

Teaching unit “non-coding RNAs and epigenetics”



Small RNAs in epigenetic inheritance in *C. elegans*

Germano Cecere

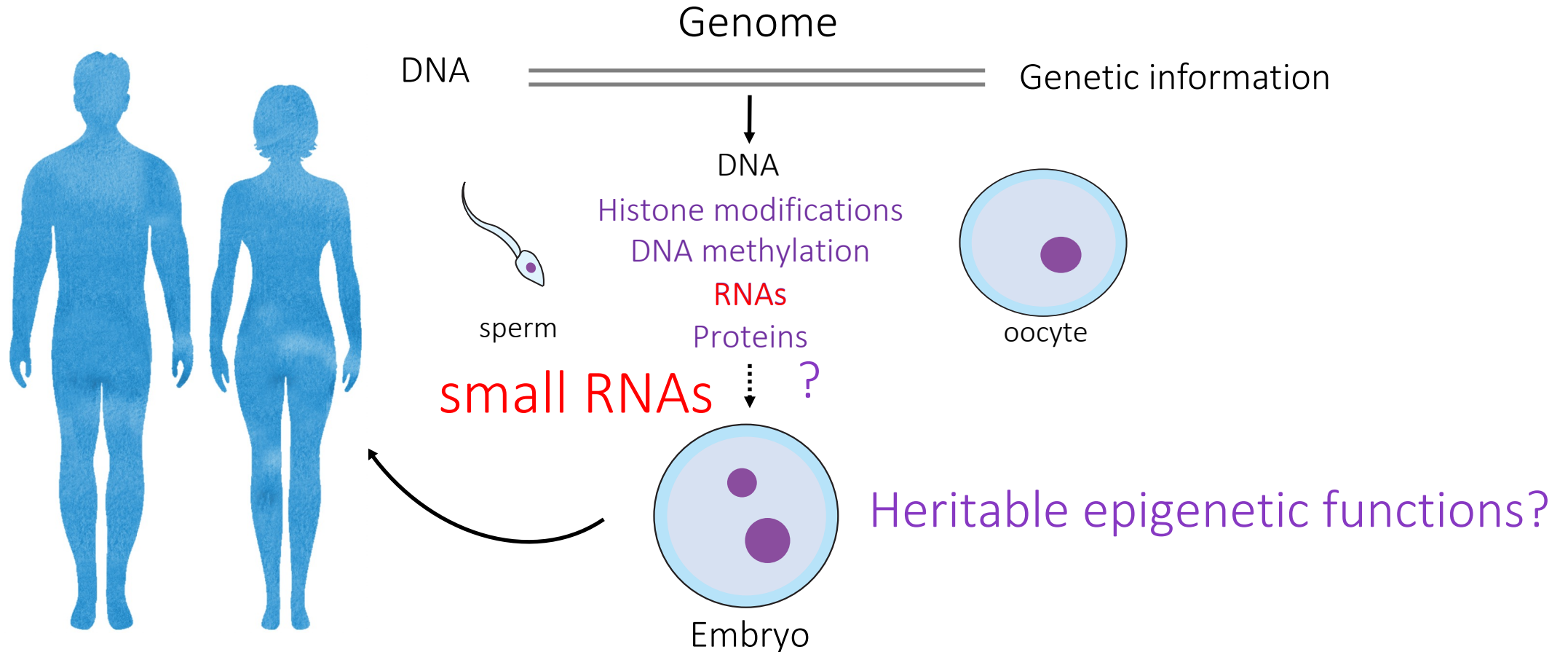
Director, Mechanisms of Epigenetic Inheritance Unit

Institut Pasteur - Paris

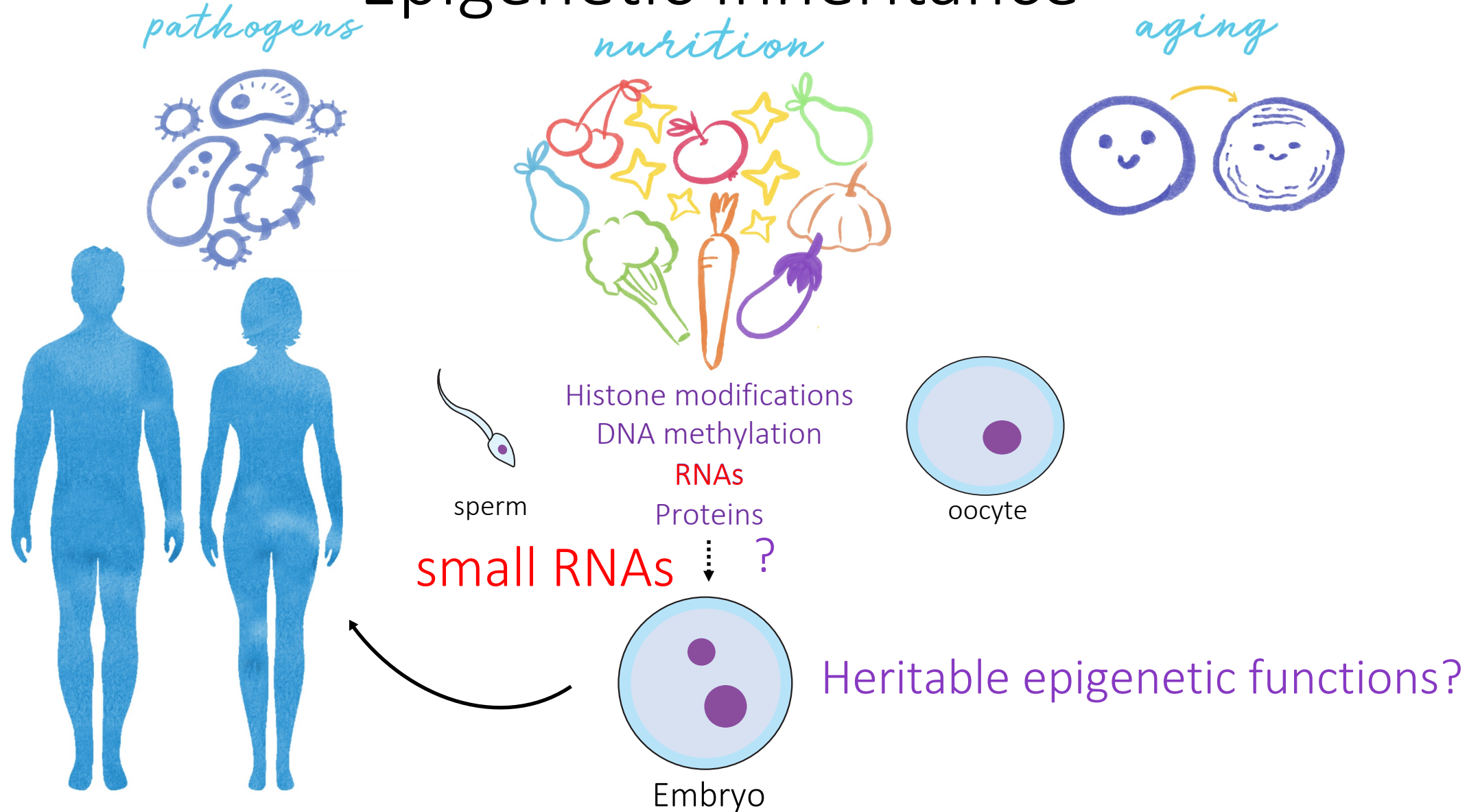
Monday, October 6th, 2025

Epigenetic Inheritance

Is the DNA the only molecule that transmits information across generations?



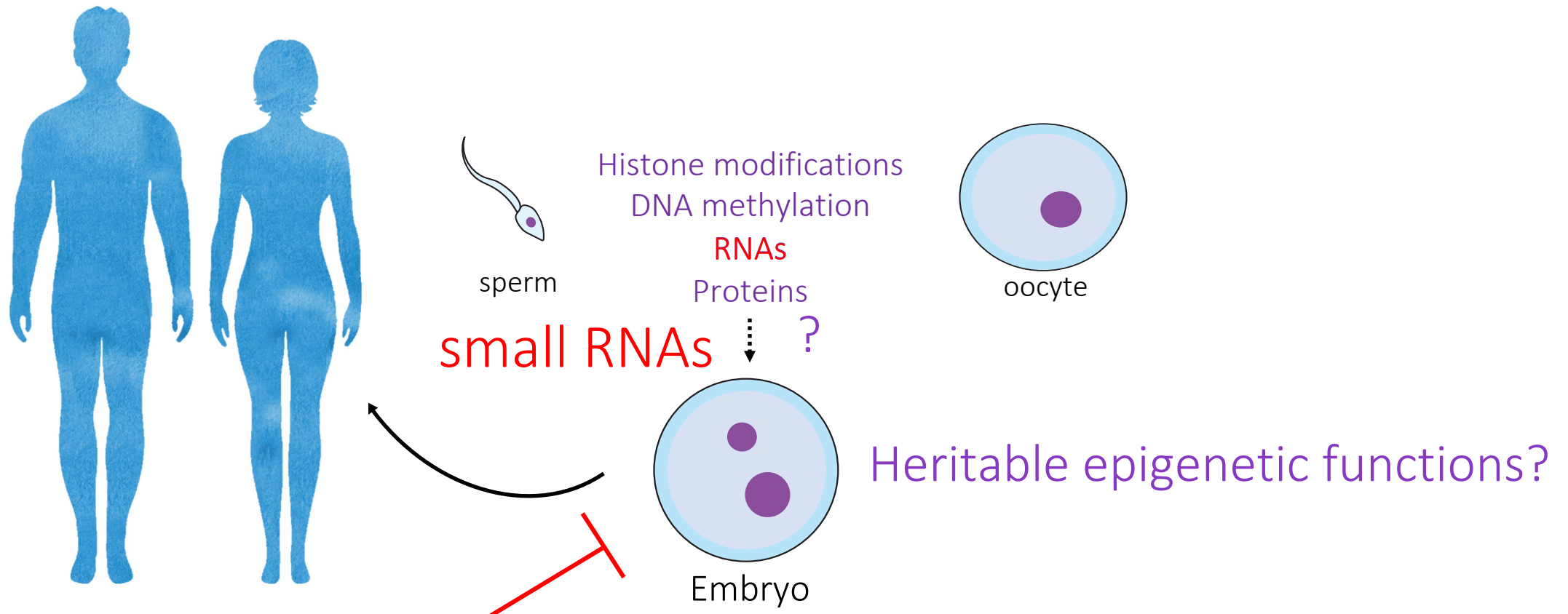
Epigenetic Inheritance



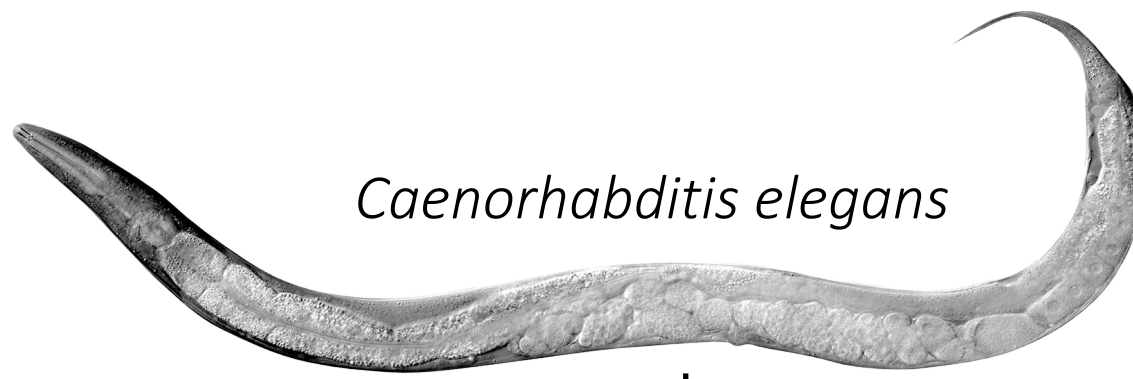
Do they transmit environmentally acquired information?

Major challenges to epigenetic inheritance

Epigenetic reprogramming in the embryo!



Erasure of epigenetic memory



Caenorhabditis elegans

small RNAs ↓ ?
Epigenetic Inheritance



Isogenic population

Generation time 3 days



Caenorhabditis elegans

Germline
small RNAs

Nobel Prize, 2006



small RNAs ↓ ?
Epigenetic Inheritance

RNAi is heritable!



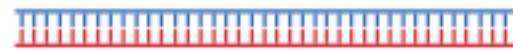
RNAi is an epigenetic phenomenon

RNA interference (RNAi)

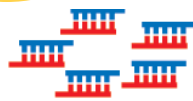
Nobel Prize, 2006



Foreign double strand RNA
(dsRNA)

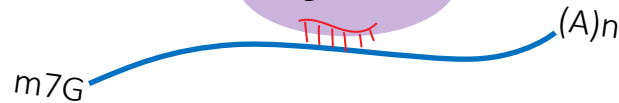


Dicer



small interfering RNAs (siRNAs)
20-24nt

Argonaute



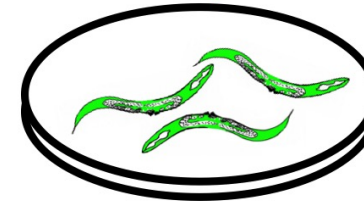
Transgenerational inheritance
of gene silencing

Fire et al., Nature 1998

Grishok et al., Science 2000

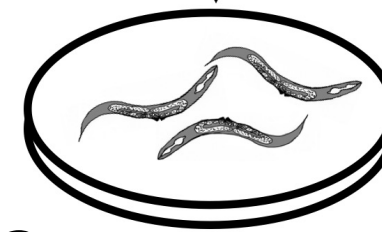
(Works in Mello and Fire labs)

GFP reporter



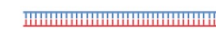
Nematode *C. elegans*

P₀



Trigger

GFP dsRNA

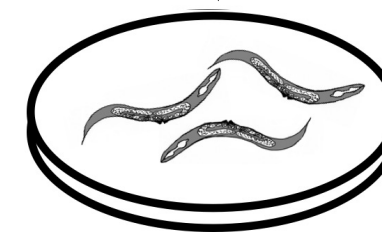


GFP siRNA



?

G₁



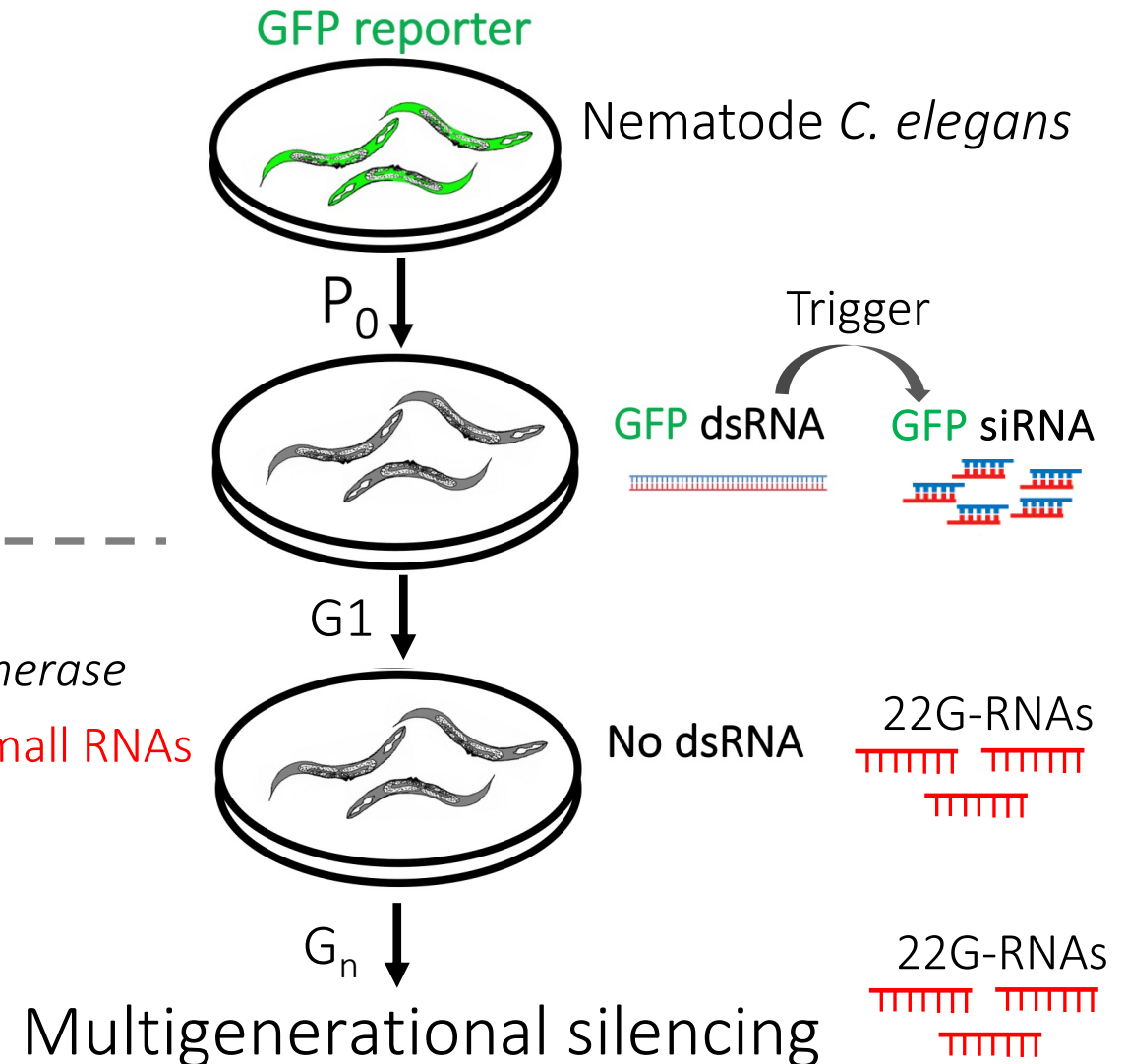
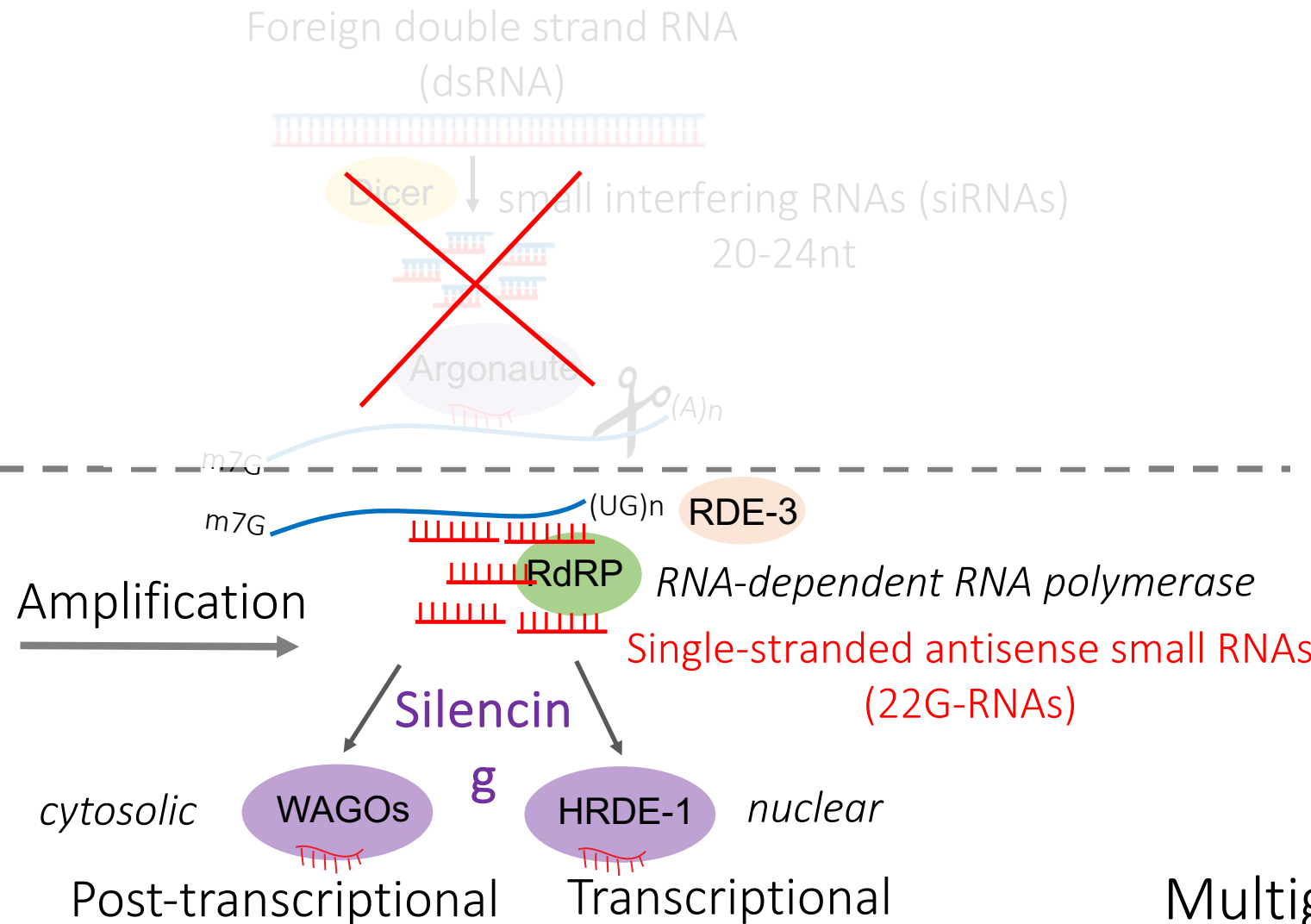
No dsRNA

G_n

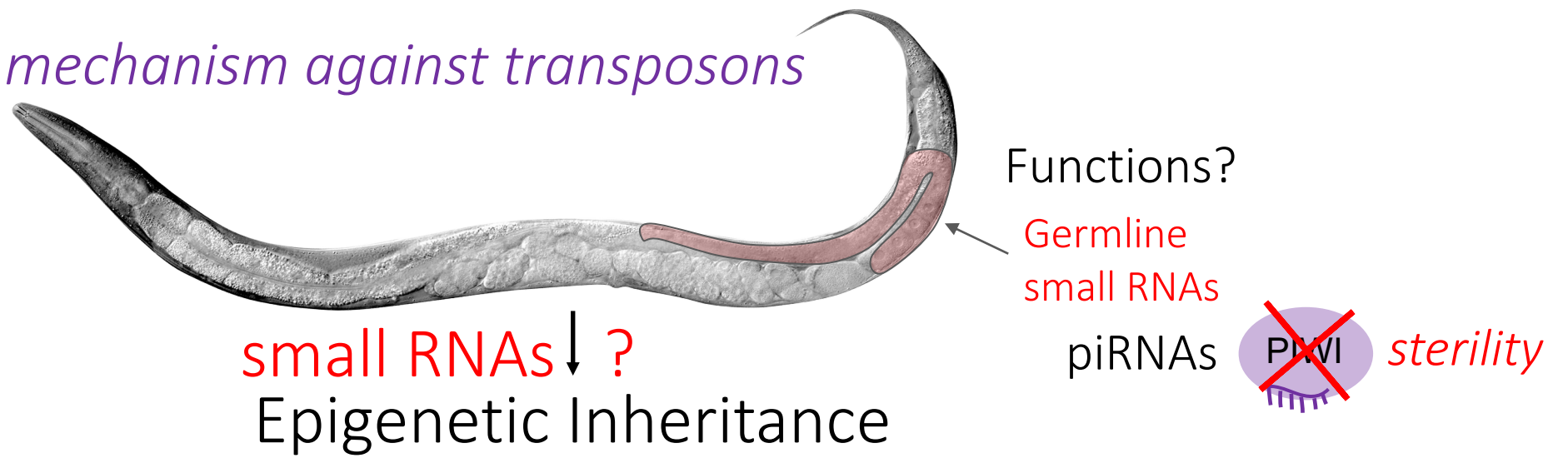
Multigenerational silencing

How is RNAi silencing transmitted across generations?

RNA interference (RNAi)



Genome defense mechanism against transposons



Ancestral small RNA-based immune system to protect the genome against nucleic acid pathogens

Genome defense mechanism against transposons



Functions?

Germline
small RNAs

piRNAs

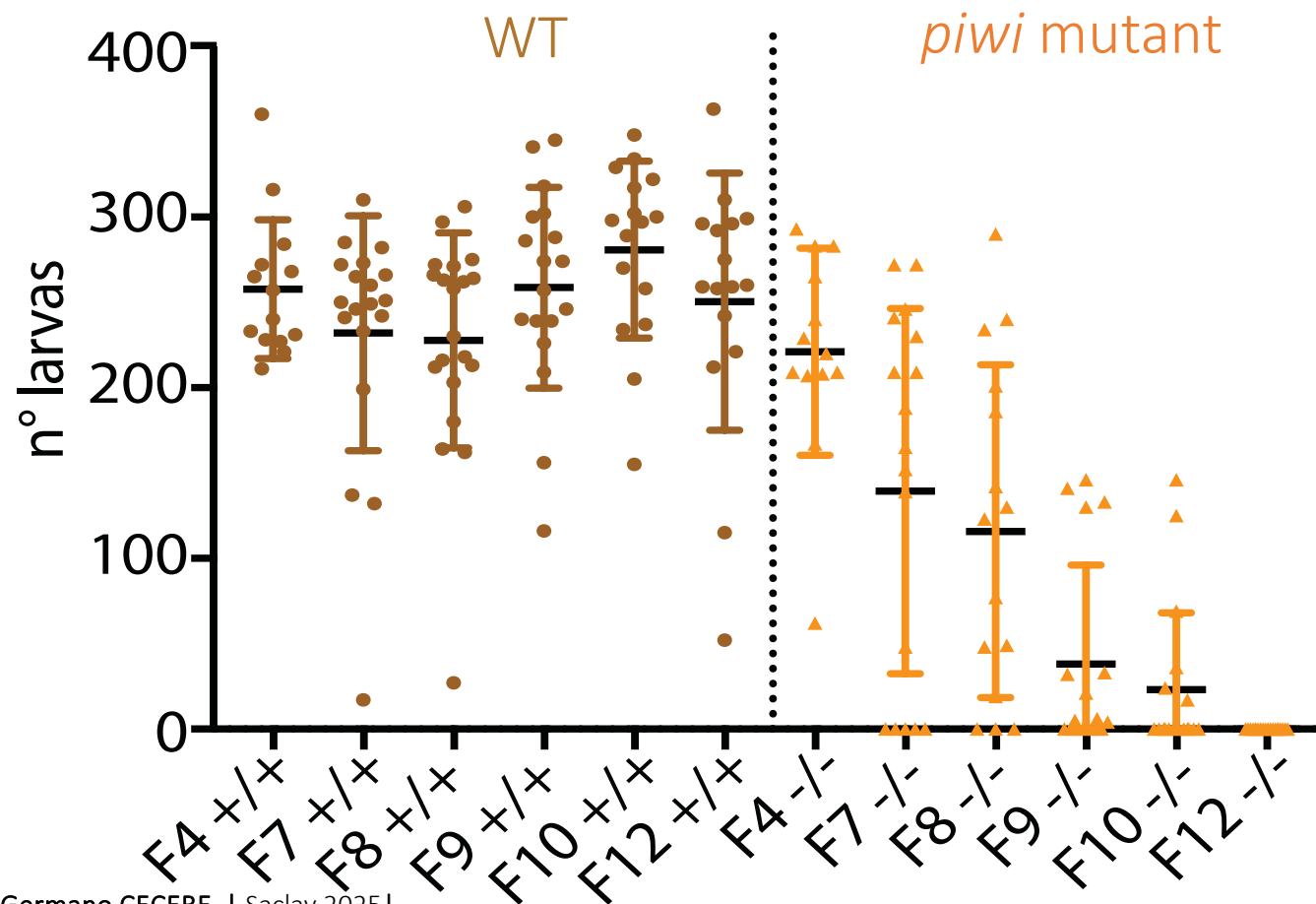


sterility



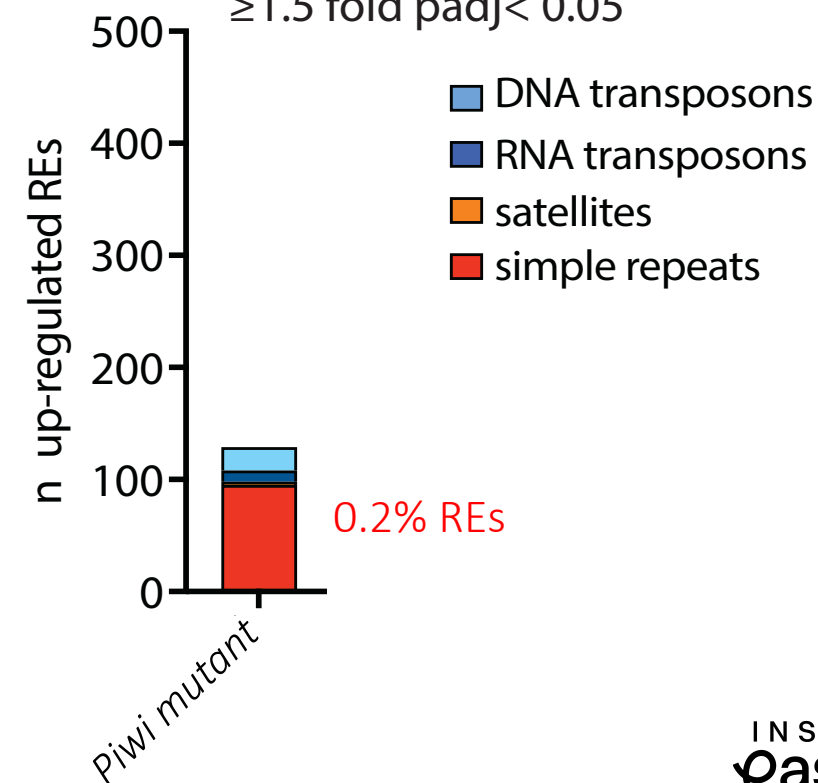
Giorgia Barucci
PhD Student

Peculiar phenotype: transgenerational loss of fertility



> 60,000 individual REs

≥1.5 fold padj < 0.05



Genome defense mechanism against transposons



Functions?

