RNA is a type of molecule in the cell. RNA is as a temporary copy of DNA sequence that can serve as a template for protein synthesis or as a regulator for gene expression. As demonstrated by this figure, RNA is required in many *many* cellular processes that occur all over the cell. For an RNA to carry out its specific function properly, it needs to be in the correct cellular location. Mislocalization of RNA can cause inefficient cell growth or cell death.

As organisms develop, certain RNAs change location in the cell, causing unique expression gradients shown in green in the left image. Mislocalization of RNA can also cause developmental defects or embryonic mortality. Similarly, certain RNAs will aggregate in neurons if mislocalized, like the pink splotches in the right image. This aggregation is thought to contribute to diseases such as ALS.

Hopefully, I’ve convinced you that RNA localization is important to study, but how do we study it?

Well, imagine that you let your black dog into your yard at night. You can’t see them. This is exactly the same idea as trying to find your favorite RNA in a cell under a microscope. However, if you give your dog a light-up collar, you can see their collar in the yard at night, so you can see them. Same idea with fluorescent RNA tools. We add a fluorescent tag to our favorite RNA and now we can see it with fluorescent microscopy!

These tools allow us to see the difference in RNA expression, localization, and behavior in the heterogeneity of different cell types. These tools also let us see the spread in a cell population. If our tool allows us to single molecules of RNA, we can even watch different molecules of our favorite RNA doing different things in the cell. And ideally, we can watch a molecule of our favorite RNA as it moves through different tasks and changes localization throughout its life cycle. Fluorescent RNA tools allow us to see our favorite RNA in live cells and explore the full heterogeneity of biology.

Riboglow is one such fluorescent RNA tool. It is composed of two parts: a Riboglow aptamer and a Riboglow probe. The aptamer is a piece of RNA that folds into a 3D shape and binds a small molecule tightly and specifically. In this case, the aptamer binds cobalamin (Cbl). The probe is cobalamin linked to a fluorophore, such that the aptamer can also tightly and specifically bind the probe. If we add the Riboglow aptamer to the end of our favorite RNA and add in the probe, we have fluorescently tagged our favorite RNA (in the same way that you would put a light-up collar on your dog). We can add multiple aptamers onto our favorite RNA so that we can bind multiple probes to our favorite RNA, so that the “light-up collar” is super super bright. We call this series of aptamers an array.

Now, cells form protein-RNA aggregates called stress granules when they are stressed out. These granules are held together by many weak interactions between protein and RNA molecules. These granules help the cell deal with an of immediate stressor. If the stressor is removed, these granules dissolve in healthy individuals. However, prolonged stress means long-lived stress granules which may serve as a starting point for ALS.

In preliminary experiments, an RNA tagged with Riboglow does get recruited to these stress granules under stress. In the top left image is the stress granule marker and in the top right, the Riboglow probe which binds to the Riboglow-tagged RNA. If we plot the fluorescence intensity of these two images, we get something like the plot on the bottom. As you can see, Riboglow probe accumulates in the stress granule with the Riboglow-tagged RNA. This means that Riboglow COULD BE a good tool to study stress granules in both healthy cells and cells that mimic cells in ALS patients.

This brings us to the main purpose of this project. If I am going to use Riboglow to study stress granules, how efficiently are Riboglow-tagged RNAs recruited to stress granules? What proportion of these stress granules contain the Riboglow probe? Will I be studying the full set of stress granules or is Riboglow only getting into some for some reason? Also how enriched is Riboglow probe in the stress granules compared to background probe levels in the cytosol? Basically, how easy will it be to study stress granules with Riboglow? Finally, there are two versions of Riboglow. Does the newer version perform better in this stress granule experiment than the original Riboglow system?

So, Rosie and I will have three-channel images as our input. These images have the stress granule marker, the Riboglow probe, and a marker for expression of the Riboglow-tagged RNA. I want to know what fraction of stress granules contain the Riboglow probe. I also want to know the signal-to-background ratio or the enrichment of Riboglow probe in the granules. I also would like to compare the new version of Riboglow to the original version. In this, Rosie and I will design a system in about six parts.

We will first find the outlines of our cells in the images. Then we will find the outlines of the granules in the images. Then we will count the number of granules and get the probe intensity inside the granules. Then we will perform calculations to get the fraction of granules that contain Riboglow probe and the enrichment of Riboglow probe in granules. Finally, we will perform some stats to see if the new version of Riboglow performs better than the original. And we will graph everything to demonstrate the spread of our data and allow us to visually compare the two versions. This will allow me to determine which version of Riboglow I should use going forward.

In the first cell segmentation portion, we will design this function to work on each image in each group of images for the versions of Riboglow. Our inputs will be the three-channel images, specifically the channels for the Riboglow probe and the expression marker for the Riboglow-tagged RNA. Our output will be a mask, or a logical list of lists, of cells in the images that have both the Riboglow probe and are expressing the Riboglow-tagged RNA. These are the only cells that we will want to look at for the remainder of our analysis.

Then, in the granule detection portion, we will input those images, specifically the channels for stress granule marker and the Riboglow probe, and the mask of the cells that we are interested in for each of our images. Then we will create a mask of granules that show up in the granule marker channel and a mask of granules that show up in the Riboglow channel.