

Differential Regulation of Drosha and Dicer Homologues by Stress in *Tetrahymena thermophila*

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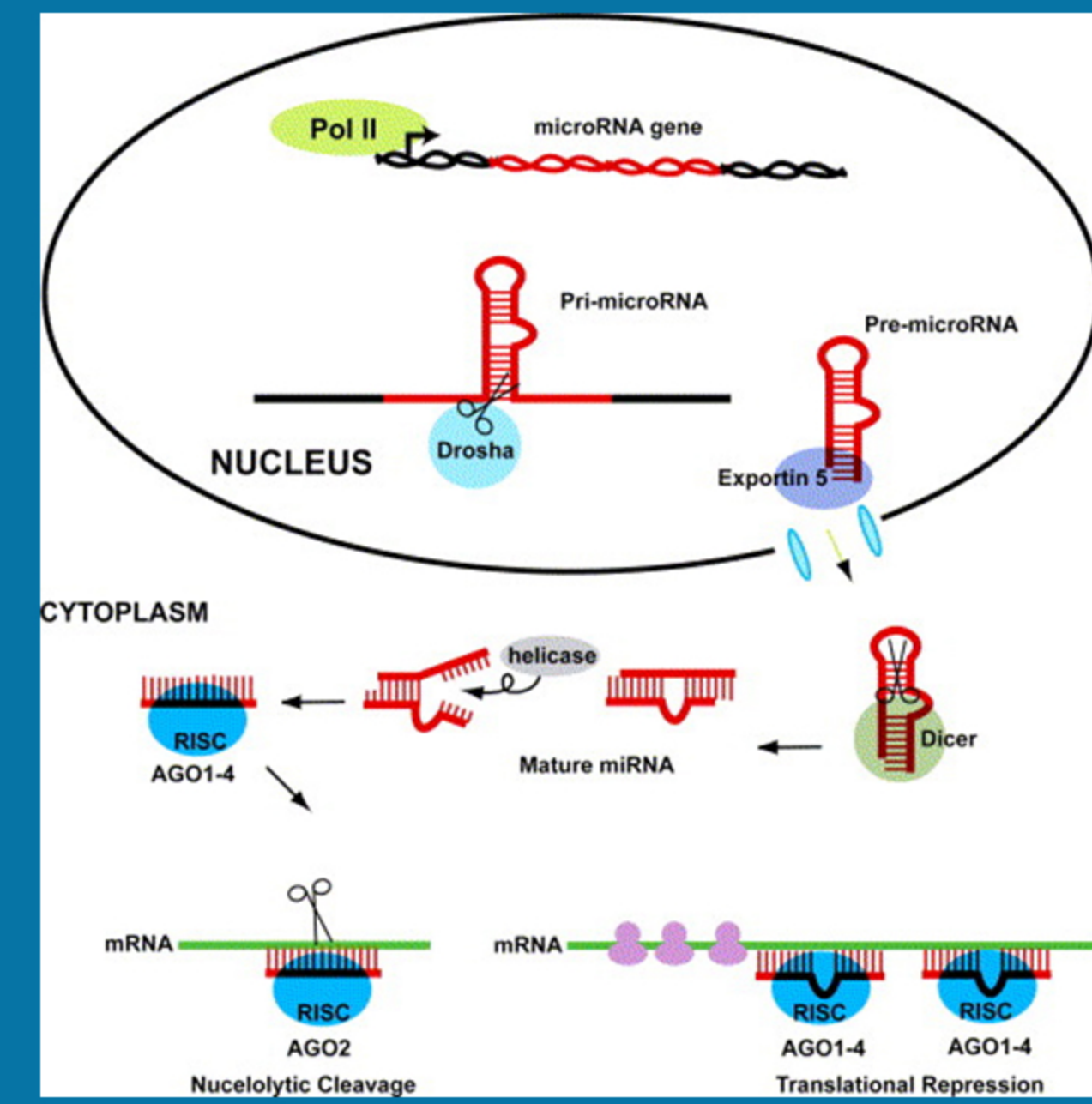
Carr, Tevia; Gannon, Kyle; Powell, Jennifer; Rose, David; Silveira, Nicholas; Vonlehmden, Georgia; Wachter, Emily; Ward, Anna; and Kuruvilla, Heather G., "Differential Regulation of Drosha and Dicer Homologues by Stress in *Tetrahymena thermophila*" (2021). *The Research and Scholarship Symposium*. 21.
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Presenters

