BIOCHEMISTRY

Modeling the synapse

A combination of experimental protein analysis and imaging techniques is used to create a three-dimensional model of a synaptic bouton.

The synaptic bouton, the compartment found at the axon terminals of a nerve cell, is an amazing structure responsible for rapidly transmitting signals to the dendrites of other neurons. In the process of neuronal signaling, vesicles are filled with neurotransmitters and then transported to a location within the synaptic bouton known as the active zone, where the vesicles dock at the plasma membrane and await an action potential. When this electrical signal arrives, it triggers the release of the neurotransmitter contents of the primed vesicles, thus rapidly transmitting the signal to the next neuron in line. The vesicles are then retrieved by endocytosis and recycled, filling up once again with neurotransmitters. This process is exquisitely controlled, enabling neurons to swiftly send signals to other cells and allowing a complex organism to respond to its environment and coordinate its movements.

Through a heroic effort involving a potpourri of different protein analysis and imaging methods, Silvio Rizzoli of the European Neuroscience Institute in Germany and his colleagues now present a three-dimensional (3D) model of an average synaptic bouton that displays the structures and locations of about 300,000 individual proteins. This model provides atomic-level insights into the mechanisms of neurotransmitter release and synaptic vesicle recycling.

Rizzoli's team isolated synaptic boutons from the brains of rats using the classical method of density-gradient centrifugation. They used electron microscopy to verify that they had indeed isolated synaptic boutons and to determine the boutons' size, shape and volume. They used quantitative immunoblotting and fluorescence microscopy to estimate the copy numbers of 62 individual proteins per synapse. They also utilized label-free quantitative mass spectrometry to determine the abundance of about 1,100 additional synaptic proteins. Together, the quantitative immunoblotting and mass spectrometry analysis accounted for about 88% of the total protein weight in the synaptic bouton preparation.

SENSORS AND PROBES

LIVE REPORTING OF KINASE DYNAMICS

A simple reporter tracks kinase activity in a living cell.

Kinases are message bearers of the cell, passing information between molecules by adding a phosphate group to target proteins. Understanding their regulatory roles calls for tools to follow activity *in vivo*. Most kinase reporters require two fluorophores to carry out fluorescence resonance energy transfer (FRET) and can be tricky to implement. Markus Covert and his team at Stanford University have recently come up with simple alternatives to report on kinase dynamics in single cells.

The idea was born from their studies of fluorescently labeled NF-κB movement to the nucleus during signaling. Postdoc Sergi Regot wondered whether nuclear fluorescence could serve as a readout for other regulatory events and focused on kinases. In the JNK kinase substrate c-Jun, he noticed a nuclear export signal sequence modified by two phosphorylation sites. Regot attached this sequence and the kinase docking site to a fluorescent protein and found that phosphorylation enhanced movement out of the nucleus.

The team engineered variations of the nuclear export sequence and added a weak nuclear localization signal, improving the signal-to-noise ratio and dynamic range of the resulting 'kinase translocation reporters'. Covert likes their simplicity. Unlike with FRET probes, researchers "can pop a kinase reporter right into what they're doing—it's just one color, so there's not a big overhead," he says.

The reporters appear to work whether kinases are located in the nucleus or cytoplasm. "The misconception is thinking that this is a static system," says Covert; reporters continually shuttle across the nuclear envelope, and reporter activation simply shifts the balance of this movement. However, the shuttling also means that reporters will not work normally when the nuclear envelope breaks down during cell division.

Kinases can change the localization of some endogenous targets, and reporters have been used before to visualize this movement in response to kinase activity.

A close-up view of the active zone of a synaptic bouton. From Wilhelm, B.G. *et al*. Composition of isolated synaptic boutons reveals the amounts of vesicle trafficking proteins. *Science* **344**, 1023–1028 (2014). Reprinted with permission from AAAS.

The researchers then focused on the 62 proteins of high interest and determined their positions within the synaptic bouton by using immunostaining and stimulated emission depletion (STED), a super-resolution fluorescence microscopy technique. By marking active-zone proteins and a vesicle protein, synaptophysin, they were able to localize 60 proteins in relation to the vesicle release site. Combining this enormous amount of experimental information with the known atomic structures of the 60 localized proteins, Rizzoli's team then created a 3D model of the architecture of an average synaptic bouton.

Interestingly, the 3D model shows that the synaptic bouton is a rather crowded space, which would limit the diffusion of proteins and vesicles. The researchers found that SNARE proteins involved in neurotransmitter vesicle exocytosis at the active zone were present in particularly high concentrations, at up to about 26,000 copies per synaptic bouton. They hypothesized that this may be a result of evolutionary selection to ensure fast neurotransmitter release under the constraints of limited diffusion. In support of this theory, they found that copy numbers of clathrin and dynamin proteins involved in vesicle endocytosis, a much slower process, were correspondingly lower (at around 4,000 and 2,300 copies per synaptic bouton, respectively).

This detailed window into the atomic structure of the synapse will serve as a highly useful tool for other researchers wishing to test different hypotheses about the regulation of neuronal signaling.

Allison Doerr

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Wilhelm, B.G. *et al.* Composition of isolated synaptic boutons reveals the amounts of vesicle trafficking proteins. *Science* **344**, 1023–1028 (2014).

Covert's team has shown that this behavior can be exploited systematically in probe design. But effective designs do require "a pretty good read on the docking site," says Covert. Some reporters are more difficult to make because specificity is determined by phosphorylation site context, so export and import signals need to be carefully 'folded' into the endogenous sequence, he says. This was the case for an AGC kinase reporter that they designed.

Kinases can have multiple targets, and multiple kinases can modify the same target. Determining the rules governing kinase specificity can be an attractive use of the reporters, as the ease of introducing mutations into a single construct will make it possible to test many hypotheses about specificity.

To convert reporter localization to a quantitative measure of activity, the researchers developed a model based on the four reporter states—active or inactive, nuclear or cytoplasmic—with different rate constants for shuttling and activation. They estimated these parameters by stimulating cells to trigger activation and by imaging JNK reporters bearing mutations that constantly either prevent phosphorylation or mimic it. "That was really exciting to me as a model-driven guy," says Covert. "Now we can go from looking at a reporter to … the active kinase concentration in an individual cell."

These reporters have begun to shed light on cellular heterogeneity and dynamic activity in unstimulated cells and could be used to screen for kinase-modulating drugs. They can also be used to study how signaling elements are coordinated in the cell. In a potent demonstration, the researchers coexpressed different-colored reporters they created for ERK, p38 and JNK kinases, finding different activation dynamics when stimulating cells or adding kinase inhibitors. "We see some cross-talk," says Covert. "It's happening in the same individual cells, and to me that is a beautiful result."

Tal Nawy

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Regot, S. *et al*. High-sensitivity measurements of multiple kinase activities in live single cells. *Cell* **157**, 1724–1734 (2014).