Biogenesis of small RNAs in animals

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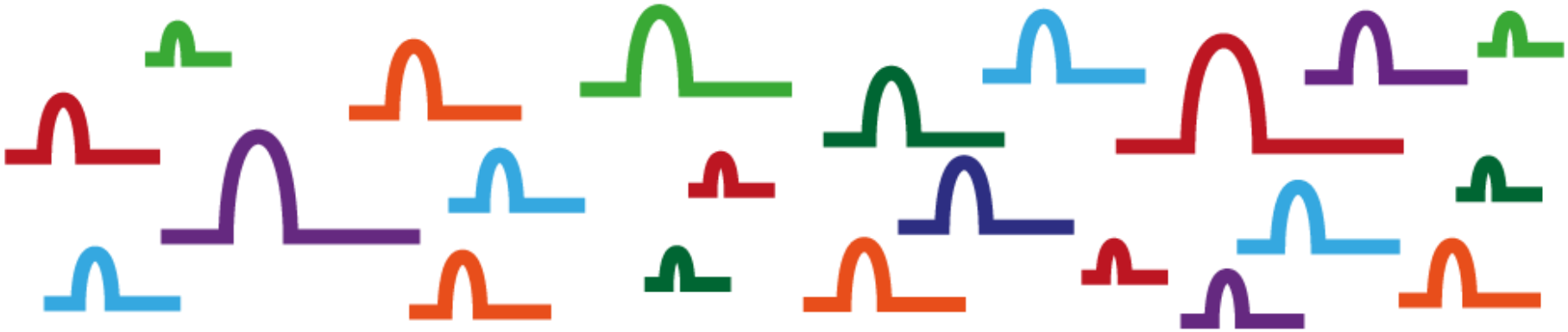


Background

1. Development of deep –sequencing technologies and computational rediction methods

2.Function range from heterochromatin formation to mRNA destabilization and translational control.
Involved in almost every biological process

3.Arbitrary term：non-coding RNAs，not related to eukaryotic small RNAs

Compare

classes	length	Associate protein	Cell type	Dicer /Drosha protein	Originatio -n	function
miRNAs	~22nt	Ago-subfa mily proteins	all	Dicer AND Drosha	local hairpin structures	post-transcri ptional regulators
Endo-siRNAs	~21nt	Ago-subfa mily proteins	Germ cells	Dicer	long dsRNAs	post-transcri ptional regulators
piRNAs	~24-31nt	Piwi-subfa mily proteins	D.Melanogaster Oocytes and ES of mousce	Drosha	intergenic repetitive elements	transposon silencing

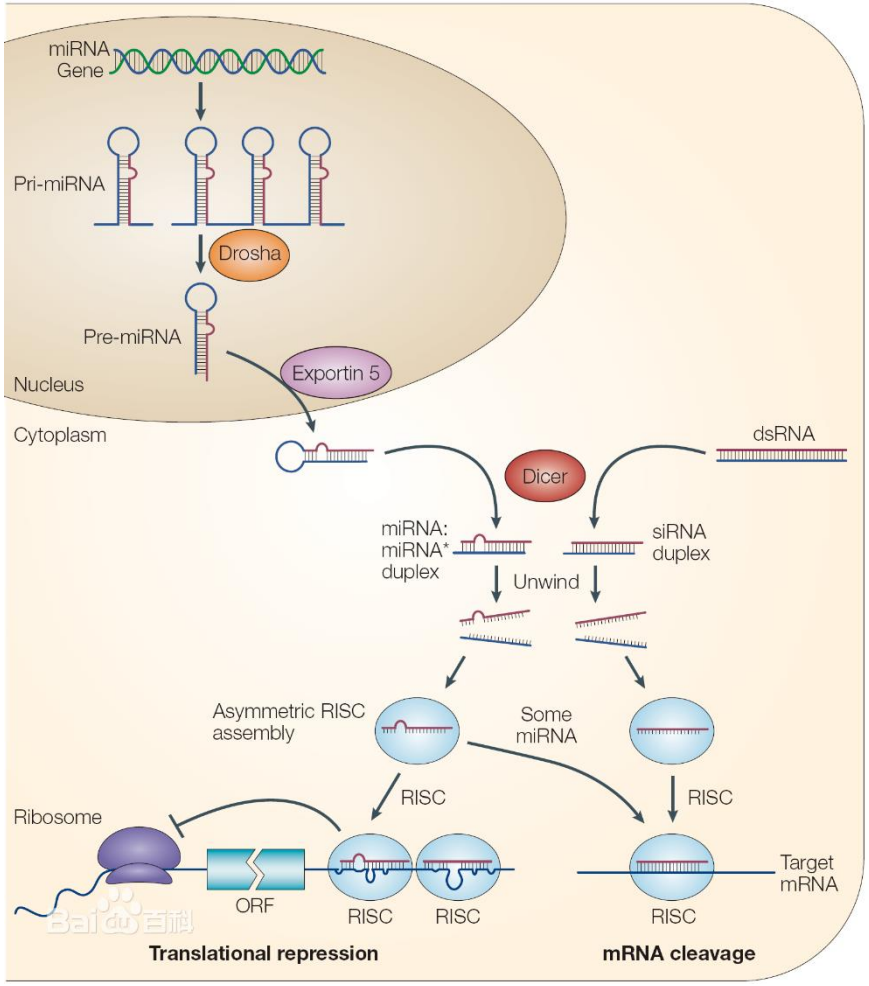
Table 1 | types of small RNA-associated Argonaute proteins, and the origin and mechanism of action of small RNAs

miRNA

1. microRNA biogenesis
    - 1.1 *miRNA genes and their transcription*
    - 1.2 *Nuclear processing by Drosha*
    - 1.3 *Nuclear export by exportin 5*
    - 1.4 *Cytoplasmic processing by Dicer*
    - 1.5 *Argonaute loading*

2. Regulation of miRNA biogenesis
    - 2.1 *Transcriptional control*
    - 2.2 *Post-transcriptional regulation*
    - 2.3 *Feedback circuits in miRNA networks*

miRNA



single-stranded RNAs (ssRNAs) of ~22 nt in length

generated from endogenous hairpin-shaped transcript

Guide molecules

Translational repression，exonucleolytic mRNA decay，translational activation，heterochromatin formation

miRNA

1.1 *miRNA genes and their transcription*

(1)phylogenetically conserved

(2) Most mammalian miRNA genes have multiple isoforms(paralogues) that are probably the result of gene duplications.