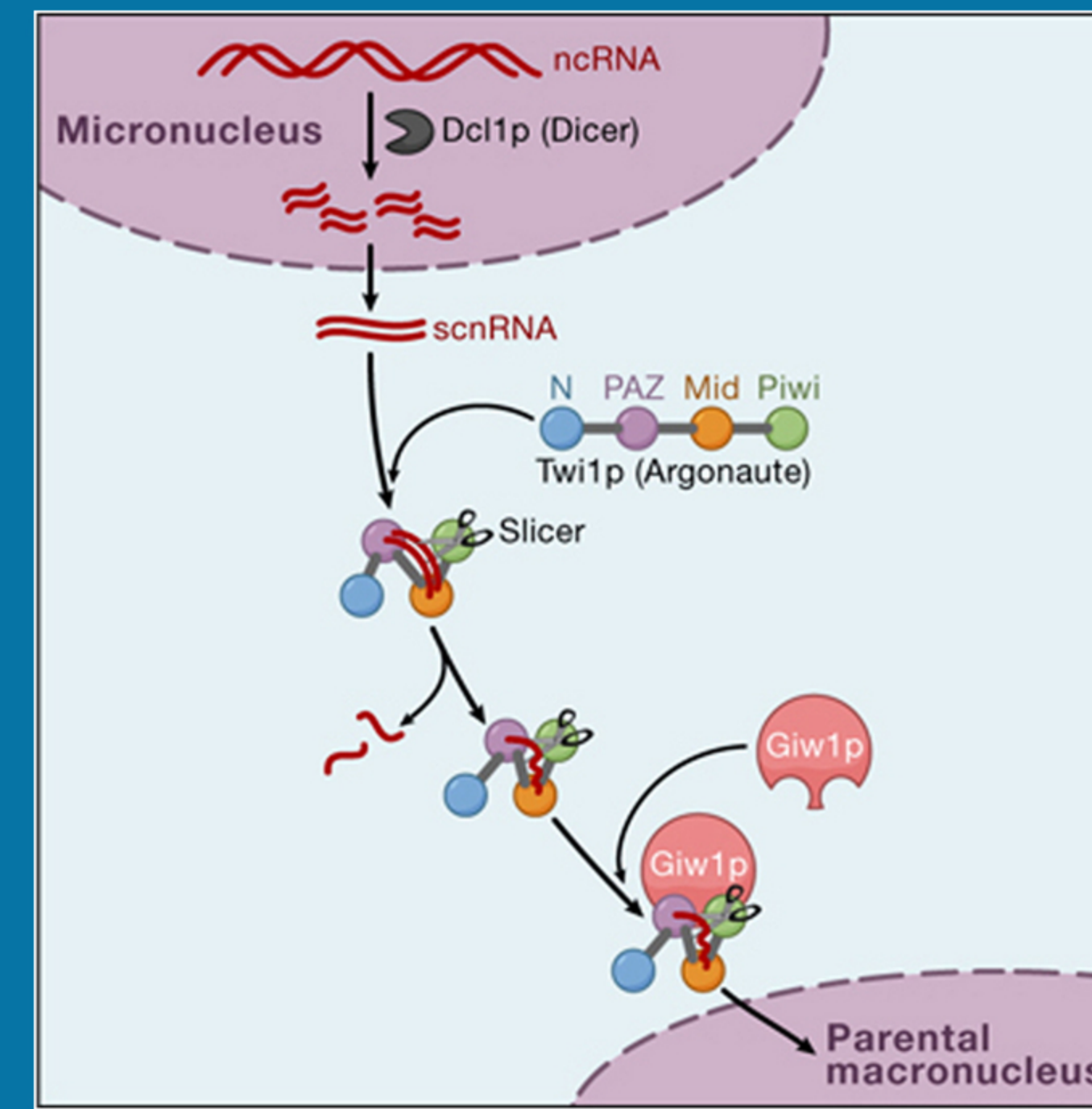
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Does stress affect expression of Dicer or Drosha homologues in *Tetrahymena*?



Mammalian miRNA processing
Image Credit: Sciencedirect.com

How does mammalian miRNA processing compare with small RNA processing in *Tetrahymena*?



Tetrahymena use Dicer-like proteins to delete the macronucleus after conjugation.