Germline
small RNAs

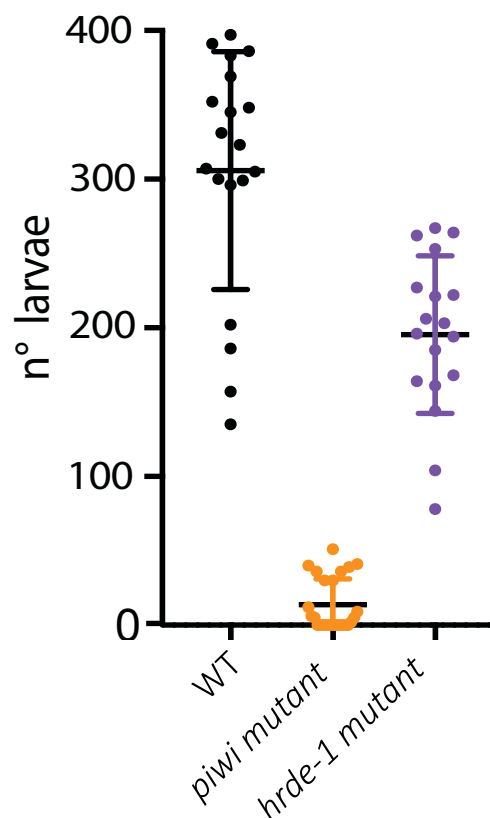
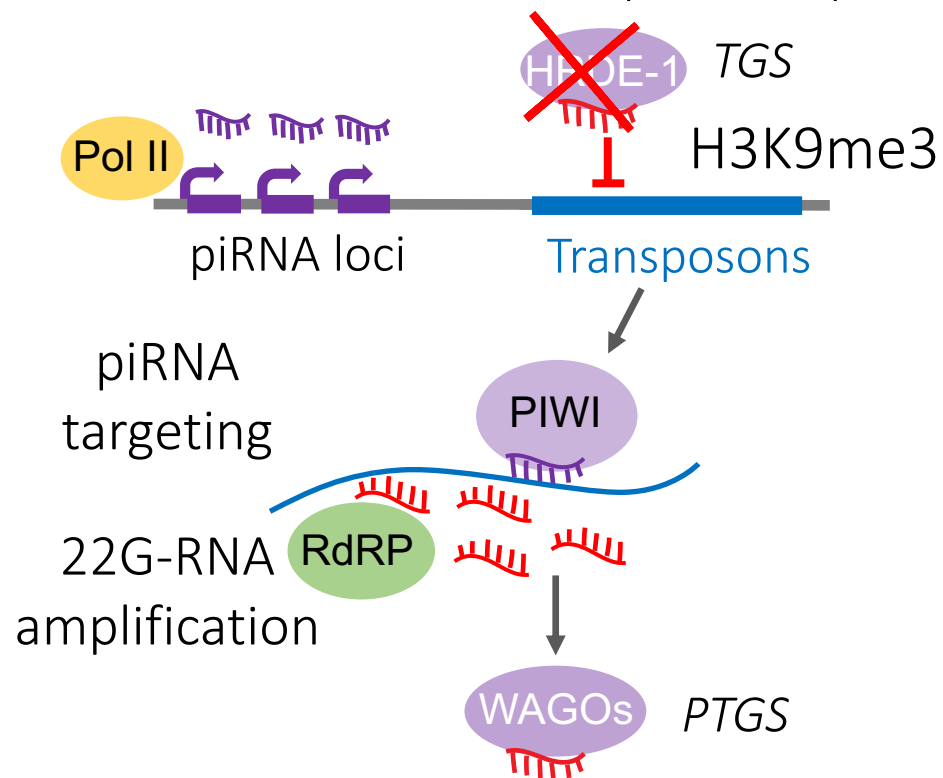


Giorgia Barucci
PhD Student

piRNAs ~~PIWI~~ sterility

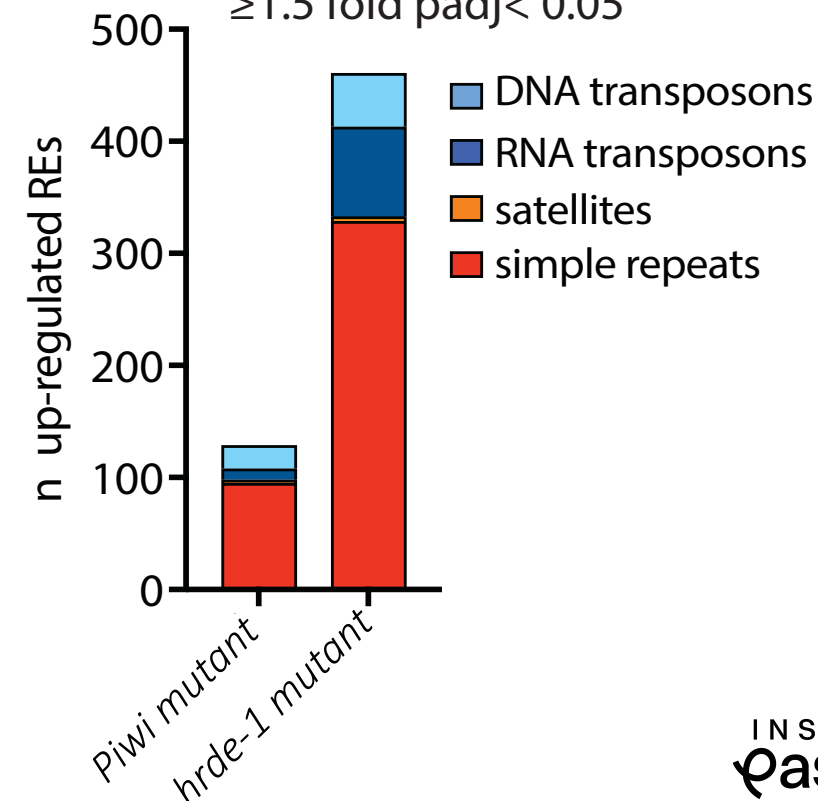
piRNAs are required to initiate the silencing of REs

More than 15,000 individual piRNA sequences



> 60,000 individual REs

≥1.5 fold padj < 0.05



(Cecere, Claycomb, Ketting, Mello, Miska, Sarkies, and other labs)

Genome defence mechanism against transposons



Functions?

Germline
small RNAs

piRNAs

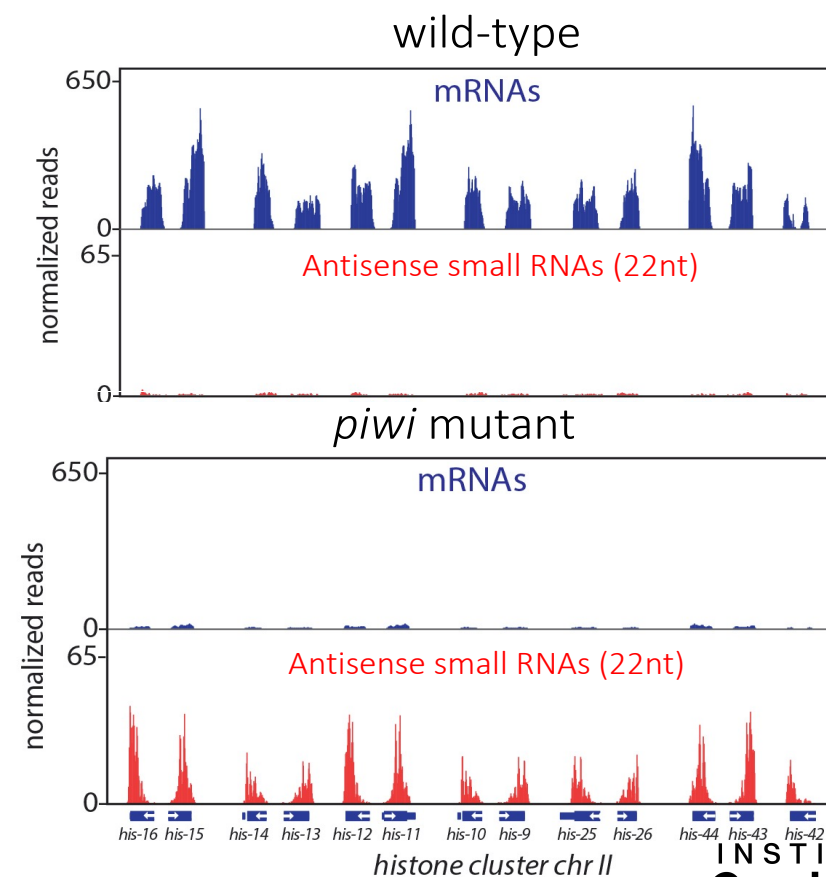
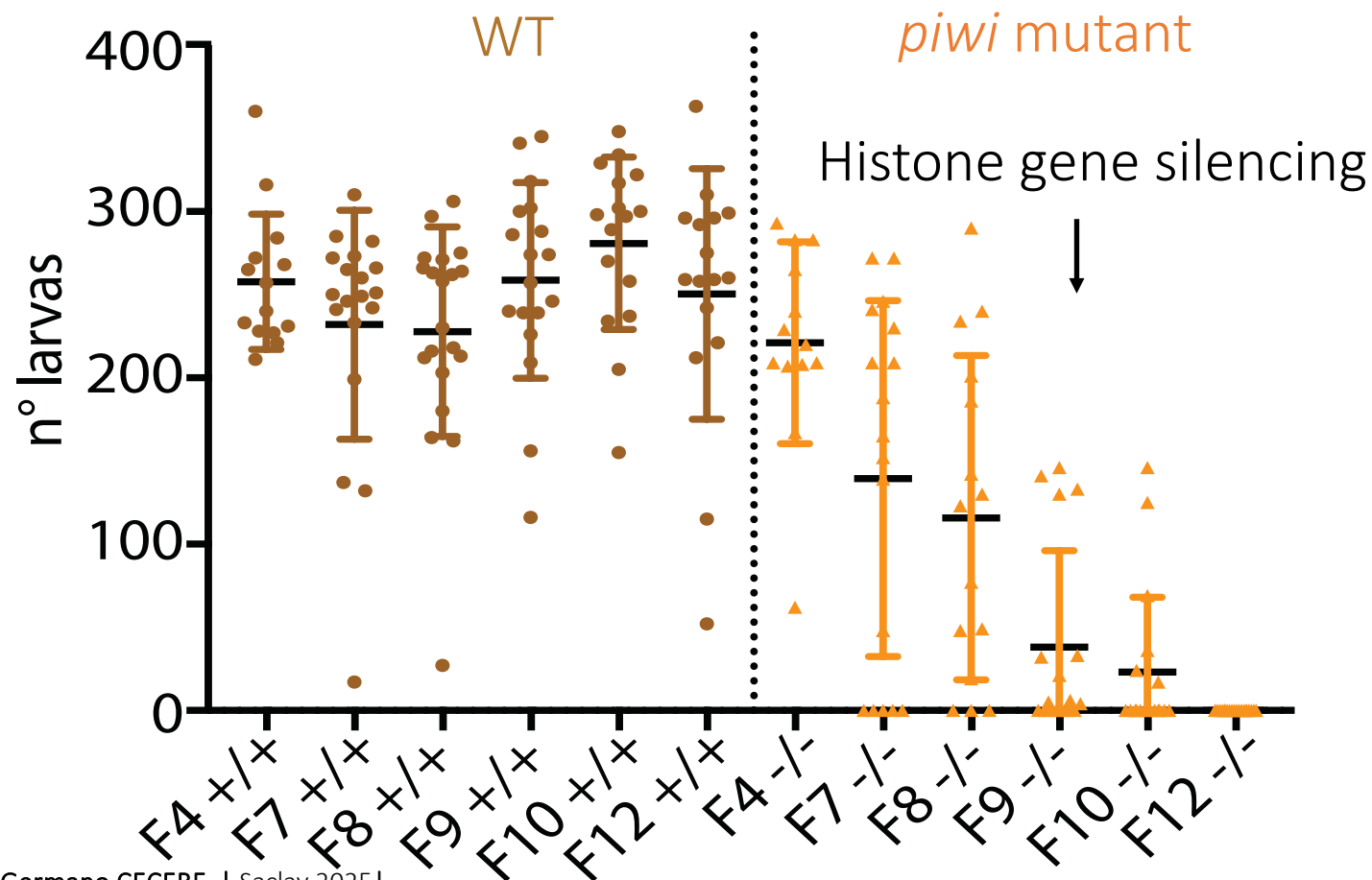


sterility



Giorgia Barucci
PhD Student

Peculiar phenotype: transgenerational loss of fertility



How are histone mRNAs silenced?



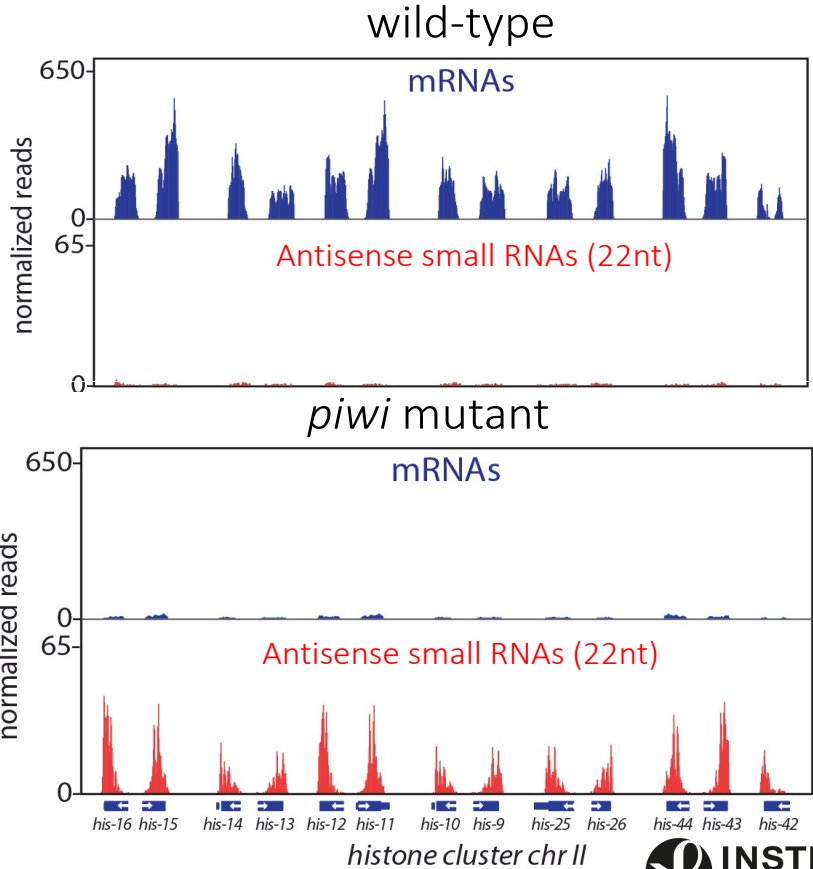
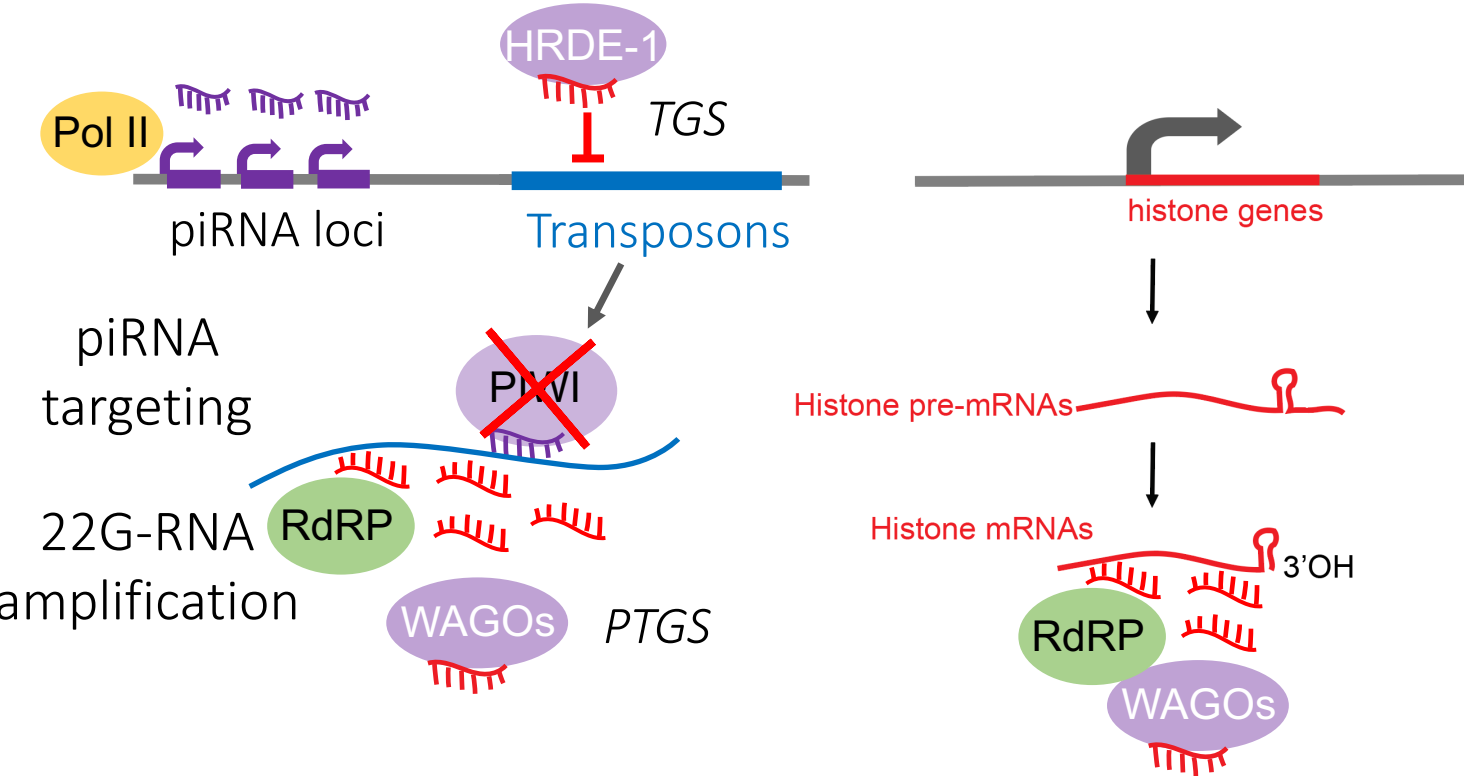
Giorgia Barucci
PhD Student

Functions?

Germline
small RNAs

piRNAs ~~PIWI~~ *sterility*

More than 15,000 individual piRNA sequences



Downstream components of the piRNA pathway relocate on histone mRNAs

Can we restore the expression of histone mRNAs in *piwi* mutant?



Functions?

Germline
small RNAs



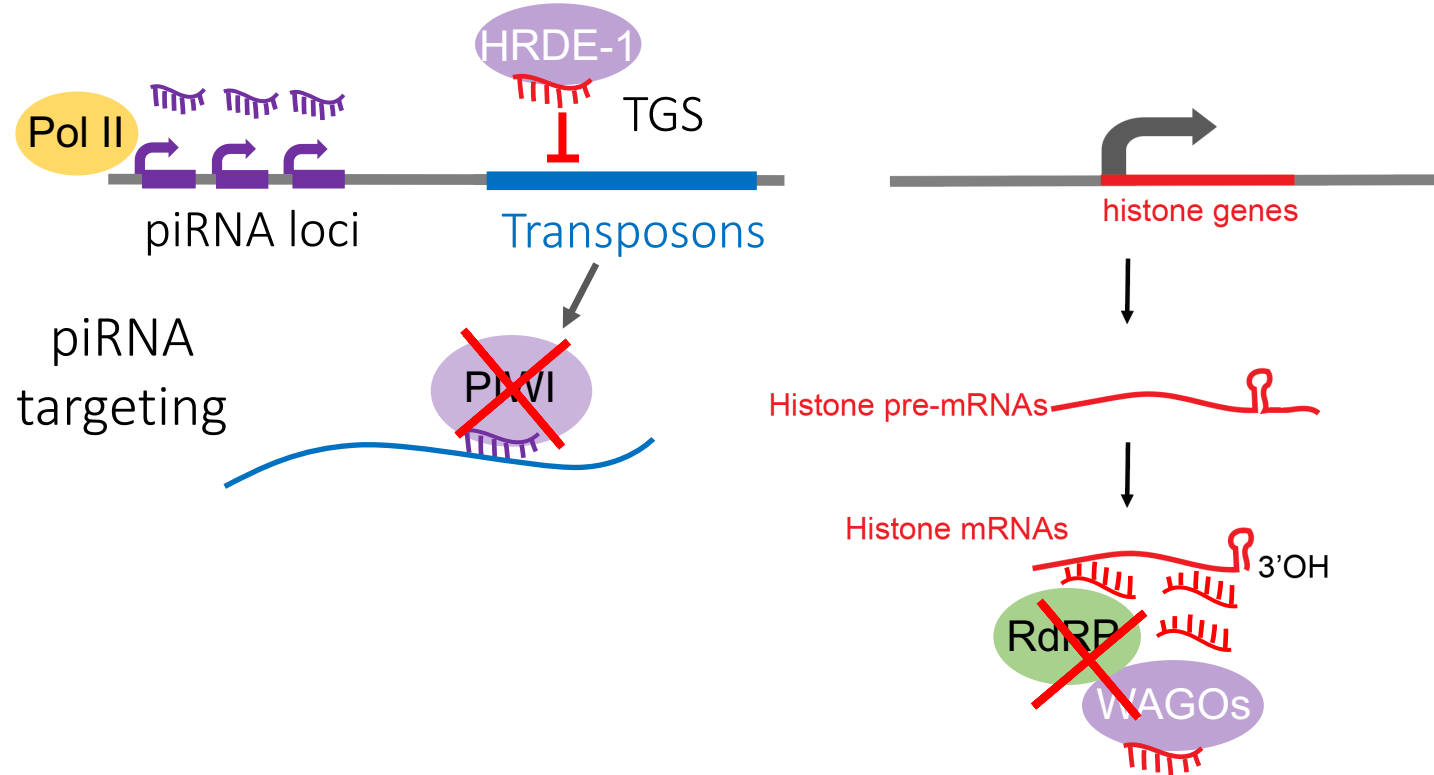
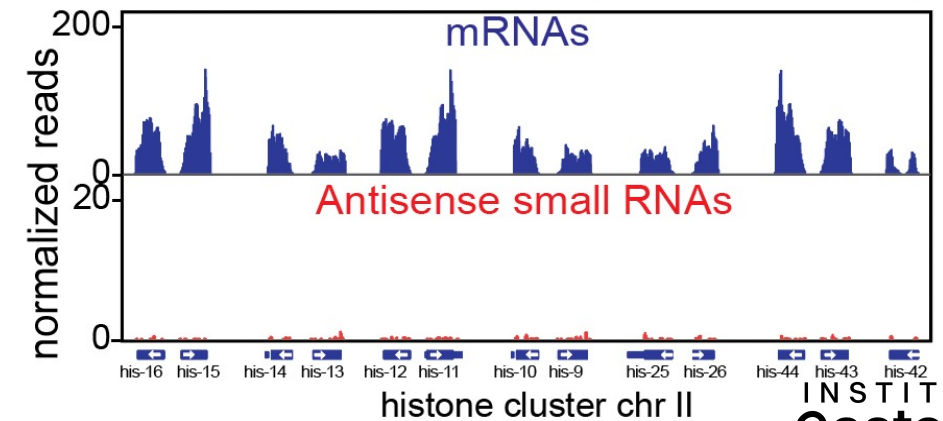
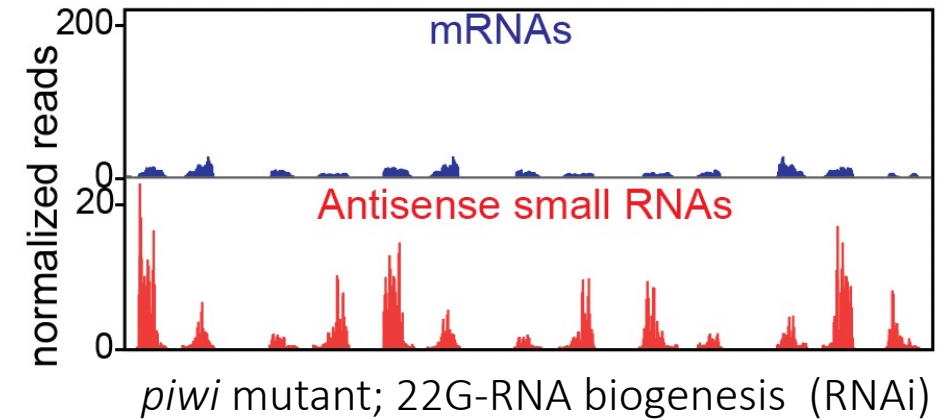
Giorgia Barucci
PhD Student

piRNAs



sterility

piwi mutant



Does the removal of histone 22G-RNAs restore the fertility of *piwi* mutant?

Inheritance of small RNAs antisense to histones



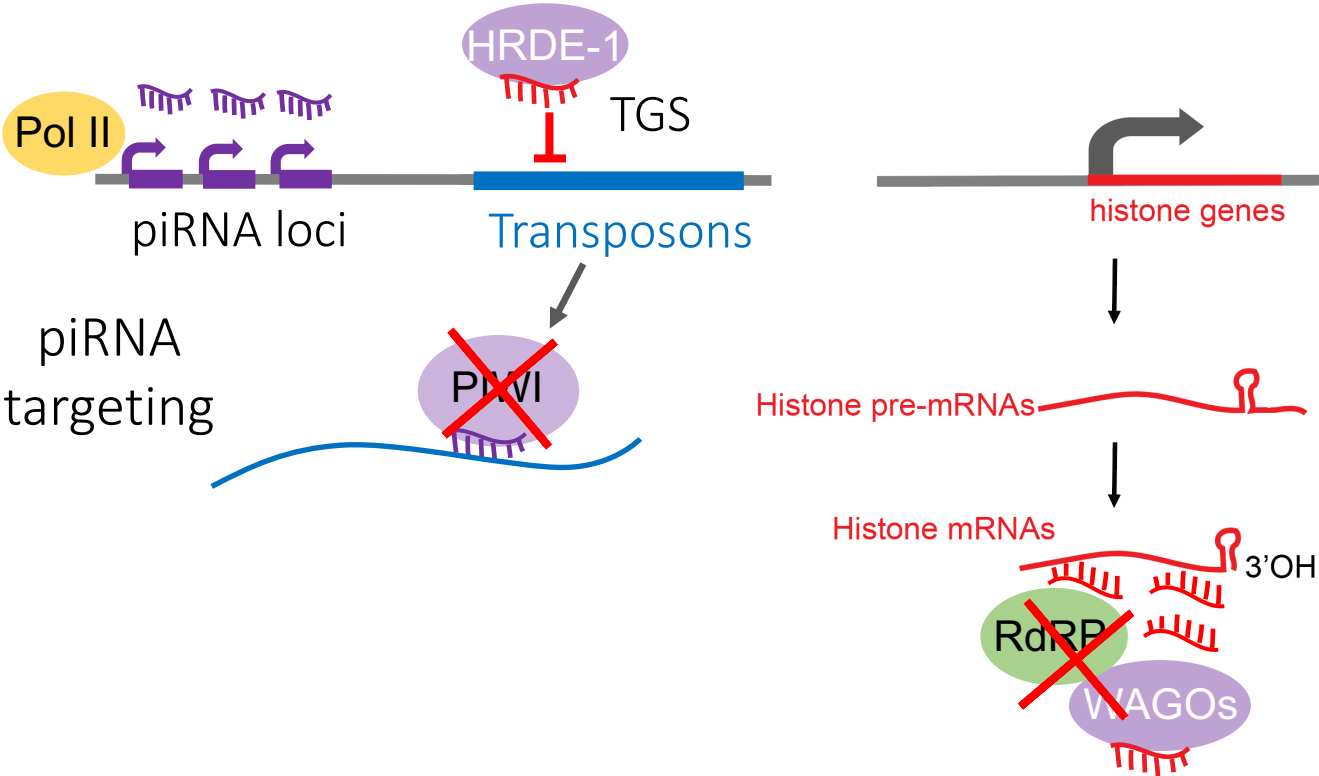
Giorgia Barucci
PhD Student

Functions?

Germline
small RNAs

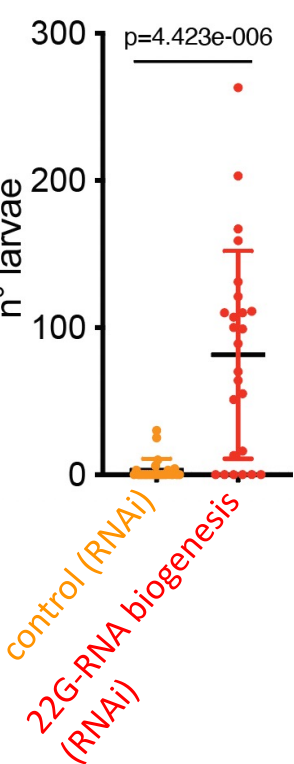
piRNAs ~~PIWI~~ *sterility*

More than 15,000 individual piRNA sequences



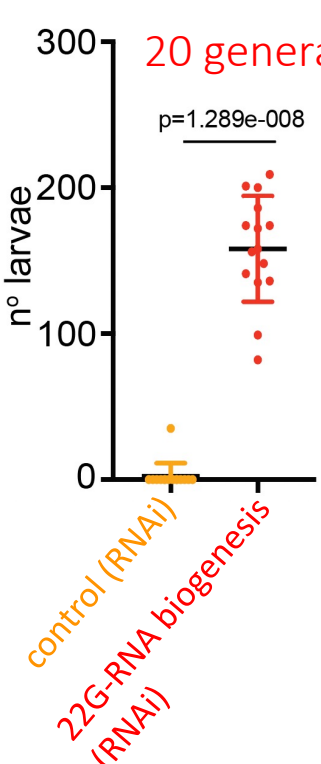
Restore fertility

piwi mutant F12



Prevent sterility

piwi mutant F1



Does the removal of histone 22G-RNAs restore the fertility of *piwi* mutant?

