Secrets of Secretion Revealed

An extraordinary confluence of three lines of research, culminating last month, shows that the same mechanism governs key communications processes in cells from yeast to the human brain

In order to carry out its necessary functions, a cell must not only make a large number of proteins, but also get them delivered precisely to the parts of the cell where they are needed. This task is complicated by the fact that the cell is more than a bag of watery cytoplasm—it is a maze of compartments, each bounded by a fatty membrane similar to the membrane surrounding the cell itself. Each compartment represents a destination for a specific set of proteins, and accurate delivery of these proteins depends on a "membrane traffic" system made up of a trusty fleet of freight carriers, in the form of little membrane-bound packets known as vesicles.

Biologists have long been trying to figure out the molecular machinery that directs the vesicles to the correct cellular addresses and enables them to fuse with the target membranes, transferring their cargo. For a decade, geneticists, cell biologists, and neuroscientists have been addressing the question independently, using experimental systems ranging from yeast to the mammalian brain. They assumed that, although the general scheme of vesicle trafficking was similar in these systems, the precise molecular mechanisms might well turn out to be quite different. But to their surprise, over the past year the three main lines of research on membrane traffic and secretion have converged on the very same molecular machinery—which has changed surprisingly little over the billion years of evolution separating our brains and the budding yeasts.

"What is really exciting...is how all these different fields of vesicular traffic are coming together," says Pietro DeCamilli, who studies neurosecretion at Yale. The findings "show the extraordinary universality of molecular constituents," adds Columbia University neuroscientist Eric Kandel, who attended a recent conference sponsored by the Howard Hughes Medical Institute where the new discoveries were discussed. "We're dealing with a molecular machine in the brain that is about as distinctive as you could get...and yet when you analyze the details of the components, you find that a surprisingly large number of them are shared...not only [with] other parts of the cell, but [with] all organisms, all the way down to yeast."

Of all the traffic patterns in the cell, one of the best known is the route taken by a protein destined for secretion. After being synthesized on ribosomes attached to a complex structure known as the endoplasmic reticulum (ER), the protein is deposited inside that structure. It subsequently leaves the ER enclosed in a vesicle formed from ER membrane, which is directed by unknown molecular signals to the next destination: the Golgi apparatus, a collection of membrane-bound structures resembling a stack of pancakes. There, the vesicle docks and fuses with the Golgi membrane, discharging the protein into the Golgi, where it is modified. Other vesicles shuttle the protein between Golgi compartments, until a vesicle containing the finished protein buds off and is directed to the cell surface, where the protein is released.

This route from ER to Golgi to cell membrane is present in all cell types, but not all forms of secretion rely on it exclusively. For example, neurons, which are constantly releasing neurotransmitter into the synapses that divide them from neighboring neurons, have a recycling system that pumps neurotransmitter back into the cell, where it is loaded into empty vesicles formed locally from excess cell membrane and released from the cell once again. Neurons have many other kinds of specializations as well, such as their unique coupling system that allows neurotransmitter to be released less than a millisecond after the arrival of a nerve impulse. Those features, says Salk Institute neuroscientist Chuck Stevens, led some researchers to think "neurotransmission is so special that it might have an entirely special apparatus."

The recent flurry of papers, however, shows that neurons aren't unique—at least in terms of membrane traffic and secretion. That conclusion stems from the weaving together of three independent research programs: a search for mutant yeast strains defective in secretion; an effort to reconstruct and biochemically analyze vesicle trafficking in the test tube; and a characterization of the key membrane proteins found at nerve endings.

Parallel pursuits

The first of the three lines of research to identify the proteins involved in membrane trafficking was the genetic study of yeast, begun in the late 1970s by Randy Schekman and his colleagues at the University of California, Berkeley. Schekman's strategy was to isolate yeast mutants with defects in secretion and characterize the mutant genes to learn their role in the process.

Schekman and others have found more than 50 mutant strains. Some are blocked in transport from ER to Golgi, others are blocked within the Golgi, and still others are unable to discharge the contents of their vesicles at the cell surface. These mutants "define the pathway of transport," says Schekman, since each mutant is defective in a gene whose protein product is required for at least one step in membrane trafficking. The normal

Traffic Patterns: Mammalian Proteins and Their Yeast Counterparts

Protein	Alternate names	Location	Yeast counterparts
NSF		Cytoplasm	SEC18
SNAPs (α, β, γ)		Cytoplasm	SEC17(α)
VAMP	Synaptobrevin	Vesicle membrane	BET1 and SLY2 (ER to Golgi) SNC1 and 2 (Golgi to plasma membrane)
Syntaxin	HPC-1	Plasma (target) membrane	SED5 (ER to Golgi) SS01 and 2 (Golgi to plasma membrane) PEP12 (Golgi to vacuole)
SNAP-25		Cytoplasm and plasma membrane	

counterparts of many of those mutant genes have been cloned over the years, but initially very few showed sequence similarity to known proteins—hardly surprising, since at the time virtually nothing was known about the proteins that control secretion.

