miRNA biogenesis

miRNA

- Genomically encoded
- Frequently are complementary to mRNA at the 3’UTR
  - Usually imperfect complementarity
- Can be encoded in clusters on the genome, or individually
- Sequences are relatively conserved between species
- Can have multiple unlinked targets
- miRNAs usually interact with the 3’ UTR
- Can alter mRNA stability, and translational initiation

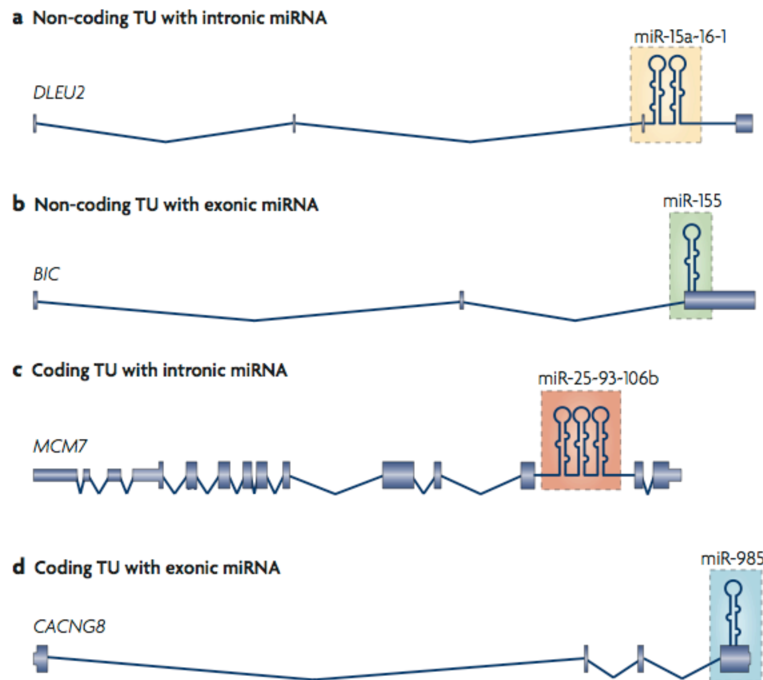
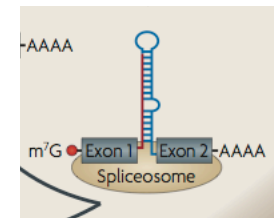
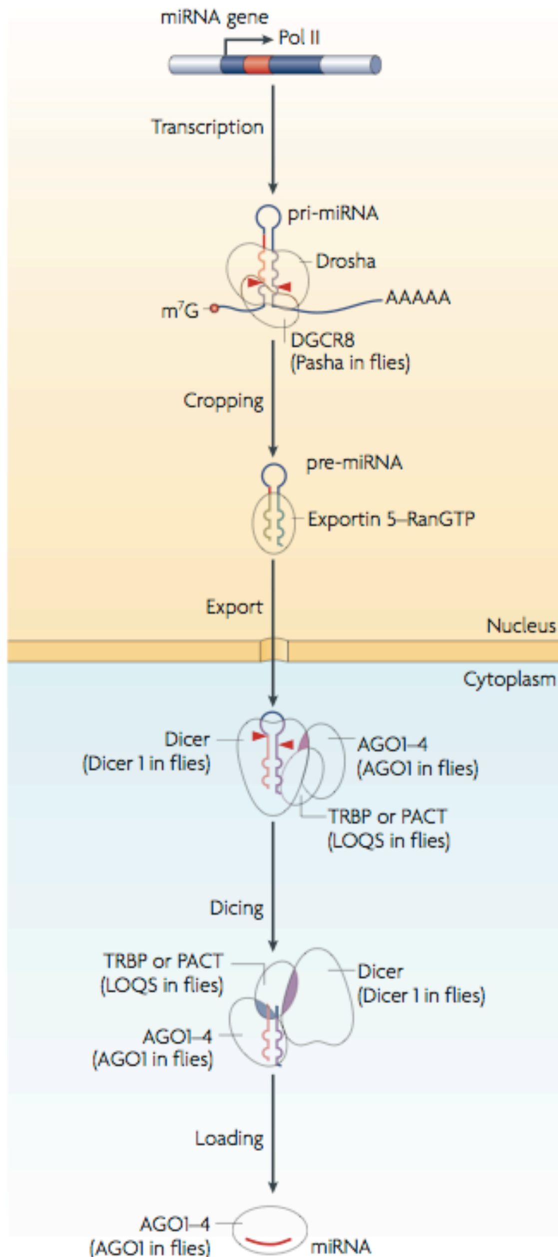


Figure 1 | **Genomic location and gene structure of miRNAs.** MicroRNAs (miRNAs) can be categorized into four groups according to their genomic locations relative to exon and intron positions. **a** | Intronic miRNAs in non-coding transcripts, such as the miR-15a~16-1 cluster. The miR-15a~16-1 cluster is found in the intron of a well-defined non-coding RNA gene, *DLEU2* (REF. 197). **b** | Exonic miRNAs in non-coding transcripts. miR-155 was found in a previously defined non-coding RNA gene, *BIC*[198]. **c** | Intronic miRNAs in protein-coding transcripts. Shown here as an example is the miR-25~93~106b cluster, which is embedded in the intron of the DNA replication licensing factor *MCM7* transcript. **d** | Exonic miRNAs in protein-coding transcripts. The miR-985 hairpin is found in the last exon of *CACNG8* mRNA. The hairpins represent miRNA stem-loops. Blue boxes indicate the protein-coding regions. This figure is roughly to scale. TU, transcription unit.

