A Heterochromatin-Specific RNA Export Pathway Facilitates piRNA Production

Authors: Mostafa F. ElMaghraby, Peter Refsing Andersen, Florian Pu¨ hringer, ..., Thomas Lendl, Laszlo Tirian, Julius Brenneck

PIWI-Interacting RNA in Drosophila:
Biogenesis, Transposon Regulation, and Beyond

Authors:Haruna Yamashiro and Mikiko C. Siomi

PIWI- interacting RNAs: small RNAs with big functions

Authors: Deniz M. Ozata, Ildar Gainetdinov, Ansgar Zoch, Dónal O'Carrolland Phillip D. Zamore

piRNA Biogenesis in Drosophila Melanogaster

Xiawei Huang, Katalin Fejes Tóth and Alexei A. Aravin

RAHHALI KARIM
BONGIOVANNI WILLIAM
PETRINI ELENA
CANARUTTO GIULIA

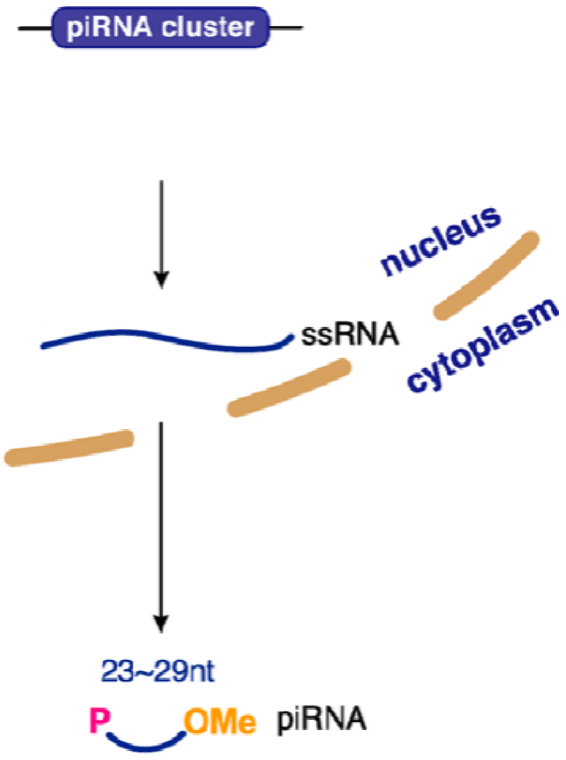
piRNAs

Introduction
-piRNAs
-Processing
-Interactors
-Canonical functions and other functions
-piRNA clusters and their export from the nucleus

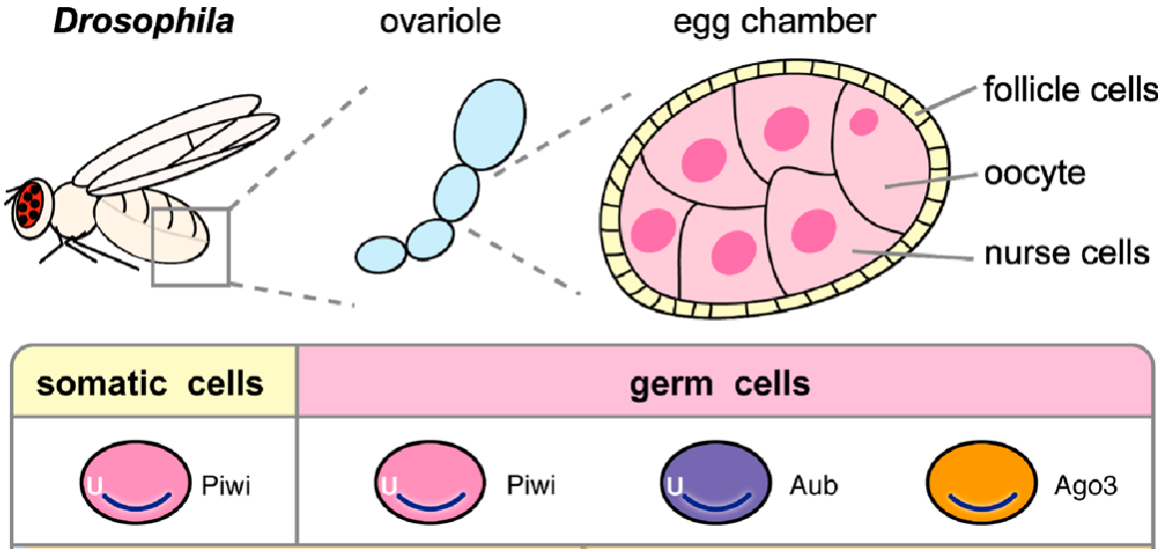
Paper
-Introduction to the study of the paper
-Mains experiment of the paper describing the research
-Conclusion

KEY PLAYERS

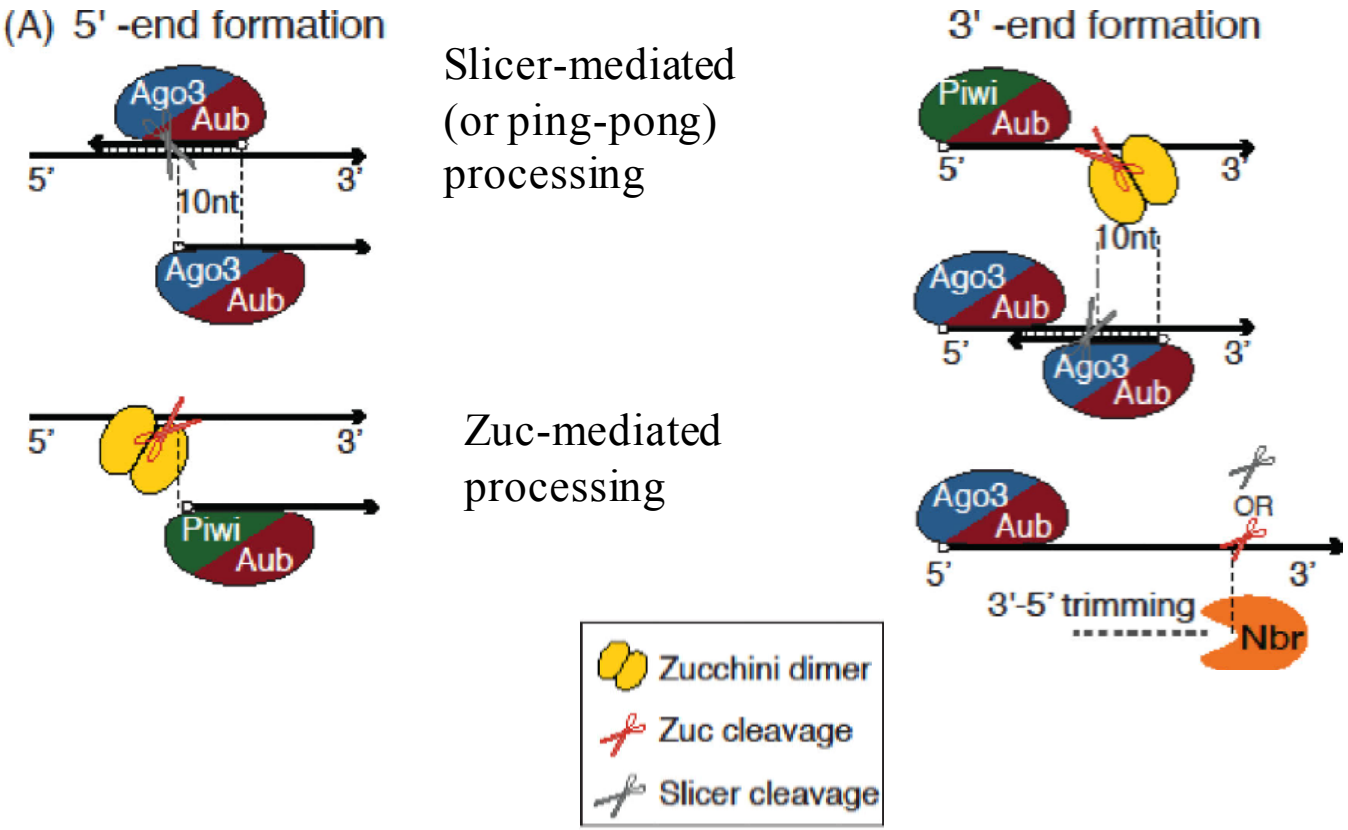
piRNA



PIWI proteins in Drosophila ovaries:
expression and transposon control mechanism



Processing of the 5'- and the 3'-end of piRNA



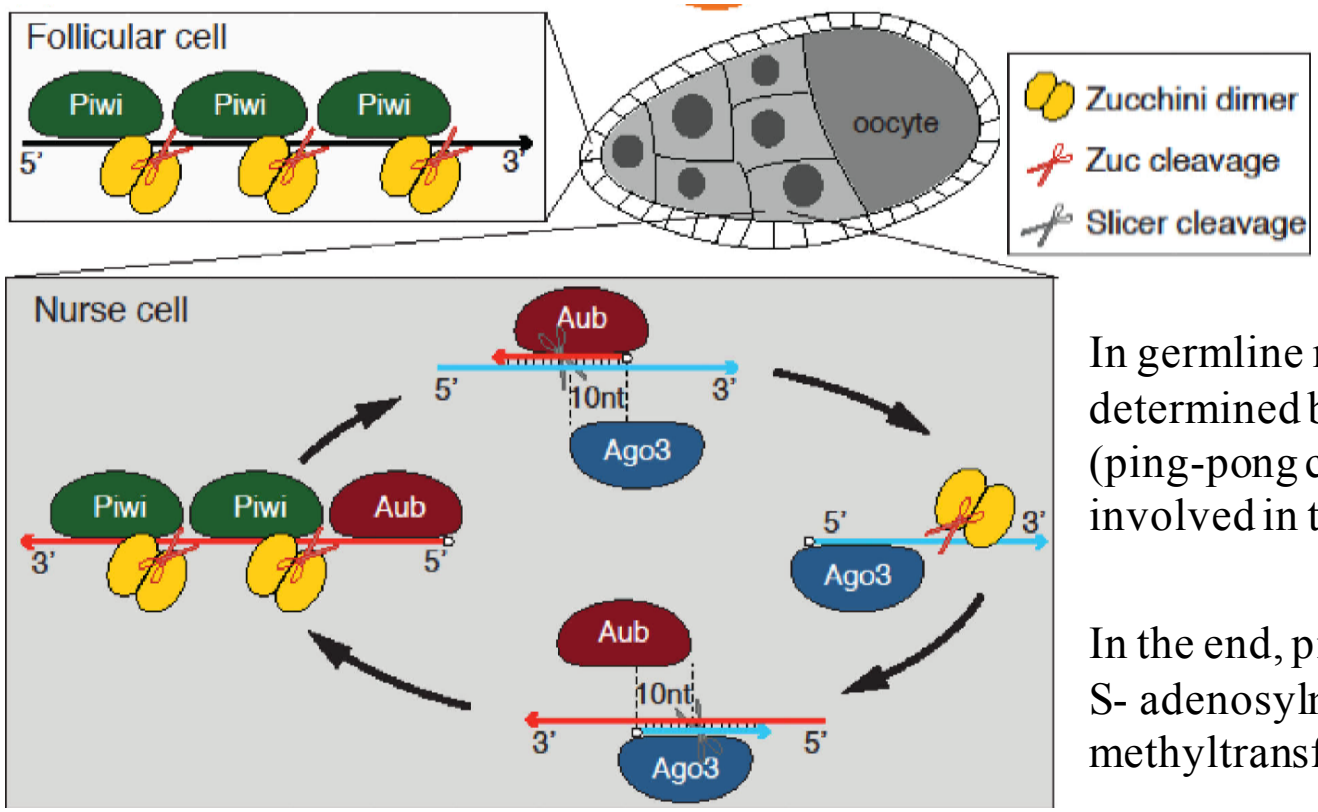
Slicer-mediated (or ping-pong) processing

