线虫里小调节性RNA的产生、功能与机制

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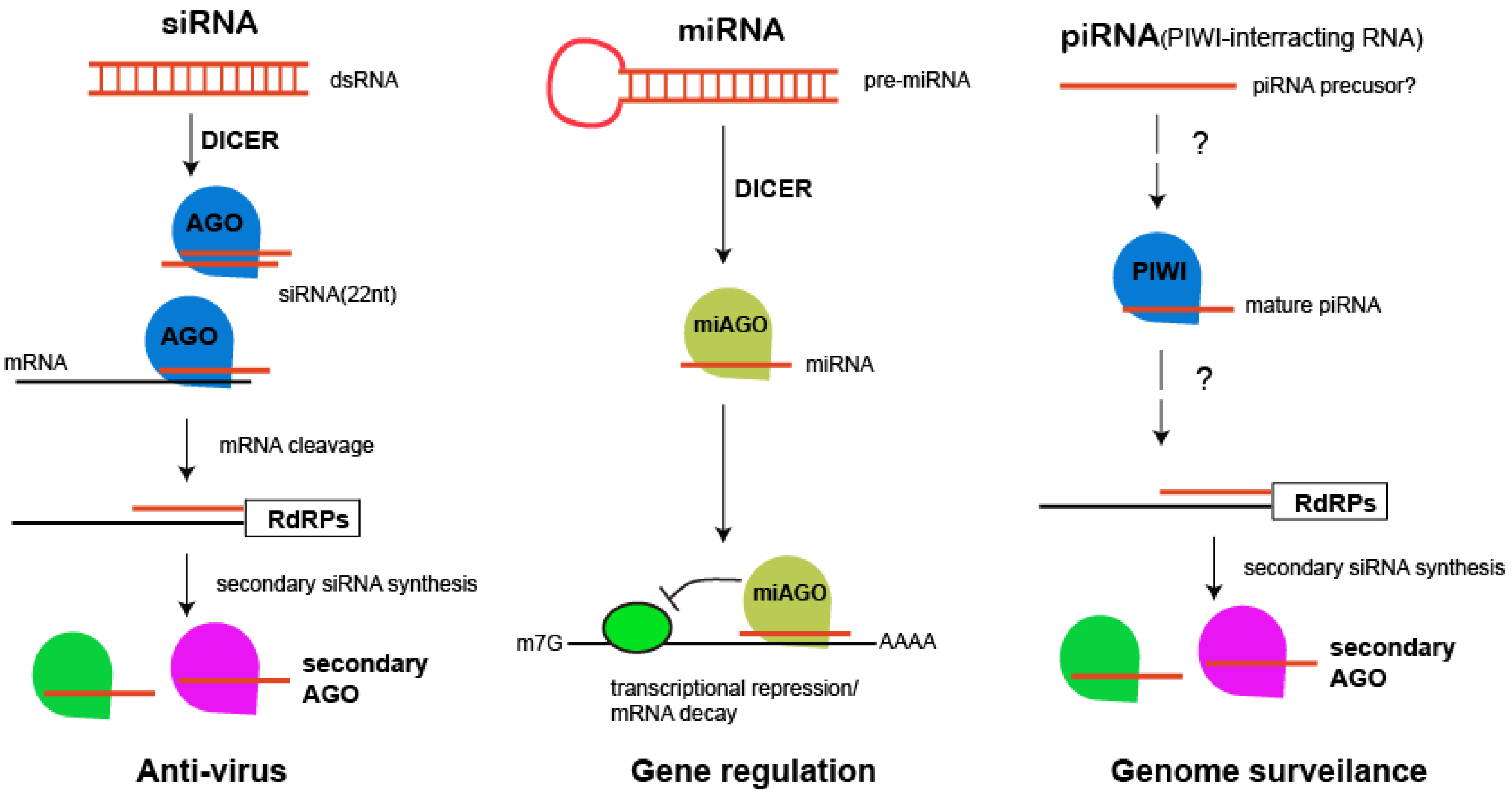
2021-10-27

非编码RNA多种存在方式

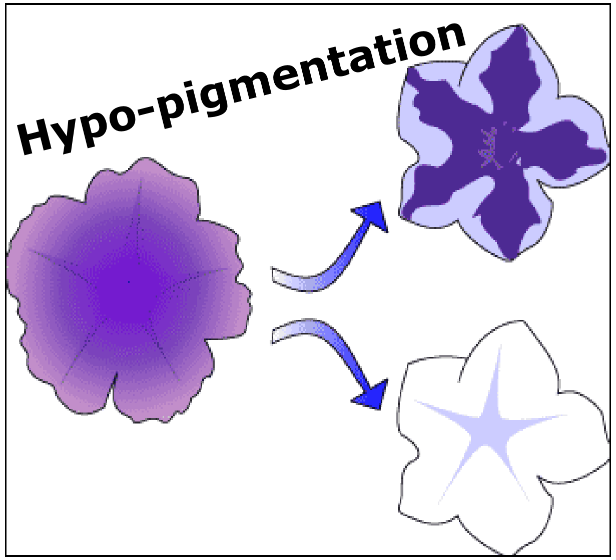
ribosomal RNA (rRNA)
transfer RNA (tRNA)
small nuclear RNA (snRNA)
small nucleolar RNA (snoRNA)
microRNA (miRNA)
small interference RNA (siRNA)
piwi interacting RNA (piRNA)
trans-acting siRNA (tasiRNA)
long interspersed ncRNAs (lincRNAs)
scan RNA (scnRNA)
promoter-associated sRNAs (PASRs)
terminator-associated sRNAs (TASRs)
cryptic unstable transcripts (CUTs)
stable unannotated transcripts (SUTs)
circular RNA (circRNA)

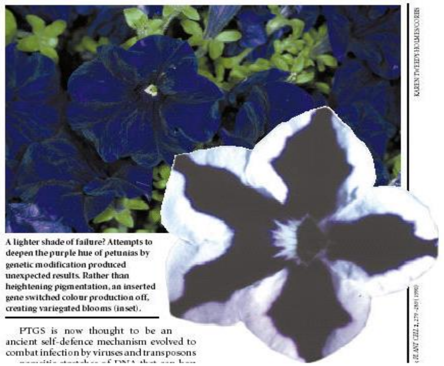
参与RNA干扰通路的小RNA



RNA干扰的发现

- 1990年，Rich Jorgensen将由强启动子控制的Chalcone synthase gene转入淡紫色的矮牵牛花->加深紫色
- Hypopigmentation：许多花出现杂色，甚至紫色消失，变成白色
- Co-suppression





RNA干扰：线虫

- 1995年，Su Guo and Kenneth J. Kemphues利用反义RNA技术阻断线虫中的par-1基因。在对照实验中给线虫注射正义RNA以期观察到基因表达的增强。（擦肩而过!）

- 然而，正义RNA和反义RNA都能够有效地抑制基因的表达？！

- 沉默的效应能够在被注射的动物及其后代中保持，虽然RNA转录本在胚胎早期就发生降解

双链RNA抑制与其序列同源的基因的表达

The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

Photo: L. Cicero/Stanford

Photo: R. Carlin/UMMAS

Andrew Z. Fire

1/2 of the prize

USA

Stanford University School of Medicine
Stanford, CA, USA

Craig C. Mello

1/2 of the prize

USA

University of Massachusetts Medical School
Worcester, MA, USA

Andrew Z. Fire
Craig C. Mello

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

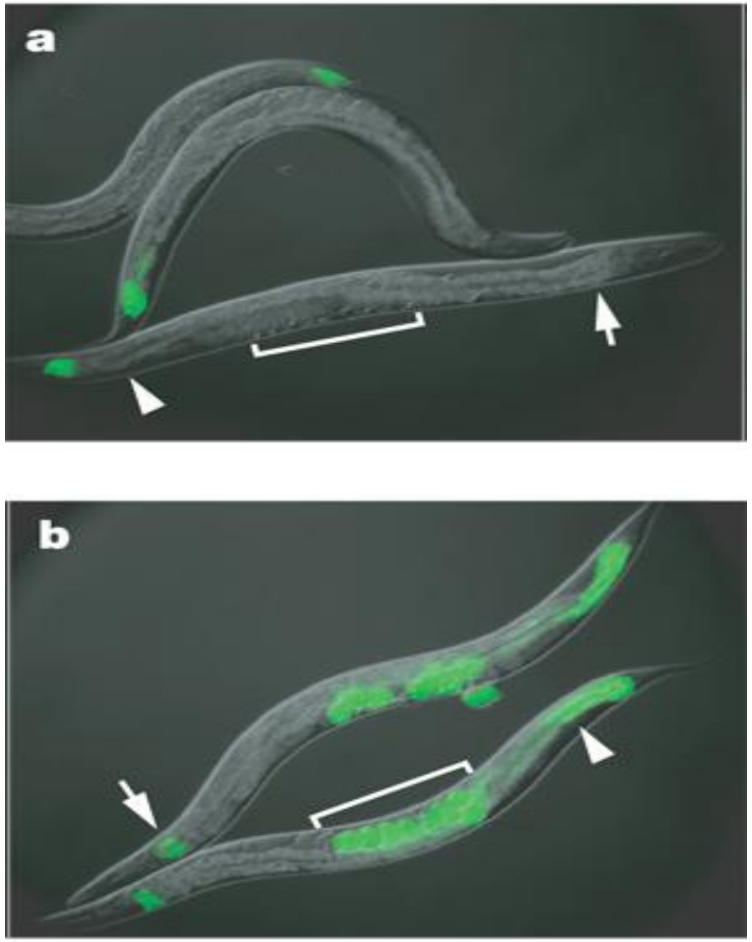
NATURE | VOL 391 | 19 FEBRUARY 1998

RNA干扰

- “RNA Interference”: 注入dsRNA能够有效地、长期的阻断基因的表达

- 给线虫喂食表达GFP dsRNA的细菌，线虫的GFP表达被抑制 (a)，但存在RNAi缺陷的则不被抑制 (b)

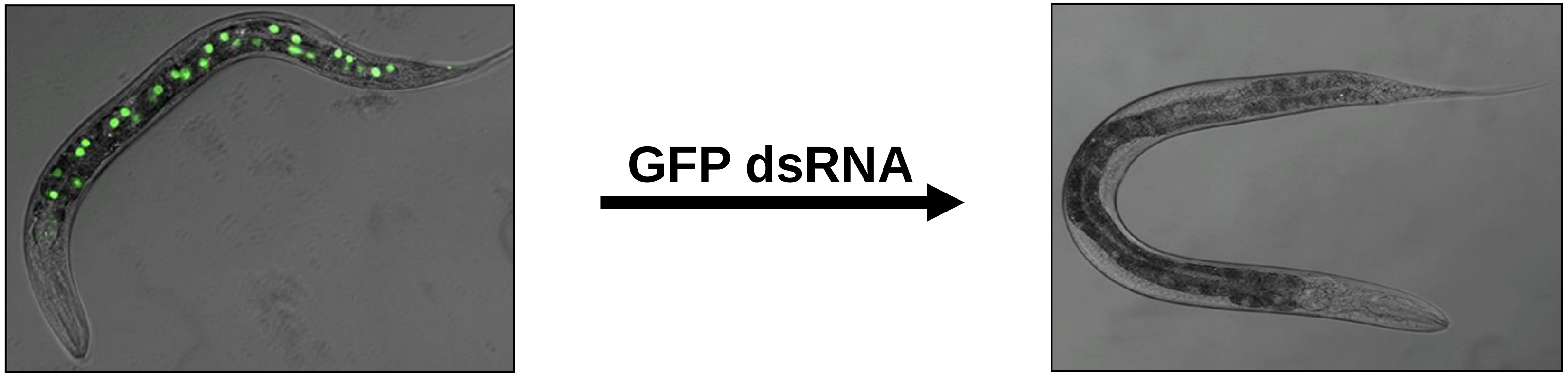
- 之前的正义RNA抑制基因表达的现象，可能是由于体外转录所得RNA中污染了微量双链RNA而引起

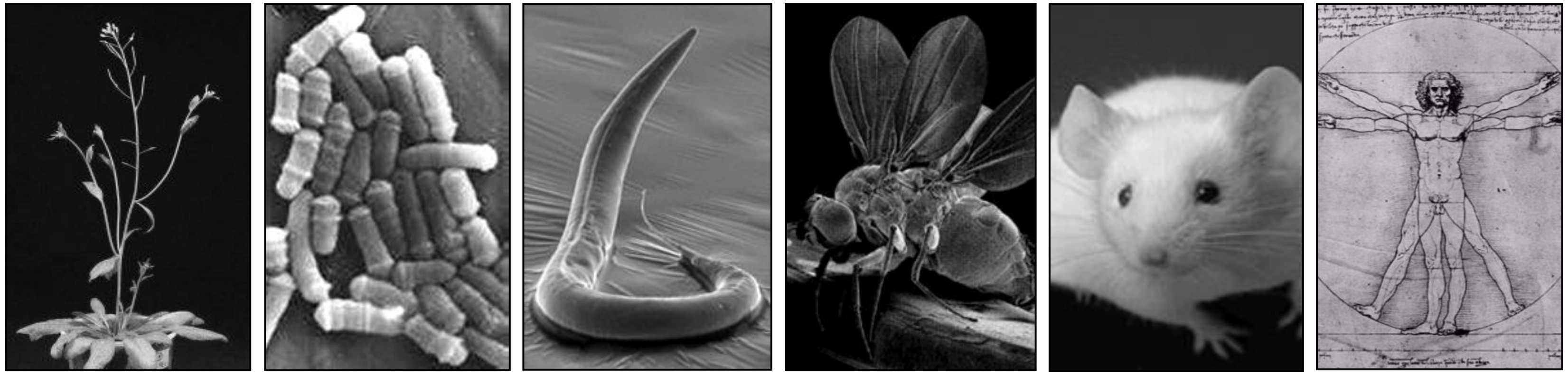


- First, dsRNA segments corresponding to various intron and promoter sequences did not produce detectable interference.
- Second, we found that injection of dsRNA produces a pronounced decrease or elimination of the endogenous mRNA transcript.
- Third, dsRNA-mediated interference showed a surprising ability to cross cellular boundaries. Interference was seen in the progeny.

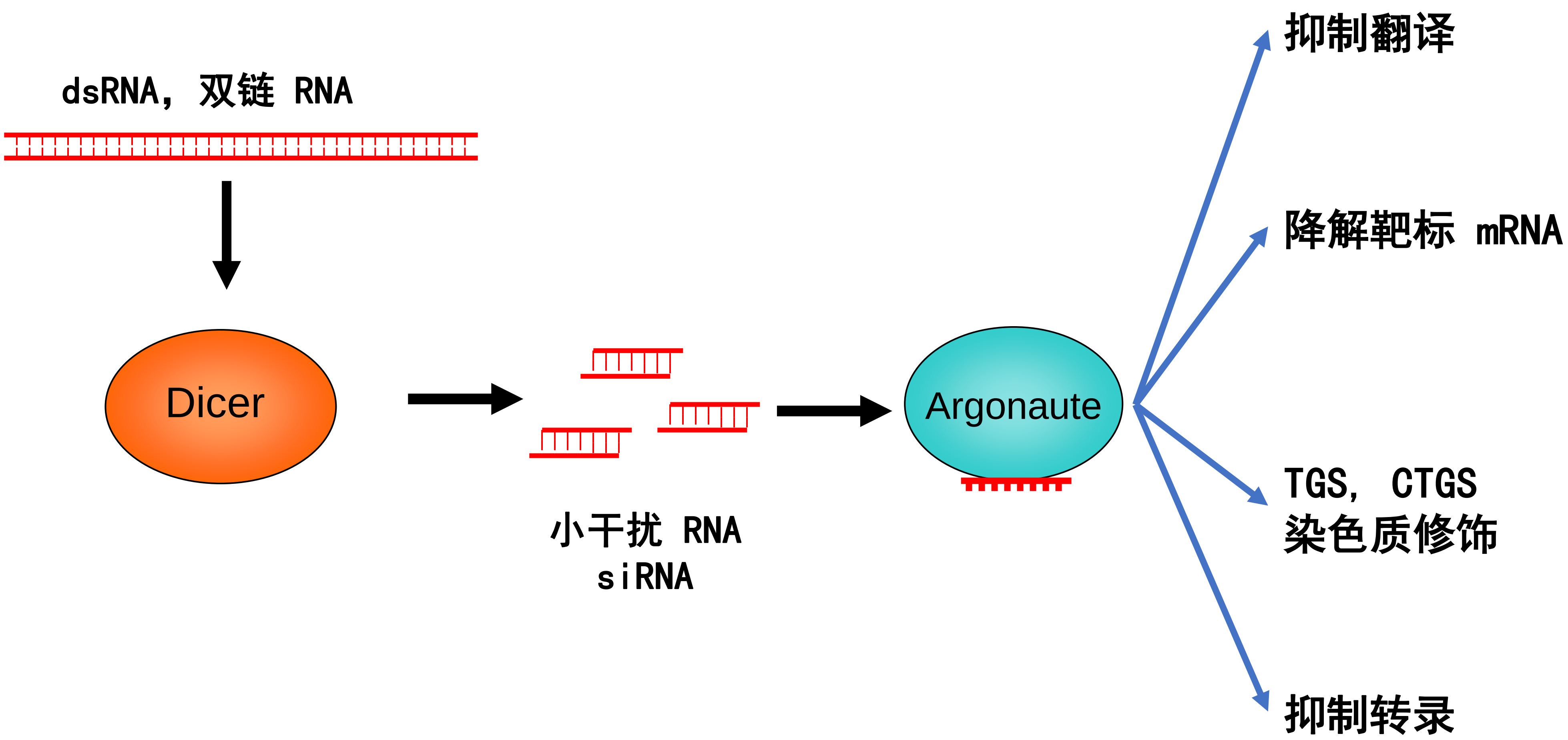
- Double-stranded RNA could conceivably mediate interference more generally in other nematodes, in other invertebrates, and, potentially, in vertebrates. RNA interference might also operate in plants.

RNA 干扰是一个保守的生物学现象





RNAi 的分子机制



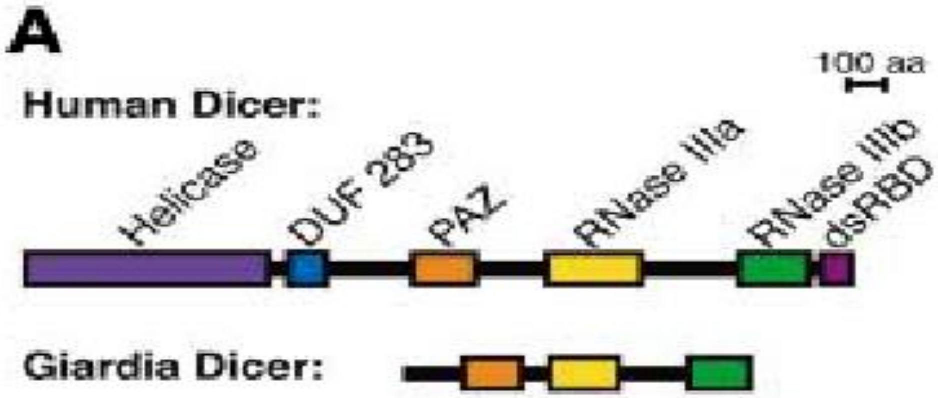
dsRNA，双链 RNA
Dicer
小干扰 RNA
siRNA
Argonaute
抑制翻译
降解靶标 mRNA
TGS，CTGS
染色质修饰
抑制转录

RNA干扰的分子机器

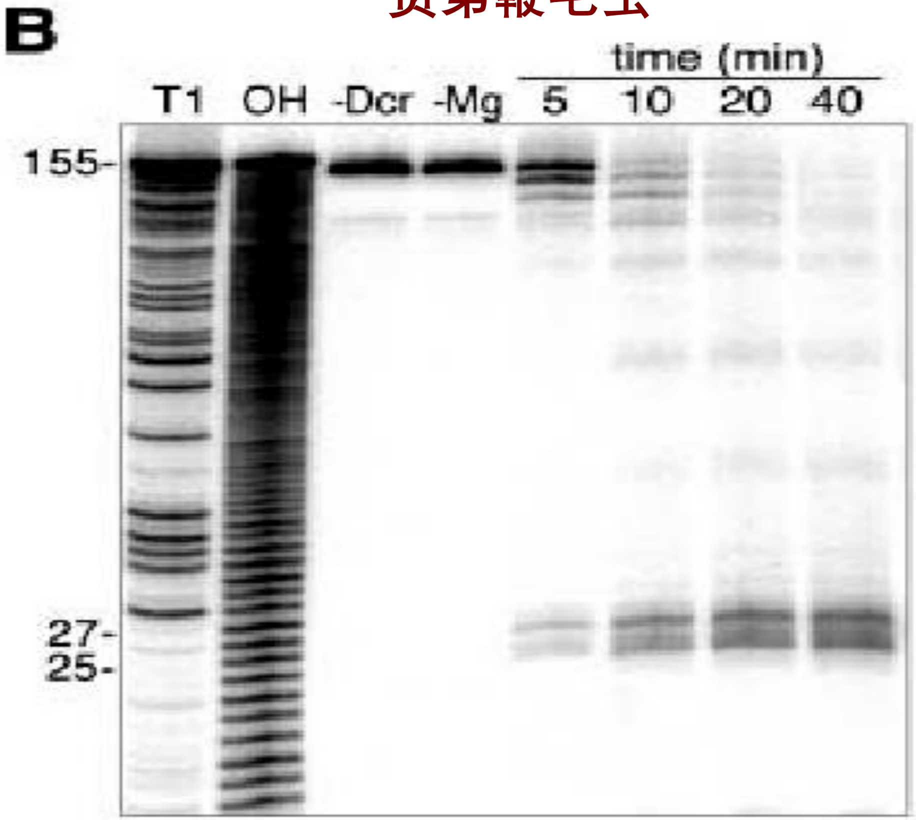
- 1. Dicer: RNase III类似的，包含多个功能结构域的核糖核酸酶，将dsRNA切割成小的short interfering RNAs (siRNAs) or microRNAs (miRNA)，并将这些产物加载到RISC上
- 2. RISC (RNA induced silencing complexes) (RNA诱导的沉默复合体): 包含多个蛋白的复合物，将与之连接的siRNA或miRNA定位到其靶点并抑制靶基因的表达

Dicer

- 序列的结构组成:
- 1. 一个PAZ结构域，与dsRNA的末端结合
- 2. 两个RNase III结构
- 3. 其他的功能结构域



贾第鞭毛虫



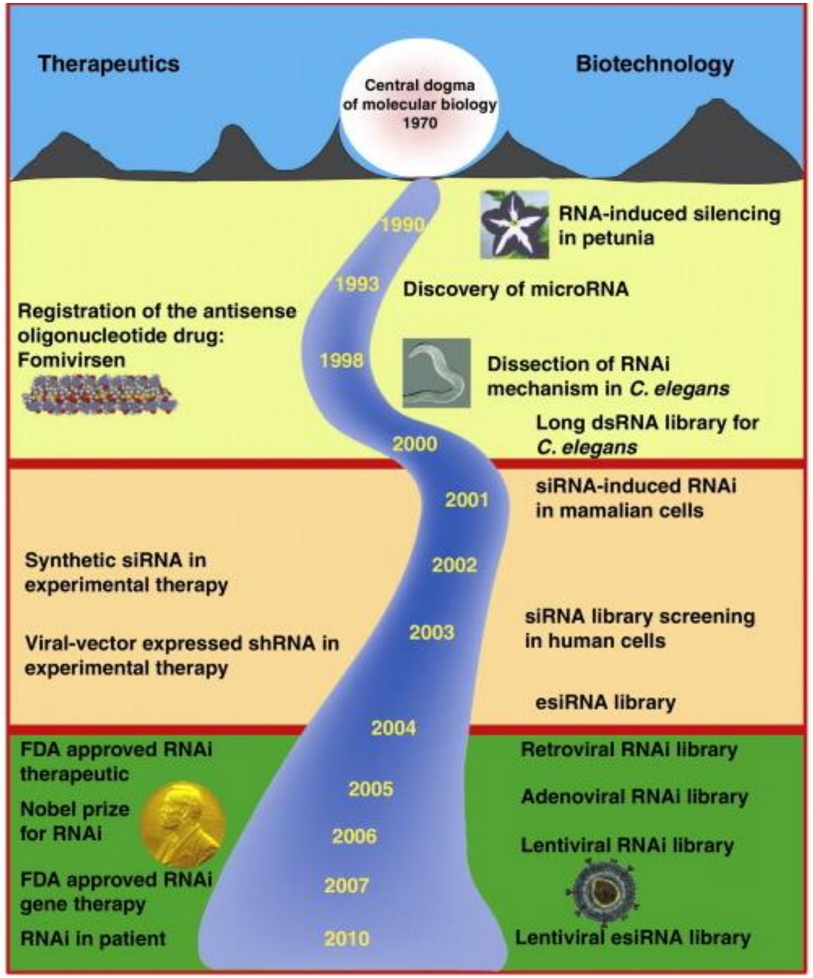
Argonaute (AGO)：RISC的核心成员

- Argonaute (AGO): 大的蛋白质家族，为RISCs的核心成员
- AGO蛋白质一般包含PAZ和PIWI两种功能结构域
- PAZ与双链siRNA 3’-端露出的两个核苷酸结合
- PIWI负责将双链siRNA切成单链
- PAZ和PIWI对于siRNA与底物mRNA之间的相互作用是必须的，并负责底物的断裂或转录抑制
- 不同的AGO具有不同功能，例如人类AGO2负责的RISCs能够割裂底物mRNA，而AGO1和AGO3则不能

RNA 干扰的应用

- Gene knockdown
- Functional genomics
- Genome-scale RNAi screening
- Medicine
- Biotechnology

疾病治疗 基因功能 农作物改良



RNA 药物

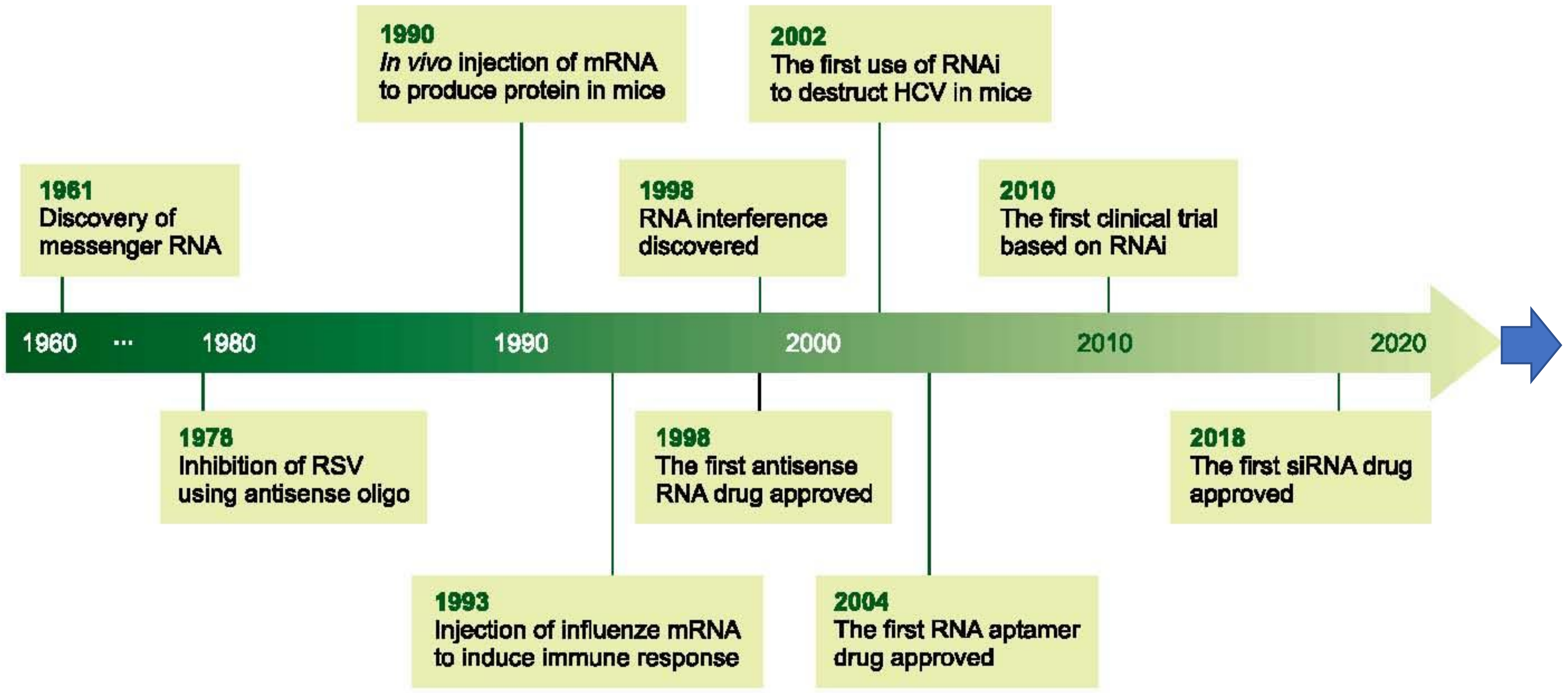
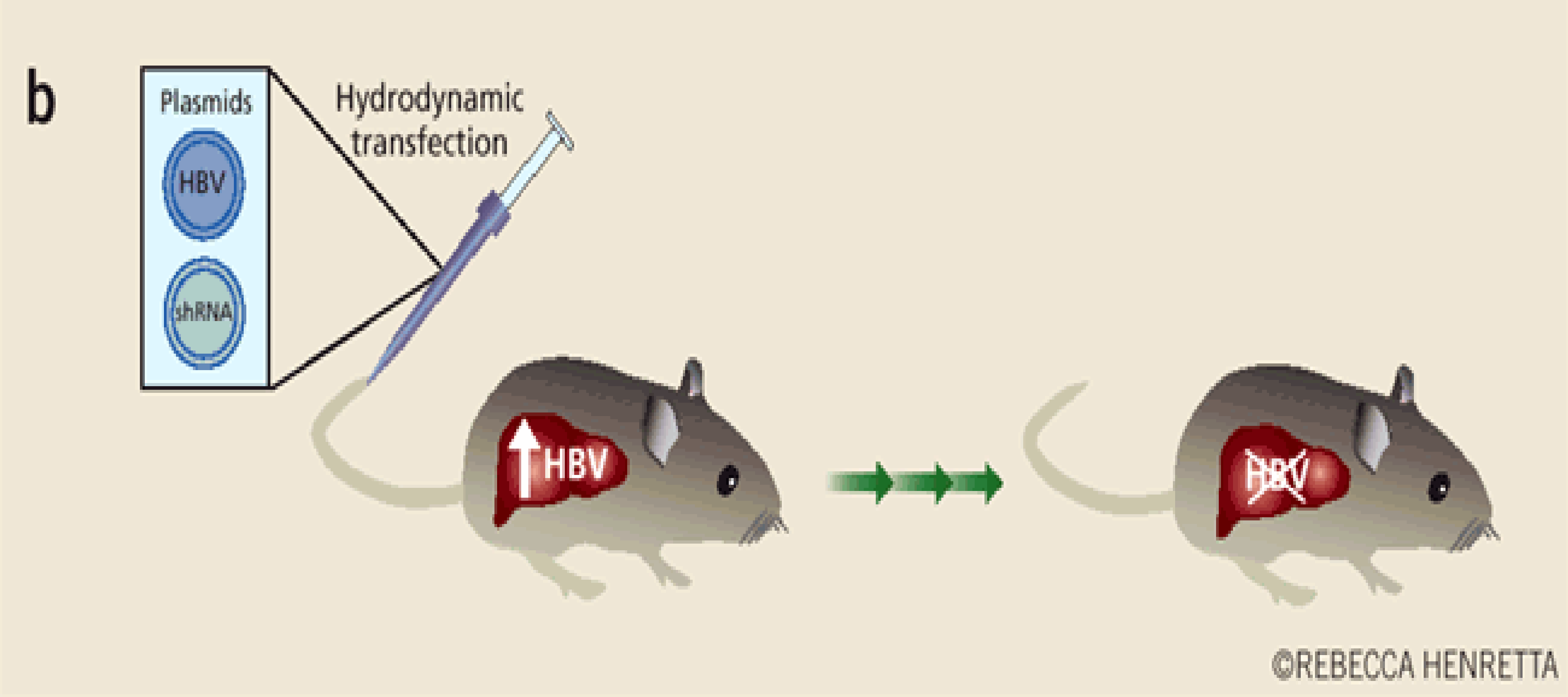


FIG. 1. Timeline of key discoveries in RNA therapy. See the text for details.