c1 *Coding transcription unit with intronic miRNA (mirtrons)*

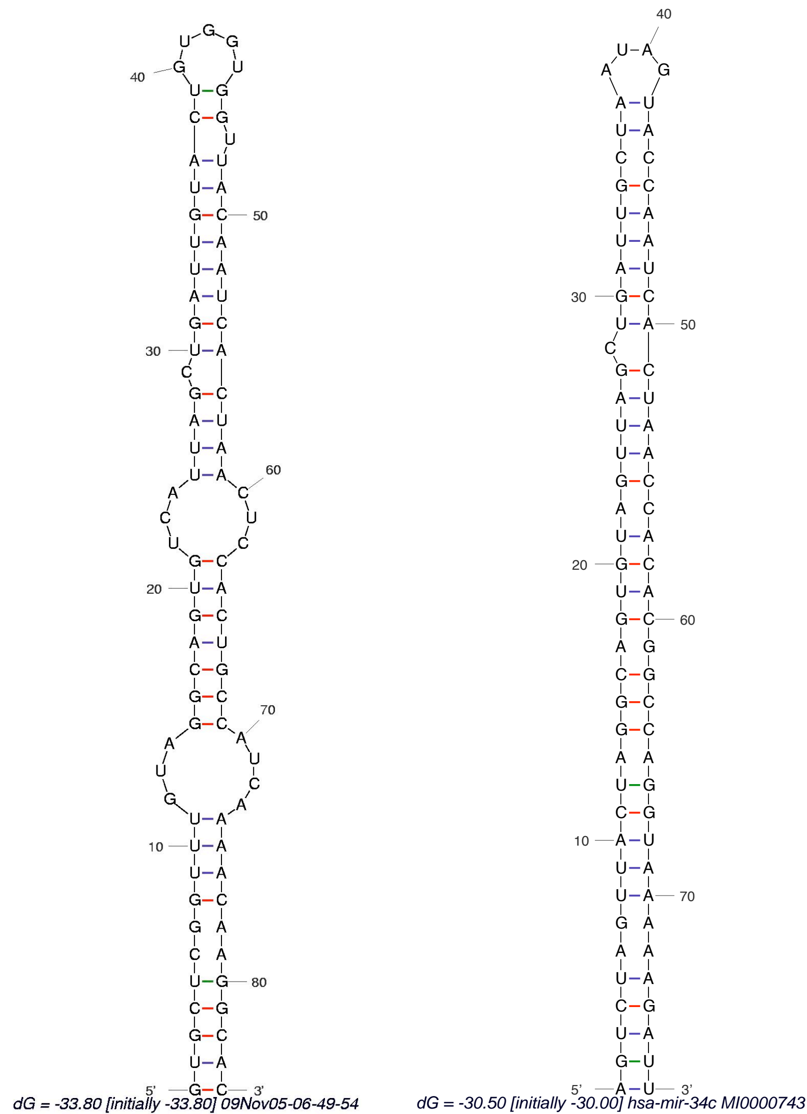




- pri miRNA is the nascent miRNA transcript , are cropped in the nucleus by the Microprocessor Complex (MC). The MC contains Drosha/DRCR8 plus other proteins (RBPs and helicases etc). Cropping generates the
- pre miRNA which is approx 70 bp long and is diced in cytoplasm by Dicer plus Ago and TRBP.

- DROSHA and Dicer are Rnase III enzymes
- Ago proteins .The PIWI domain can adopt an RNaseH structure

