Form follows function: Structure of cell growth regulator GATOR2 finally revealed

July 14 2022, by Madeleine Turner

A 3D rendering of GATOR2. Credit: Kacper Rogala/Whitehead Institute

An important function of our cells' signaling pathways is to coordinate growth with environmental cues, such as the availability of nutrients in the cell's environment. Cell growth plays an important role in normal cell

function—for example, building muscle in response to exercise— and it also goes awry in diseases such as cancer. The protein complex GATOR2 plays an important role in sensing and relaying information to the mammalian target for rapamycin complex 1 (mTORC1), an assembly of proteins that together control cell growth in response to environmental conditions. Through protein complexes such as GATOR2, mTORC1 senses nutrient levels in the cell, and either stymies or promotes growth accordingly. In a paper published July 13 in *Nature*, Whitehead Institute postdoc Kacper Rogala and Massachusetts Institute of Technology (MIT) graduate student Max Valenstein reveal the structure of GATOR2 and offer a glimpse into its function. This research was done in the lab of former Whitehead Institute Member David Sabatini.

"In biology, form follows function. Understanding what something looks like can tell us a tremendous amount about how it might work," Valenstein says. "We're trying to learn as much as we can about the GATOR2 complex. What is it doing and how is it activating mTORC1?"

Previous work from the Sabatini lab showed GATOR2 is composed of five different protein subunits. But no one understood if multiple or single copies existed, or how each copy fit together to form the larger protein complex. "We actually had a lot of very confusing data," Valenstein says. The GATOR2 subunits contain several copies of a structure called the RING finger domain. These RING domains—RING stands for "Really Interesting New Genes"—are a common structure that reliably plays a role in ubiquitin-related function. Ubiquitin is a regulatory protein found throughout most tissues in eukaryotic organisms (hence its name). By attaching to other proteins, it can alter their activity. In early work, Valenstein expected GATOR2 to act as a ubiquitin ligase, promoting the ubiquitylation of other proteins, but experiment after experiment failed to show this mechanism in action.

In 2019, Rogala joined Valenstein in an effort to determine the 3D structure of GATOR2 using cryogenic electron microscopy, or cryo-EM. "Everything else was a dead end," Rogala says. "We just decided, 'okay,' we have to take it apart and take a look under the microscope…maybe that will give us some clues on how to crack its function.'"

The project required extracting GATOR2 from cells in adequate quantity and quality to be useful for cryo-EM imaging, Valenstein says. It was a process that took about two years. MIT undergraduate Pranav V. Lalgudi played a significant role in the project. When the researchers finally visualized GATOR2 in 3D form, previously confusing data began to make sense. Individual proteins in GATOR2 come together by crossing their RING domains, forming a "spartan handshake," like two hands clasping the opposite forearm. In other words, the researchers revealed that instead of tagging other proteins with ubiquitin, RING domains are critical for holding GATOR2 together, a role that was previously undocumented.

Their studies showed that GATOR2 is a cage-like structure, with proteins forming an octagonal frame. The frame is composed of three subunits: MIOS, WDR24 and WDR59, which are further supported by several copies of two additional subunits, SEH1L and SEC13. These extra subunits are positioned around the frame, reinforcing the octagon.

While RING domains serve to hold the octagonal scaffold together, eight pairs of WD40 beta-propellers are affixed to the structure and associated with function. True to their name, these WD40 structures are shaped like propellers on a plane, with blade-shaped pieces pointing inwards (although they do not spin). The researchers also worked to understand how the beta-propellers interacted with protein complexes previously associated with GATOR2. They found that in GATOR2, there are three classes of beta-propellers which serve different functions. Two interact with proteins that sense the levels of specific amino acids in the cell.

The third type of beta-propellers interacts with other protein complexes involved in the pathway, known as GATOR1 and KICSTOR. GATOR1 acts like a hand brake, halting activity in the mTOR pathway, and GATOR2 is like the hand brake operator, sensing nutrients in the cell, then either intervening or pulling the brake.

Rogala describes their published work as only the first "episode" in a series. In the future, Rogala and Valenstein hope to pin down the specifics of how the subunits in GATOR2 communicate with each other. The researchers are also interested in understanding how cancer can derail normal functions of GATOR2, as well as the mTOR growth pathway as a whole.

By delving into the structure and function of GATOR2, researchers may begin to uncover new targets for drugs that act by regulating mTOR.

"Drugs are small molecules that bind in the crevices of proteins," Rogala says. "Any type of structural work is, in a way, a blueprint for drug discovery."

More information: Max L. Valenstein et al, Structure of the nutrient-sensing hub GATOR2, *Nature* (2022). DOI: 10.1038/s41586-022-04939-z

Provided by Whitehead Institute for Biomedical Research