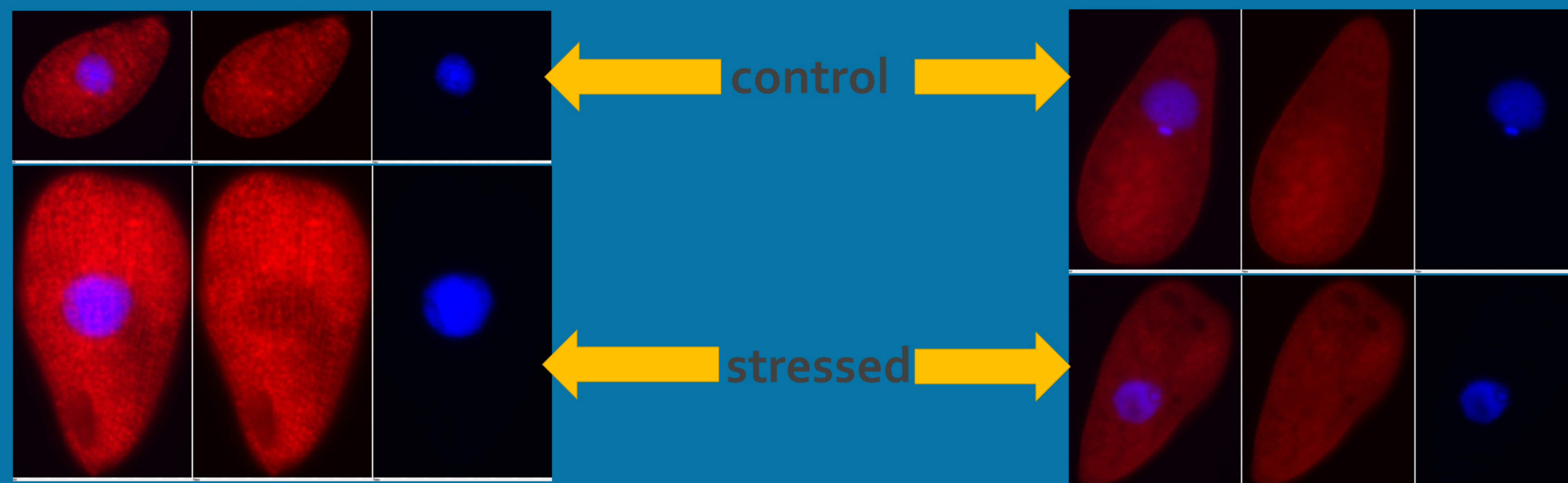
Image Credit: Noto, T. et al., 2010
[https://www.cell.com/fulltext/S0092-8674\(10\)00125-X](https://www.cell.com/fulltext/S0092-8674(10)00125-X)

Are Dicer homologues present in *Tetrahymena*?
BLAST analysis conducted in Uni-Prot

Dicer homologue	Predicted Molecular Weight
Dcr1	172,246
Dcr2	218,356
Dcr2p	233,170
Dcl1p	150,260
Dcr1p	78,017