Genetic + Epigenetic = Sterility



Germline
small RNAs

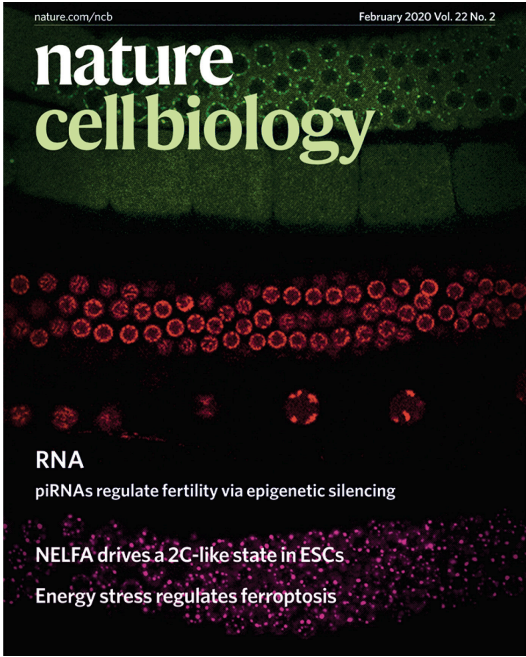


Giorgia Barucci
PhD Student

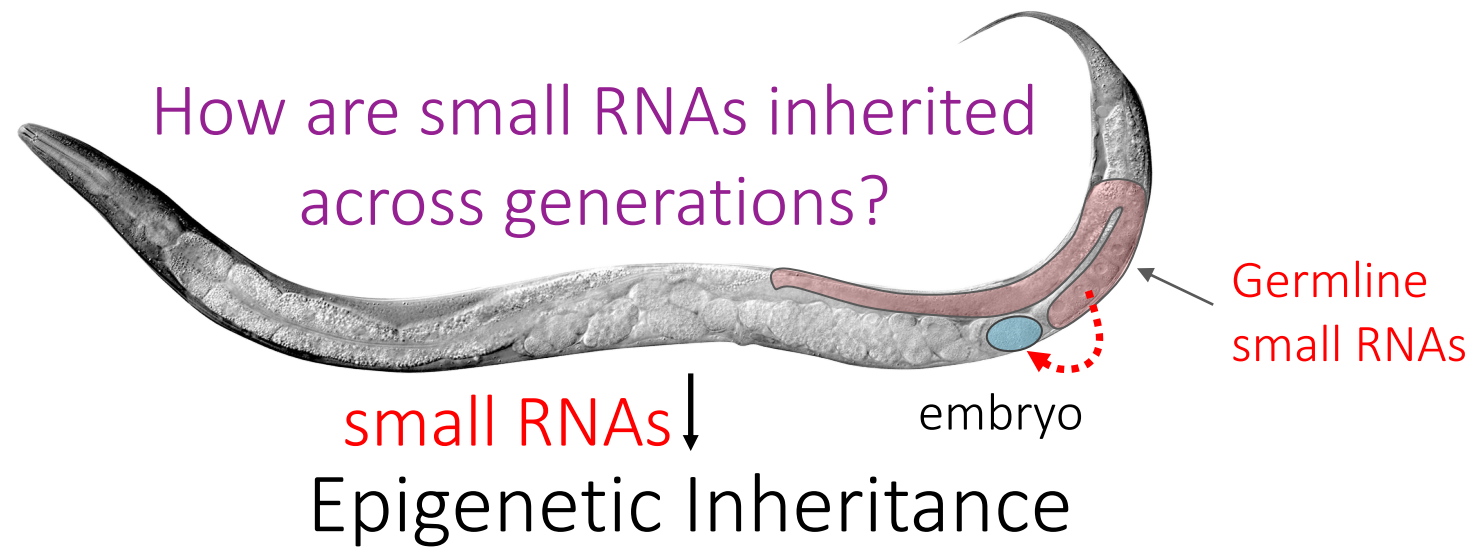
small RNAs ↓
Epigenetic Inheritance

piRNAs ~~PWI~~ sterility

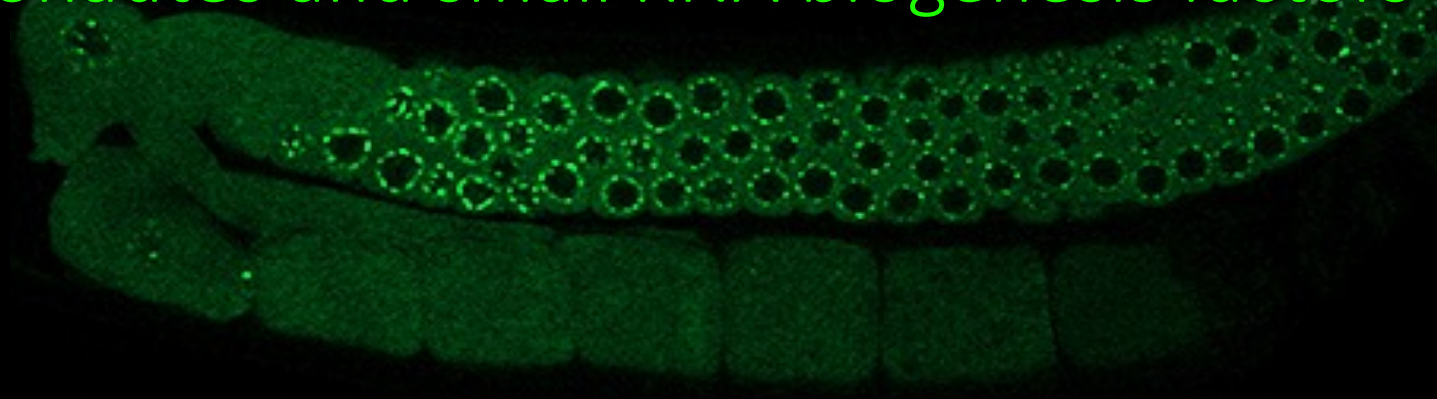
small RNAs can transmit heritable
phenotypes across generations



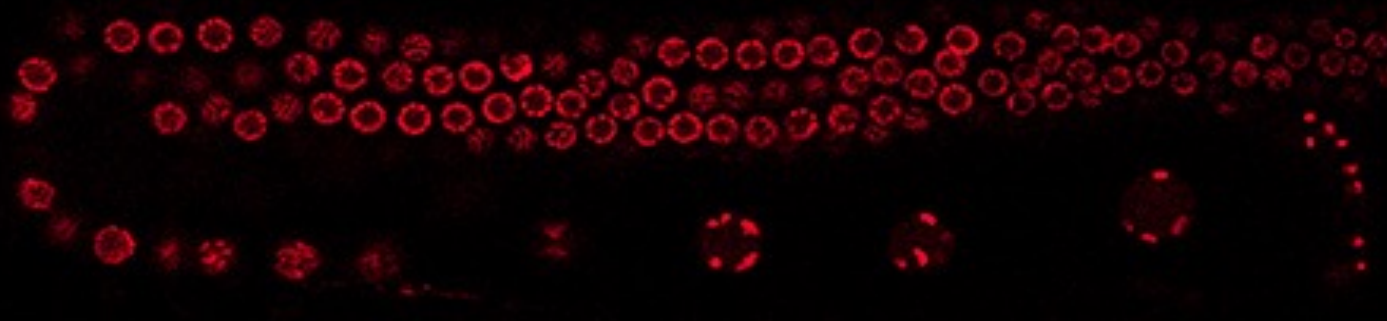
Barucci et al., Nat Cell Biol 2020



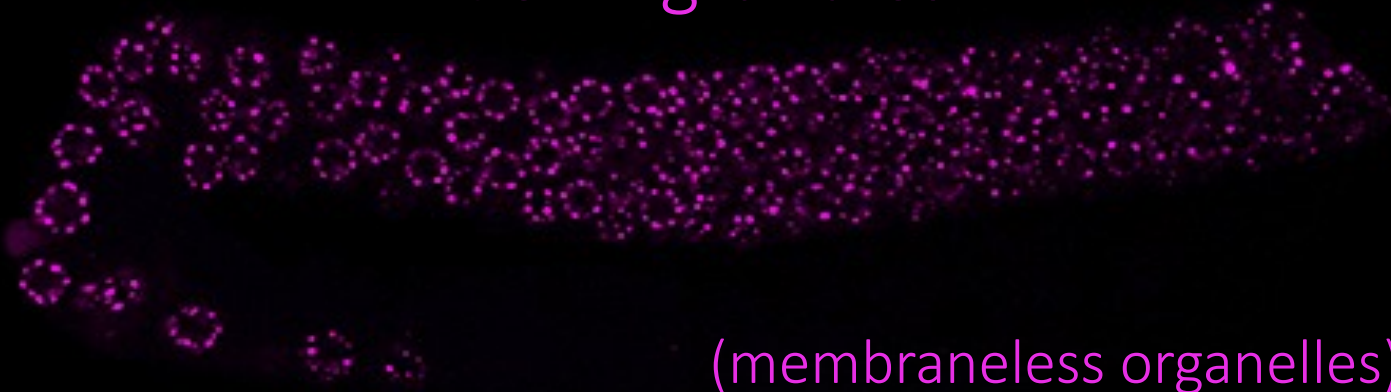
Argonautes and small RNA biogenesis factors (RdRPs)



DNA

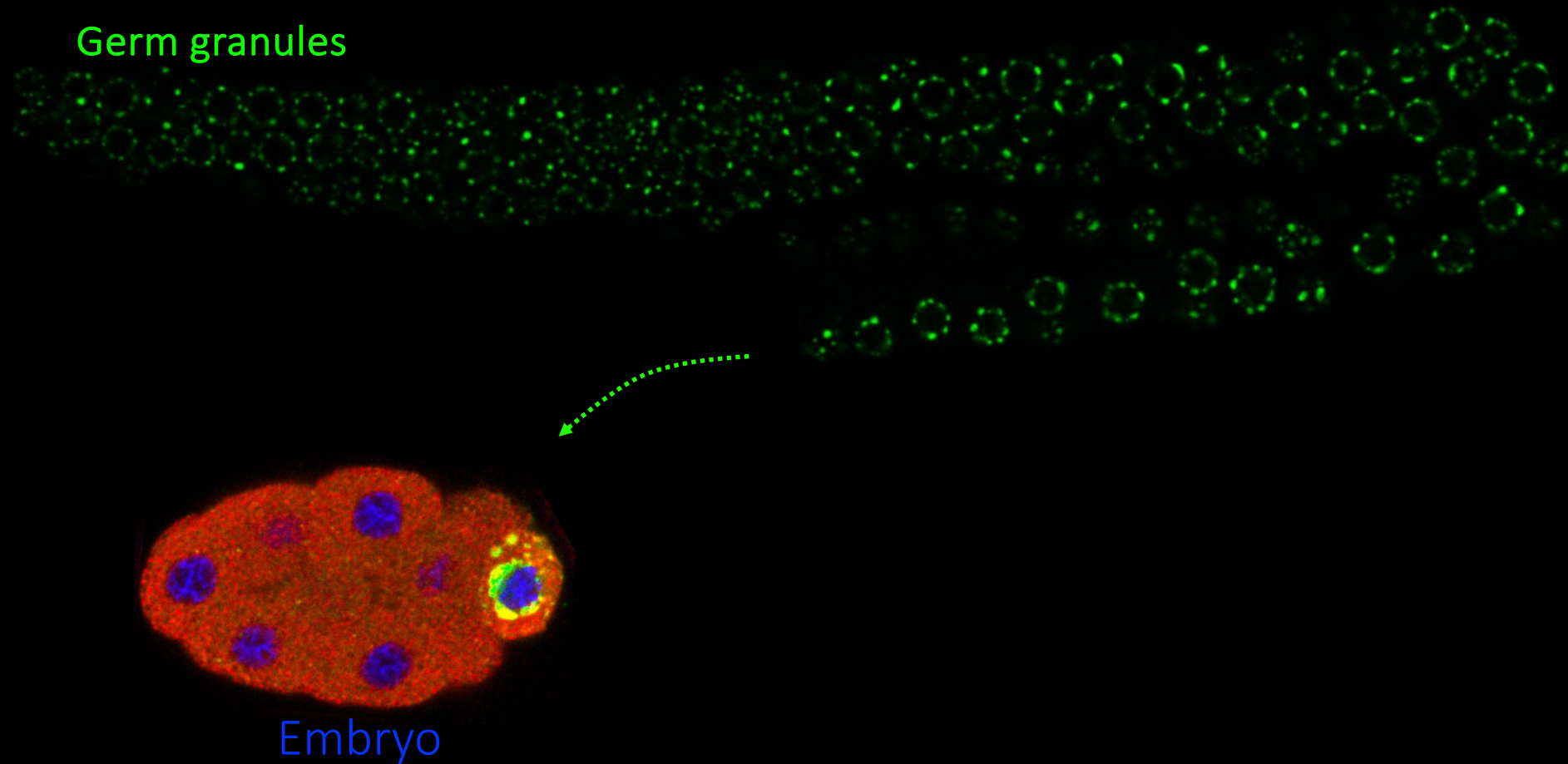


Germ granules



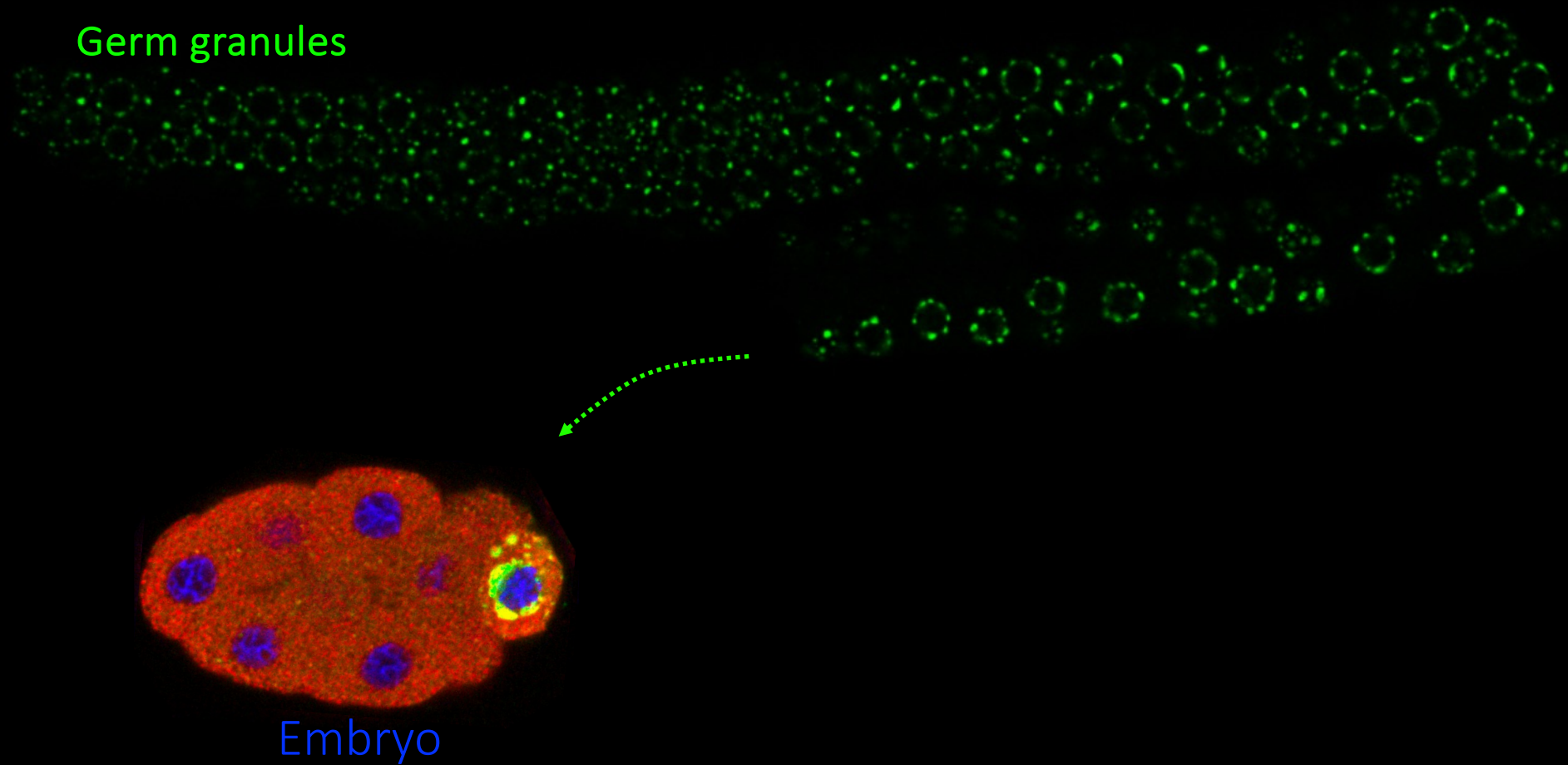
(membraneless organelles)

Germ granules are maternally inherited



Germ granules are essential for
small RNA inheritance in embryos

Germ granules are maternally inherited



Germ granules might protect small RNAs and mRNA templates from other biochemical activities in embryos

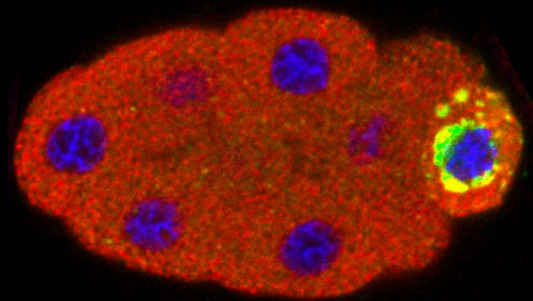
Maternal mRNAs degradation in somatic cells

Germ granules

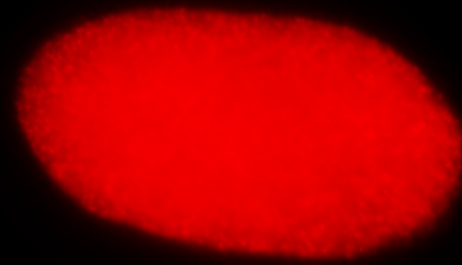
1. Not engaged in translation
2. Not newly transcribed
3. The removal of germ granules impairs small RNA accumulation

RdRP-dependent *de novo*
small RNA synthesis

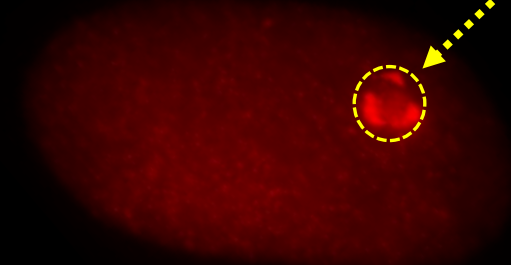
maternal RNAs are not
degraded in granules
maternal mRNA (smFISH)



Embryo



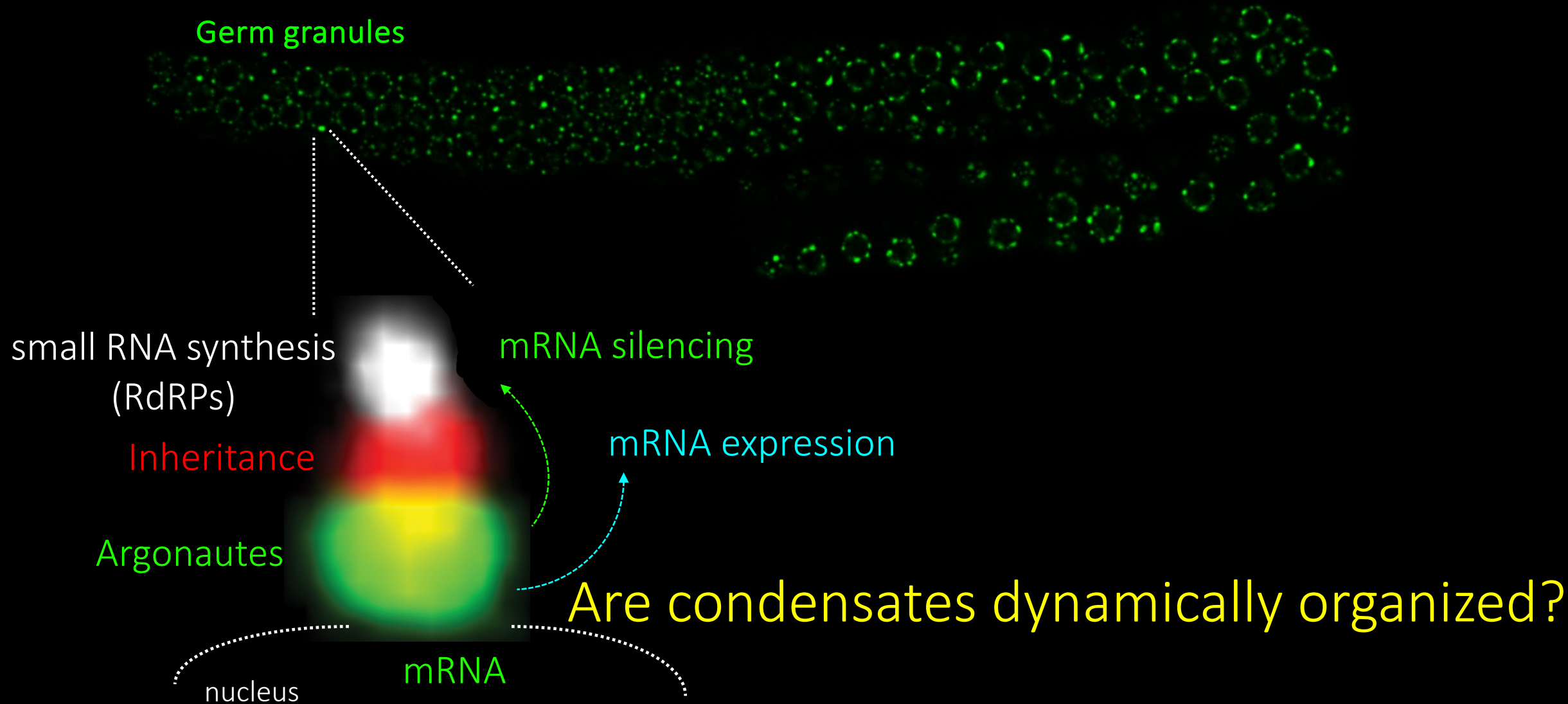
1-cell



>100-cells

Germ granules might protect small RNAs and mRNA templates from other biochemical activities in embryos

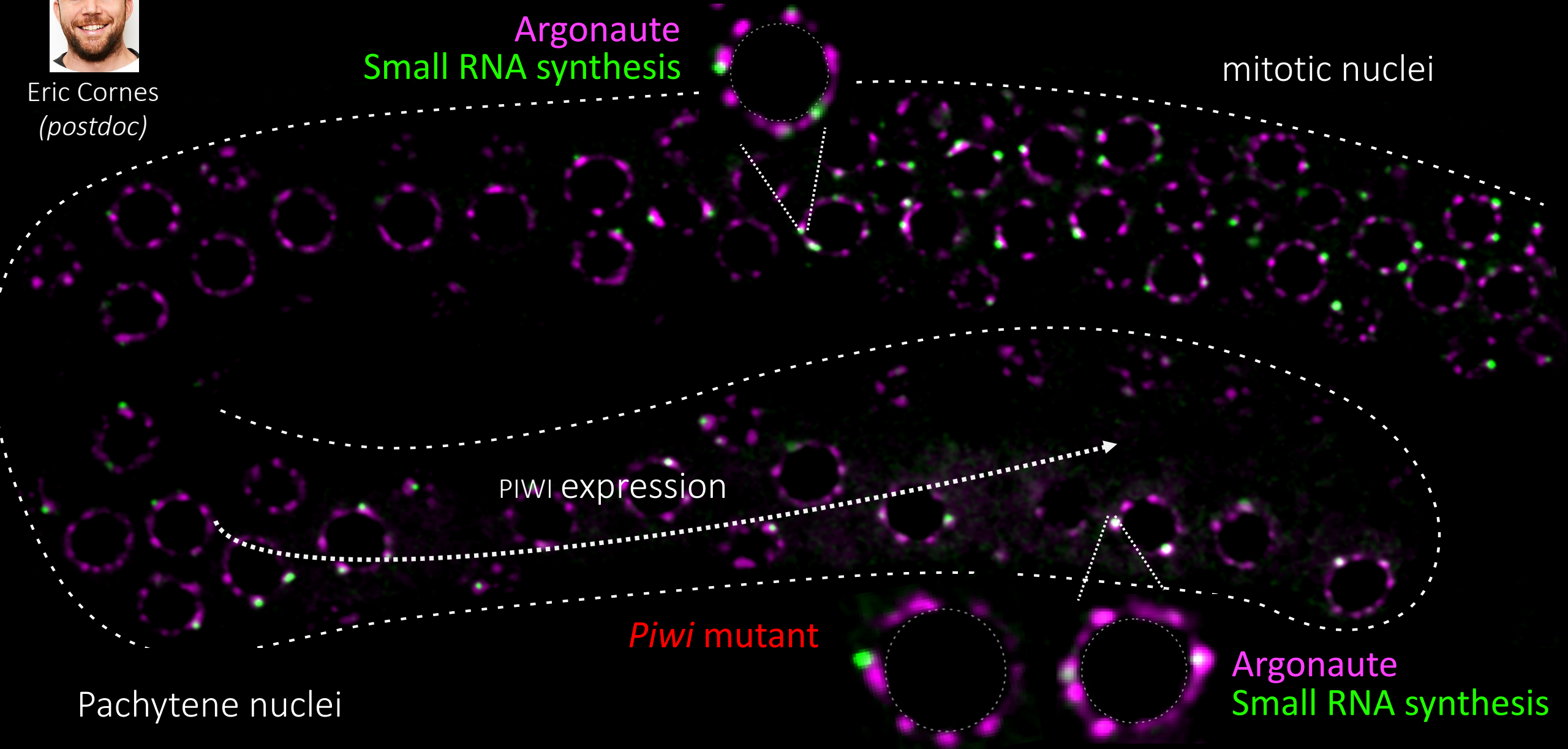
Small RNA processes are highly organized in germ granules





Eric Cornes
(postdoc)

Dynamic organization of germ granule compartments





Spermatogenic transcription during gametogenesis

Eric Cornes
(postdoc)

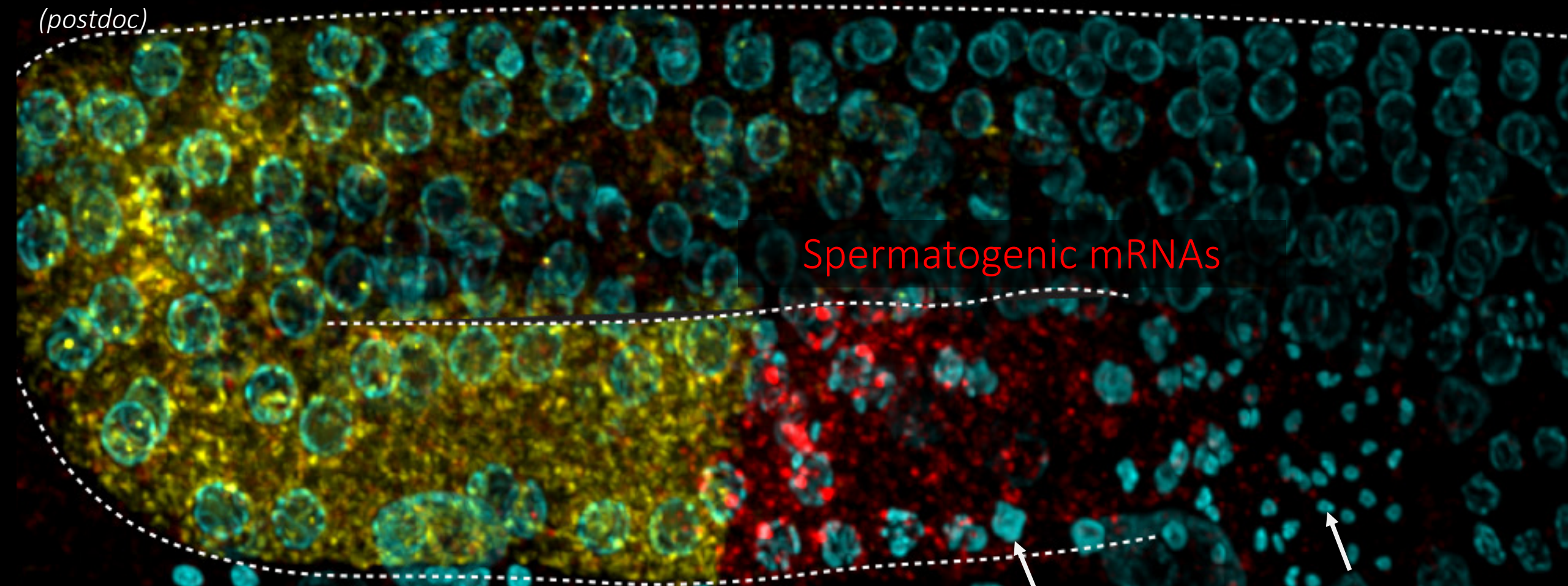
Oogenic mRNAs

Spermatogenic mRNAs

Wild-type

spermatocytes

mature sperm





Spermatogenic transcription during gametogenesis

Eric Cornes
(postdoc)