Zuc-mediated processing

endonucleolytic cleavage by Zuc or slicer

by 3’ to 5’ trimming of longer precursors by the 3'-to-5’ Nibbler exonuclease

piRNA processing in somatic follicular and germline cells of Drosophila ovary

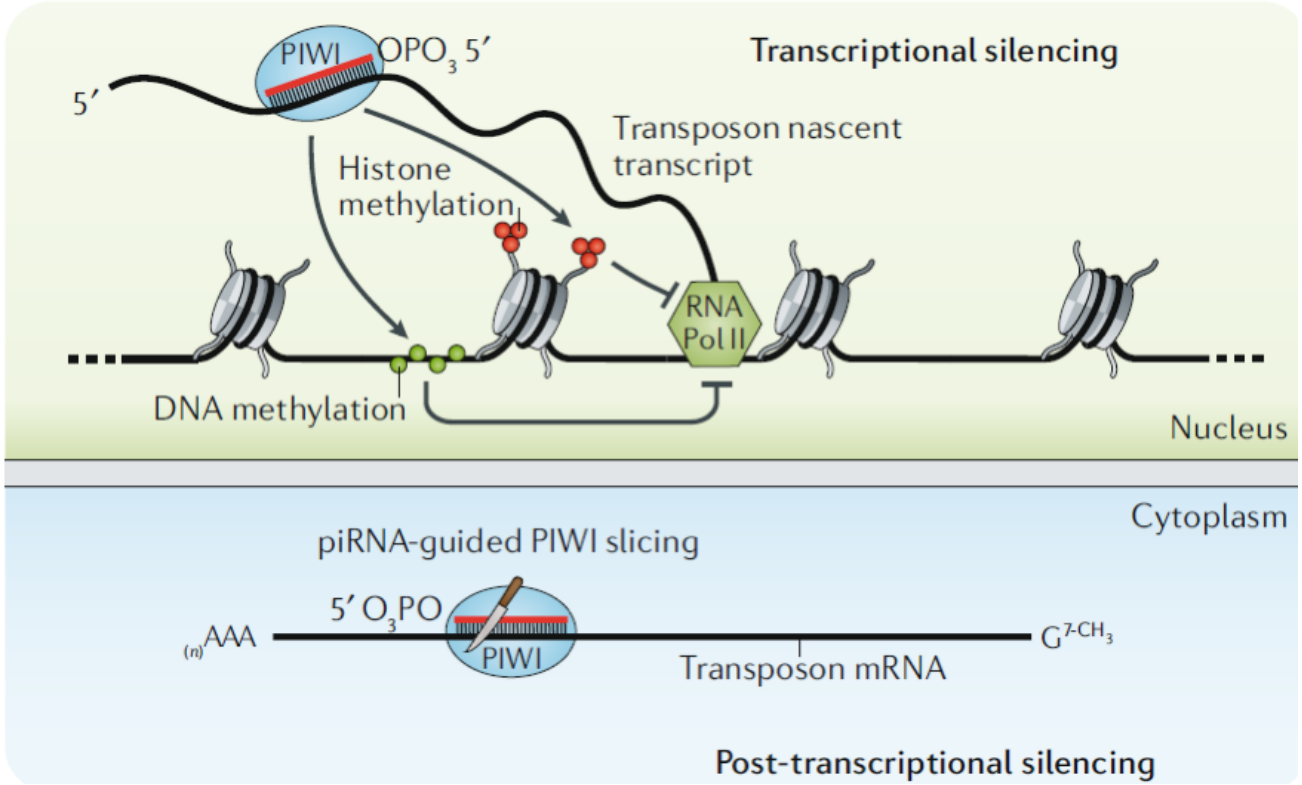


Only Piwi is expressed in follicular cells. In these cells both ends of mature piRNAs are formed exclusively through Zuc-mediated processing.

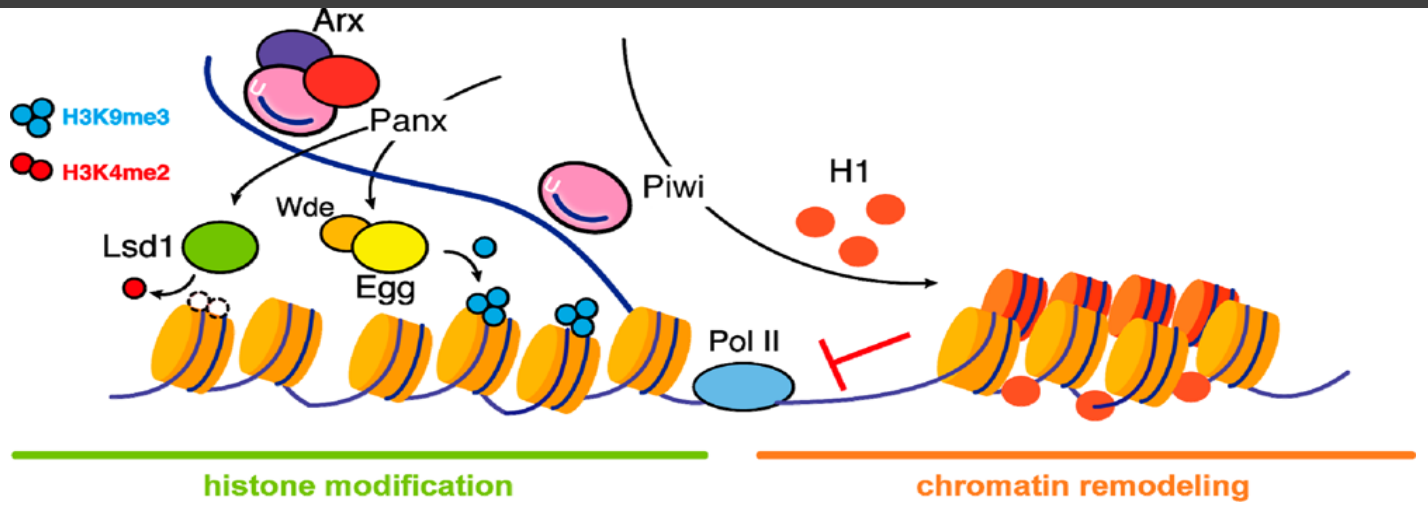
In germline nurse cells the 5'-end of piRNAs is determined by the Slicer activity of Aub/Ago3 (ping-pong cycle) while Zuc and Nbr exonuclease are involved in the 3'-end formation.

In the end, piRNA 3'ends are 2'-O- methylated by an S- adenosylmethionine (SAM)-dependent methyltransferase (Hen1 in flies).

PIWI- interacting RNAs (piRNAs) silence transposons transcriptionally and post- transcriptionally.



Transcriptional silencing of transposons in the nucleus.



The piRNA pathway provides features of both innate and adaptive immunity against transposons

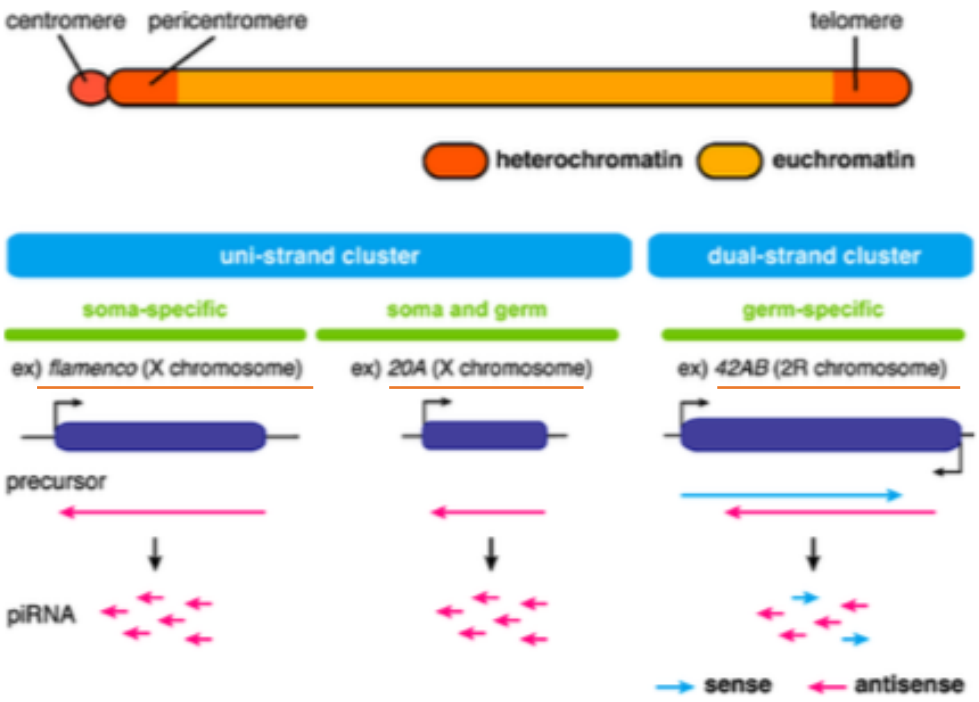
Hybrid Dysgenesis: a transposon carrying male mated to a naive female produces sterile offspring