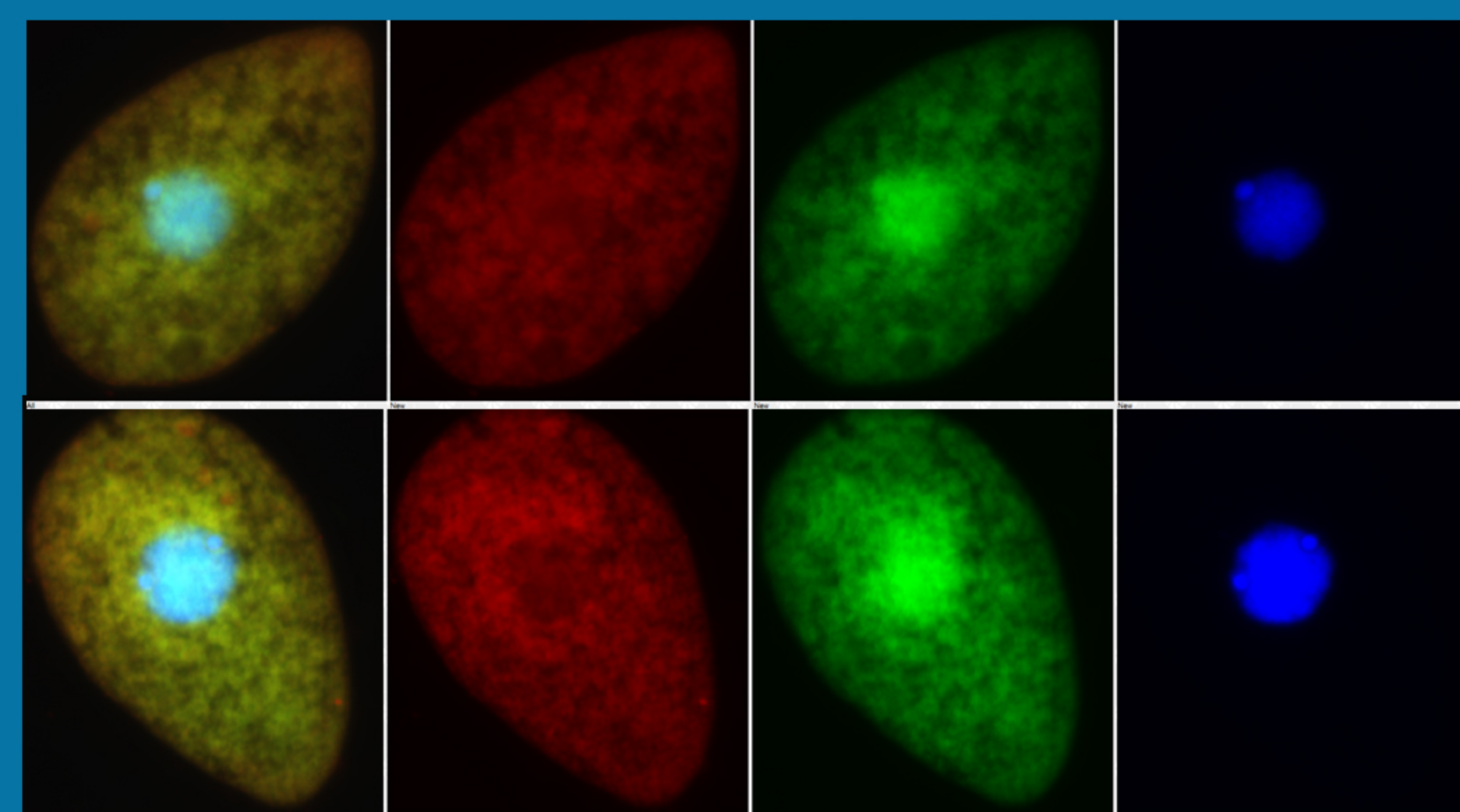
Are Drosha homologues present in *Tetrahymena*?
BLAST analysis conducted in Uni-Prot