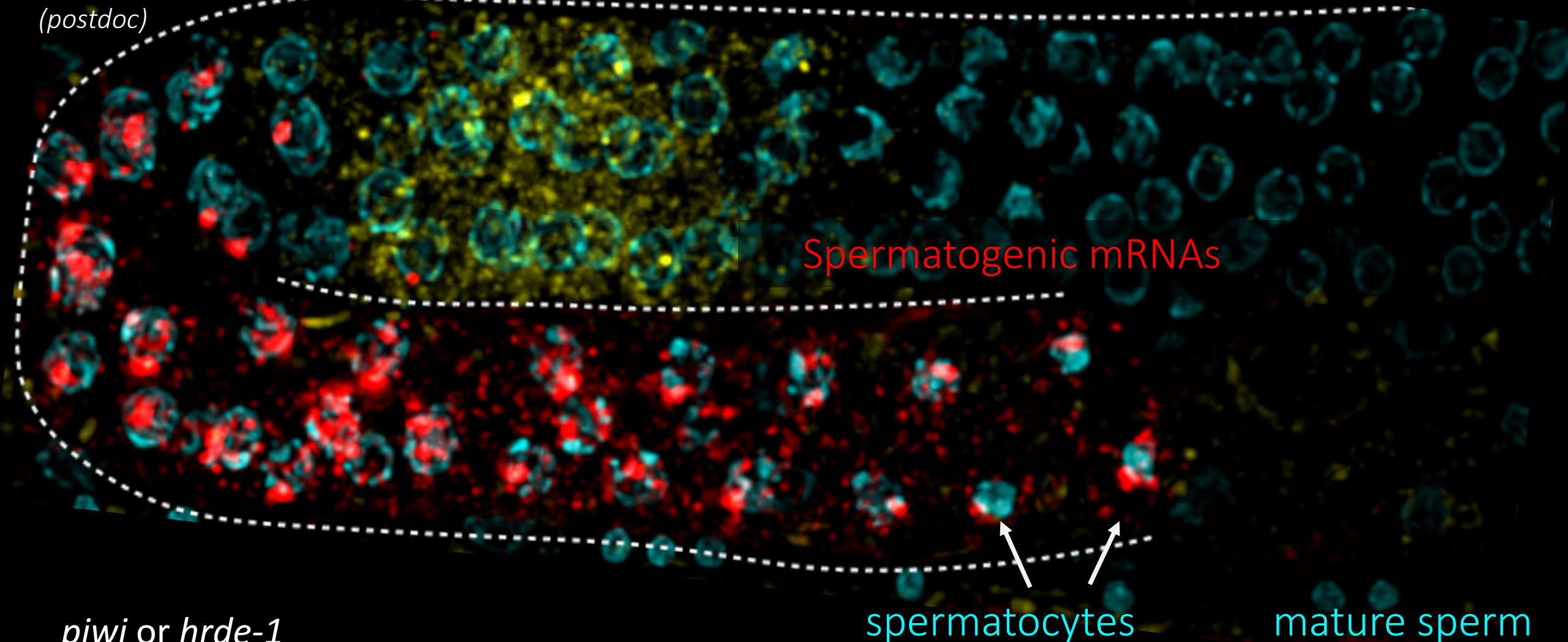
Oogenic mRNAs

Spermatogenic mRNAs

piwi or *hrde-1*

spermatocytes

mature sperm

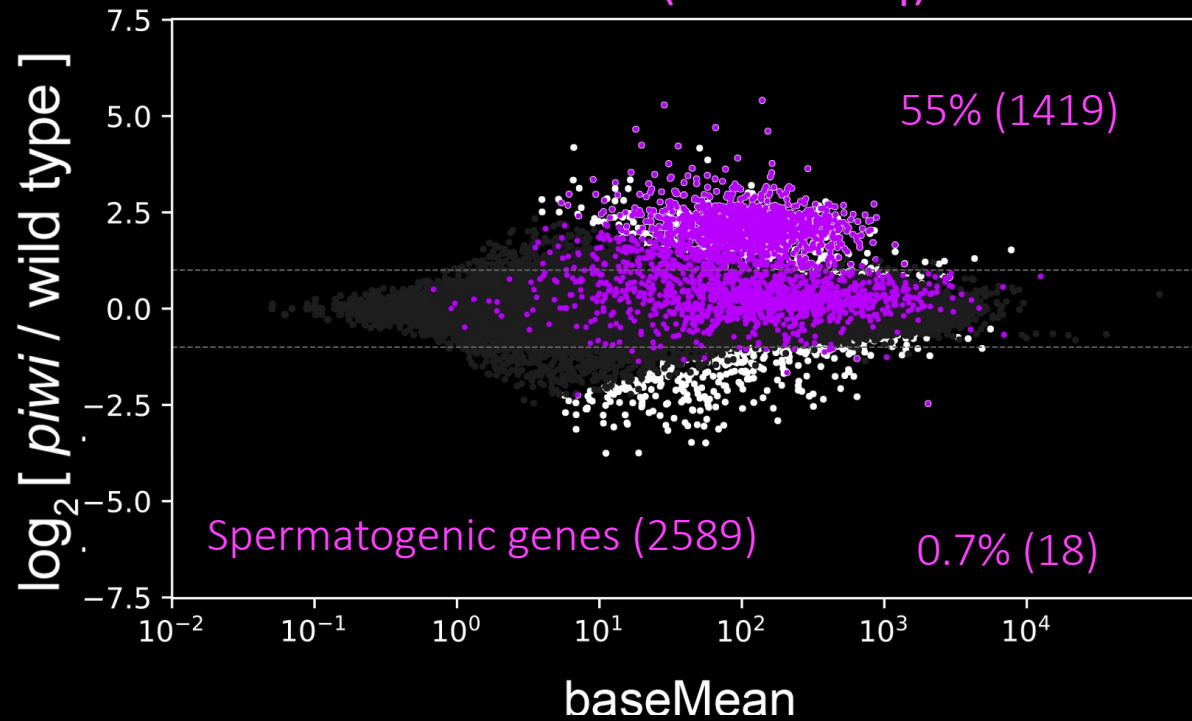




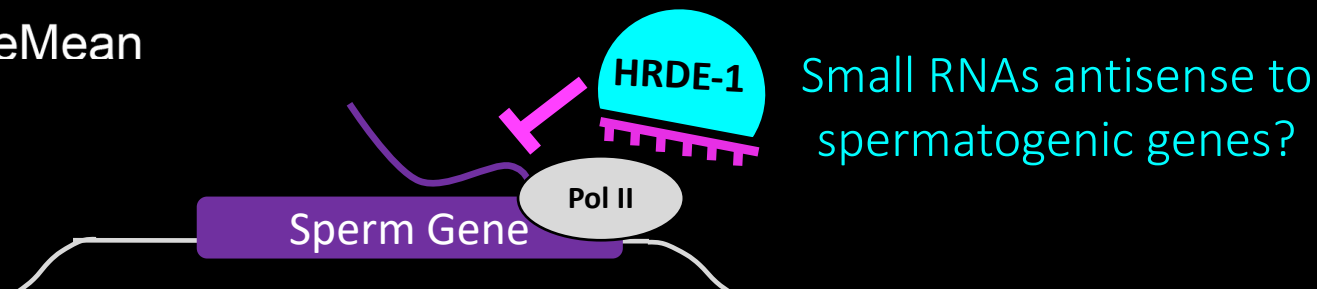
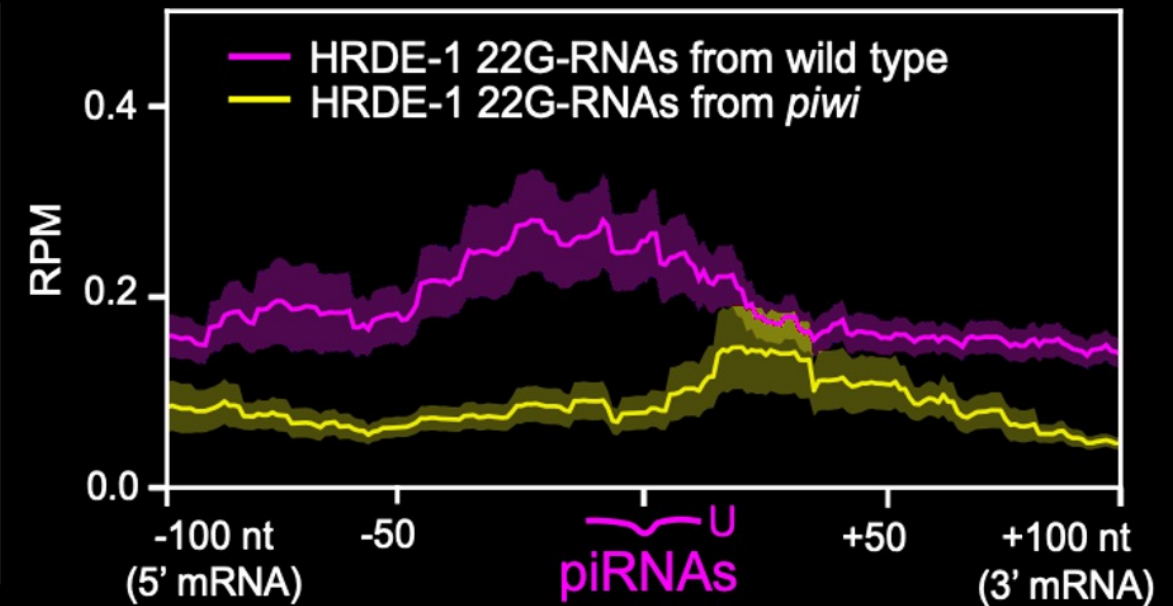
Do piRNAs directly silence spermatogenic transcription?

Eric Cornes
(postdoc)

Nascent transcription in *piwi* or *hrde-1* mutants (GRO-seq)



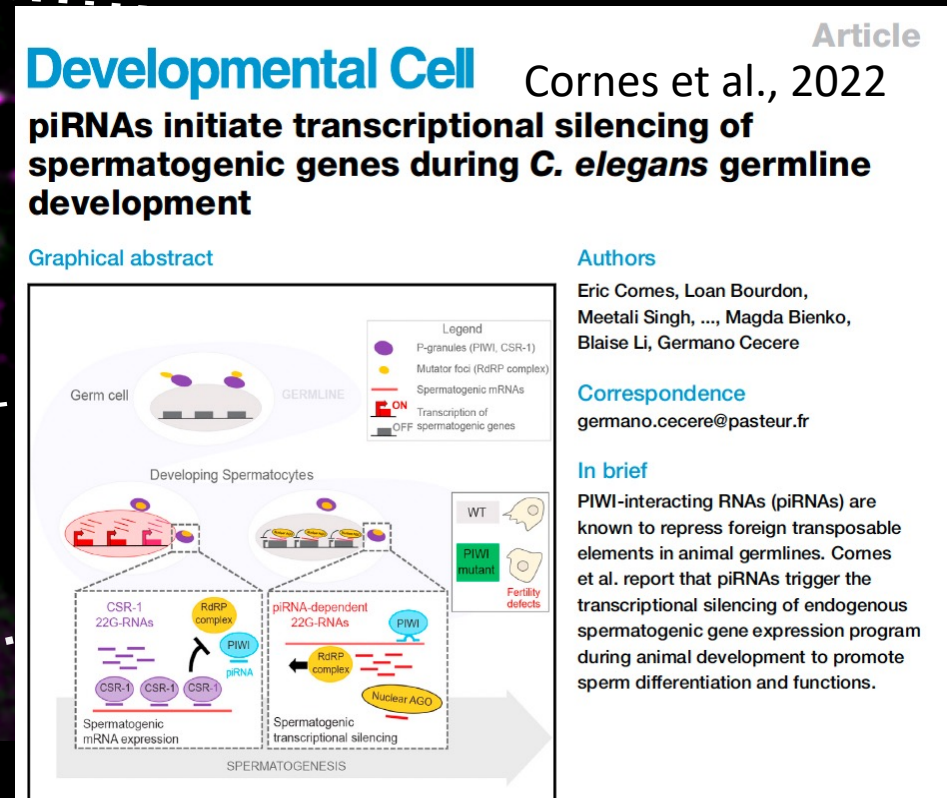
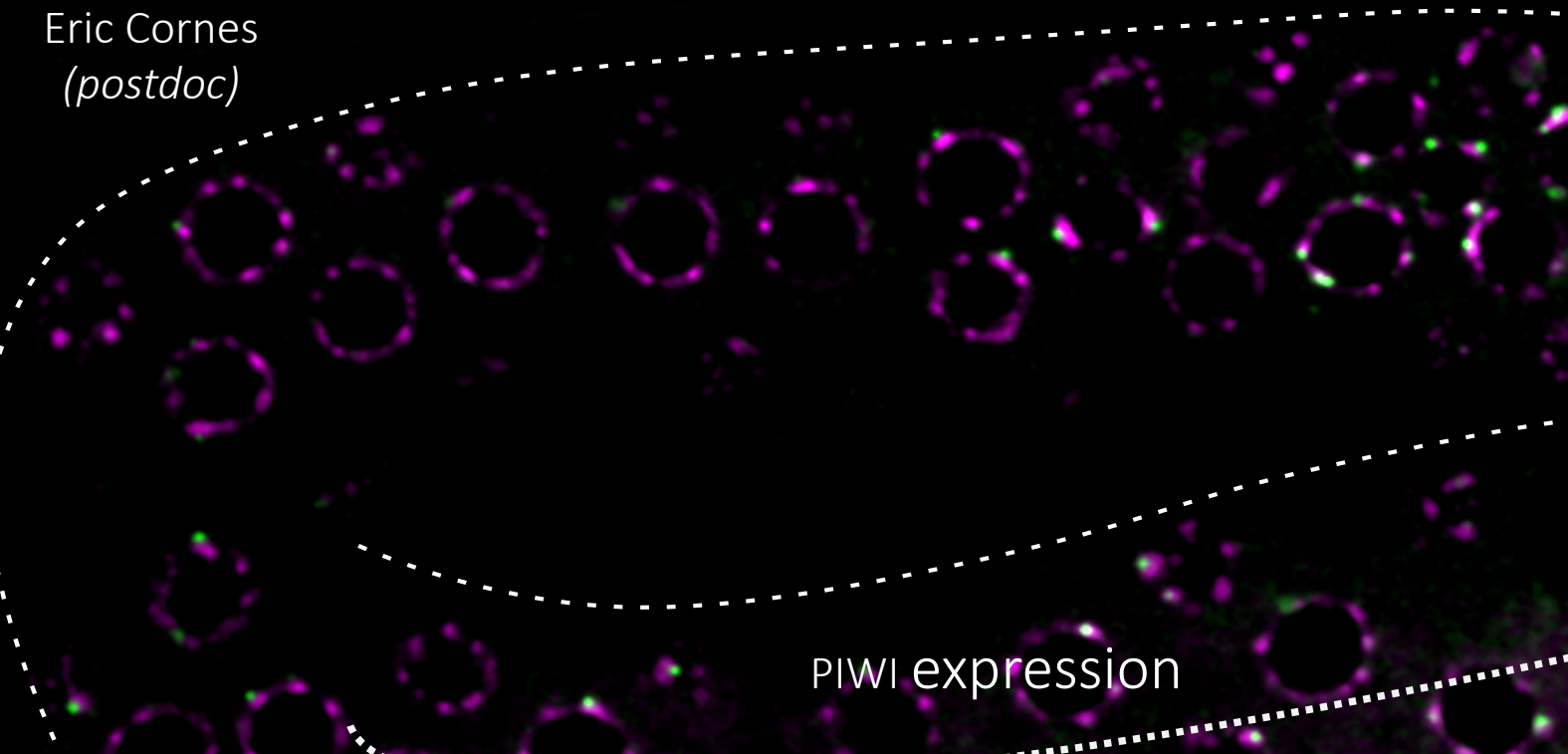
22G-RNAs antisense to spermatogenic genes





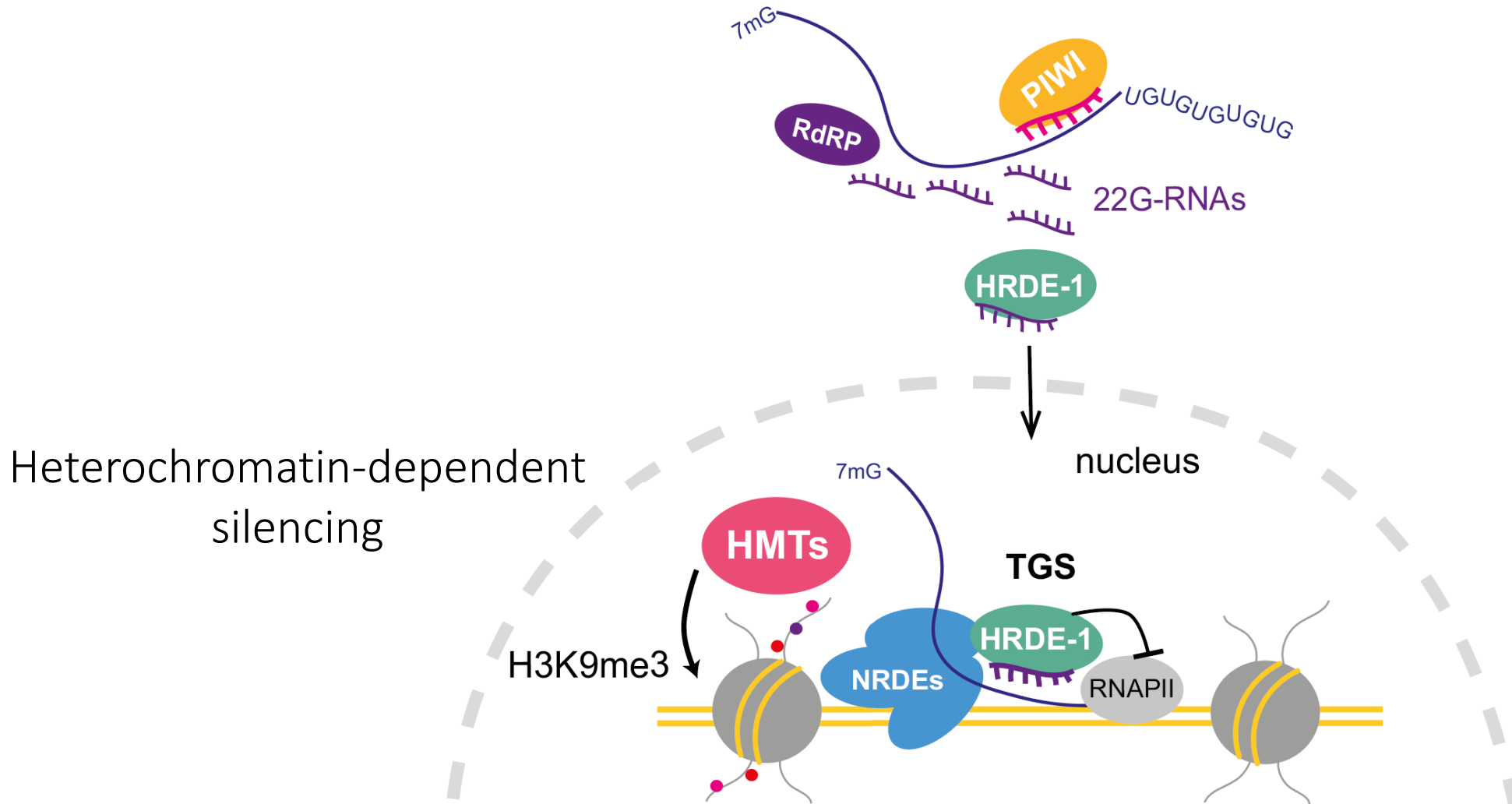
Eric Cornes
(postdoc)

Novel function of piRNAs in repressing spermatogenic transcription to allow sperm differentiation



Genome defense mechanisms can be co-opted to regulate essential developmental functions

How does nuclear Argonaute initiate and maintain nuclear silencing?





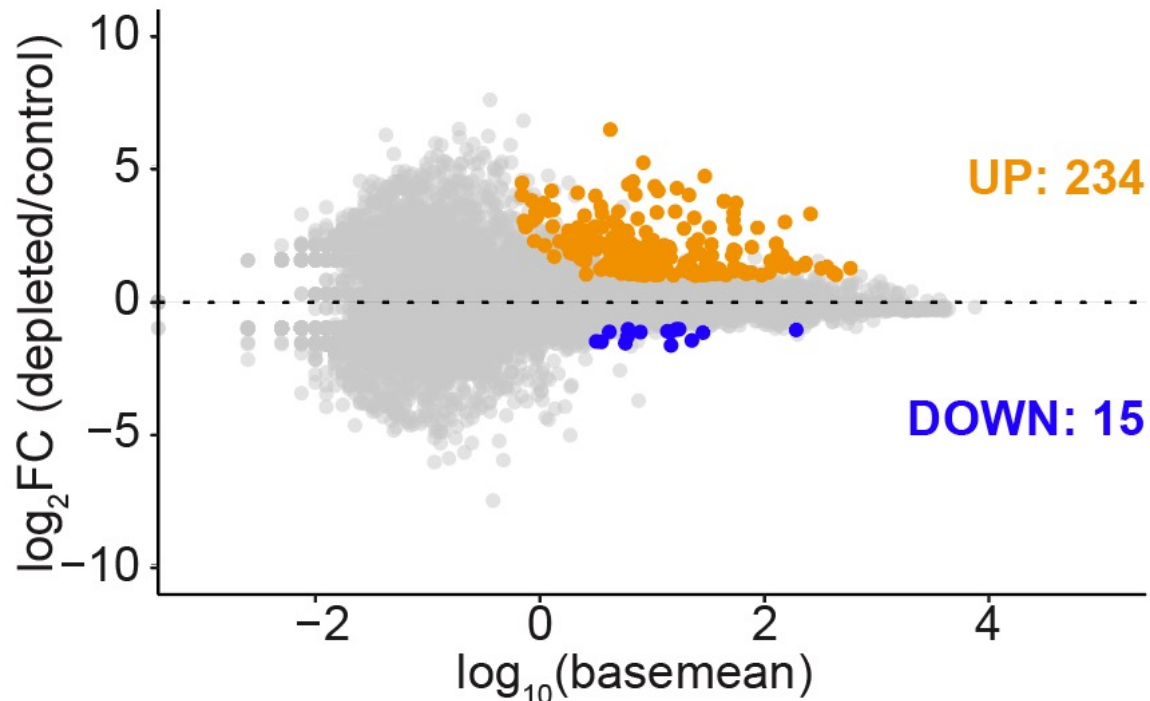
Arantxa Rojas
Postdoc

HRDE-1 mediates transcriptional silencing independent of heterochromatin

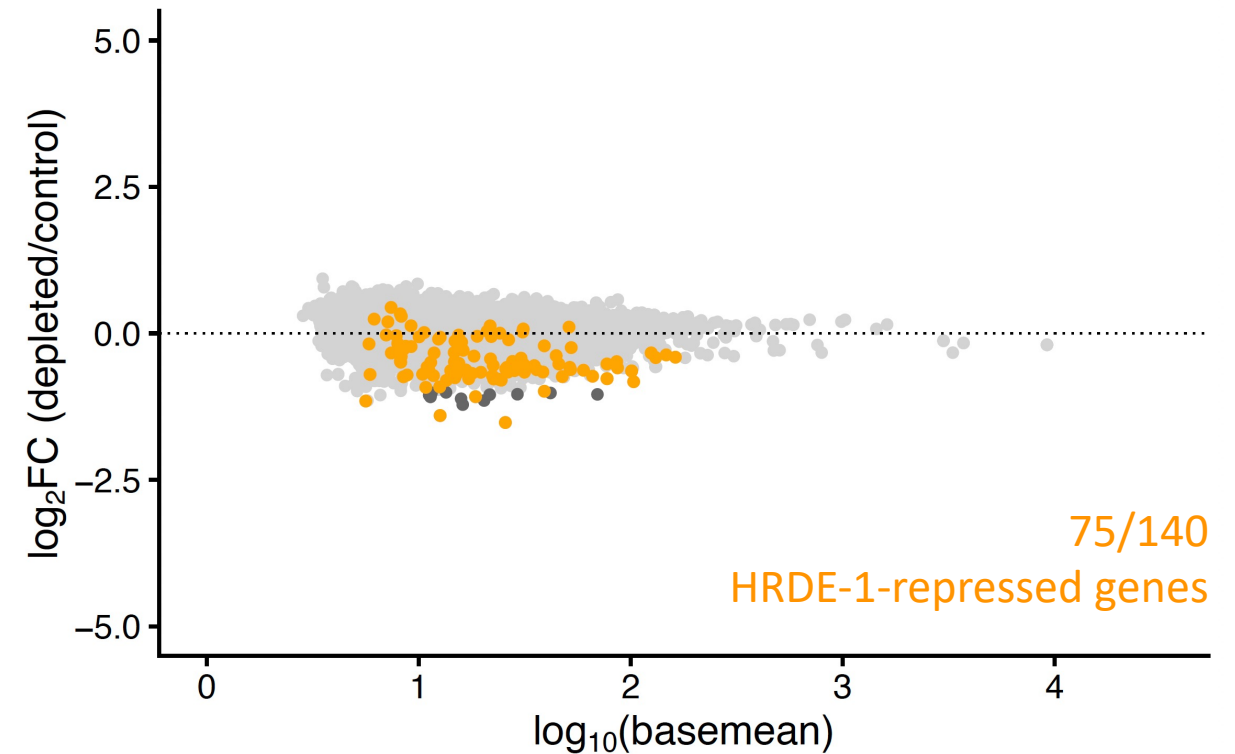


Rojas et al., Biorxiv (2025)

Depletion of HRDE-1 (G0) RNA-seq



G0 - H3K9me3 in absence of HRDE-1





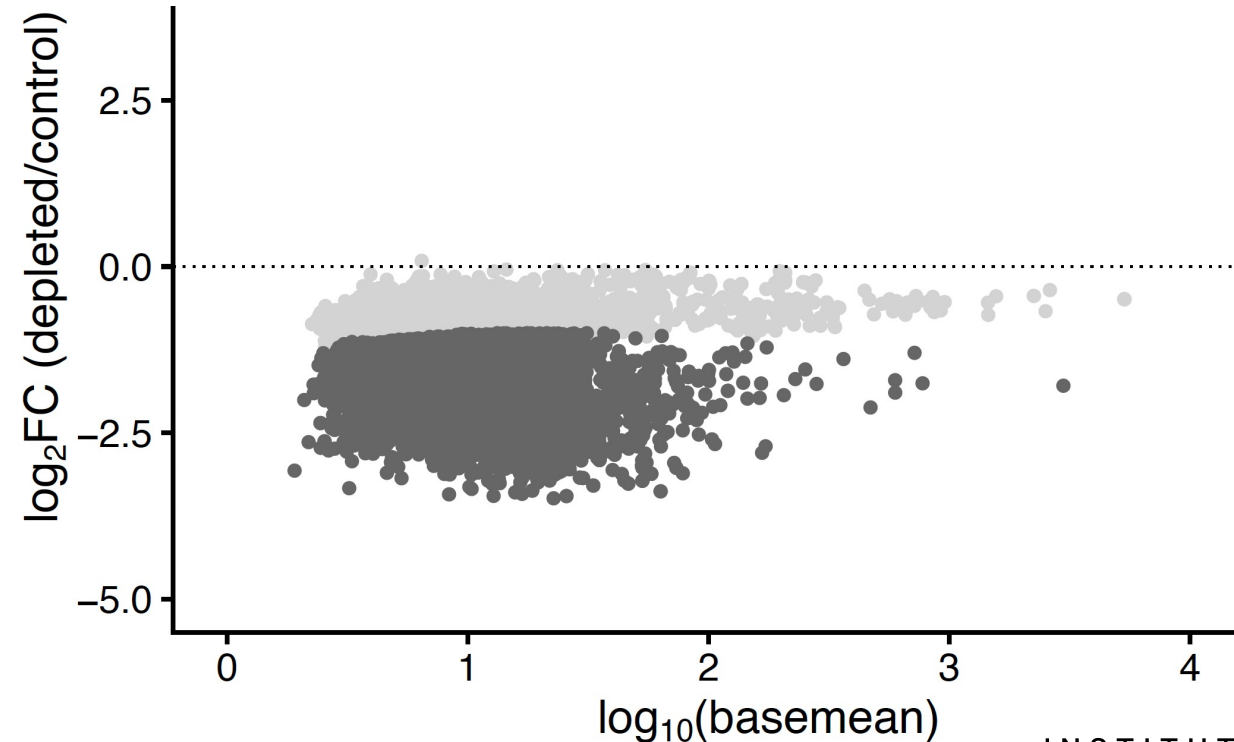
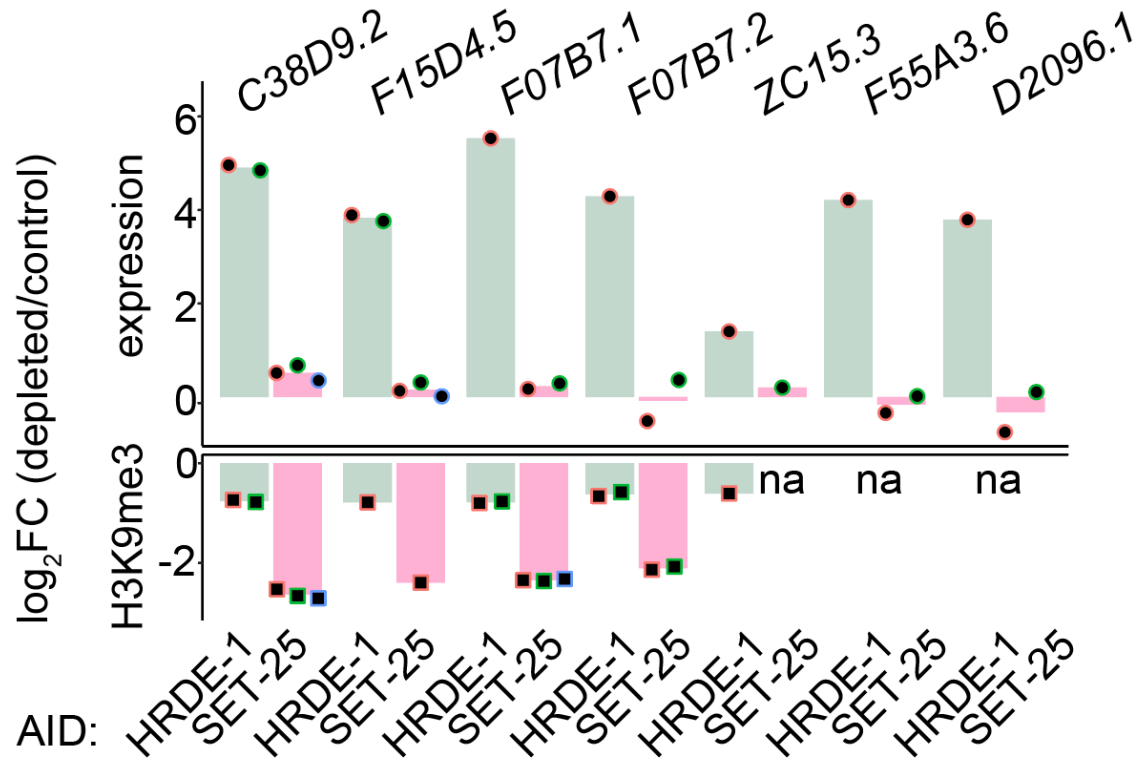
Arantxa Rojas
Postdoc

HRDE-1 mediates transcriptional silencing independent of heterochromatin



Rojas et al., Biorxiv (2025)

G0 – RT-qPCR in absence of SET-25 or HRDE-1 G0 - H3K9me3 in absence of SET-25





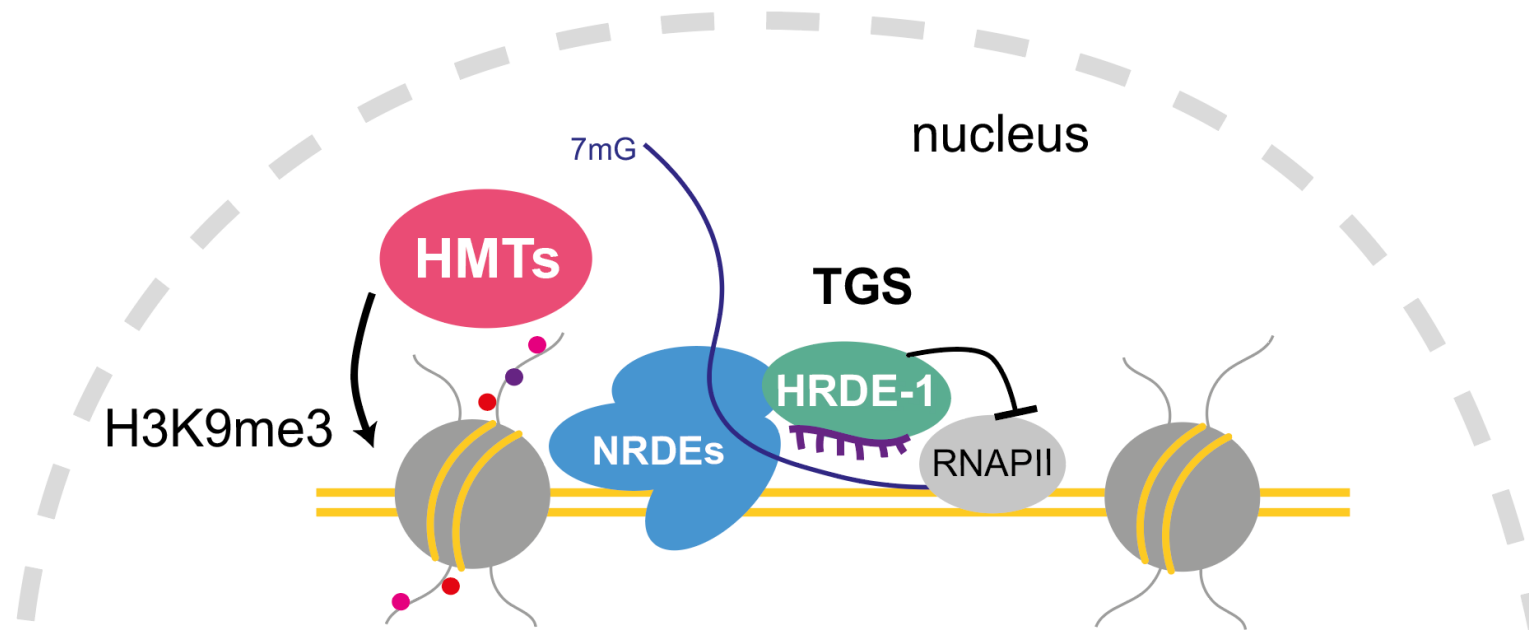
Arantxa Rojas
Postdoc

How does nuclear Argonaute initiate and maintain nuclear silencing?