(3) **Polycistronic transcription unit**

An RNA transcript that includes regions that represent multiple gene products.

(4) transcription of most miRNA genes is mediated by RNA polymerase II (Pol II),although a minor group of miRNAs that are associated with Alu repeats can be transcribed by Pol III.

miRNA

1.2 *Nuclear processing by Drosha*

Pol II：local stem-loop structures

↓

cleavage at the stem of the hairpin structure

↓

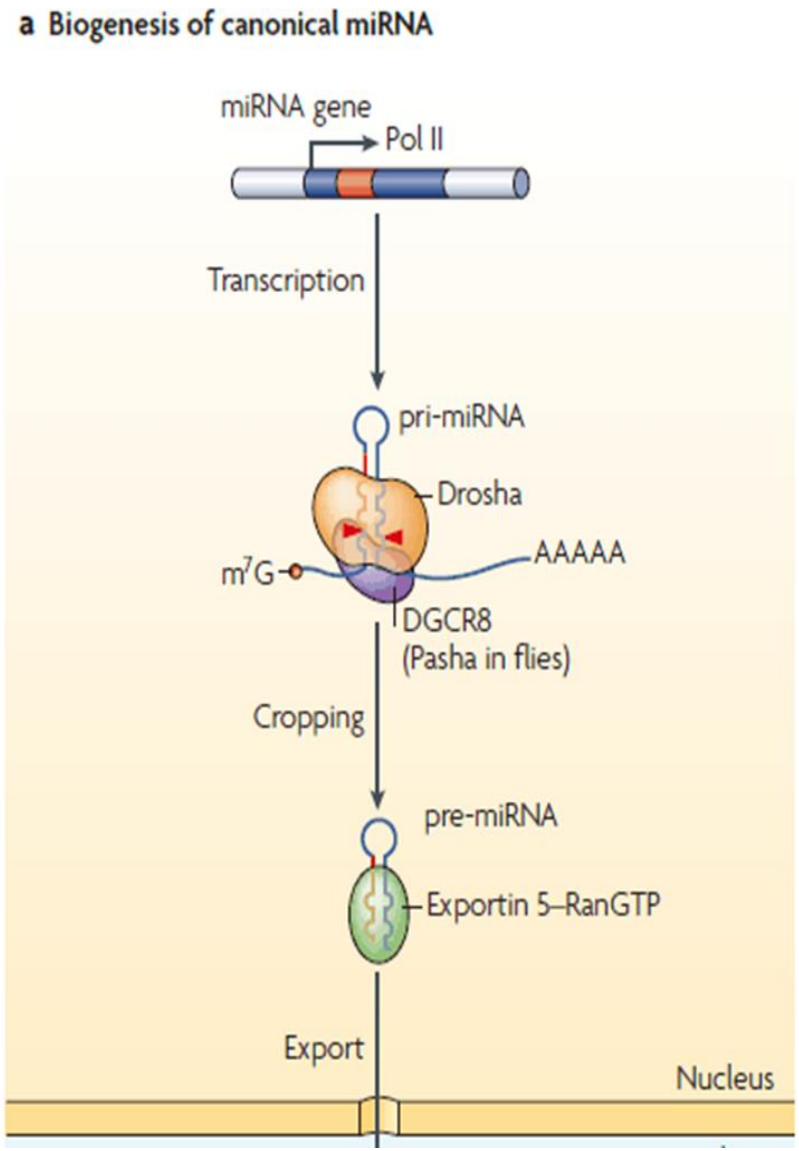
Drosha+DGCR8=Microprocessor complex

↓

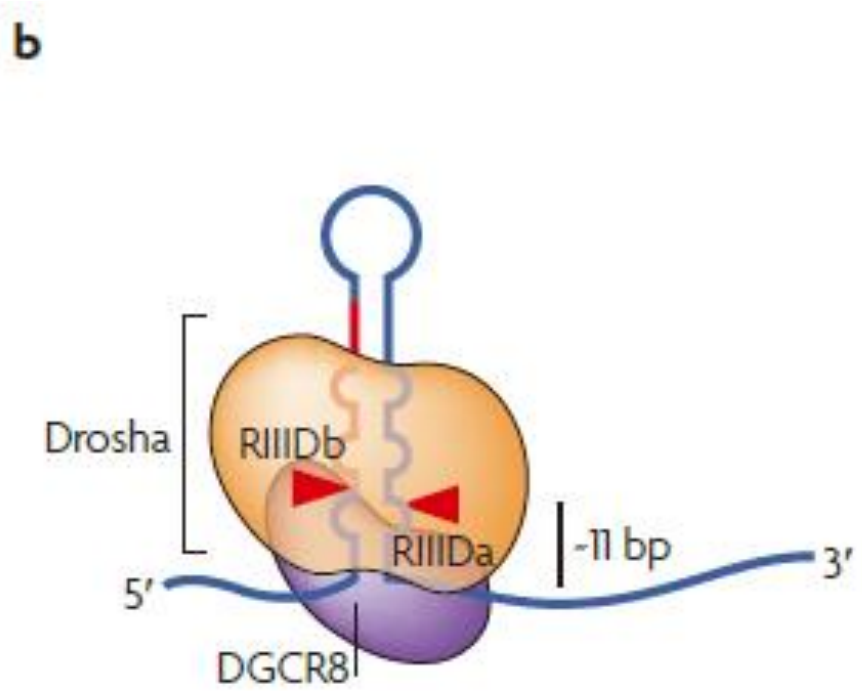
cleave the substrate ~11 bp away
from the ssRNA–dsRNA junction

↓

exported to the cytoplasm by exportin 5



Two types of RNase III are found in animals: Drosha and Dicer. Both proteins have two tandem RNase III domains (RIIIDs) and a double-stranded RNA-binding domain (dsRBD); Two RIIIDs interact with each other to make an intramolecular dimer in which the two catalytic sites are located closely to each other.