Other functions in Drosophila

Protein-coding gene repression by piRNAs

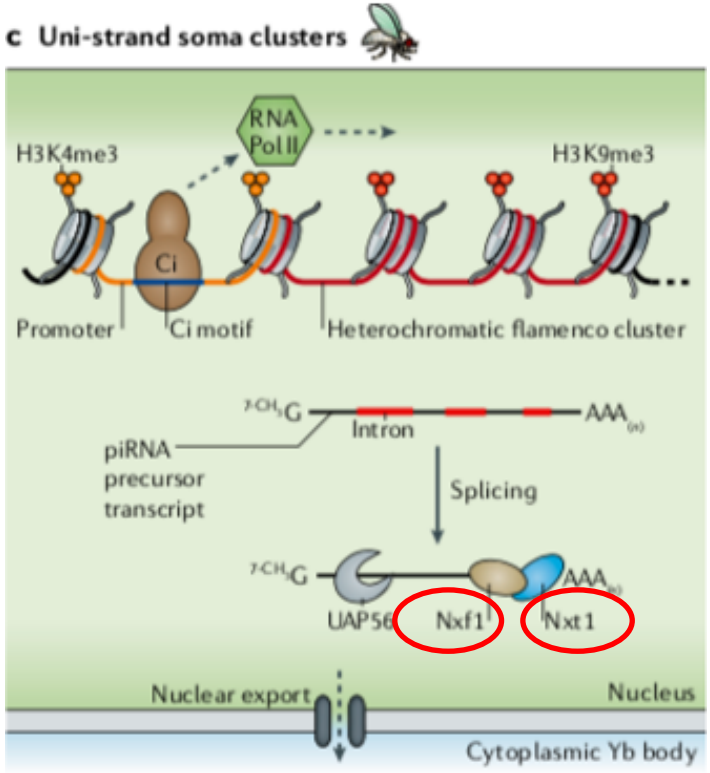
Maternal piRNAs specify the use of piRNA clusters in adult ovaries

piRNA clusters



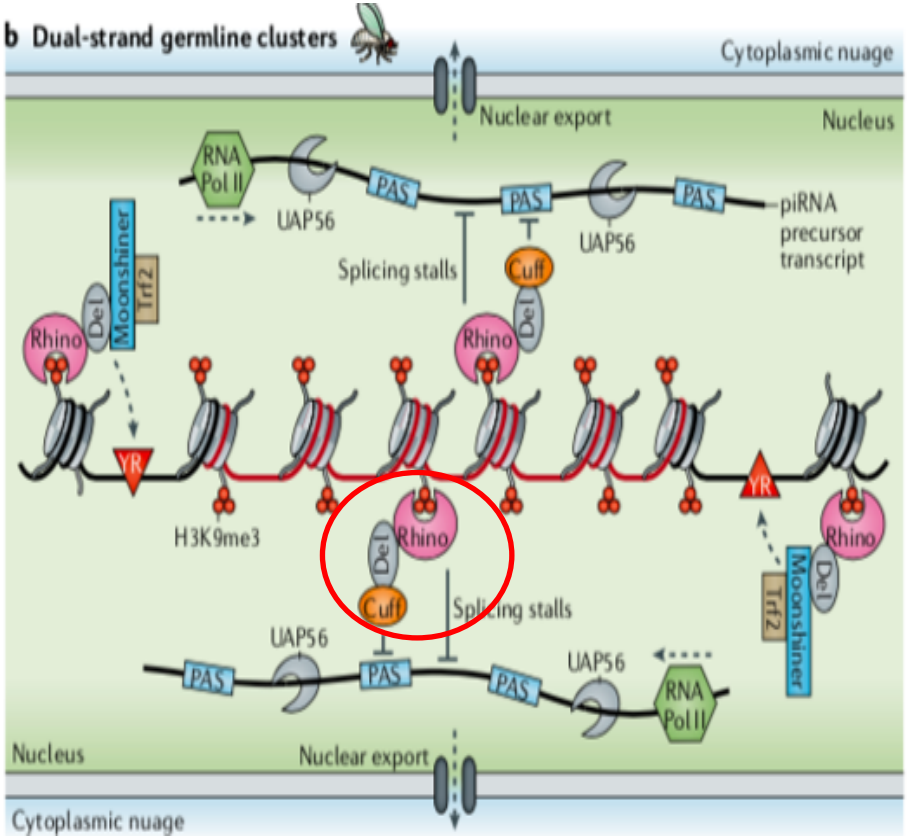
- piRNA clusters are the primary origin of piRNA in Drosophila
- Located in centromeric and pericentromeric region
- Transcribed by RNA pol II
- Two subgroups

Unistrand piRNA cluster



- Transcription requires cubitus interruptus factor (not specific for piRNA)
- RNA transcript are processed to have a 5'CAP and a 3' poly(A) and are even spliced
- Exported by Nxf1/Nxt1

Dual strand piRNA cluster



- Unique in Drosophila germ cells
- Do not possess their own promoter
- Rhino binds to H3K9me3
- Cutoff blocks splicing
- Moonshiner promotes initiation of transcription

Article

Cell

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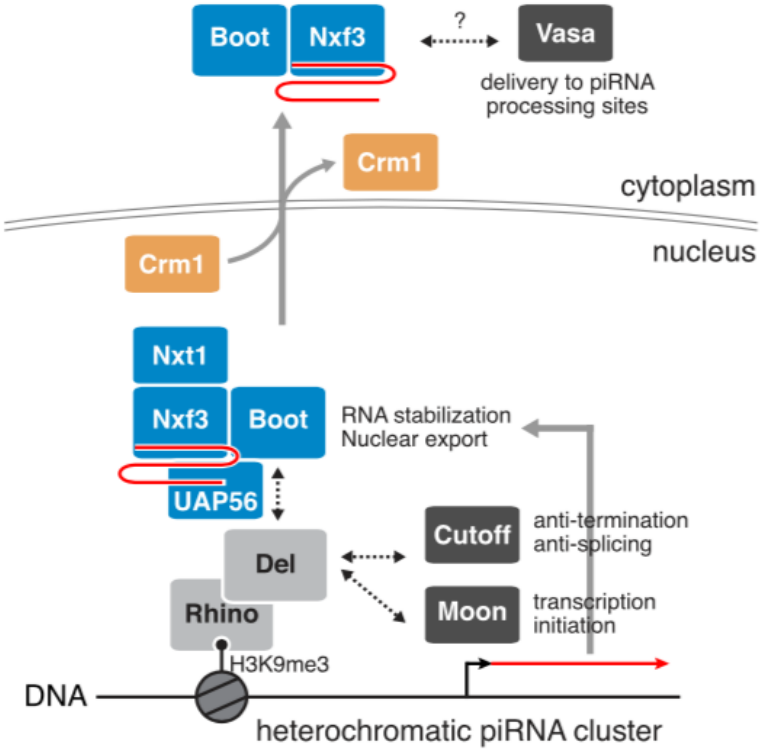
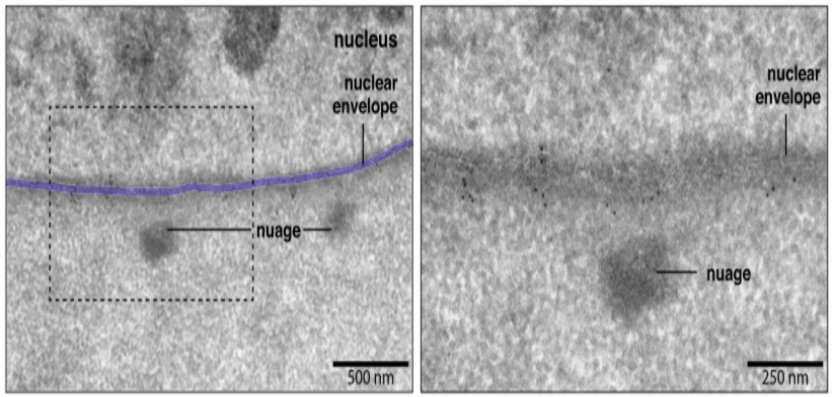


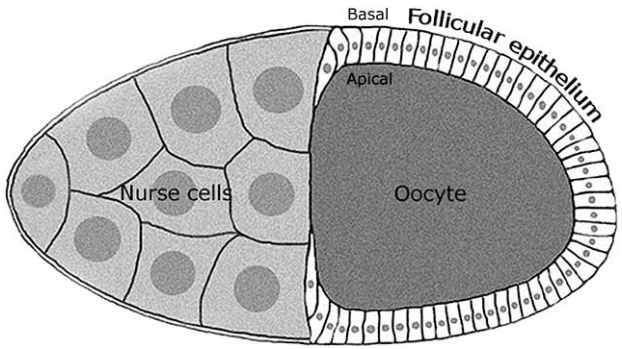
- All piRNA biogenesis factors are detected in cytoplasm
- Nuclear export is needed
- Rhino/Ded interact with Boot that recruits Nxf3/Nxt1 and UAP56 to piRNA precursors
- Export through Crm1 to peri-nuclear nuage

Nuages



- Nuages are piRNA processing site.
- Membraneless structures they are polymer condensate where piRNA precursors and proteins for their processing are highly enriched
- In perinuclear region

Transport route of Rhino dependent piRNA precursors in ovaries

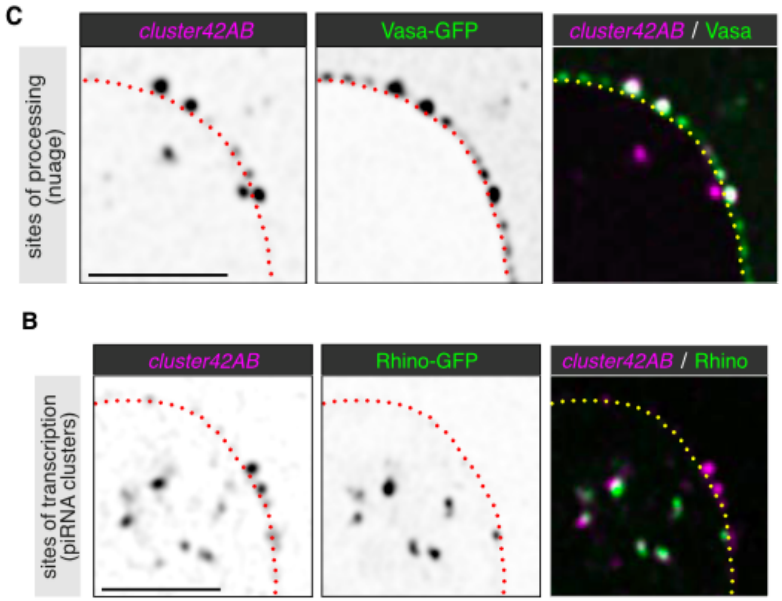


Where? Nurse cells

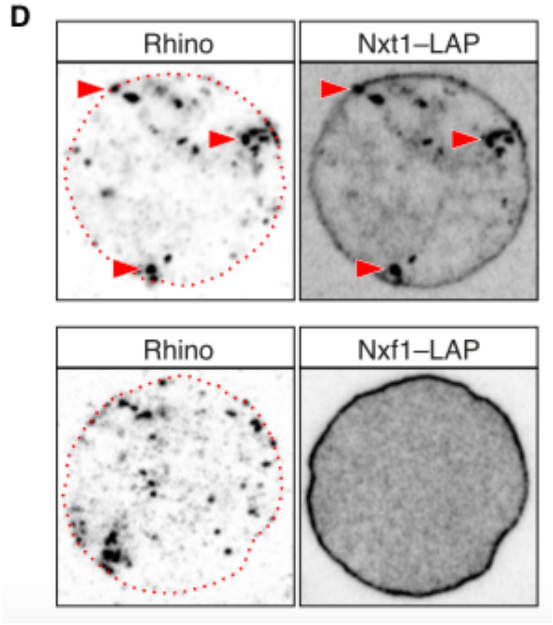
Why? Nurse cells dump their cytoplasm containing RNAs and proteins into the oocyte

- RNA FISH against piRNA cluster 42AB

Conclusion - Cluster enriched in nuclear foci and in cytoplasmic loci



Nxt1 for mRNA and piRNA export



Heterodimeric nuclear receptor Nxt1/Nxf1 is essential for mRNA export

Is Nxt1/Nxf1 important also for piRNA export pathway?