Rojas et al., Biorxiv (2025)

Heterochromatin-dependent silencing



Environment



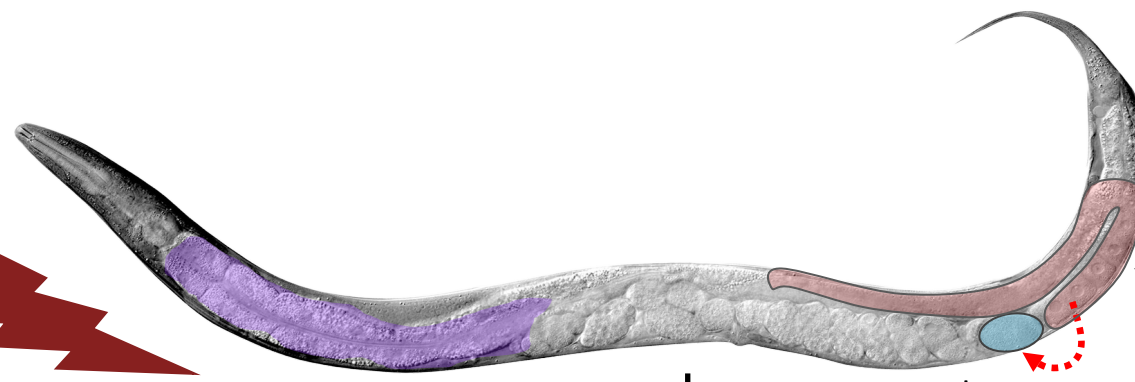
Infection



Chemicals



Diet



small RNAs ↓

Epigenetic Inheritance

Germline
small RNAs

Embryo

Stress, Infection, nutrition

Heritable germline small RNAs control
animal fertility and embryonic development

Barucci et al., Nature Cell Biol 2020

Quarato et al., Nature Comms 2021

Singh et al., Nature Comms 2021

Cornes et al., Dev Cell 2022

Soma-to-germline small RNA transfer?

Environment



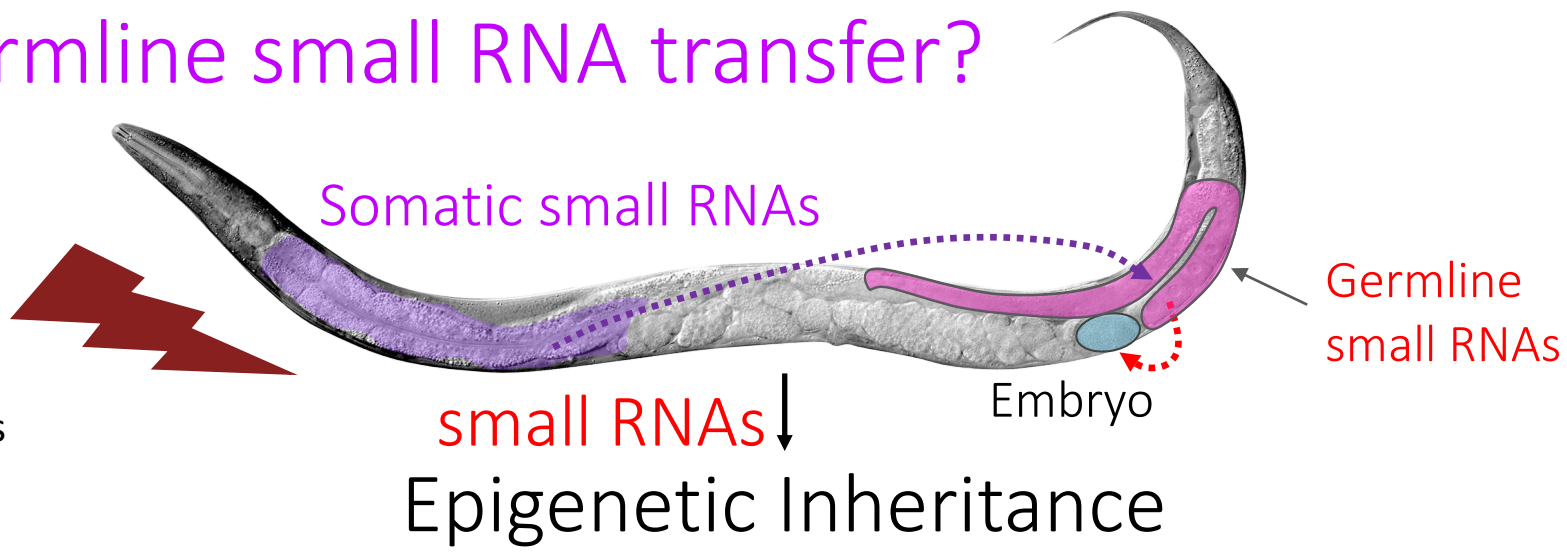
Infection



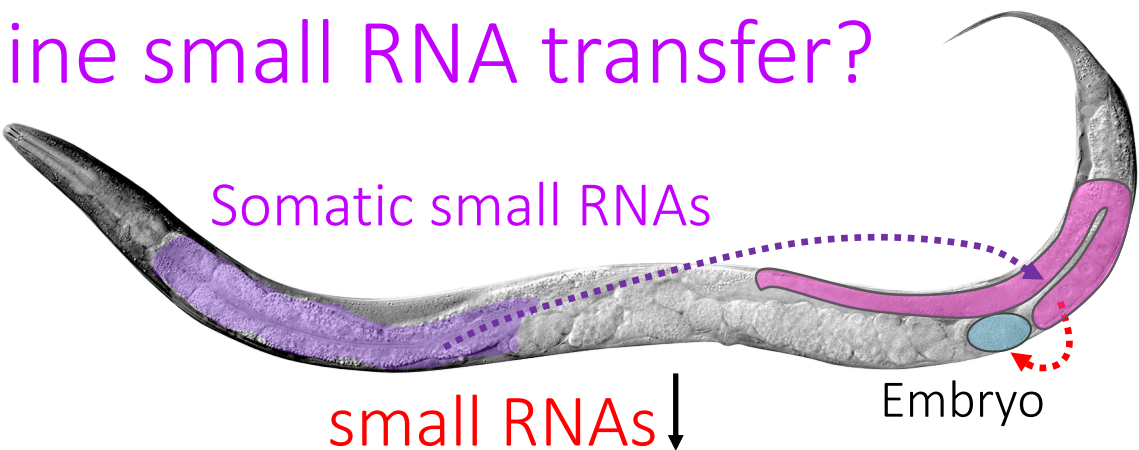
Diet



Chemicals

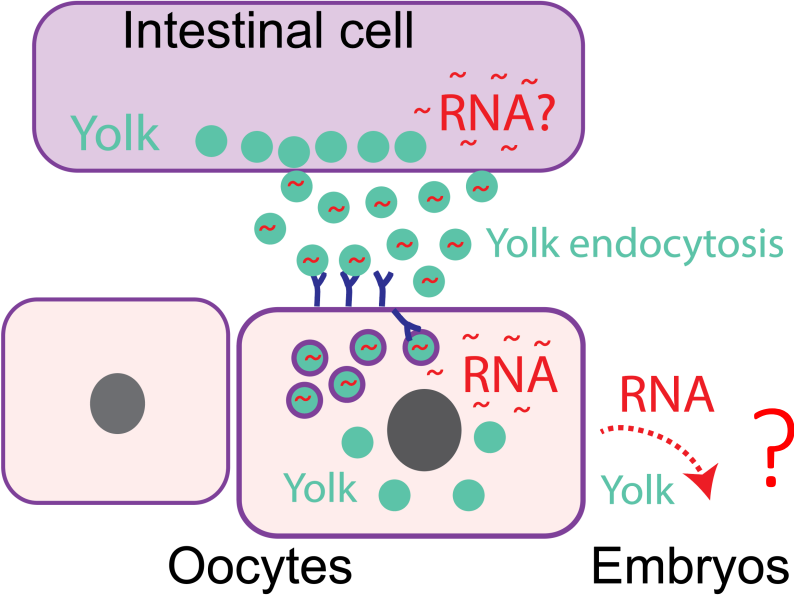


Soma-to-germline small RNA transfer?



Epigenetic Inheritance

The Yolk Pathway



IJAE

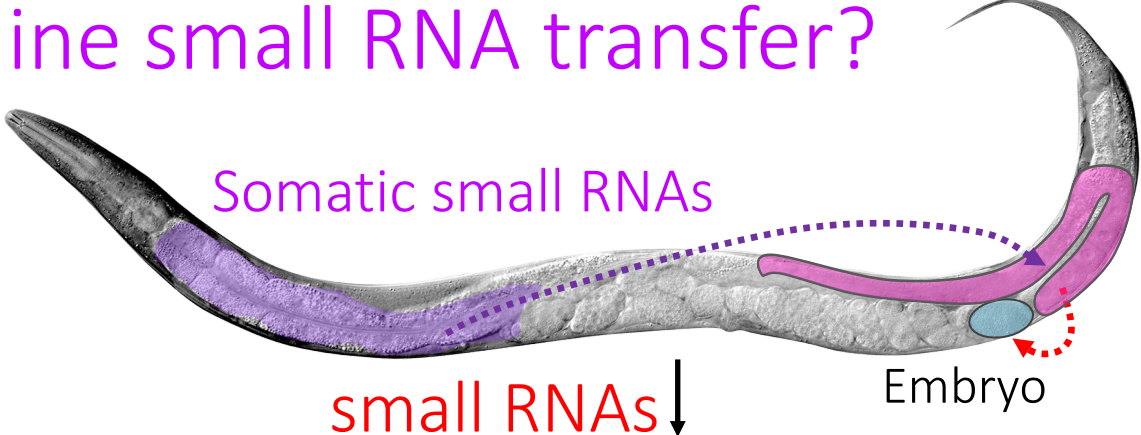
Italian Journal of Anatomy and Embryology

Official Organ of the Italian Society
of Anatomy and Histology

Origine dei mitocondri durante lo sviluppo embrionale di Rana esculenta. Studio al microscopio elettronico

G Lanzavecchia, A Le Coultre - Arch. ital. anat. embriol, 1958

Soma-to-germline small RNA transfer?



Epigenetic Inheritance

STUDIES ON AMPHIBIAN YOLK

III. A RESOLUTION OF YOLK PLATELET COMPONENTS

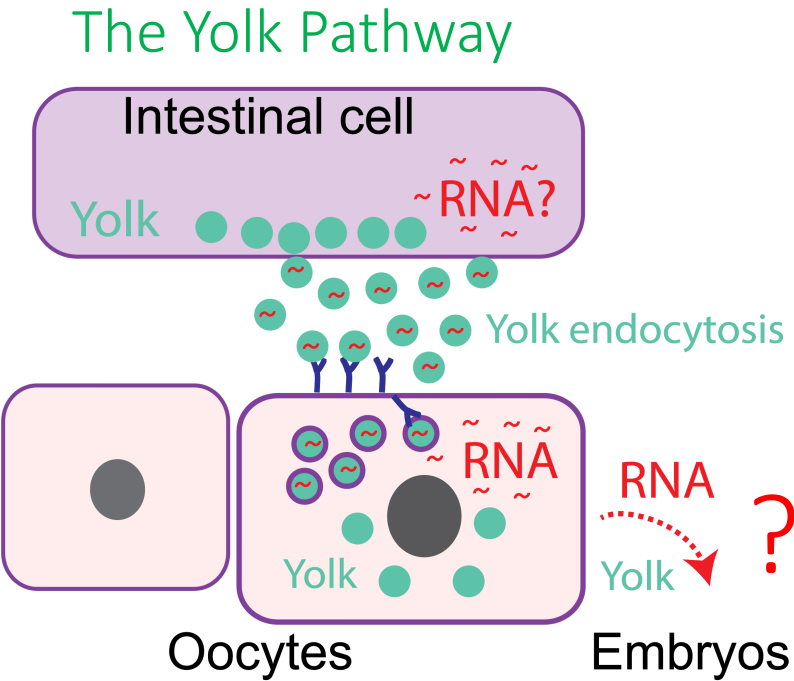
ROBIN A. WALLACE*

*Biology Division, Oak Ridge National Laboratory**, Oak Ridge, Tenn. (U.S.A.)*

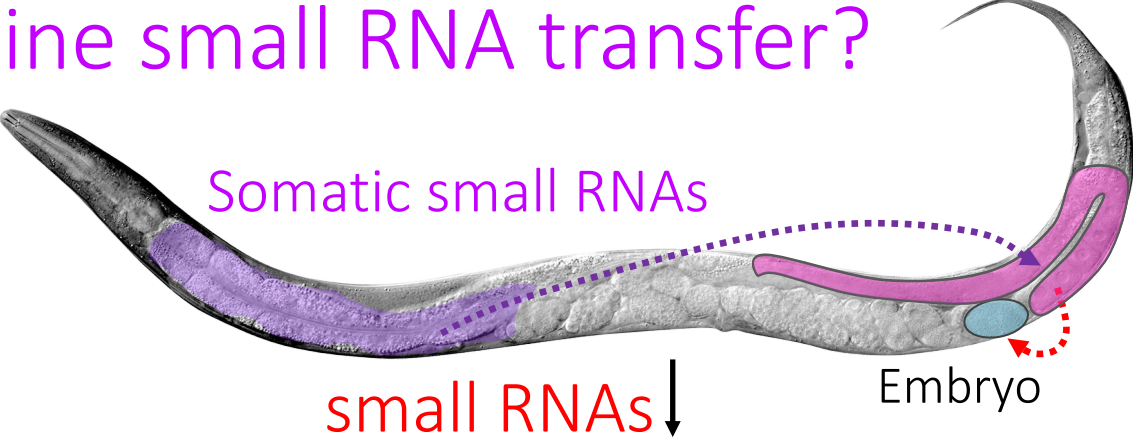
(Received November 30th, 1962)

SUMMARY

Intact isolated yolk platelets have been analyzed for nucleic acids, lipid, hot tri-chloroacetic acid-extractable carbohydrate and water-extractable constituents. It is concluded that yolk platelets *in situ* contain no RNA, but the amount of DNA which they contain is still uncertain. Also, no evidence thus far has been obtained for a readily extractable polysaccharide. When dissolved in strong salt solutions under non-oxidative conditions, the main part of the yolk platelet (water-insoluble) resolves as two components in the ultracentrifuge. A method for separating these two components in quantitative yield is indicated.

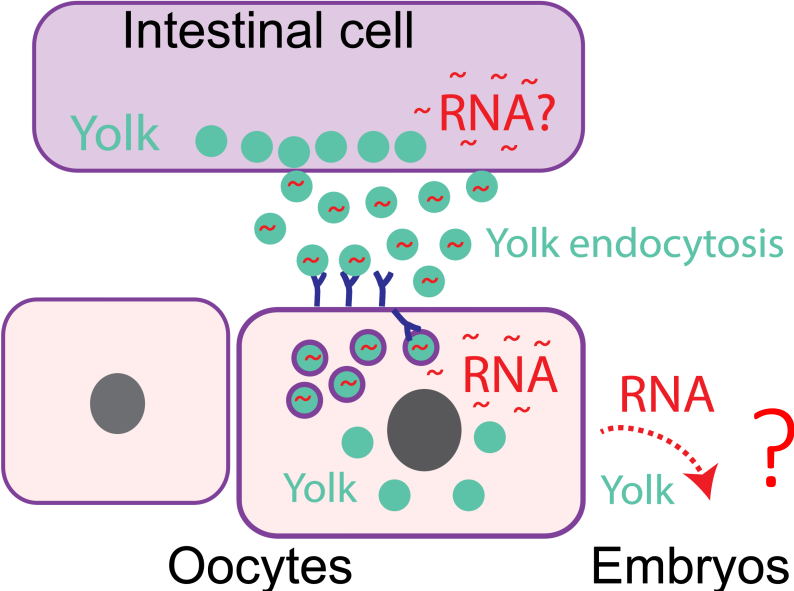


Soma-to-germline small RNA transfer?



Epigenetic Inheritance

The Yolk Pathway



Yolk platelets isolated from *Xenopus* gastrulae

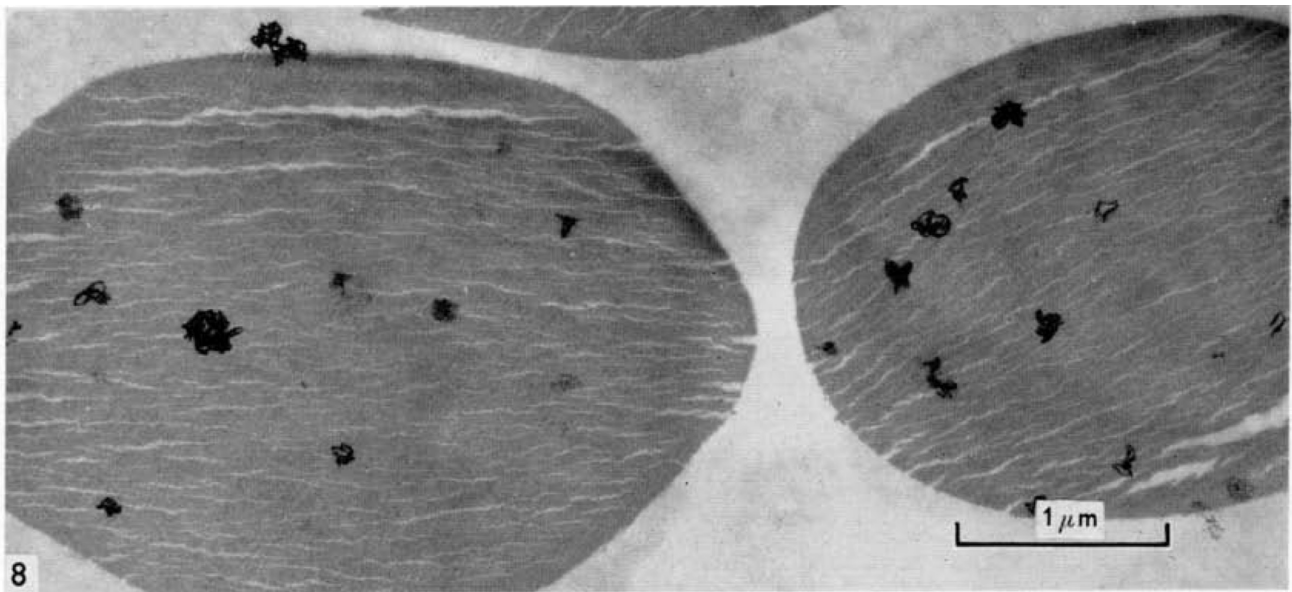
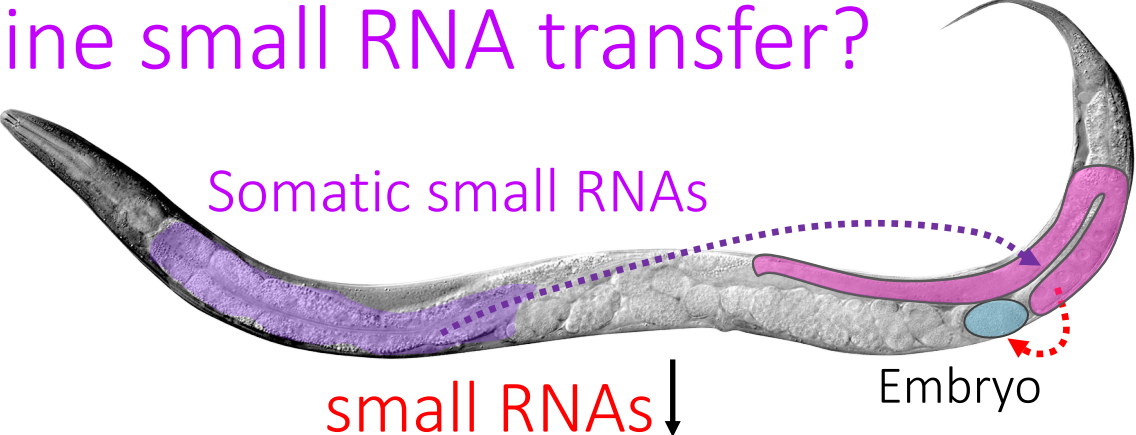
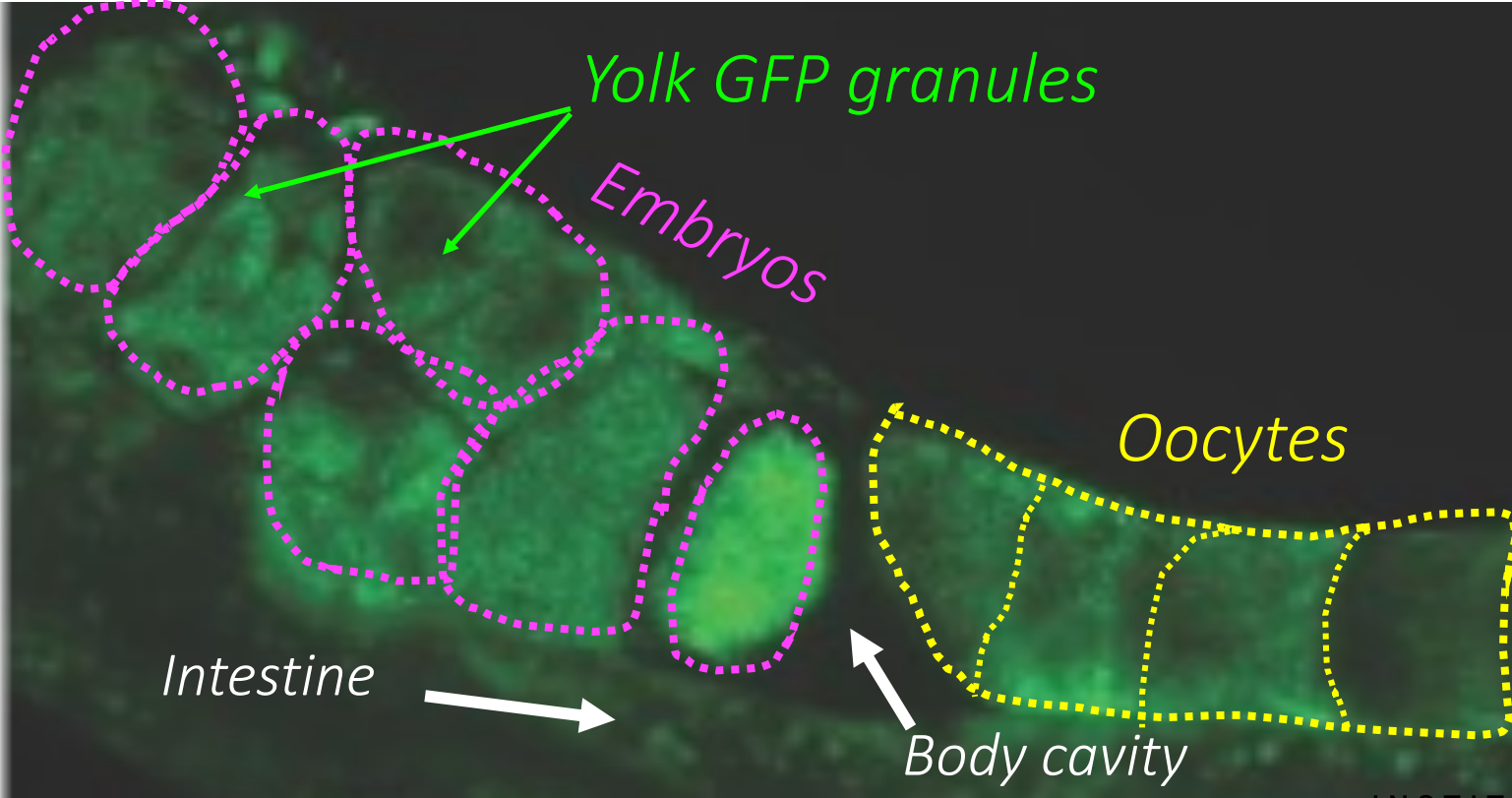
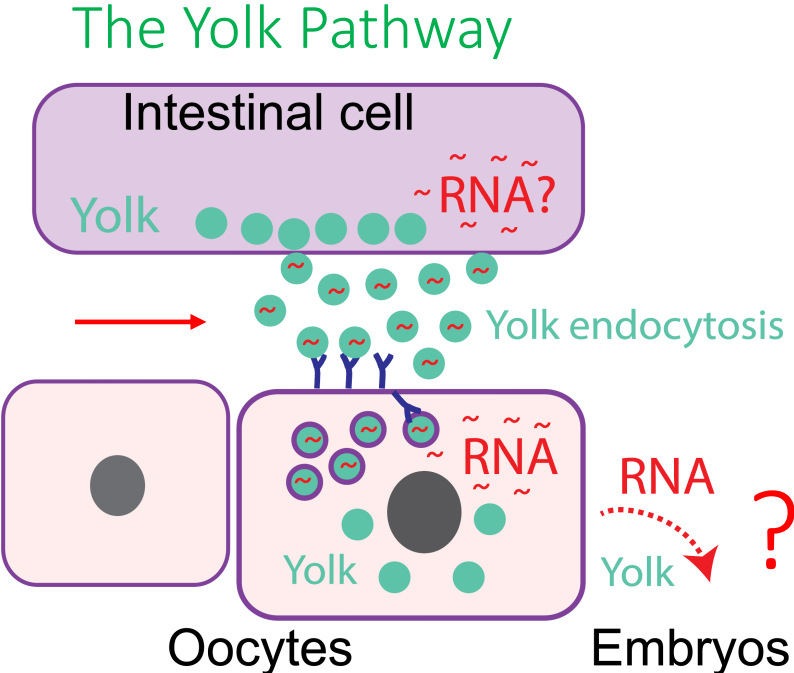


Fig. 8. Autoradiograph of platelets isolated from mid-gastrula cells cultured in [³H]uridine for 1 h. × 33000.
Kelley et al., Development 1971

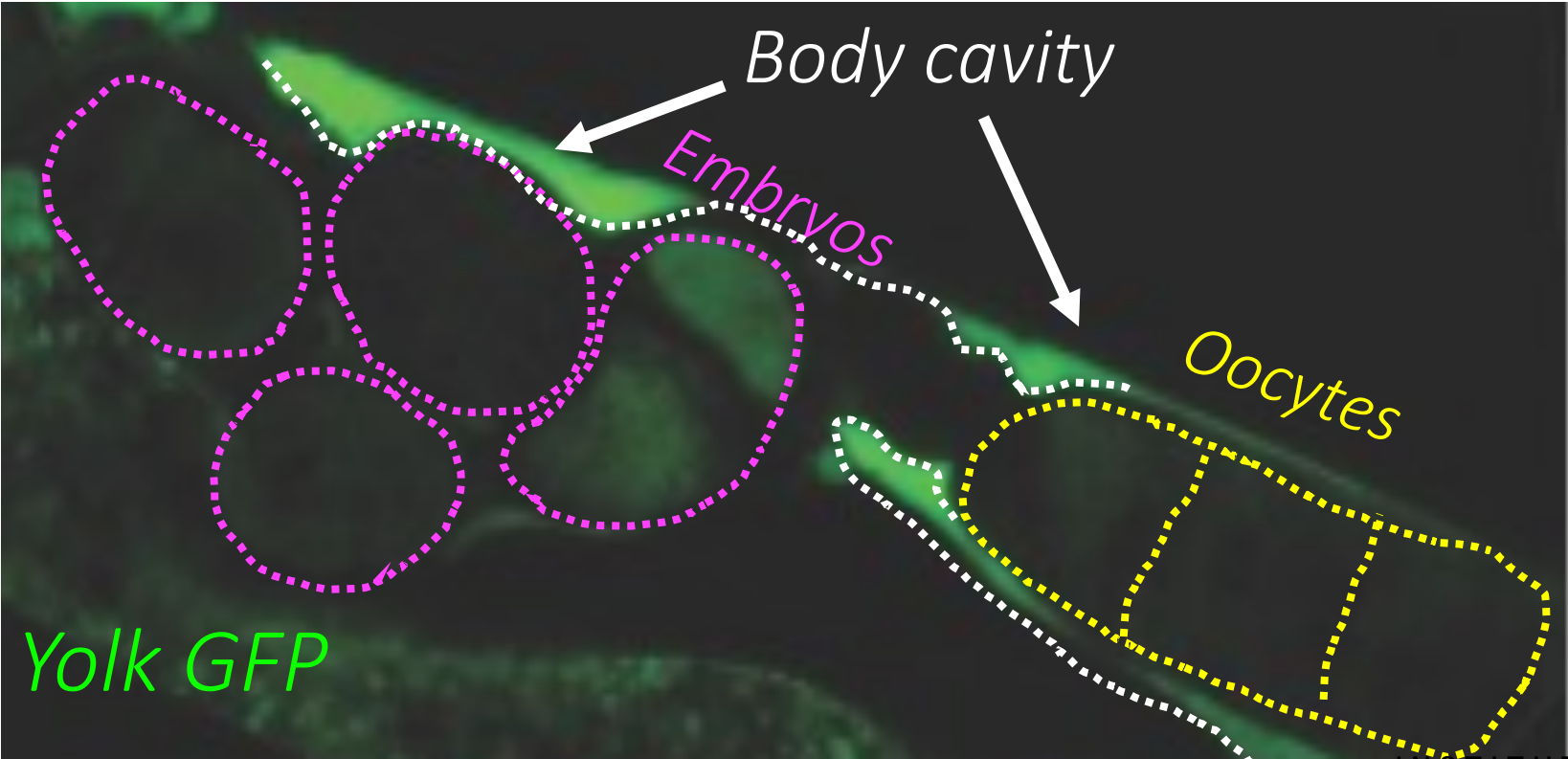
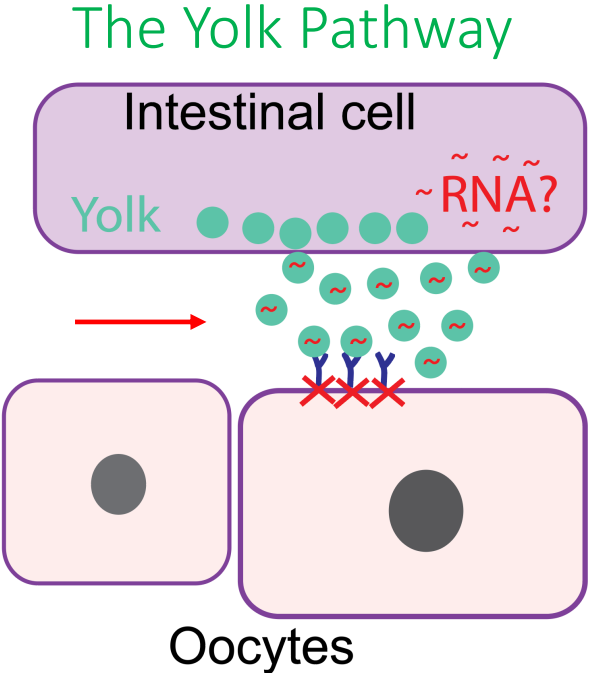
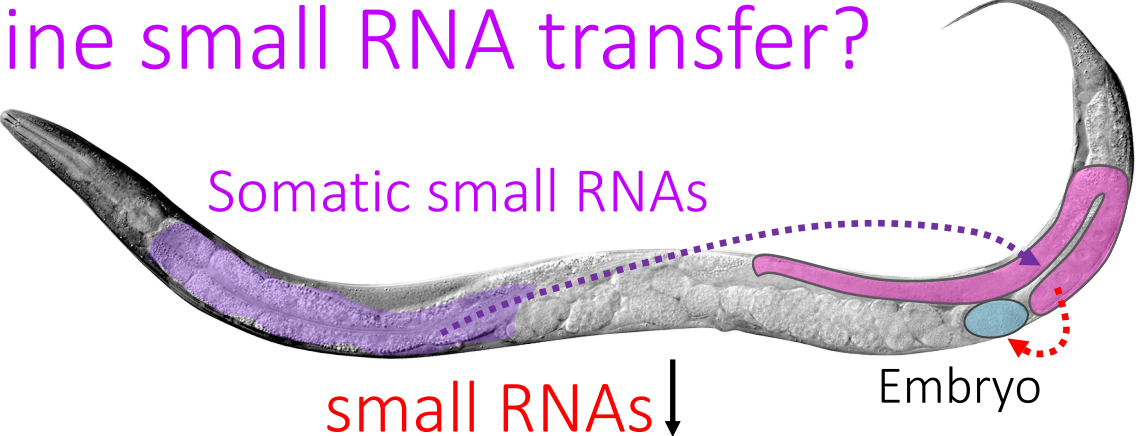
Soma-to-germline small RNA transfer?



small RNAs ↓
Epigenetic Inheritance



Soma-to-germline small RNA transfer?



