Immunolocalization of a Drosha-Like Protein in Tetrahymena thermophila

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Abstract

Drosha is an enzyme used by animals to process pri-miRNA into pre-miRNA. This processing normally occurs in the nucleus. The partly processed RNA molecule is then exported to the cytosol, where it is fully processed and then used in gene regulation. While much is known about this process in animals, less is known about how unicellular organisms process miRNA. We wanted to compare miRNA processing in the unicellular eukaryote, *Tetrahymena thermophila* with miRNA processing in the animal kingdom. In our past studies, we have successfully purified miRNA from *Tetrahymena thermophila*. Our searches of the *Tetrahymena Genome Database* indicated the presence of multiple genes with very high levels of homology to the miRNA processing enzymes Drosha and Dicer. Because of these data, we postulated that an anti-Drosha antibody would bind to a number of proteins in *Tetrahymena.* We hypothesized that a Drosha-like protein would immunolocalize to the nucleus of *Tetrahymena*, as seen in animal systems.

Materials and Methods

Immunofluorescence was carried out using a modified protocol obtained from cellsignal.com. Cells were fixed in 3.7% formaldehyde for 15 minutes, then rinsed twice in PBS and blocked for an hour in 3% BSA. Cells were then rinsed in PBS and incubated overnight at room temperature in primary antibody at a dilution of 1:100 in the presence of BSA. After rinsing three times in PBS, cells were incubated in a 1:100 dilution of secondary antibody for 1–2 hours at room temperature in the dark. Cells were then rinsed three times in PBS. 5 ml of cell suspension was then applied to a slide and mixed with 5 ml of DAPI. Cell suspension was then observed under a fluorescence microscope at 400X. Mean fluorescence of each group (approximately 10 cells) was compared using a two-tailed T test.
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Results

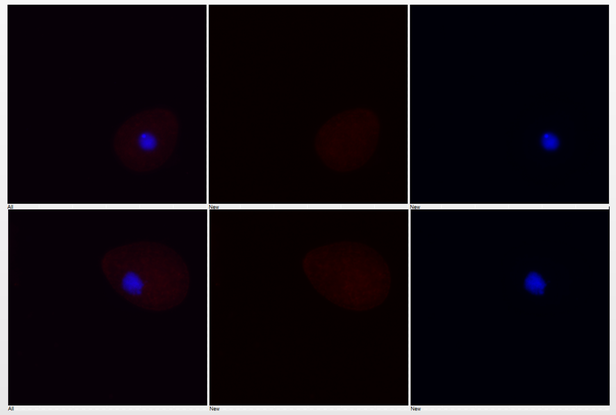

Figure 1. Immunofluorescence using secondary antibody alone shows low levels of fluorescence that show diffuse localization to the cytosol.

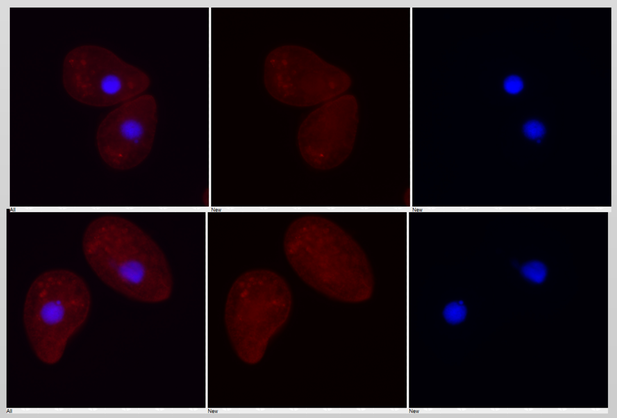

Figure 2. Immunofluorescence using anti-Drosha antibody shows fluorescence throughout the cytosol, including some staining of cytosolic granules. Contrary to our hypothesis, no Drosha staining is seen in the nucleus.

Conclusion

- Staining with anti-Drosha antibody is significantly higher than staining with secondary antibody alone. Anti-Drosha antibody localizes to the cytosol, and some cytosolic vesicles.
- Further analysis of Drosha-like proteins will be conducted via Western blotting. A preliminary Western blot showed low levels of signal, and stained a band at approximately 150 kD (data not shown). We plan to concentrate our protein by immunoprecipitation prior to performing additional Western blotting.
- While Drosha appears to have its activity in the nucleus of animals, the anti-Drosha antibody shows no nuclear staining. If this protein is processing pri-miRNA, it appears that the processing occurs in the cytosol of this organism.
- We are working on a new fixative protocol that will allow us to stain for RNA and see if the RNA colocalizes to the granular structures stained by the anti-Drosha antibody.

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