2021

2021
mRNA vaccine

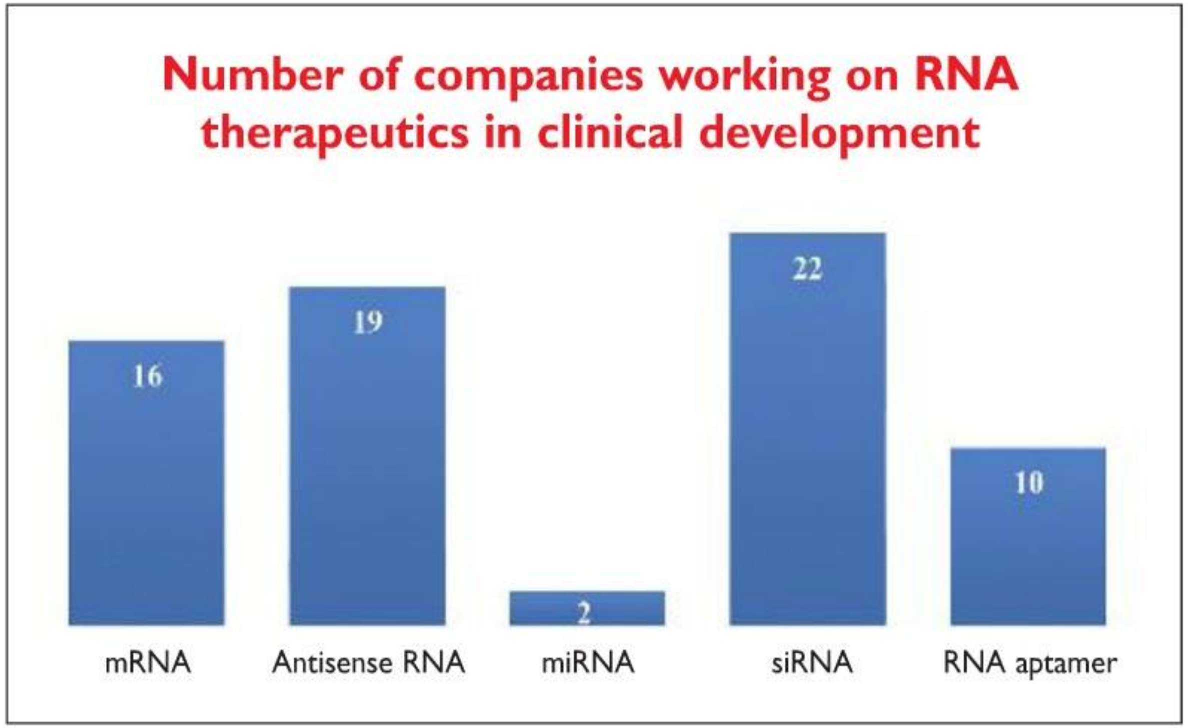
Inhibition of hepatitis B virus in mice by RNA interference



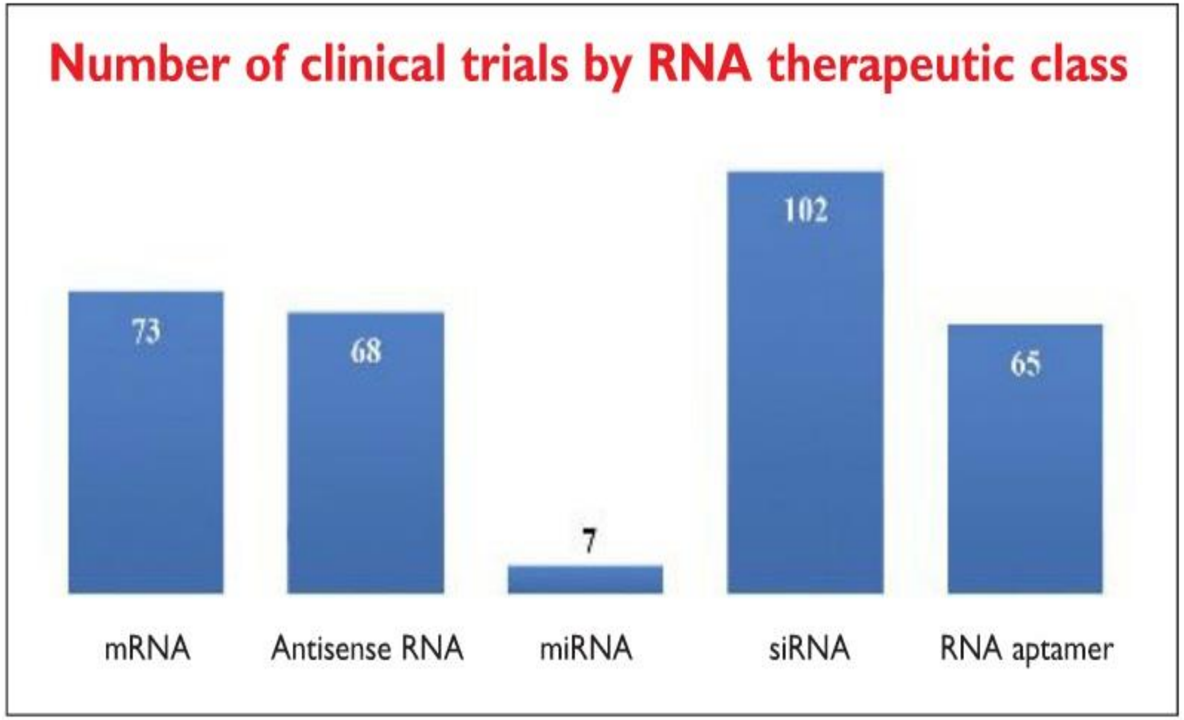
Nature Biotechnology 2003
Hepatology 2003

TABLE 1. List of clinically approved RNA drugs by the United States Food and Drug Administration

Category	Drug	Brand name	Approved year	Target molecule	Treatment result	Target disease
Antisense RNA (single-stranded RNA)						
	Fomivirsen	Vitravene	1998	IE2 mRNA	Binds to IE2 mRNA and blocks its translation	CMV retinitis
	Mipomersen	Kynamro	2013	ApoB mRNA	Binds to ApoB mRNA and induces its degradation by RNase H	Familial hypercholesterolemia
	Nusinersen	Spinraza	2016	SMN2 mRNA	Modulates the alternative splicing of SMN2 mRNA and increases the SMN protein level	Spinal muscular atrophy
	Eteplirsen	Exondys 51	2016	Dystrophin mRNA	Induces the exclusion of exon 51 of dystrophin mRNA during splicing to produce a functional protein	Duchenne muscular dystrophy
	Inotersen	Tegsedi	2018	Transthyretin mRNA	Binds to Transthyretin mRNA and induces its degradation by RNase H	Hereditary transthyretin amyloidosis
	Golodirsen	Vyondys 53	2019	Dystrophin mRNA	Induces the exclusion of exon 53 of dystrophin mRNA during splicing to produce a functional protein	Duchenne muscular dystrophy
Small interfering RNA (double-stranded RNA)						
	Patisiran	Onpattro	2018	Transthyretin mRNA	Suppresses the hepatic production of transthyretin protein through RNA interference	Hereditary transthyretin amyloidosis
	Givosiran	Givlaari	2019	ALAS1 mRNA	Reduces the hepatic production of ALAS1 protein through RNA interference	Acute hepatic porphyria
RNA aptamer						
	Pegaptanib	Macugen	2004	VEGF protein	Binds specifically to the 165 isoform of VEGF and blocks its function	Age-related macular degeneration



Graph 1: Companies developing RNA-based therapeutics in the clinic (as of July 2018). Data provided by GlobalData Plc

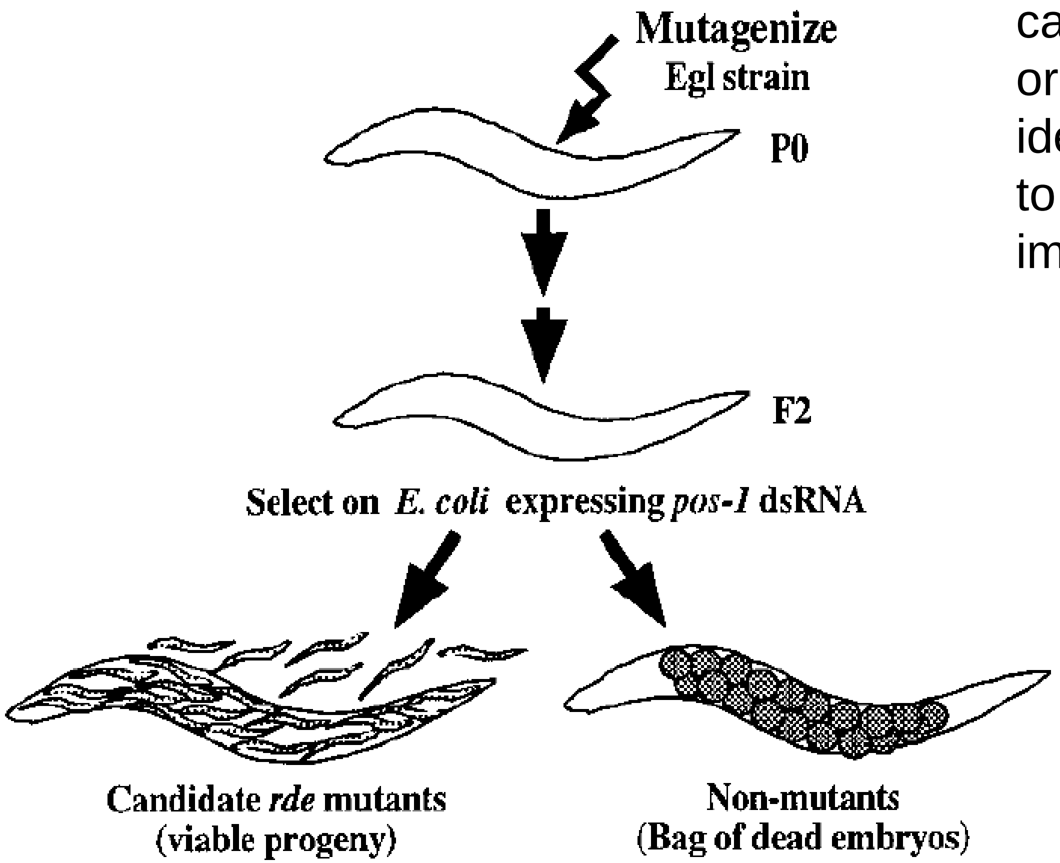


Graph 2: Number of RNA-based therapeutics in clinical trials (as of July 2018). Data provided by GlobalData Plc

科学问题

调节性小非编码 RNA 是**怎样产生**的，通过什么样的**分子机制**起作用，其**生物学功能**是什么，是怎么样参与**性状遗传**的？

Screening for RNAi-deficient mutants



Tabara H. 1999. Cell, Vol. 99, 123–132,

The first mutants in the RNAi pathway identified by Tabara and Mello were called RNAi deficient (rde). These original screens were aimed at identifying of viable mutants, resistant to RNAi targeting pos-1, a gene important for viability.

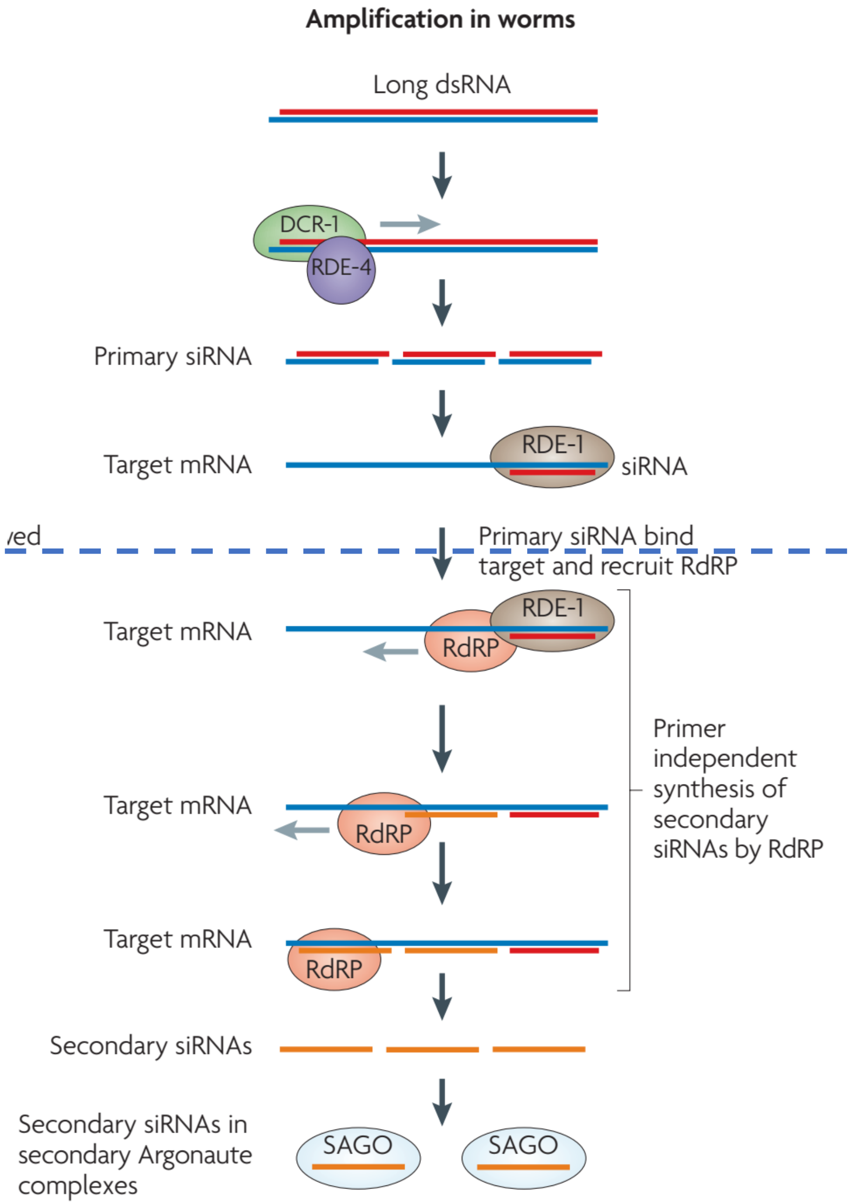
rde-1 non
rde-2 Ste/him/mutator
rde-3 Ste/him/mutator
rde-4 non
mut-2 Ste/him/mutator
mut-7 Ste/him/mutator

↓

Existence of related silencing pathways with distinct triggering mechanisms

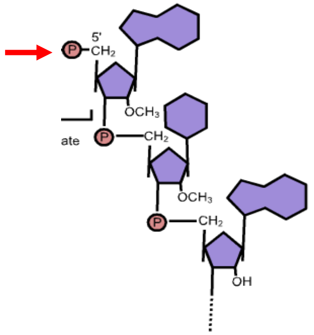
外源RNA干扰通路

初级生成

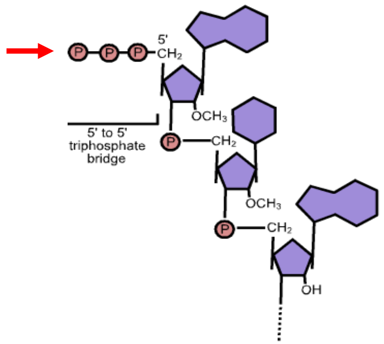


次级扩增

Primary siRNA



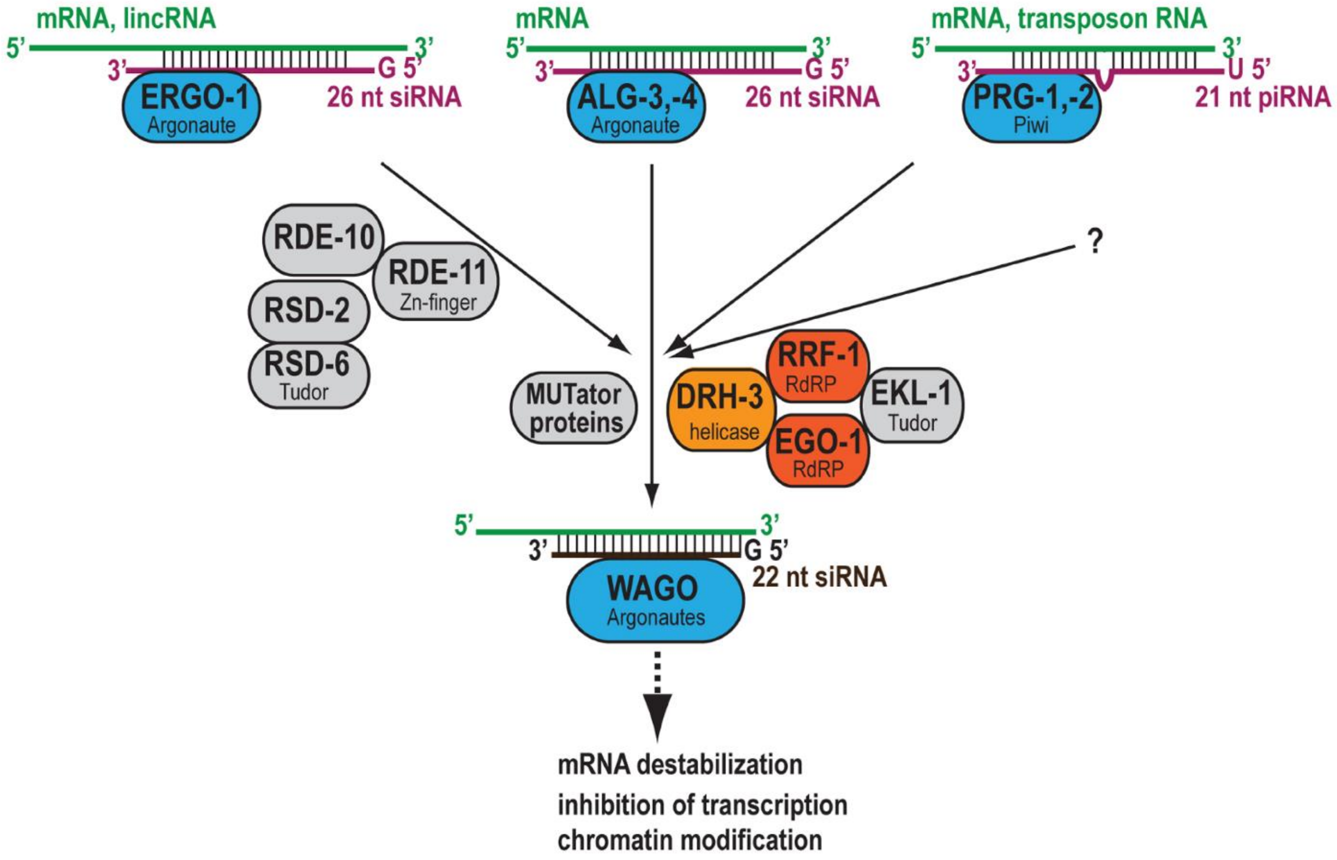
Secondary siRNA



RISC: RNA诱导的沉默复合体
RdRP: RNA依赖的RNA聚合酶
siRNA: Short interfering RNA

Ghildiyal and Zamore *et al*., 2009

内源RNA干扰通路



Billi *et al.*, 2014

Part I: nuclear RNAi and nucleolar RNAi

1.1: nuclear RNAi

1.2: nuclear RNAi & transgenerational inheritance

1.3: antisense ribosomal siRNA (risiRNA) and nucleolar RNAi

Part II: piRNA biogenesis

2.1: USTC 复合物与 piRNA 转录

2.2: PICS复合物与 piRNA 加工

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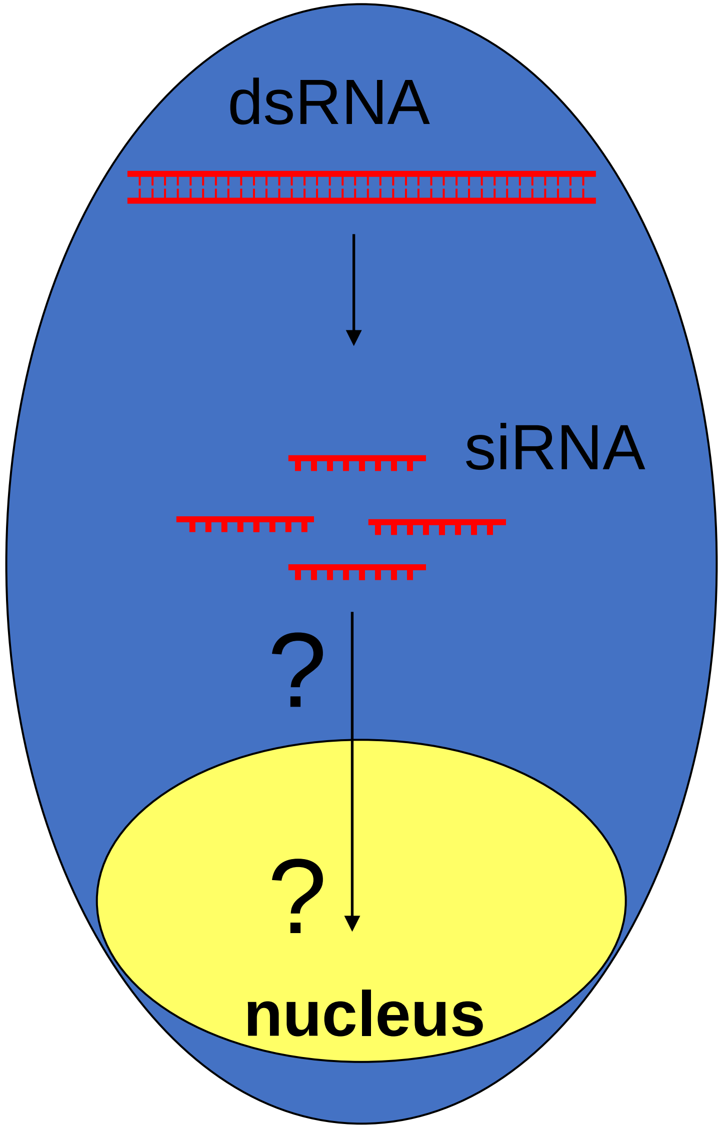
科学问题

- 小干扰 RNA是否在细胞核中有作用？
- 如果有，需要哪些关键因子？
- 细胞核RNA 干扰的分子机制是什么？

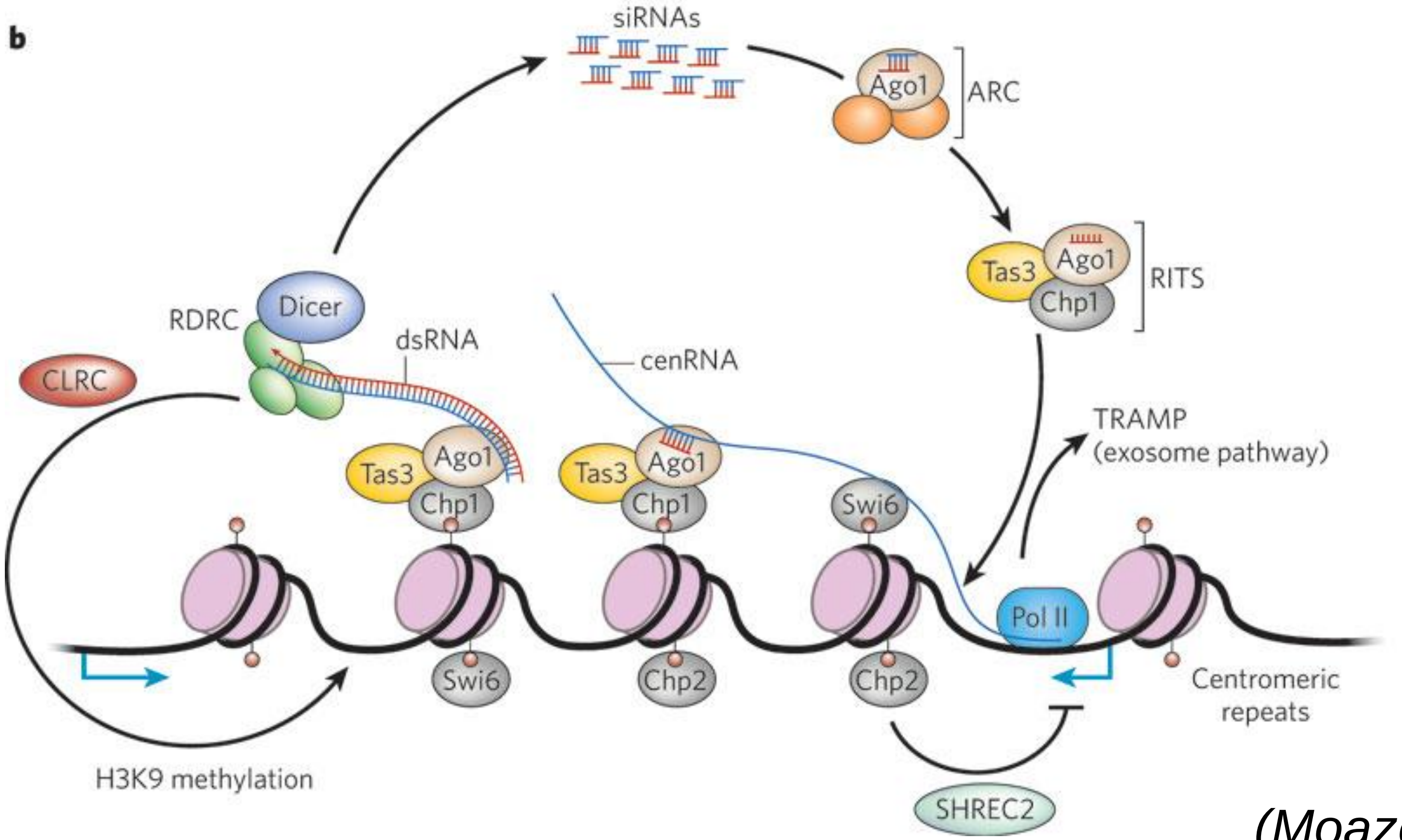


RNAi与异染色质：植物

- dsRNA与染色质沉默密切相关
- 植物的转基因被同源dsRNA的表达所沉默，并使同源DNA区域发生甲基化
- 由siRNA介导，甲基转移酶能够被招募到靶基因的位点

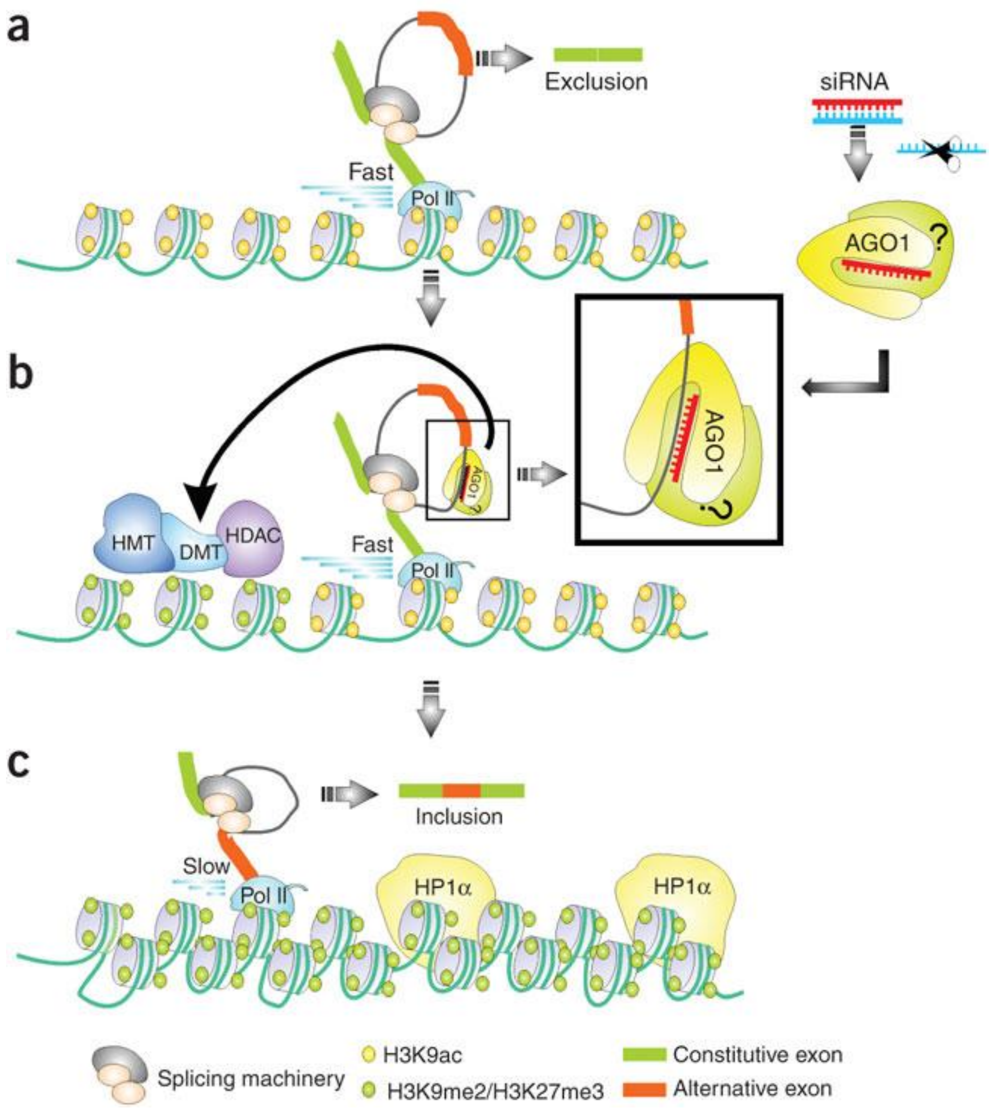
多个蛋白复合物参与异染色质的调控

Co-transcriptional gene silencing in *S. pombe*

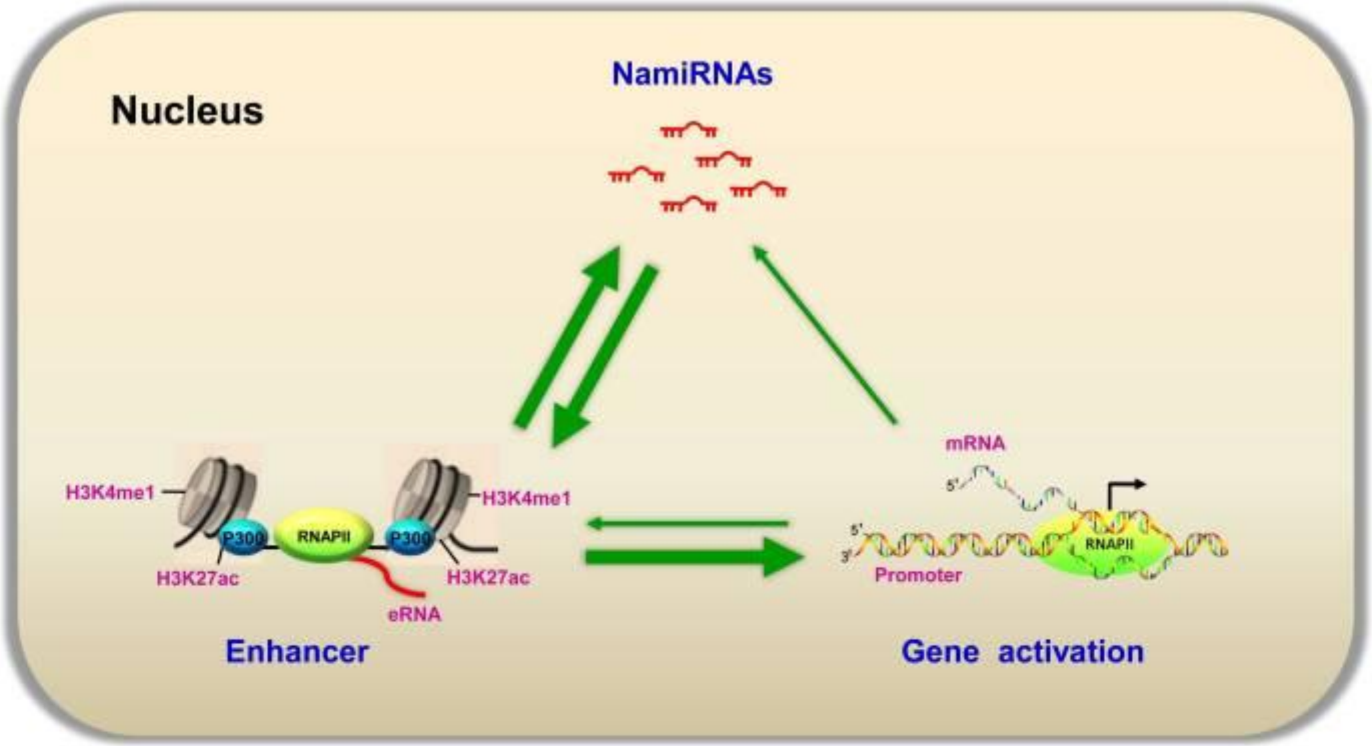


(Moazed 2009)