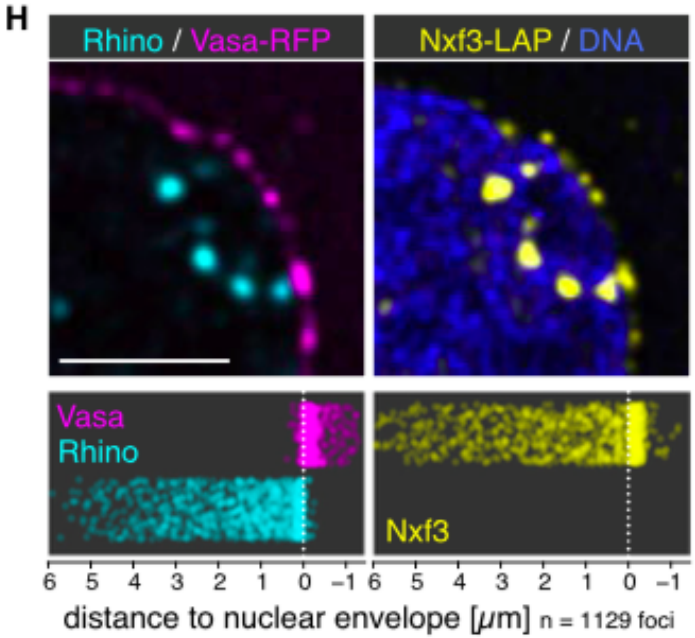
Only Nxt1-LAP was enriched at nuclear Rhino foci, while Nxf1-LAP was dispersed in the nucleus but located in the nuclear envelope.

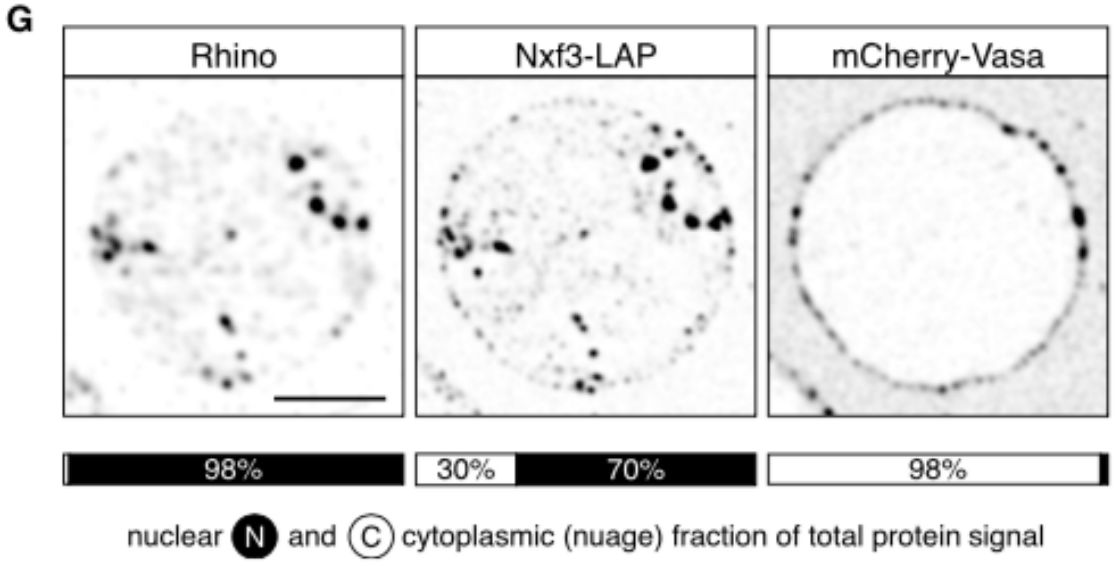
Conclusion - Nxt1 is involved in Rhino-dependent piRNA clusters export

Nxf3 Localizes to piRNA Transcription and Processing site

- Affinity purification
Nxf3 highly enriched in Nxt1-LAP eluates

- Expression of Nxf3-LAP specially occurs in the nucleus co-localize with Rhino as well as in cytoplasmic foci (Vasa factor positive foci)





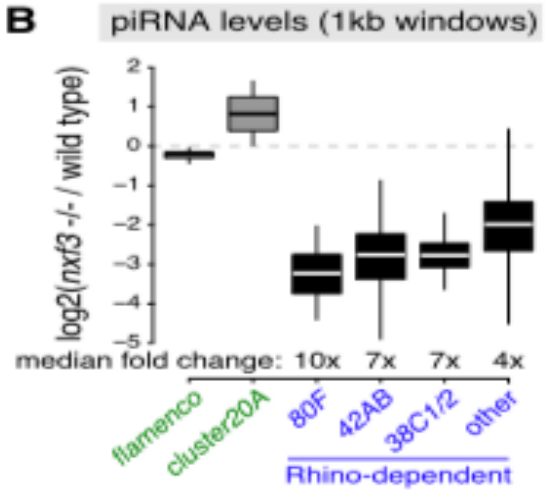
Of all known proteins localizing to Rhino-dependent piRNA precursor, Nxf3 is the only one with dual localization

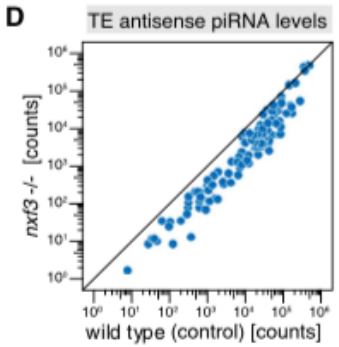
Conclusion - Nxf3 is essential to transport piRNA precursors from nucleus to cytoplasmic processing site

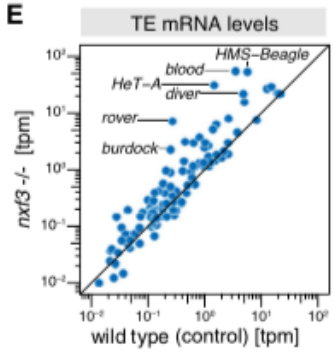
nxf3 mutant cells show in de-repression of transposons

Flies carrying nxf3 null-alleles show a 10 fold reduced levels of piRNA originating from Rhino-dependent piRNA source loci

Rhino-independent piRNA source was unaffected







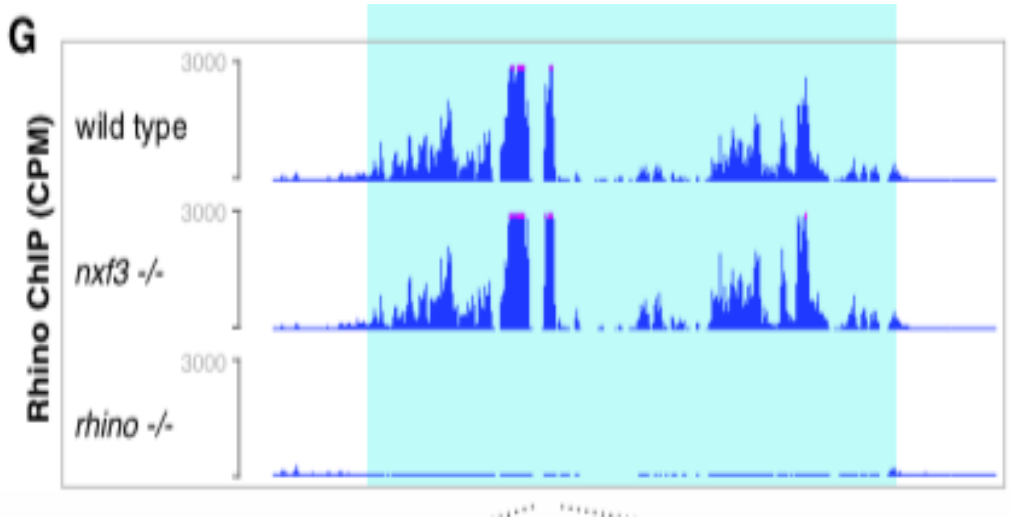
Transposons are derepressed

Nxf3 null-alleles flies were female sterile due to severe oocyte DNA damage

Rhino localization was unaffected in Nxf3 null-alleles

Rhino's genome wide enrichment at piRNA source loci was unchanged in nxf3 null cells

Conclusion - piRNA loss is due to defects downstream pathway of piRNA cluster specification and transcription

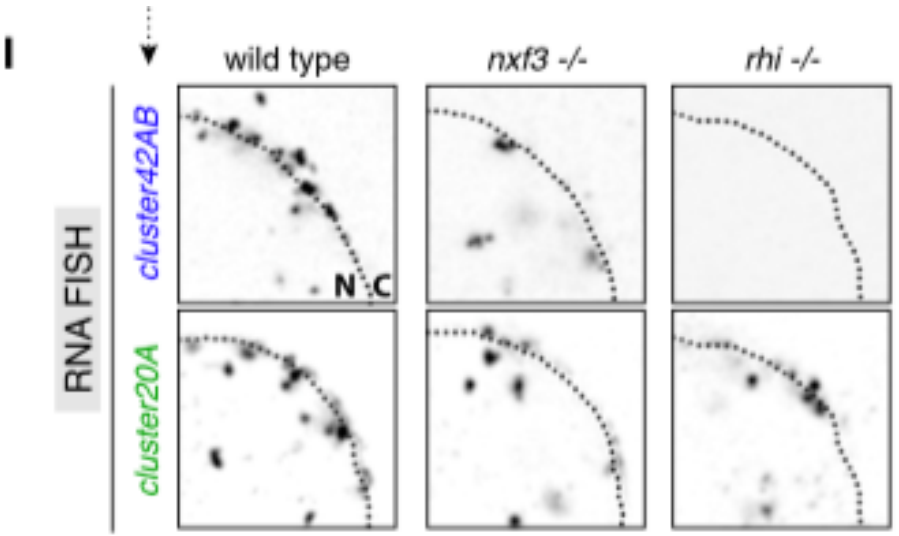


piRNA Precursor Export Requires Nxf3

Subcellular localization of *cluster42AB* with the Rhino-independent *cluster20A* serving as a control