Folding of pri miRNAs and RNAseIII cleavage sites

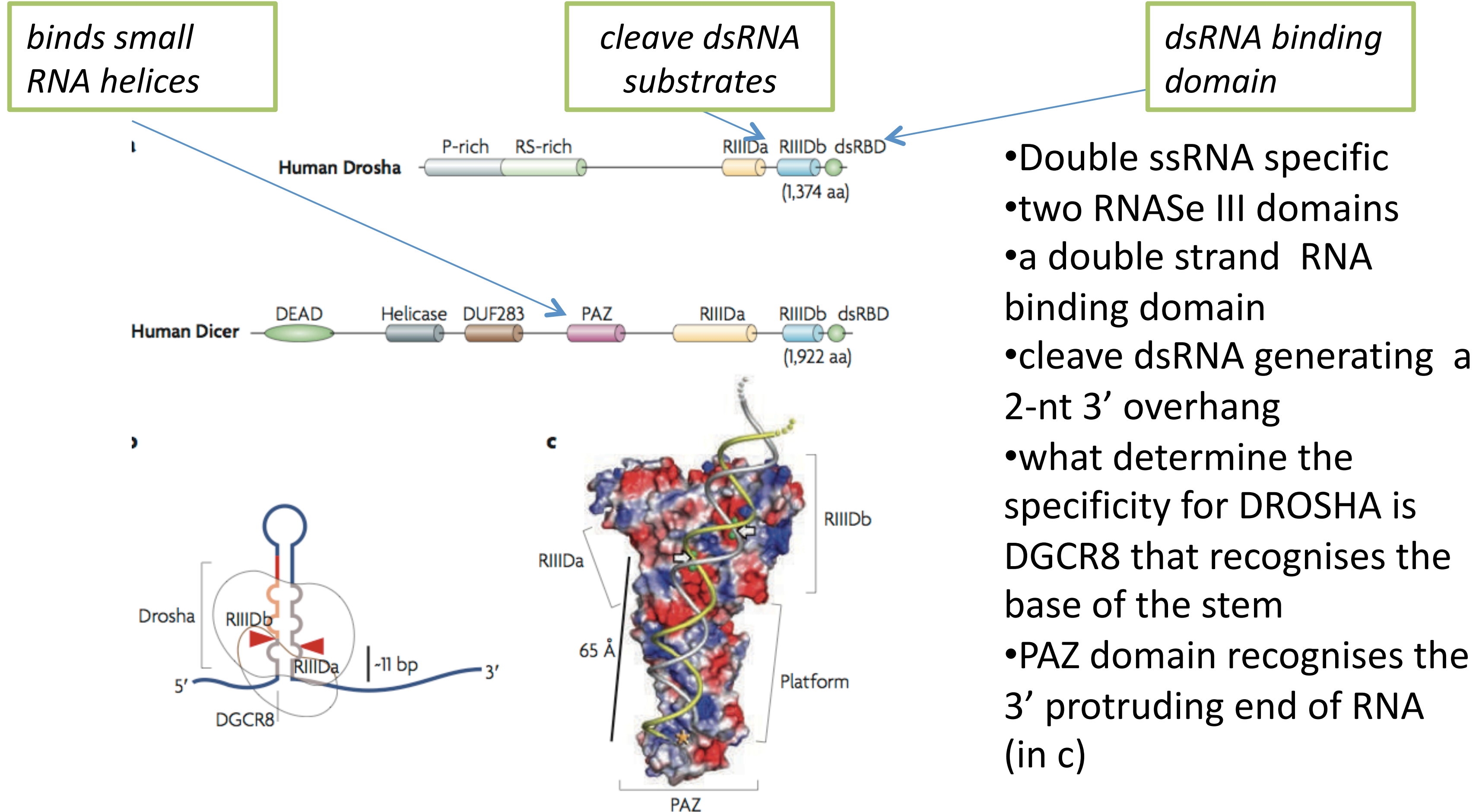


DICER cleavage

- imperfectly paired stem of approx 33bp
- terminal loop
- flanking segments

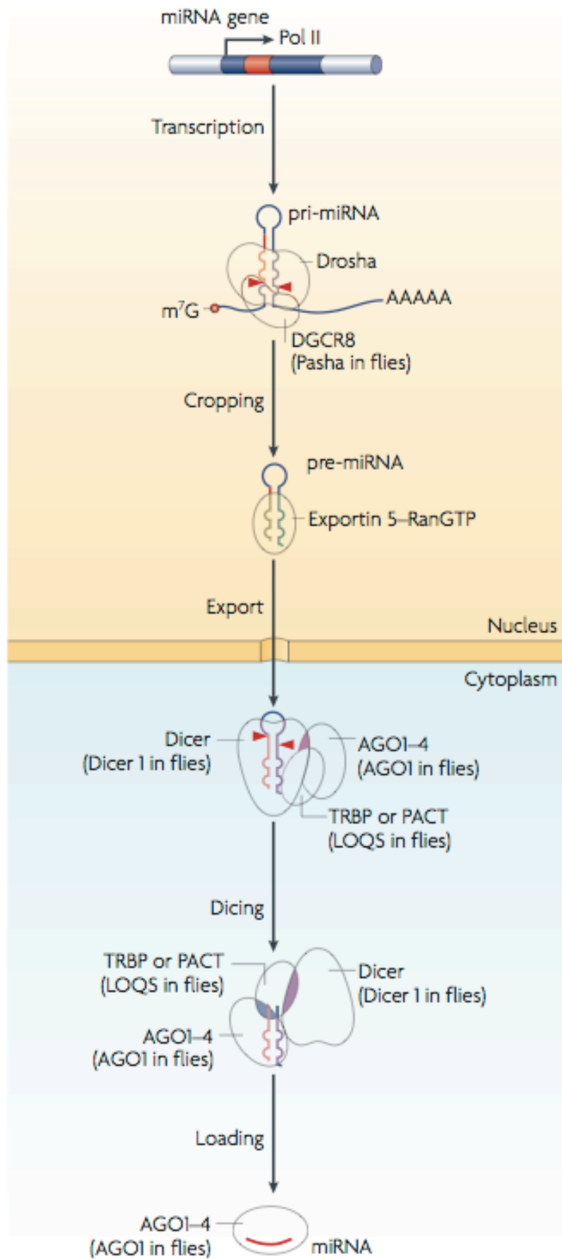
DROSHA cleavage

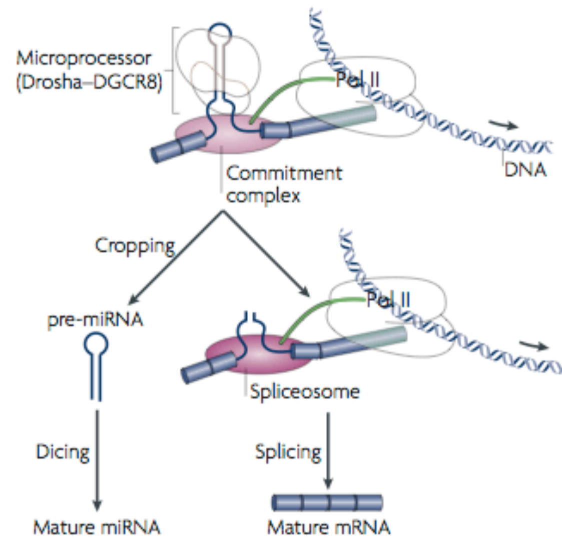
RNAse III proteins and their mechanism of action