Control of alternative splicing through siRNA-mediated transcriptional gene silencing



namiRNAs activate gene expression in nucleus

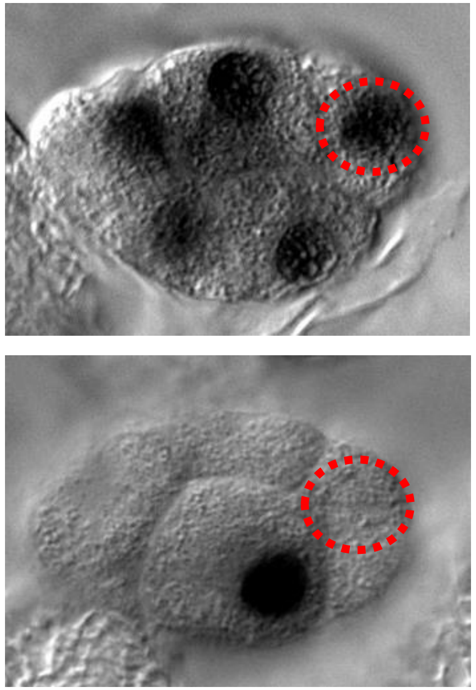


RNAi silences a nuclear localized RNA

WT

Control

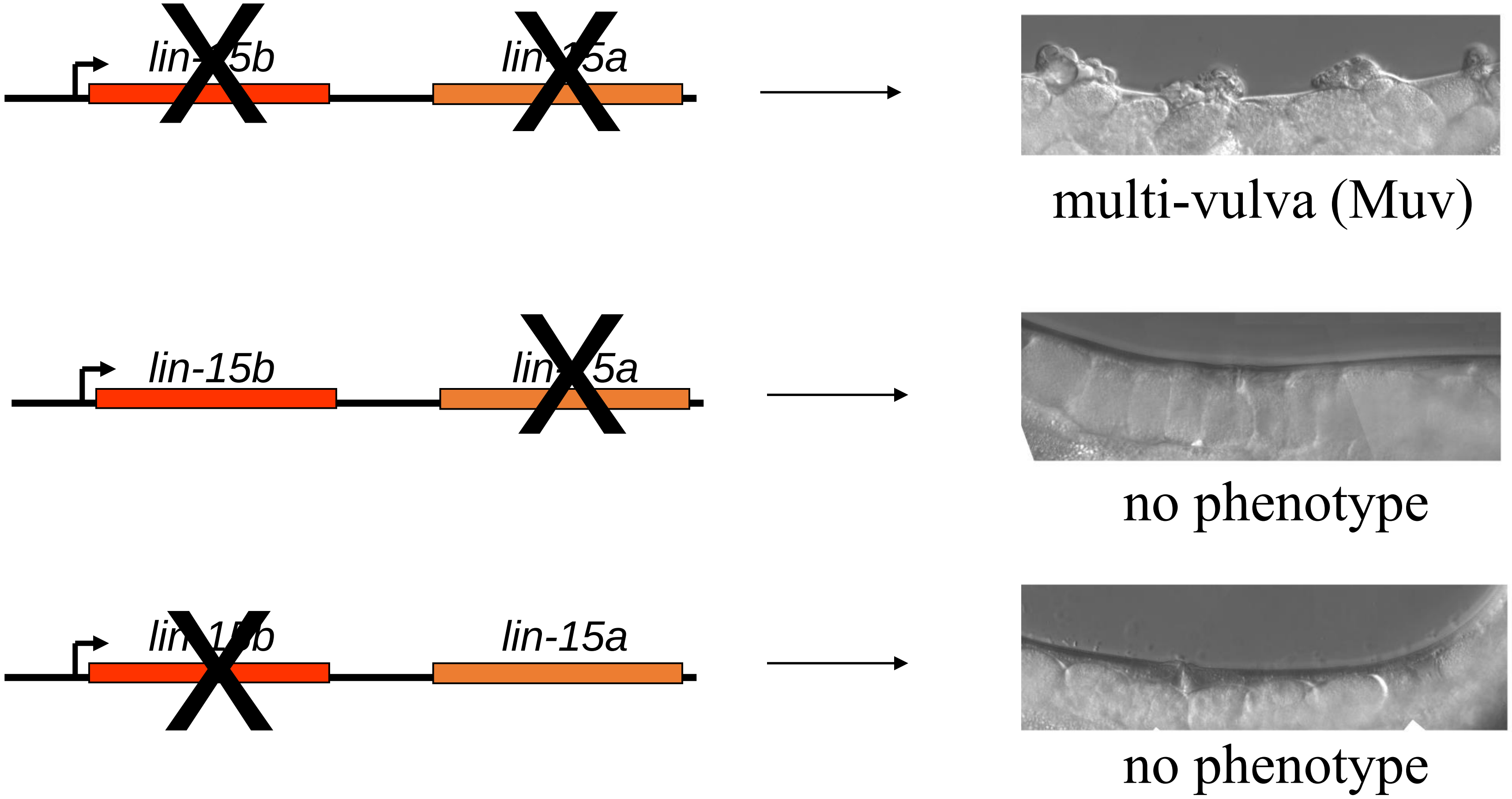
RNAi



RNA in situ

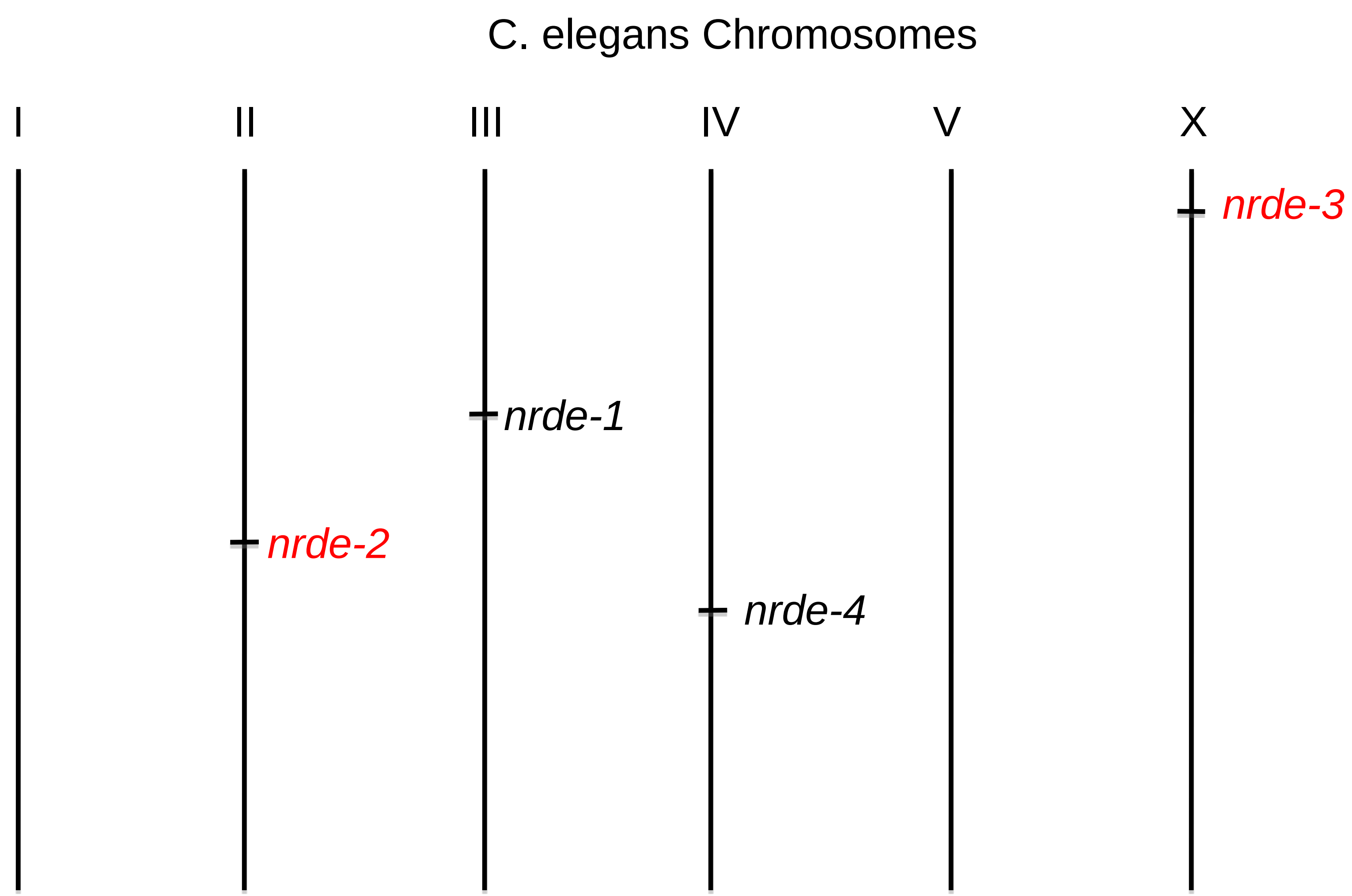
Science 2008

Mutations of both *lin-15a* and *lin-15b* result in a synthetic multi vulval (Muv) phenotype

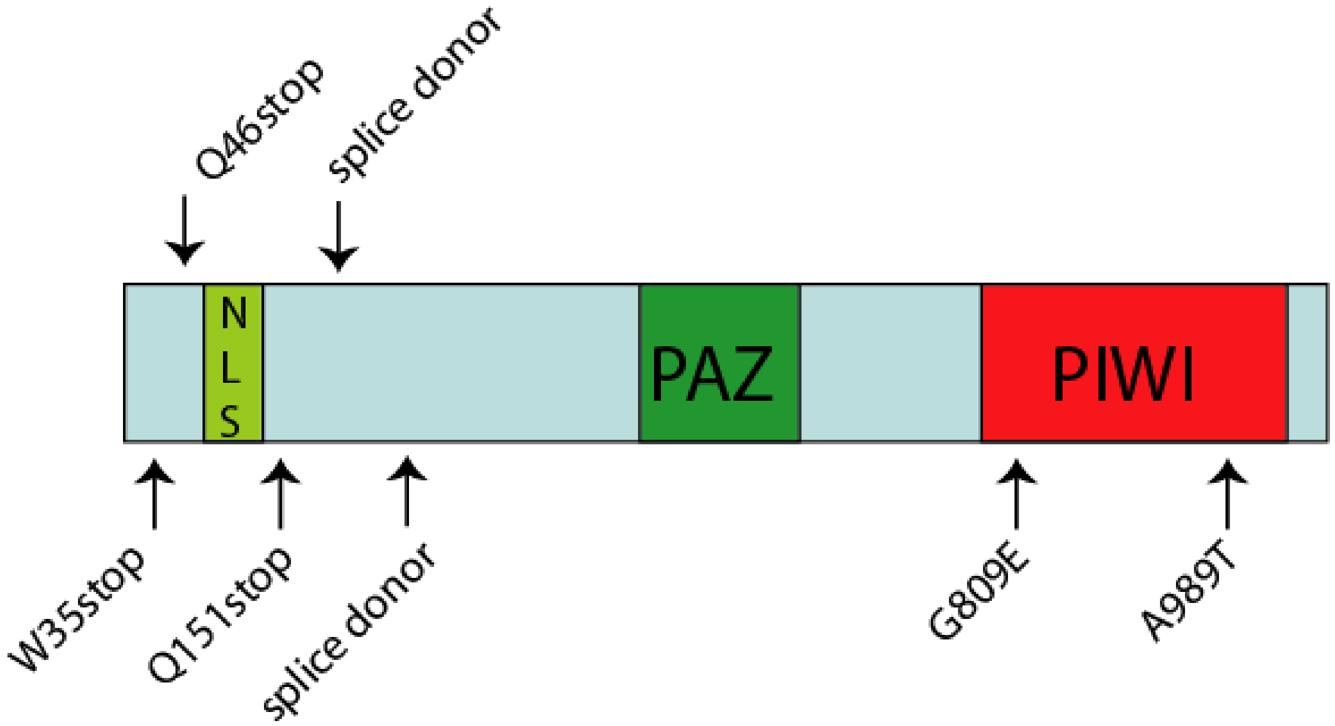


Does RNAi of *lin-15b* give a Muv phenotype?

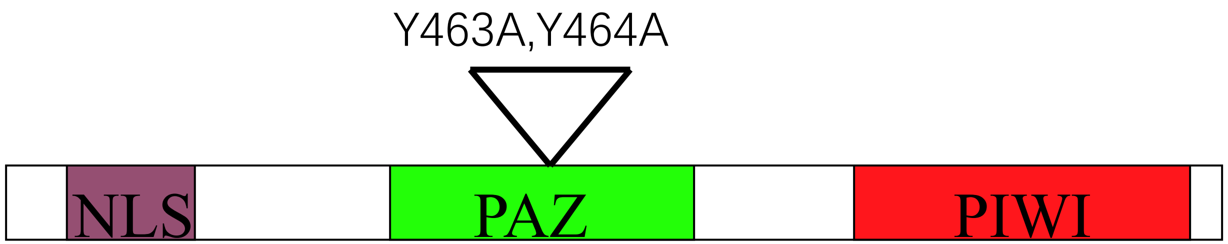
A forward genetic screening identifies Nuclear RNAi Defective genes (NRDE)

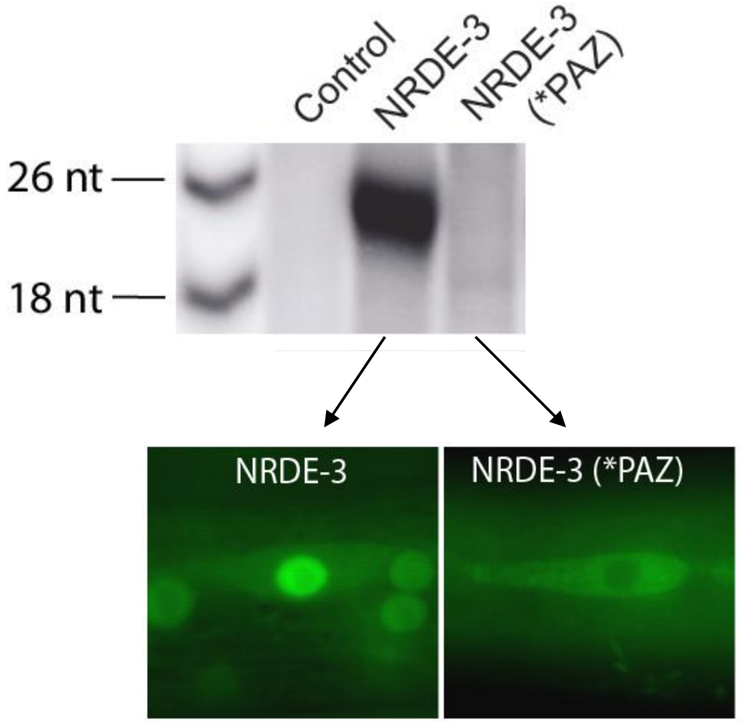


在秀丽线虫中发现细胞核 RNA 干扰关键因子 NRDE-3
（Nuclear RNAi Defective -3）



Binding siRNA is required for NRDE-3 nuclear localization and function





- IP GFP::NRDE-3

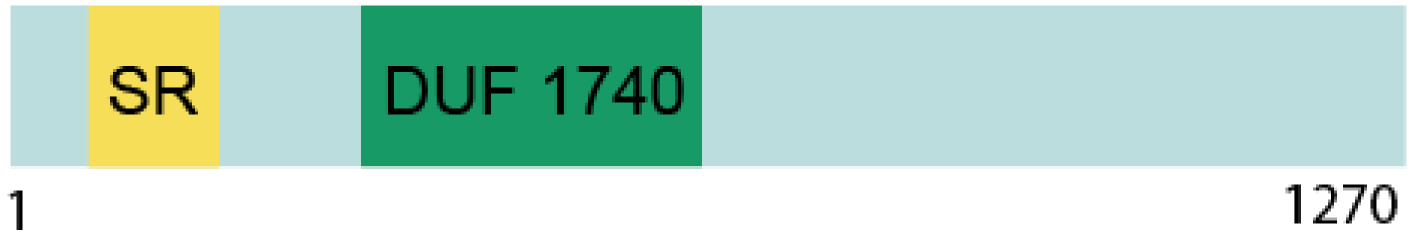
↓

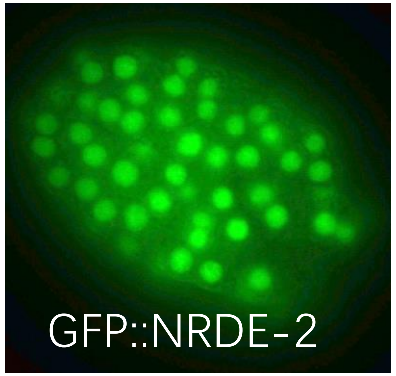
- Extract small RNAs

↓

- Label with γ-p^{32}-ATP

nrde-2 encodes a conserved protein required for nuclear RNAi

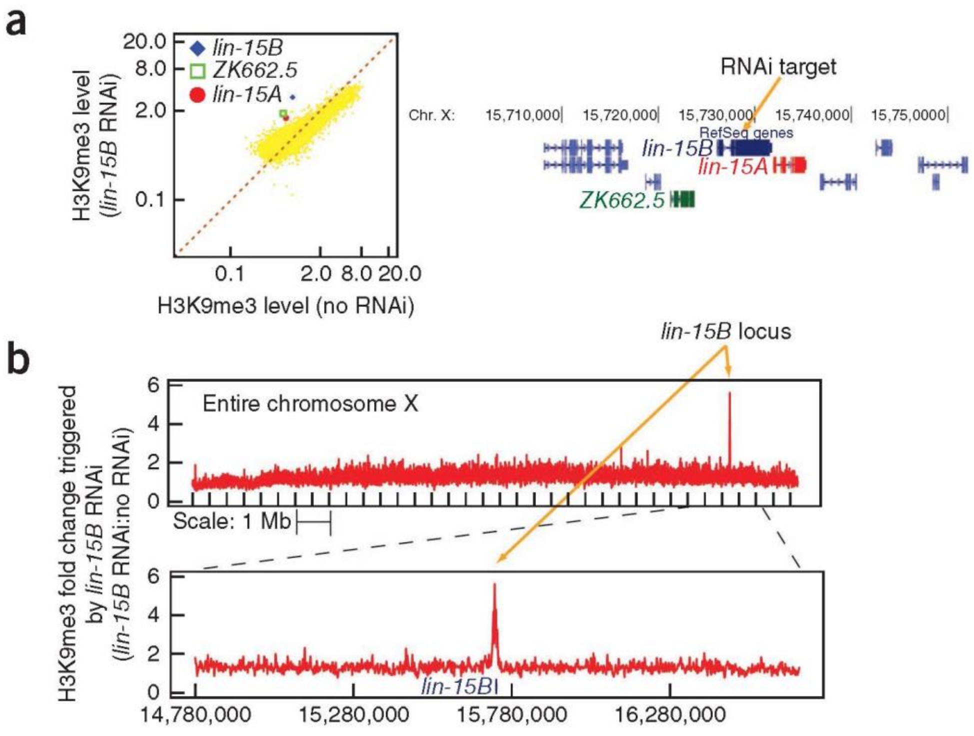


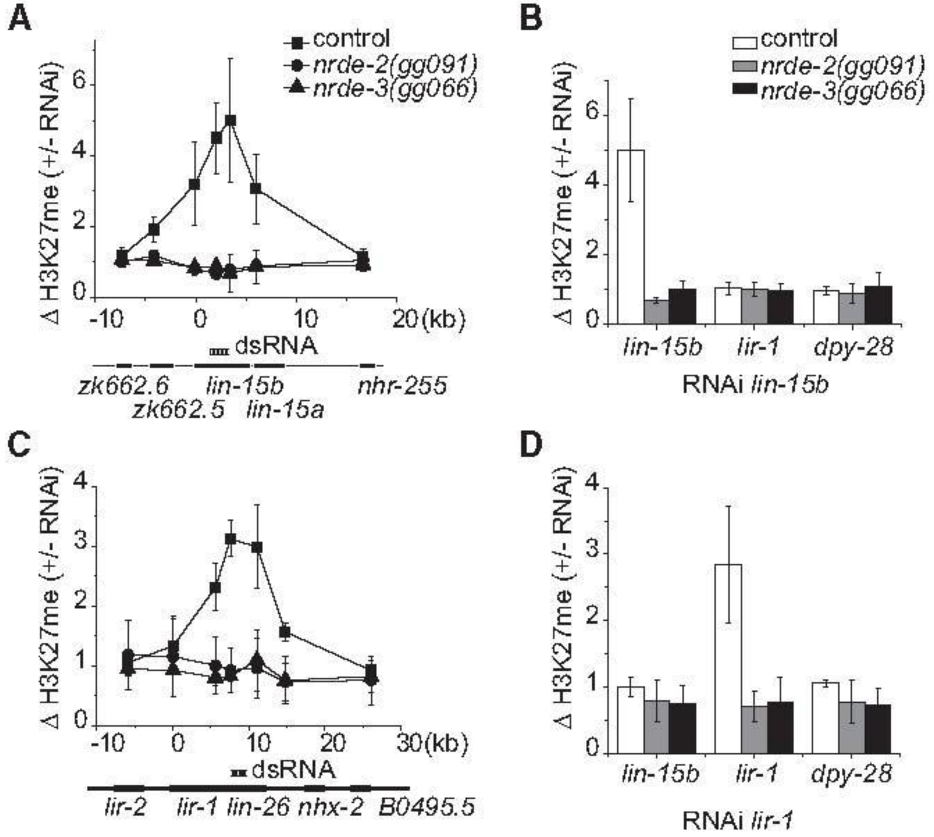


~200 cell embryo

Nature 2010

细胞核 RNA 干扰诱导靶标位置的 H3K9me3 和 H3K27me3

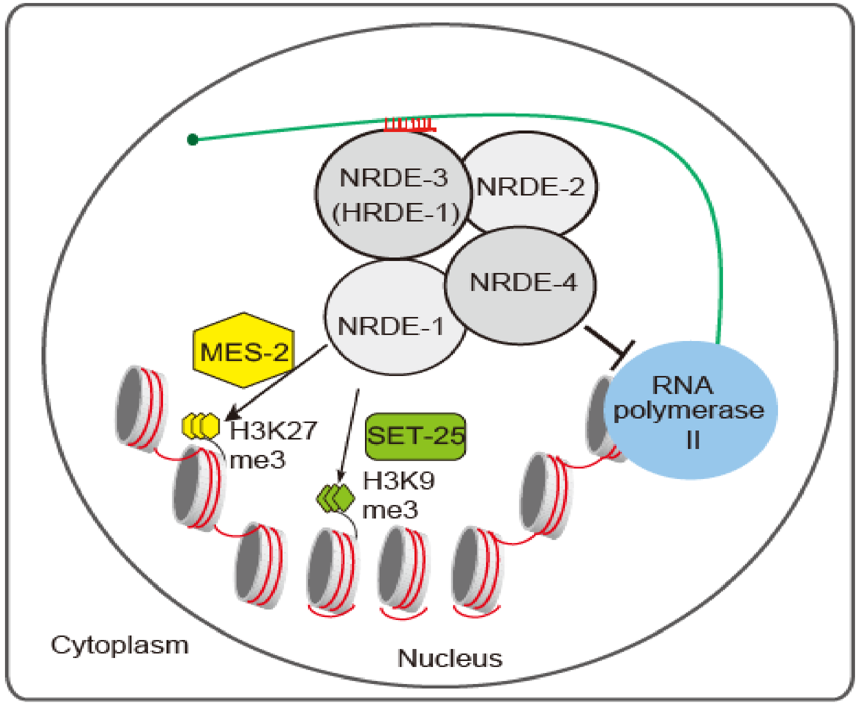




Nature Genetics 2012

Current Biology 2015

细胞核 RNA 干扰通路



Science 2008;
Nature 2010;
PLoS Genetics 2011;
Nature Genetics 2012;
Genetics 2014;
Current Biology 2015

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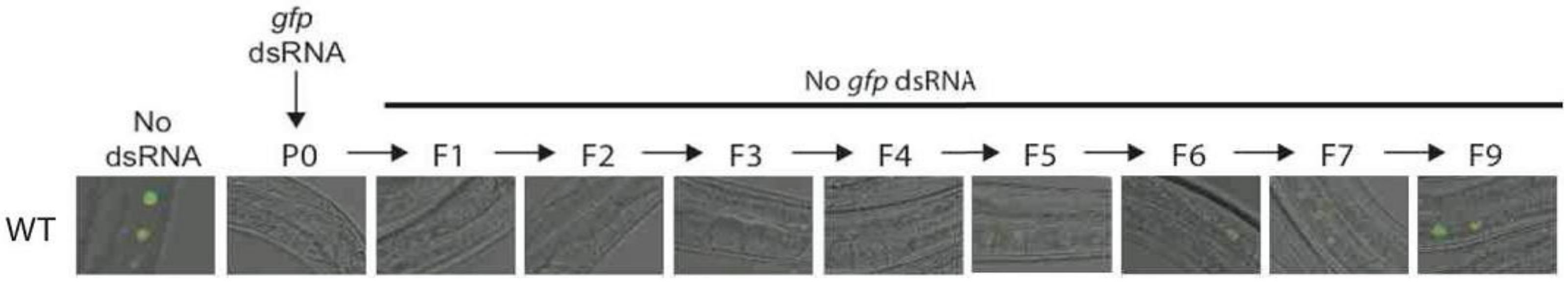
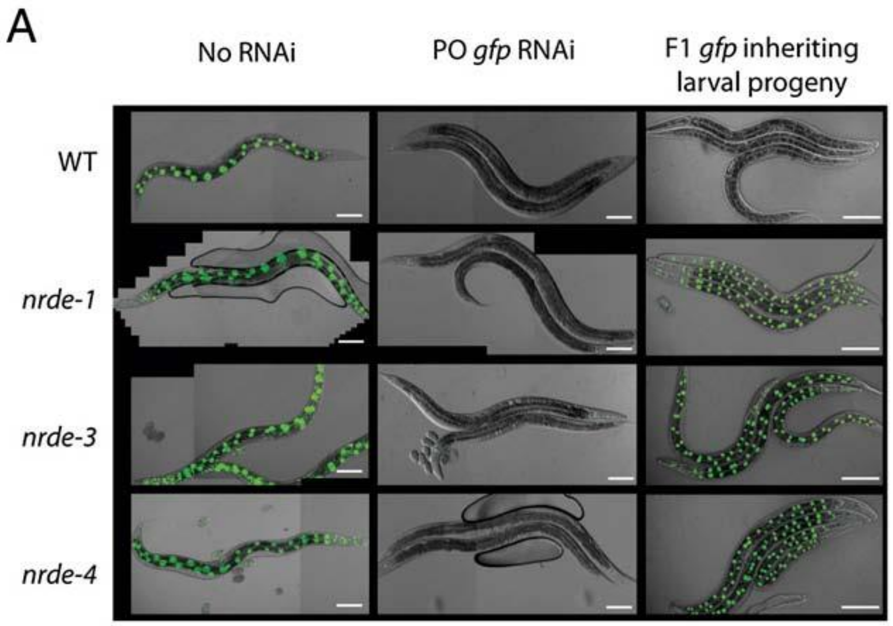
RNA干扰性状可以被多代遗传，细胞核RNA干扰通路起关键作用

