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Structure Reveals Details of Cells' Cargo-Carriers

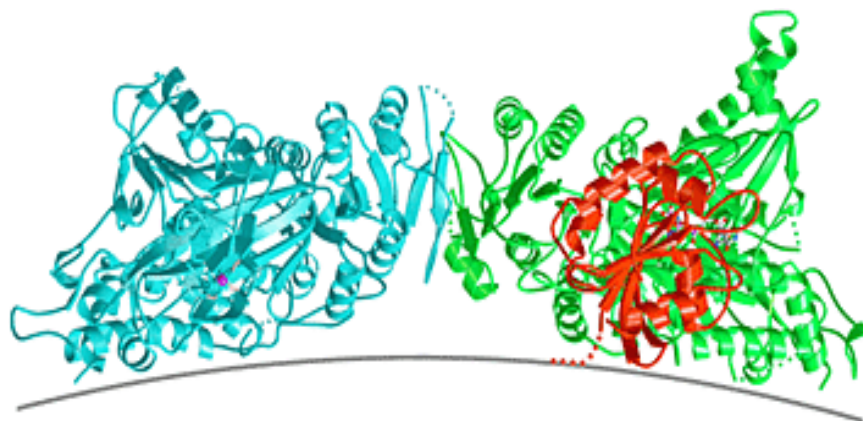


Image Title: Picture of the pre-budding complex, with Sar1 (red), Sec23 (green), and Sec24 (blue). The complex is curved to conform to the spherical shape of a vesicle the gray line indicates the position of the vesicle surface. - Goldberg Laboratory

Using x-ray crystallography, researchers have produced the first images of a large molecular complex that helps shape and load the small, bubble-like vesicles that transport newly formed proteins in the cell. Understanding vesicle budding is one of the prerequisites for learning how proteins and other molecules are routed to their correct destinations in the cell.

In an article published in the September 19, 2002, issue of the journal *Nature*, Howard Hughes Medical Institute (HHMI) investigator Jonathan Goldberg, Xiping Bi and Richard Corpina at Memorial Sloan-Kettering Cancer Center unveil the intricate architecture of the pre-budding complex, which is a set of proteins that participates in the formation of vesicles on the cells endoplasmic reticulum (ER). The pre-budding complex is the triggering component of a protein coat called COPII that grabs a section of the ER membrane, pinches it off to form the vesicle and packages the protein cargo to be transported.

"The structure developed by Bi, Corpina and Goldberg makes an important contribution to the understanding of vesicle formation -- a process central to the transport of newly formed proteins," said HHMI investigator Randy

Schekman, a pioneer in vesicle studies at the University of California, Berkeley. It illuminates in detail the mechanism by which the core complex of the COPII protein coat assembles on the ER membrane to initiate the process of membrane cargo capture and vesicle budding. Schekman and James Rothman of Memorial Sloan-Kettering Cancer Center, working independently, have identified many of the fundamental details of protein transport and secretion.

Goldberg said the entire pre-budding complex was considered an important structure to solve because of COPII's role in protein transport. "What makes the COPII coat unique is that encoded in its proteins is much of the information that tells it to go to the endoplasmic reticulum and which cargo to take up from the ER," said Goldberg. Also, COPII selects the appropriate fusion machinery, to ensure that the vesicle fuses with its correct target, a structure called the Golgi complex.

"In order to understand the process of vesicle formation and transport in molecular terms, one must begin with the initiating event -- with the multi-component pre-budding complex," Goldberg said. "We had to get a clear structural picture of the intact particle so that we can understand the first event in budding, which begins the process of selecting the protein cargo," he said.

Bi, Corpina and Goldberg produced crystals of the entire complex and analyzed the structures of the proteins using x-ray crystallography. Their studies revealed how each of the components of the complex works: A component called Sar1 launches the budding process by anchoring itself to the ER membrane. Sar1 accomplishes this feat by changing its shape through a chemical reaction called GTP binding.

This shape change also enables Sar1-GTP to recruit a second component called Sec23/24, which attaches to form the pre-budding complex, Sec23/24-Sar1. The structure produced by Goldberg and his colleagues reveals how the change in Sar1's shape enables Sec23/24 to recognize Sar1 and attach to it.

The scientists discovered that the pre-budding complex has a concave surface that hugs the ER membrane, conforming to the spherical shape that the vesicle will ultimately assume. According to Schekman, the structure reveals the mechanism by which the complex anchors to the ER membrane and how its curvature might impart curvature to the membrane; and in doing so initiate the shape change that accompanies vesicle budding.

Goldberg's group also identified the part of the complex that faces away from the ER membrane, which includes components that attract another molecule that knits together, or polymerizes, the coat, pinching off the vesicle from the ER membrane like a mold. The new structure hints at how the coat disassembles itself by, in effect, breaking the mold around the vesicle, and

freeing it to carry its protein cargo away to be released at the right place in the cell.

Now that they have solved the structure of the pre-budding complex, Goldberg and his colleagues can begin to explore another central question -- how do the vesicles know which proteins to take on as cargo?

"We suspect -- and it is a model that Randy Schekman put forward several years ago -- that the COPII coat is selecting many of the proteins directly," said Goldberg. "As we explore the coat structure further, I suspect we will see lots of binding-site crevices that specific cargo can plug into and thereby enter the vesicle. So, our next task is to look for those crevices."