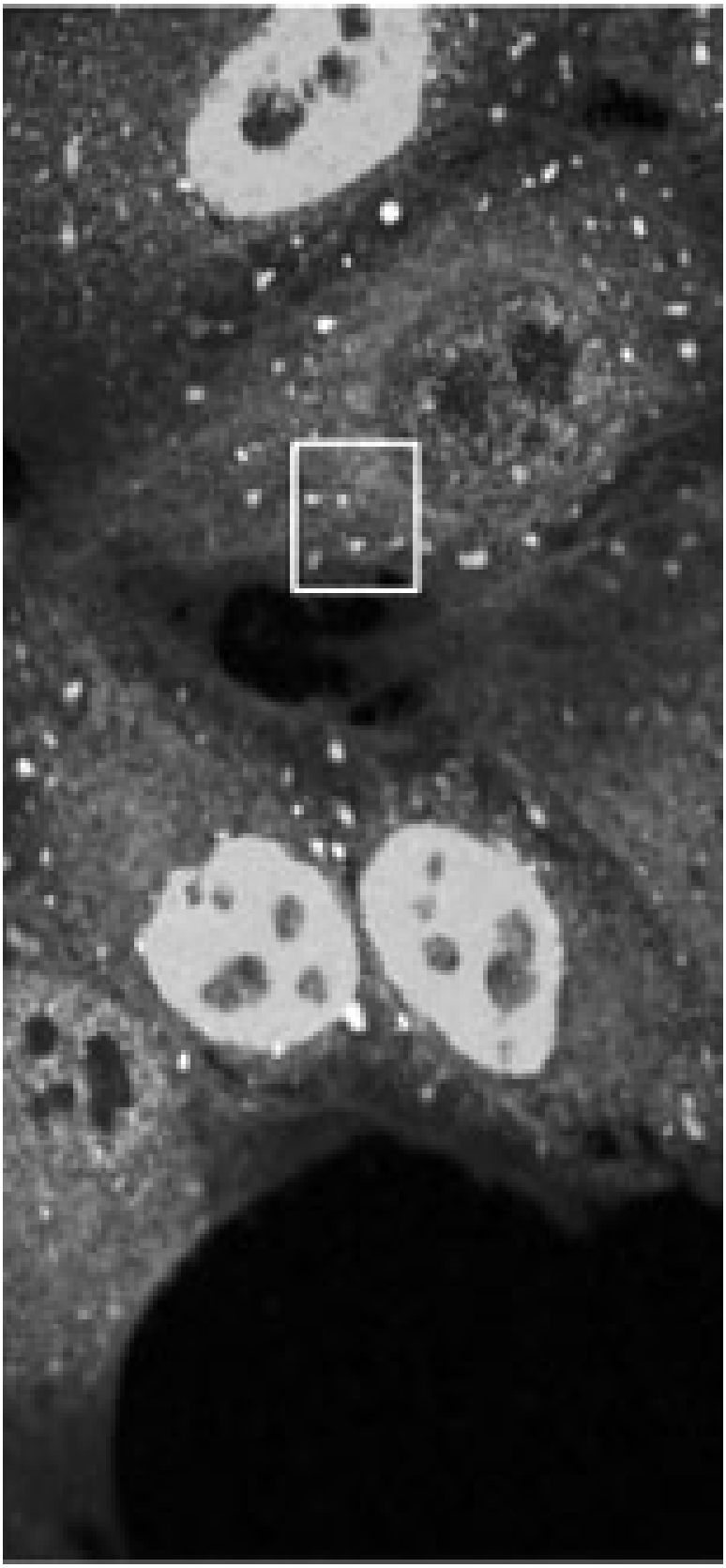

Figure 1: Stress granules: Upon exposure to different stresses, cells develope specific stress granules in response, an example highlighted in the box above.
Photo courtesy of Open-i from the National Library of Medicine.

DEALING WITH STRESS: THE CELL EDITION

How cells learned to deal with different stresses through the dynamic formation of stress granules

BY ALEXANDRA ROJEK

Stress: it's a term that every college student is all too familiar with. Whether there's an exam you haven't studied nearly enough for, a problem set that's due in an hour but is still mostly blank, or a paper with a tangled set of ideas, the common factor is stress. We deal indirectly with stress by complaining to friends, procrastinating (a futile solution), or maybe going for a run, but ultimately, these are all just ways to mitigate our stress level without actually solving the problem: the only way to do that is to put all else on hold and resolve the problem, whether it be studying for that exam, completing that problem set, or writing the last sentence of a paper.

Just like college students, every organism has ways of dealing with stress – usually in a more efficient manner than the former. Stresses can come from external or internal sources for a cell and can take a number of different forms, but all cells must develop ways in which they can limit the amount of damage induced by the stress, functionally counteract the stress until it is gone, and maintain a delicate balance with other critical processes while doing so.