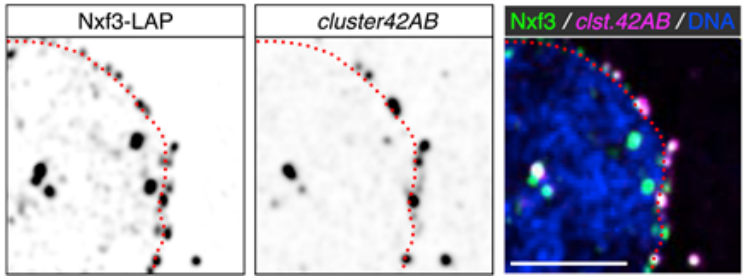
In Rhino mutants nuclear and cytoplasmic foci of cluster42AB was lost but nuclear localization was unaffected in nxf3 null cells

Conclusion - Nxf3 has a direct role in stabilization and nuclear export of Rhino-dependent piRNA cluster



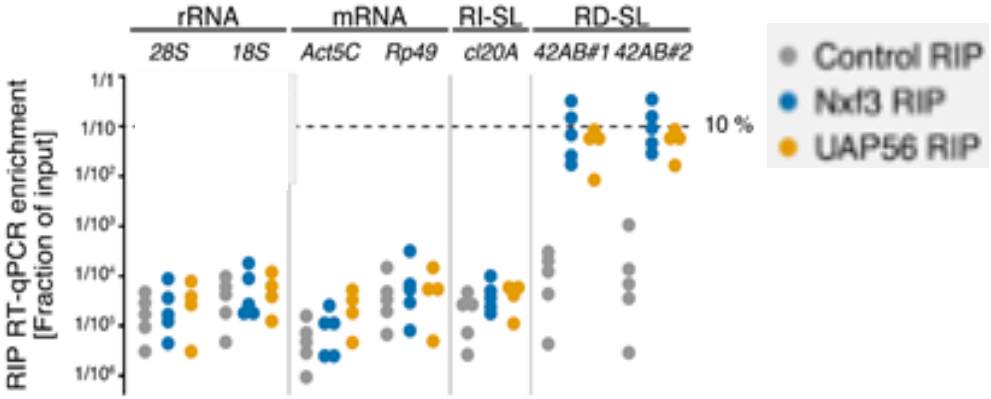
Investigating Nxf3 interaction with piRNA precursors

Using CRISPR-Cas9 to generate Nxf3-LAP and observing with RNA FISH:

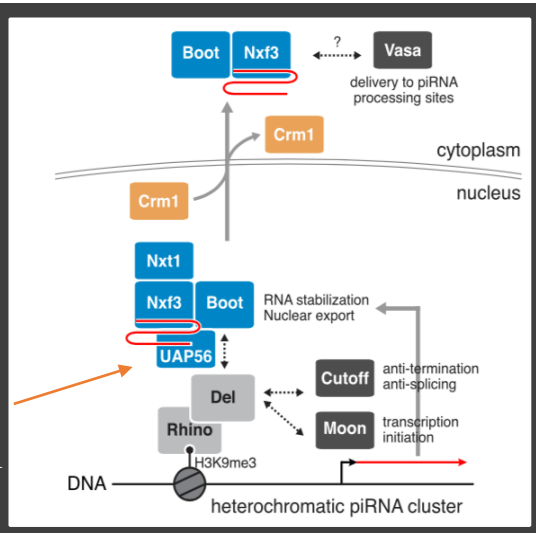


direct interaction between Nxf3 and the piRNA precursor RNAs

RNA immune-precipitation anti Nxf3 and UAP56, and qRT-PCR on the elution:

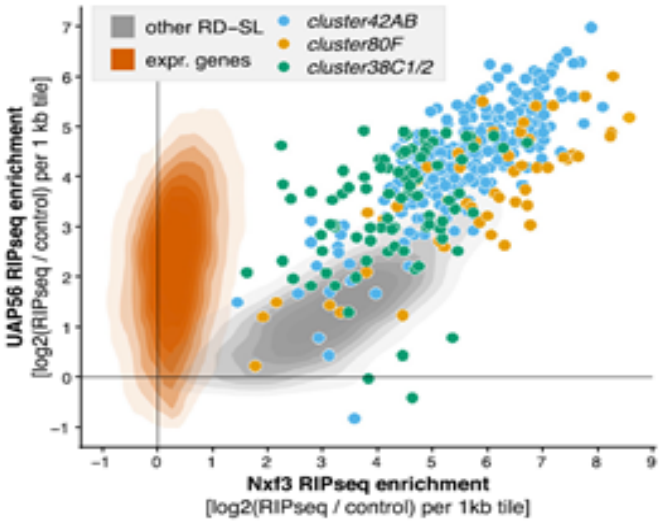


UAP56 is an helicase which binds Rhino-dependant piRNA precursors



Investigating Nxf3 interaction with piRNA precursors

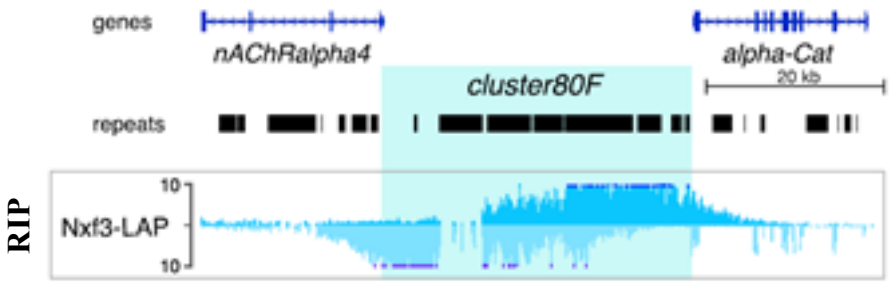
Sequencing of the libraries derived from RIP



Transcripts of cluster Rhino-dependent interact with both Nxf3 and UAP56

Transcripts of cluster Rhino-independent interact only with UAP56 (interacting with Nxf1)

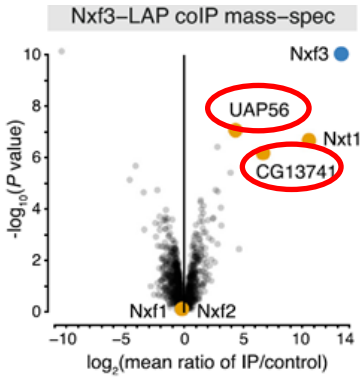
Observing the distribution of the transcript bound by Nxf3



The top strand and the bottom strand precursors are asymmetrically distributed following a model where high Rhino levels in the cluster drive bidirectional transcription initiation

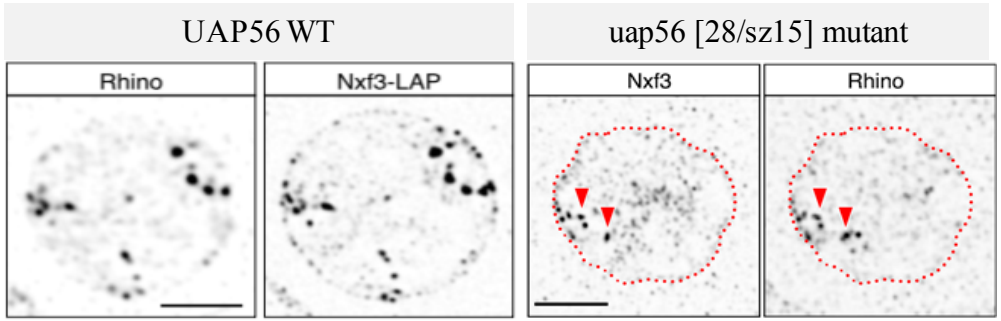
Molecular mechanism of Nxf3

Immuno-purification of Nxf3-LAP to identified co-eluting proteins



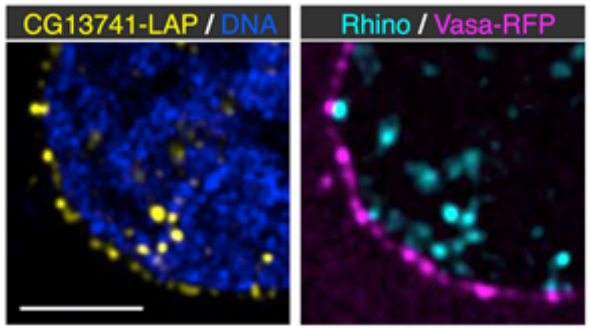
UAP56

uap56[28/sz15] mutation

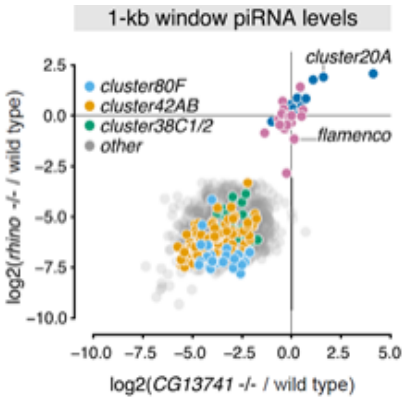


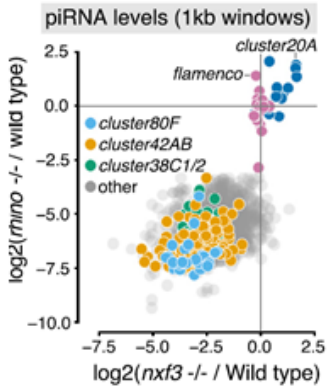
CG13741

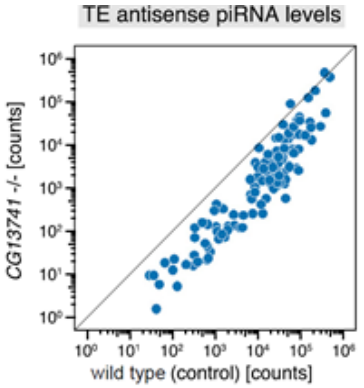
Using CRISPR-Cas9 to generate CG13741-LAP and observing with RNA FISH

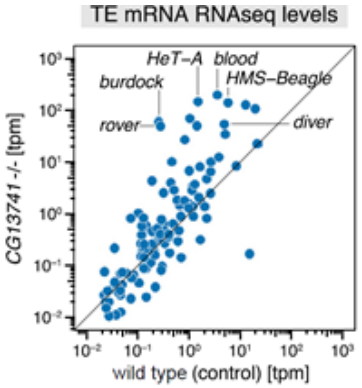


Generation of CG13741 frameshift-null alleles





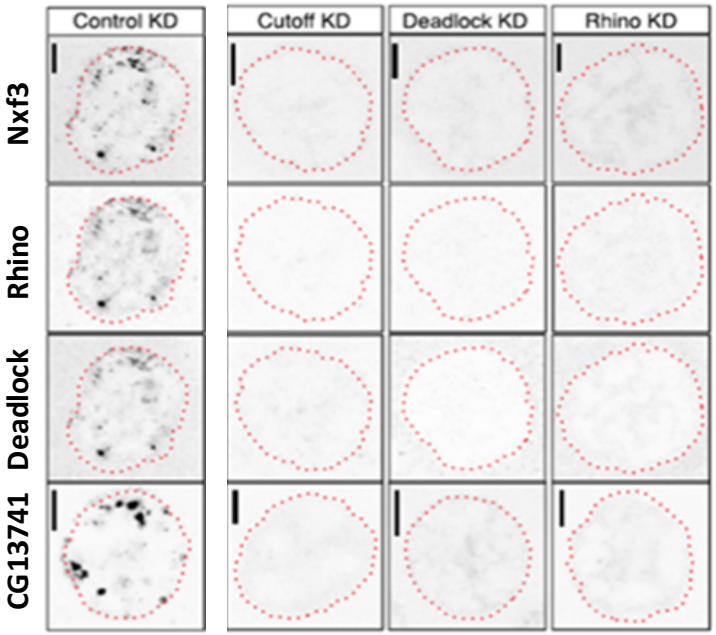




Determination of the dependency between CG13741 and the other proteins of the export machanism