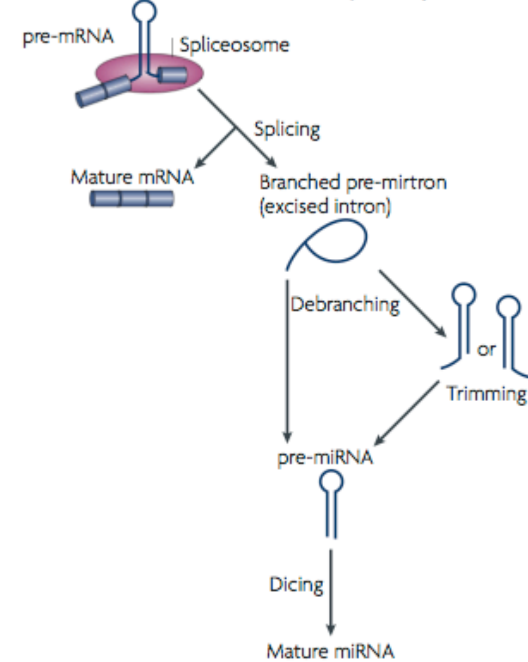


- •Double ssRNA specific
- •two RNASe III domains
- •a double strand RNA binding domain
- •cleave dsRNA generating a 2-nt 3’ overhang
- •what determine the specificity for DROSHA is DGCR8 that recognises the base of the stem
- •PAZ domain recognises the 3’ protruding end of RNA (in c)

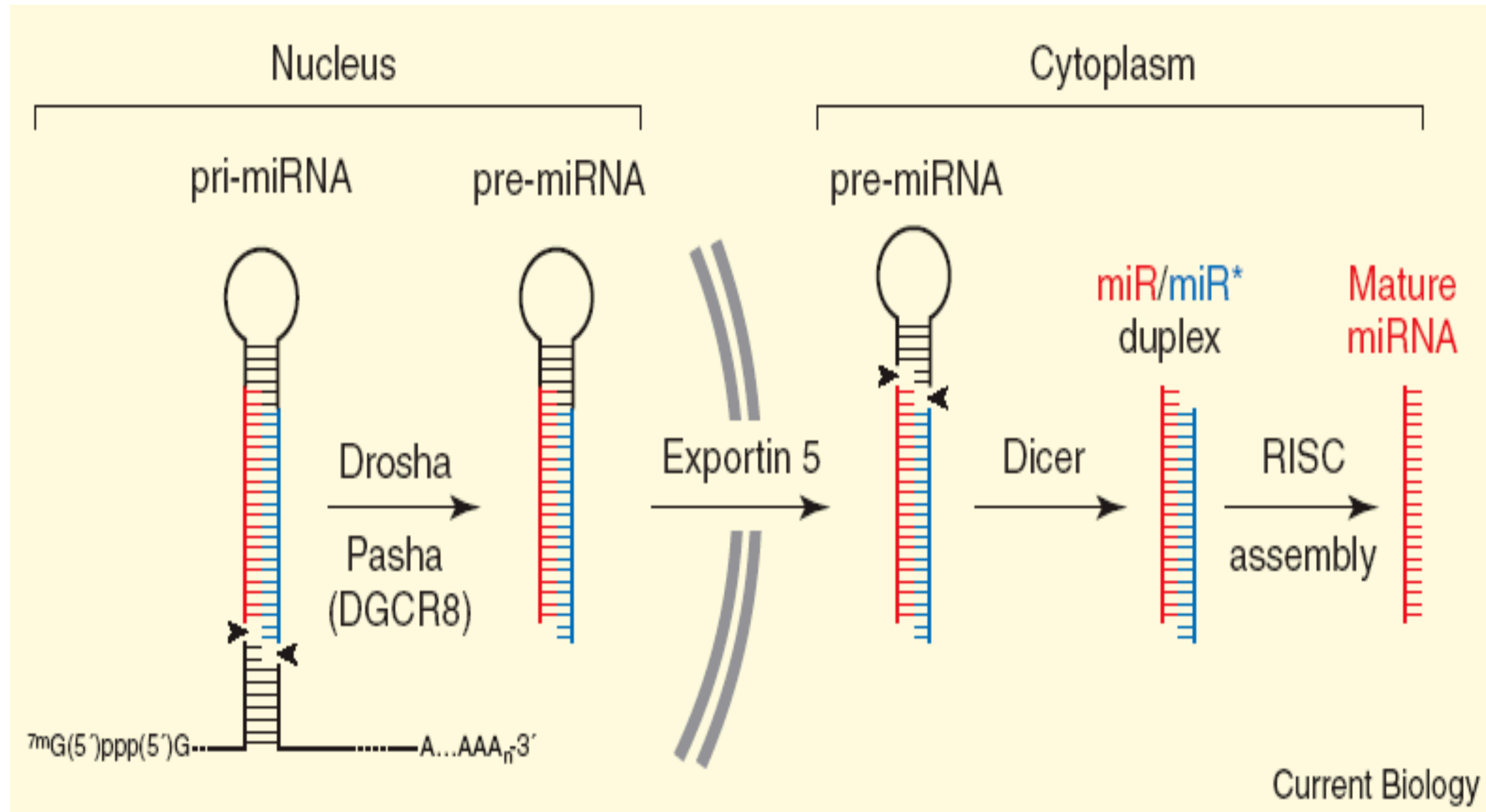
Two types of RNase III are found in animals: Drosha and Dicer. Both proteins have two tandem RNase III domains (RIIIDs) and a double-stranded RNA-binding domain (dsRBD; see the figure, part **a**). Two RIIIDs interact with each other to make an intramolecular dimer in which the two catalytic sites are located closely to each other. The first RIIID cuts the 3′ strand of dsRNA, whereas the second RIIID cleaves the 5′ strand, generating a 2-nucleotide (nt) 3′ overhang[38,189,190] (see the figure, parts **b**,**c**).