Human:Drosha
D.Melanogaster and C.elegants:Pasha

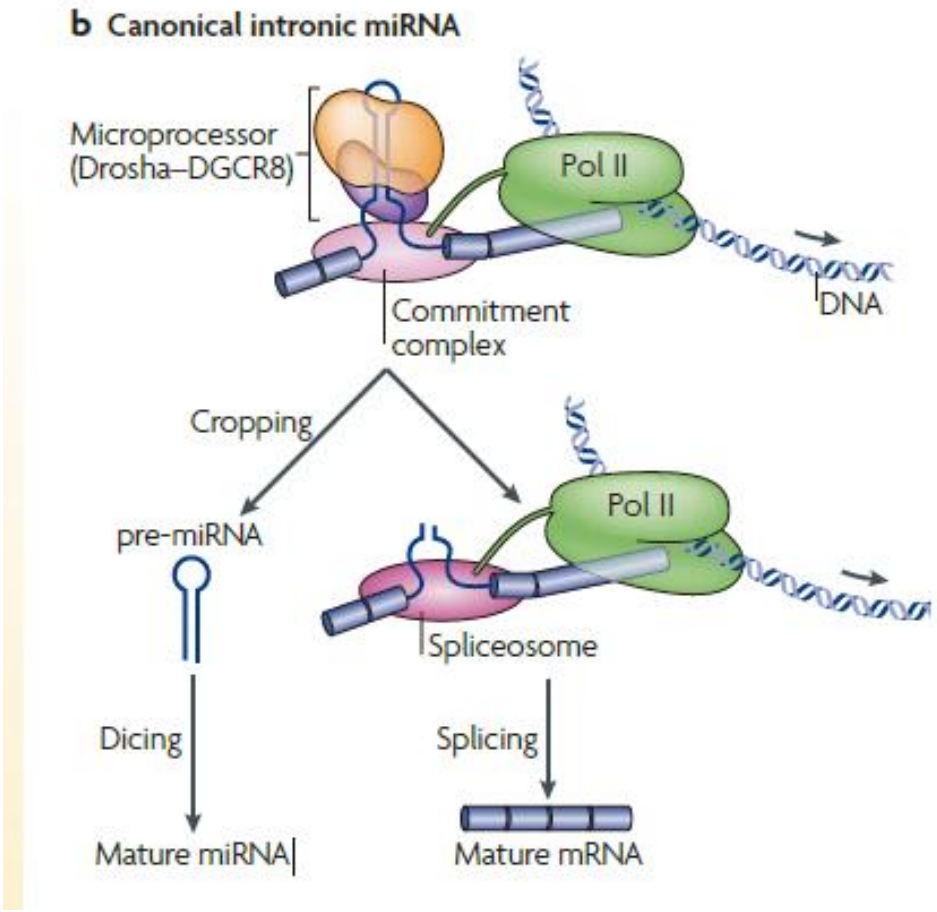
ES-cell function

miRNA： Exon-tethering model

co-transcriptional process

the exons of Pol II transcripts are co-transcriptionally assembled into the spliceosome.

Drosha processing might take place after the transcript is tied to the splicing commitment complex (also known as the early spliceosome complex), but before the intron is excised.



miRNA： non-canonical pathways

feed pre-miRNAs into the miRNA pathway through Drosha-independent processes：

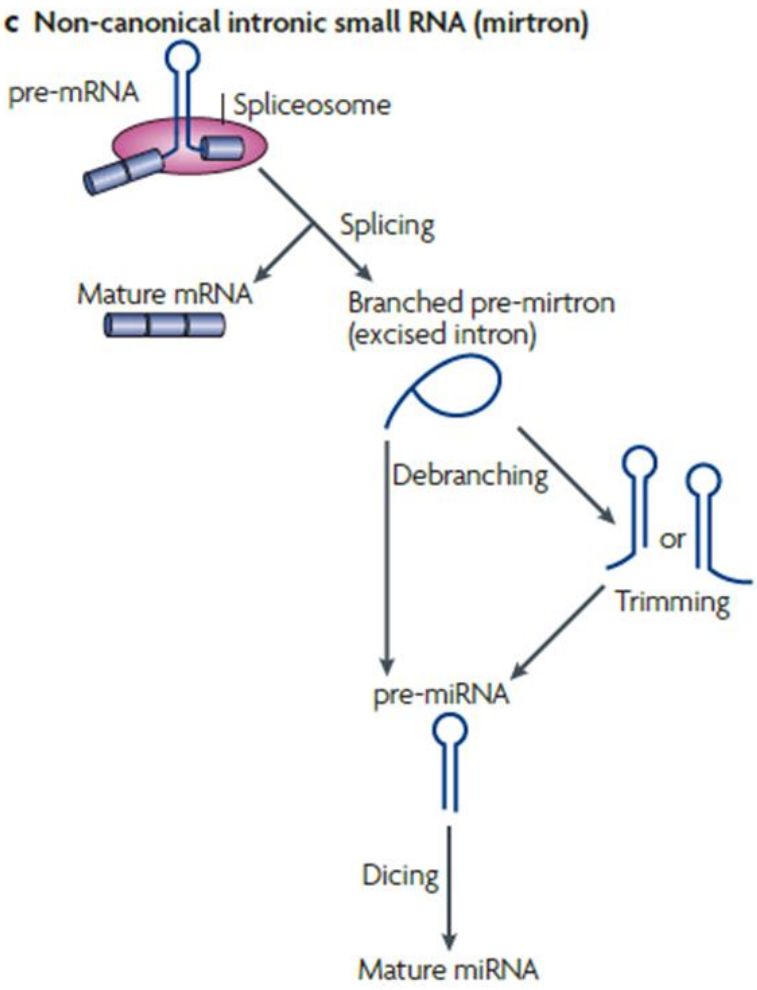
lariat-shaped intron ： resolved and the debranched

↓

intron forms a hairpin structure that resembles pre-miRNA

↓

exonucleolytic trimming： tails at either the 5′ or 3′ end



miRNA

1.3 *Nuclear export by exportin 5*

Function

Mediates the nuclear export of proteins bearing a double-stranded RNA binding domain (dsRBD) and double-stranded RNAs (cargos). XPO5 in the nucleus binds cooperatively to the RNA and to the GTPase Ran in its active GTP-bound form. Proteins containing dsRBDs can associate with this trimeric complex through the RNA. Docking of this complex to the nuclear pore complex (NPC) is mediated through binding to nucleoporins. Upon transit of a nuclear export complex into the cytoplasm, hydrolysis of Ran-GTP to Ran-GDP (induced by RANBP1 and RANGAP1, respectively) cause disassembly of the complex and release of the cargo from the export receptor. XPO5 then returns to the nuclear compartment by diffusion through the nuclear pore complex, to mediate another round of transport. The directionality of nuclear export is thought to be conferred by an asymmetric distribution of the GTP- and GDP-bound forms of Ran between the cytoplasm and nucleus. Overexpression may in some circumstances enhance RNA-mediated gene silencing (RNAi). Mediates nuclear export of isoform 5 of ADAR/ADAR1 in a RanGTP-dependent manner.

