



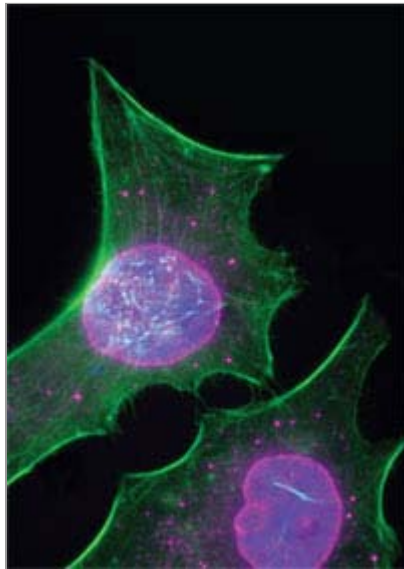
► RESEARCH

A New View of Translational Control

How P-bodies, stress granules, and other cytoplasmic foci manage the cellular currency

By **Charles Q. Choi**

Courtesy Edward K.L. Chan



INTERFERING BODIES:

Immunofluorescent image of HeLa cells stained for GW182. These so-called GW bodies are important cytoplasmic foci for RNA interference.

The bank note that Dominique Weil used to buy ice cream for her family at the beach this past summer may have traveled a long way. The note, a product of international treaties and detailed artistry, could have crossed a dozen international borders or more. Complicated decisions from individual consumers to corporate accountants and government agencies determined its path from bank to pocket to cash register and back – possibly saved for days or years in case of a rainy day. After getting too old, worn, or torn, treasury inspectors may destroy and replace the note. Or if inflation is too high, it could be removed from circulation altogether.

Organized distribution is crucial to smooth operation in everything from economies to trash disposal. When efficiently optimized, such systems borrow from a logic as simple as that of the assembly line: Concentrate the right resources in the right places. Local, specialized processing centers should recycle where possible, prepare for emergencies, and dispose of waste promptly. Why would anyone expect something as elegant as a cell to act any differently?

RNA FROM MINT TO CIRCULATION

Yet as recently as 15 years ago, researchers viewed the nucleus as just "a bag of goo," with a disorganized, free-floating genome, says Greg Hannon of Cold Spring Harbor Laboratory in New York. Fluorescent tags and electron microscopy have since revealed how membraneless structures organize nucleic-acid metabolism. High concentrations of RNA polymerases appear in anywhere between 500 and 10,000 nuclear foci. Elsewhere in the nucleus, dozens of splicing speckles aggregate pre-mRNA transcripts and splicing factors, playing their part in the assembly line of posttranscriptional modification.

But after transcripts are spliced, capped, polyadenylated, and ejected through the nuclear pore, "the trail goes cold," says Kenneth Kosik of the University of California at Santa Barbara. In other words, "We might know a lot about how the money is made," says Weil, a cell biologist at the National Center for Scientific Research (CNRS) in Paris, but "there remains a lot we don't know about how the money gets distributed or recycled afterwards."

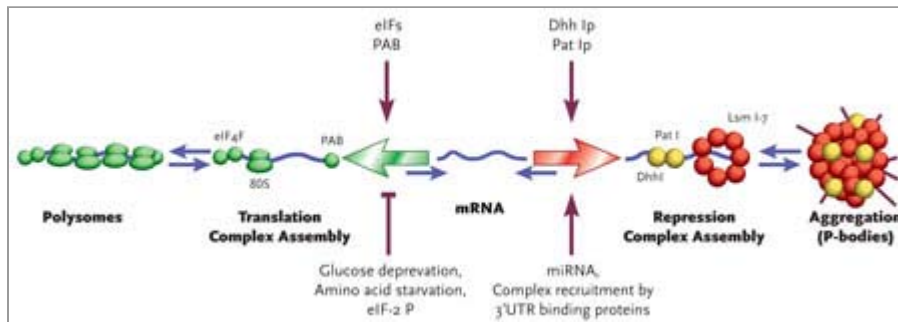
Now that's changing. Researchers are rapidly uncovering so-called granules in the cytoplasm that cluster function-specific proteins for RNA storage, silencing, reuse, destruction, and perhaps even splicing. Apparently related to the well characterized maternal mRNA granules that jumpstart embryogenesis, these neighborhood processing centers serve important functions in adult cells, including shaping synaptic plasticity and responding to stress. Moreover, their existence may mandate a reevaluation of how microarray results are interpreted.