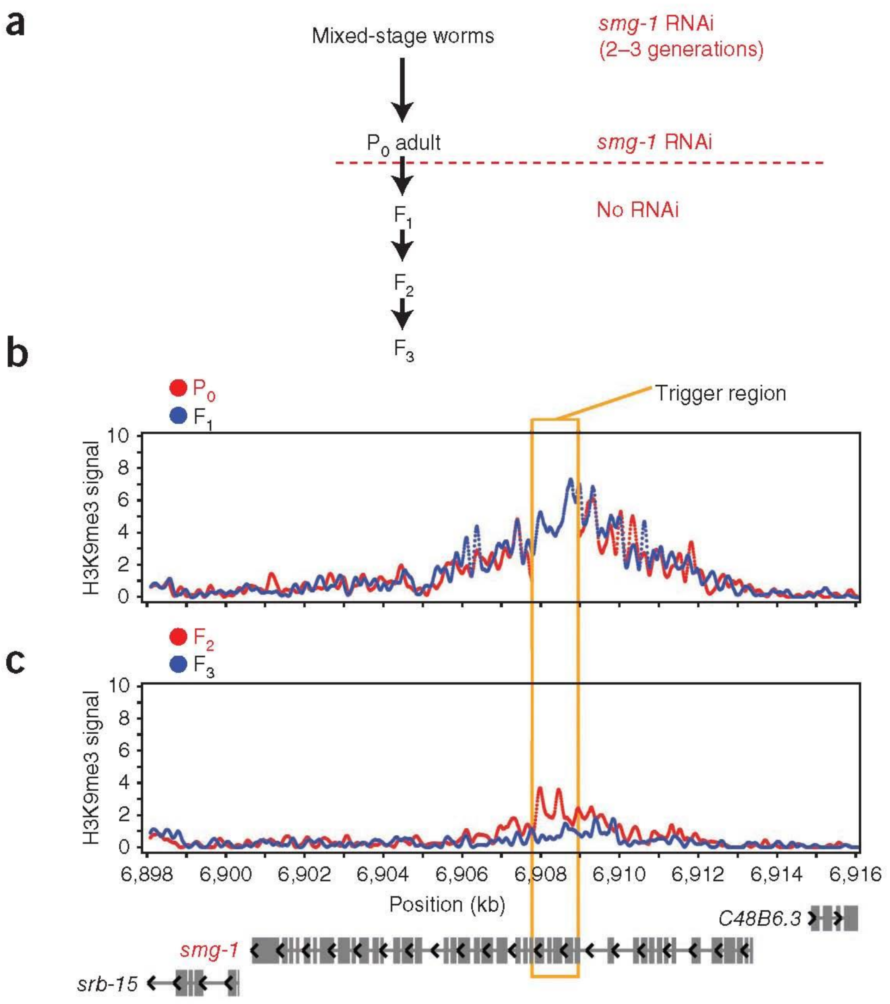
Exogenous dsRNA trigger



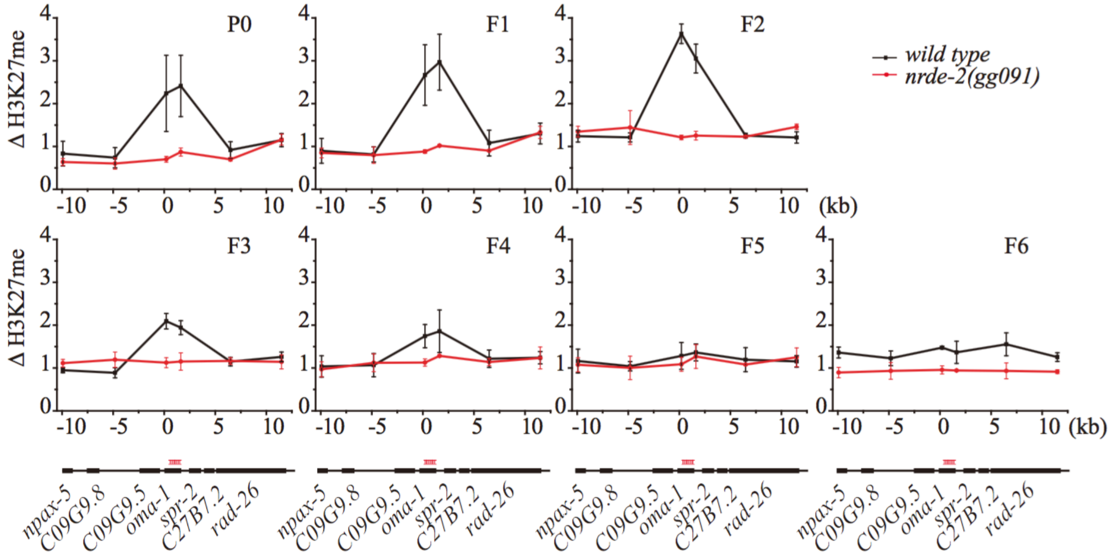


Nick O. Burton, Kirk B. Burkhart, and Scott Kennedy （2011）PNAS

Nuclear RNAi 介导的 H3K9me3 和 H3K27me3可以多代遗传

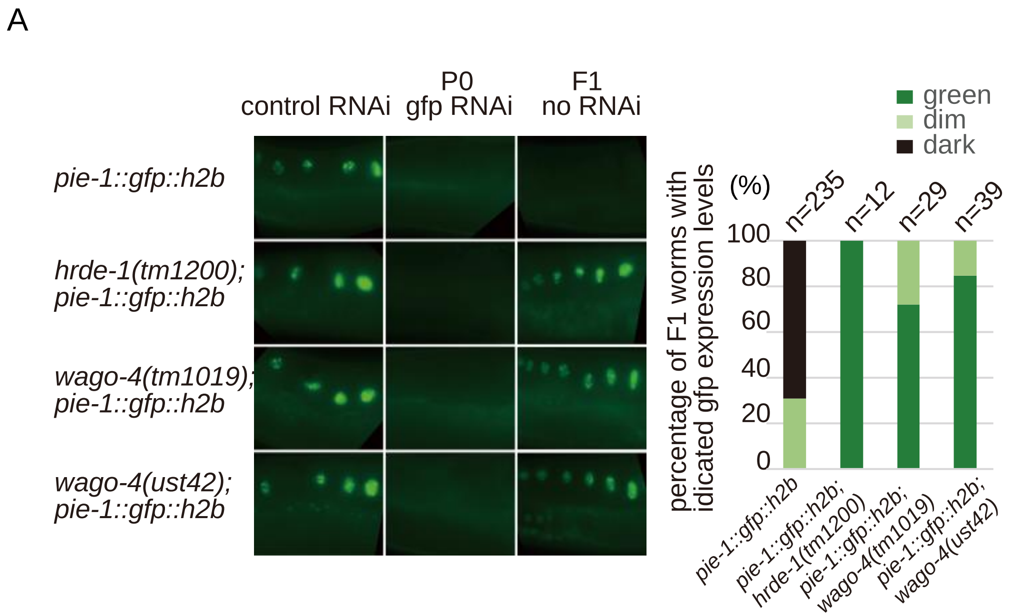


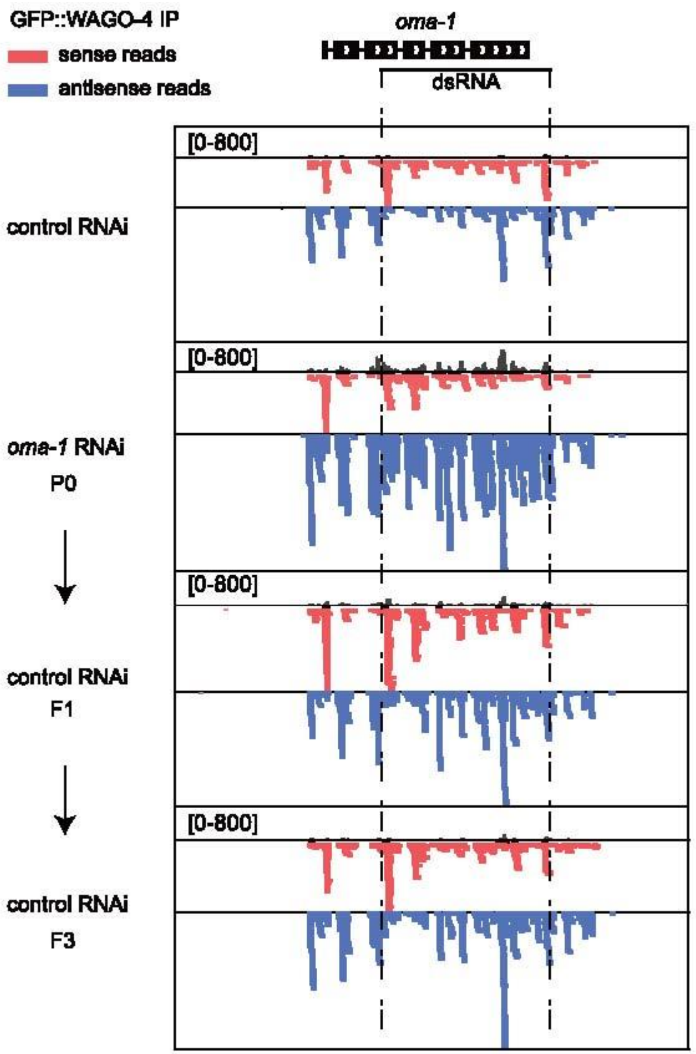
Nature Genetics 2012



Current Biology, 2015

WAGO-4 和细胞质 RNA 干扰机器参与获得性遗传

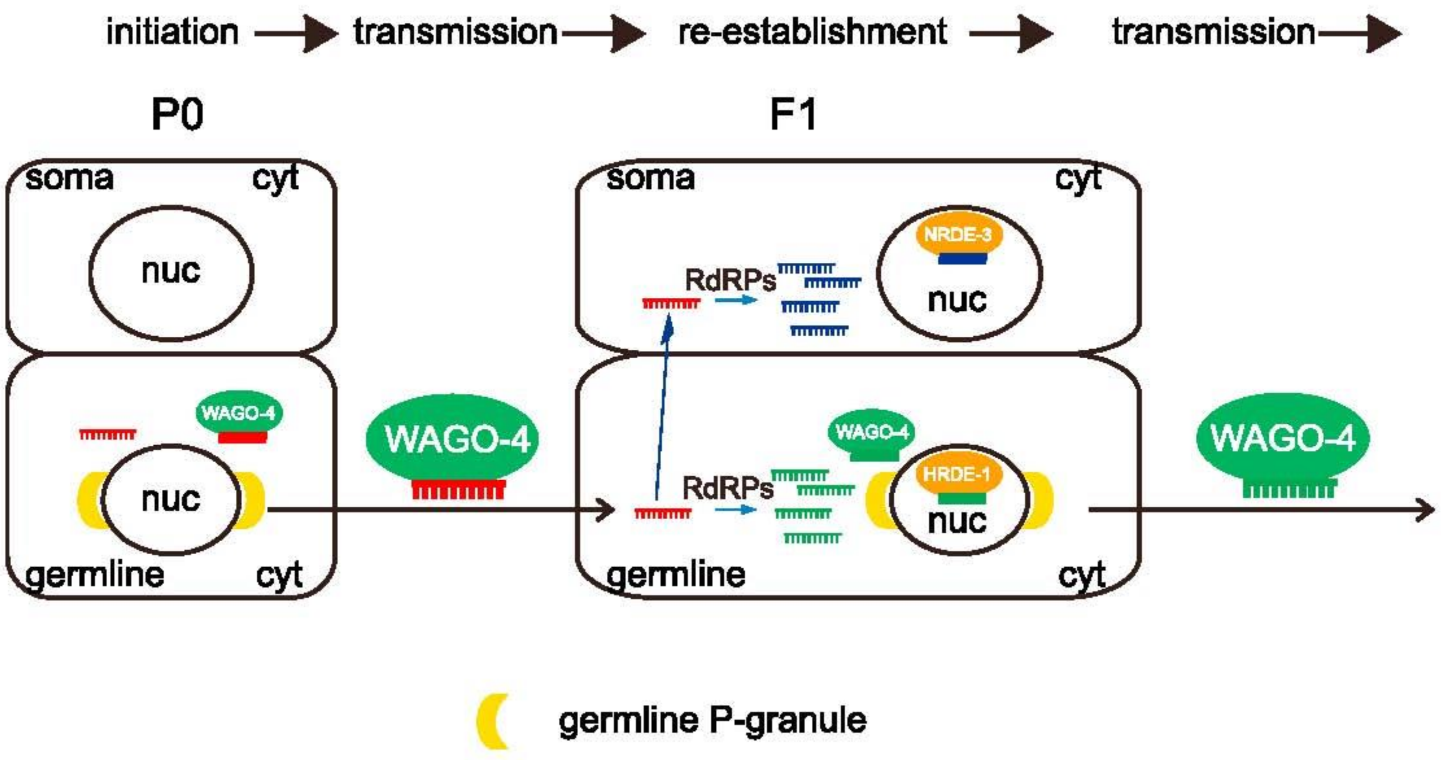




Cell Reports 2018

Wan G, Fields BD, Spracklin G, Shukla A, Phillips CM, Kennedy S. (2018) Nature

获得性遗传的三个步骤


initiation
transmission
re-establishment
transmission
P0
F1
soma
cyt
nuc
WAGO-4
nuc
germline
cyt
WAGO-4
soma
cyt
NRDE-3
RdRPs
nuc
WAGO-4
HRDE-1
RdRPs
nuc
germline
cyt
WAGO-4
germline P-granule

Part I: nuclear RNAi and nucleolar RNAi

1.1: nuclear RNAi

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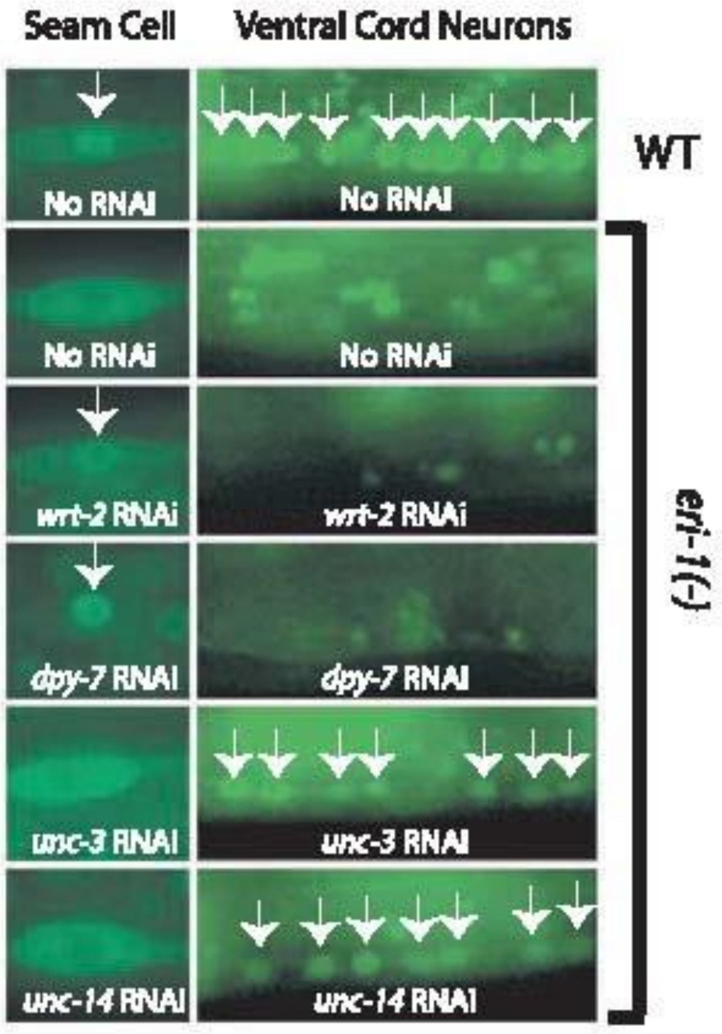
2.2: PICS复合物与 piRNA 加工

NRDE-3 transports siRNAs to the targeted pre-mRNA

NRDE-3 可以作为基因表达的时空指示



Science 2008

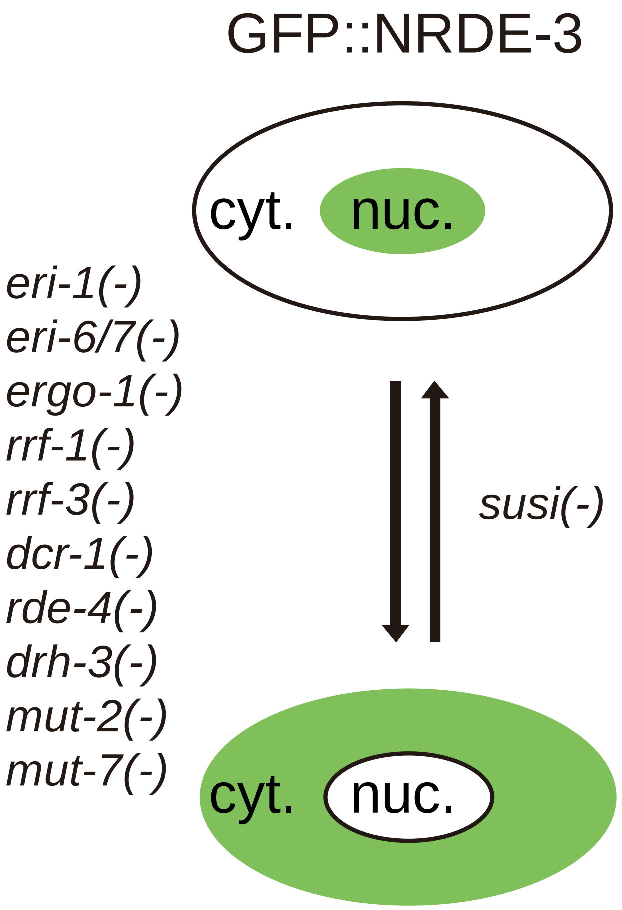
Published online 9 June 2021

Nucleic Acids Research, 2021, Vol. 49, No. 15 ***e86***
https://doi.org/10.1093/nar/gkab469

Imaging of native transcription and transcriptional dynamics *in vivo* using a tagged Argonaute protein

Amel Toudji-Zouaz, Vincent Bertrand* and Antoine Barrière*

Forward genetic screening identified *suppressor of siRNA (susi)* mutants

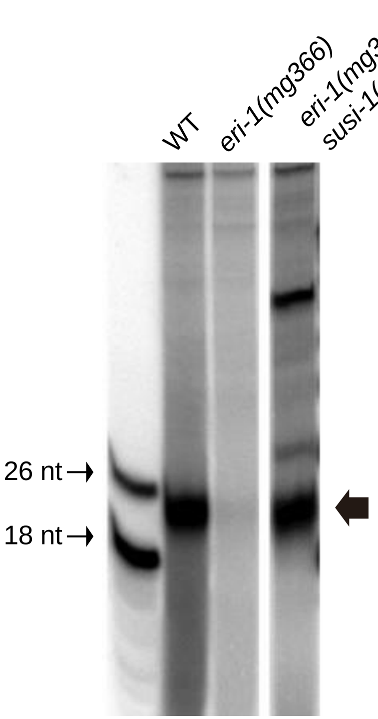


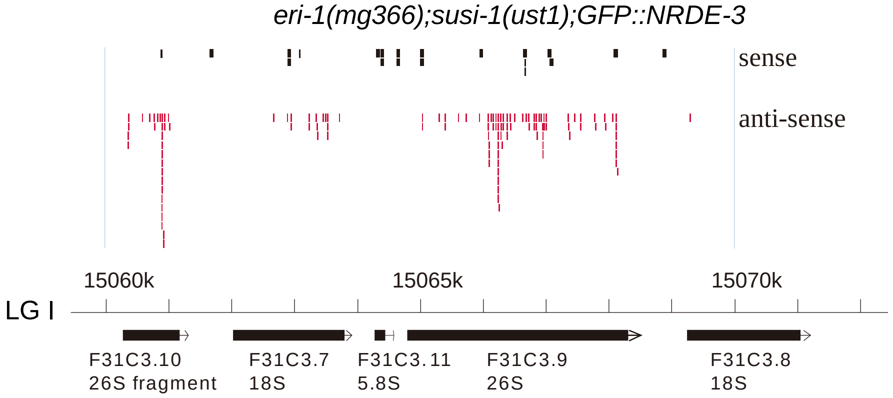
suppressor of siRNA:

susi-1
susi-2
susi-3
susi-4
susi-5

反义核糖体小干扰 RNA (risiRNA) 是一类新的小干扰 RNA

IP NRDE-3





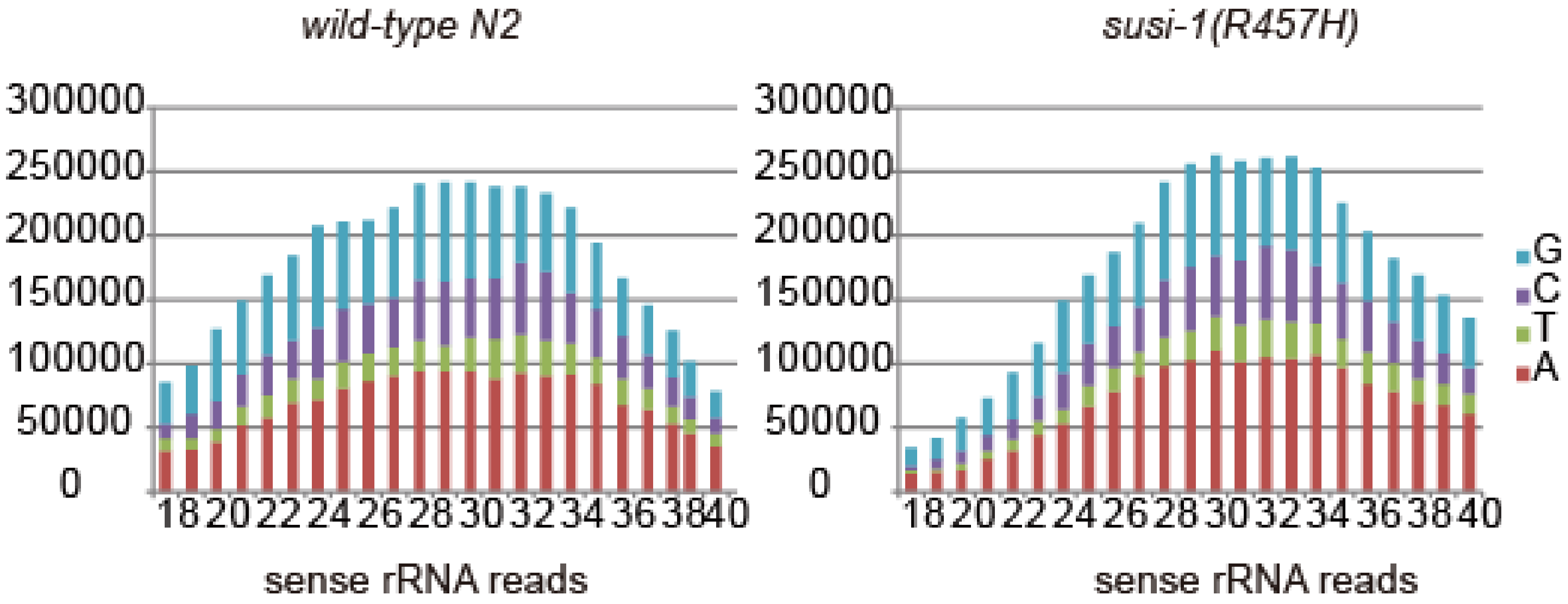
siRNA sequencing

risiRNA: antisense ribosomal siRNA

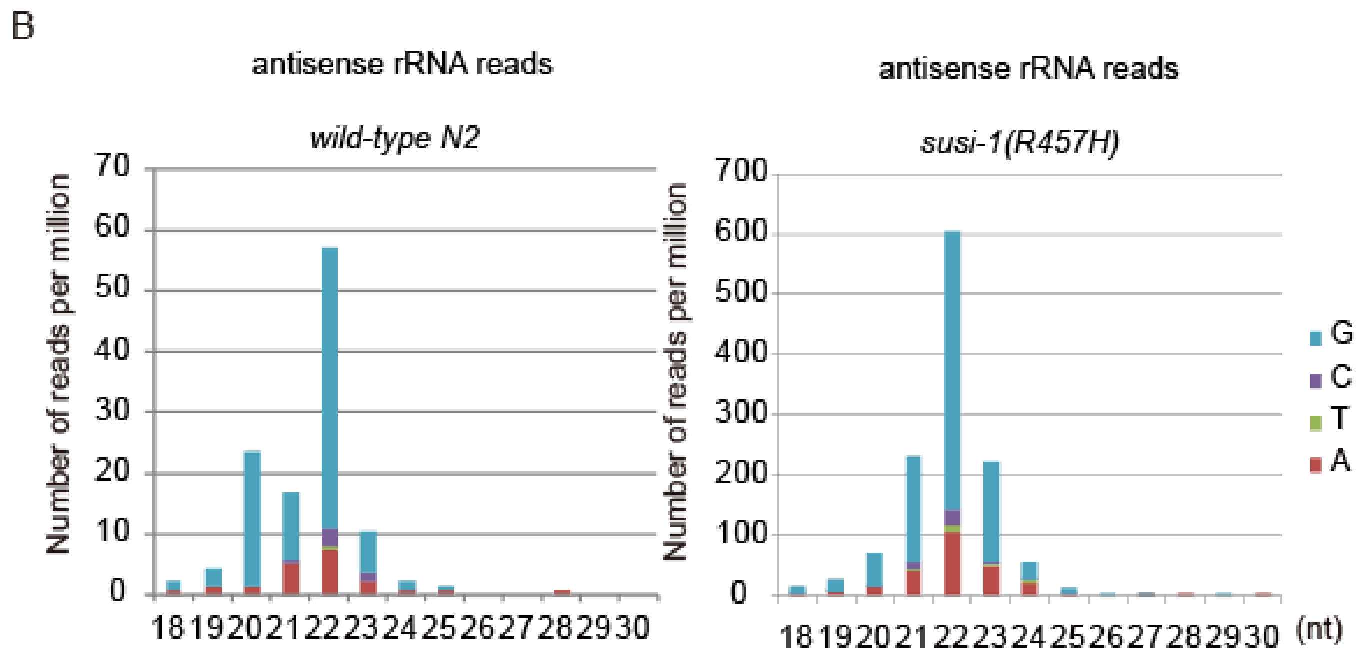
Nature Structural & Molecular Biology 2017

risiRNA is not degradation product of rRNA

Deep-seq



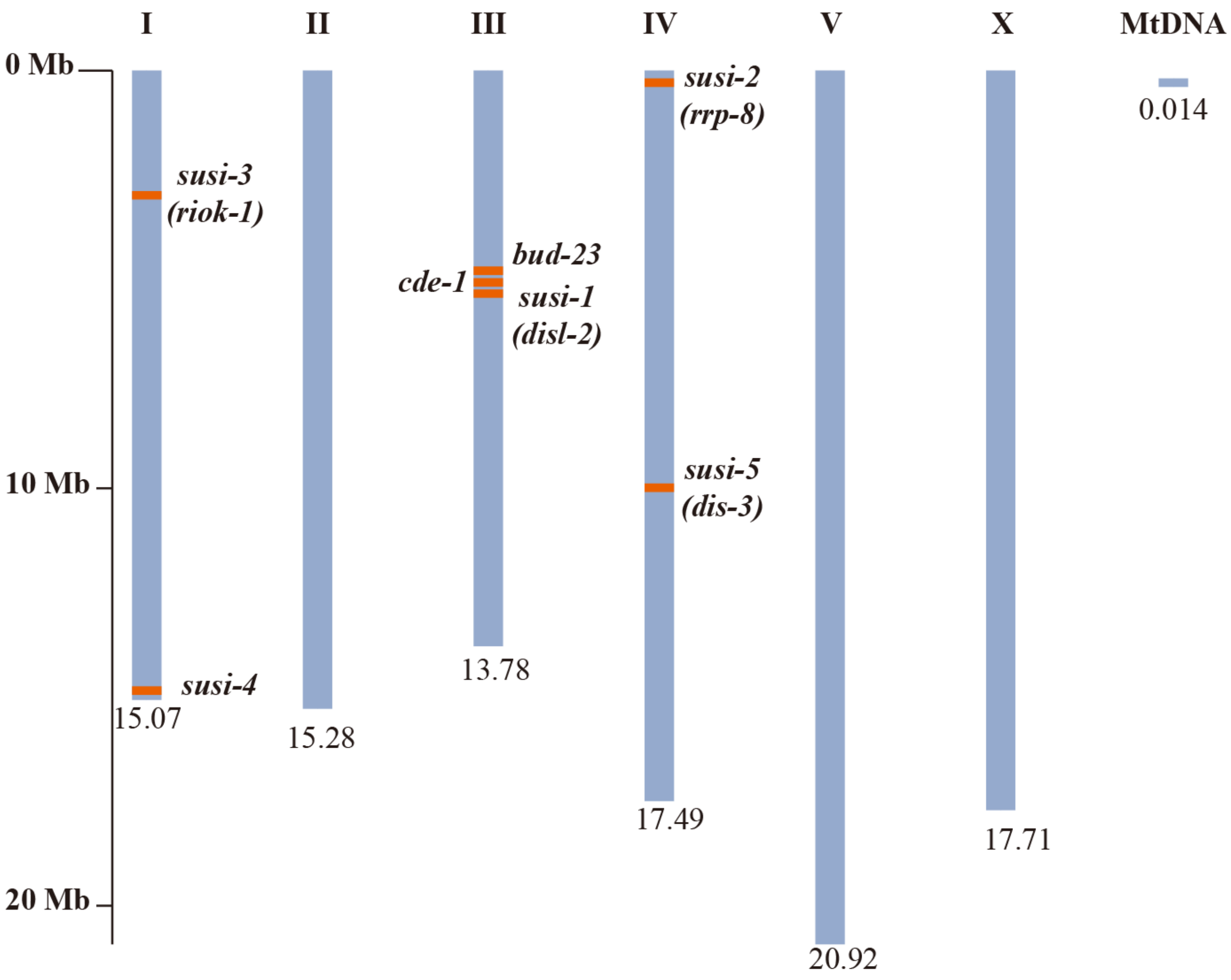
反义核糖体小干扰 RNA (risiRNA) 是一类新的小干扰 RNA



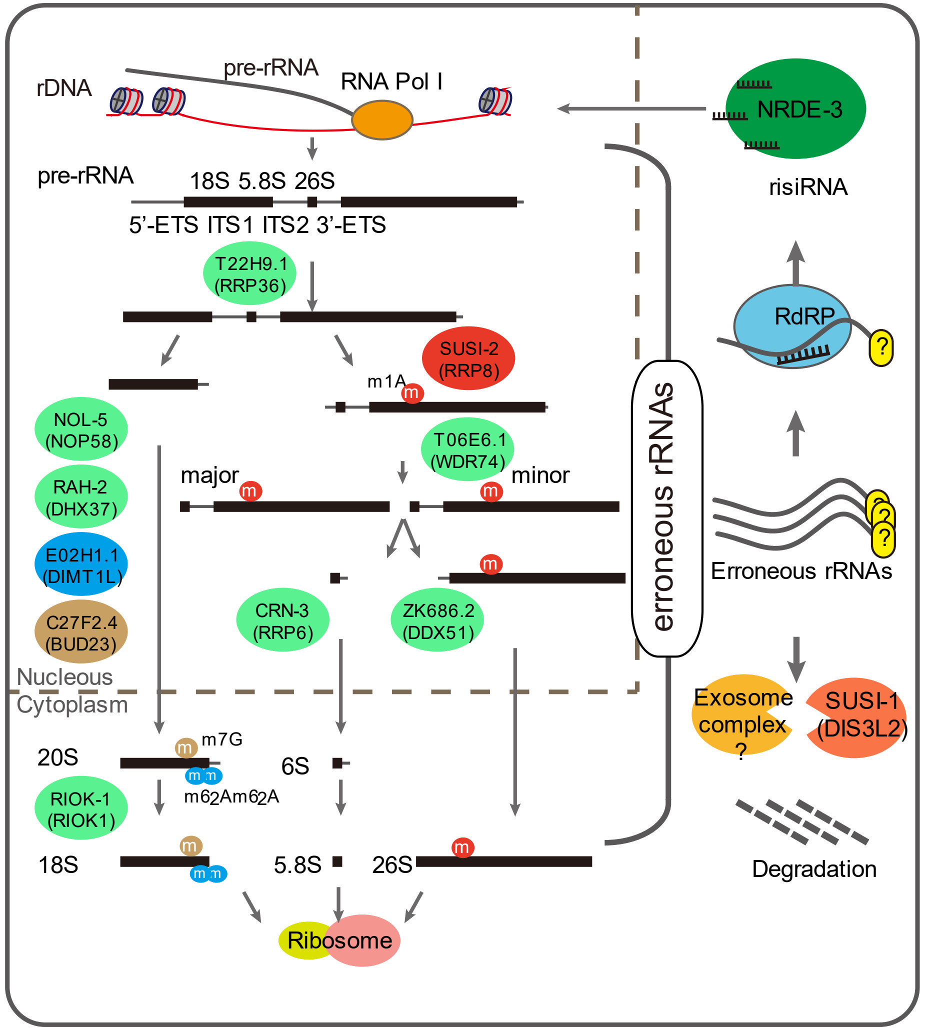
risiRNA 广泛存在于不同物种里

- Buhler M, Spies N, Bartel DP, Moazed D. TRAMP-mediated RNA surveillance prevents spurious entry of RNAs into the Schizosaccharo-myces pombe siRNA pathway. *Nat Struct Mol Biol 2008*
- Lee HC, Chang SS, Choudhary S, Aalto AP, Maiti M, Bamford DH, Liu Y. qiRNA is a new type of small interfering RNA induced by DNA damage. *Nature 2009*
- Cao M, Du P, Wang X, Yu YQ, Qiu YH, Li W, Gal-On A, Zhou C,Li Y, Ding SW. Virus infection triggers widespread silencing of host genes by a distinct class of endogenous siRNAs in Arabidopsis. *Proc Natl Acad Sci U S A 2014*
- You C, He W, Hang R, Zhang C, Cao X, Guo H, Chen X, Cui J, Mo B. FIERY1 promotes microRNA accumulation by suppressing rRNA-derived small interfering RNAs in Arabidopsis. *Nat Commun. 2019*