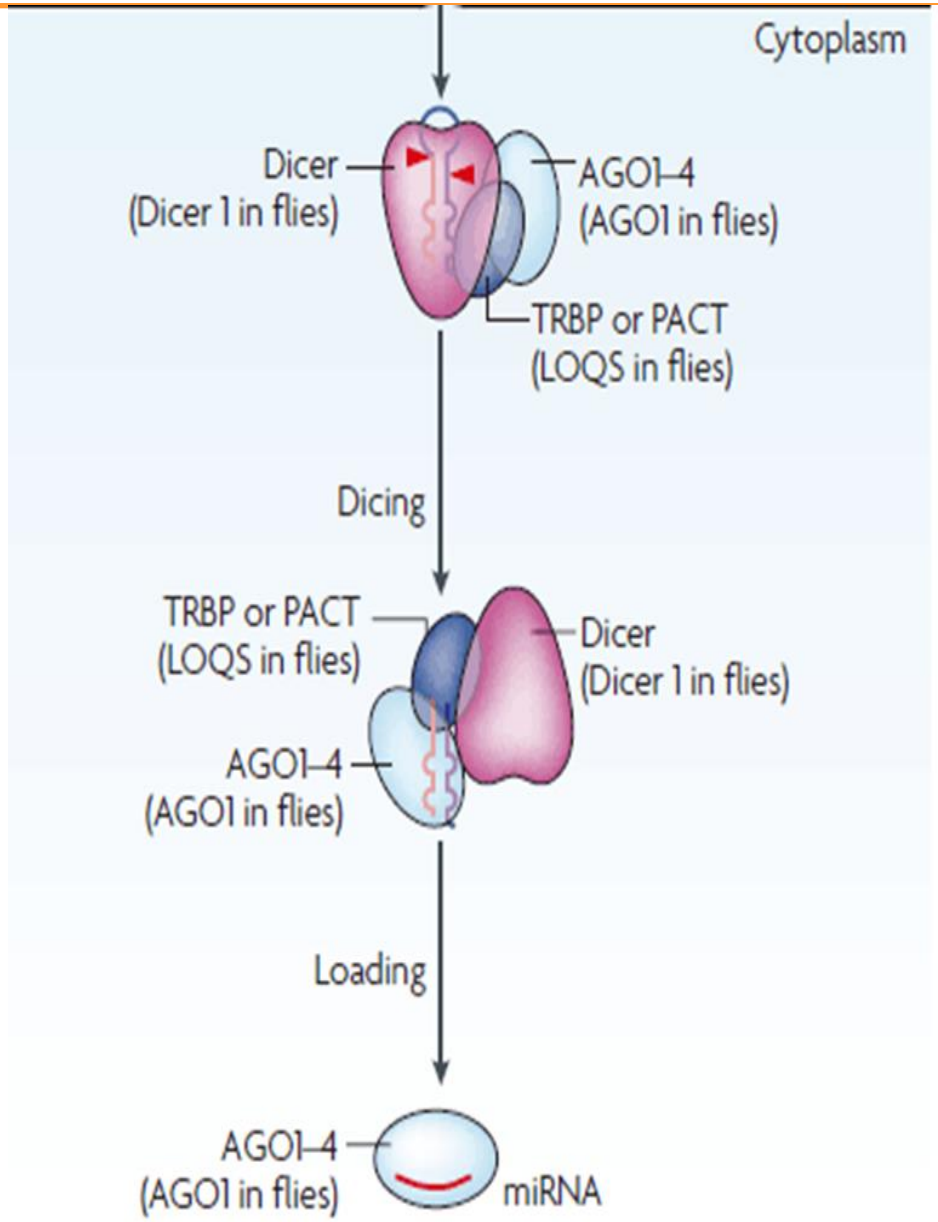
Mediates the nuclear export of micro-RNA precursors, which form short hairpins. Also mediates the nuclear export of synthetic short hairpin RNAs used for RNA interference, and adenovirus VA1 dsRNA. In some circumstances can also mediate the nuclear export of deacylated and aminoacylated tRNAs. Specifically recognizes dsRNAs that lack a 5'-overhang in a sequence-independent manner, have only a short 3'-overhang, and that have a double-stranded length of at least 15 base-pairs. Binding is dependent on Ran-GTP.

miRNA:1.4 Cytoplasmic processing by Dicer

pre-miRNAs are cleaved near the terminal loop by Dicer, releasing ~22-nt miRNA duplexes

