Team shows a protein modification determines enzyme's fate

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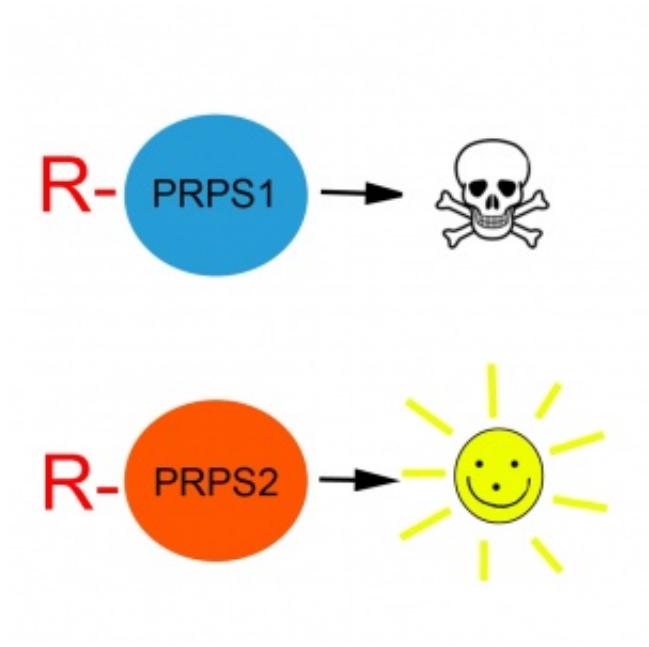

PRPS1 and PRPS2 are nearly identical. But an arginine tag flags PRPS1 for degradation, while PRPS2 survives the process.

The human genome encodes roughly 20,000 genes, only a few thousand more than fruit flies. The complexity of the human body, therefore, comes from far more than just the sequence of nucleotides that comprise our DNA, it arises from modifications that occur at the level of gene, RNA and protein.

In a new study, researchers from the University of Pennsylvania School

of Veterinary Medicine show how one of these modifications, which occurs after RNA is translated into proteins, has the power to greatly influence the function of an enzyme called PRPS2, which is required for human life and can become hyperactive in cancer.

This type of protein modification, known as arginylation because it attaches an arginine molecule to the protein, has only recently been characterized, but likely plays an important role in regulating not only PRPS2, but a plethora of other proteins that are necessary for life.

"This is the first time anyone has demonstrated that arginylation can regulate an enzyme's activity," said Anna Kashina, senior author on the paper and a professor of biochemistry at Penn Vet. "I think mechanisms like this one likely regulate a host of important genes."

The Penn Vet's team findings also hold significance for cancer treatment strategies. The process of arginylation could be a target for intervention, and it's possible that gene therapy modifications to PRPS2 could prevent the uncontrolled cellular expansion seen in metastatic growths.

Kashina led the work along with co-lead authors Fangliang Zhang, formerly of Penn Vet and now at the University of Miami, and Devang M. Patel of the University of Miami. Additional coauthors included Penn Vet's Kristen Colavita and Sougata Saha, who is now at India's Tezpur University; Irina Rodionova, David Scott and Andrei Osterman of Sanford Burnham Medical Research Institute; Brian Buckley and Mikhail Chernov of Roswell Park Cancer Institute and Akhilesh Kumar of the University of Miami.

The research is reported this week in the journal *Nature Communications*.

Kashina has spent the past several years studying arginylation and has

found that it often regulates proteins that are involved in basic, but critical, functions in the cell, and are represented by multiple copies in the genome. They're often referred to as "housekeeping genes." In 2010, she and colleagues published a paper in Science showing that arginylation regulates an actin protein, which is required for cell movement and for maintaining cellular structure.

"People used to assume that the reason for families of identical proteins was because they were so essential that the genome built in some redundancy," Kashina said. "But as people started studying these protein families, they realized that virtually identical family members often have different functions and that they are not actually redundant at all."

The question remained: how can seemingly identical proteins take on different functions?

To find out, Kashina's team examined two members of one such family of highly similar proteins: PRPS1 and PRPS2. Both are involved in the production of new nucleotides, the building blocks of DNA. The researchers had previously learned that PRPS2 is arginylated while PRPS1 is not. The team also knew from prior work that PRPS2 plays a role in cancerous growth, as cancer cells reproduce rapidly and need a constant source of nucleotides in order to keep up a fast rate of growth.

In the current study, the team got further clues that arginylation played a role in the different roles of PRPS1 and PRPS2 when they examined cells in which arginylation had been blocked by making the enzyme that adds arginine, ATE1, inoperative. They found that these cells had problems with making purine nucleotides, the As and Gs of the DNA sequence. The genetically modified cells also made more serine and glycine amino acids than normal cells, a further sign that purine synthesis was impaired. Because both PRPS proteins are involved in purine nucleotide synthesis, they were clear candidates for being subject

to regulation by arginylation.

In additional experiments, the researchers found that overall PRPS protein levels were higher in cells in which ATE1 was inactive, and that PRPS2 in particular was nearly three times more active when it was arginylated.

Many post-translation protein modifications target proteins to be broken down and destroyed. When the team introduced arginylated forms of PRPS1 and PRPS2 into cells, they found that PRPS1 was unstable and quickly degraded, while PRPS2 survived arginylation.

Although PRPS1 and PRPS2 are highly identical when it comes to their amino acid sequences, the researchers surmised their differences in regulation may be attributed in part to slight differences in their nucleotide sequences, which affect how mRNA strands are shaped. The structure of PRPS1 mRNA is likely to slow down the process of translation, the researchers believe, perhaps making that protein vulnerable to degradation once it is arginylated. Because PRPS2 mRNA lacks the same obstructions to translation, it appears able to survive arginylation without being targeted for degradation.

"To me an exciting part of this work is that it seems possible to regulate the protein function just by coding sequence," Kashina said. "We used to believe that coding sequence encodes only the sequence of amino acids but actually it encodes a lot more."

Knowing that PRPS2, which has been implicated in driving cancer, is regulated by arginylation provides a potential "switch" for tuning that activity. And the importance of mRNA structure in how a protein is eventually treated could give another way of targeting PRPS2's activity and turning it down to prevent the uncontrolled growth seen in malignancies.

"If we know that coding sequence affects post-translational modifications, this could become a potential new strategy for gene therapy by making substitutions that don't alter the amino acid sequence of a protein but alter this regulation," Kashina said.

Looking ahead, Kashina hopes to examine the role of arginylation for a number of other housekeeping protein families.

"I believe it's a very global process," she said.

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