whether their growth is linear or exponential. For linear growth, a cell would enlarge at a constant rate until it divides. But for exponential growth, the increase would be proportional to the cell's girth. In the latter case, the small but natural variations in size between two cells resulting from division would rapidly expand to create a population of cells with large size differences. So if cells are growing exponentially, researchers reason, they must have a checkpoint to rein in growth and keep themselves within the observed narrow size range.

Determining what type of growth various kinds of cells actually undergo can be extremely difficult, however. The disparity in growth rates between a linearly growing cell and an exponentially growing one will be only about 6%, Kirschner notes.

Four years ago, cell biologist Alison Lloyd of University College London and colleagues gauged how quickly nervous system cells enlarged. Their results suggested that growth in these cells was linear. "Do mammalian cells have a checkpoint? We say no, the cell doesn't need it," Lloyd says.

In a *Science* paper published 2 years ago (10 July 2009, p. 167), Kirschner and colleagues applied a statistical approach to reach the opposite conclusion about immune system cells. The next year, Kirschner teamed up with Scott Manalis of the Massachusetts Institute of Technology (MIT) and colleagues to weigh individual cells, herding them one by one onto the microscopic equivalent of a scale. Again, they found that the cells, which included bacteria and immune cells, displayed exponential growth, suggesting that checkpoints are operating. The identity of the molecules that

detect the size of the cells remains unclear, however, Kirschner says.

His group and Lloyd's could both be right, Kirschner suggests: The two teams studied different kinds of cells that might behave differently. Umen agrees. Fast-growing immune cells might be more like yeast, relying on their own size-measuring mechanism, whereas nervous system cells mainly heed external cues, he speculates.

Although researchers have made some progress toward understanding how dividing cells sense and control their size, that issue is part of a broader question, says molecular biologist David Sabatini of MIT. Most of the cells in our bodies don't divide, yet they can continue to grow. How these cells sense when they are big enough to stop expanding is also a mystery, he says. Biologists who want to decipher cell size control still have some big questions to answer.

—MITCH LESLIE

How Does the Cell Position Its Proteins



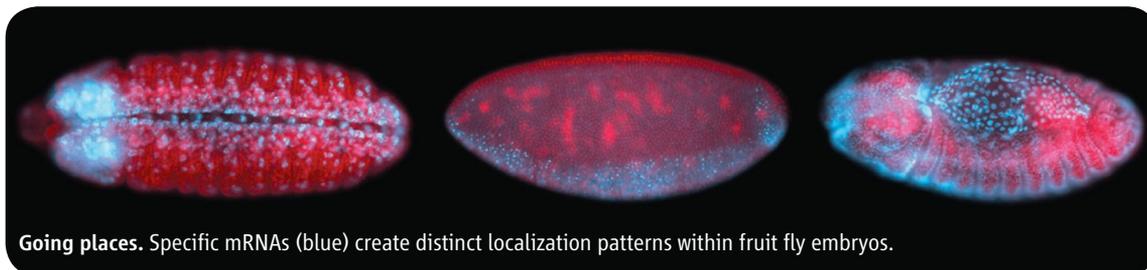
If you think air traffic controllers have a tough job guiding planes into major airports or across a crowded continental airspace, consider the challenge facing a human cell trying to position its proteins. The latest analyses suggest that some of our cells make more than 10,000 different proteins. And a typical mammalian cell will contain more than a billion individual protein molecules. Somehow, a cell must get all its proteins to their

the cytoplasm is a homogeneous goo."

As organelles were discovered and specific proteins were found to be localized to them and to other cellular compartments, there came a need to explain how the molecules get to specific homes. Enter cell biologist Günter Blobel of Rockefeller University in New York City, who with colleague David Sabatini theorized in 1971 that proteins intended for the endoplasmic reticulum carried among their

find specific homes or molecular partners within the relatively vast spaces of a cell. So does the cell have other ways to concentrate proteins in the right spots? Undoubtedly, biologists say. "The Blobel hypothesis in a sense kept blinders on people for a long time," notes developmental biologist Henry Krause of the University of Toronto in Canada. "The common perception was that proteins knew how to get places."

Biologists are finding other tricks cells use to place newly minted proteins, but another emerging story is that a cell begins to position proteins even before they are made. Starting in the mid-1980s, a few biologists began to



Going places. Specific mRNAs (blue) create distinct localization patterns within fruit fly embryos.

correct destinations—and equally important, keep these molecules out of the wrong places. While research addressing this challenge has already produced a Nobel Prize, biologists stress that the mystery of how cells place their protein repertoire is far from solved.

Protein localization within the cell wasn't always recognized as a fundamental puzzle. Before the advent of powerful microscopes and tools to label individual proteins, scientists typically considered cells simple bags of freely diffusing molecules. People were taught, says cell biologist James Wilhelm of the University of California, San Diego, "that

amino acids a localization, or sorting, signal. In work that won him a Nobel Prize in 1999, Blobel subsequently proved that conjecture, identifying sorting signals that direct newly synthesized proteins to various organelle membranes. "The concept that a sequence on a protein carried information relevant to its final destination was novel. ... It was very speculative," says cell biologist Karl Matlin of the University of Chicago, who is writing a history of that period.