Forward genetic screening identified *suppressor of siRNA (susi)* mutants

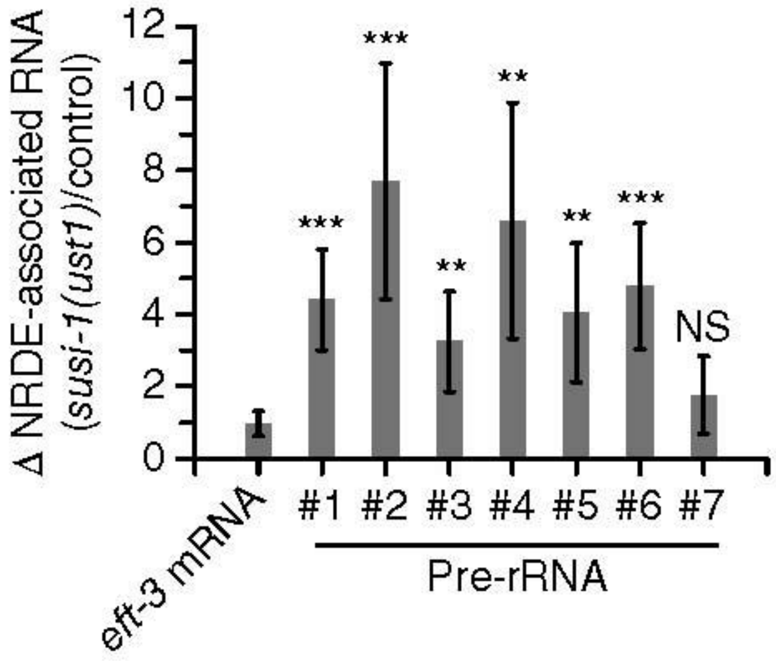


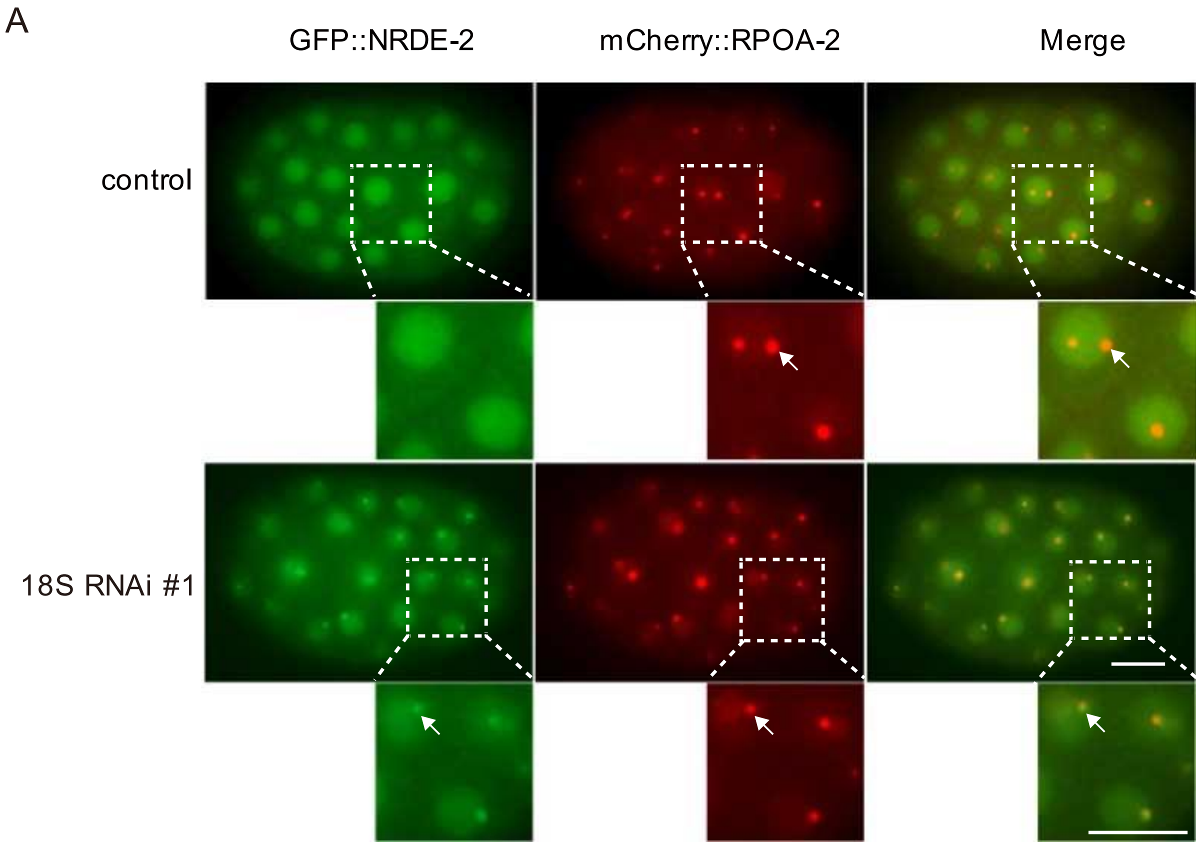
错误的核糖体 RNA 诱导 risiRNA 产生



PNAS 2018

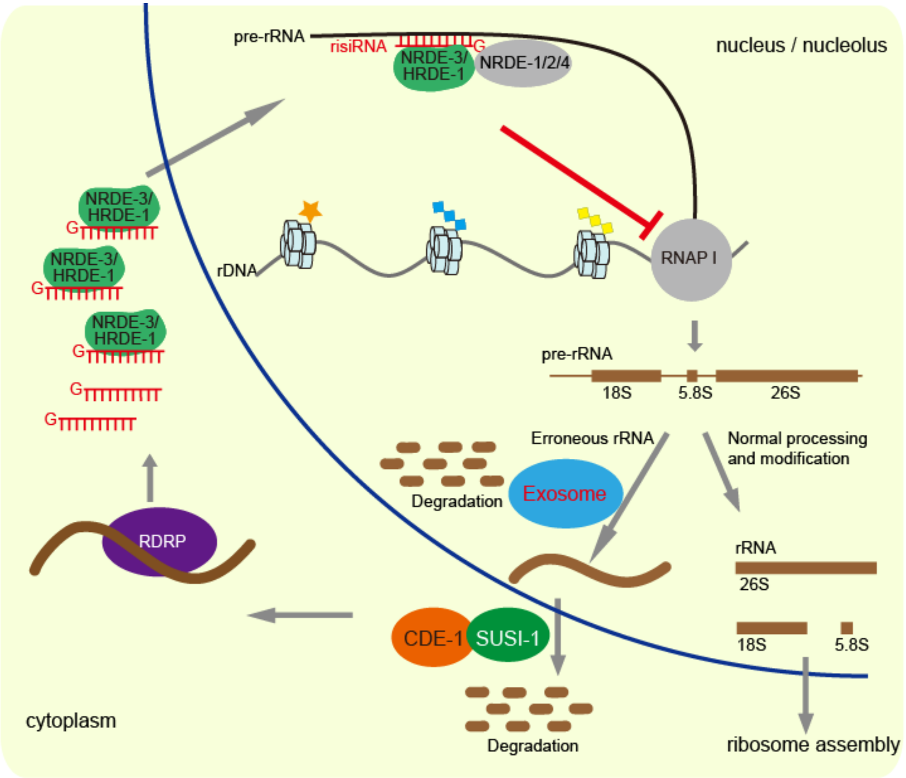
risiRNA 诱导核仁 RNA 干扰通路 (nucleolar RNAi)





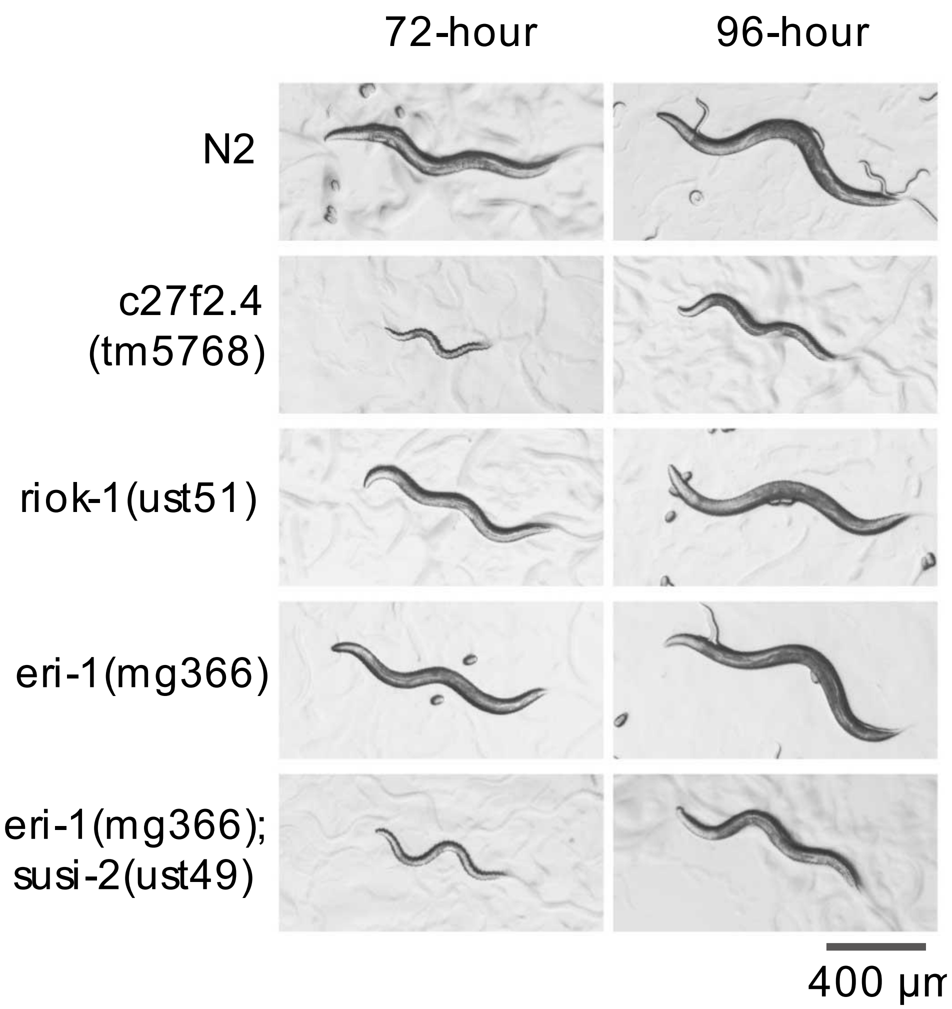
Nucleic Acids Research (2021)

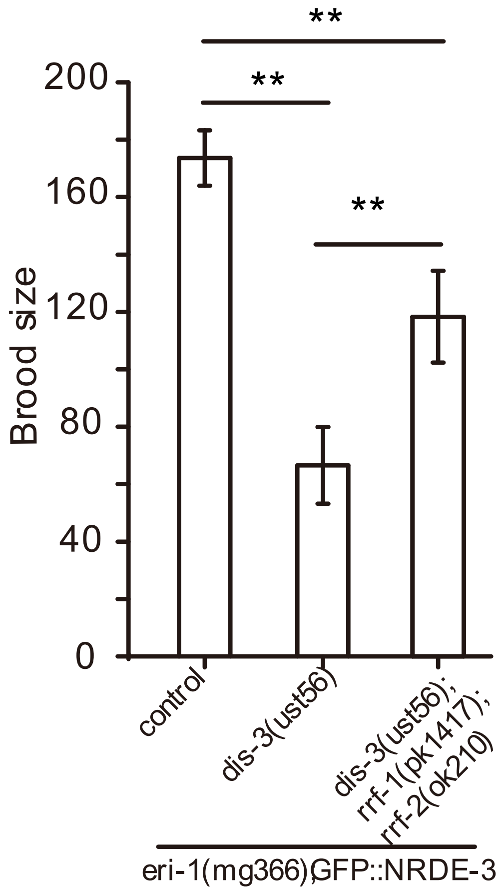
核仁 RNAi 的分子机制



NSMB 2017;
PNAS 2018;
NAR 2021

risiRNA is important for growth and fertility



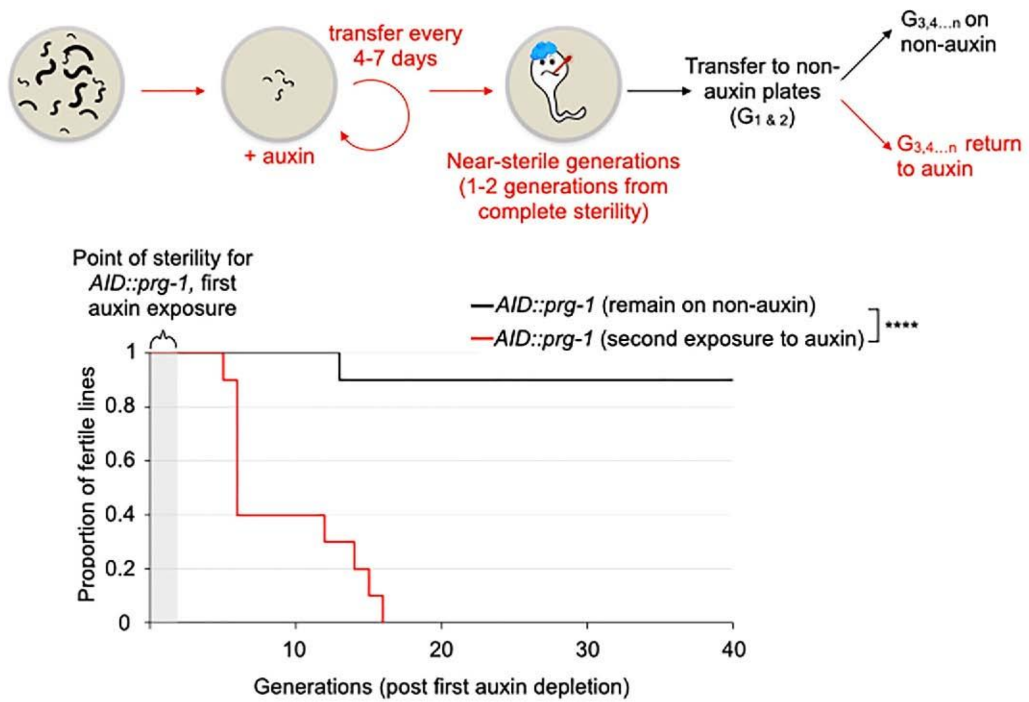


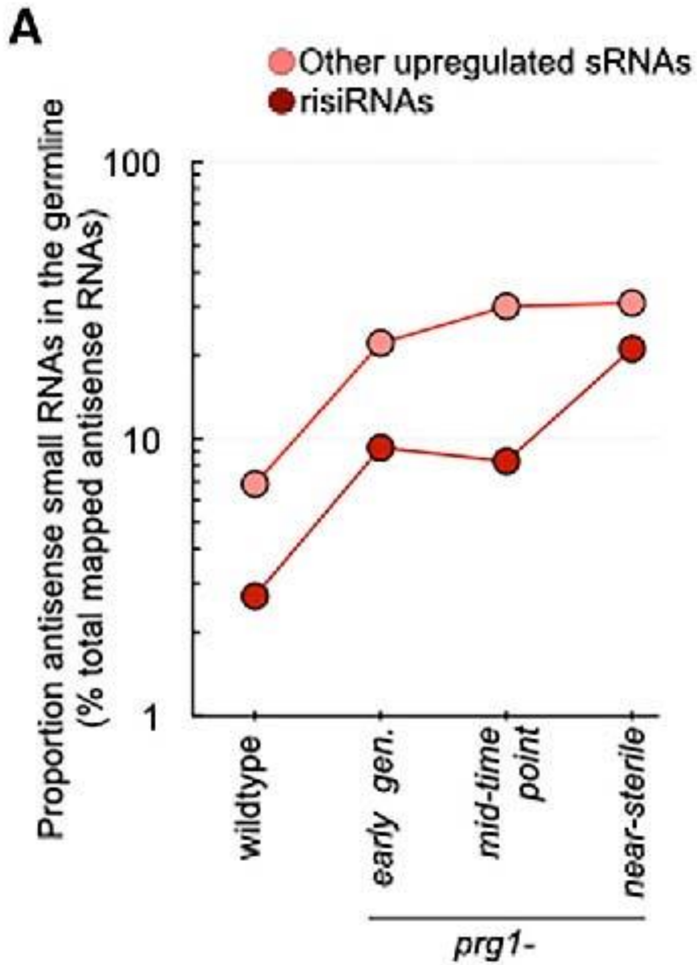
Developmental Cell (2021)

Article

An essential role for the piRNA pathway in regulating the ribosomal RNA pool in *C. elegans*

Lamia Wahba,[1] Loren Hansen,[1,2] and Andrew Z. Fire[1,3,*]





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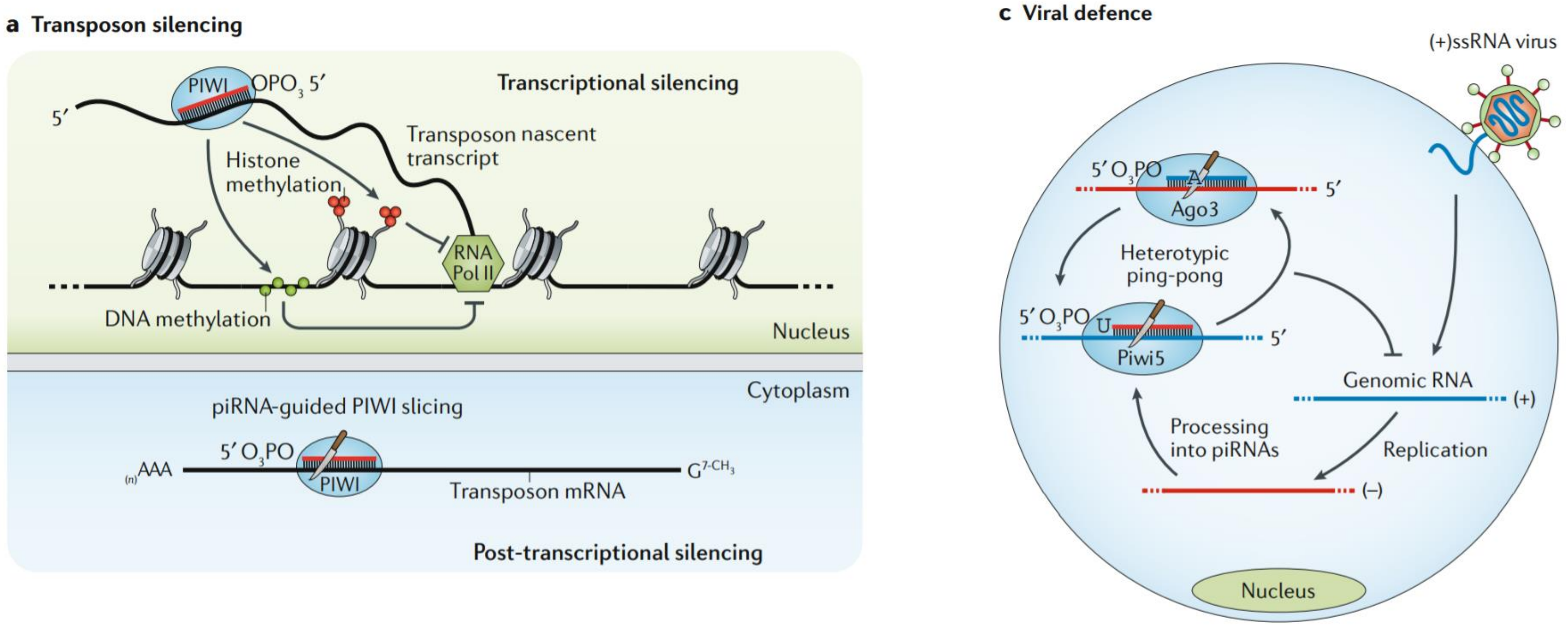
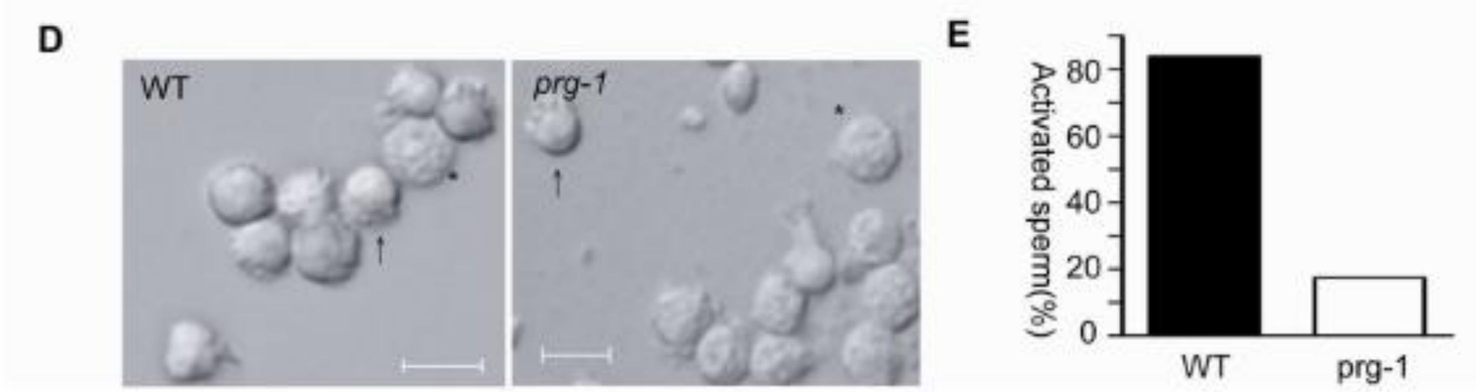
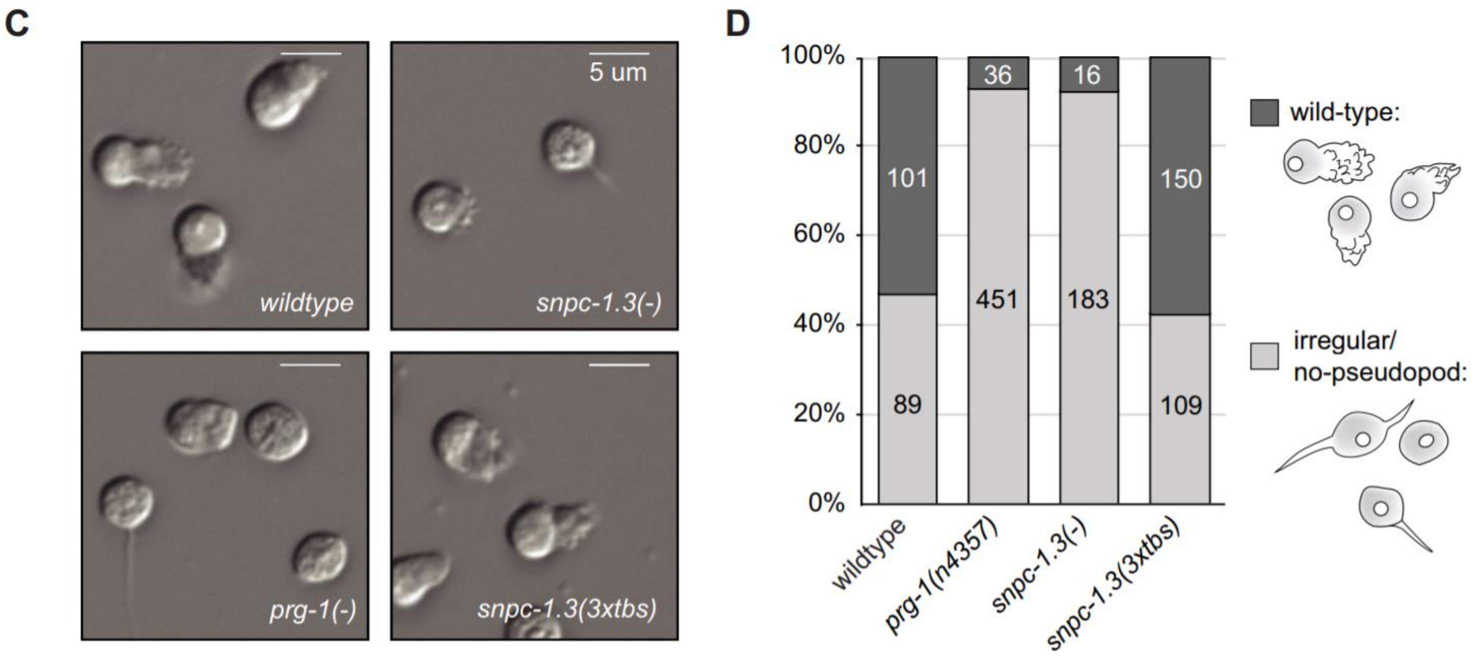
piRNA参与沉默转座子和外源序列的表达



