RAP tag: A new protein purification approach

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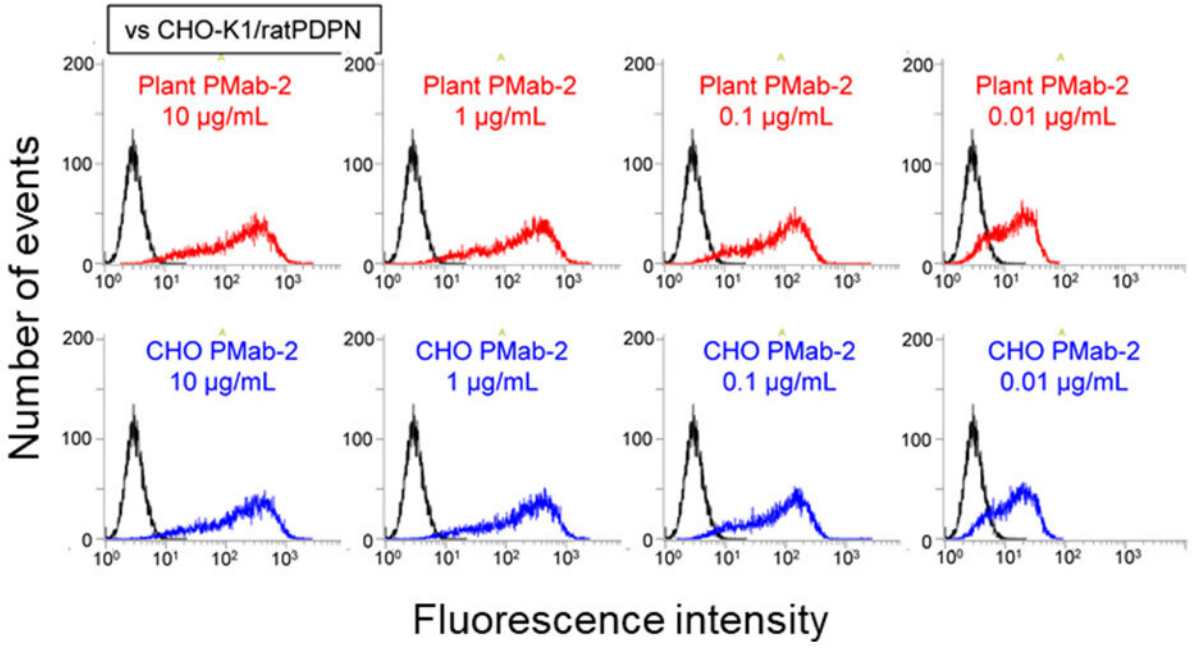

Credit: University of Tsukuba

Whether it's our diets, building strength, or as part of medical advancements, it is no secret that proteins form an important part of our lives. Tracking how proteins work and move in cells, and purifying engineered proteins, are important tools for researchers. Traditional approaches to label proteins of interest, called 'tagging,' have the

disadvantage of interfering with protein characteristics, including function and localization. Sometimes, these tags can also cross-react, which makes the information they provide nonspecific. A successful protein tagging system needs to be highly specific and have high affinity.

In a study published in September 2020 in *Frontiers in Plant Science*, researchers from the University of Tsukuba, led by Professor Kenji Miura, have described a new tagging system for detecting and purifying proteins in plant cells. This approach uses a short sequence called a 'RAP tag' to label proteins. An antibody, PMab-2, is then able to specifically recognize the RAP tag and can be used to purify the proteins of interest.

In describing this approach, Professor Miura says, "The high affinity and specificity of immunoaffinity chromatography using monoclonal antibodies makes it a very powerful tool, especially for the purification of proteins expressed at low levels." A hurdle to applying this approach, however, is the high cost of reagents, especially that of antibodies.

To get around this, Professor Miura and colleagues explored whether they could produce the PMab-2 antibody in the plant model Nicotiana benthamiana, a relative of the tobacco plant. Not only could they successfully produce PMab-2, they went on to show that the plant-produced PMab-2 behaved similarly to that produced in animal cells. This discovery opens the door to reducing the cost of antibody production, and could be applied more widely across scientific fields.

Testing the feasibility of a RAP-tagged/ PMab-2 affinity purification approach, the researchers then expressed RAP-tagged proteins in plant cells. They found that these tagged proteins could be specifically identified using the PMab-2 antibody. Moreover, RAP-tagged recombinant proteins, involving the fusion of sequences from more than one protein, and protein complexes were also expressed in these cells and identified by PMab-2. These proteins could also be purified from

plant cells using the PMab-2 antibody, indicating that the RAP tag can be used for both protein detection and purification from soluble plant extracts.

"Plants are an extremely valuable resource for molecular biology," explains Professor Miura. "They can be used as bioreactors to produce large amounts of proteins because they are unlikely to suffer from contamination issues faced by bacterial and mammalian cell systems."

The results presented by the team show that this approach has the potential to be widely applied across the molecular sciences.

More information: Kenji Miura et al, RAP Tag and PMab-2 Antibody: A Tagging System for Detecting and Purifying Proteins in Plant Cells, *Frontiers in Plant Science* (2020). DOI: 10.3389/fpls.2020.510444

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