Research describes missing step in how cells move their cargo

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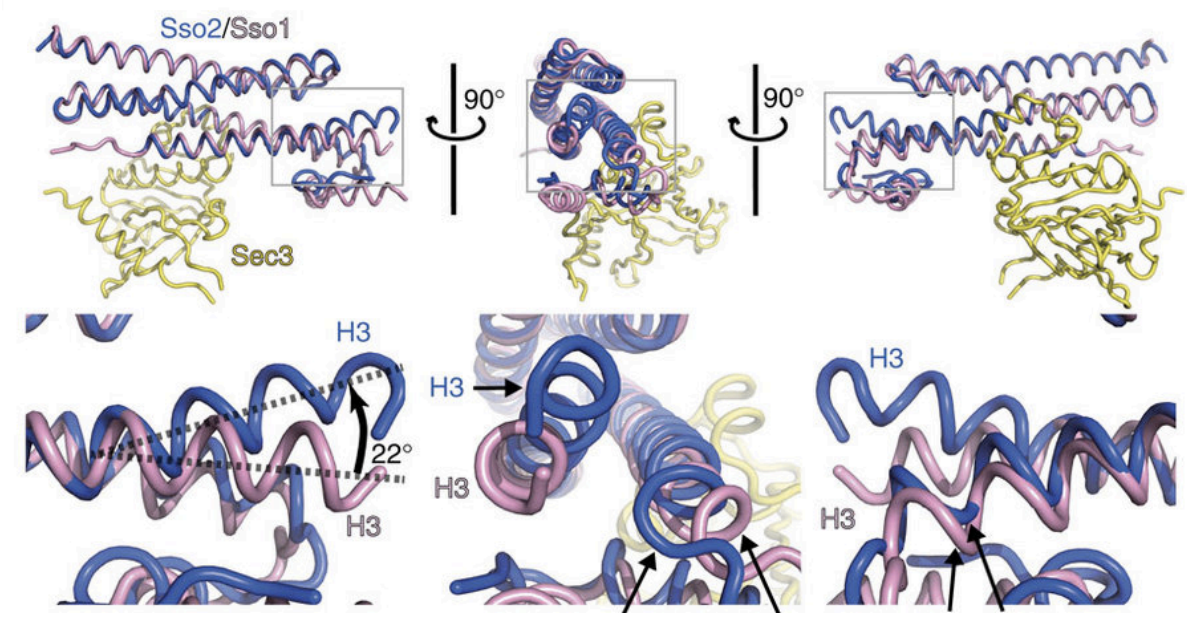


The researchers discovered that Sec3 allows the t-SNARE to relax, opening it up and allowing it to form a complex with another t-SNARE and eventually a v-SNARE to complete membrane fusion. Credit: University of Pennsylvania

Every time a hormone is released from a cell, every time a neurotransmitter leaps across a synapse to relay a message from one neuron to another, the cell must undergo exocytosis. This is the process responsible for transporting cellular contents via lipid-encapsulated vesicles to the cell surface membrane and then incorporating or secreting

them through membrane fusion. Insights into this cellular cargo transport system won three Americans the Nobel Prize in 2013.

Now, a study led by Wei Guo, a professor of biology in the University of Pennsylvania's School of Arts & Sciences, has identified a key activation mechanism that leads to vesicle fusion to the membrane.