a Biogenesis of canonical miRNA
miRNA gene
Pol II
Transcription
pri-miRNA
Drosha
m7G
AAAAA
DGCR8
(Pasha in flies)
Cropping
pre-miRNA
Exportin 5–RanGTP
Export
Nucleus
Cytoplasm
Dicer
(Dicer 1 in flies)
AGO1–4
(AGO1 in flies)
TRBP or PACT
(LOQS in flies)
Dicing
TRBP or PACT
(LOQS in flies)
Dicer
(Dicer 1 in flies)
AGO1–4
(AGO1 in flies)
Loading
AGO1–4
(AGO1 in flies)
miRNA


b Canonical intronic miRNA
Microprocessor
(Drosha–DGCR8)
Pol II
DNA
Commitment
complex
Cropping
pre-miRNA
Pol II
Spliceosome
Dicing
Splicing
Mature miRNA
Mature mRNA


c Non-canonical intronic small RNA (mirtron)
pre-mRNA
Spliceosome
Splicing
Mature mRNA
Branched pre-mirtron
(excised intron)
Debranching
or
Trimming
pre-miRNA
Dicing
Mature miRNA

Another view



miRNA function

- Mechanism of action is unknown
- Interacts with the 3’UTR of the target mRNA
- Inhibits translation from a mRNA without inducing mRNA degradation
- Can form a ribonucleoprotein complex (miRNP)
- Can be complexed with ribosomes and target mRNA
- Also important for silencing retrotransposons and endogenous retroviruses
- Important for development and differentiation