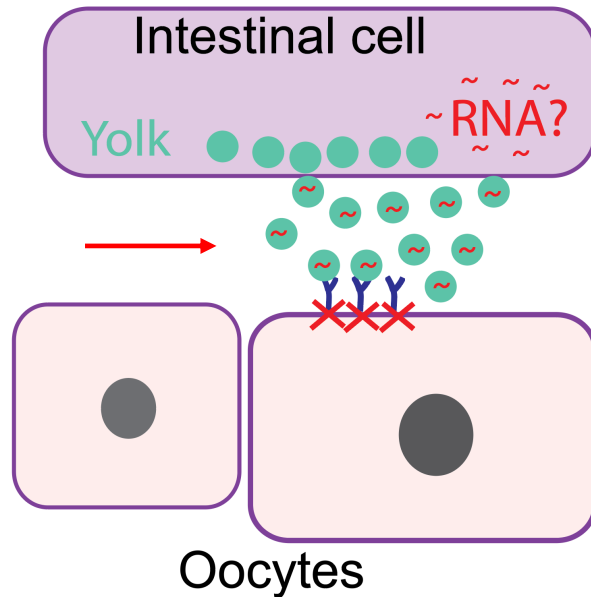
Soma-to-germline small RNA transfer?



Somatic small RNAs

Embryo

The Yolk Pathway



Yolk purification
(ultracentrifugation)

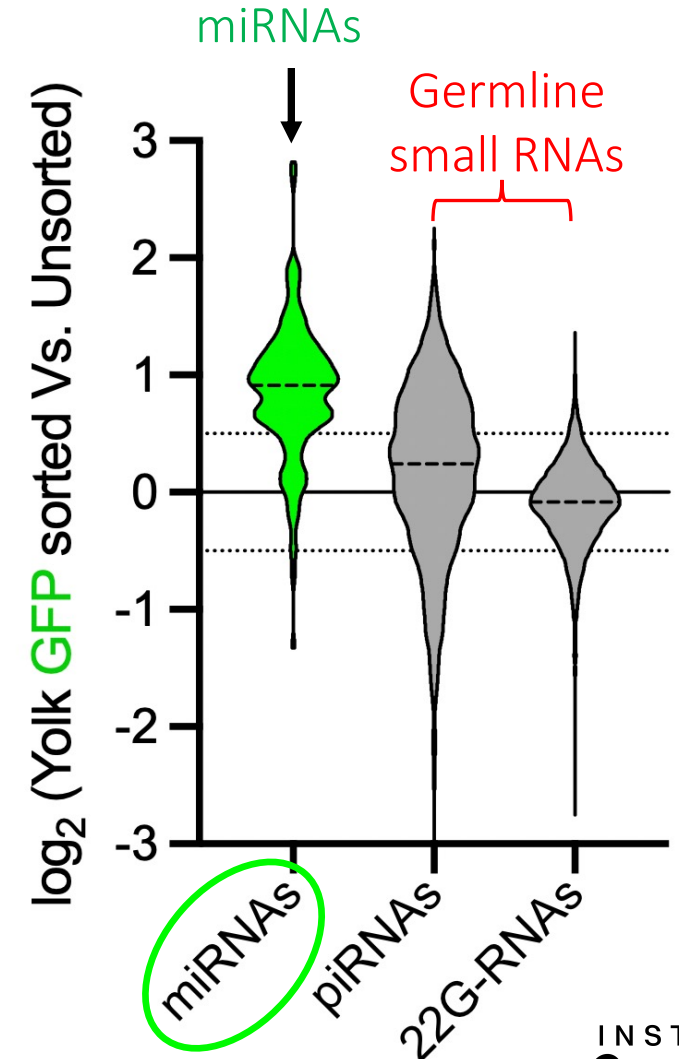
Unsorted
(total)

small RNA-seq

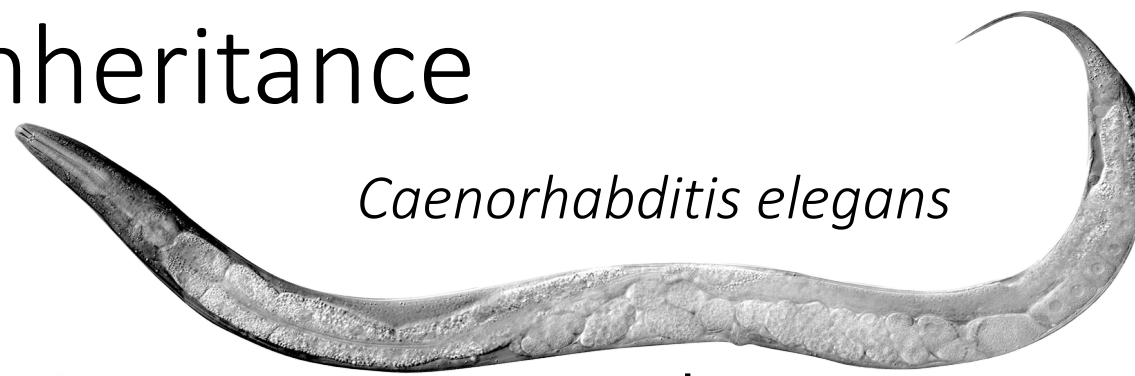
FACS sorting of VIT-2::GFP

Sorted
(yolk)

small RNA-seq



Epigenetic Inheritance



Caenorhabditis elegans

The Nobel Prize in Physiology or
Medicine 2024



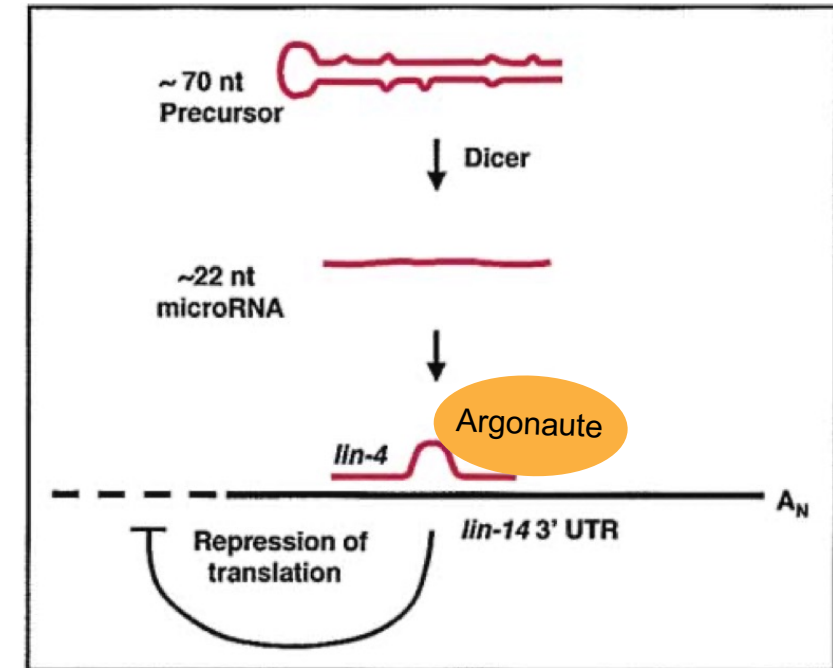
Ill. Niklas Elmehed © Nobel Prize
Outreach
Victor Ambros
Prize share: 1/2



Ill. Niklas Elmehed © Nobel Prize
Outreach
Gary Ruvkun
Prize share: 1/2

miRNAs ↓ ?

microRNAs are global negative
regulators of gene expression during
animal development



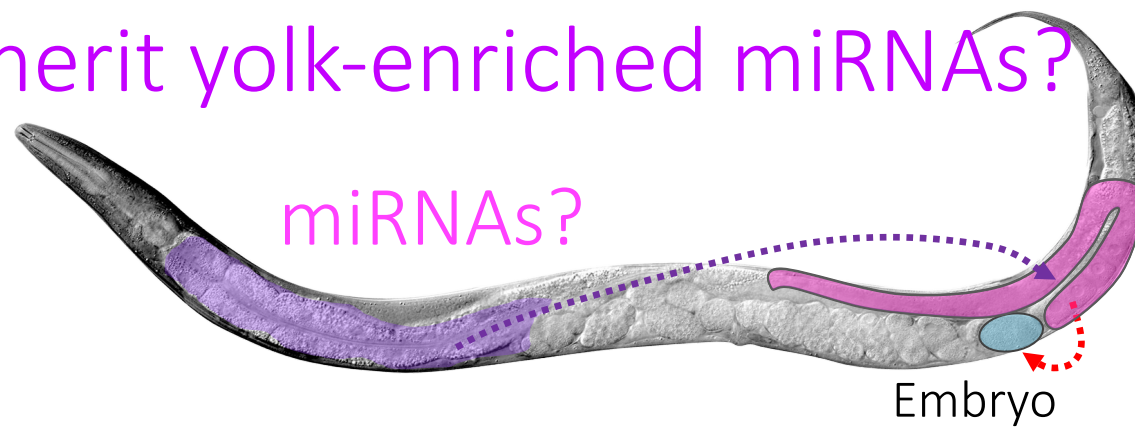
Adapted from Ambros, Cell 2001

Lee et al., Cell 1993 (*lin-4*)

Wightman et al., Cell 1993 (*lin-14*, target)

Reinhart et al., Nature 2000 (*let-7*)

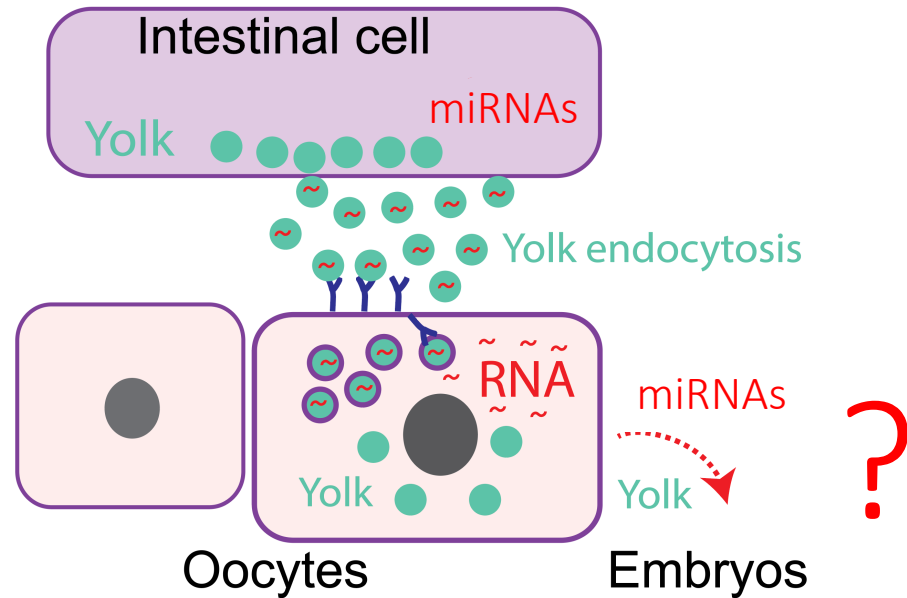
Do embryos inherit yolk-enriched miRNAs?



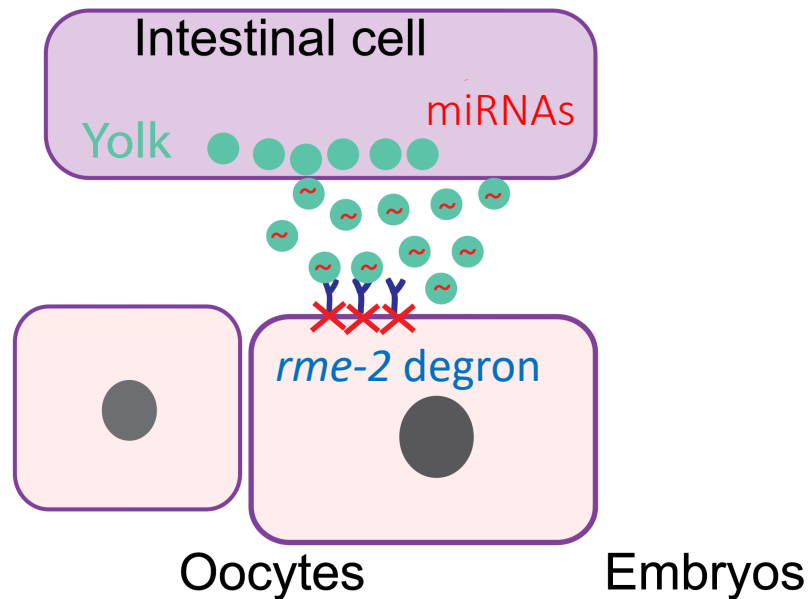
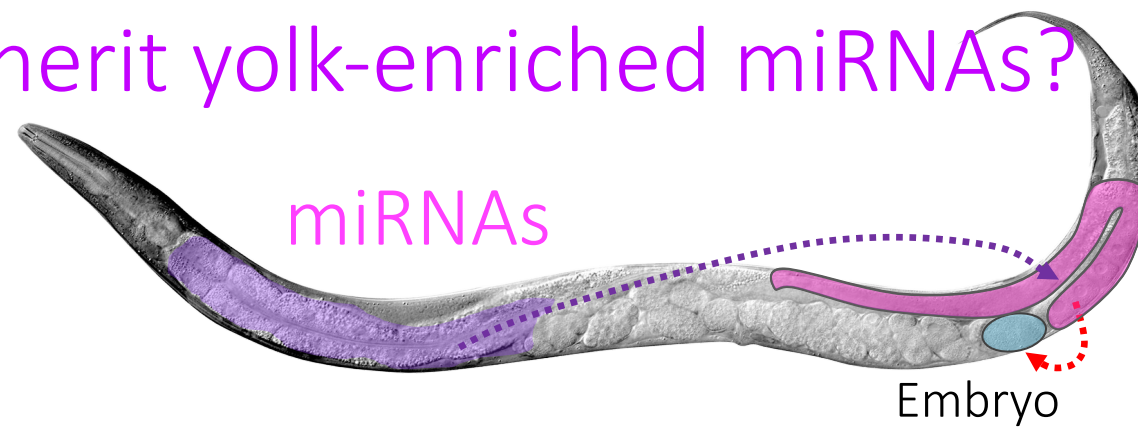
Meetali Singh
Postdoc



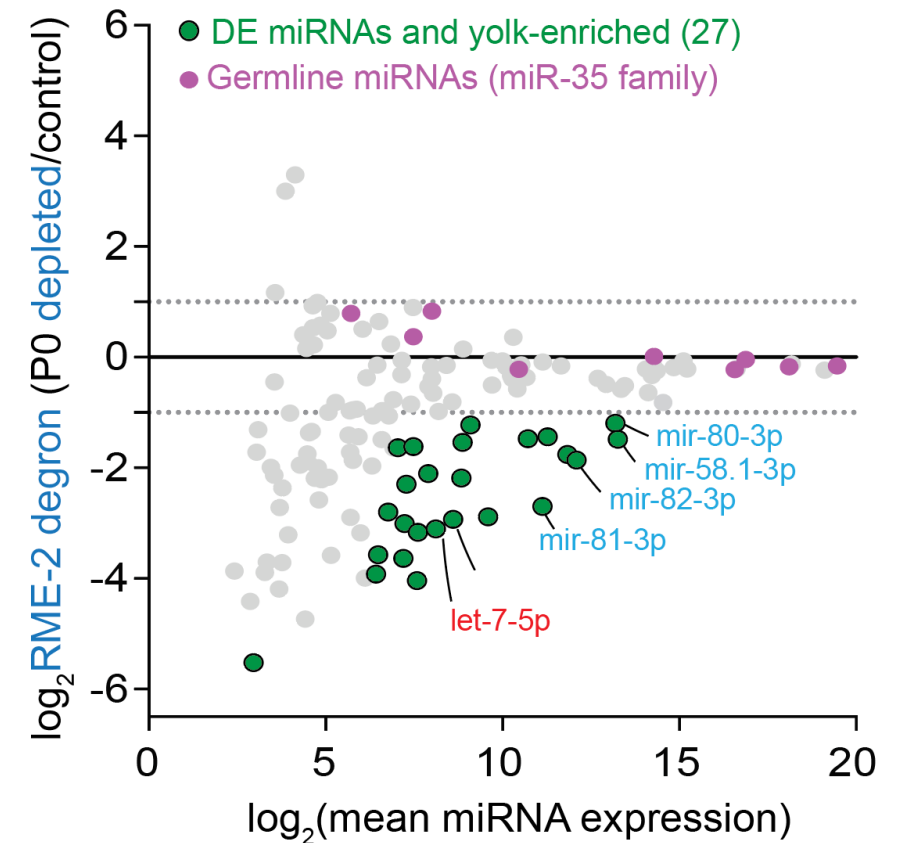
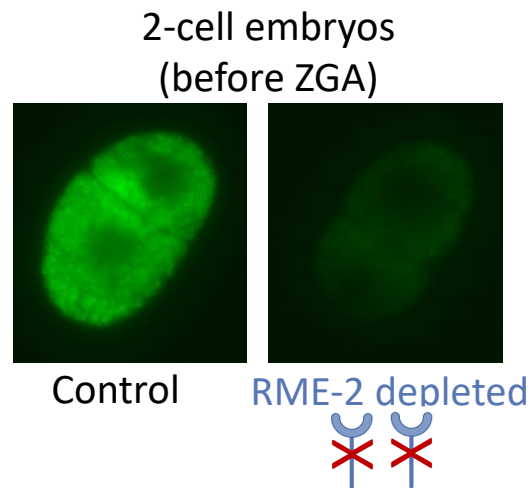
N  v   Aup  rin
Ph.D. student



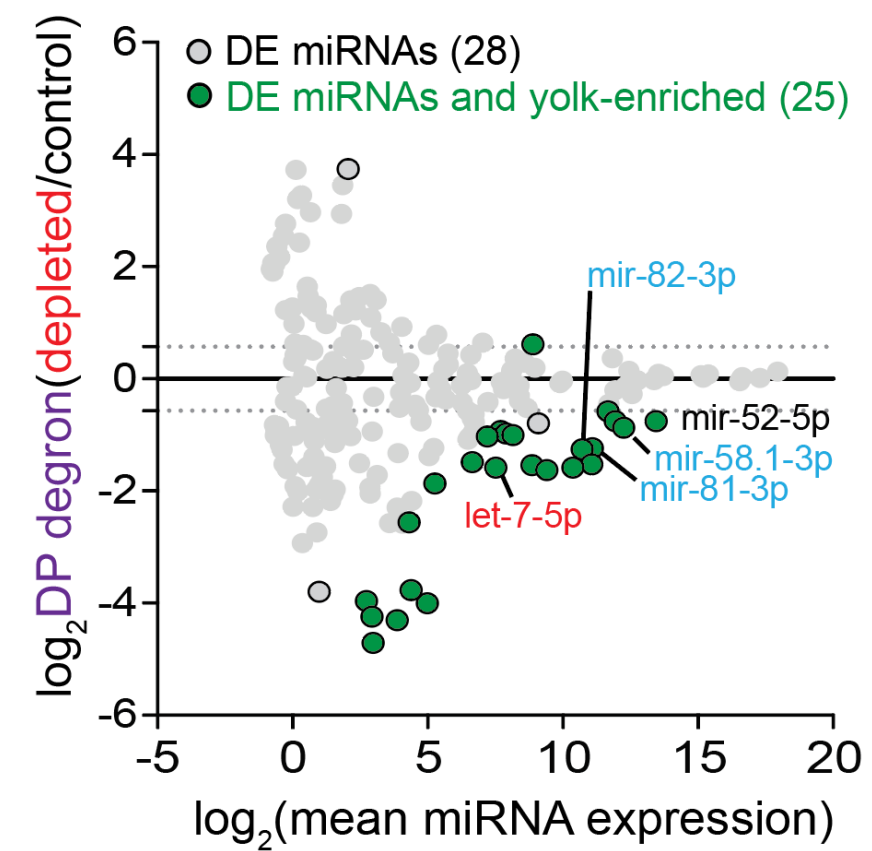
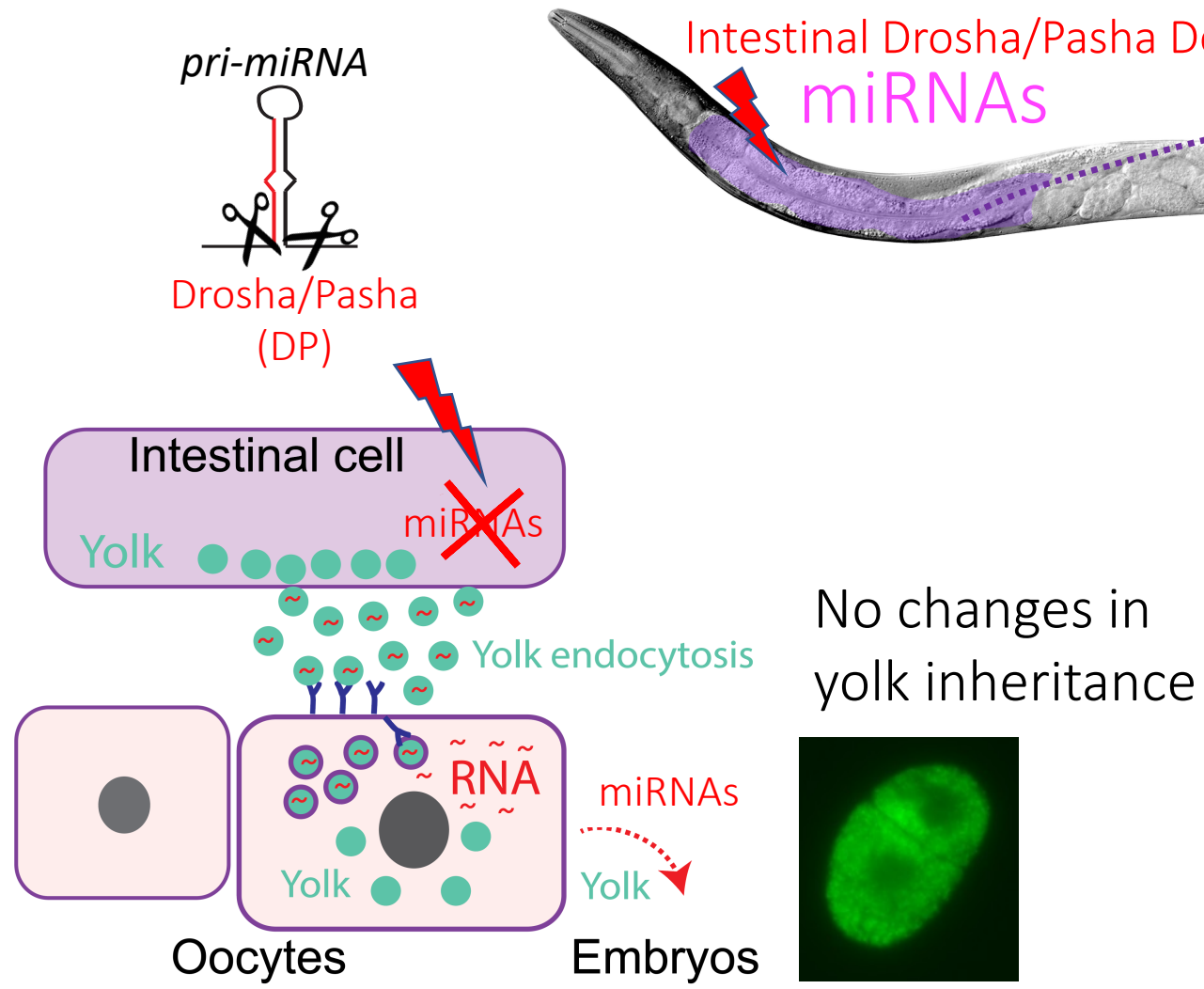
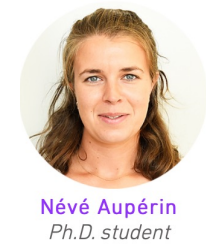
Do embryos inherit yolk-enriched miRNAs?



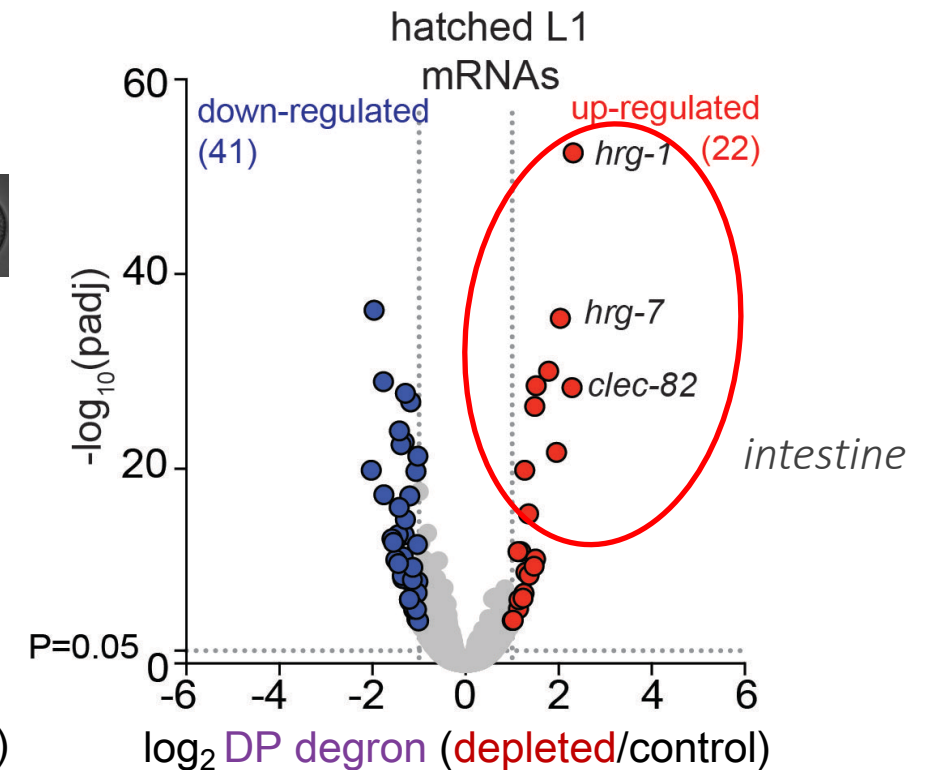
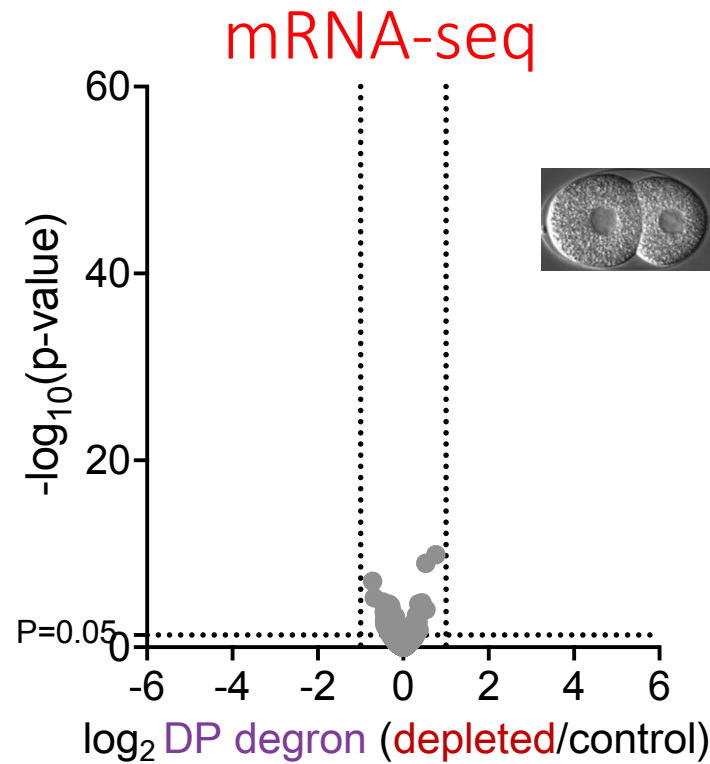
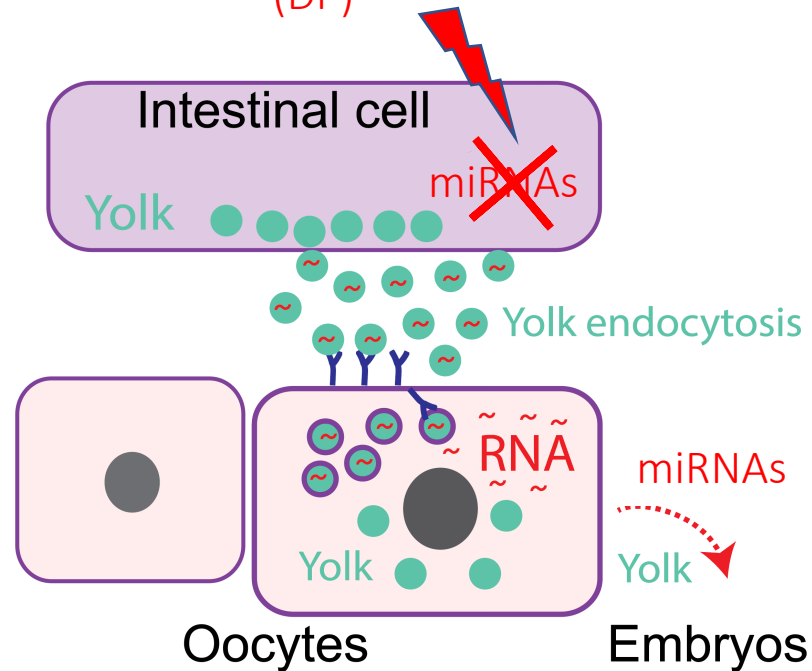
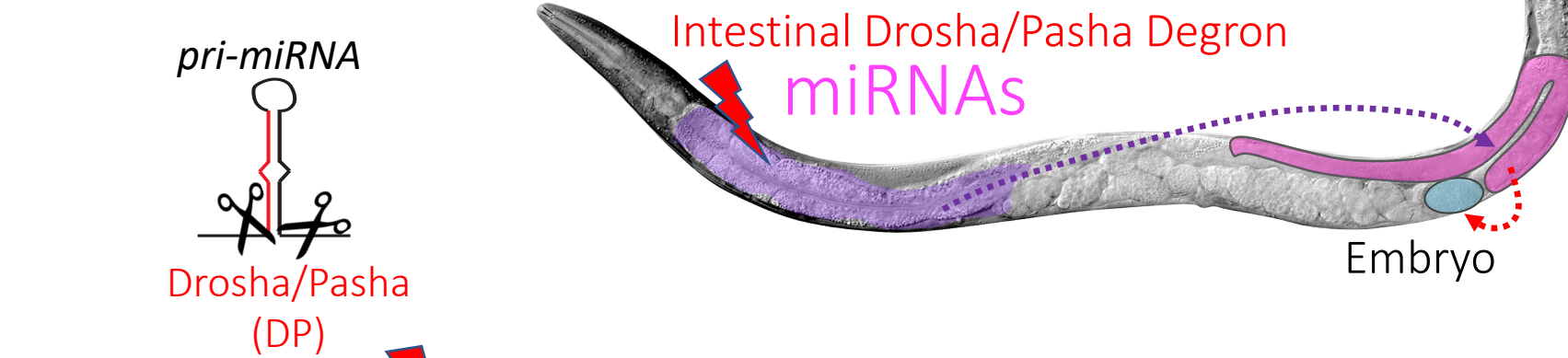
miRNA-seq