Deniz *et al.*, 2019

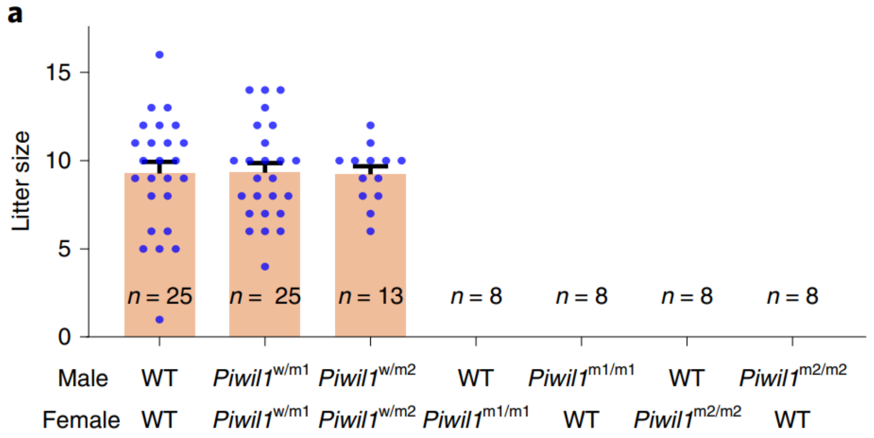
piRNA生成缺陷导致线虫精子发生异常且雄虫不育

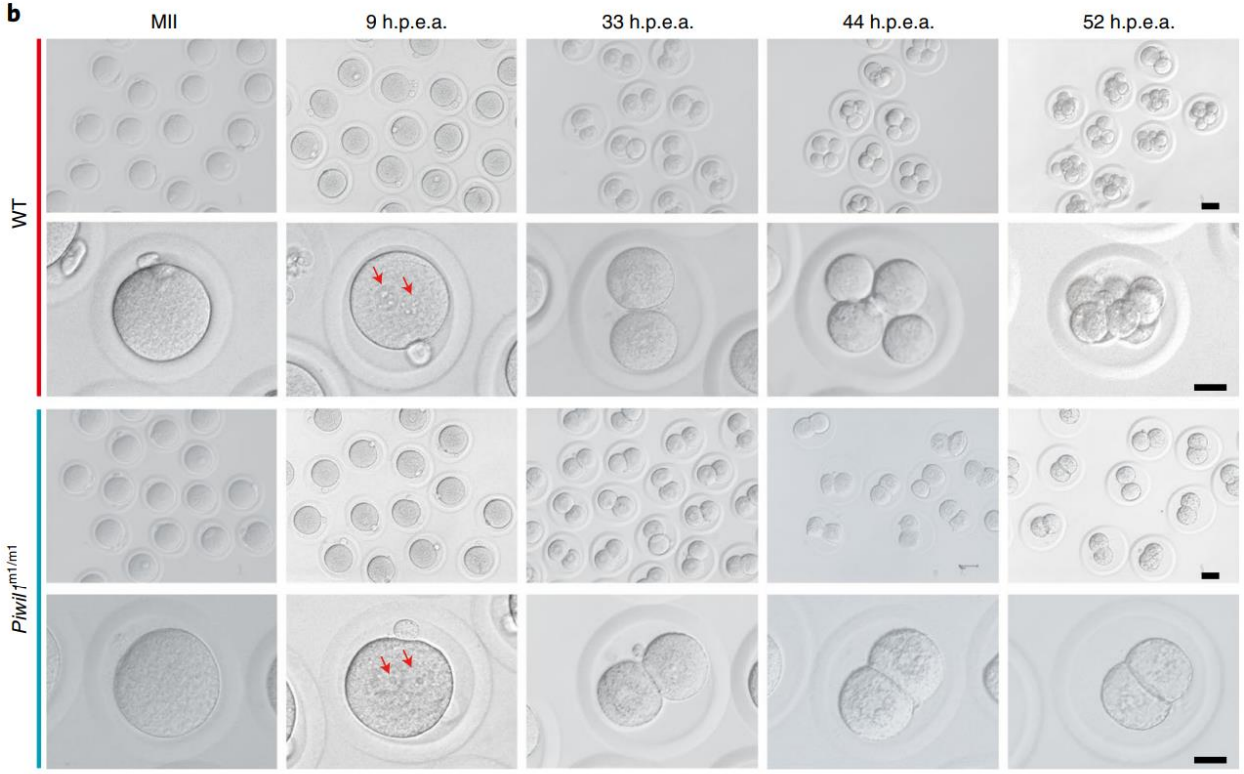


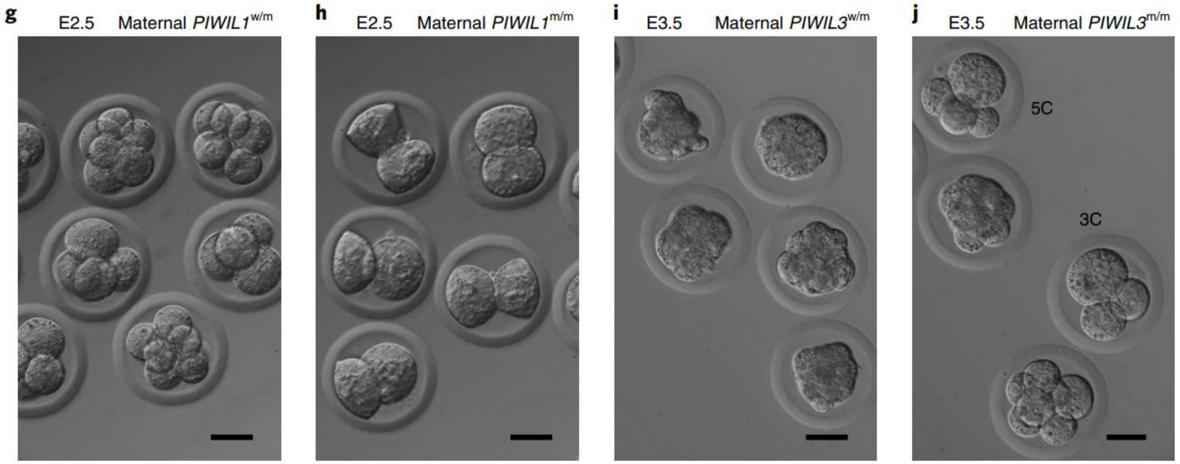


Wang *et al*., 2008; Charlotte *et al*., 2021

piRNA通路在金黄地鼠雄性和雌性生殖中发挥重要作用

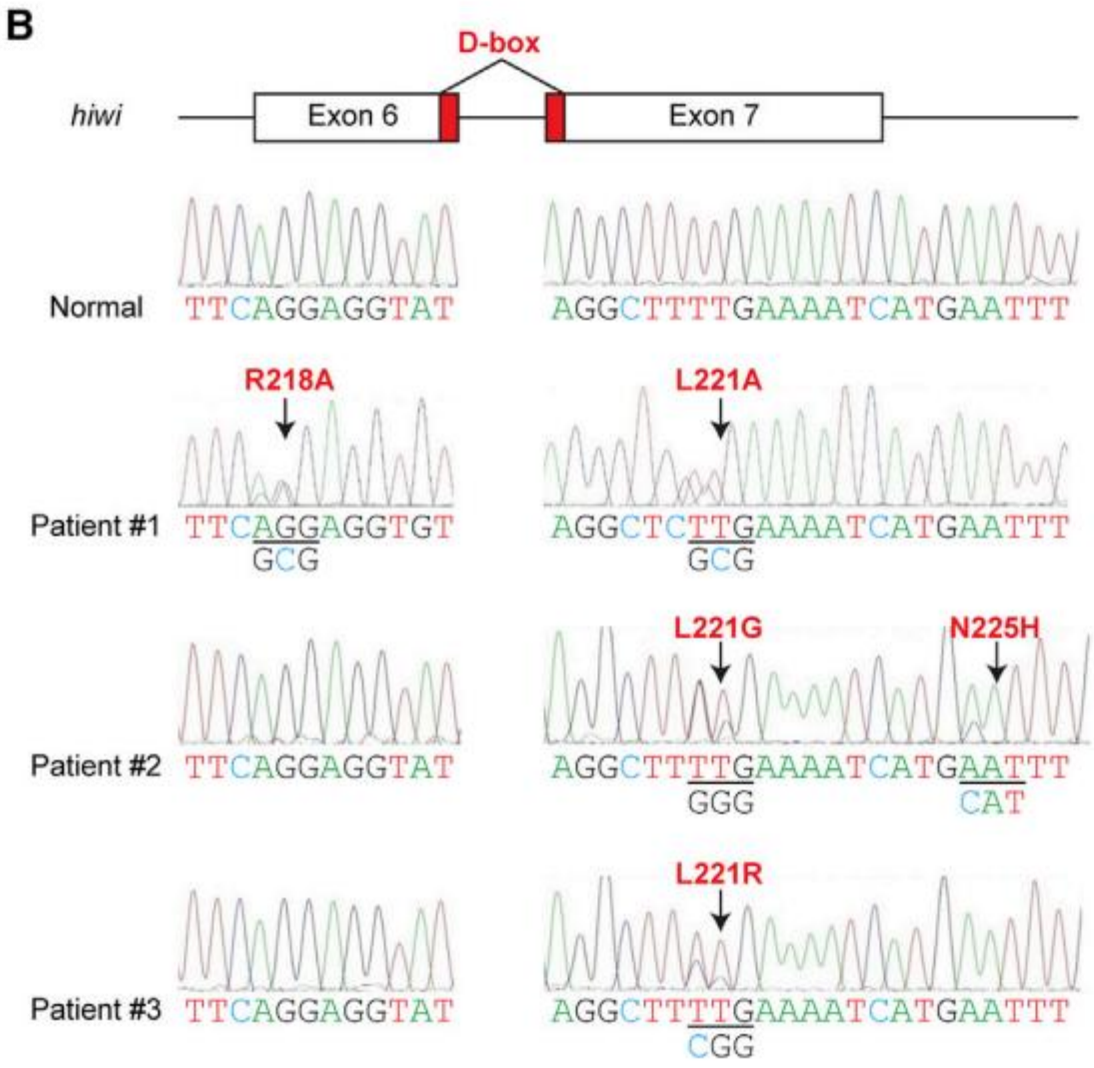


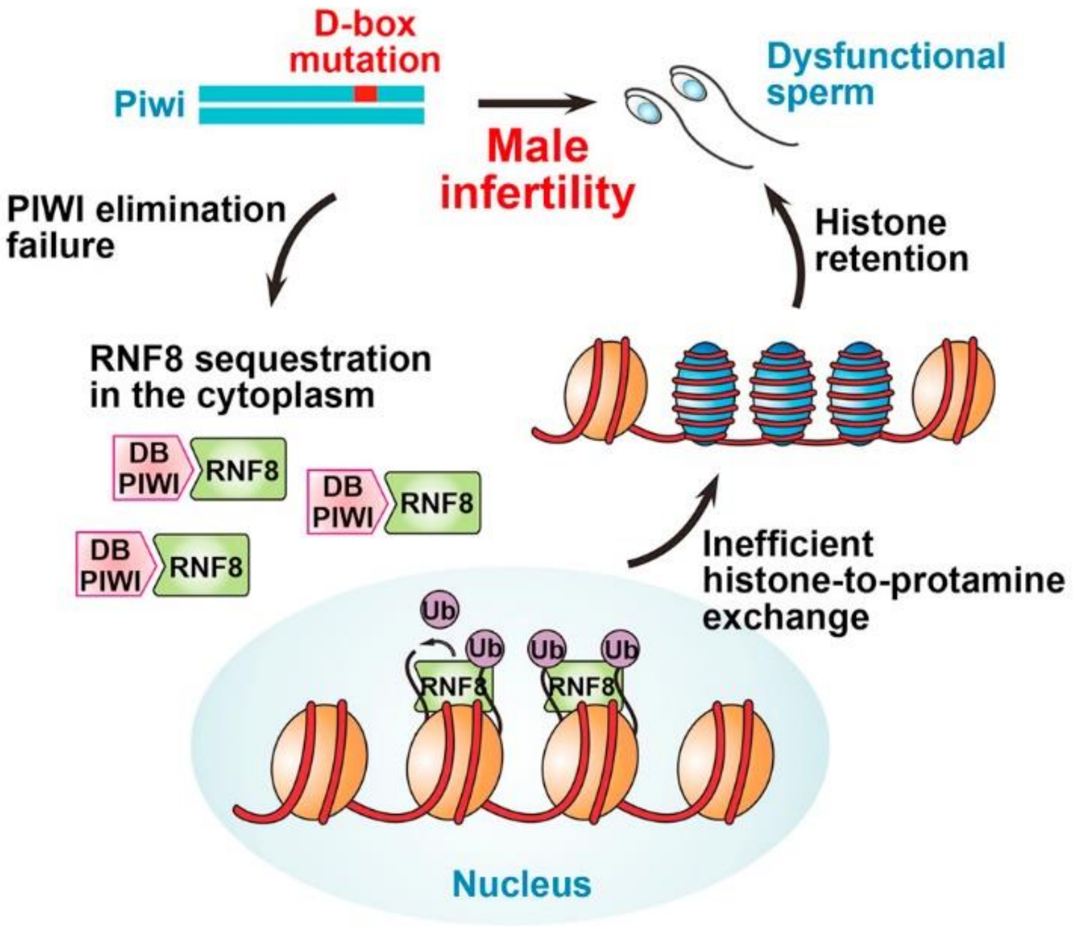




Hasuwa *et al*., 2021; Zhang *et al*., 2021

人类Piwi蛋白突变造成男性不育

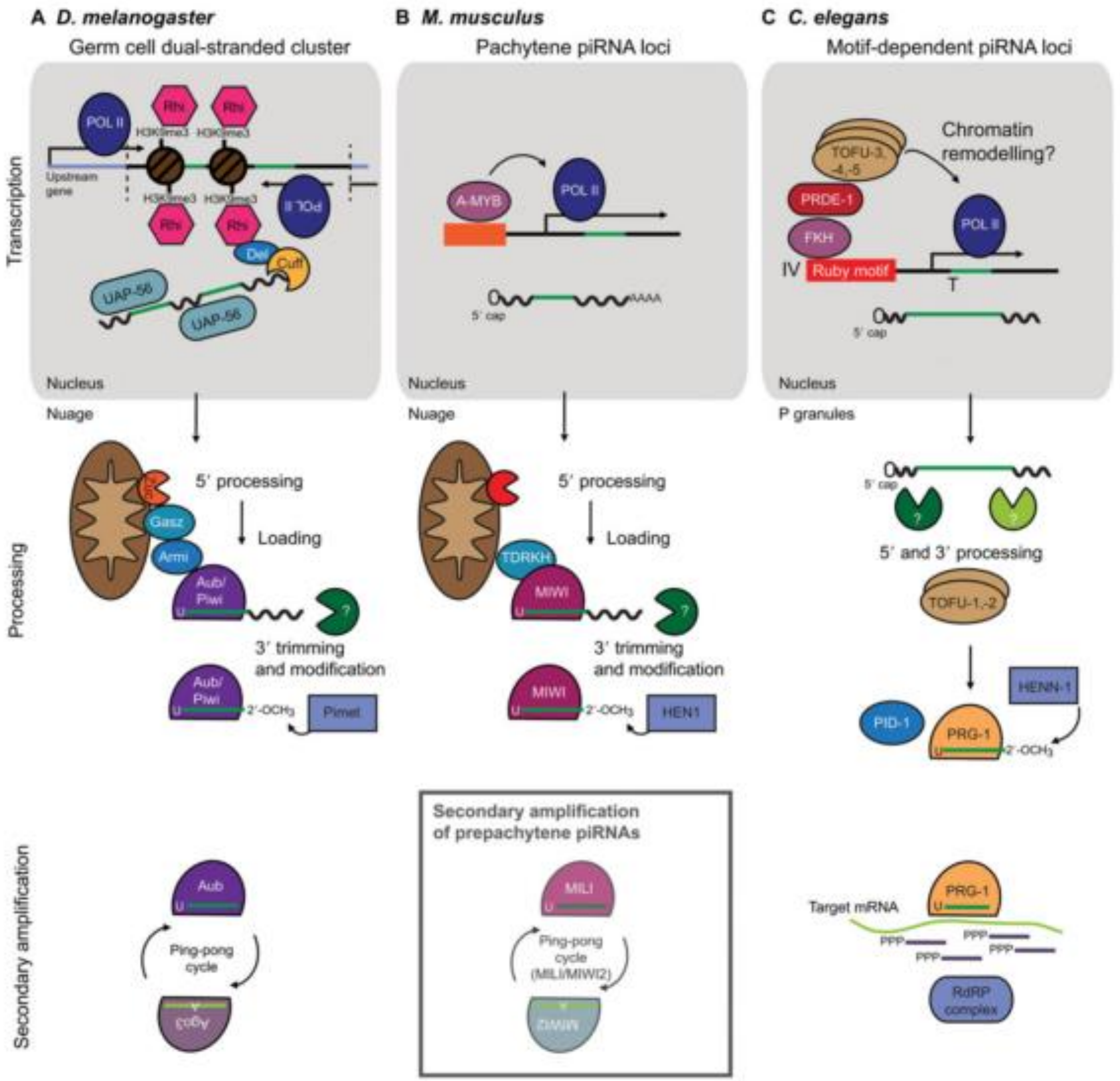




Gou *et al.*, 2017

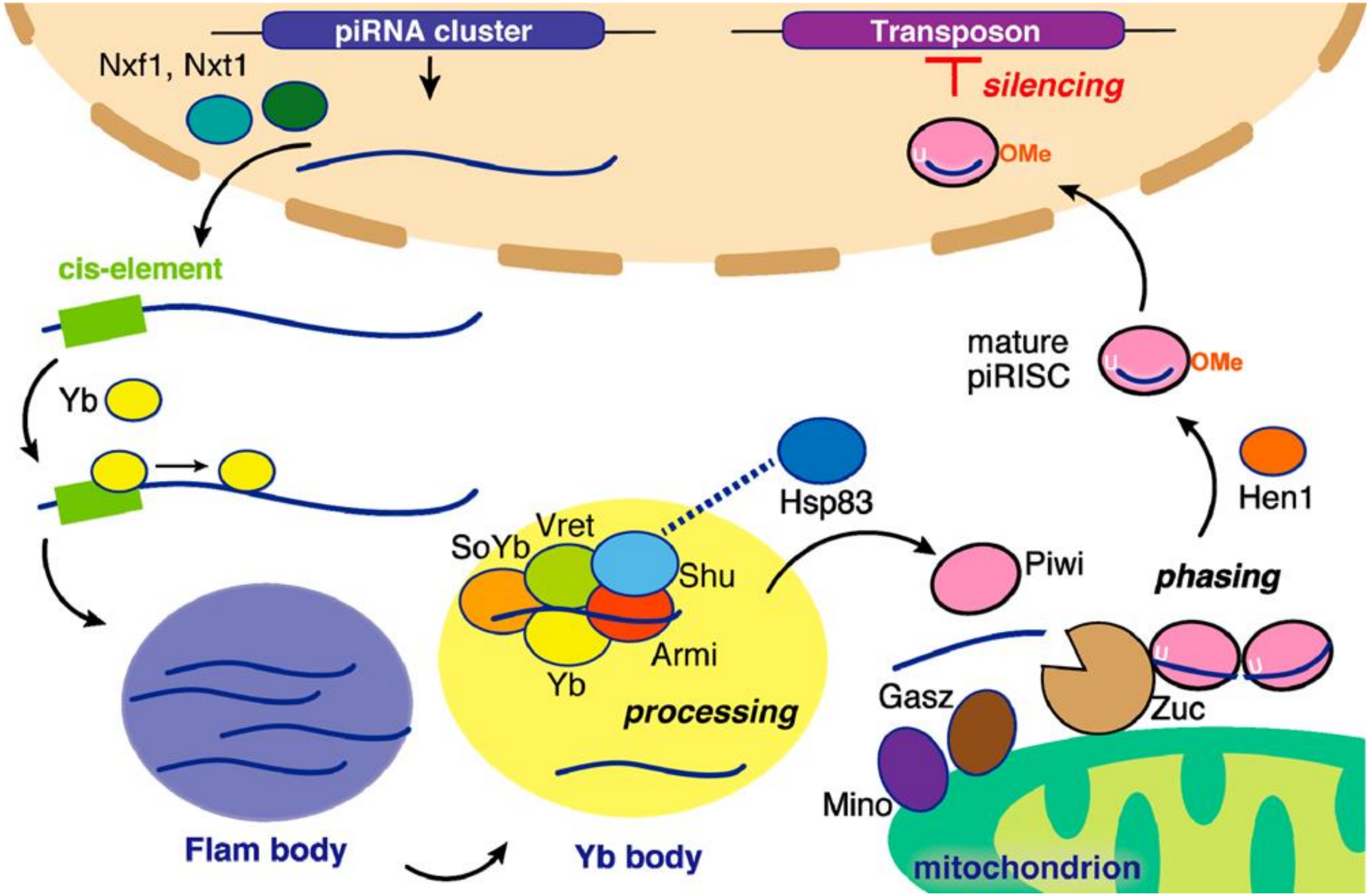
研究背景

piRNA的产生与加工机制并不完全清楚



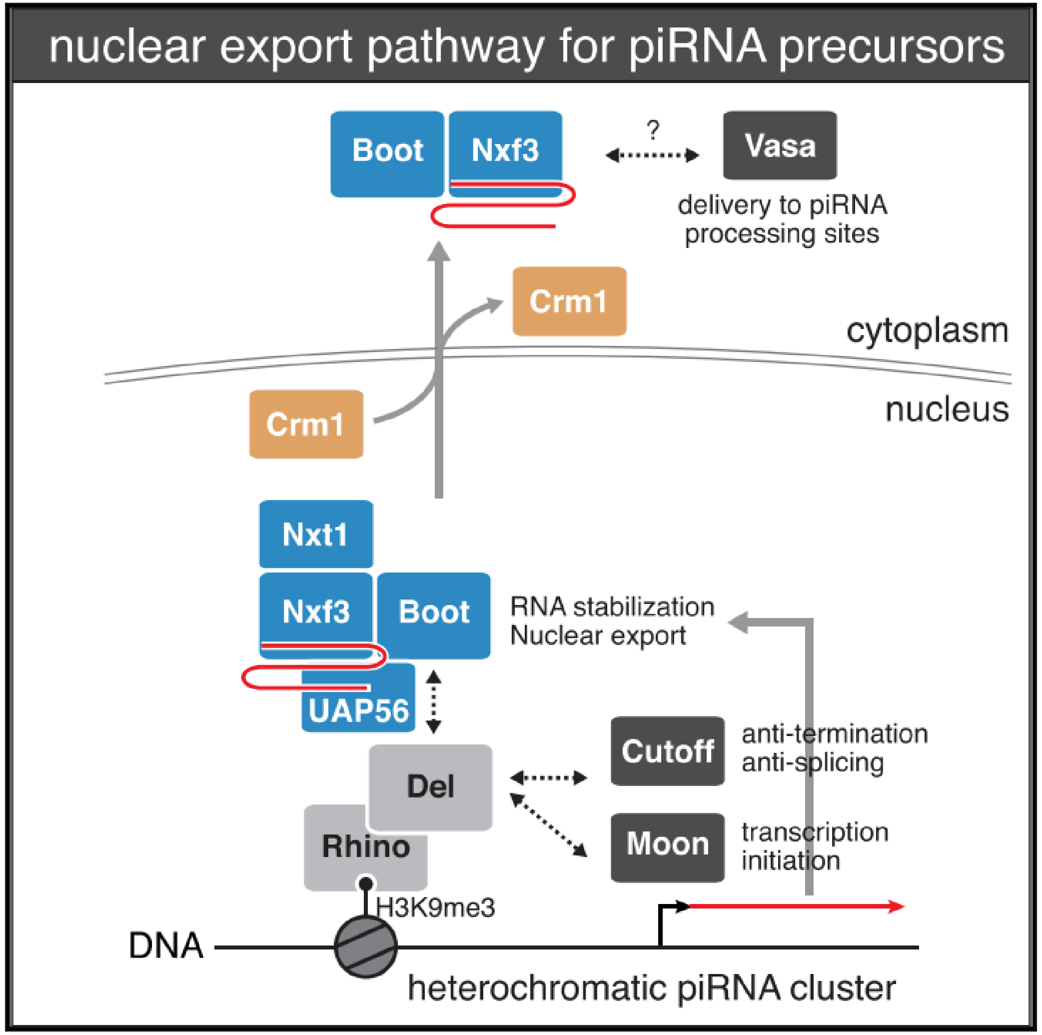
Eva-Maria, *et al.* 2014

果蝇单向转录piRNA的生成



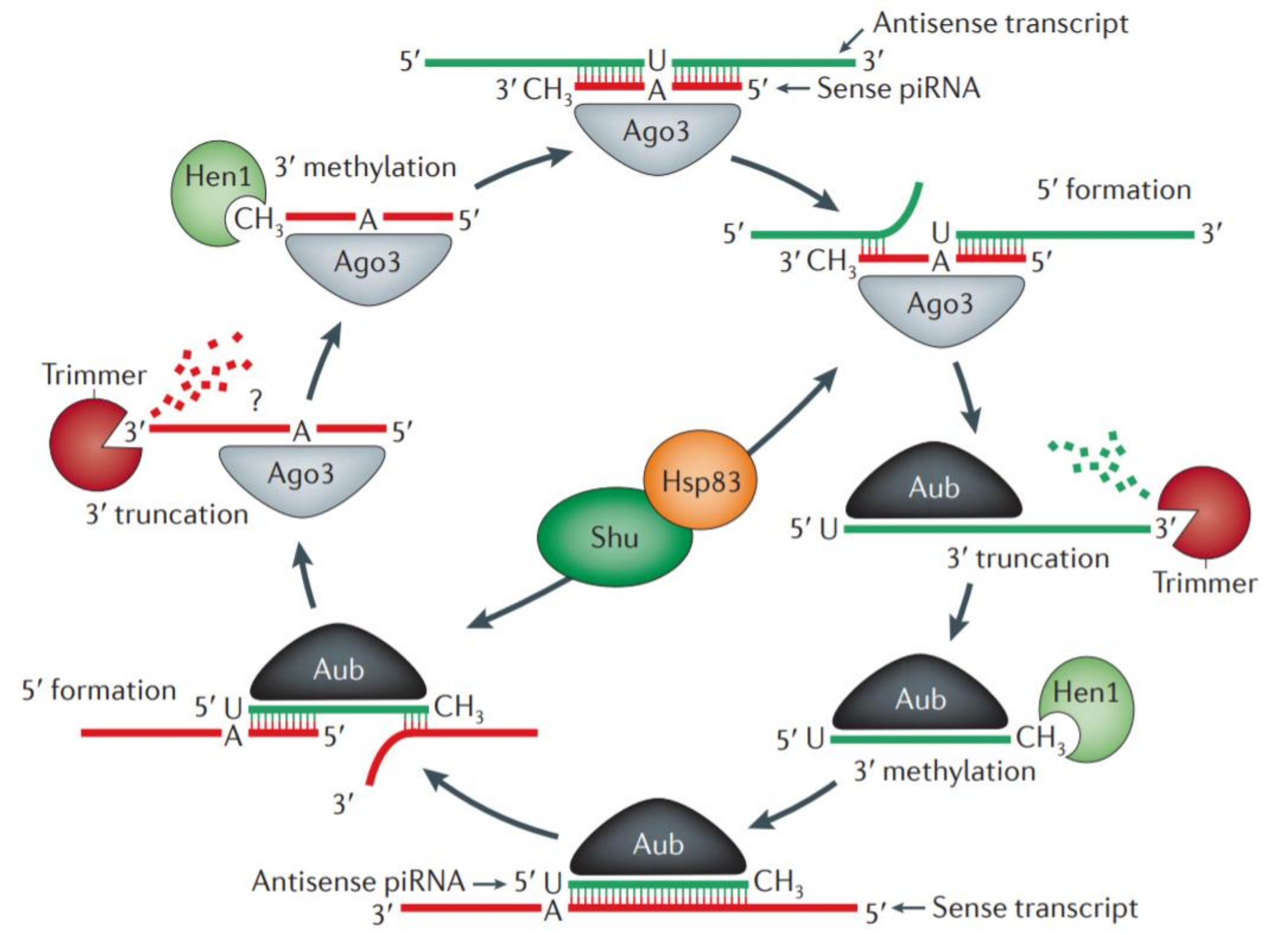
Haruna *et al.*, 2018

果蝇双向转录piRNA的生成



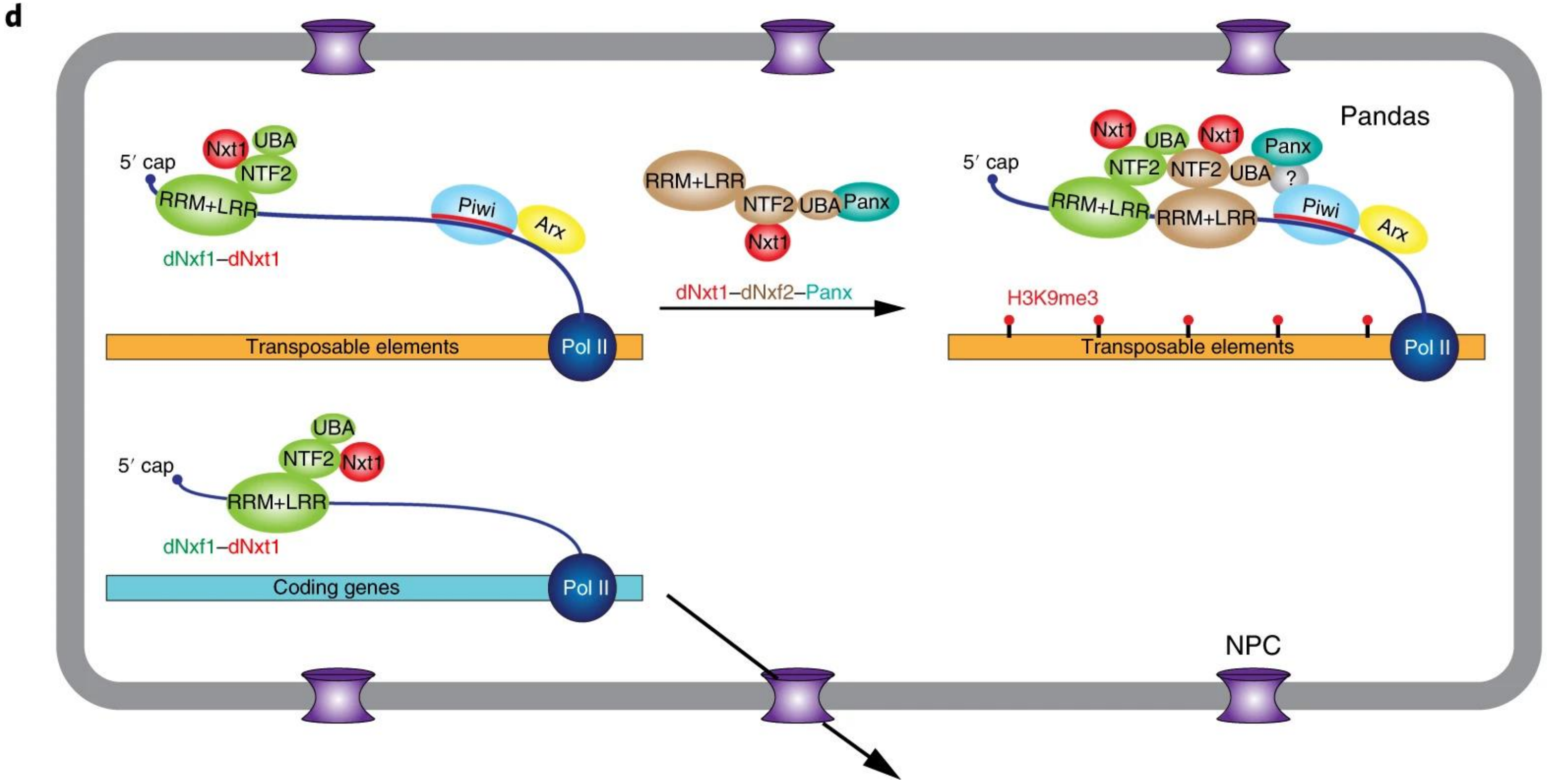
Mostafa *et al.*, 2019

果蝇次级piRNA的生成 - Ping-Pong循环



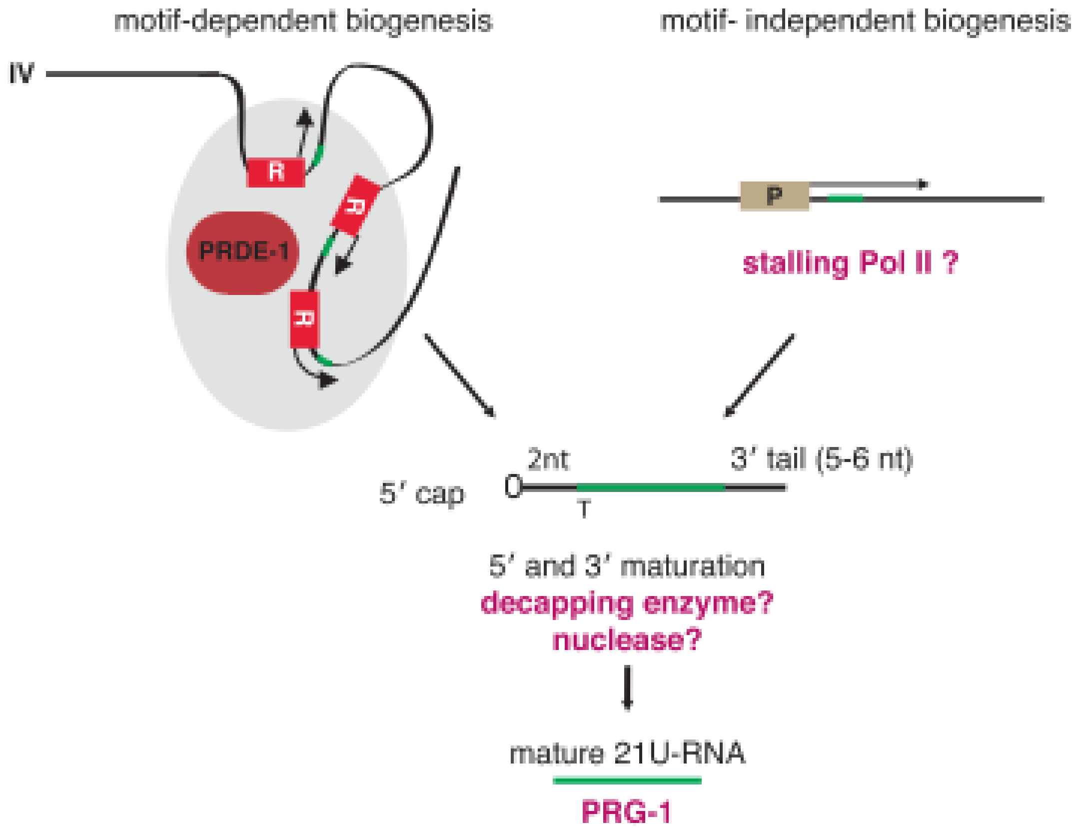
Maartje *et al.*, 2013

A Pandas complex adapted for piRNA-guided transcriptional silencing and heterochromatin formation



Kang Zhao & Yang Yu (2019) Nat. Cell Biol.

线虫 piRNA 的转录与加工成熟



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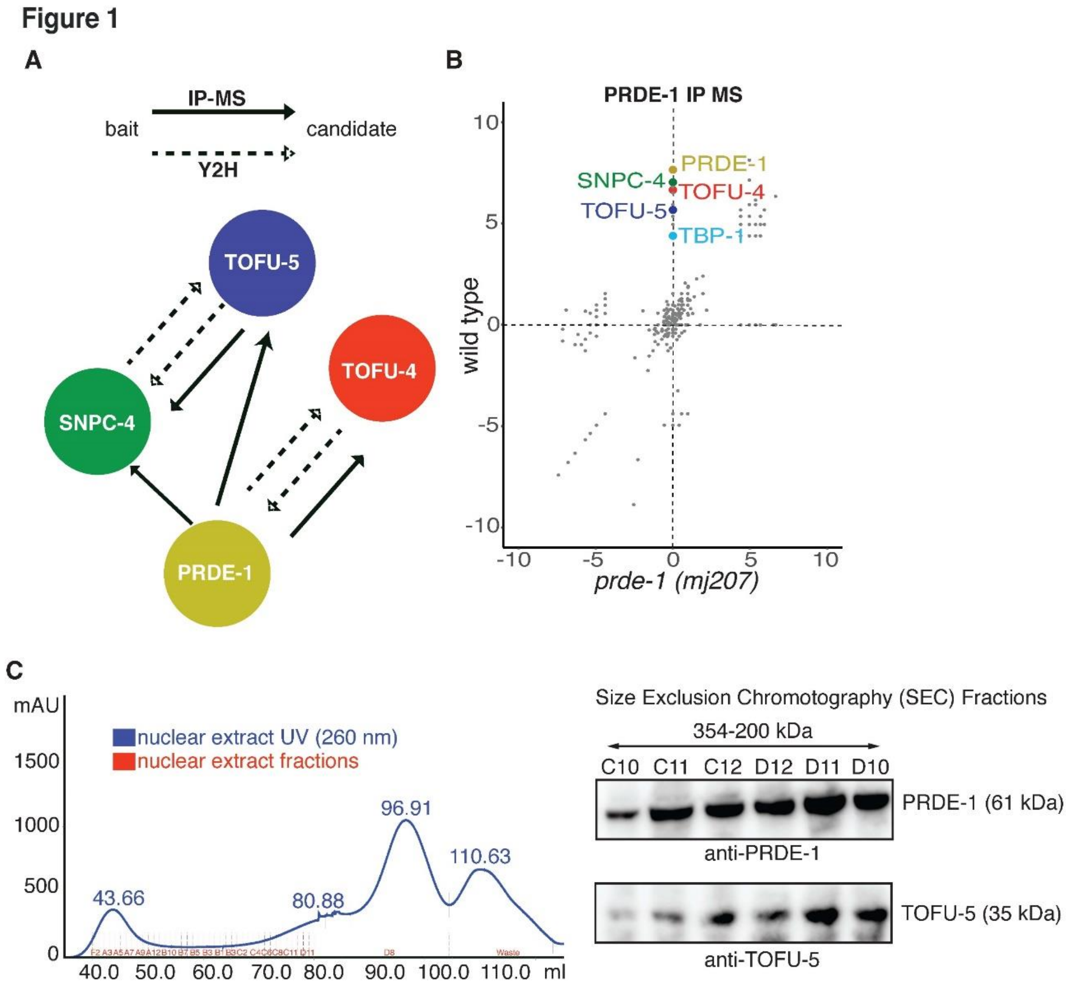
Functional proteomics identified a USTC complex

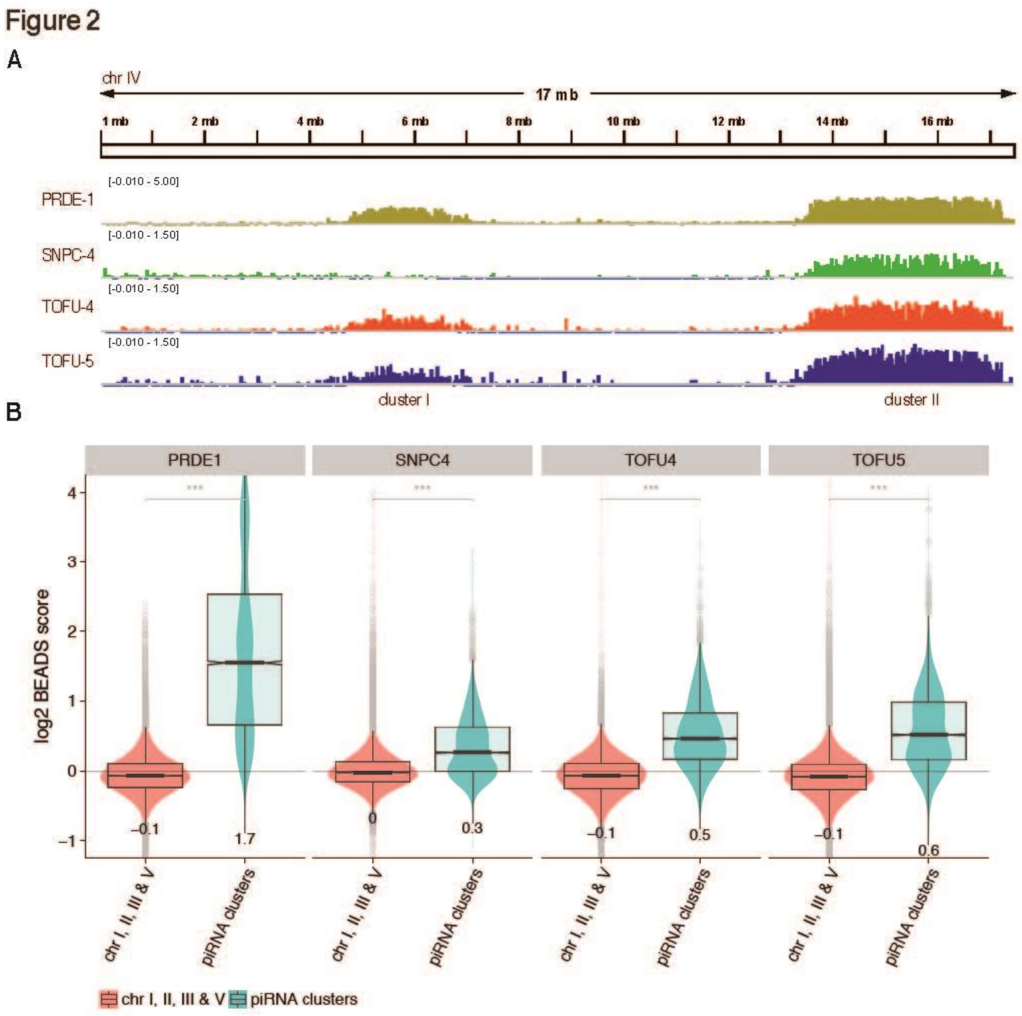
IP-MS
&
Y2H



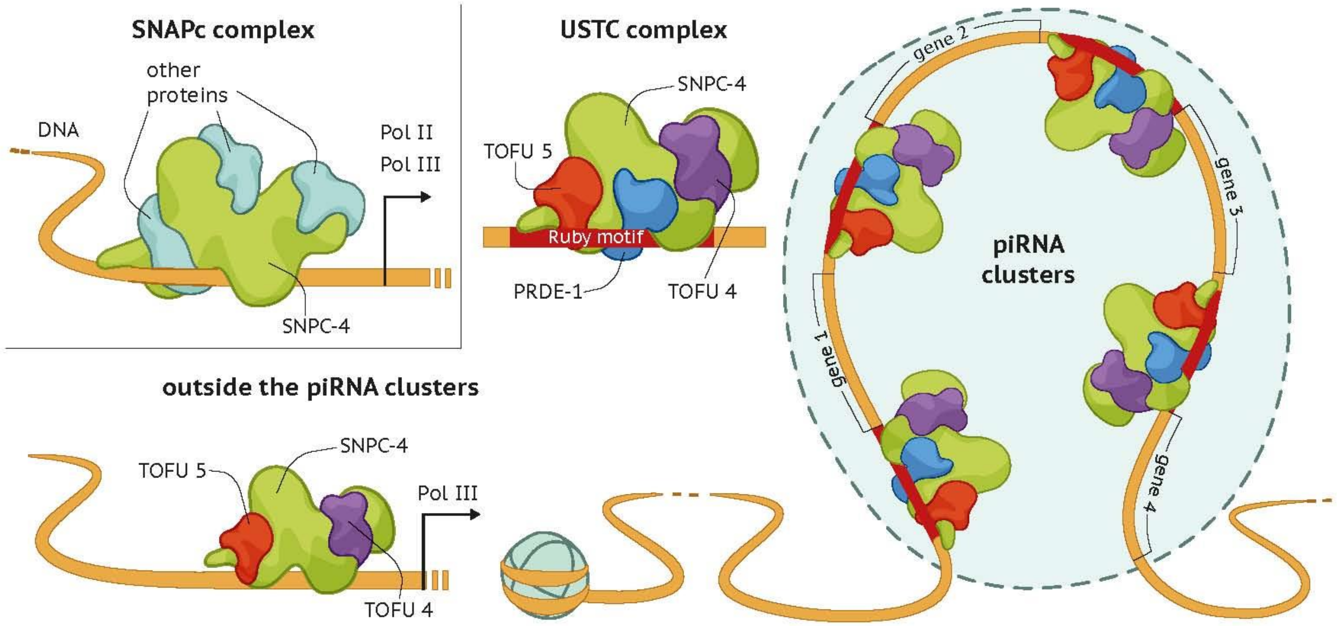
USTC:
upstream
sequence
transcription
complex