The most critical process to any cell or organism is the production of proteins, which are translated from messenger RNAs (mRNAs). The levels of specific mRNAs in a cell are under constant regulation by other factors (also proteins) at the transcriptional level, where the DNA code for specific proteins is transcribed into mRNA to be exported out of the nucleus. It is in a situation of stress that the protection of mRNAs is most vital: without accurate mRNA translation into protein, the cell would not be able to function for very long, but at the same time it needs to protect the mRNA transcripts so that translation can resume when the stressor is gone. The cell deals with such a situation much like a trauma surgeon would: triage*(1)* .

Anatomy of a stress granule

The physiological manifestation of the cell's triage process is stress granules. Formally, stress granules are messenger ribonucleoprotein particles, or accumulations of specific mRNAs and proteins. It is thought that stress granules in eukaryotes form directly as a result of translational pausing, which can occur for a variety of reasons. Although the direct mechanisms remain unknown, it has been suggested that pauses within a very specific window of the translation initiation process and the presence of specific factors lead to stress granule assembly *(2)*. Errors in the stress response pathway have the potential to provoke many disease processes, including cancer, microbial infection, diabetes, and inflammatory disease *(3)*.

The hallmark of stress granules is the effective arresting of translation in the cell, because actively translating mRNAs and their initiation factors are sequestered in the stress granule. After translation initiation is stalled, polysomes are disassembled, the first factors are aggregated and stress granules are nucleated. Next, there may be involvement of other proteins, mRNA triage, and finally stress granule disassembly when the stress has been dealt with *(1)*.

Although many details about stress granule assembly in different stresses and organisms remain unknown, three contributory factors have emerged across studies. First, posttranslational modifications to different components of stress granules is an appealing option, as it would allow for the quick reversibility *(2)*. The prion-like aggregation of other proteins also suggests that they may be able to quickly self-aggregate and induce stress granule formation. Many RNA metabolism proteins contain prion-like domains, although some details remain unclear as to how specificity could be achieved *(4)*. Heat shock proteins, or chaperones, are also known to regulate these prion-like proteins during stress granule formation by possibly allowing for 'prion seeds' to form after partially disaggregating them, but also allowing for their disaggregation after the heat stress is over *(5)*. The microtubule network in a cell has also been shown to be vital to stress granule assembly: from an intuitive level it makes sense that appropriate movement of all these components to one locus is necessary. It is known that microtubules are needed for assembly, but are not strictly required once this is accomplished *(6)*. The indication that factors necessary for assembly contain microtubule-interacting regions support this theory *(7)*.

Although the above three factors are some common characteristics of stress granule assembly, it is important to note that most stress granules are very dynamic in nature: not only do they vary by composition and requirements between organisms, but also within an organism by stress type. For example, in yeast some factors are required for stress granule assembly under glucose starvation *(8)* but different ones are needed for a proper heat shock response *(9)*.