- miRNA:mRNA (miRNP) targeted to p-bodies

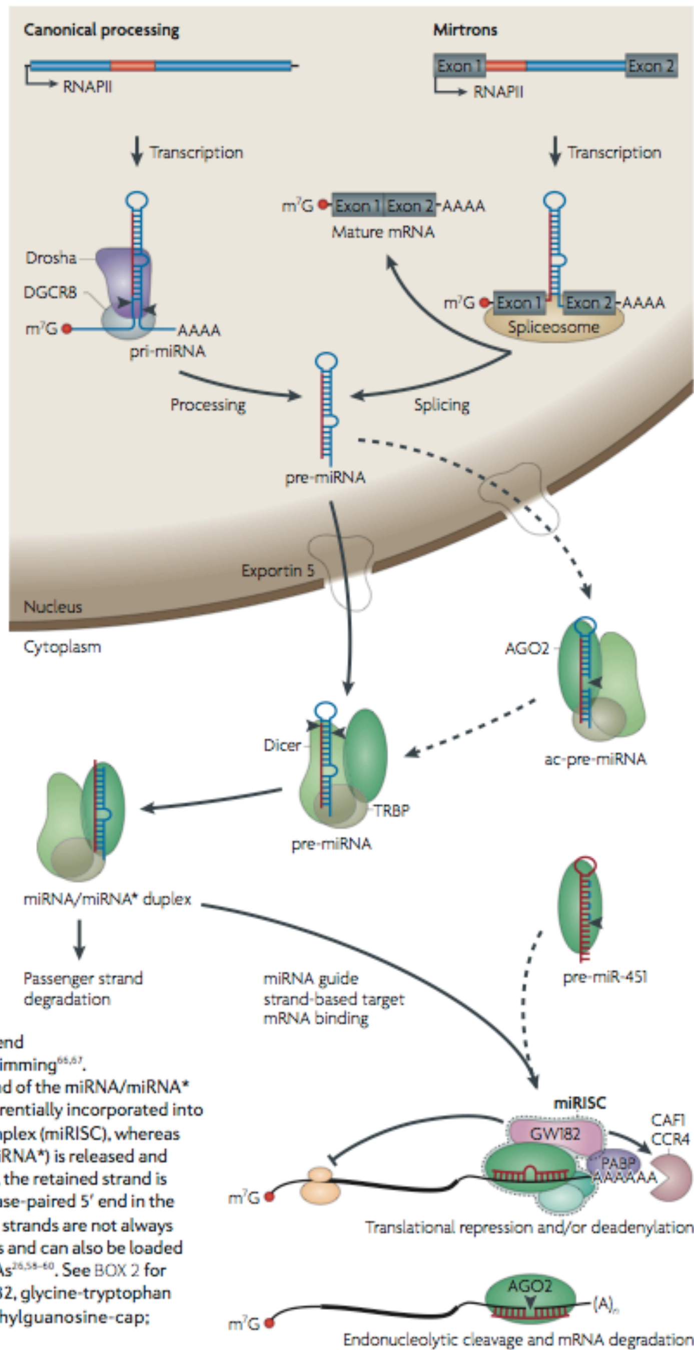
A more recent view

MicroRNAs (miRNAs) are processed from RNA polymerase II (RNAPII)-specific transcripts of independent genes or from introns of protein-coding genes[2,5]. In the canonical pathway, primary precursor (pri-miRNA) processing occurs in two steps, catalysed by two members of the RNase III family of enzymes, Drosha and Dicer, operating in complexes with dsRNA-binding proteins (dsRBPs), for example DGCR8 and transactivation-responsive (TAR) RNA-binding protein (TRBP) in mammals.

In the first nuclear step, the Drosha–DGCR8 complex processes pri-miRNA into an ~70-nucleotide precursor hairpin (pre-miRNA), which is exported to the cytoplasm. Some pre-miRNAs are produced from very short introns (mirtrons) as a result of splicing and debranching, thereby bypassing the Drosha–DGCR8 step. In either case, cleavage by Dicer, assisted by TRBP, in the cytoplasm yields an ~20-bp miRNA/miRNA* duplex.

In mammals, argonaute 2 (AGO2), which has robust RNaseH-like endonuclease activity, can support Dicer processing by cleaving the 3′ arm of some pre-miRNAs, thus forming an additional processing intermediate called AGO2-cleaved precursor miRNA (ac-pre-miRNA)[70]. Processing of pre-miR-451 also requires cleavage by AGO2, but is independent of Dicer and the 3′ end is generated by exonucleolytic trimming[65,67].

Following processing, one strand of the miRNA/miRNA* duplex (the guide strand) is preferentially incorporated into an miRNA-induced silencing complex (miRISC), whereas the other strand (passenger or miRNA*) is released and degraded (not shown). Generally, the retained strand is the one that has the less stably base-paired 5′ end in the miRNA/miRNA* duplex. miRNA* strands are not always by-products of miRNA biogenesis and can also be loaded into miRISC to function as miRNAs[26,58–60]. See BOX 2 for details of miRISC function. GW182, glycine-tryptophan protein of 182 kDa; m^{7}G, 7-methylguanosine-cap; PABP, poly(A) binding protein.



The players: summary

Drosha and DGCR8 are part of the “Microprocessor” protein complex (~600-650kDa)

Drosha and Dicer are RNase III enzymes

DRCR8 is a dsRNA binding protein

Exportin 5 is a member of the karyopherin nucleocytoplasmic transport factors that requires Ran and GTP

Argonautes contains a PIWI domain (in Ago2 adopt an RNase H structure)

cropping on pri miRNA	DROSHA/DGRC8
dicing on pre miRNA	DICER
slicing on mRNA	Ago2

Regulation of miRNA biosynthesis

- at the level of transcription
- at the level of processing
  - cropping efficiency of pri miRNA
  - dicing efficiency of pre miRNA
- post-transcriptional modifications

Box 3 | Regulation of microRNA gene transcription

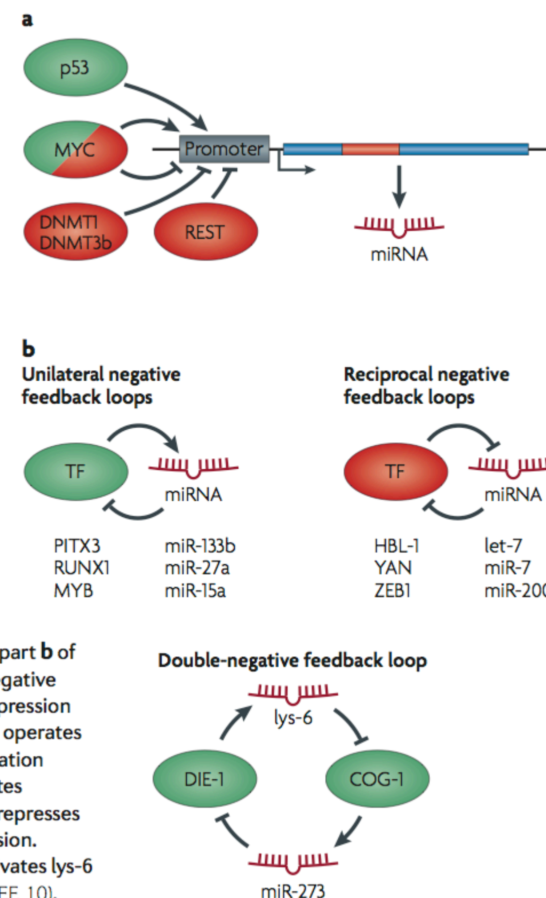
The promoter regions of autonomously expressed microRNA (miRNA) genes are highly similar to those of protein-coding genes[152,153]. The presence of CpG islands, TATA box sequences, initiation elements and certain histone modifications indicate that the promoters of miRNA genes are controlled by transcription factors (TFs), enhancers, silencing elements and chromatin modifications, which is similar to protein-coding genes.