"I think we'll learn that how cells control the destruction and translation of messenger RNAs through these structures will be a fundamental part of the control of genetic expression," says Roy Parker at the University of Arizona in Tucson. In the past two years, Parker has found cytoplasmic structures containing mRNA decapping and degradation enzymes. These compartments first appeared to serve as an mRNA junkyard: Transcripts with shortened poly(A) tails, or those otherwise no longer needed were relegated here for destruction. Parker dubbed them processing bodies, or P-bodies.¹

SILENCING AND SAVING

Parker and others speculated that given these structures' role in mRNA degradation, they might also be involved in RNAi. Parker's team, along with Helen Blau's group at Stanford² and Hannon's lab,³ showed that in mammalian cells Argonaute proteins 1 and 2, signature components of RNAi, also congregate in these compartments. Moreover, mRNAs targeted for repression by microRNA become concentrated in P-bodies. In addition, Edward Chan at the University of Florida in Gainesville, collaborating with Marvin Fritzler at the University of Calgary, found the 182-kilodalton protein GW182 in these structures. Chan calls them GW bodies, and his research suggests small interfering RNAs (siRNAs) localize within these compartments. Knocking out the structures inhibits RNAi.⁴

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CHECKING THE BALANCE:

Roy Parker, at the University of Arizona, Tucson, posits a general active repression machinery that exists in competition with translation. Any number of events can tip the balance one way or the other to set the relative translation rate for an mRNA. (From J. Collier, R. Parker, *Cell*, 122:875–86, 2005.)

"It makes sense to have compartments for degradation. It's not just RNA randomly floating around with an enzyme happening to find it," says Keith Blackwell at Joslin Diabetes Center in Boston. But P-bodies may be more than just centralized paper shredders; they may store mRNA for later use. In September, when Parker and colleagues blocked translation in yeast cells by depriving them of glucose, the number of free-floating ribosome complexes known as polysomes decreased, and P-bodies grew in size as mRNAs went to them.⁵ But instead of

being degraded, mRNAs accumulated. When glucose was restored, P-body size decreased and polysome number rose, suggesting that mRNAs were getting reused for translation. Reusing old mRNAs is likely more efficient and faster than making new ones, says John Rossi at the Beckman Research Institute of the City of Hope in Duarte, Calif.

Such results speak to a greater role in translational control. Normally, glucose deprivation or amino-acid starvation represses translation in yeast. Parker's team has shown that mutant cells lacking two key P-body proteins don't undergo this repression.⁶ Overexpressing either of these two proteins repressed mRNA translation and inhibited cell growth by sending mRNAs from polysomes to P bodies.

"We believe what we have here is a competition between translation and repression, an ongoing battle between mRNAs sent to the protein synthesis machinery or pulled into a silenced, sequestered state," Parker says.

Thus, "banking" of mRNA transcripts may be P-bodies' fundamental role. Scientists for decades have known of granules in egg cells that store maternal mRNAs. These produce proteins that drive much of development up to the 4- or 8-cell embryo, prior to transcriptional activation. So-called maternal granules may be P-bodies, Blackwell speculates. Key P-body components such as Dhh1 are found in them, and he and his colleagues found that putative *Caenorhabditis elegans* maternal granules drift around the cell like P-bodies.

Beyond the embryonic stage, only the germline cells in *C. elegans* seem to have these granules. Germline cells are also the only stem cell-like population in the worm, Blackwell notes. "We're excited over the possibility whether these RNA storage pathways are used specifically in other types of stem cells in higher organisms, where one could use them to store up a sort of posttranscription memory of what a cell is supposed to be about," he says.

In neurons, mRNA granules seem to influence synaptic plasticity (the variability in a synapse's signal strength), which appears fundamental to memory formation and learning. Kosik and colleagues found that granules store translationally silent mRNAs in dendrites.⁷ When the cell is depolarized, Kosik hypothesizes that the granules release their mRNAs to polysomes, resulting in localized protein changes. "They make sure that translation is directed to specific locations and not in the wrong place," he explains. The importance of such systems is hard to predict, Kosik says: "We could be talking about a branch of biology as extensive and intricate as the study of how proteins are directed to their destinations." Parker notes that neuronal and maternal granules have proteins in common and says he's looking to see if neuronal granules also possess P-body proteins.