While Schekman and others were isolating yeast mutants, cell biologist James Rothman of the Memorial Sloan Kettering Cancer Center in New York was developing a second, completely different, line of research, albeit one directed at a similar goal. Since the late 1970s, Rothman, then at Stanford, had wanted to purify the proteins governing vesicle traffic in animal cells. To achieve that goal, he and his colleagues set out to reconstitute the process in the test tube, so that they could observe the effects of adding or subtracting individual proteins.

By the early 1980s, Rothman and his coworkers had completed the first step: reconstitution. When they combined purified Golgi membranes, ATP (as an energy source), and proteins from the cell's cytoplasm, they got vesicles to transport proteins between Golgi compartments. By 1988 they had used chemical inactivation by a compound called NEM to identify a cytoplasmic protein needed for vesicles to fuse with their target membranes, and they had purified the protein, which they called NSF (for NEM-sensitive fusion protein). With NSF in hand, they went on to find other proteins that aided the process, which they called "soluble NSF attachment proteins," or SNAPs.

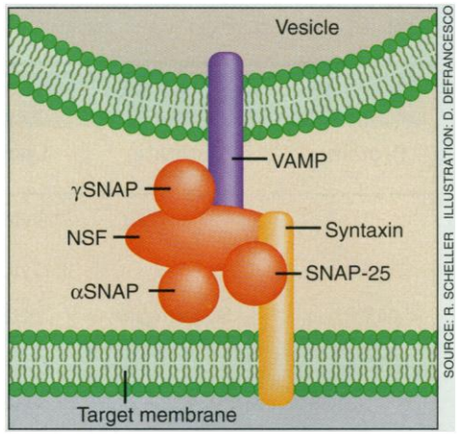
The third avenue of research was taken by neurobiologists in several labs who wanted to understand the machinery responsible for the release of neurotransmitter-filled "synaptic vesicles" at the nerve terminals where neurons communicate with one another. These neurobiologists took advantage of the fact that nerve terminals are chock full of vesicles. "Vesicles are so abundant in the brain," says Richard Scheller, a Stanford biologist who studies neurosecretion, "that we can purify large amounts of them, and characterize all their proteins." Which is just what a number of labs proceeded to do. In the past few years, the resulting list of synaptic proteins purified from neurons has grown to nearly a dozen. From the amino-acid sequences of the proteins, researchers could postulate how they might play a role in the fusion of vesicles to their target membranes.

Despite this progress, concrete evidence that the proteins the neuroscientists were finding are actually involved in vesicle fusion remained sparse. In addition, the amino-acid sequences of these proteins were unlike those of other known proteins. "All these proteins we were identifying seemed specific for synaptic vesicles," says Reinhard Jahn of Yale University, who in collaboration with Thomas Südhof of the University of Texas Southwest Medical Center isolated several vesicle proteins. That seemed to mean one of two things, says Jahn. Either neurotransmitter release is a unique process, using a unique set of proteins, or "we hadn't really gotten the players yet, and what we were looking at was some kind of abundant [proteins] which had something to do with neuron functions," but not with vesicle fusion.

Filling the gaps

The neuroscientists had to live with those doubts for several years. But developments were afoot in the two other areas that would soon link their story to the trafficking process common to all cells—and provide some answers about the proteins they had found.

In 1989, after cloning the NSF gene found in mammalian cells, Rothman postdoc Duncan Wilson found that NSF was similar in sequence to SEC18, a yeast protein that had come out of the Schekman group's screen for secretion mutants. More exciting yet, Wilson found that the yeast SEC18 protein could actually substitute for mammalian NSF in a test-tube assay. The two proteins "were not only similar, they were functionally interchangeable," Rothman says, adding that "this was the first strong hint that the mechanisms in yeast and animal cells were going to be very similar."



Togetherness complex. Vesicle docking seems to involve the formation of a complex that includes proteins from the cytoplasm as well as proteins permanently inserted in the vesicle and target membranes.