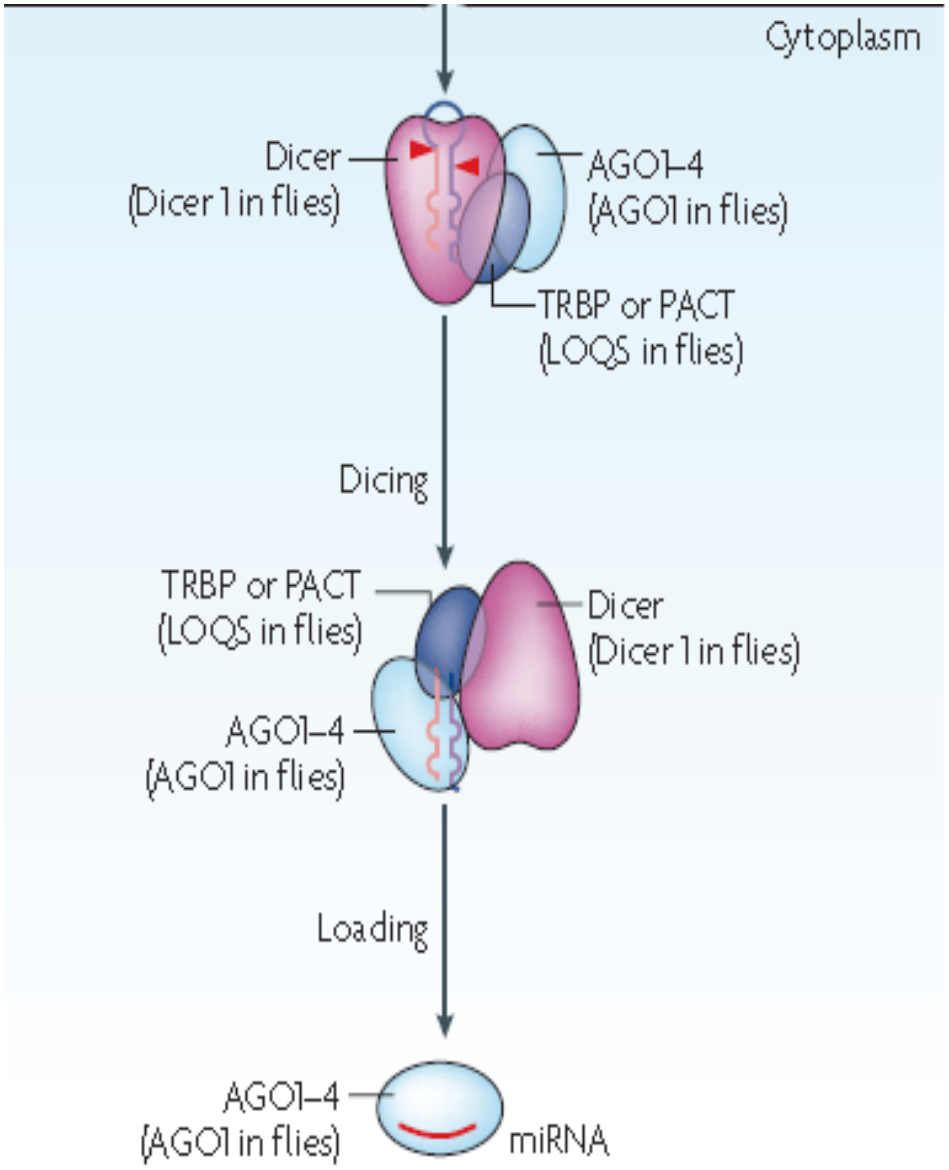
Dicer： highly conserved protein
different Dicer isotypes have distinct role

dsRNA-binding proteins：
D. melanogaster Dicer 1： loquacious (loQS)
Human Dicer： TRBP (TAR RNA-binding as TARBP protein and PACT (PRKRA), contribute to formation of the RNA-induced silencing complex (RISC)



miRNA: 1.5 *Argonaute loading*

one strand
of the ~22-nt RNA duplex remains in Ago as a mature miRNA (the guide strand or miRNA), whereas the other strand (the passenger strand or miRNA*) is degraded.
Thermodynamic stability :relatively unstable base pairs at the 5′ end typically survives
RLC Ago
often not a stringent process
R2D2



miRNA:

multiple choices of Ago proteins during RISC assembly

D. melanogaster:
the structure of the precursor—miRNA central mismatches+AGO1, perfectly matching siRNA+AGo2

C. elegans:
AlG-1 and RDE-1 associate with small RNAs from mismatched precursors and perfect dsRNA precursors respectively.

Humans:
all four Ago proteins, AGo1–4, bind to miRNAs with only marginal differences in miRNA repertoire.

miRNA

http://v.youku.com/v_show/id_XMTU1ODc4NjEzMg==.html?qq-pf-to=pcqq.discussion

miRNA

2. Regulation of miRNA biogenesis

2.1 *Transcriptional control*

2.2 *Post-transcriptional regulation*

2.3 *Feedback circuits in miRNA networks*

miRNA

2.1 *Transcriptional control*

Pol II-associated transcription factors:
MyoD1

tumour-suppressive or oncogenic transcription factors:
P53

Epigenetic control -DNA methylation:
miR-203

miRNA

2.2 *Post-transcriptional regulation*

(1)Drosha processing

heterogeneous ribonucleoprotein

nuclear RNAbinding proteins influence miRNA processing through specific interactions with a subset of pri-miRNAs.

miRNA

(2) The let-7 miRNAs :
lIN28, is responsible for the suppression of let-7 biogenesis
interfering with pre-let-7 processing and/or by inducing terminal uridylation of pre-let-7. The u tail (~14 nt) that is added to the 3′ end of pre-let-7 blocks Dicer processing and facilitates the decay of pre-let-7 .

miRNA

(3)RNA decay enzymes

target not only mature miRNAs but
also the precursors (pri-miRNAs and pre-miRNAs).

small RNA degrading nuclease (SDN) proteins
were recently reported to affect the stability of miRNAs inplants.

miRNA

(4) RNA editing ：

Because the modified pri-miRNAs or pre-miRNAs become poor substrates of RNase III proteins,editing of the precursor can interfere with miRNA processing.

Editing can also change the target specificity of the miRNA if it occurs in miRNA sequences.

miRNA

2.3 Feedback circuits in miRNA networks

Two types:observed: single-negative feedback and double-negative feedback.

Single-negative feedback:stable or oscillatory expression of both components--levels of Drosha and Dicer are controlled by singlenegative feedback to maintain the homeostasis of miRNA production

double-negative feedback :mutually exclusive expression.
--effective genetic switch of specific miRNAs during differentiation. (let-7 and lIN28)

1.Piwi-interacting RNAs

1.1 *piRNA biogenesis in flies*

1.2 *piRNA biogenesis in mice*

piRNA

1. 24-29nt in D.melanogaster
   24-31nt in mice

2. Intergenic repetitive elements,including retrotransposon
   Repeat-associated small interfering RNAs(rasiRNAs,重复相关小RNA) in D.melanogaster

3. Be associated with PIWI proteins

4. Be produced without Dicer(The requirement for Drosha has not been formally tested)

piRNA: *flies*

1.1 *piRNA biogenesis in flies*

1.1.1 piRNA clusters

The differences in subcellular localization, the expression patterns and the associated proteins might influence the selective piRNA association.

