Researchers pin down long-elusive protein that's essential to 'life as we know it'

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A team of researchers is being recognized for devising a new way to study a human protein that long has evaded close scrutiny by scientists investigating its role in the communication of important genetic messages inside a cell's nucleus to workhorse molecules found elsewhere.

Last year, the team, led by J. Andrew Hockert, at the time a doctoral researcher at the Texas Tech University Health Science Center School of Medicine, put in its crosshairs the protein known as CstF-64. Today, in what has been named a "Paper of the Week" for the Jan. 1 issue of the Journal of Biological Chemistry, the team is reporting its successes.

Short for "cleavage stimulation factor-64," CstF-64 carries out countless activities required to keep its parent cell alive and well. Hockert's team was particularly interested in the protein because it controls polyadenylation, an essential step in gene communication that involves tacking on information to genetic messages.

For years, CstF-64 refused to give up its secrets when scientists zeroed in. The protein has so many important duties that tweaks prompted a sort of murder-suicide: It killed its own parent cell and, so, died with it.

"Previously, it had been very hard to examine the functions of most of the polyadenylation proteins in cells because polyadenylation is essential for 'life as we know it.' If we perturbed polyadenylation in any way, the cells died, and we could not measure anything," says Hockert, who is

now an assistant professor at the University of the Cumberlands in Williamsburg, Ky.

Undeterred, Hockert set out to observe - in a living cell - how the elusive CstF-64 gets from its home base in the cytoplasm, or outer region of a cell, and into the nucleus, where genetic messages originate.

For CstF-64, it's all about location, location, location. Only once in the nucleus can it start doing its job in the polyadenylation process, getting the messages ready to be taken out to worker molecules in the cytoplasm.

But to make his observations, Hockert had to employ what co-investigator Clinton MacDonald calls "a trick."

"Andrew realized we can make a version of the protein that is different than the regular version already in the cell. We can mutate it," says MacDonald, an associate professor at Texas Tech who oversaw Hockert's work. "And, if you put that mutated version of the protein in the cell, it only works on the genes we tell it to work on and not the rest. So, it doesn't kill the cell."

Having come up with a clever way to study and measure different aspects of the protein in a living cell, MacDonald says, the team then had to pick one in particular on which to focus.

"The feature Andrew chose to examine was how CstF-64 interacted with another polyadenylation protein and how that interaction allowed both those proteins to work inside the nucleus," MacDonald says.

As important as CstF-64 is to gene expression, it doesn't exactly have "VIP" status when it comes to gaining access to the nucleus. Lacking what is known as a nuclear localization signal, it has to rely on its partner

protein, CstF-77, to lead the way to and get in the door.

"We already knew the sequence of our protein, CstF-64, and so we knew it didn't have a special signal to get it in the nucleus. So, we hypothesized something else was dragging it in, and the most likely thing was a partner protein working alongside it," Hockert explains.

With the mutant version of the protein in place, the team soon discovered their hypothesis was correct: CstF-64 had to bind with CstF-77 to get into the cell's command center. Furthermore, MacDonald says, the team was able to report which piece of CstF-64 binds with its partner — "the hinge domain."

Having overcome the cell-death obstacle and having confirmed the significance of the "hinge" domain for nuclear localization, the researchers expect their technique will be used by future scientists to monitor a variety of protein-protein interactions in living cells and better understand the cell's polyadenylation machinery.

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