Genes & Development 2019
与董梦秋，Eric Miska合作

USTC binds to piRNA clusters

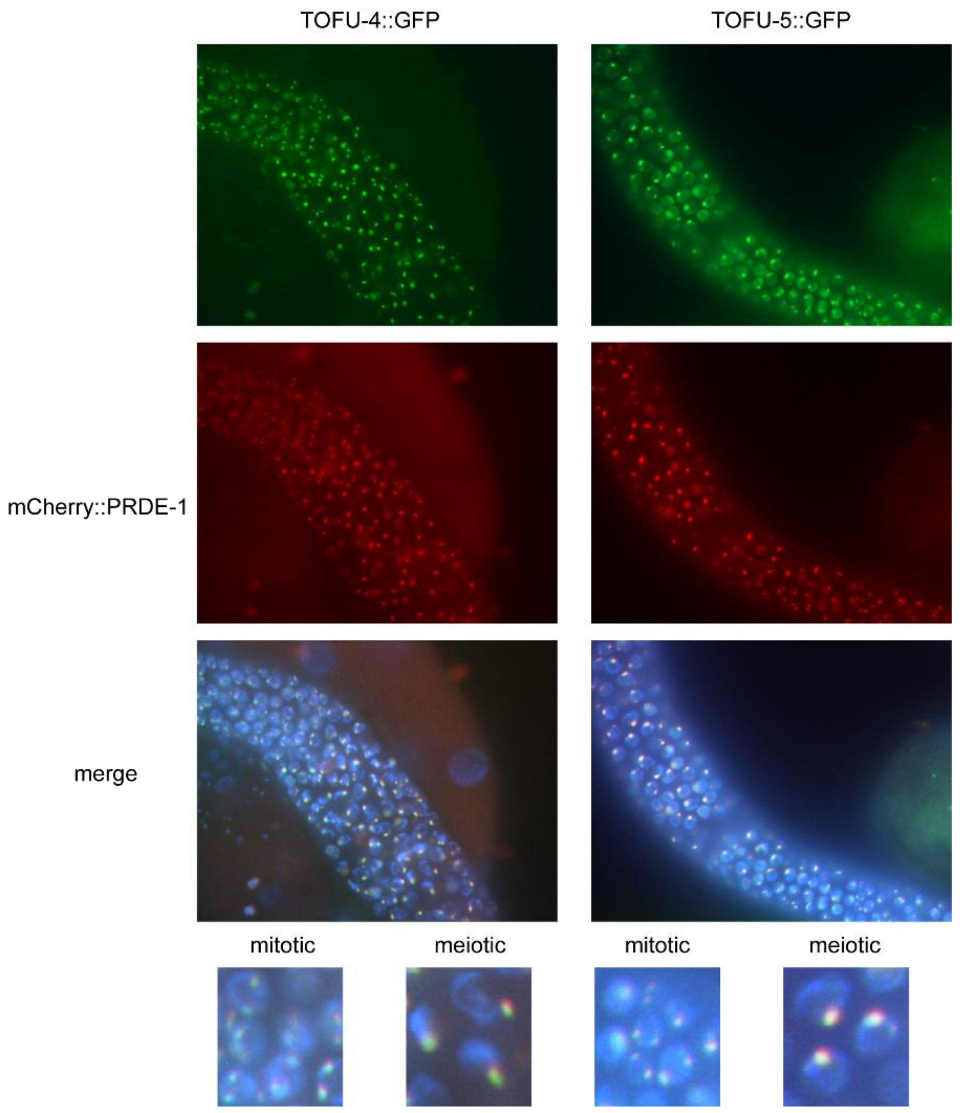


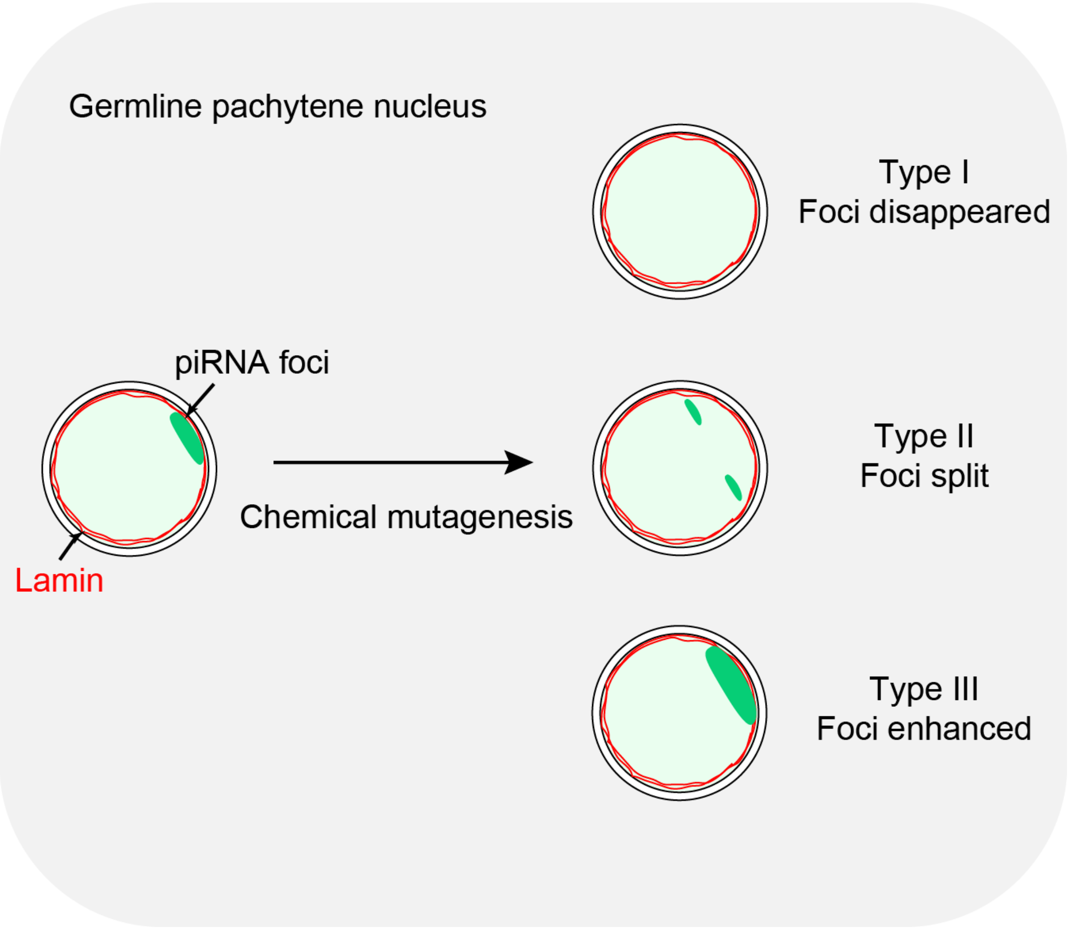
上游序列转录复合物(USTC)介导 piRNA的转录



USTC: upstream sequence transcription complex

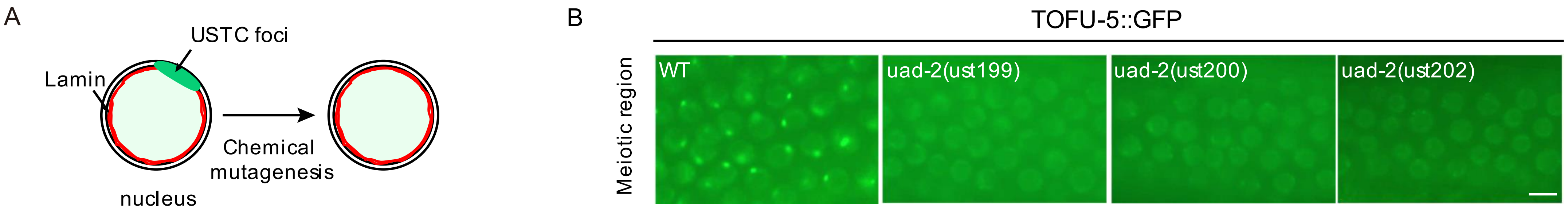
利用USTC复合物进行正向遗传学筛选

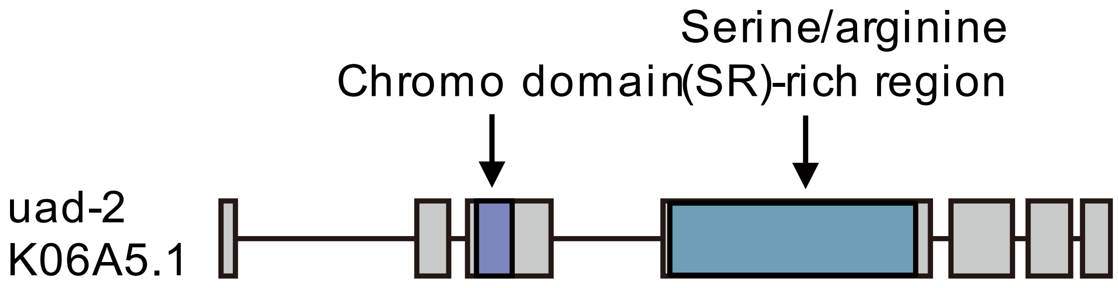




Weng *et al*., 2019

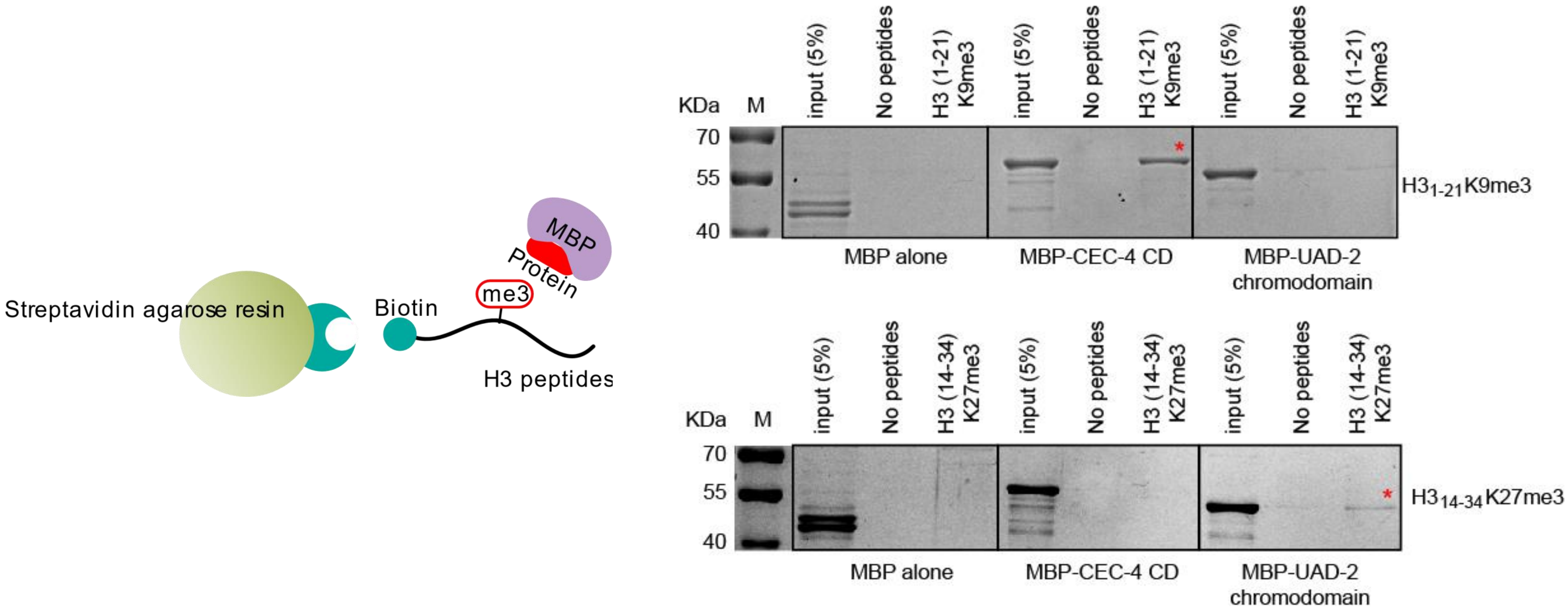
正向遗传学筛选发现 *USTC association dependent (uad)-2* 基因对 piRNA focus 形成是必需的



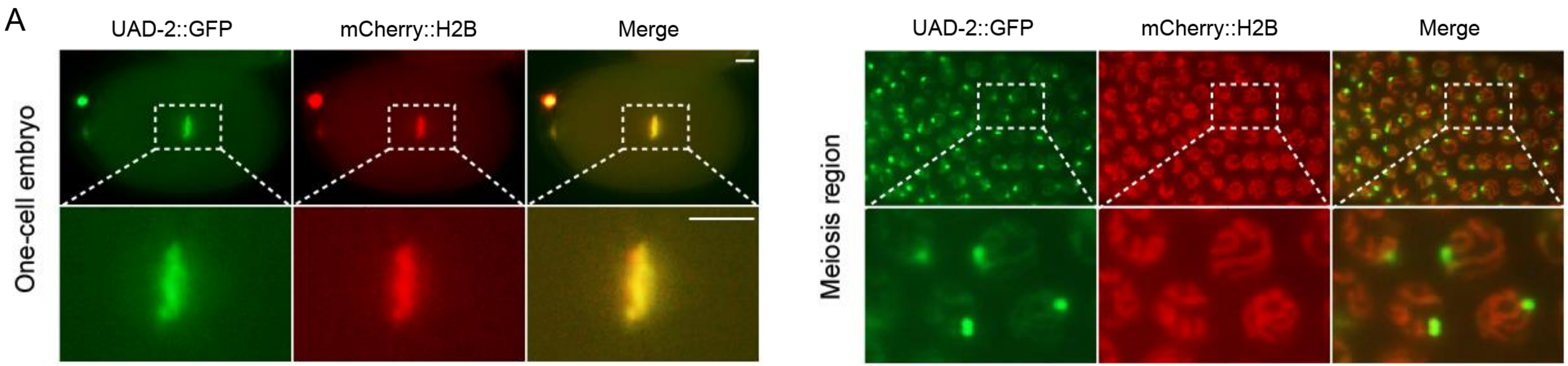


PNAS (2021)

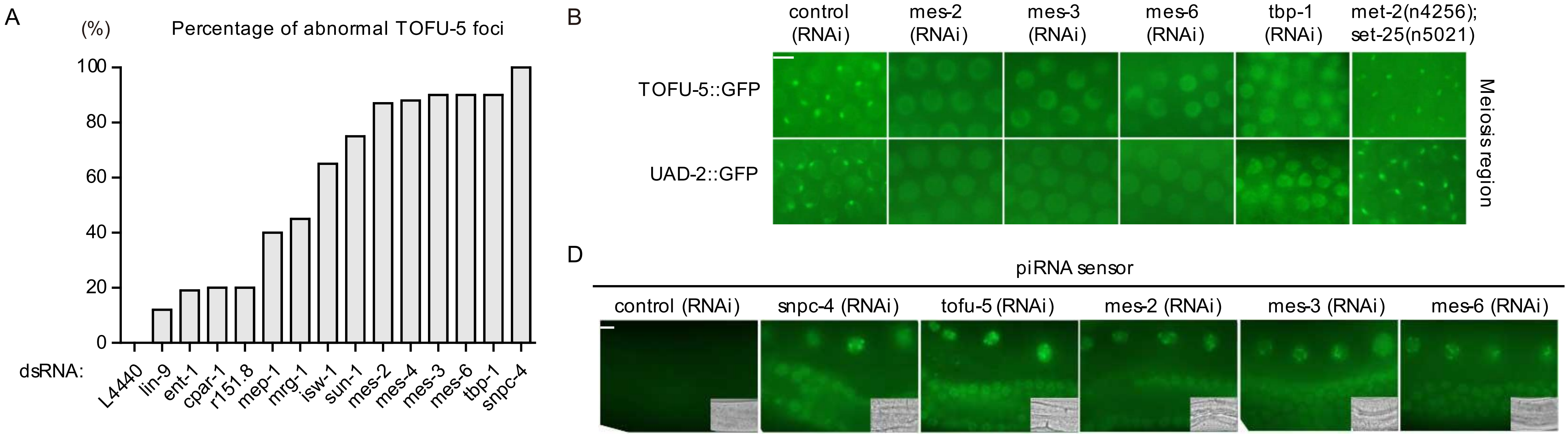
UAD-2 结合 H3K27me3



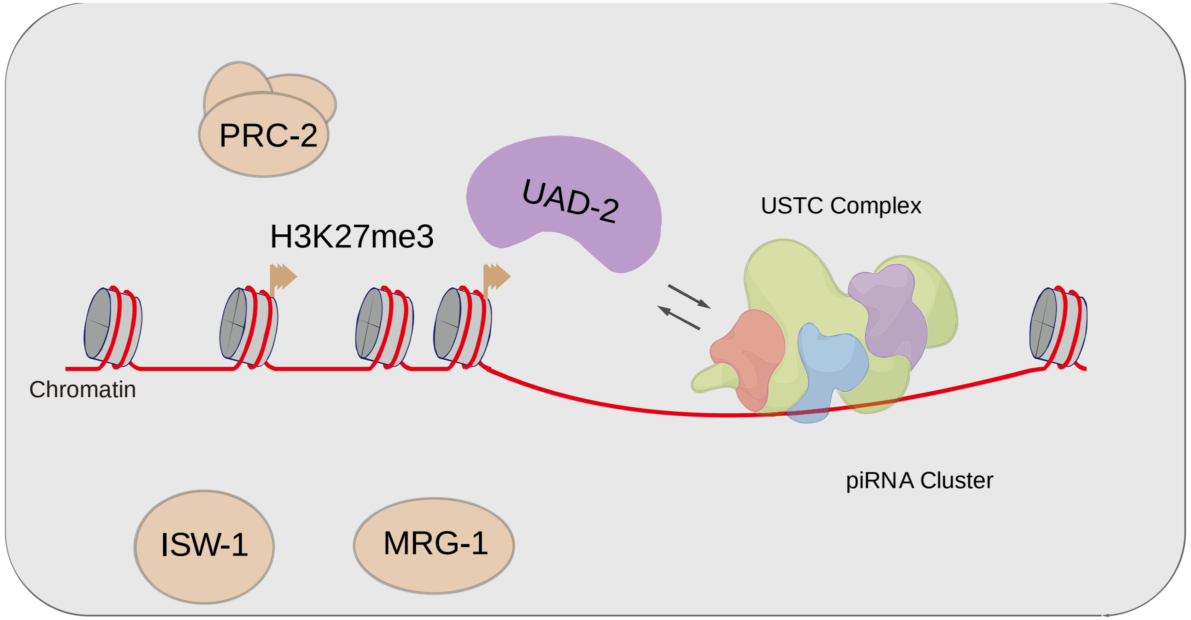
UAD-2结合在染色体上



反向遗传学发现染色质微环境介导 piRNA focus形成和 piRNA 的生成



上游序列转录复合物结合依赖 *(uad)-2* 基因介导 piRNA转录



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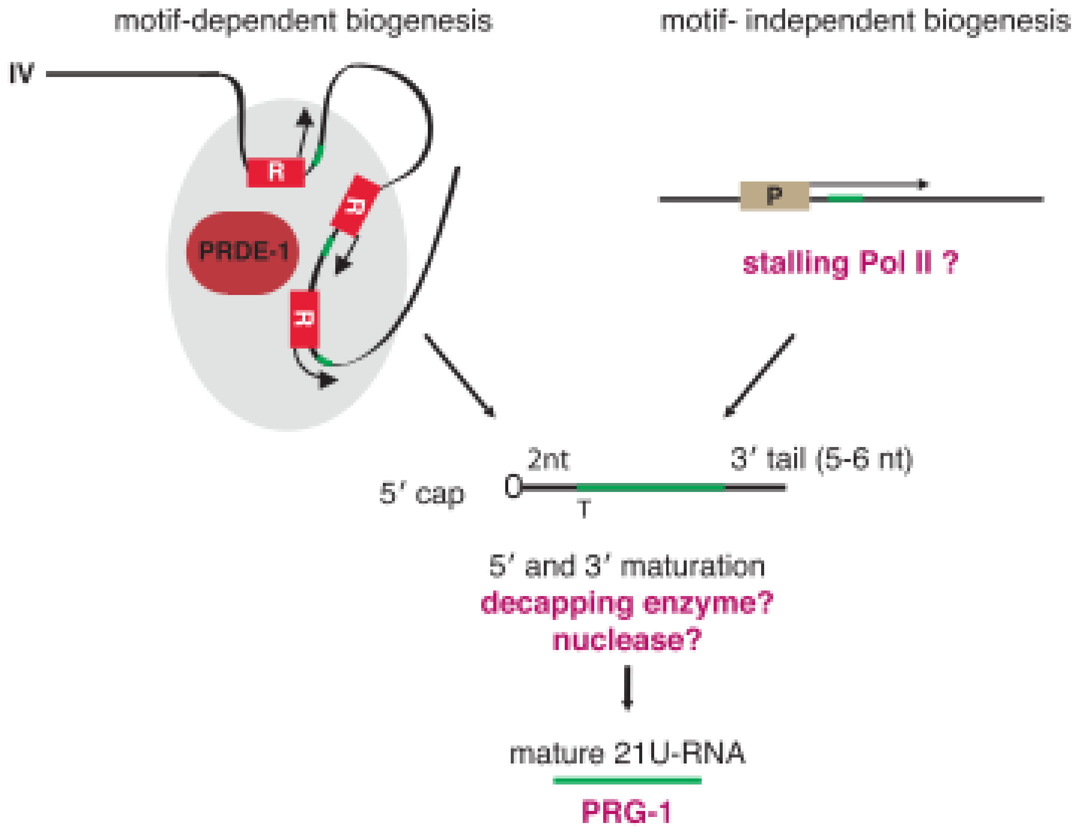


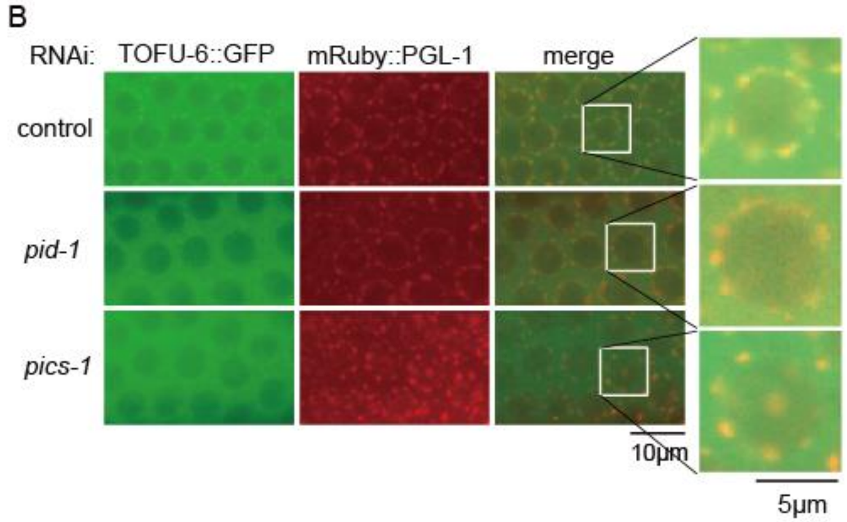
Functional proteomics identified TOFU-6 interactors

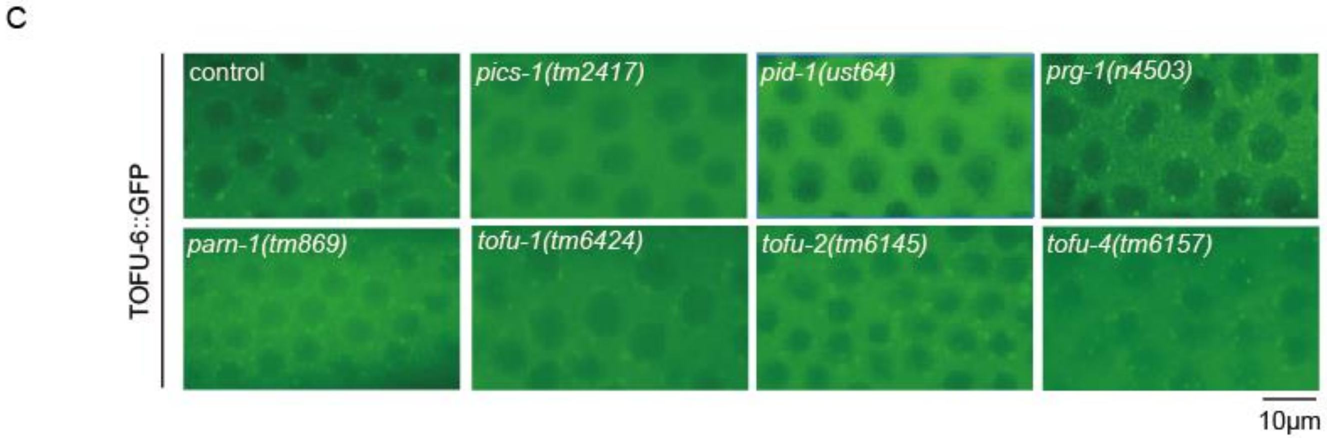
IP-MS
&
RNAi screening

A

TOFU-6::GFP IP-MS

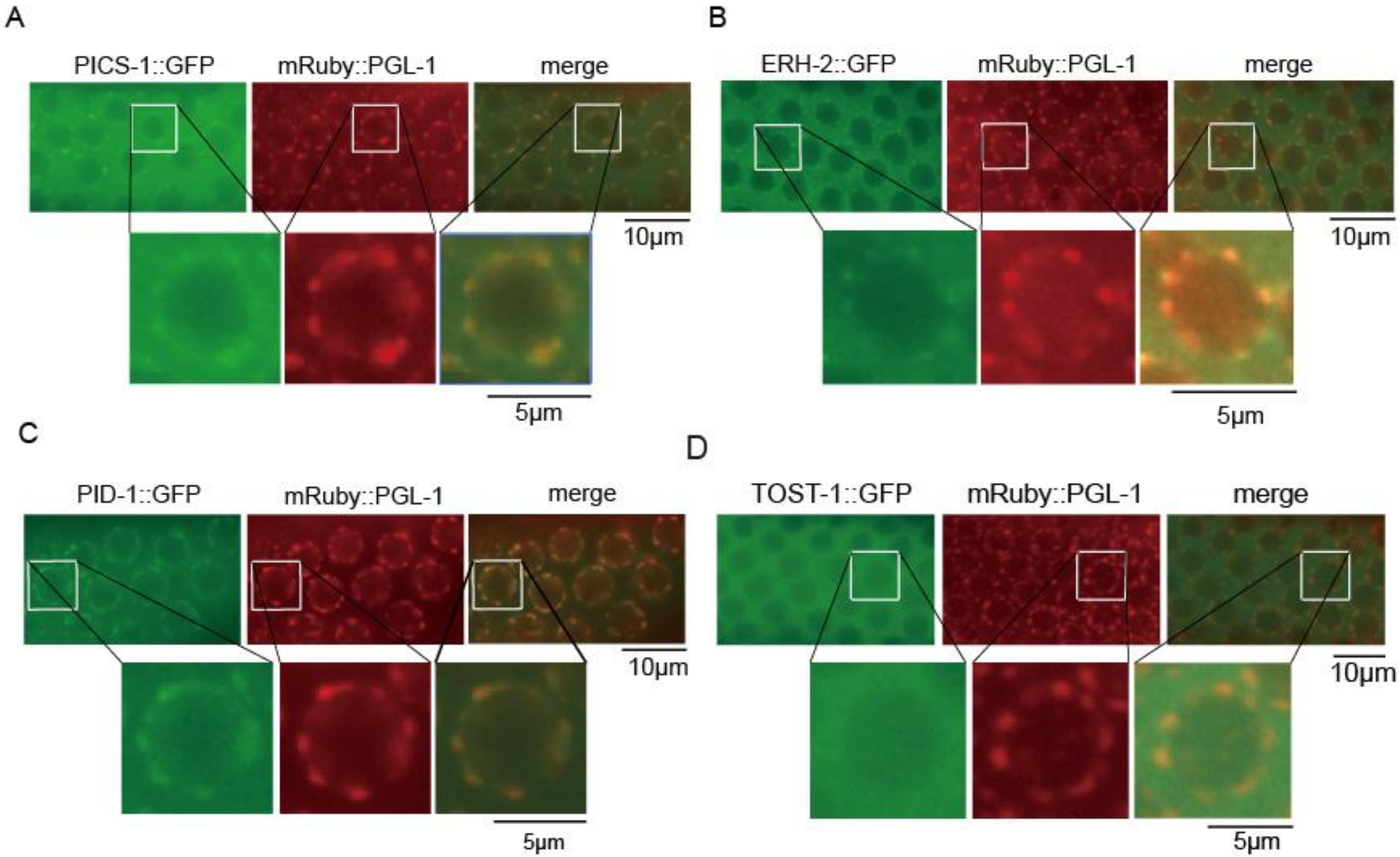
Gene name	gene ID	Domains/processes
tost-1	C35D10.13	——
pid-1	F18A1.8	piRNA defective
tofu-6	EEED8.1	RRM
pics-1	Y23H5A.3	RNA binding
erh-2	F35G12.11	homolog of ERH