Activators and repressors of miRNA transcription

Many TFs regulate miRNA expression positively or negatively in a tissue-specific or developmental-specific manner (see part **a** in the figure; transcriptional activators or repressors are shown in green and red, respectively). For example, MYC and MYCN both stimulate expression of the miR-17-92 oncogenic cluster in lymphoma cells[154] and miR-9 in neuroblastoma cells[155], but inhibit expression of several tumour suppressor miRNAs (for example, miR-15a), which promote MYC-mediated tumorgenesis[156]. p53 stimulates the expression of miR-34 and miR-107 families, which enhances cell cycle arrest and apoptosis[157]. The RE1 silencing transcription factor (REST) recruits histone deacetylases and methyl CpG binding protein MeCP2 to the *mir-124* gene promoter, preventing its transcription in neuronal progenitors and non-neuronal cells[158]. REST is downregulated upon differentiation, allowing for high miR-124 expression in post-mitotic neurons. Transcription of miR-148a, miR-34b/c, miR-9 and let-7 is dependent on their gene promoter methylation status, which is regulated by the DNMT1 and DNMT3b DNA methyltransferases[159].

Regulatory networks of miRNA expression

miRNAs frequently act in regulatory networks with TFs, which can drive or repress the expression of the miRNAs. A few examples of autoregulatory feedback loops are shown in part **b** of the figure, with examples of specific miRNAs and TFs indicated. Unilateral or reciprocal-negative feedback loops (single or double loops) result in oscillatory or stable mutually exclusive expression of the TF and miRNA components. The double-negative feedback loop shown in the figure operates in the chemosensory neurons of *Caenorhabditis elegans*. Here, proper transcriptional activation and/or inactivation is accomplished by spatially controlled miRNA expression, and facilitates establishment of the left–right asymmetry of 'ASE' chemosensory neurons. The COG-1 TF represses the left ASE (ASEL) cell fate in the right ASE (ASER) neuron and stimulates miR-273 expression. miR-273 targets the DIE-1 transcription factor in ASER but not in ASEL, in which DIE-1 activates lys-6 expression and promotes the ASEL-specific cell fate. In ASEL, COG-1 is blocked by lys-6 (REF. 10).



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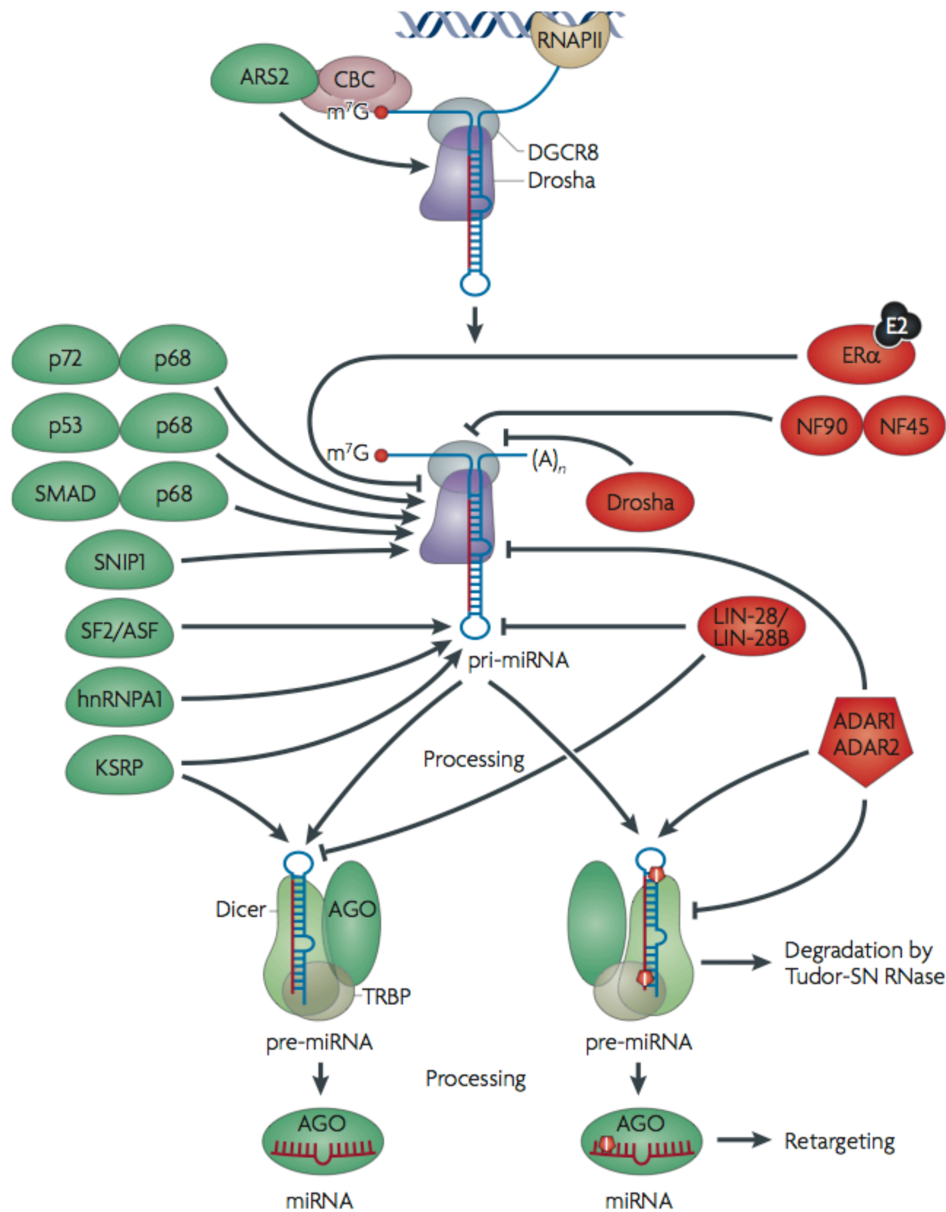

