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Silent RNAs express themselves

Study finds that RNA molecules housed in cytoplasmic granules are active.

RNA molecules stowed in cytoplasmic granules are generally thought to be silent. But a protein mutated in some amyotrophic lateral sclerosis (ALS) patients forms granules that permit translation of stored RNAs, Yasuda et al. reveal (1). The finding identifies a new mechanism that could contribute to the pathology of the disease and suggests that RNAs in other types of granules also may not be silent.

RNAs are gregarious. They cluster with other RNA molecules and proteins to form ribonucleoprotein complexes (RNPs). The protrusions extended by migrating cells, for example, sport RNPs that contain several kinds of RNAs and proteins, including the tumor suppressor APC (2). These protein–RNA clusters might help control the cells' movement. Granules are larger, more complex structures that contain RNPs. There are several kinds of granules, including stress granules, which appear when cells are under duress, and P-bodies, which help break down unneeded RNAs (3, 4). Researchers thus far have thought that RNAs in granules are not translated, although some findings have hinted otherwise (5). Large-scale protein–RNA conglomerations might have a role in disease. One protein, known as Fus, that induces formation of these structures is faulty in some cases of ALS. Cells from these patients contain molecular globs, or inclusions, that resemble stress granules, suggesting that RNA metabolism goes awry in the disease.

Yasuda et al. took a closer look at the functions of Fus. The researchers found the protein in the APC-containing RNPs in cell protrusions. Even in the absence of Fus, RNA molecules gathered in the protrusions, suggesting that the protein isn't necessary to recruit RNAs. However, levels of the protein Kank2, whose mRNA collects in the APC-carrying RNPs, fell after Fus depletion.

Focal Point

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(Top row, left to right) Kyota Yasuda, Ian Macara, Stavroula Mili, and colleagues (not pictured) probed the activities of the protein Fus. The researchers found that Fus promotes the translation of mRNAs in cell protrusions. The protein is mutated in some people with ALS, and the researchers discovered that these faulty Fus variants induce formation of cytoplasmic granules in which RNA translation occurs. The researchers demonstrated this effect by dosing cells with puromycin, which sticks to proteins being synthesized on ribosomes. An anti-puromycin antibody (green) labels mutant Fus granules (red) in the presence (left) but not the absence (right) of puromycin.

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That finding indicates that Fus spurs translation of RNAs in the protrusion RNPs. The team tested this idea by adding two molecules, azidohomoalanine and homopropargylglycine, that cells plug into proteins instead of the amino acid methionine. Furnishing the molecules to cells at different times enabled the researchers to distinguish newly synthesized proteins from older polypeptides. The technique revealed reduced levels of translation in the protrusions of cells lacking Fus, suggesting that Fus promotes translation mainly within the protrusions.

But the abnormal versions of Fus found in ALS patients have broader effects. Cells engineered to make Fus mutants harbor cytoplasmic granules that are similar to the inclusions found in patients' cells. Yasuda et al. showed that the mutant varieties of Fus drew APC-containing RNPs into the granules. The researchers anticipated that the RNAs in these RNPs would be silent. Instead, they discovered that the cells translated several of the RNAs into proteins. "That was unexpected," says senior author Stavroula Mili. To confirm the result, the team used two different methods to visualize the site of protein synthesis. In one technique, labeling with azidohomoalanine revealed where newly made proteins accumulated. The researchers also highlighted translation sites by adding puromycin, which adheres to proteins being made on ribosomes. Both methods supported the idea that the mutant cells manufacture proteins in the granules.

The findings raise the possibility that translation occurs in granules under other circumstances, including in healthy cells. Other studies have provided indications that it does. Researchers have observed granules and ribosomes in close proximity in mammalian cells, for example, and in *Drosophila* translation occurs at the margin of P-body–like structures. The results also offer a new potential mechanism that drives the cellular phenotype of ALS. "The study suggests that it's not silencing of RNAs but misdirection of their translation that might contribute to disease," Mili says. One question researchers need to answer is how Fus switches on translation. How the inappropriate production of certain proteins in granules promotes ALS pathology also remains unclear.

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