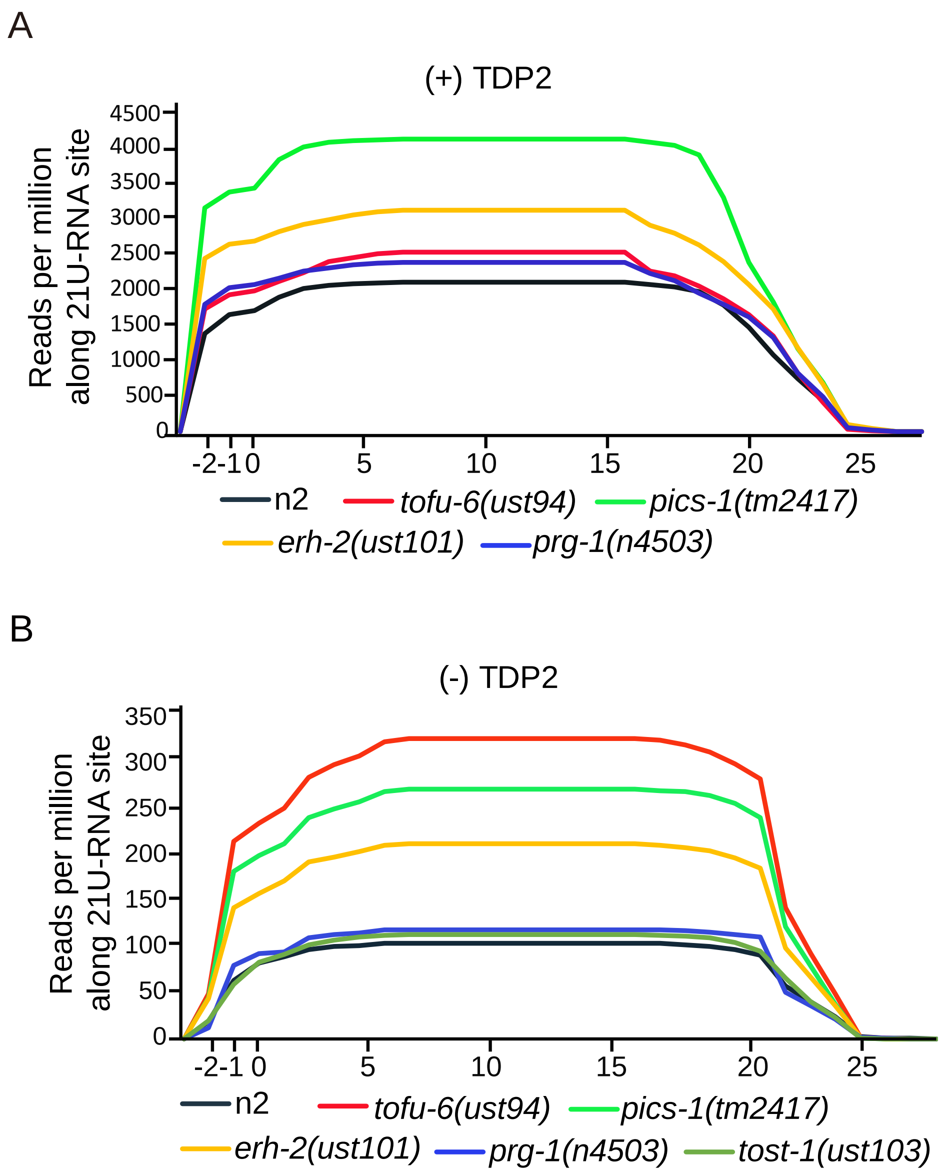


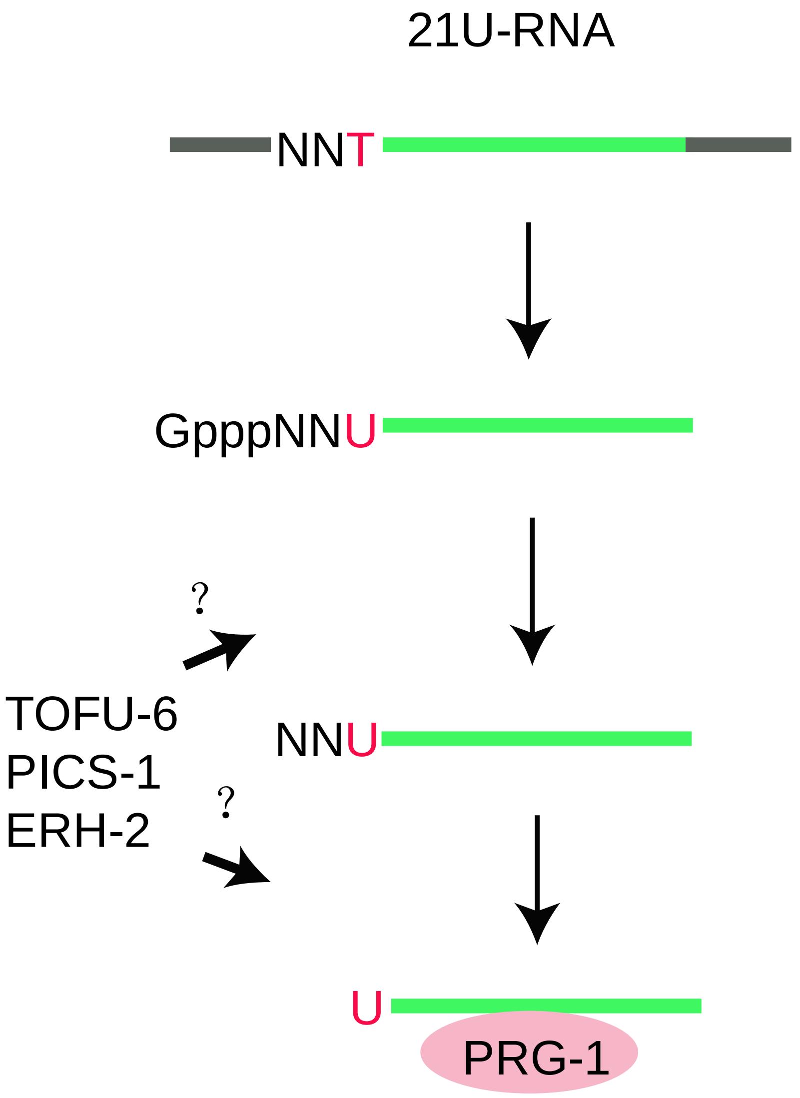
Cell Reports 2019
与许超, 董梦秋合作

Subcellular localization of PICS factors

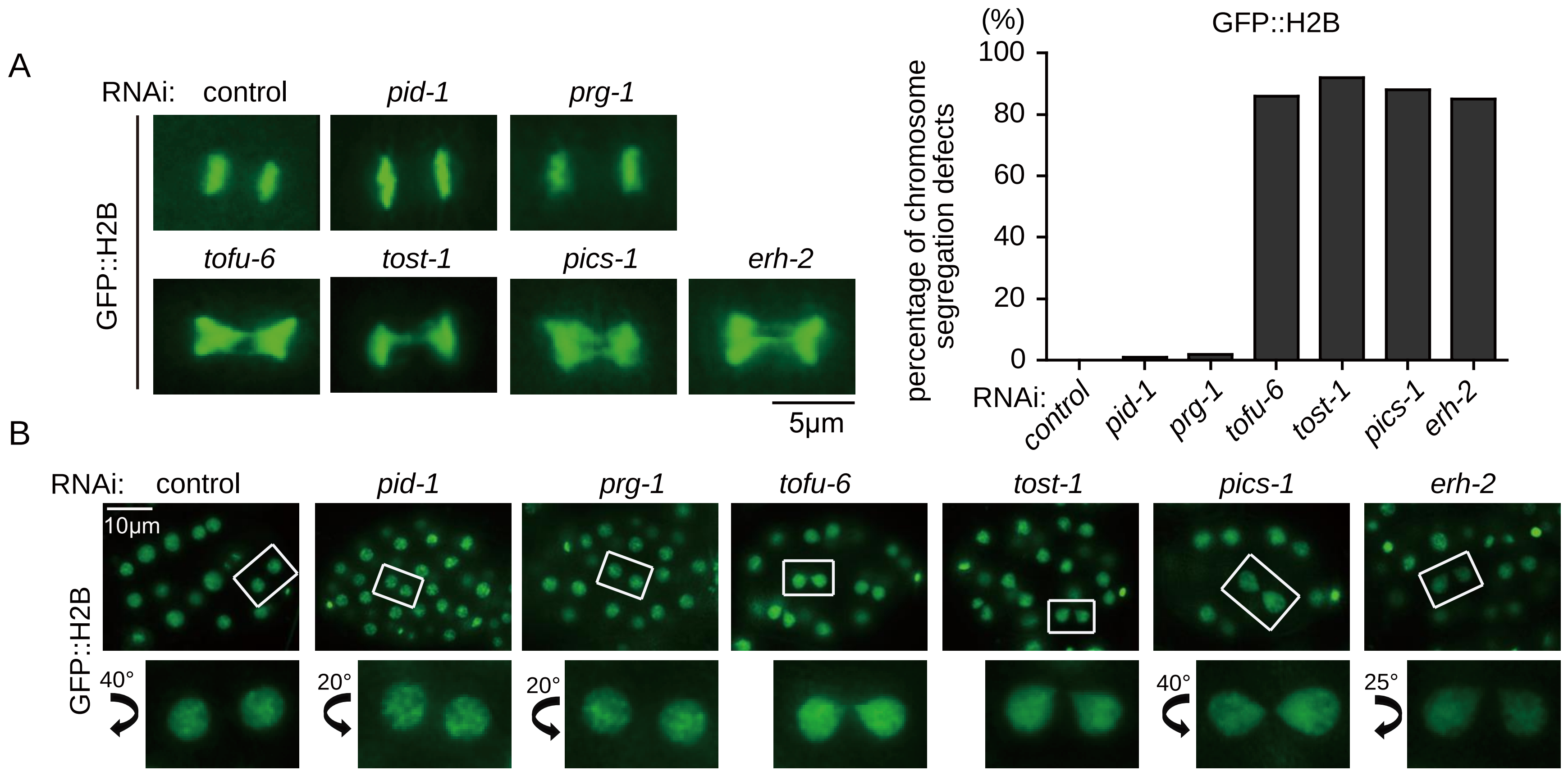


PICS complex is required for piRNA maturation

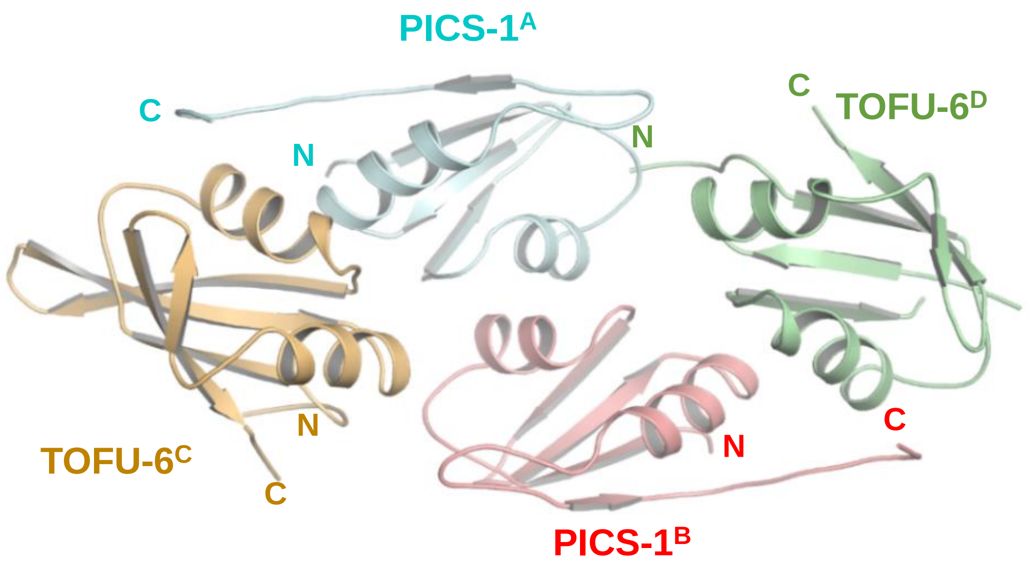


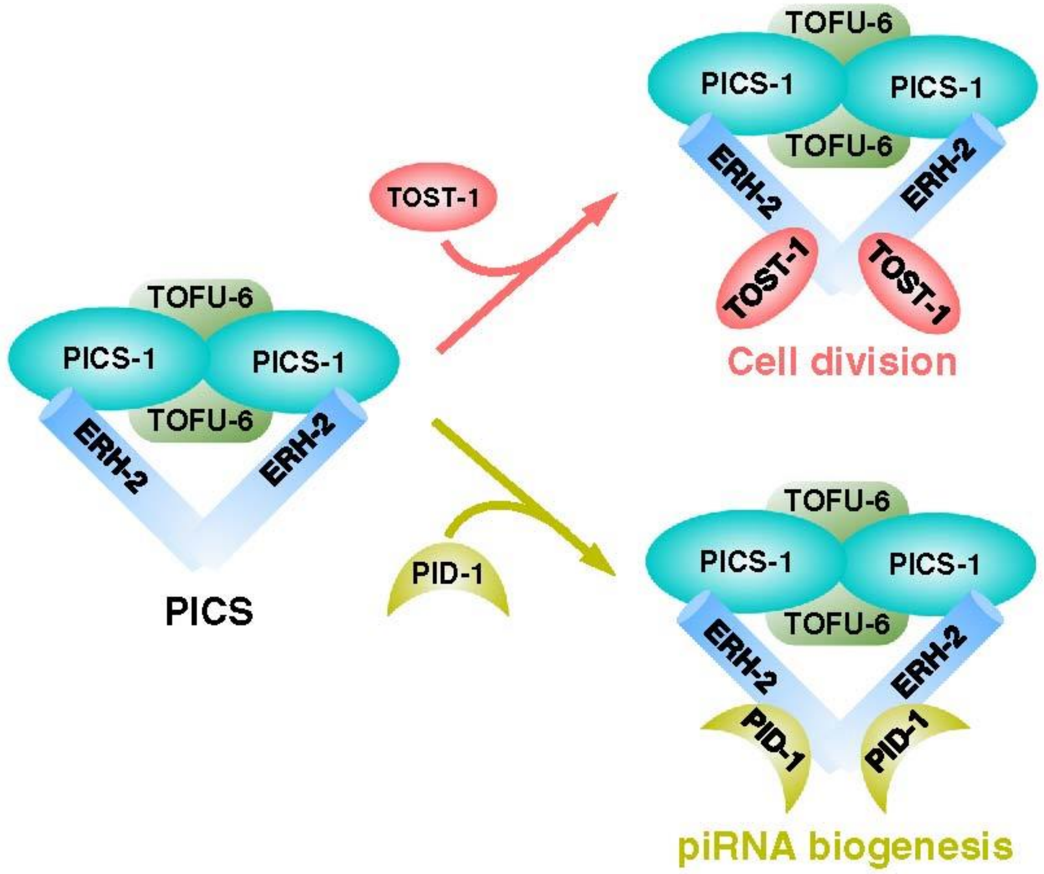


TOFU-6, PICS-1, ERH-2 and TOST-1 are required for chromosome segregation and cell division



PICS 复合物的结构解析





Nature Comm. (2021)
与许超合作

Published online 9 October 2020

Nucleic Acids Research, 2020, Vol. 48, No. 19 11097–11112
doi: 10.1093/nar/gkaa827

ERH facilitates microRNA maturation through the interaction with the N-terminus of DGCR8

S. Chul Kwon[1,2,3,†], Harim Jang[1,2,†], Siyuan Shen[4,†], S. Chan Baek[1,2], Kijun Kim[1,2], Jihye Yang[1,2], Jeesoo Kim[1,2], Jong-Seo Kim[1,2], Suman Wang[4], Yunyu Shi[4], Fudong Li[4,*] and V. Narry Kim[1,2,*]

Mol Cell. 2020 April 16; 78(2): 289–302.e6. doi:10.1016/j.molcel.2020.01.026.

MicroRNA Clustering Assists Processing of Suboptimal MicroRNA Hairpins Through the Action of the ERH Protein

Wenwen Fang[1,2,3], David P. Bartel[1,2,3,4,*]

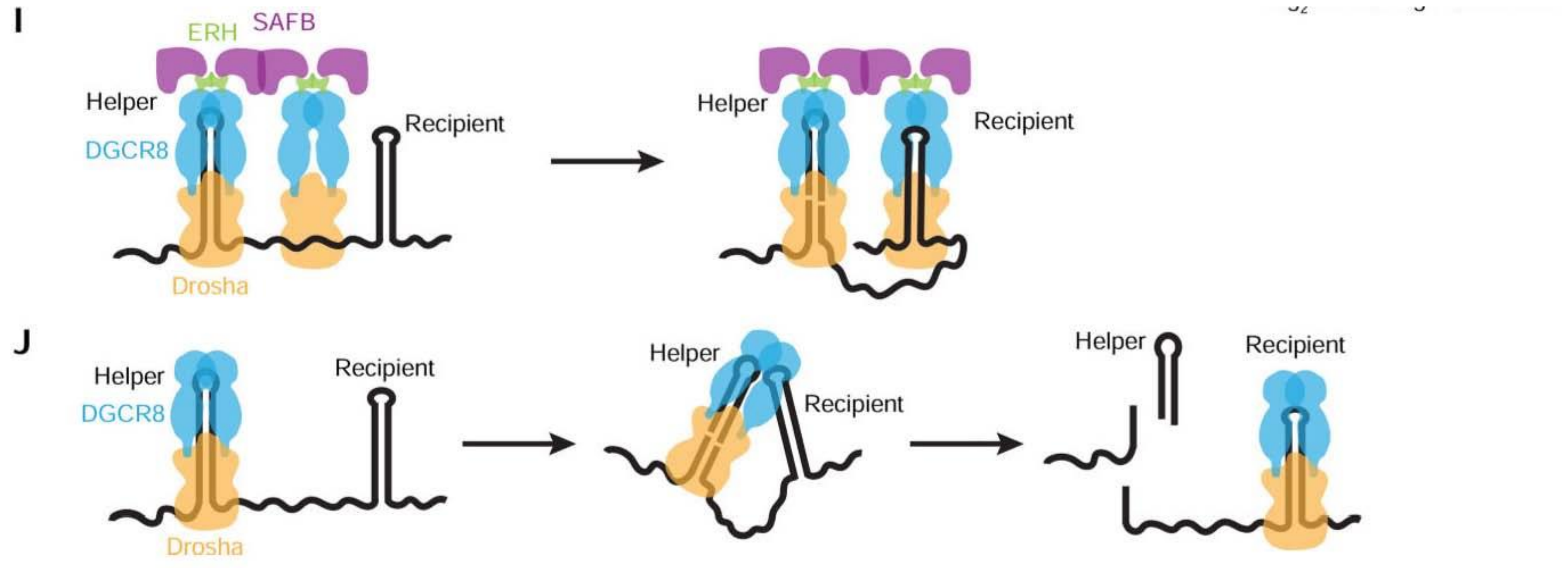
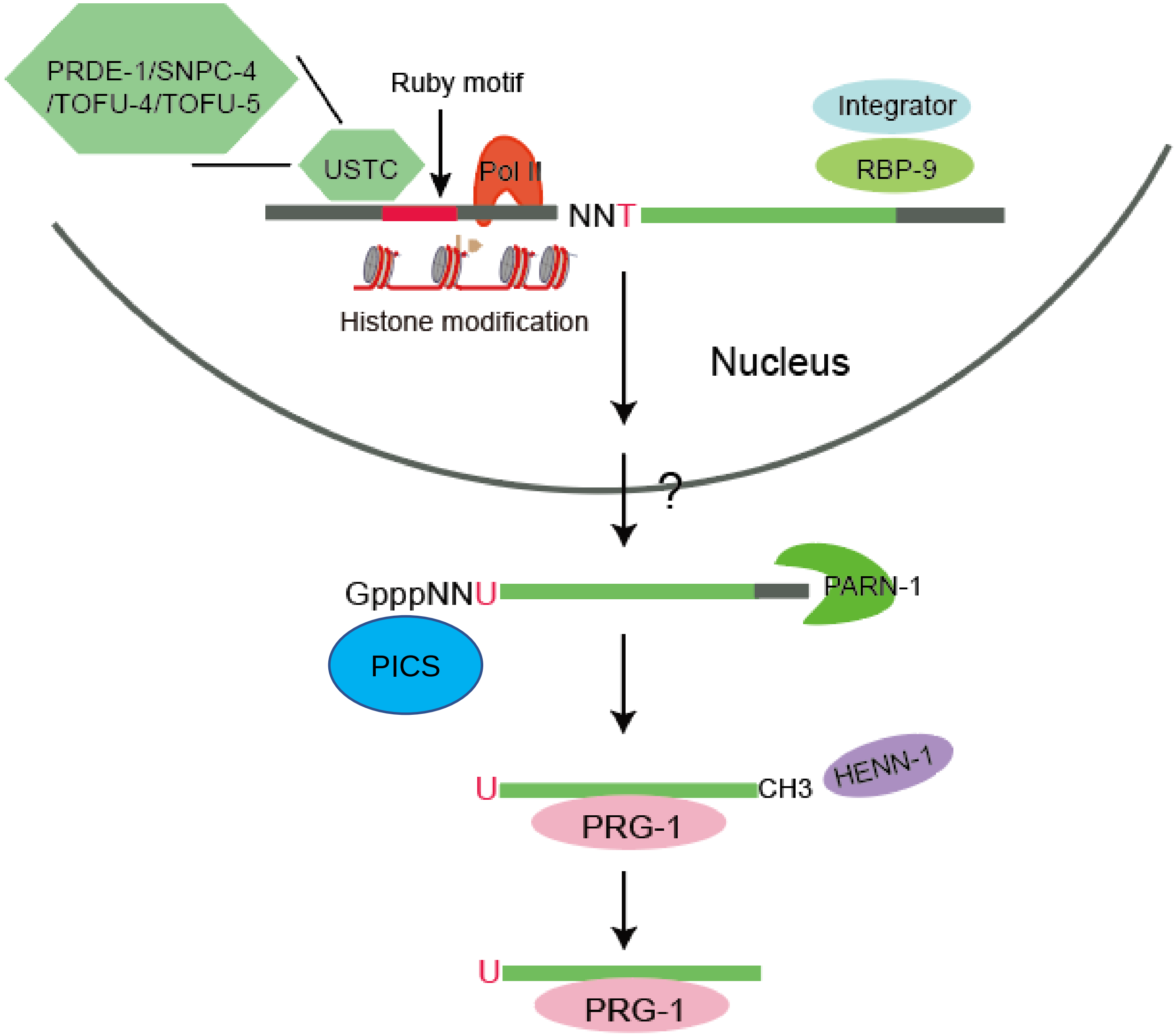


Figure 7. ERH Copurifies with Microprocessor and Helps Mediate Cluster Assistance

线虫中的piRNA通路



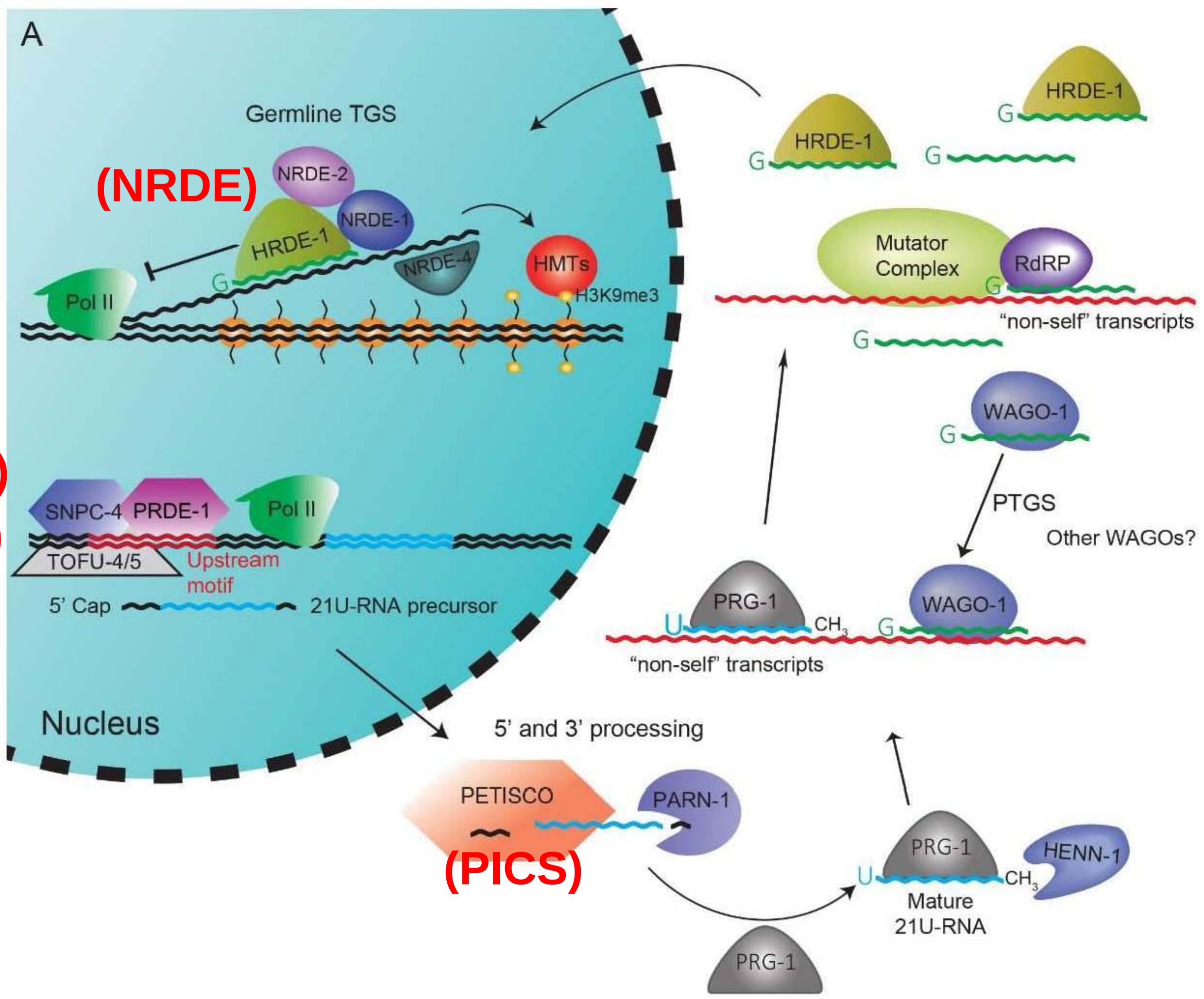
G&D 2019
Cell Reports 2019
PNAS 2021
Nat. Comm. 2021

线虫里小 RNA的产生,功能与作用

2005

2021

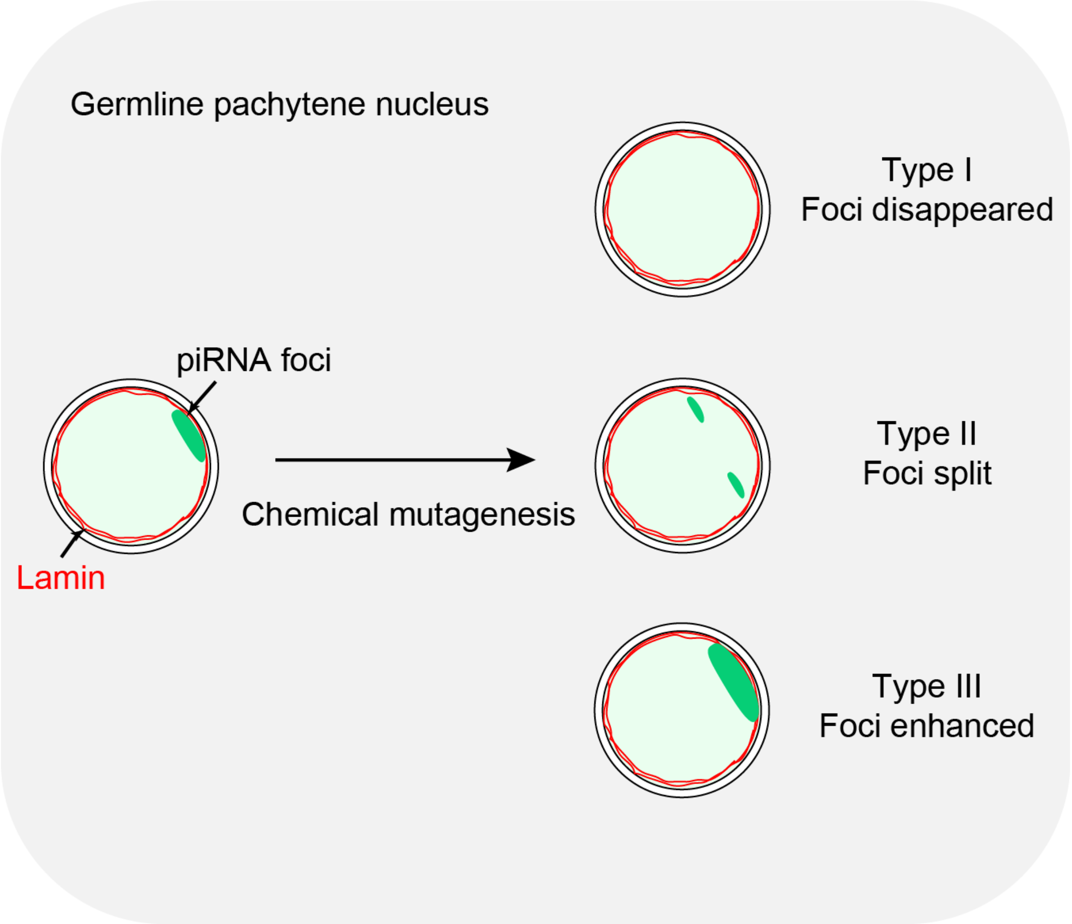
(UAD-2)
(USTC)

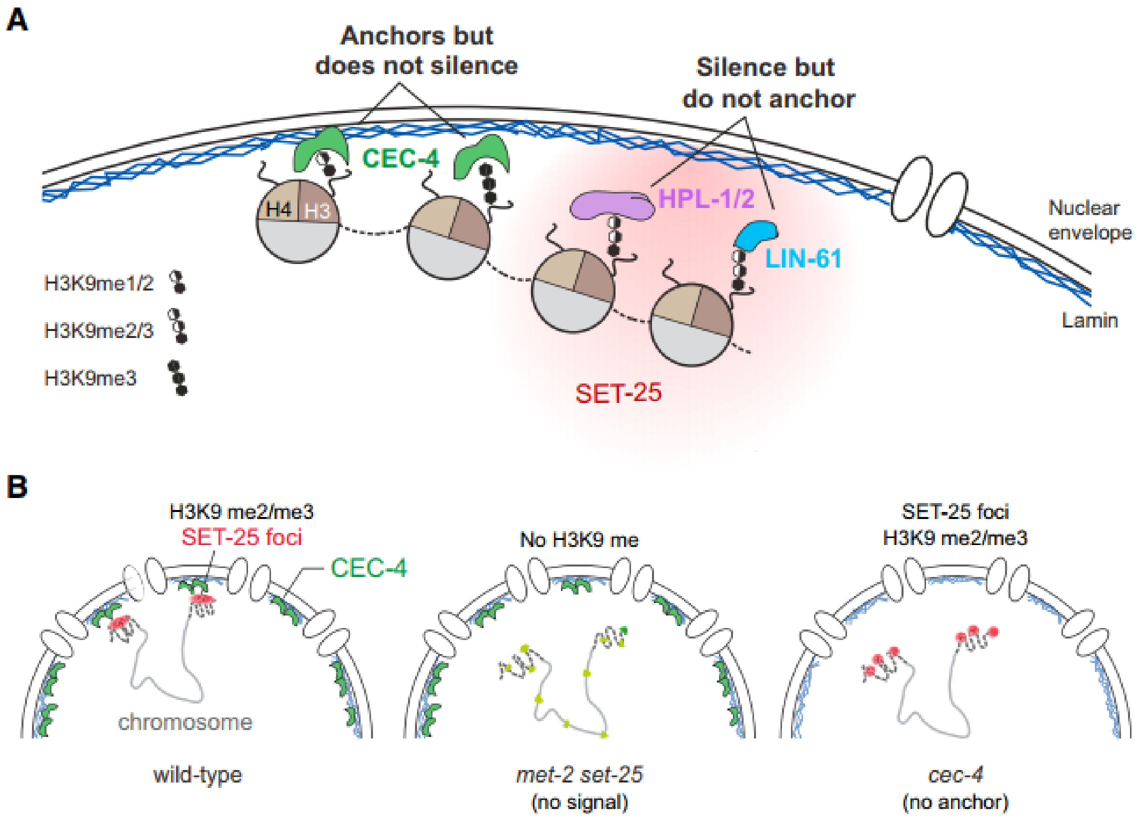


1.Nat. Comm. (2021)
2.Nucleic Acids Res. (2021b)
3.Nucleic Acids Res. (2021a)
4.PNAS (2021)
5.Cell Reports (2019)
6.Genes & Development (2019)
7.PNAS (2018)
8.Cell Reports (2018)
9.Nat. Struct. & Mol. Biol. (2017)
10.Current Biology (2015)
11.Genetics (2014)
12.Nature Genetics (2012)
13.PLoS Genetics (2011)
14.Nature (2010)
15.Science (2008)

Miguel Vasconcelos Almeida, Miguel A. Andrade-Navarro, and René F. Ketting （2019）Noncoding RNA

利用USTC复合物和UAD-2进行正向遗传学筛选





Gonzalez-Sandoval *et al.*, 2019

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