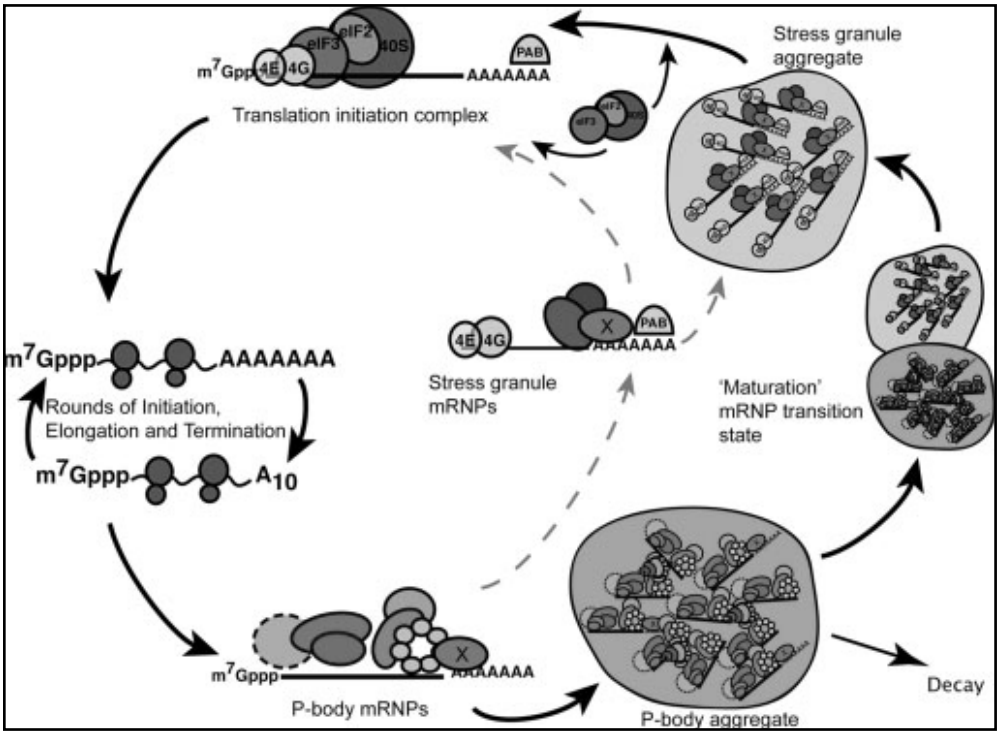

Figure 2: Stress granule assembly: Various signals, some unknown, triage mRNAs first into P-body aggregates that can then develop into stress granules, protecting existing mRNAs while the cell deals with a given stress.
Photo courtesy of Open-i from the National Library of Medicine.

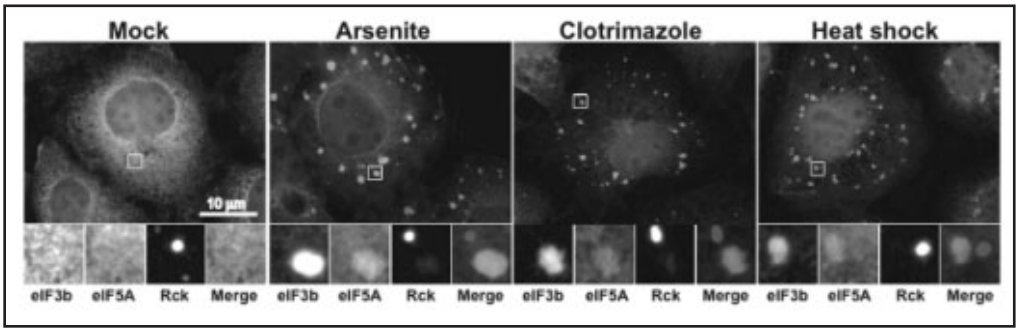

Figure 3: Various stressors all promote stress granule (SG) assembly: Upon stress induction by arsenite, clotramizaole, or heat shock, assembly of stress granules can be seen, as identified by the colocalization of several key factors identified to be essential for SG assembly.
Photo courtesy of Open-i from the National Library of Medicine.

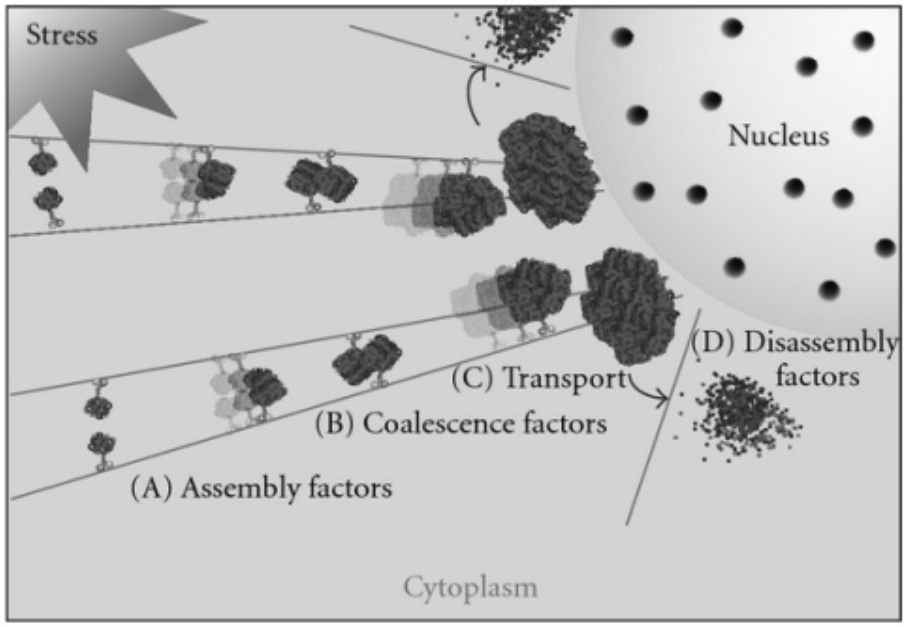

Figure 4: Role of microtubules: Microtubules are necessary components for stress granule assembly, presumably to accurately transport SG components, and are not required to maintain SG existence. ***Photo courtesy of Open-i from the National Library of Medicine.***

Triage and disassembly

Once a stress granule is assembled and other regulatory processes have recruited additional proteins, the process of RNA triage occurs. Although it was originally thought that stress granules protected mRNA from any changes until the cellular environment was once again 'safe' for normal translation to ensue, it was found that many vital stability-associated proteins move in and out of the cell while stress granules are intact. For example, a protein that stabilizes the poly-A tail of all eukaryotic mRNA transcripts, Pabp, was found to move in and out of stress granules rapidly *(10)*, arguing against a static model for mRNA sequestration. Other proteins, both destabilizing and stabilizing, have also been found in stress granules, facilitating either the reassembly into polysomes or decay of the mRNA transcript *(1)*.