To study the interaction of CG13741 and the other proteins we used different knock-out models

Rhino/Deadlook + Cutoff induce a recruitment of Nxf3/Nxt1 and UAP56 to piRNA precursors in an unknown way



Rhino, Dedlook, Cutoff

⬇

CG13741 + Nxf3-Nxt1

Determination of the dependency between CG13741 and the other protein of the export machanism

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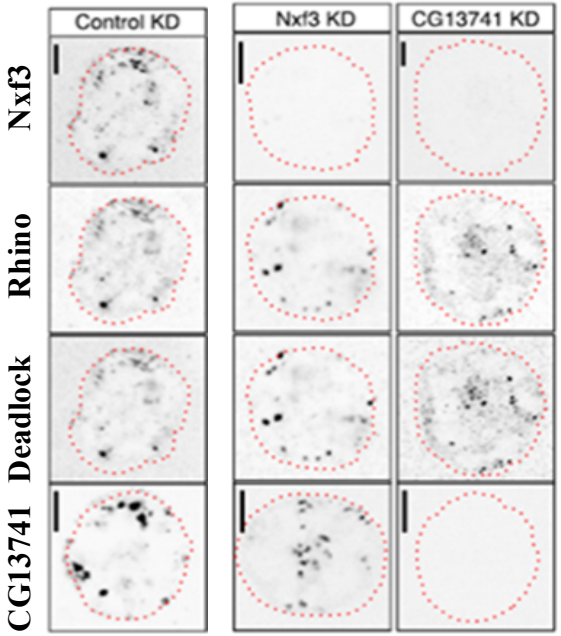

Rhino, Dedlook, cutoff

⬇

CG13741

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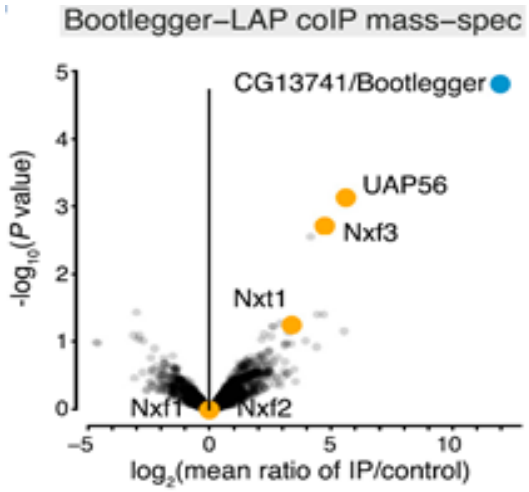
Nxf3-Nxt1

CG13741 is named Bootlegger

What is the connection of Bootlegger to Rhino-Deadlock-Cutoff complex?

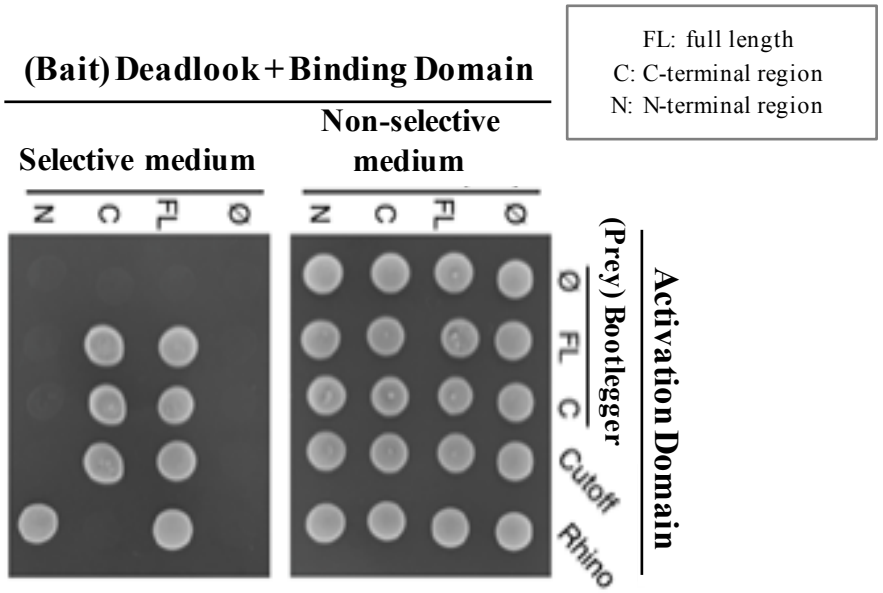
Immuno-purification of Bootlegger-LAP



No results

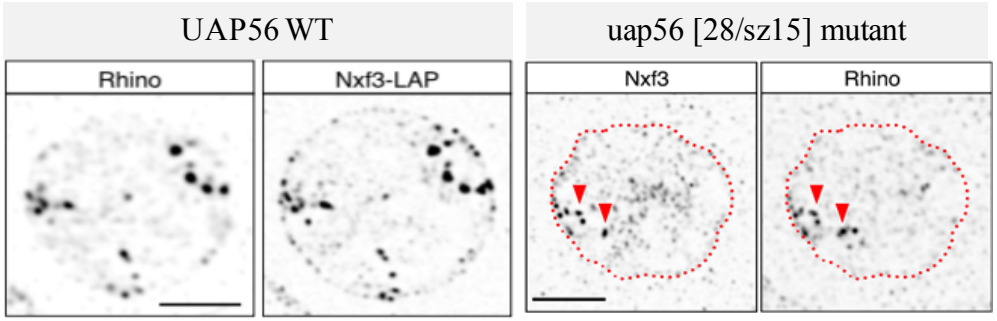
Yeast two-hybrid screen with Deadlock as bait

It act as adaptor of Rhino to recruit Cutoff in the compex

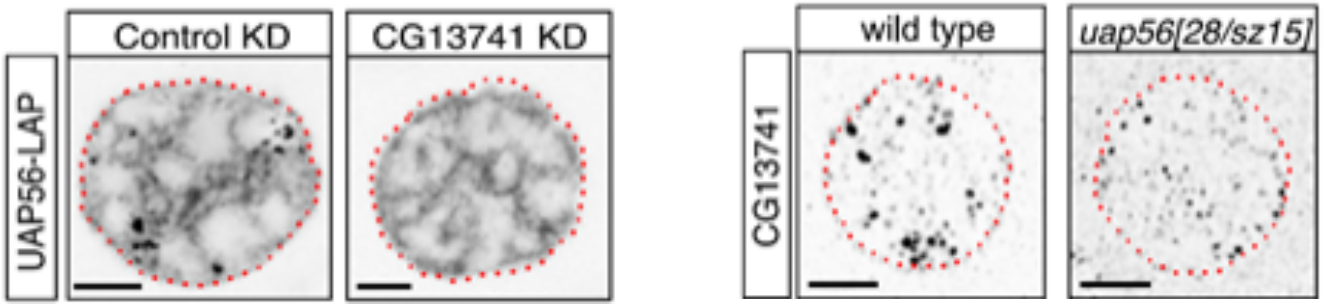


C-terminal Deadlook interacted with the C-terminal of Bootlegger

There is a contribution of Nxf3 or of Bootlegger to recruit UAP56?

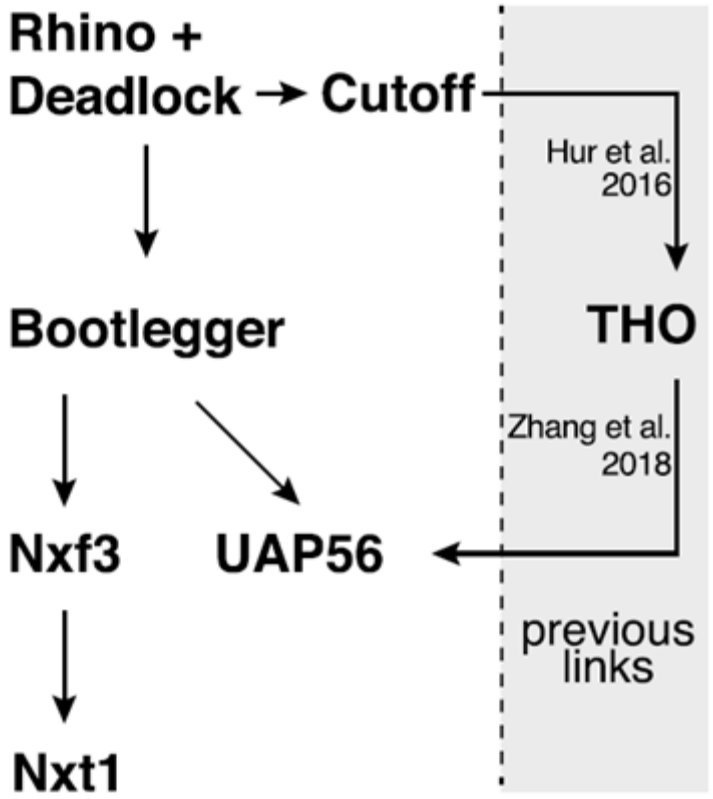


Nxf3 needs UAP56 to be present



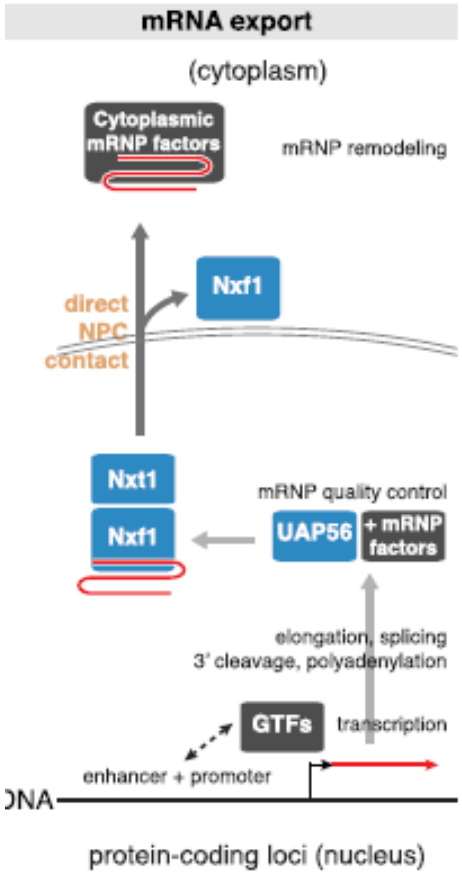
UAP56 needs Bootlegger to be present

Recruitment Hierarchy



Nxf1 role and characteristics

1. Nxf1-Nxt1 hetero-dimer
2. mRNA export receptor



- Nxf1/Tap and its binding partner Nxt1/p15 are the most prominent mRNAs export receptor in cells