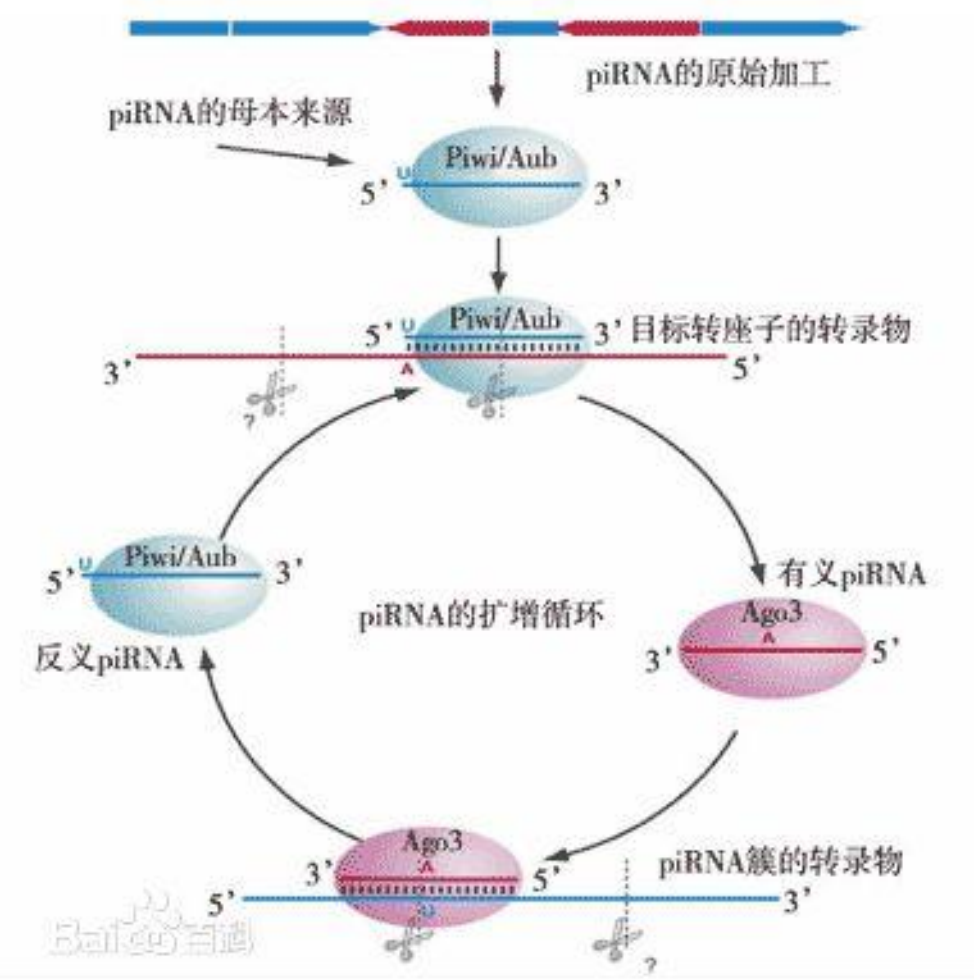
1.1.2 nucleotide bias

1.1.3 ping-pong cycle

1.1.4 How does such a piRNA biogenesis cycle initiate during development?

germline transmission

piRNA: *flies*



1.AGO3-associated piRNAs mostly have adenine at nucleotide 10

2.AuB- and PIWI-associated piRNAs show strong preferences for uracil at their 5′ ends

3.AuB-associated piRNAs frequently show complementarity to AGo3-associated piRNAs in their first 10 nt

4.Piwi cleaves target RNA at between positions 10 and 11 relative to the 5′ end of the associated small RNAs

piRNA: *flies*

1. Mutations in several genes, such as those that encode the putative nucleases Squash and Zucchini, and others such as *Spindle-E*14, *Krimper*159 and *Maelstrom*160, cause the depletion of piRNAs in fly ovaries, suggesting that they are involved in piRNA biogenesis. However, the precise roles of these proteins remain elusive.

2. In *D. melanogaster*, at least AuB and possibly PIWI are deposited for the next generation by germline transmission
A recent study shows that piRNAs that are maternally inherited to embryos have an epigenetic regulatory role in transposon silencing

piRNA:mice

1.2 *piRNA biogenesis in mice*

1.2.1 In mammals, two classes of Piwi-interacting RNAs (piRNAs) have been identified

1.2.1.1 pre-pachytene piRNAs is expressed before meiotic pachytene and is derived from repeat- and transposon-rich clusters。

1.2.1.2 pachytene piRNAs remain an enigma: >80,000 distinct species are derived from large genomic clusters of up to 200 kilobases. These piRNA clusters exhibit a marked strand asymmetry, as if the piRNAs are processed from one or a few huge transcripts.说明同一簇piRNA可能来源于同一长初始转录物

1.2.2 pre-pachytene piRNAs ping-pong cycle , However, the different compositions of the embryonic, neonatal and adult piRNAs suggest that the biogenesis cycle does not continue throughout male germ-cell development