And there was more to come. Schekman's and Rothman's groups subsequently showed that α-SNAP, one of the three SNAP proteins associated with NSF, also has a yeast counterpart: SEC17, another of Schekman's secretory mutants. In a separate bit of convergence, Peter Novick of Yale cloned and sequenced the yeast secretory mutant SEC4 and found it had homology to the oncogene *ras*. Like the *ras* protein, Novick found, SEC4 was a GTP-binding protein, and GTP binding by SEC4 was in fact crucial for vesicles to fuse with the cell membrane.

Novick's observation "set off a flurry of activity all over the world, looking for homologs," says Schekman. The result: A whole family of proteins related to the yeast mutant SEC4 was found to be associated with different types of vesicles in animal cells. Armand Tavitian's group at the INSERM Faculté de Medecine Lariboisiere-Saint Louis in Paris found many of them, and called them *rab*, for *ras* genes from rat brain.

At this point the unification moved to a new level. The three protein families that had so far been linked in yeast and mammals—NSF, SNAPs and Rabs—are all cytoplasmic proteins that associate transiently with membranes. But researchers knew there had to be more to the story. They knew there must be proteins permanently embedded in the membranes to act as markers and anchors for the cytoplasmic proteins to recognize and latch onto. And, they reasoned, these integral membrane proteins must come in two flavors: one for the vesicle membrane, one for the target, or "acceptor" membrane.

Some of the yeast proteins involved in secretion were integral membrane proteins, but the researchers with the corner on integral membrane proteins in animal cells were the neuroscientists, because their approach to the whole problem had been to purify membranes from nerve terminals and characterize the proteins they contained.

Yet as recently as a year ago, no homology had emerged between these neuronal membrane proteins and the membrane proteins in yeast. And for that matter, there was no hard evidence that the neuronal proteins really play a role in vesicle docking and fusion. But in the past 9 months all that has changed, as the neuroscience story has linked up with the other two.

An early move toward the linkage came last summer, when Mark Bennett, a postdoc with Scheller at Stanford, identified a protein from neuronal cell membranes, which he calls syntaxin. Scheller hypothesized that syntaxin is involved in vesicle docking, because it binds a protein called synaptotagmin, which is found in secretory vesicle membranes. At first, syntaxin was in a class by itself, as most of the other vesicle proteins seemed to be. But in the case of syntaxin, the isolation didn't last long. "When syntaxin was published [last July], there were no homologs in the [DNA] database," says Scheller, "but within 6 months, there were three homologous genes identified in yeast."

The yeast homologies provided indirect evidence that syntaxin was indeed involved in vesicle trafficking. Hugh Pelham of the Medical Research Council (MRC) in Cambridge, England, was the first to see the homology, when he realized last summer that the amino-acid sequence of syntaxin was similar to that of a yeast protein called SED5, which his group had just found to be essential for vesicle transport from the ER to the Golgi.

The similarities went beyond mere sequence data to include location: Both proteins seemed to be found not on the vesicle membrane, but on the acceptor membrane. "We got excited," says Pelham, "because these are the only [known] integral membrane proteins on the acceptor side of the fusion events."

The implication was that if syntaxin looks like SED5, and sits in the acceptor membrane as SED5 does, then it probably plays a role in trafficking, as SED5 does. Moreover, Pelham soon realized that SED5 resembled two other yeast proteins that are involved in vesicle fusion steps at the plasma membrane, and at a yeast organelle called the vacuole. The emerging pattern, Pelham says, is that there seems to be a family of proteins in yeast—with a counterpart in neurons—whose members play related roles at different steps in the membrane trafficking process.

Meanwhile, it was becoming apparent that a similar family of proteins exists in yeast vesicle membranes, and it too has a counterpart in neurons. Jeffrey Gerst at the Mt. Sinai School of Medicine found a pair of proteins he calls SNC1 and 2 in secretory vesicle membranes in yeast, which are 40% identical to the mammalian vesicle-associated membrane protein known as VAMP. Genetic experiments in yeast showed that the SNCs are essential for secretory vesicles to fuse with the plasma membrane. Again, the implication is that VAMP may be important for vesicle fusion in neurons, since its yeast counterparts have been shown to have such a role.