- Nxf1-Nxt1 hetero-dimer exports mature mRNAs and unistrand piRNA

- Few piRNAs precursors in Drosophila are spliced and polyadenilated and thus require the mRNA export receptor Nxf1-Nxt1

Most of them lack these processing marks so there must be another export pathway

Nxf3 and Nxf1: differences and analogies

1. RNA cargo specificity
2. RNA cargo fate after NPC passage

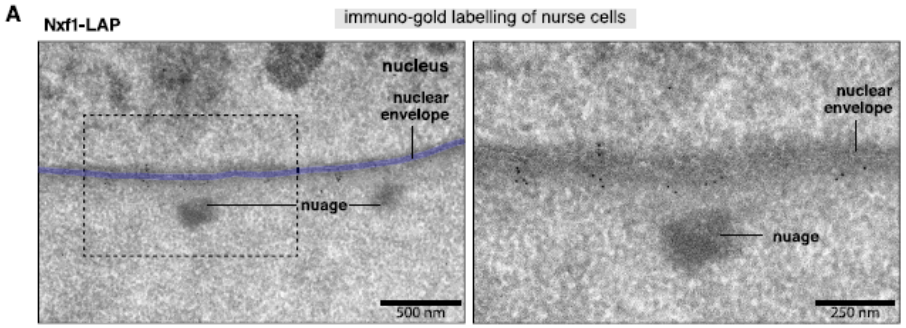


Fig. S6A: Trasmission electron microscopy images of nurse cells expressing Nxf1-LAP at different magnifications.

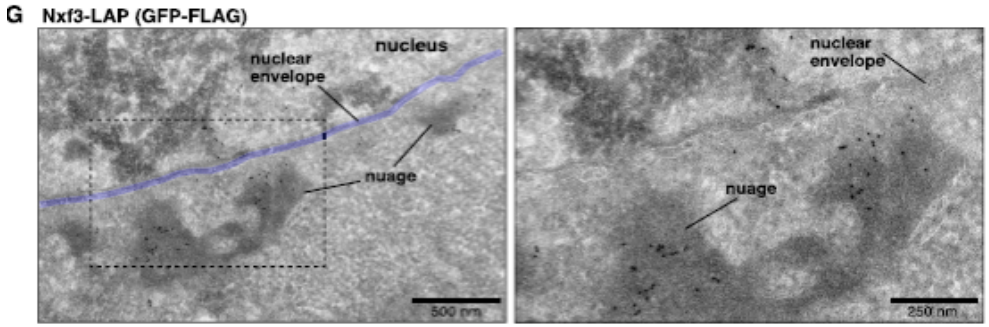


Fig. S1G: Trasmission electron microscopy images of nurse cells at different magnifications.

How is nuclear export attained?

- Nxf1
  - ➡ Two nucleoporin binding sites
    1. Nfx1 ubiquitin-associated domain (UBA)
    2. NTF2-like domain

- Nxf3
  - ➡ No nucleoporin binding sites

Investigation of the role of Nxt1

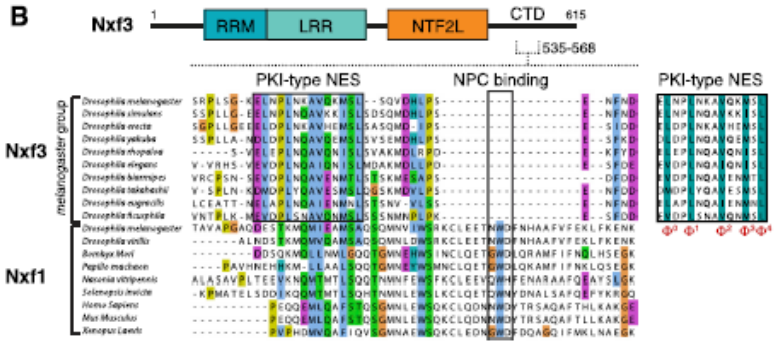


Fig. 6A: Protein domain composition of Nxf1, Nxf3, and Nxt. The PKI type NES in Nxf3 is indicated.

Nxt1 can bind Nxf3's NTF2-like domain.

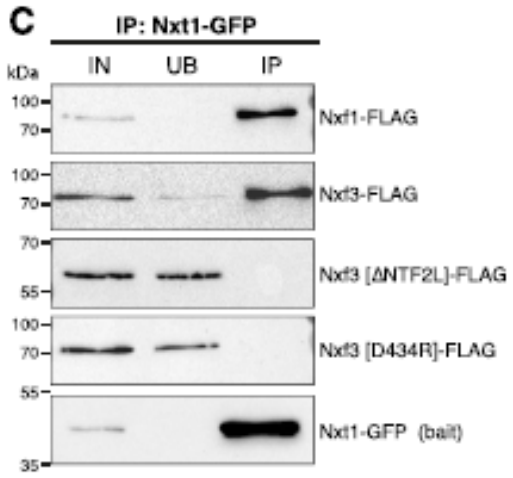


Fig. S6C: western blot analysis of Nxt1-GFP co-immuno-precipitation.

Mutation introduced in Asp-434 causes the loss of the interaction between NTF2-like domain and Nxt1.

Consequences of D434R engineered locus

1. Reduced levels of Nxf3 *in vivo*
2. Cannot bind Nxt1 above background
3. Interaction with UAP56 and Bootlegger present

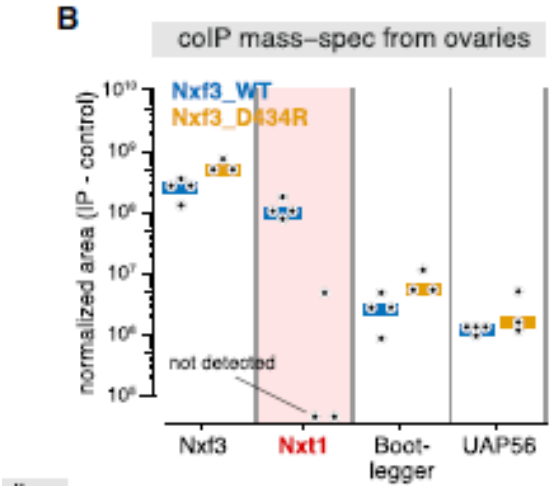


Fig. 6B: Absolute peptide peak intensities for Nxf3 interactors from co-IP of Nxf3-LAP (WT) and D434R.

4. Nxf3 localized in the nuage in a reduced matter
5. Nxf3 co-localized with Rhino at piRNA clusters in the nucleus

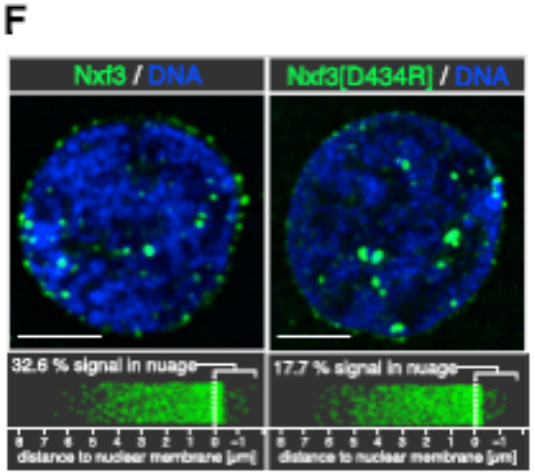


Fig. S6F: Localization of indicted Nxf3-LAP proteins.

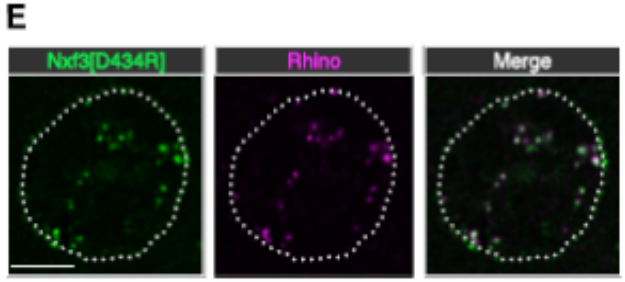


Fig. S6E: Showing co-localization of Nxf3-LAP [D434R] and Rhino.

Consequences of D434R engineered locus

6. Subfertile Nxf3 mutants

7. Transposon de-silencing

8. Reduced presence of cluster42AB transcripts

9. Reduced presence of piRNA from Rhino-dependent source loci transcripts

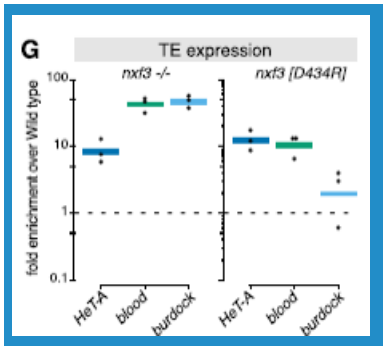


Fig. S6G: Changes in RNA levels of indicated transposons.

Human NXF3

1. Orphan variant
2. Generated through duplication
3. NPC translocation using a NES, recognized by Crm1

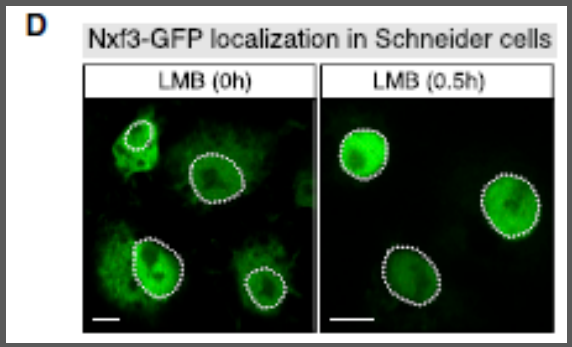


Fig. 6D: Sub-cellular localization of Nxf3-GFP in untreated (left) or LMB-treated (right) S2 cells.

nxf3[M553P] mutants

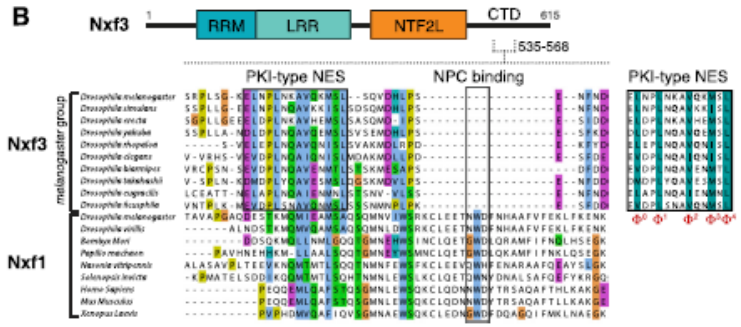


Fig. S6B: Protein sequence alignment displaying the nucleoporin interacting motif in Nxf1 as well as the position of the PKI-type NES in Nxf3.

