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Researchers develop powerful new technique to study protein function

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In the cover story for the journal *Genetics* this month, neurobiologist Dan Chase and colleagues at the University of Massachusetts Amherst describe a new experimental technique they developed that will allow scientists to study the function of individual proteins in individual cell types in a living organism.

The advance should allow deeper insights into protein function, Chase says, "because we can only get a true understanding of what that single protein does when we isolate its function in a living organism. There was no tool currently available to do this."

The journal's cover art uses a jigsaw puzzle of a worm to illustrate how knockdown strategies in this organism have evolved over time to achieve more and more cell-type specificity, culminating in the new approach developed by the Chase lab, which can restrict knockdown to a single cell type.

"This strategy is super cool and it works great," he says. "We've already used it to tease apart some of the mechanisms of dopamine signaling, but the strategy can be adapted to study the function of any protein involved in any biological process."

There are more than 1 trillion cells in the human body, yet only 20,000 to 25,000 genes are expressed in them, Chase explains, so each gene must be expressed in many different cells. Understanding the function of 20,000 genes and whether this differs by cell type has been difficult, but

over the last 10 years, he adds, "we have learned that the answer to this last question is a resounding yes. Gene function can differ by cell type."

Pursuing this further, however, was hampered by the fact that traditional approaches for studying protein function rely on genetic mutations that act on DNA, so they disrupt protein function in ALL cells. And to understand what a protein really does, it must be studied in an individual cell in a living organism.

Specifically, Chase's lab uses the roundworm *C. elegans* to explore how dopamine modulates the activities of specialized neurons. The worm is a useful model because it has only 302 neurons instead of billions in mammals. Despite its simplicity, the worm's basic neurotransmission mechanisms are also found in humans.

In the quest to identify genes that regulate dopamine signaling, the UMass Amherst researchers quickly recognized that dopamine acts through proteins used by other neurotransmitters in other nervous system cells. "So we couldn't use traditional genetic tools to study dopamine signaling. We needed to develop a new method to study protein function in individual cells in multicellular organisms," Chase notes.

The technique they developed takes advantage of nonsense-mediated decay (NMD), a surveillance mechanism present in all eukaryotic organisms. NMD destroys aberrant mRNA molecules that can arise naturally through mutation during transcription or mRNA processing.

"In our strategy, we replace the normal copy of a gene with a tagged version that targets the gene's mRNA for destruction by NMD," Chase explains. "We then remove NMD from all the organism's cells. Without NMD present, the replacement gene is expressed normally in all cells. We then knock down expression of the gene cell-specifically by restoring NMD activity only in cells we select."

He adds, "This cell-specific restoration of NMD activity is easy and can also be controlled in time. Thus, using NMD we can not only remove gene function in individual cell types, we can control exactly when gene function is removed in that cell type. This gives complete control of gene expression and allows one to investigate the function of any gene in any cell type at any time."

"With this very powerful new technique, now you can identify an individual gene and you can ask whether it plays a role in the behavior of interest. All these genes are expressed in our brains, so we are learning about all sorts of fascinating interactions in the worm and we can begin to translate the meanings to humans. Dopamine signaling is something you really can't study in the human brain very well, but with this approach we are having success."

To measure how much RNA is left in the animals after NMD is activated, microbiologist Aishwarya Swaminathan in John Lopes' group at UMass Amherst used quantitative polymerase chain reaction (PCR), an amplification technique, allowing the researchers to precisely measure the efficiency of the strategy.

Chase says, "It turns out that the NMD-mediated knockdown is super good, better than anything else available. And anybody can use it, it's straightforward molecular biology."

Provided by University of Massachusetts Amherst