Nobel Prize—case closed on protein localization? Not so fast. Many proteins operate outside organelles but still need to

gather evidence that cells localized proteins by directing the corresponding messenger RNAs (mRNAs) that encode their production to a specific destination, so the proteins are manufactured where they are needed. One of those pioneers, Robert Singer, now at Albert Einstein College of Medicine in New York City, initially showed that fibroblast cells position the protein beta-actin's mRNA to facilitate cell movement. Other scientists followed, revealing mRNA localization in fruit fly and frog eggs and in various animal neurons, for example. But a perception lingered that these cases were

rare or oddities. “It was awfully lonely for a decade or so,” Singer recalls, noting that a meeting on mRNA localization he organized in 1994 drew only about 30 scientists.

There are seemingly obvious advantages to mRNA localization for positioning proteins. Efficiency, for example. Rather than requiring a cell to move many copies of a protein, Singer notes, “one mRNA could make thousands of proteins, and in the right place.” Localizing mRNAs could also prevent dangerous, unintended interactions as proteins move through a cell. “It seems easier to sort the information for a molecule than the molecules themselves,” Wilhelm says.

It’s increasingly clear that cells agree with Wilhelm. He, Singer, and others point to a 2007 study by Krause’s group as some of the first compelling evidence that mRNA localization is pervasive. In that work, the scientists labeled RNA with fluorescent tags and systematically observed the positioning of more than 3000 different types of mRNA during early fruit fly development. More than 70% exhibited clear localization. That’s a “staggeringly large number,” Singer says. “It’s almost as if every mRNA coming out of the nucleus knows where it’s going.”

Thanks to this and similar work, many biologists are taking a new look at mRNA localization. And to an extent, history is repeating itself. Much as Blobel did with proteins, biologists are now identifying specific short RNA sequences—Singer calls them Zip Codes—that direct mRNA strands to various parts of the cell. Even proteins that have sorting signals of the type Blobel studied may also have mRNAs with built-in Zip Codes. “It looks like the RNA is localized first,” Krause says.

Krause suspects that cells needing to position proteins turn to RNA in additional ways. He speculates that certain RNA strands serve as focal points within the cytoplasm around which specific complexes of proteins form.

Clifford Brangwynne of Princeton University may have uncovered something along those lines while probing how cells position so-called P granules, poorly understood assemblages of proteins and RNA that help specify germ cells during development in nematodes. Once the fertilized worm egg breaks symmetry, P granules move to one half of the single-cell embryo, from which germ cells will arise. In 2009, Brangwynne reported that P granules behave like liquid droplets within cytoplasm—the drops can fuse to one another, drip out of dissected

cells, and “wet” the surfaces of organelles such as the nucleus—and that specific RNA-binding proteins control localized “condensation” of these drops.

The biophysicist suspects that cells often use this strategy to condense RNAs and proteins into dynamic “microreactors” that encourage molecular interactions, perhaps between an enzyme and its substrate, for example. “People usually think about cellular organization being accomplished largely by membrane-bound compartments, which are bathed in a homogeneous cytoplasmic fluid,” he says. “The work by us and others in this area is beginning to paint a picture in which this

cytoplasmic fluid is actually highly structured, which is useful for putting things in the right place at the right time.”

Cell biologist Timothy Mitchison of Harvard Medical School in Boston has recently collaborated with Brangwynne and found that nucleoli, RNA-protein bodies in a cell’s nucleus, similarly represent such droplets. “It’s fair to say Cliff has discovered a new state of biological matter,” he says.

It’s clear that biologists have a long way to go before they fully understand the protein traffic control system within a cell. A Nobel Prize doesn’t mean a mystery is solved, Blobel says with a laugh. “There’s a lot left to be learned.”

—JOHN TRAVIS

Online

sciencemag.org

Podcast interview
with author
John Travis.

How Do Hungry Cells Start Eating Themselves?



Hungry cells take recycling to the extreme. When a cell runs out of raw materials, it sprouts an internal membrane that encapsulates some of its contents and breaks them down for reuse. The process is called autophagy: literally, “self-eating.” Cell biologists have long wondered where this membrane comes from. Now, they might be close to an answer.

To devour part of itself, a cell initially fashions the equivalent of a mouth: a membrane pocket known as a phagophore. The expanding phagophore swallows a portion of cytoplasm, trapping proteins and even organelles such as mitochondria. After it closes, this membrane receptacle, now termed an autophagosome, docks with the cell’s version of a stomach—the lysosome—which digests the delivery, recycling the material it contains.

Even in good times, when nutrients are plentiful, cells rely on autophagy to rid themselves of worn-out or defective organelles and molecules. More and more evidence suggests that when this self-cannibalism goes awry, cellular—and overall—health suffers. Faltering autophagy might promote a range of ill-

nesses, from neurodegenerative disorders such as Huntington’s disease to diabetes, and it could also spur aging.

Since researchers discovered autophagy in the 1950s, they’ve worked out many of its molecular details, but the origin of the autophagosome membrane has slipped through their grasp. And that’s frustrating because determining where the membrane comes from could give a boost to researchers who hope to combat diseases by managing autophagy.

The autophagosome “wasn’t following the rules,” says cell biologist Jennifer Lippincott-Schwartz of the National Institute of Child Health and Human Development in Bethesda,

Maryland. Most organelles, she explains, hew to a “like from like” rule. A new mitochondrion, for example, is born when an existing one cleaves. But autophagosomes apparently don’t spring from other autophagosomes.

That leaves two options. Proteins inside the cell could build the phagophore from scratch, synthesizing fresh membrane in the cytoplasm. Alternatively, the cell could borrow lipids from another location;



Gobble, gobble. In this model, lipids form a membrane mouth (blue) that envelops cell contents.