

## WHERE AND WHEN TO LEAVE THE CELL

In cells that have a nucleus, proteins are sent to various destinations by a complex sorting and delivery system. This involves transport in small membranous vesicles that move from one intracellular membrane to the next. The vesicles bud from the departure membrane and then fuse with the destination membrane, depositing their contents. This system requires highly coordinated and specific addressing of vesicles involving complexes of proteins at both ends of the journey. In yeast, a complex that regulates the targeting of vesicles to the plasma membrane for extracellular delivery of proteins, a process known as exocytosis, has been identified. The structure of one of these proteins, Sec3 (Fig. 1), was recently solved at IMCA-CAT beamline 17-BM at the APS. The structure reveals important information about how Sec3 acts as a coincidence detector for exocytosis and identifies a new subtype of pleckstrin homology (PH) domain and new proteins that share this new domain, information that will be instrumental in uncovering their functions.

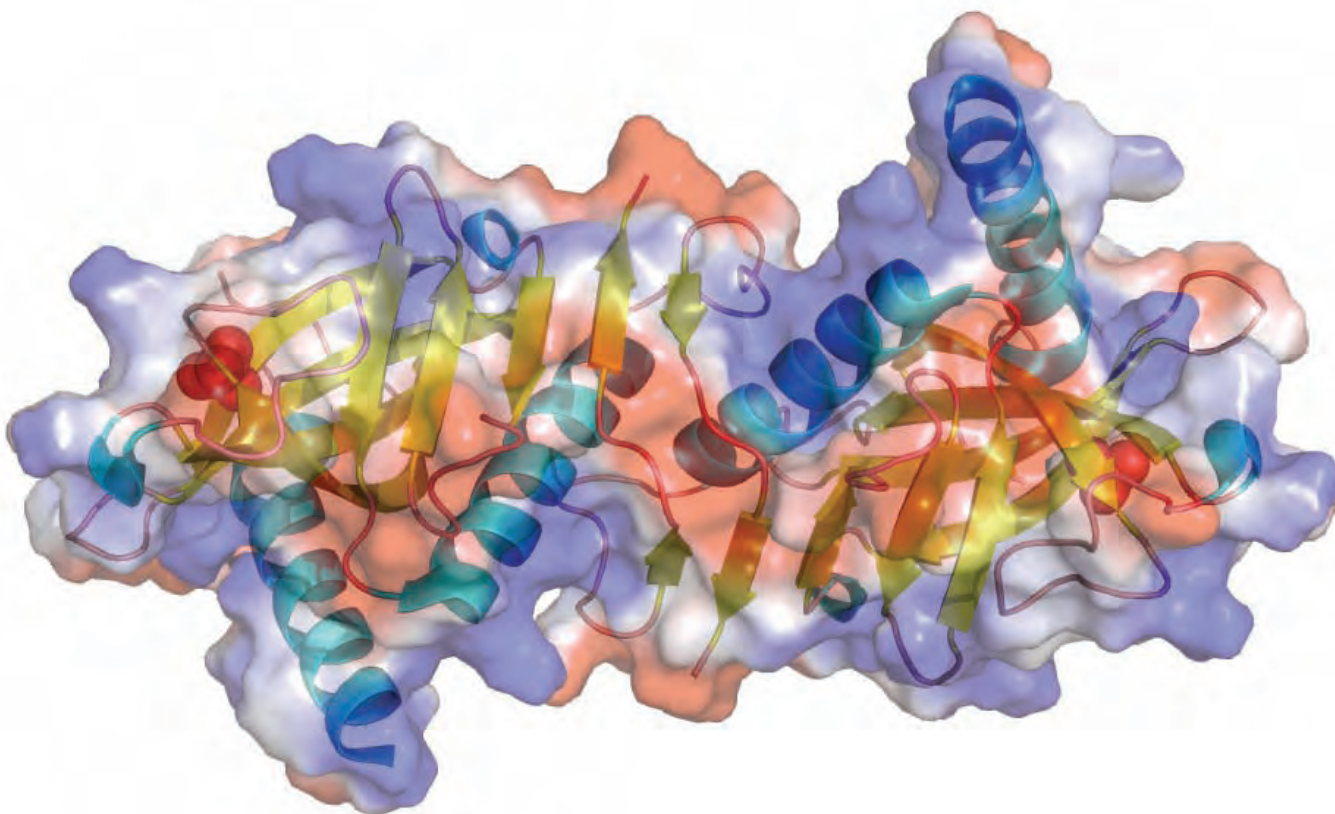


Fig. 1. Structure of the Sec3 PH-domain dimer. The view is down the pseudo two-fold axis of the dimer. A transparent electrostatic surface representation (positively charged: blue; negatively charged: red) is shown along with a ribbon diagram of the structure (helices: cyan; strands: yellow; loops: firebrick). The phosphate groups bound in the phospholipid-binding pockets are shown as red spheres.

## This finding provides a new paradigm for understanding the function of PH domain-containing proteins

What is a coincidence detector? This is a term used for proteins that detect the presence of more than one molecule and perform a function when both are present at a specific location. This task is crucial for coordination of exocytosis which requires the formation of a complex of proteins at the cell membrane at a specific time. The team was interested in the N-terminal section of the Sec3 protein because it had been identified as important in vesicle targeting and exocytosis, and had been shown to bind to phospholipids of the plasma membrane and small G proteins that regulate spatiotemporal control of the process.

Their first task was to crystallize the protein. After some trial and error, they were able to get crystals for a segment of Sec3 from amino acid 71 to 241 that contained the regions of interest. There were four copies of the Sec3 segment in the asymmetric unit and, surprisingly, these seemed to comprise two dimeric units mediated through  $\beta$ -sheet interactions in the C-terminal part of the protein fragment. Although dimerization had not been detected by other methods, the presence of two dimeric units in the crystal structure suggested the possibility of dimeric association of Sec3 at the plasma membrane where a high local concentration might favor such interactions.

After the team solved the structure of the Sec3 protein fragment to a resolution of 2Å, their analysis revealed that Sec3 formed part of a new subfamily of PH domains, a fold that is relatively abundant but whose presence in Sec3

was unsuspected. Searching of the database for sequence similarity revealed a number of proteins with homology to this new domain subtype that were not previously suspected to have PH domains. In particular, a protein with a known function in exocytosis called amisyn. This finding provides a new paradigm for greater understanding of the function of PH domain-containing proteins and will be important in discovering the functions of other proteins like amisyn.

Examination of the conserved region of the PH domain revealed possible phospholipid contact points, coinciding with the presence of a bound phosphate ion in the structure, which enabled the team to make mutations at those positions and determine their importance to exocytosis in secretion assays in yeast. Although they were unable to co-crystallize Sec3 in the presence of phospholipids, they were able to determine that the amino acids they identified in the structure were important in Sec3 function in exocytosis. This work, combined with earlier work that showed that this N-terminal region binds to small G proteins, shows that Sec3 mediates interactions between membrane phospholipids and G proteins to act as a coincidence detector. This has also been shown for a growing number of other PH domain-containing proteins and represents an important new paradigm for vesicle trafficking.

The team plans to follow up on their discovery of a new type of PH domain by determining whether the

other exocytosis protein identified by this study, amisyn, also acts as a coincidence detector. — *Sandy Field*

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