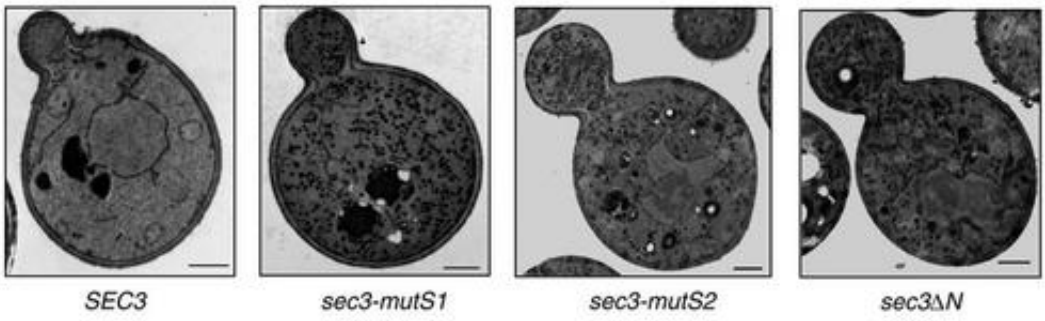
"Knowing the steps of this process is important for many physiological processes: endocrinology, neurotransmission, immune response," Guo said. "This was a fundamental question in cell biology."

The work appears in the journal *Nature Communications*. Peng Yue, a former graduate student at Penn, was the study's lead author. Kunrong Mei, Shaoxiao Wang and Yueyao Zhu of Guo's lab were coauthors, along with the Medical University of Vienna's Yubo Zhang, Johannes Lesigang and Gang Dong.

Exocytosis involves a complex dance of proteins called SNAREs, both v-SNAREs, on the vesicle, and t-SNARES, on the "target" membrane, which in the case of this study is the cell's plasma membrane. The interaction of v-SNAREs and t-SNAREs allows the vesicle to fuse with the membrane, a prerequisite for unloading the vesicle's contents.

Much of the research in this area has been done in yeast cells, a model system commonly used in studying exocytosis. It's known that the SNARE complex in yeast forms between two t-SNAREs that together form a complex with one v-SNARE. But before this occurs, one of the t-SNAREs, syntaxin, tends to form what's known as an autoinhibitory conformation, essentially a closed door that blocks it from interacting with the other t-SNARE. Guo and colleagues sought to find out exactly how this "door" was opened to allow the SNARE complex to fully assemble, enabling vesicles to fuse with the plasma membrane.

"This autoinhibition is a big barrier to overcome for the cell," Guo said, "and if you think about a process like hormone secretion, which takes place in seconds, there has to be something that is speeding the process along."



In a cell with a mutant Sec3, vesicles were unable to fuse to the membrane and instead accumulated within the cell. Credit: University of Pennsylvania

This mystery took years for Guo and his team to solve. They first narrowed in on the exocyst, a protein complex that tethers a vesicle to the membrane before fusion occurs. After screening the eight subunits of the exocyst protein complex to see if any interacted with syntaxin responsible for autohibition, they found one that did: Sec3.

When the researchers mixed Sec3 with syntaxin, and then added the other t-SNARE to which syntaxin binds, they found that it significantly accelerated the binding. They also found that its interaction was fleeting; it acted almost like an enzyme, catalyzing the reaction and then departing. They also confirmed that Sec3 sped membrane fusion using a

chemically reconstituted lipid system.

Investigating the crystal structure of the interaction allowed the team to determine that Sec3 was able to relax one of the alpha helices at the "hinge" of the autoinhibited t-SNARE, opening it.

"After binding to Sec3, the autoinhibition is relieved," said Guo. "Then the other t-SNARE comes in quickly to assemble the SNARE complex."

The researchers created Sec3 mutants and found that the resulting yeast cells were unable to properly complete exocytosis. Vesicles intended for the plasma membrane instead accumulated within the cell's interior.

Furthermore, the researchers determined that one part of Sec3 was responsible for fusion, while another part was involved in vesicle docking. Mutating different domains of Sec3 led to one defect or the other.

In future work, Guo's lab looks to investigate how the other subunits of the exocyst function in membrane fusion and to move one step deeper to understand how Sec3 is controlled to start the fusion process.

More information: Peng Yue et al, Sec3 promotes the initial binary t-SNARE complex assembly and membrane fusion, *Nature Communications* (2017). DOI: 10.1038/ncomms14236

Provided by University of Pennsylvania