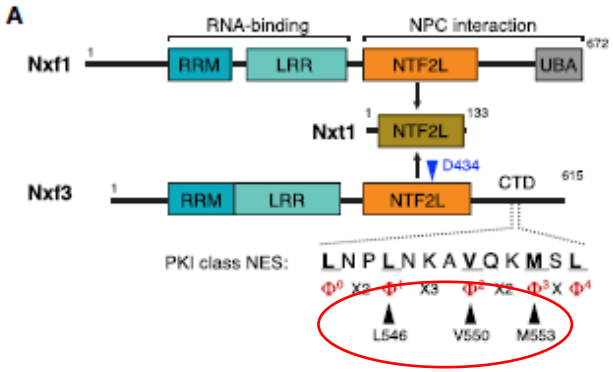


Fig. 6A: Protein domain composition of Nxf1, Nxf3, and Nxt. The PKI type NES in Nxf3 is indicated.

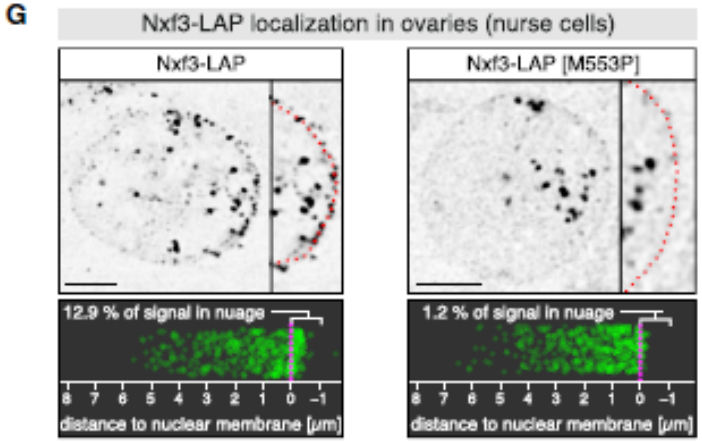


Fig. 6G: Localization of Nxf3-LAP (left) or Nxf3-LAP [M553P] (right) in nurse cells.

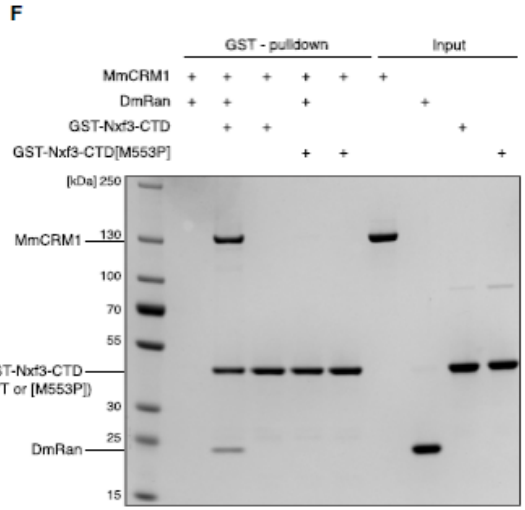


Fig. 6F: SDS-PAGE gel showing in vitro binding assay of GST-tagged Nxf3-CTD peptides, MmCRM1, and DmRanQ69L.

nxf3[M553P] mutants

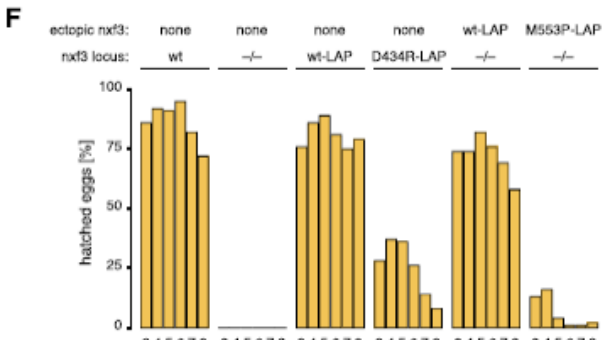


Fig. S7F: Bar plot summarizing hatching rate (in percent) of eggs.

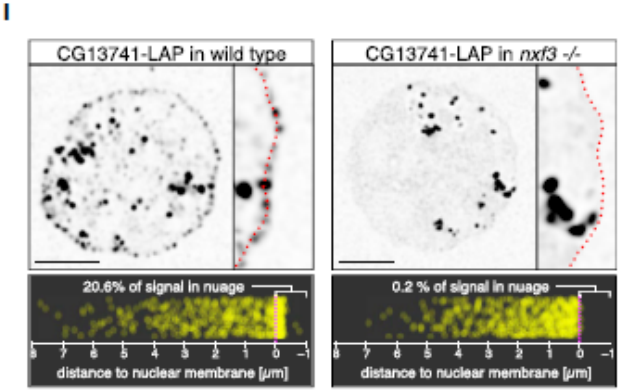


Fig. 6I: Localization of Bootlegger.

Nxf3-Bootlegger pathway

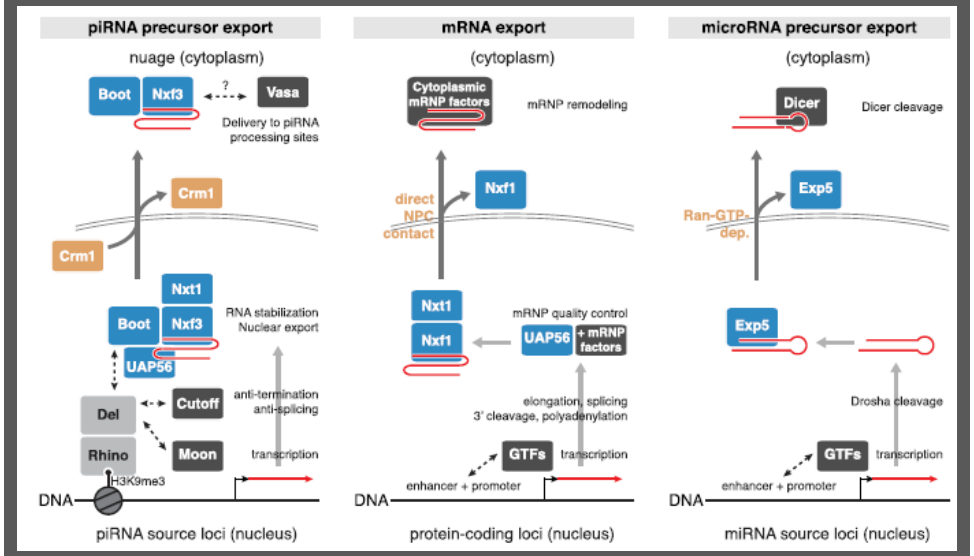
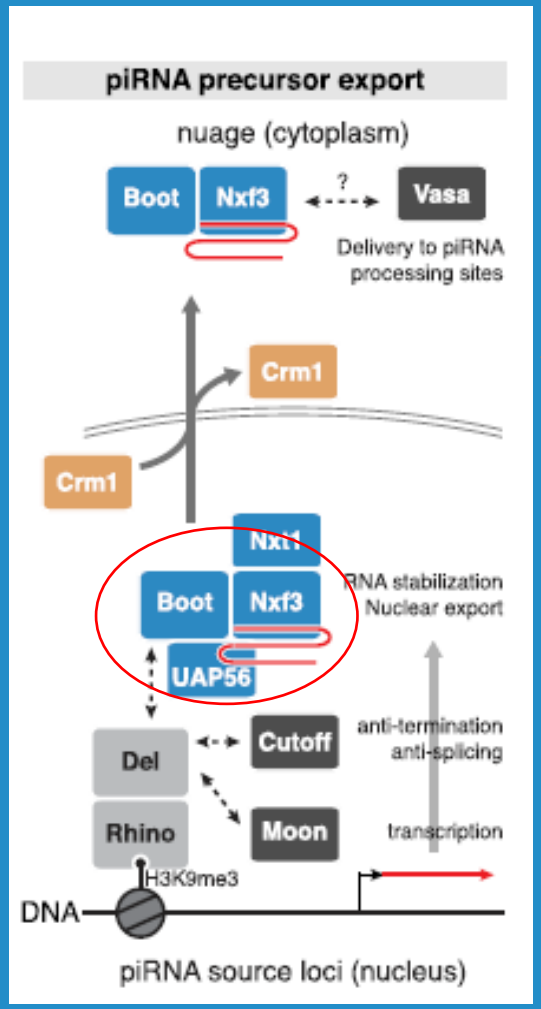
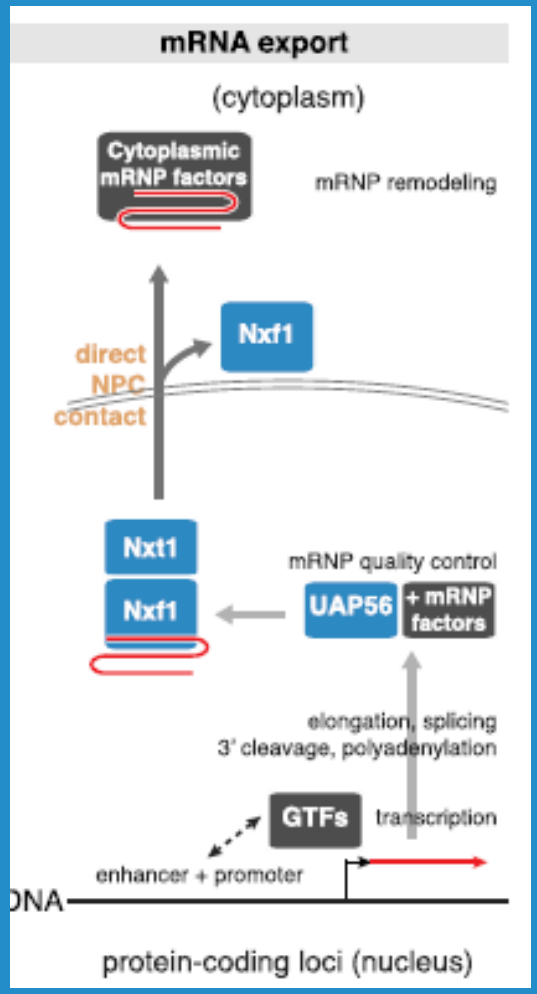


Figure 7: Nxf3-Bootlegger pathway in comparison to mRNA export and pre-miRNA export.

NXF protein family

1. Hotspot for genetic innovation and neo-functionalization
2. Mammal NXF2 and NXF3 present in gonads
3. Nxf2 mice mutants are sterile





Nxf3-Bootlegger pathway - Conclusions

- Insight on gene expression
- Insight on piRNA biogenesis
- How biological information is sorted and distributed inside cells

Possible future developments

Investigate NXF role in mammal and possibly in human infertility, examining whether there are transposons modification and de-regulation with a resulting major incidence of pathologies (ex. hemophilia, cancer…)

Thank you for your attention

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