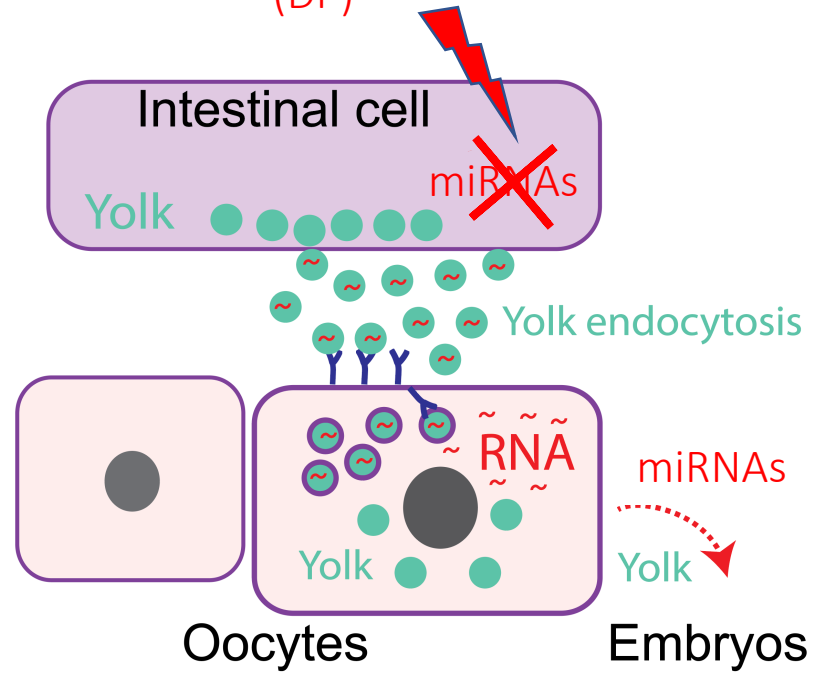
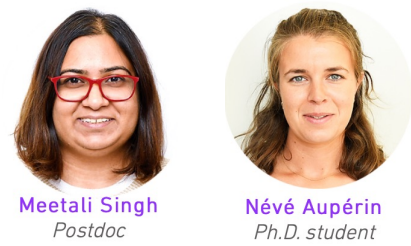
Are miRNAs transferred from the intestine?



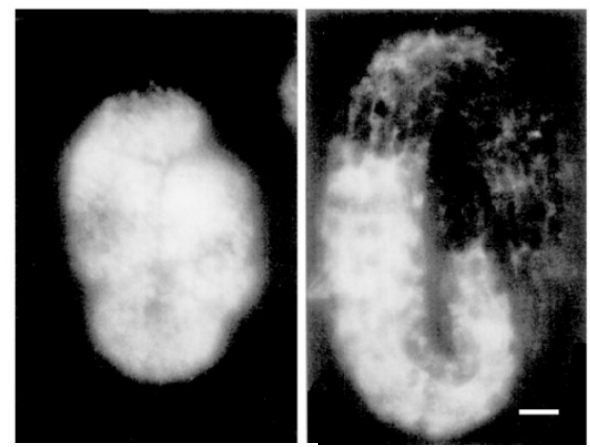
Do yolk-miRNAs regulate genes in progenies?



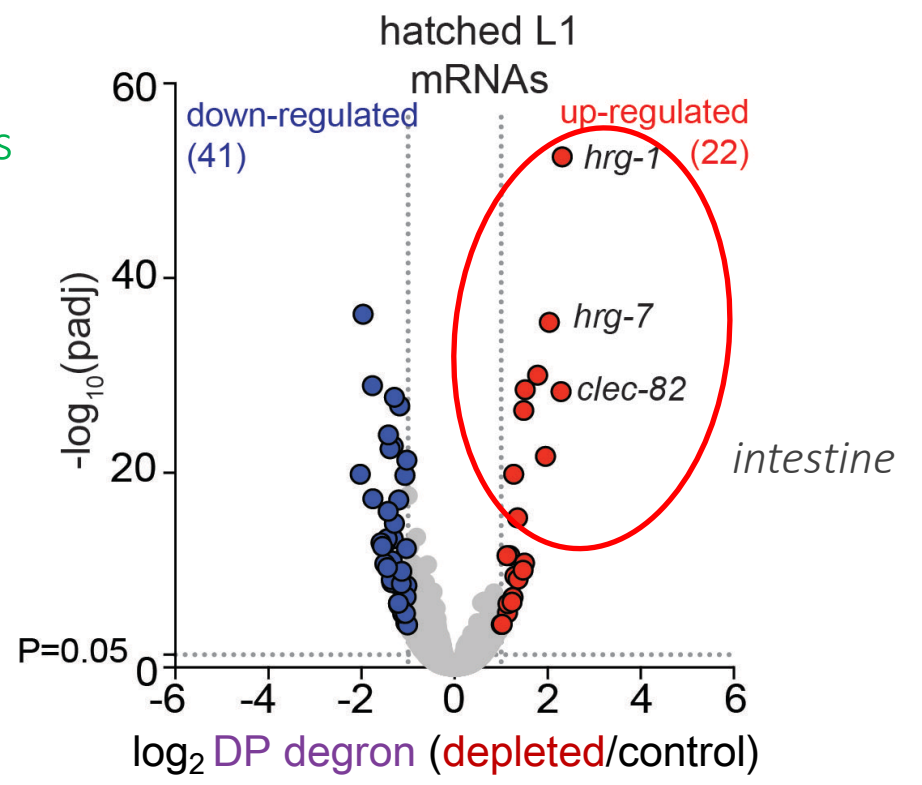
Do yolk-miRNAs regulate genes in progenies?



Yolk persistance in intestinal cells



4-cell embryo Larvae
(Adapted from Sharrock, 1983)



What is the physiological relevance of this miRNA transfer?

Somatic
small RNAs

miRNAs

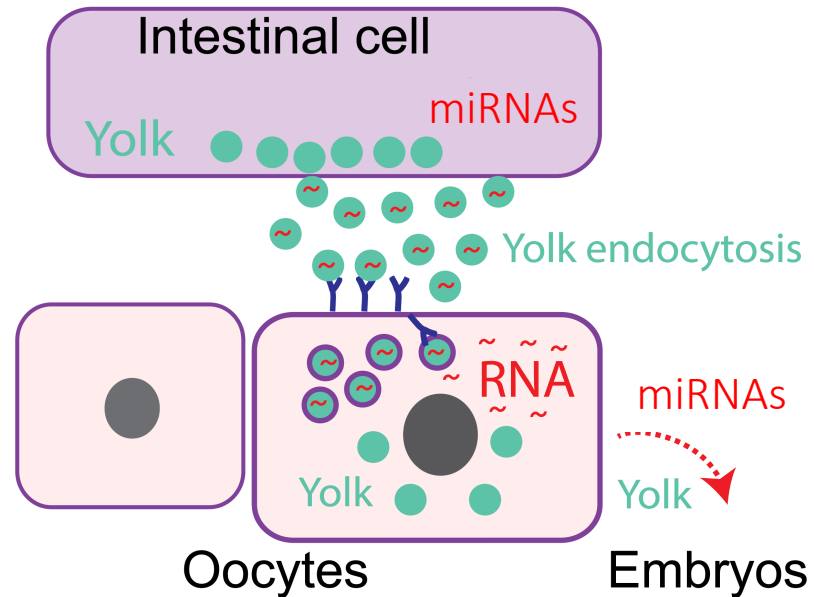
Embryo



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Postdoc



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Ph.D. student



What is the physiological relevance of this miRNA transfer?



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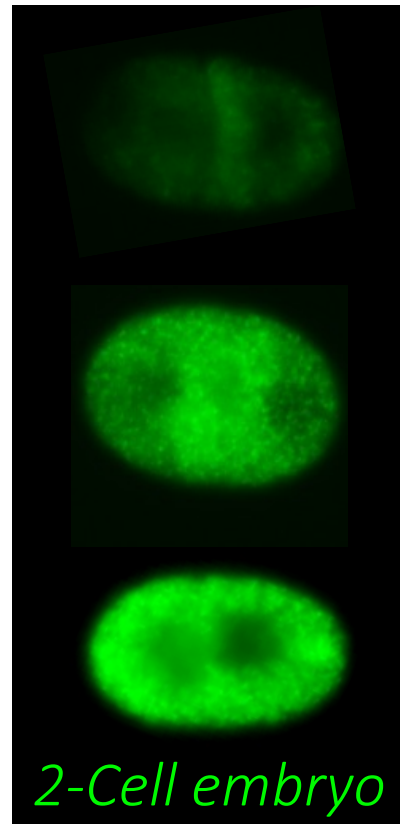
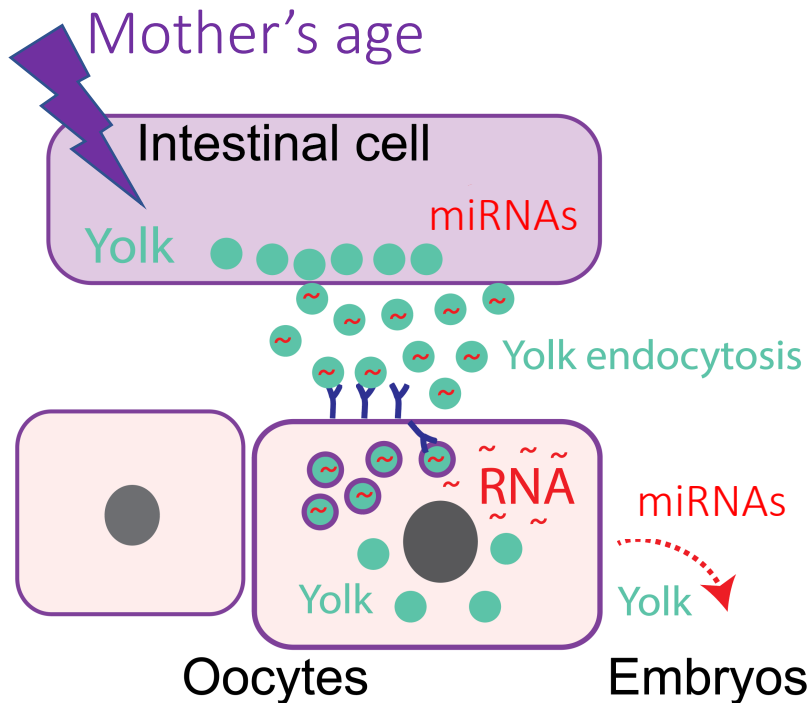


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Somatic
small RNAs

miRNAs

Embryo



Day 1
mother

Day 2
mother

Day 3
mother

2-Cell embryo

Maternal age generates
phenotypic variation in *C. elegans*

Perez et al., Nature 2017

What is the physiological relevance of this miRNA transfer?



Somatic
small RNAs

miRNAs

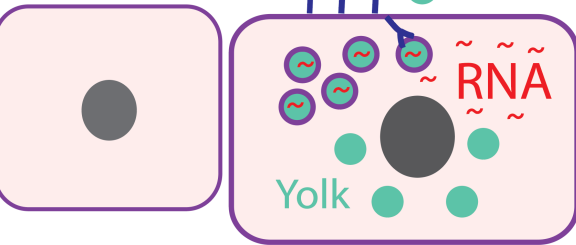
Embryo

Mother's age

Intestinal cell

Yolk miRNAs

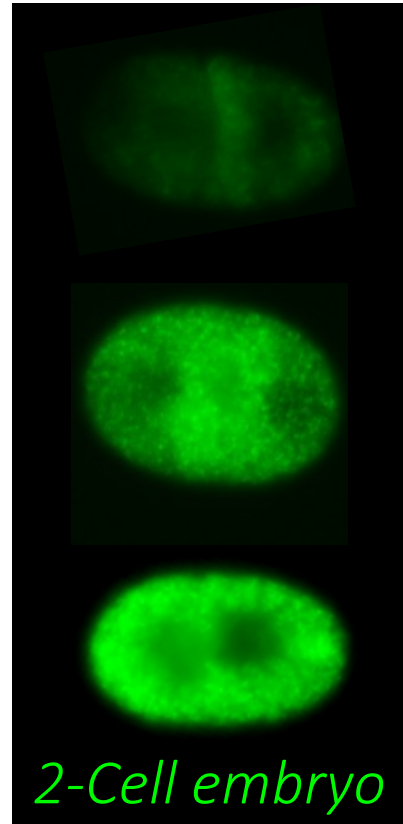
Yolk endocytosis



Oocytes

miRNAs

Embryos

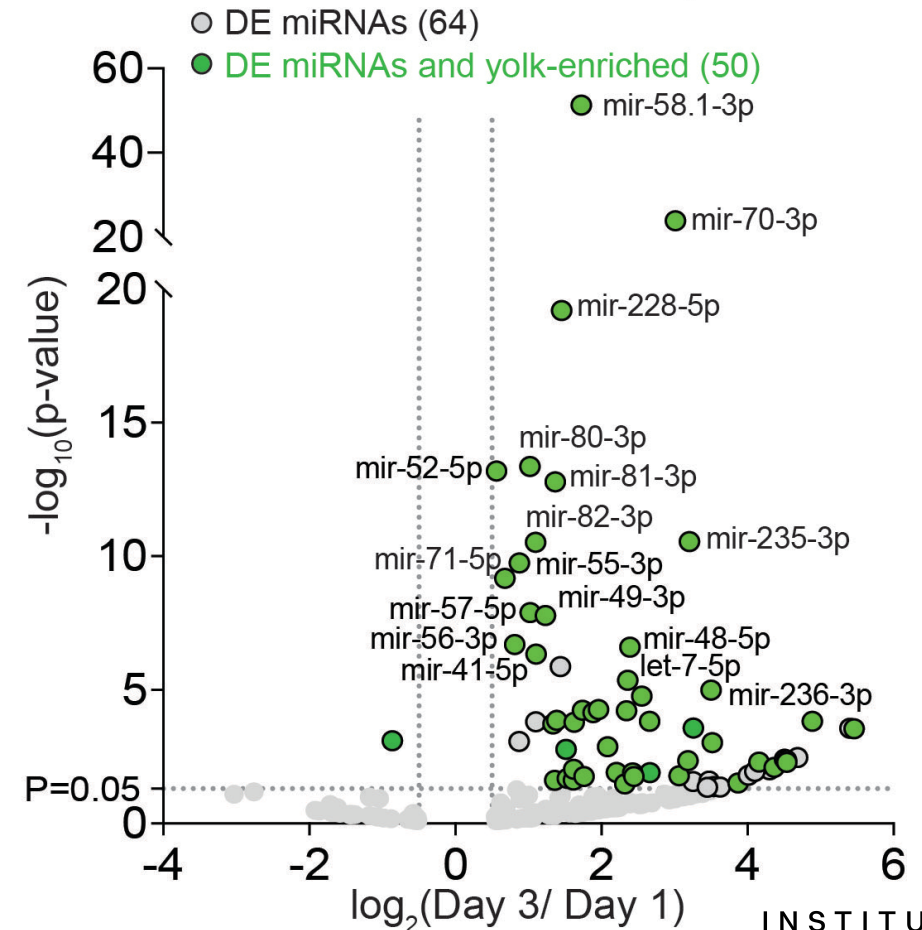


Day 1
mother

Day 2
mother

Day 3
mother

miRNAs in 2-cell embryos



What is the physiological relevance of this miRNA transfer?



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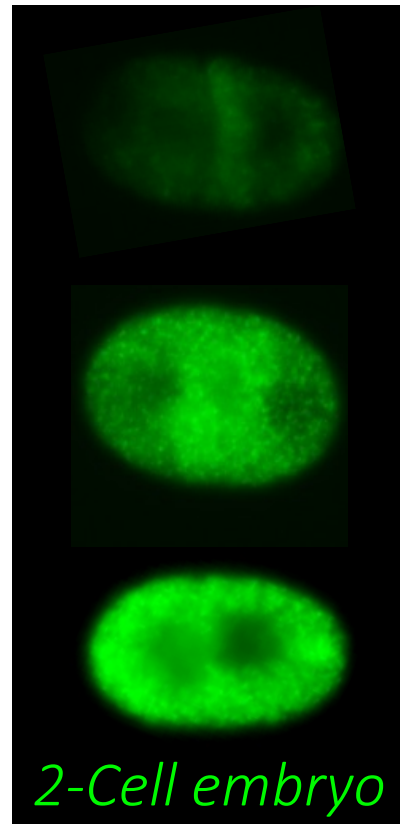
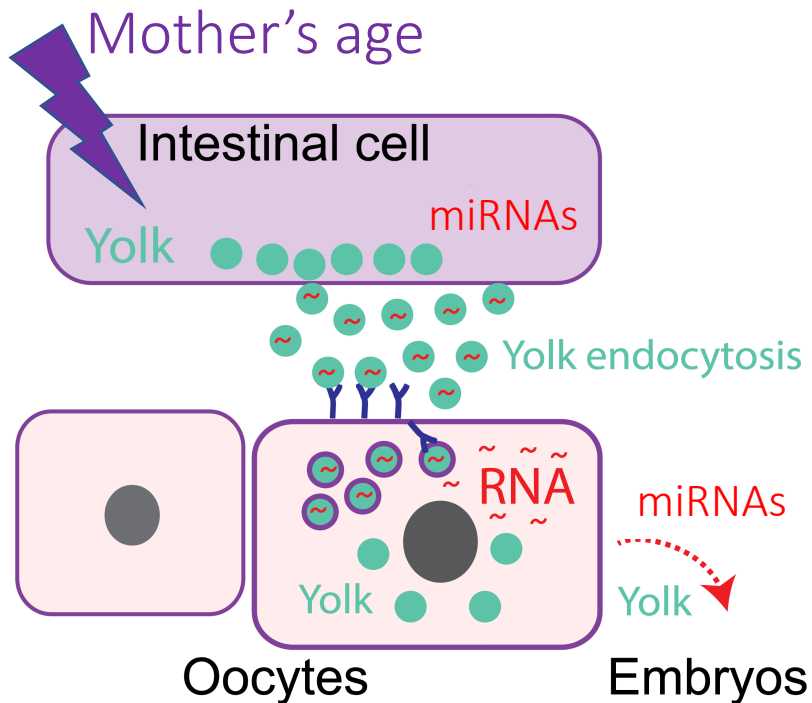


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Somatic
small RNAs

miRNAs

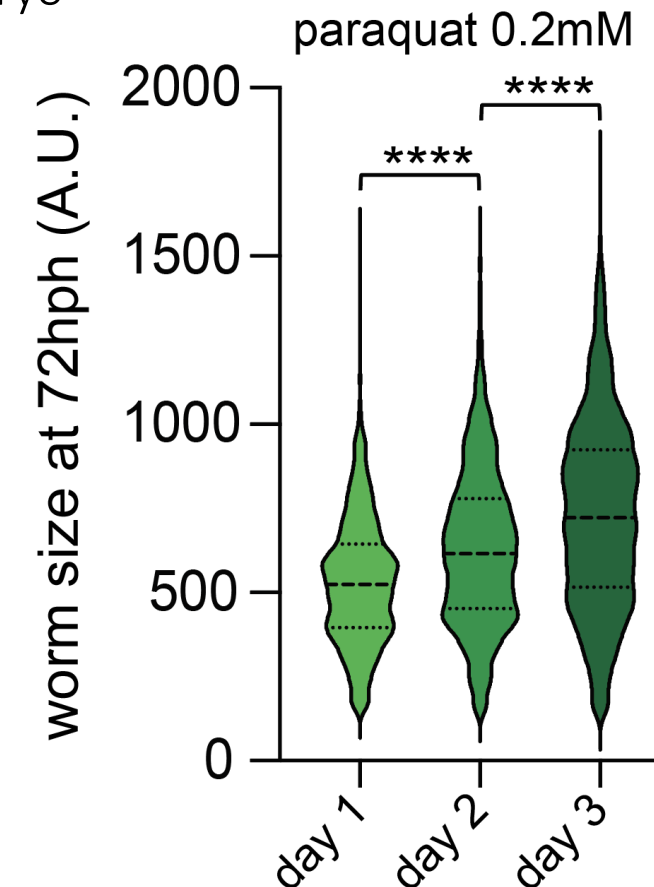
Embryo



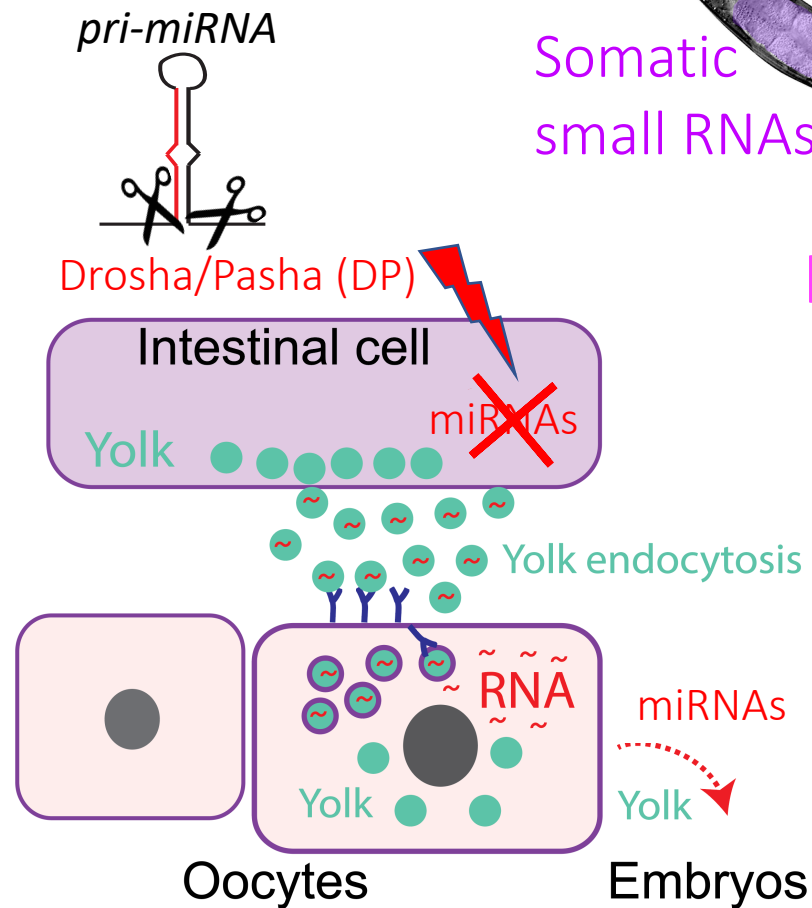
Day 1
mother

Day 2
mother

Day 3
mother



What is the physiological relevance of this miRNA transfer?



Somatic
small RNAs

miRNAs

Embryo

How do these larvae develop
in harsh environment?

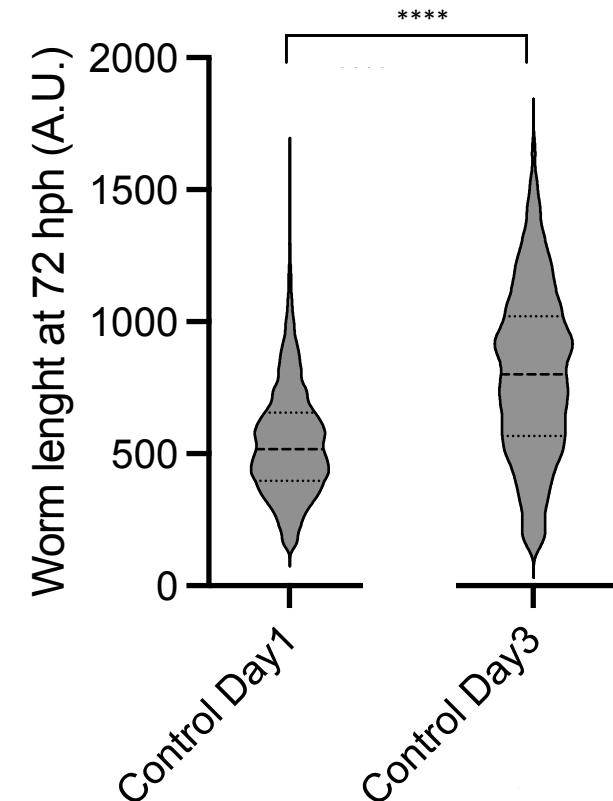
Larvae

Adults

*Development in presence
of oxidative stress*

Normal Drosha/Pasha
expression

Oxidative stress
(paraquat 0.2mM)



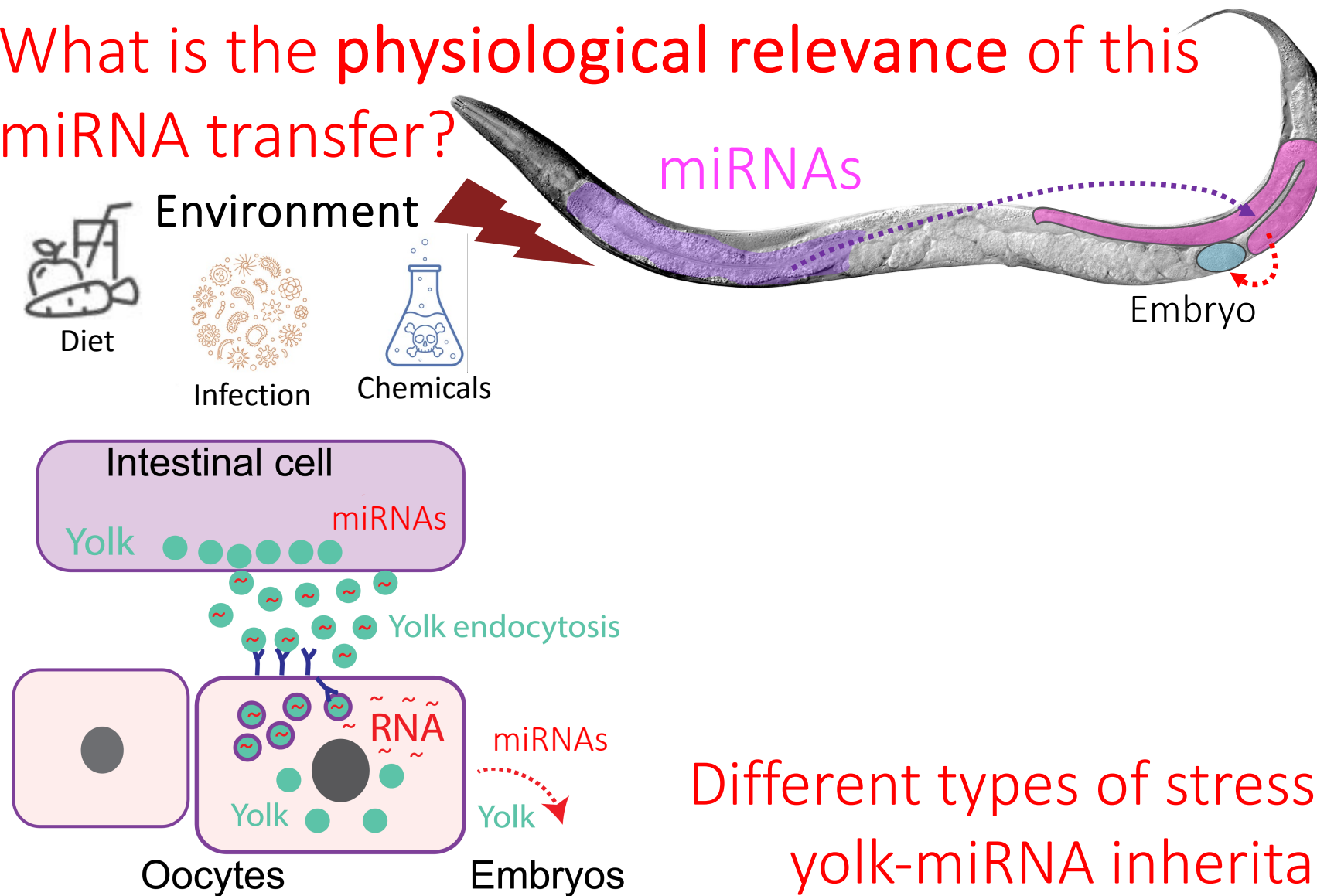
What is the physiological relevance of this miRNA transfer?



