Protein 'rescues' stuck cellular factories

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Using a powerful data-crunching technique, Johns Hopkins researchers have sorted out how a protein keeps defective genetic material from gumming up the cellular works. The protein, Dom34, appears to "rescue" protein-making factories called ribosomes when they get stuck obeying defective genetic instructions, the researchers report in the Feb. 27 issue of *Cell*.

"We already knew that binding to Dom34 makes a ribosome split and say 'I'm done,' and that without it, animals can't survive," says Rachel Green, Ph.D., a professor in the Department of Molecular Biology and Genetics at the Johns Hopkins University School of Medicine and a Howard Hughes Medical Institute investigator. "In this study, we saw how the protein behaves in 'real life,' and that it swoops in only when ribosomes are in a very particular type of crisis."

Ribosomes use genetic instructions borne by long molecules called messenger RNA to make proteins that cells need to get things done. Normally, ribosomes move along strands of messenger RNA, making proteins as they go, until they encounter a genetic sequence called a stop codon. At that point, the protein is finished, and specialized recycling proteins help the ribosome disconnect from the RNA and break up into pieces.

Those pieces later come together again on a different RNA strand to begin the process again. From Green's earlier work with Dom34, it appeared that the protein might be one of the recycling proteins that kicks in at stop codons.

To see if that was the case, Green used a method for analyzing the "footprints" of ribosomes developed at the University of California, San Francisco. In 2009, scientists there reported they had mashed up yeast (a single-celled organism that is genetically very similar to higher-order animals) and dissolved any RNA that wasn't protected inside a ribosome at the time. They then took the remaining bits of RNA—those that had been "underfoot" of ribosomes—and analyzed their genetic makeup. That sequence data was then matched to the messenger RNA it came from, giving the researchers a picture of exactly which RNA—and thus, which genes—were being turned into protein at a given moment in time.

Green and postdoctoral fellow Nick Guydosh, Ph.D., adapted this method to see what Dom34 was up to. Guydosh wrote a computer program to compare footprint data from yeast with and without functioning Dom34 genes. The program then determined where on messenger RNAs the ribosomes in cells without Dom34 tended to stall. It was at these points that Dom34 was rescuing the ribosomes in the normal cells, Guydosh says.

"What many of these 'traffic jams' had in common was that the RNA lacked a stop codon where the ribosome could be recycled normally," he says. For example, some of the problem messenger RNAs were incomplete—a common occurrence, as chopping up messenger RNAs is one way cells regulate how much of a protein is produced.

In others, the RNA had a stop codon, but something strange and unexpected was going on in these latter cases: The ribosomes kept going past the place where the stop codon was and went into a no man's land without protein-making instructions. "Ribosomes kept moving but stopped making protein, at least for a time," Guydosh says. "As far as we know, this 'scanning' activity has never been seen before—it was a big surprise."

"What these results show us is why we need Dom34 to survive: It's the only protein that can rescue ribosomes stuck on RNAs," says Green. "Without it, cells eventually run out of the ribosomes they need to make protein."

More information: *Cell* paper: www.cell.com/abstract/S0092-8674%2814%2900162-7

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