The dynamic size changes that P-bodies undergo is reminiscent of the stress granule, another structure that appears to help regulate mRNA in the cytoplasm. In the mammalian cell, these granules form and grow in size when the cell is stressed, and mRNA and proteins rapidly shuttle in and out of them, says Paul Anderson at Harvard University. While P-bodies are relatively mobile in the cell, stress granules remain relatively stationary. Weil⁸ and Anderson⁹ independently found that P-bodies will frequently dock at stress granules for hours, and that RNA-binding proteins that promote mRNA degradation promote P-body docking.

Anderson suggests that stress granules are sites of mRNA triage: If translationally silenced mRNAs bind to destabilizing factors in the granule, they might be shuttled to P-bodies, while ones that interact with stabilizing factors are stored or shuttled to polysomes. "Stress granules are likely to be one of the factors that

Courtesy Nancy Kedersha, Brigham and Women's Hospital

determine whether a stressed cell induces repair enzymes and survives or throws in the towel and dies by apoptosis," Anderson says. These granules may monitor the cellular economy, says Weil: "If the economy gets better, the consumers start to buy again. Stress granules disappear, mRNAs are reused. If the crisis gets worse, with an increasing inflation rate, the 'money' can be depreciated so much that it has to be eliminated."

OTHER ROLES, OTHER INFLUENCES

Granules may serve purposes other than storage and destruction. James Eberwine and colleagues at the University of Pennsylvania Medical Center recently found that mRNA splicing can take place outside the nucleus, in the dendrites of rat neurons.¹⁰ "I do indeed believe that there are splicing speckle-like granules in dendrites," he says.

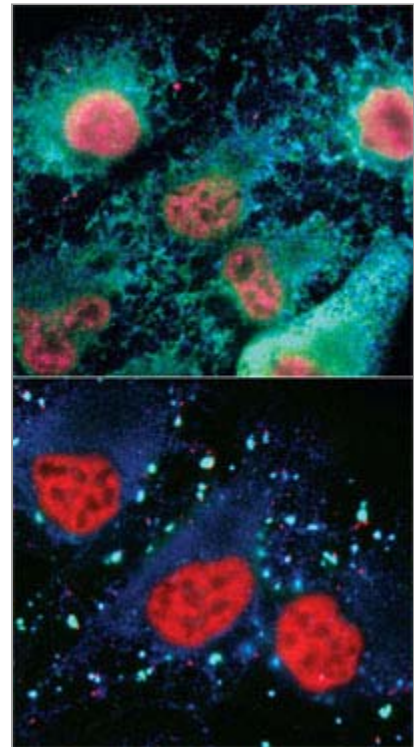
Further study of these compartments should influence genomic and proteomic studies. "Everyone looks for molecular biology or biochemistry approaches on how RNA is regulated, but that's probably useless unless you know where and when in the cell this takes place," Weil says.

Interpreting microarray results could be difficult if RNA is simply hanging around and not being used. "When people look at DNA chips, they're looking at the total level of message expressed. They miss whether the message is functional or is being stored," says Chan. "That's at least part of the reason why it's difficult to correlate mRNA levels and proteomics."

Insights into granule dynamics may benefit other studies. Researchers can see the specific mRNA and microRNAs that converge on GW bodies, notes Chan. "Then you can see which mRNAs get regulated by which microRNAs. That's not information that's quite known right now for most known microRNAs." He is currently establishing systems for such experiments.

Other research should narrow in on the signals and mechanics directing proteins and mRNA to and from these foci. Also high on priority list is a parts list for each compartment. "A lot of these structures sometimes look the same, sometimes not ... depending on the animal model they are studying," Weil says. Chan also stresses that electron microscopy work, such as his own on GW bodies, is important "to reveal how these components actually all fit together."

But just as the money in one's hand has a murky history, how an mRNA is shuttled about the cytoplasm remains hard to determine. Kosik says, "What we have here [is] a set of poorly visualized structures of even more poorly understood function that are all referred to as granules ... There's a real opportunity here to take a community of people who aren't talking with each other that much and bring them together and make this field coalesce."



STRESSED OUT:

These DU145 prostate adenocarcinoma cells have been stained red for a P-body marker, green for ribosomal subunit S6, and blue for the eIF3 pre-initiation complex component. In the bottom panel, cells are treated with sodium arsenite to induce oxidative stress, causing stress granules to assemble and P-bodies to increase in number.

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