After the stress is mitigated, it is necessary for the components of stress granules to be functional again – reproducing all the components of stress granules would not only be costly to the cell, but would also make stress granule assembly and RNA triage a futile process. It has been suggested that just as microtubules and motor proteins may play a role in stress granule assembly, they are also vital in their disassembly for redistributing the components of stress granules to resume active translation *(11)*. In addition to the redistribution of stress granule components, refolding of aggregated proteins is also a key process that is mediated through factors such as HSP70, a heat shock protein that is a chaperone *(1)*.

Prion domains and viruses

One of the key factors that was found relatively early on to be vital in stress granule formation was a RNA-binding protein, TIA-1 *(4)*. This protein contains a glutamine-rich prion-related domain (PRD) that allows it to self-aggregate through the perpetuation of conformation changes leading to aggregation. This aggregation renders TIA-1 resistant to protease cleavage, but the entire process is reversible with HSP70. Accompanying studies with a proposed prion-like aggregating protein from yeast also suggest that it may be able to induce aggregation of not only itself but other proteins as well to promote stress granule assembly *(4)*.

Viral infectious often cause reprogramming of a host cell's translational machinery, and therefore poses a significant stress on the cell. Viruses have evolved to prevent the cell from mediating such an infection by inhibiting the formation of stress granules. For example, West Nile Virus interacts with TIA-1. The virus increases the efficiency of its own replication through this association, and may either use this native protein to associate with stress granules and therefore limit nascent protein production, or by inhibiting the formation of stress granules in the first place *(12)*.

Stress granules and disease

Although aggregations of mRNAs and proteins were long known to form in the cell, it has not been until as of late that the full scope of the impact of stress granules in diseases has been explored. Debate remains about whether stress granules are the cause or the consequence of translational arrest in a cell under stress, but the correlation of stress granules with diseases as varied as viral infections (seen above), cancer, and immune responses, to name just a few, provide significant evidence for placing a greater emphasis on understanding these processes.

Current methods of cancer treatment are aimed at stressing malignant cells in the hope of killing them off. However, many treatments also often result in the induction of stress of normal cells, for example through radiation and toxic drugs. The types of stresses these responses induce often lead to apoptosis, or cell death – desirable for cancerous cells, but not for healthy cells. There are many different categories of stress, however, with different endpoints destined for each. Stresses like heat shock, hypoxia (decreased supply of oxygen), and arsenite (a poison for cells) are classified as type 1 stresses and the response is the formation of cytosolic stress granules. However, other types of stress, such as X-ray irradiation and genotoxic drugs, are

classified as type 2 stresses and induce an apoptotic response *(13)*.

Hypoxia in cancerous cells is often associated with developed resistance to chemotherapy drugs because of the inhibition of the apoptotic pathway and therefore a decrease in drug-induced senescence *(14)*. Further investigation into the interactions between the pathways of type 1 and 2 stress revealed that in type 1 stress, which includes hypoxia, the assembly of cytosolic stress granules includes the scaffold protein RACK1. This scaffold protein is a known component of the apoptotic pathway for type 2 stresses, through its binding to a key kinase cascade (MAPKKK) to facilitate the stress response upon stimulus recognition. However, when RACK1 is included in stress granules in type 1 stress conditions (which include hypoxia), it is unable to facilitate this response to type 2 stress, resulting in a failure to induce apoptosis *(13)*.

The inability of the type 2 stress response to be induced when a cell is already in type 1 stress makes it such that if a cell is under hypoxia, heat stress, or arsenite stress, for example, it is unable to respond to type 2 stresses, such as X-ray irradiation and genotoxic drugs. This repressive effect of one pathway on the other has the devastating effect of inducing chemotherapeutic drug resistance when cells are under hypoxia stress. Presence of both types of stress allows for cross talk between the two pathways, suggesting a way that cancer cells have evolved to develop resistance to chemotherapy drugs *(13)*.

Future directions

Pioneering groups working on stress granules are rapidly demonstrating how functionally vital stress granules are, and on how broad of a systematic level their impact is seen. Continuing research to further understand their components, conditions of assembly and disassembly, and their role in disease is bound to reveal new insights that have the potential to not only revolutionize our knowledge of stress and how it is mediated, but to also reveal potential therapeutic solutions in a broad range of diseases.

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Chemotherapy drug resistance: Stress granules play a role in the development of chemotherapy drug resistance: their induction in type 1 stress represses the type 2 stress response pathway to drugs, thus blocking apoptosis and allowing for cancer cell proliferation.
Photo courtesy of kirk.senate.gov .

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