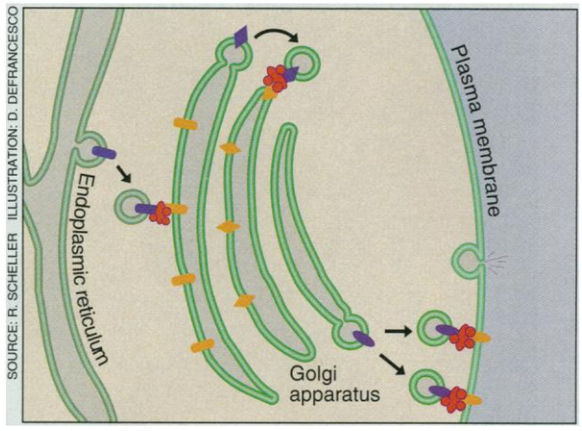
All of that evidence was intriguing—but it was also inferential. At least in the case of VAMP, however, direct evidence that the protein actually plays a role in neuronal secretion quickly followed. Last fall, the lab of Cesare Montecucco, at the Centro C.N.R. Biomembrane, in Padova, Italy, followed by Jahn and Südhof, reported that VAMP is the target of two lethal neurotoxins, tetanus and botulinum toxin, both of which are known to do their damage by blocking neurotransmission. "That was a seminal [finding]," says Yale's DeCamilli. "For the first time, a single membrane molecule was identified as playing a crucial role in the secretory machinery of neurons."

The circle closes

At that juncture it seemed clear that there were a set of membrane proteins and a set of cytoplasmic proteins, both involved in vesicle transport, in cells ranging from yeast to neurons. But were those proteins components of the same molecular machinery? Only last month, Rothman's group answered that question, and, in doing so, they brought all three lines together at a central point.

The finding originated in work undertaken by Rothman postdoc Thomas Söllner, who was searching for proteins in the membranes of vesicles and their targets that bind to SNAPs and NSF. Söllner knew that SNAPs and NSF form a biochemically active complex with other proteins that, after serving its purpose, falls apart when ATP in the complex is hydrolyzed during vesicle docking and fusion. When Sollner purified the complex from cow brains and gave it ATP to hydrolyze, the proteins that dropped off were syntaxin, VAMP, and another protein, coincidentally named SNAP-25 (for synapse-associated protein) by its discoverer, Michael Wilson of the Scripps Research Institute in La Jolla.

That finding shook the field when it appeared in *Nature* last month, suggesting as it does that the proteins that various researchers had been finding—and which were directly related from yeasts to brain—are in fact working together as a unit in the process of membrane trafficking. Although their exact role in vesicle docking and fusion remains to be learned, says Pelham of the MRC, the revelation "ties everything together and gives you a real feeling that one can begin to understand this."



Traffic signals. In this model, the cytoplasmic proteins, NSF and the SNAPs, participate in all stages of trafficking, while the membrane proteins for each step are separate members of a family of related proteins.

To many researchers, the recent findings suggest a model for vesicle docking and fusion in which the soluble proteins NSF and the SNAPs (or their yeast counterparts Sec18 and Sec17) are common to fusion complexes throughout the cell, while the integral membrane proteins VAMP and syntaxin represent protein families whose members are located on different types of vesicles and take part in specific kinds of vesicle fusion.

Despite the appealing elegance of that model, many of its elements still need testing. And even as the convergence is under way, it would be an oversimplification to conclude that secretion in neurons is simply a replica of the process in yeast, says Yale's Jahn. Neurons still have key distinctions, the most important being that their release of neurotransmitter is regulated. Unlike yeast and many types of animal cells, which secrete their products as they make them, neurons (along with other specialized secretory cells) hold their vesicles until a signal such as a nerve impulse comes along and gives the vesicles the go-ahead to fuse with the membrane and release their product. "I think what we will see in the next few years...is that synaptic vesicles use general [secretion] machinery, but in addition, neurons have specialized, added components," Jahn says. The vesicle protein synaptotagmin, which binds calcium, but is not found in yeast, may be a candidate for such a regulator, he adds.

And the identity of such regulatory proteins isn't the only outstanding mystery. Although some of its elements have been glimpsed, the biochemical functioning of the machinery common to all secretion is far from understood. For example, how do vesicle and target membranes actually fuse? Most researchers assume a protein triggers the process; SNAP-25, Rab, and another protein called synaptophysin have all been nominated as candidates. Another outstanding question is just how vesicles are directed to the right membrane among the sea of membranes within the cells. Members of the VAMP and syntaxin families could do the directing, but Rabs, of which there are many varieties dotting all the vesicle types, are another possibility.

"There are quite a few proteins lurking on synaptic vesicles, so there is still some room for discovery," says Tom Martin of the University of Wisconsin, who is searching for proteins that regulate secretion. Indeed, probably the greatest fortune is that the convergence of fields has brought so much enthusiasm and experience to bear in one place. All that energy and more will no doubt need to be put to use before the mystery of vesicle trafficking and neurotransmitter release is solved.

–Marcia Barinaga

Additional Reading

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