A new twist to an old story of cellular signaling in the eye of a fly

March 14 2018

When light falls on the eye of a fly, ripples of messages flow through its cells. These messages are carried by molecular messengers forming an intricate information system that exists in most living cells.

Since most molecular messengers are not abundant, and many are broken down during signal transfer, the cell must renew its stock of messenger molecules quickly and efficiently to maintain its signaling networks. Given this necessity, many molecular messengers are recycled, during which the breakdown products of a signaling reaction are reconstructed via a series of enzyme-catalyzed reactions to recreate the original messenger molecule.

However, a new study from the National Centre for Biological Sciences (NCBS), Bangalore, now shows that the regeneration of a membrane-bound messenger molecule, phosphatidylinositol 4,5 bisphosphate or PIP2, is not as straightforward as it was once thought to be.

Mathematical models imply that the commonly accepted closed cycle of PIP2 breakdown and reconstruction, with no net addition or loss of molecules to the cycle may not hold true. Instead, it is likely that the PIP2 cycle is an open cycle, where PIP2 breakdown products are siphoned away to other biochemical pathways, and not fed back into a loop that resynthesizes PIP2.

The work is a collaborative effort between Raghu Padinjat's and Sandeep Krishna's groups at NCBS. While Padinjat's group mainly relies

on an experimental approach using fruit flies to understand cellular signaling, Krishna's group at the Simons Centre for Living Machines in NCBS, takes a theoretical and modeling approach to explore biological phenomena. A result of their combined efforts has led to the discovery that the PIP2 cycle, formulated many decades ago, is not as simple or complete as researchers believed it to be.

Several receptors on the cell surface use PIP2 as an intermediary for information transfer. When such receptors are activated, a signaling cascade is initiated to split PIP2 into two constituents—diacylglycerol (DAG) and inositol 1,4,5 trisphosphate (IP3)—both of which further continue the information transfer. Following this, DAG and IP3 were thought to be routed back into a multistep biochemical process to resynthesize PIP2, forming a closed cycle with no net loss or addition of molecules during the process.

However, when mathematical models using this closed cycle assumption were constructed, the researchers found that the models' predictions failed to mirror experimental data.

Under such models, if an enzyme converting DAG into the next intermediate in the PIP2 cycle were to be defective, an increase in DAG levels would be expected. Surprisingly, experimental data from mutant fruit flies with such a defective enzyme showed no increases in DAG levels as predicted.

"It was quite unexpected. We began to realize that perhaps there were gaps in our current understanding of the PIP2 cycle," says Rohit Suratekar, who worked jointly with the Krishna and Padinjat groups. "After further rigorous investigation, we found that adding two additional reactions at two specific steps in the currently defined cycle could actually explain all the mutant data," he adds.

Of the two reactions Suratekar mentions, one proposes that the DAG formed as a breakdown product of PIP2 signaling is siphoned away to an undefined biochemical process or a sink instead of being funneled into the next step—the formation of phosphatidic acid (PA)—for PIP2 resynthesis. The second proposes that the PA required for PIP2 synthesis is acquired from another, as yet unknown biochemical pathway, or a source.

Hence, the traditional closed cycle for PIP2 recycling, may now need to be replaced with an open cycle where some intermediate products are removed through a sink and added through a source.

One reason for this change may lie in the fact that PIP2 resynthesis is spread across two distinct compartments of the cell. Some reactions must occur on the membranes of the endoplasmic reticulum, while others occur on the cell surface membrane. Since PIP2, DAG, and several intermediates of the cycle are membrane-bound molecules, they cannot diffuse through the water-filled cell interior, and require coordinated transfers between these compartments.

"Previous mathematical models of the PIP2 cycle have not taken note of the compartmentalized nature of cells," says Raghu Padinjat. "Our new models not only account for how biochemical reactions are organized in time, but also include information on how these reactions may be structured in space," he adds.

"The continuous two-way interaction between theory and experiment was crucial for this discovery, and was also incredibly satisfying for us intellectually," says Sandeep Krishna. "The value of this type of theoretical work lies in clarifying the assumptions that underlie our understanding of biological signaling and regulation, which suggests new experiments, which in turn suggest new models, and so on," he says, adding that this type of approach is crucial for raising entirely novel

questions that can be explored experimentally and theoretically.

"Relying on models based on the old PIP2 cycle could lead to wrong interpretations of experimental data," cautions Suratekar. "As this signaling cascade is involved in many cellular functions, studying this topic is highly important," he further emphasizes.

As Suratekar points out, the PIP2 cycle functions in many cell types. In humans, the cycle is essential for development, proper functioning in immune cells that defend the body against infections and neural tissue that is basis of human brain function. Genetic defects in elements needed to run the PIP2 cycle have been linked to various cancers, neurological diseases, and mental illness.

"Designing drugs /inhibitors to treat human disease is often aided by a thorough understanding of the biochemistry underlying the function of cells and tissues. Thus, a clear understanding of the organization of the PIP2 cycle may be crucial in designing drugs for therapy," says Padinjat, adding that in the future, his group hopes to refine their new mathematical model to predict the effects of therapeutic drugs which act via the PIP2 cycle to modify cellular functions.

Mukund Thattai, a faculty member from the Simons Centre unconnected with the study comments, "this project really reveals how theory-experiment collaborations work. It is a process, a back-and-forth conversation; it takes time for each side to become comfortable with the other. Theorists start with the least amount of detail necessary, and experimentalists are confronted with all the detail at once. What's really commendable is how Rohit Suratekar has managed to strike the perfect balance."

More information: Rohit Suratekar et al, Evidence of sinks and sources in the phospholipase C-activated PIP2 cycle, *FEBS Letters*

(2018). DOI: 10.1002/1873-3468.12998