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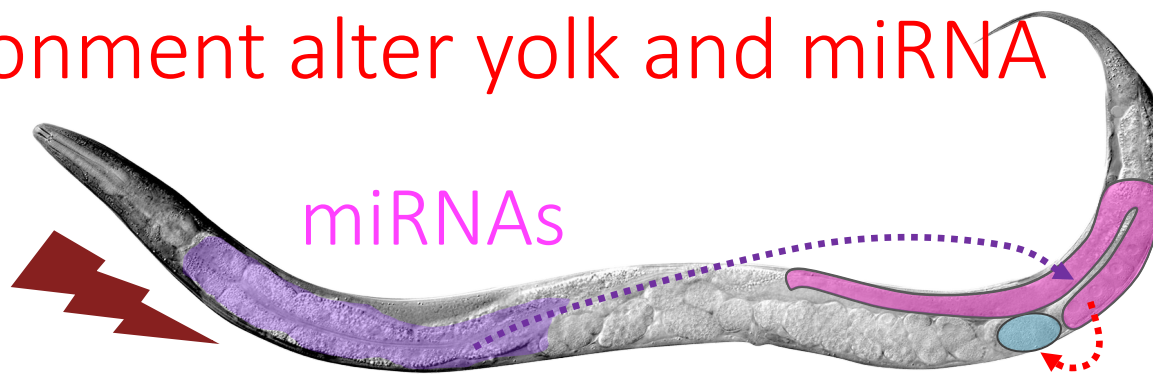


Maternal environment alter yolk and miRNA inheritance

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Postdoc

Névé Aupérin
Ph.D. student

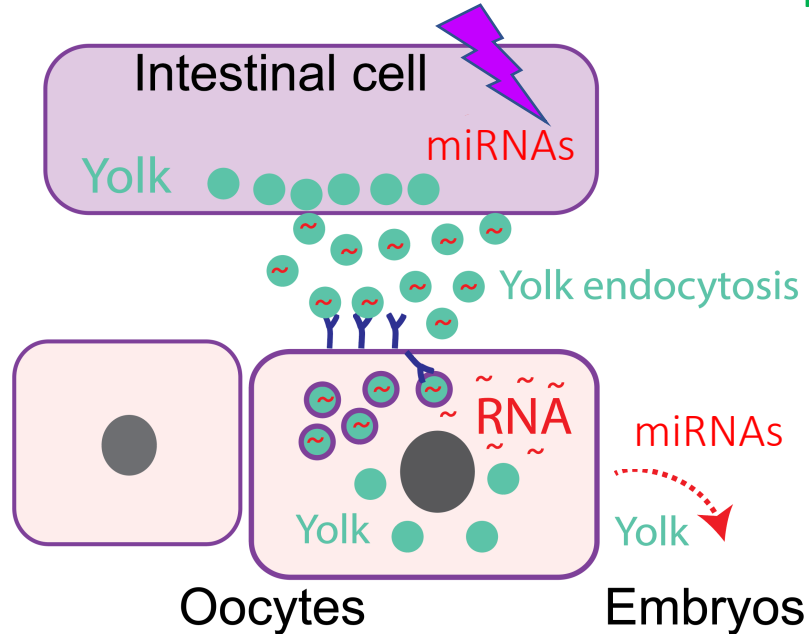
Environment



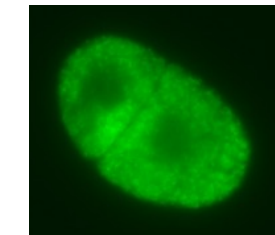
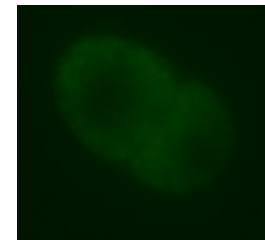
Embryo

YOLK in 2-cell embryos

osmotic stress

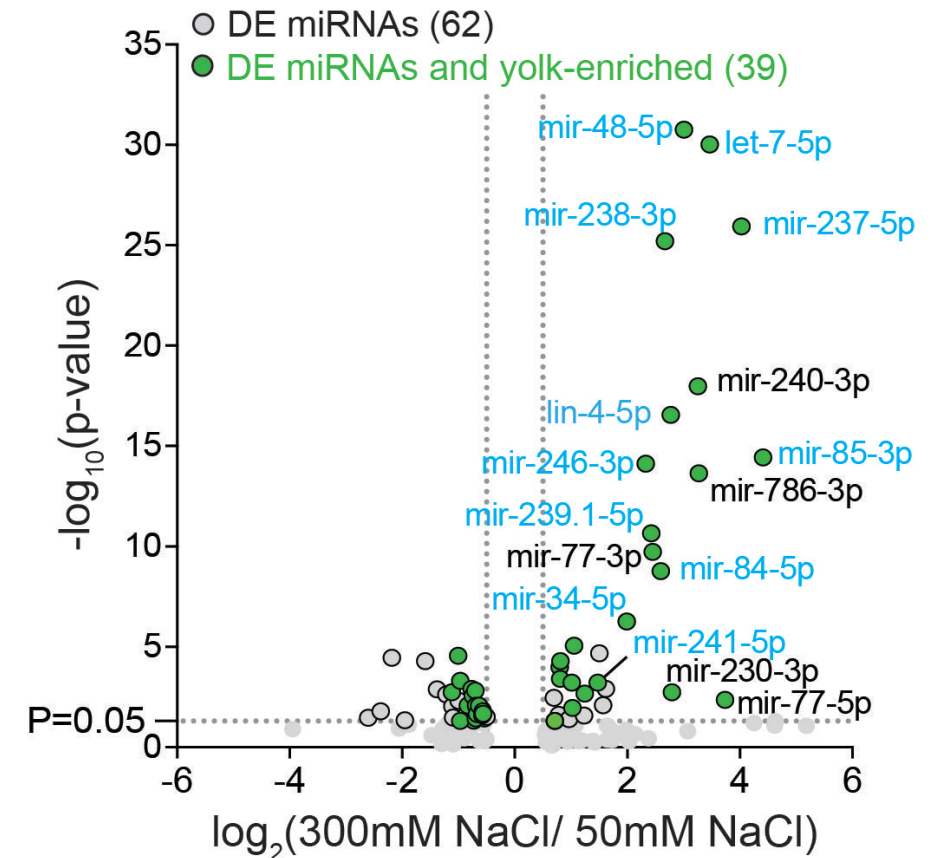


Non-stressed mother



Stressed mother

miRNAs in 2-cell embryos



Maternal environment alter yolk and miRNA inheritance

Meetali Singh
Postdoc

Névé Aupérin
Ph.D. student

Environment

miRNAs

Embryo

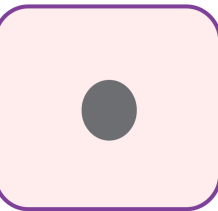
osmotic stress

Intestinal cell

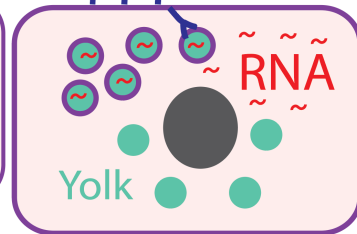
Yolk

miRNAs

Yolk endocytosis



Oocytes



Embryos

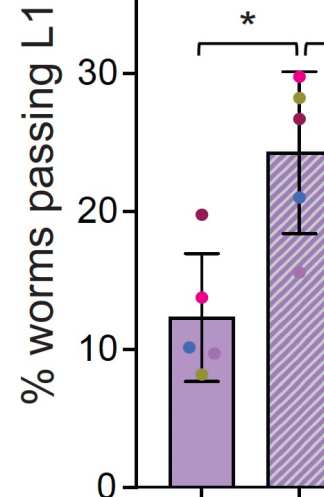
miRNAs

Yolk

Osmotic stress in mother

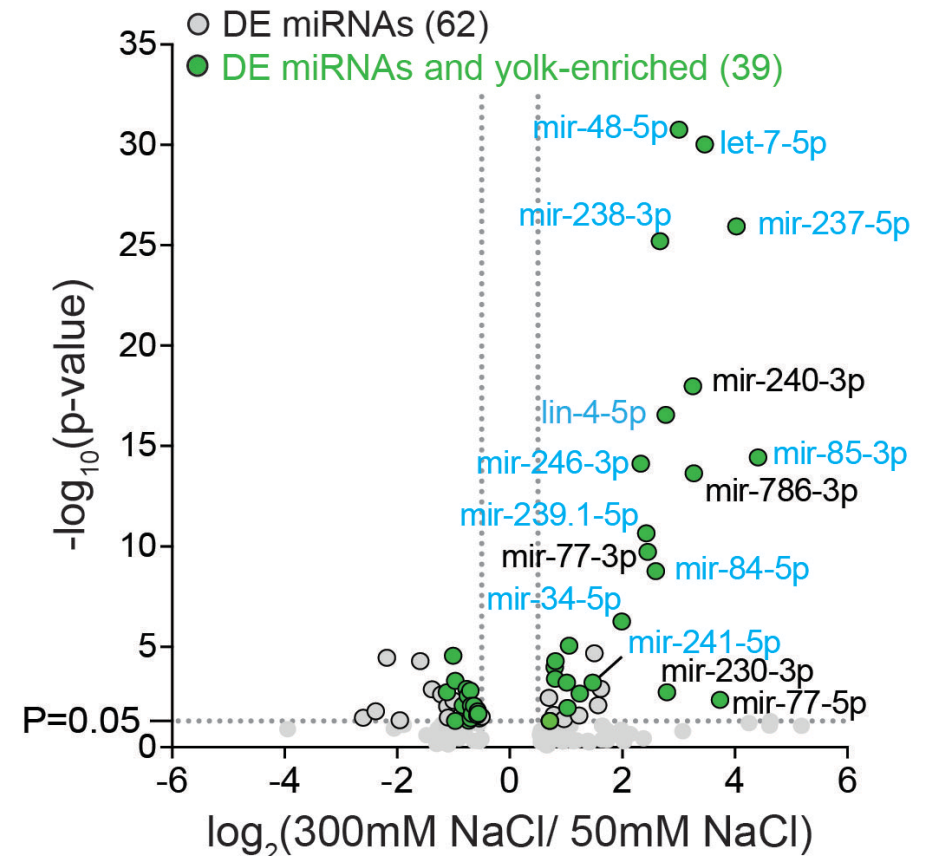
Progeny

osmotic stress
500mM NaCl



DP
degron
(control)

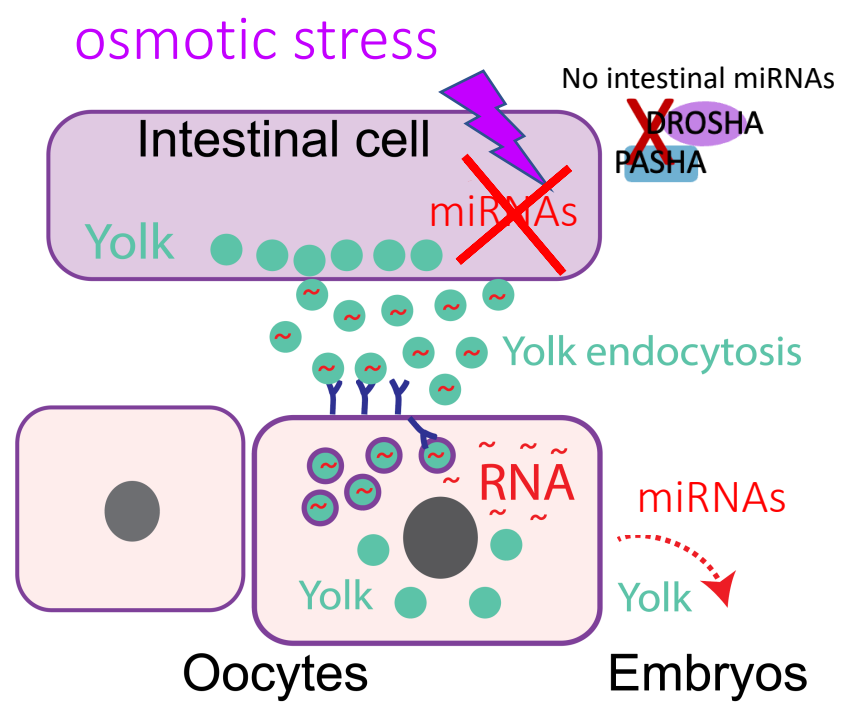
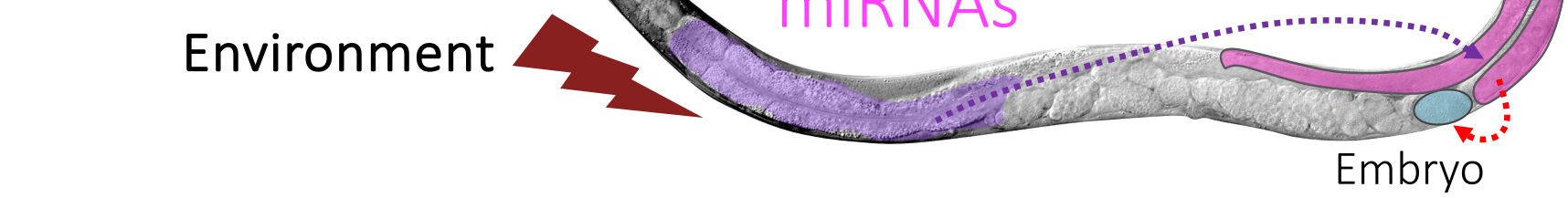
miRNAs in 2-cell embryos



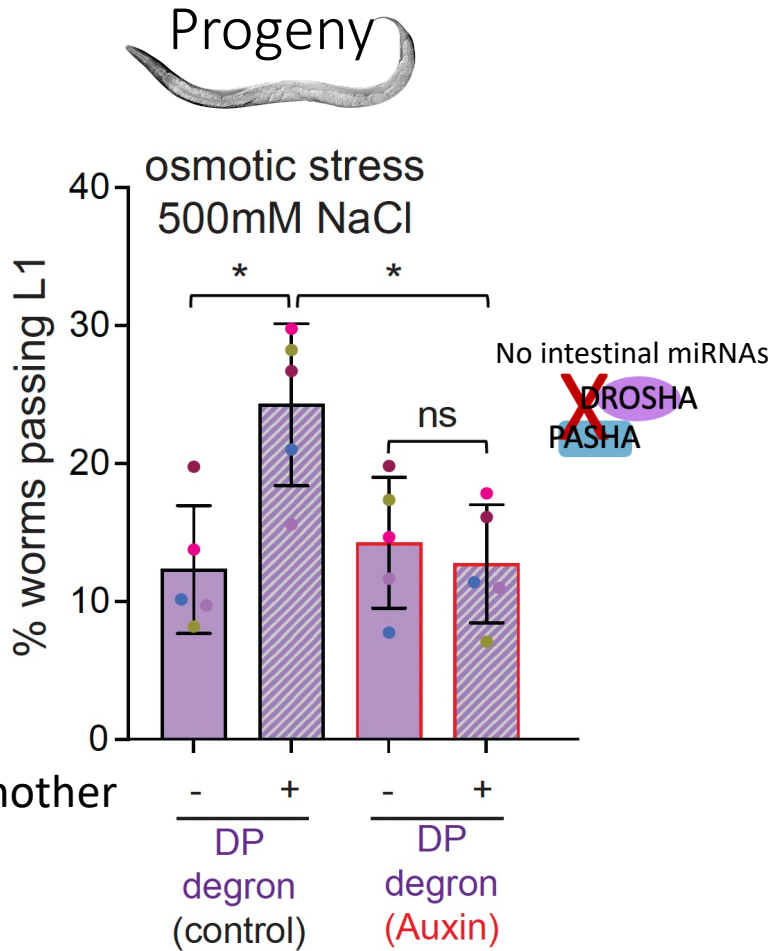
Maternal environment alter yolk and miRNA inheritance

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Postdoc

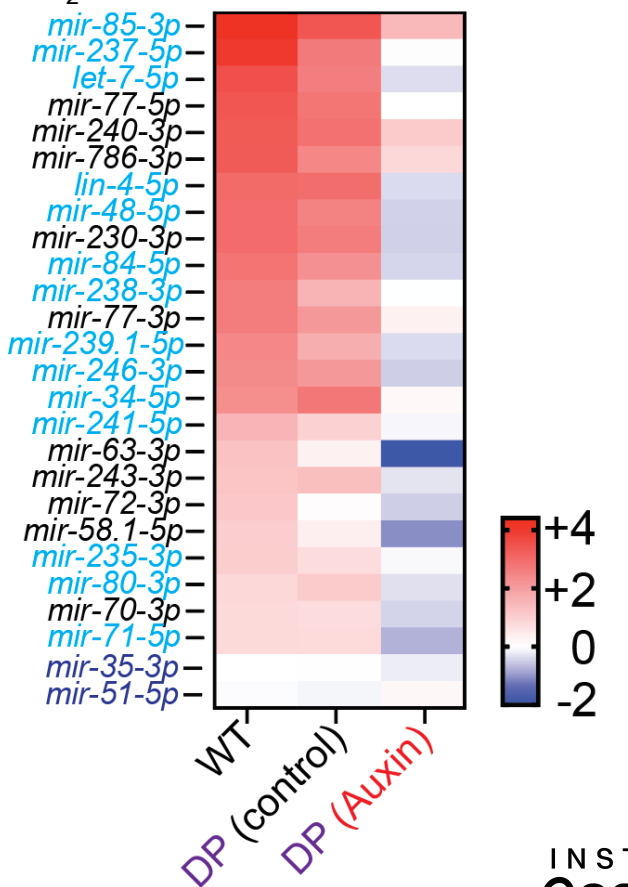
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Ph.D. student



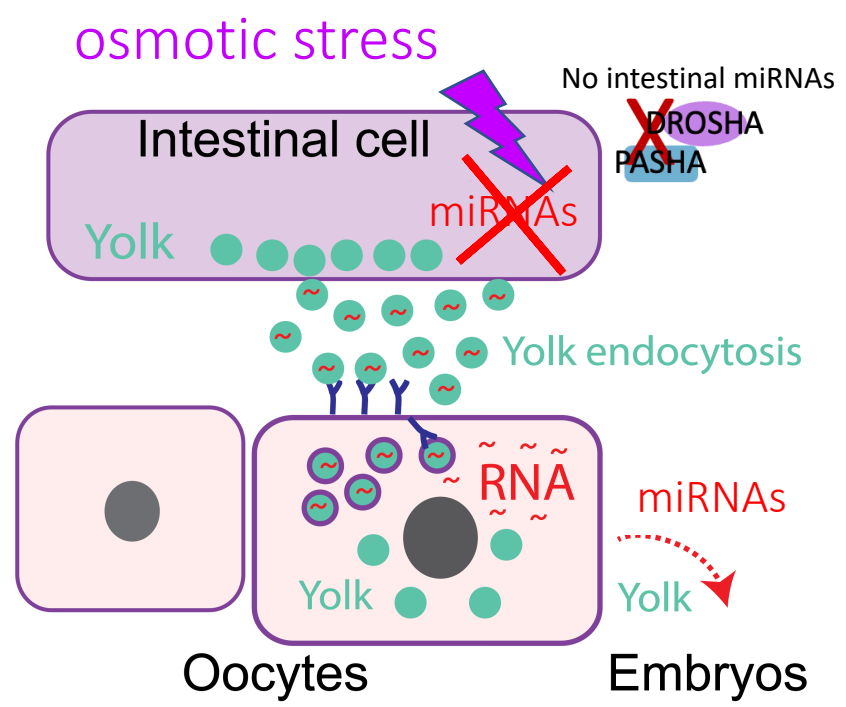
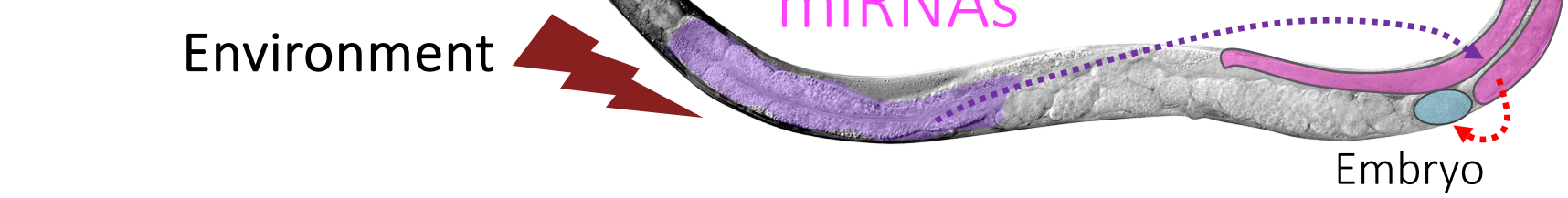
Osmotic stress in mother



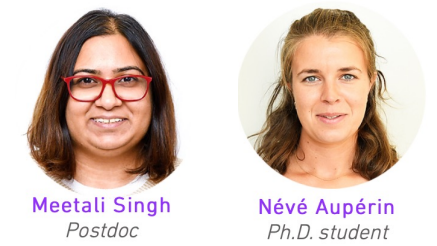
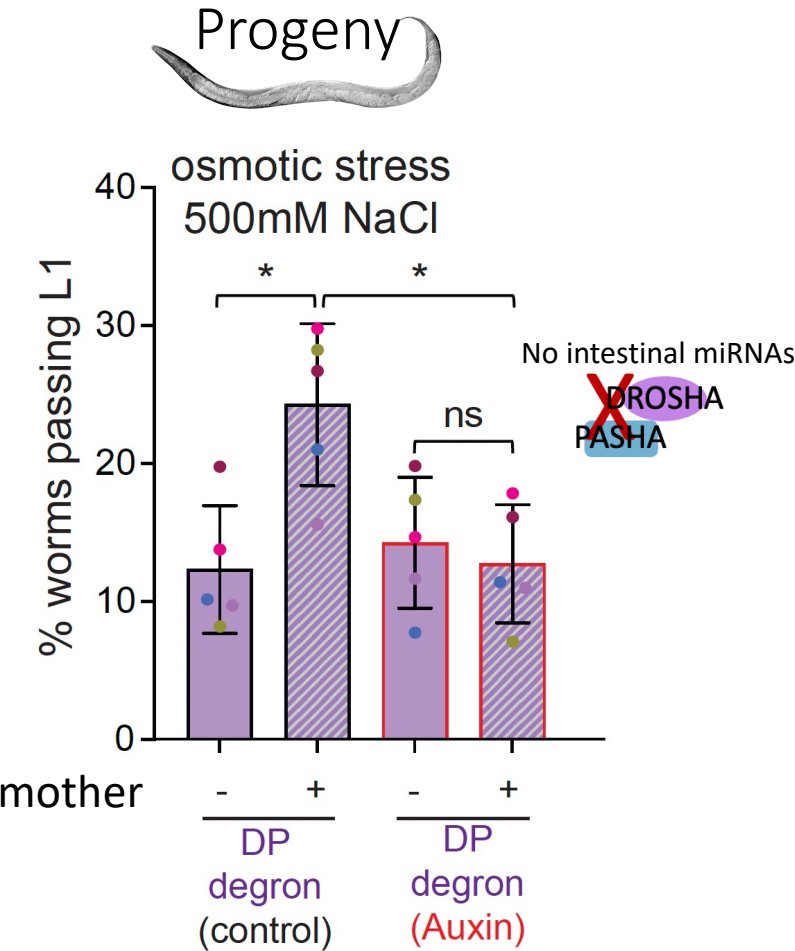
miRNAs in 2-cell embryos
log₂(300mM NaCl vs. 50mM NaCl)



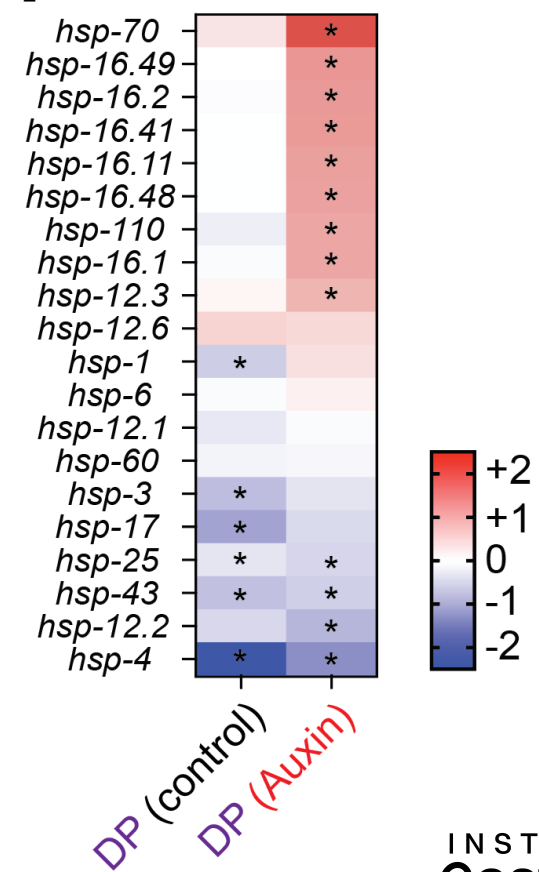
Maternal environment alter yolk and miRNA inheritance



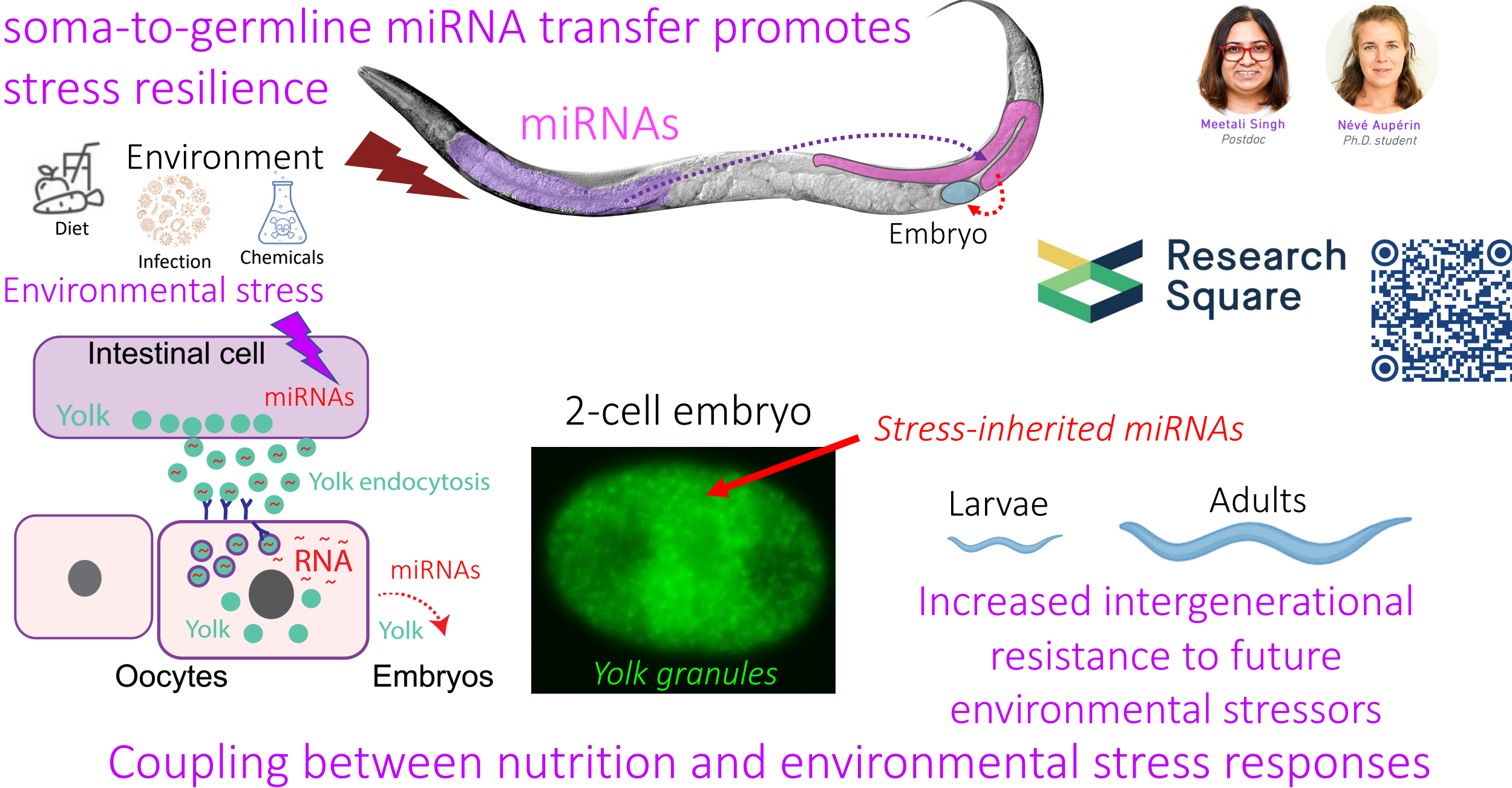
Osmotic stress in mother



RNA-seq in hatched L1
log₂(300 mM NaCl vs. 50 mM NaCl)



soma-to-germline miRNA transfer promotes stress resilience



Meetali Singh
Postdoc

Névé Aupérin
Ph.D. student



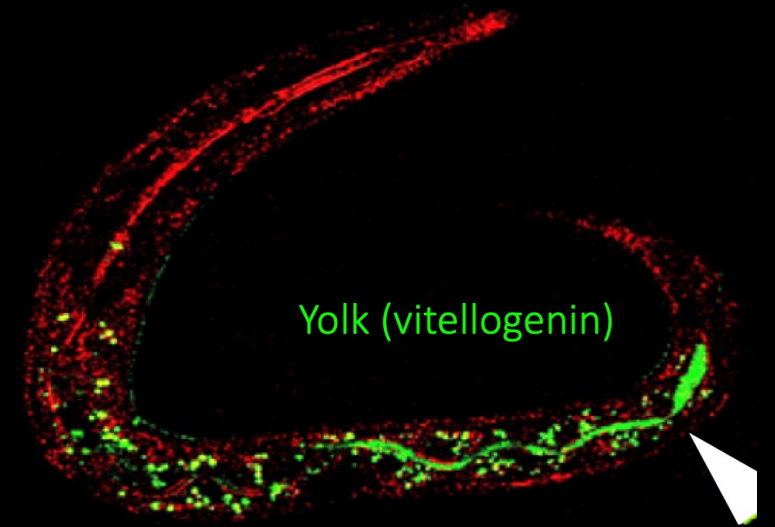
Research
Square





Kern et al., Nature Comms 2021 (David Gems Lab)

Larvae fed on vented yolk milk



Collaborations:

Marie Delattre Lab, *DNA elimination in nematodes*
(ENS, Lyon)

Marie-Anne Felix Lab, *C. elegans ecology*
(ENS, Paris)

Nicola Iovino Lab, *Drosophila chromatin epigenetics*
(Max Planck Institute Epigenetics, Freiburg)

Former Members:

Eric Cornes (postdoc) Meetali Singh (postdoc)



Group leader
Université de Bordeaux



Assistant Professor
Indian Institute of Science



Giorgia Barucci
(Ph.D. student)



Piergiuseppe Quarato
(Ph.D. student)
Now postdoc in Angelo Lombardo Lab

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