Drosha homologue	Predicted Molecular Weight
Dcr2p	233,170
Dcr1p	78,017

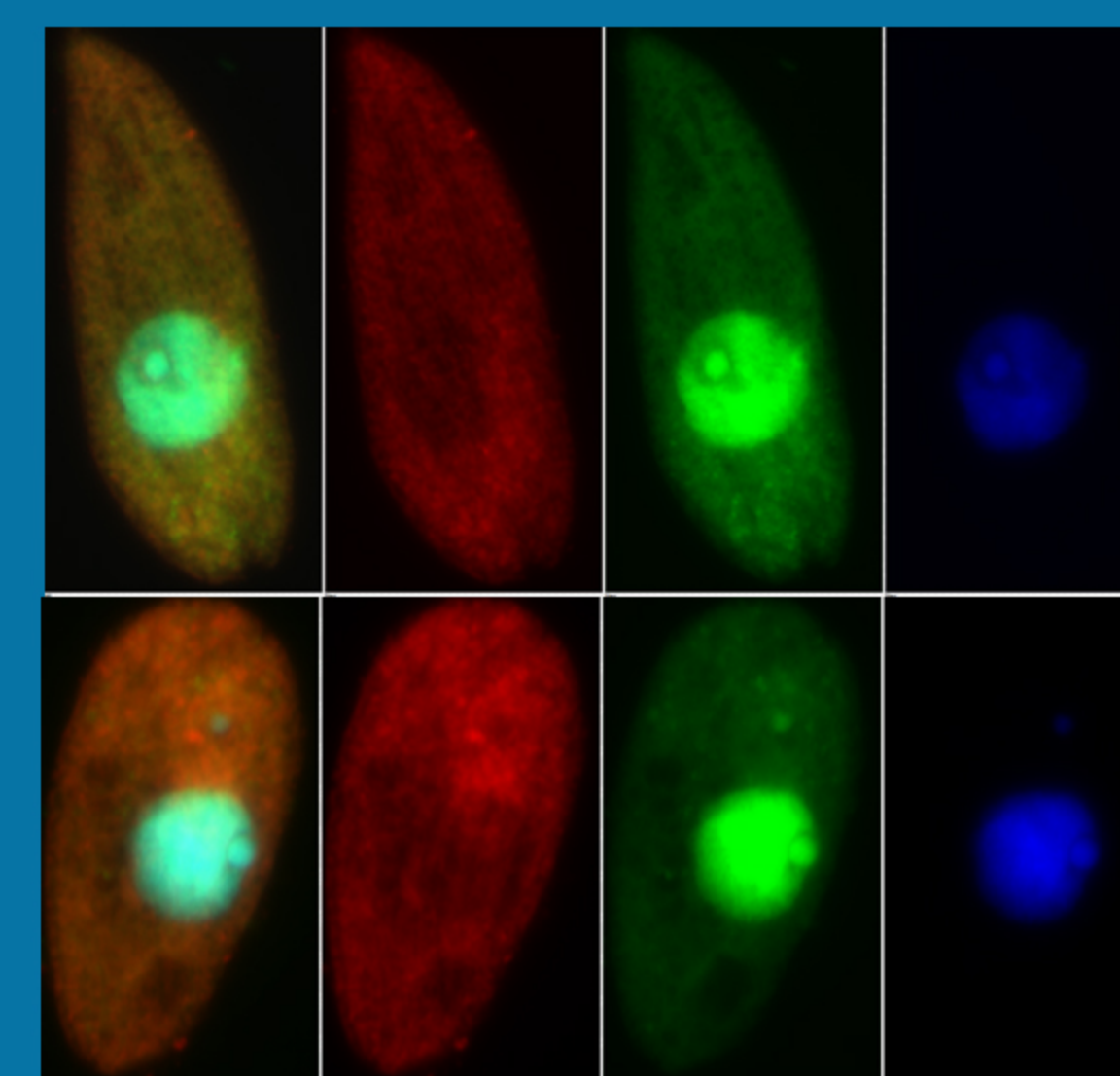


Dicer

Drosha



Dicer (red) and RNA (green) expression in **control** cells



Dicer (red) and RNA (green) expression in **stressed** cells

Differential Regulation of Drosha and Dicer Homologues by Stress in *Tetrahymena thermophila*

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Introduction

Tetrahymena thermophila are free-living ciliated protozoans that possess two nuclei; a diploid micronucleus used only for mating, and a polyploid macronucleus which governs gene regulation and cellular homeostasis. When environmental conditions are favorable, the micronucleus exists as heterochromatin and the macronucleus is active. However, when conditions are unfavorable, *Tetrahymena* will attempt to conjugate with another mating type of the same species, in order to obtain new genetic information. During conjugation, the old macronucleus breaks down and a new polyploid macronucleus is regenerated.

Macronuclear breakdown in *Tetrahymena* is governed by small RNAs, similar to miRNAs in multicellular eukaryotes. Our previous research, using the *Tetrahymena Genome Database*, along with the findings of other scientists, indicates that *Tetrahymena* possess a number of enzymes homologous to the miRNA processing enzymes, Drosha and Dicer. In our current study, we immunolocalized these enzymes in the presence and absence of cellular stress. Our immunofluorescence data indicate that Dicer-like proteins are expressed at a higher level in stressed cells than in control cells.

Conclusions

- Mean fluorescence of Dicer-like proteins in *Tetrahymena thermophila* **increased** in response to stress (2-tailed T test, $p = 0.004$). Western blots run on cell extracts to determine which Dicer isoforms were upregulated have been inconclusive to date.
- Immunofluorescence of Drosha-like proteins in *Tetrahymena thermophila* was detected at a low levels under control and stressed conditions, with **no significant difference** in mean fluorescence (2 tailed T test, $p = 0.89$).
- Dicer staining **colocalized** with **cytosolic** RNA staining in control and stressed *Tetrahymena*, suggesting that some Dicer homologues are expressed and active **prior to conjugation**. Dicer was not present in the **nucleus** of controlled or stressed cells.

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