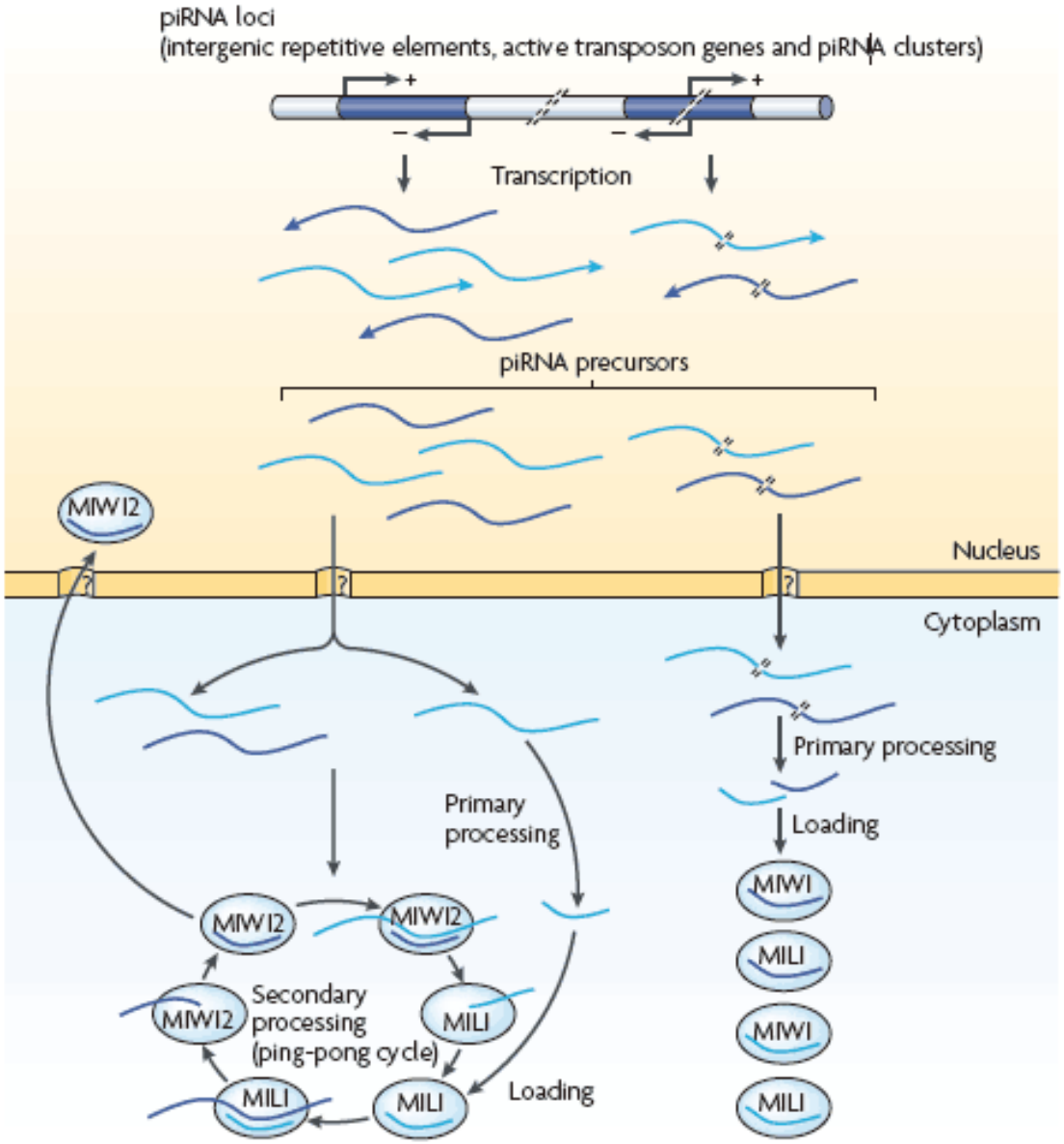


Figure 3 | **piRNA biogenesis pathway.** Piwi-interacting RNAs (piRNAs) of 24–31

piRNA:mice

1. Mutations in *Mili* and *Miwi2* eliminated DNA methylation of long interspersed nuclear element 1 (lINE1) and intracisternal A particle (IAP) and led to male infertility（雄性不育）

2.unlike animal miRNAs but similar to plant miRNAs, piRNAs have a 2′-*O*-methyl modification at their 3′ ends. This modification is carried out by homologues of *A. thaliana* HEN1 methyltransferase169 (known as HEN1 or PIMET in flies)165,166. It was also shown that HEN1 associates with Piwi proteins in ovaries.

植物miRNA2′O甲基转移酶HEN1在小鼠中的同源蛋白mHEN1，它在体外培养的睾丸中特异性表达并且使piRNA的3′端发生了甲基化（而HEN1在果蝇中的同源蛋白Pimet则介导了piRNA3′端的2′O甲基化作用）

endo-RNA

1.Endogenous siRNAs

1.1 *Endo-siRNA processing by Dicer 2 in flies*

1.2*Endo-siRNA biogenesis in mouse oocytes*

endo-RNA

1. ~21-nt
2. These RNAs are derived from transposon transcripts, sense–antisense transcript pairs and long stem-loop structures
3. Numerous types of endo-siRNAs are present in oocytes and less abundantly in ES cells(mouse)
somatic tissue, cultured cells and ovaries in *D. melanogaster*

4. Be associated with AGO2
5. Similar to exo-siRNAs, but different from miRNAs, the processing of endo-siRNAs is dependent on Dicer 2 rather than Dicer 1
6. long dsRNA structures and bulges
7. possess RNA dependent RNA polymerases (RdRPs)（plants and worms）
RdRP-independent(flies and mammals lack RdRP)

endo-RNA:*flies*

Endo-siRNA processing by Dicer 2 in flies

1.Endo-siRNA precursors are mainly produced from sense–antisense pairs derived from transposons. They can also arise from convergent transcription of protein-coding genes and from unannotated regions of the genome

2.~20% of AGO2-associated endo-siRNAs in S2 cells show sequence substitutions. As most of the mutations found were A–G substitutions, this is probably due to RNA editing by ADAR. The editing activity of ADAR is restricted in the nucleus and accepts only dsRNAs as the substrate.
This posttranscriptional nucleotide modification causes further tiny bulges in the precursors.

endo-RNA

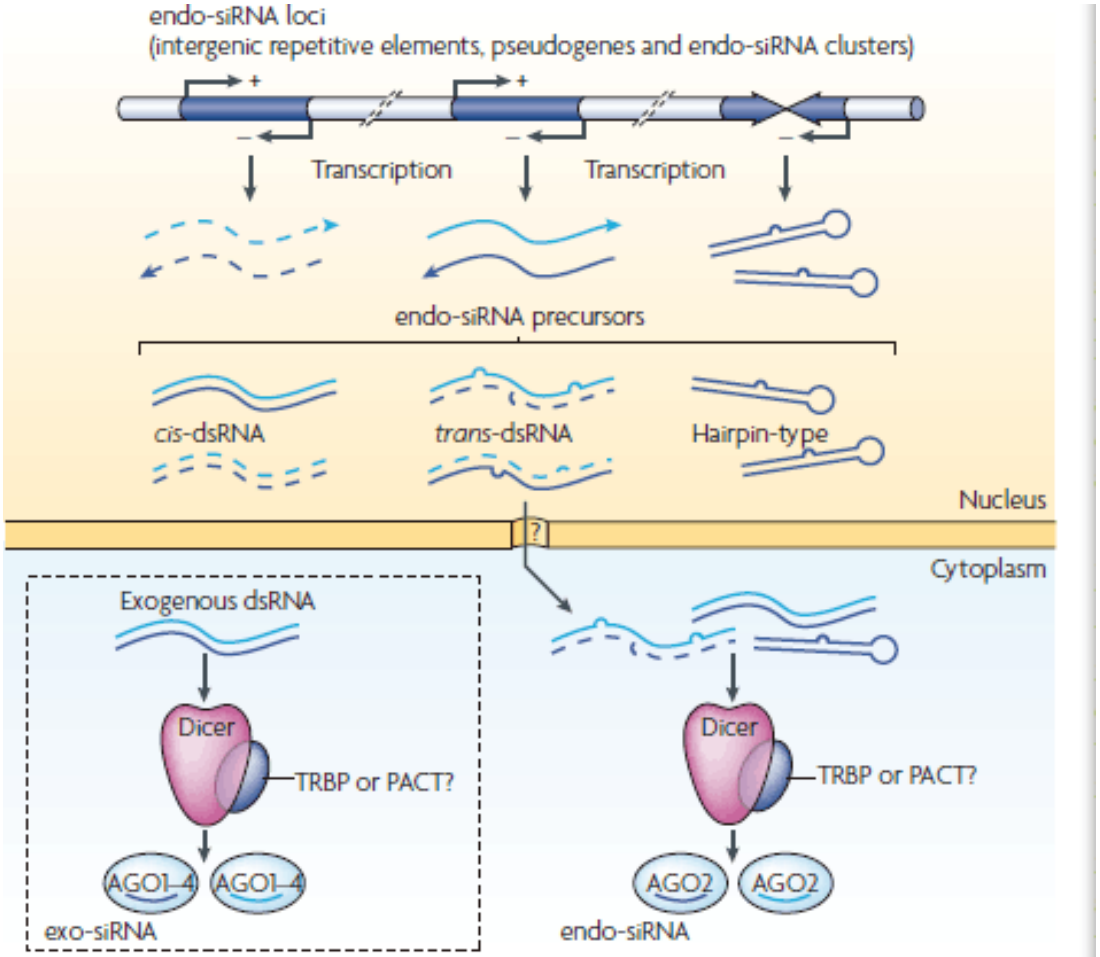


Figure 4 | **Exo- and endo-siRNA biogenesis pathway.** Exogenous small interfering RNAs

endo-RNA

Some piRNA-generating loci can be a source of endosiRNAs like piRNAs, endo-siRNAs have 2′-*O*-methyl groups at their 3′ ends in flies, which might be added by the methyltransferase HEN1

Difference

RNAs	**len**	protein partners	expressed cell	editing status	generated from dsRNAs
piRNAs	24-29nt	Piwi	mostly germ cells	NO	F
Endo-RNAs	21nt	AGo2	ubiquitous expression	can be edited by ADAR	T

endo-RNA

1.2*Endo-siRNA biogenesis in mouse oocytes*

在卵母细胞中，可通过为蛋白质编码的有义转录产物与假基因的反义转录产物配对所形成的dsRNA，产生内源siRNA

in ovaries, transposon silencing is ensured by two pathways: piRNA and endo-siRNA pathways ,the endo-siRNA pathway might have been lost in males

在小鼠中，任何PIWI家族成员的删除都会导致发生以丢失生殖细胞为特征的雄性不育。这种生殖细胞丢失与转座子活化有相关性，而转座子一般是受生殖细胞piRNA的抑制

类似于piRNA的另一种小RNA途径在卵母细胞中起作用

Conclusions

- miRNAs can exert broad and strong effects，seem to serve as hubs of gene networks that are rich in information flow.

原因

1、miRNA靶向多个基因

2、miRNA经常与其他调节因子一起参与反馈回路

Conclusions

- 对small RNA的深入了解将帮助我们更好地了解基因网络，并获得更安全和更有效的遗传操作策略。
- 归功于测序效率的提高，数以万计的small RNA已经被发现，还有许多即将被发现。但面临的挑战是，难以区分有功能的small RNA 与无功能的“noises”
- 实际上数据库中许多small RNA是被误认，只是其他的长RNA降解的产物。

Conclusions

- Small RNA是否具功能性需要实验证明
- Confirmation of their expression and their dependence on the Ago proteins will first be needed to validate individual small RNAs. Dependence on other biogenesis factors, such as Drosha, DGCR8 and Dicer, will also be useful in validating small RNAs as well as in classifying them.

首先确认其表达以及对AGO蛋白的依赖性，以确保是small RNA。根据对其他生物因子（如Drosha、DGCR8、Dicer等）的依赖性也能对其进行验证和分类。

Conclusions

- how small RNA path-ways are connected to other aspects of RNA metabolism, including transcription, pre-mRNA splicing and mRNA decay. Given that intronic miRNAs are processed co-transcriptionally, it is tempting to speculate that these processes (transcription, splicing and Drosha processing) are coupled by specific factors.

  还有一个问题是：如何将small RNA pathway与RNA代谢的其他方面联系起来，包括转录、前体mRNA剪接和mRNA衰减。鉴于内含子miRNA是共转录加工的，所以推测这些过程（转录，剪接和Drosha加工）是通过特定因子偶联的。

Conclusions

此外

- 还存在许多低丰度、非规范途径产生、难以分类small RNA，对它们的特异性功能了解尚少。
- piRNA途径很大程度上难以捉摸。乒乓模型不解释所有特征，还存在未知的发生途径
- piRNA与异染色质形成机制有关联，也可能为PIWI相关蛋白的鉴定和分析提供线索。

Conclusions

- It is also of note that animal RNase III, Drosha and Dicer are involved not only in small RNA pathways but also mRNA stability control of hairpin-containing mRNAs. further studies are needed to identify

  动物的RNase III，Drosha和Dicer不仅参与small RNA途径，而且涉及含有发夹结构的mRNA稳定性控制。

展望

- The miRNA biogenesis pathway is well studied in comparison to piRNA and endo-siRNA pathways, although many questions remain unanswered.

  相比piRNA、siRNA， miRNA的生物发生途径被很好的研究，但还有许多详细的机制问题没有清楚，例如：

展望

- Microprocessor, EXP5 and Dicer–RISC in association with the substrate RNAs

- additional protein factors in the pathway need to be identified，the identities of the factors that are involved in miRNA turnover in animals

展望

- the significance and enzymology of the modifications of small RNAs, such as uridylation and adenylation of miRNAs
- the auxiliary factors that regulate miRNA maturation
- how miRNA biogenesis is controlled by these factors in response to various cellular signals

  总之就是miRNA如何受各种因子调节控制、相应细胞信号。

THANKS!