Figure 1 | **Regulators of microRNA processing.** Several activators and repressors regulate microRNA (miRNA) biogenesis through either protein–protein or protein–RNA interactions. Arsenite-resistance protein 2 (ARS2) supports Drosha processing of pri-miR-21, pri-miR-155 or pri-let-7, providing functional coupling of pri-miRNA transcription and processing[47,48]. The p68 and p72 helicases, identified as components of the Drosha Microprocessor complex, are thought to stimulate processing of one-third of murine pri-miRNAs[154]. p68 and p72 interact with various proteins and possibly act as a scaffold that recruits other factors. The SMAD–p68 complex, or a SMAD nuclear interacting protein 1 (SNIP1), enhances processing of pri-miRNAs, as well as the accumulation of mature miRNAs, like pri-miR-21 (REFS 43,44). Splicing factor SF2/ASF promotes Drosha-mediated processing of pri-miR-7 (REF. 50). Operating by direct protein–RNA interactions, heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) binds to the loop regions in pri-miR-18a and facilitates its Drosha-mediated processing, possibly by inducing a re-arrangement of RNA structure[51]. The splicing regulatory protein KSRP binds to a subset of pri-miRNAs that have GGG triplet motifs in their terminal loops and enhances processing by Drosha. KSRP also promotes Dicer-mediated processing of some pre-miRNAs in the cytoplasm[52]. While the LIN-28 repressor effect seems to be restricted to let-7 family members, the nuclear factor NF90–NF45 heterodimer blocks maturation of a broader range of pre-miRNAs[175]. NF90–NF45 interacts with the stem of pri-miRNAs in a sequence-independent way and prevents DGCR8 binding[175]. The estrogen receptor α (ERα) interacting with p68 and p72 helicases[176] and Drosha[177] affects the Drosha complex formation and represses processing of several pri-miRNAs[177]. Drosha can also negatively regulate miRNA processing by decreasing DCGR8 levels. Editing of pri-miRNAs or pre-miRNAs by adenosine deaminases that act on RNA (ADAR1 and ADAR2) affects accumulation of mature miRNAs, and might also influence miRNA target specificity[54–57]. AGO, argonaute; CBC, cap-binding complex; m^{7}G, 7-methylguanosine-cap; RNAPII, RNA polymerase II; TRBP, transactivation-responsive (TAR) RNA-binding protein.

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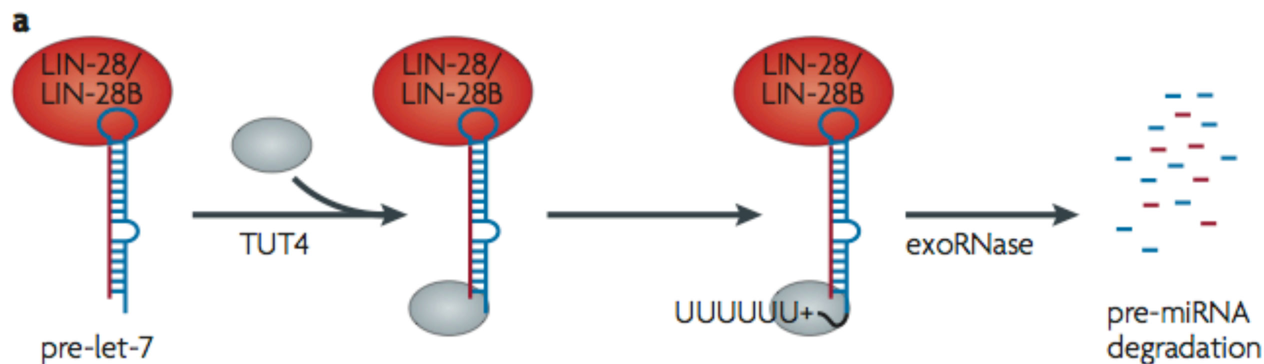

Figure 2 | **Modification at the 3′ end of microRNAs regulates stability.**
The post-transcriptional addition of non-genome-encoded nucleotides to the 3′ end of either pre-microRNA (miRNA) or mature miRNA affects miRNA stability or abundance. **a** | The RNA-binding protein LIN-28 promotes uridylation of pre-let-7 in *Caenorhabditis elegans* and mammalian cells by recruiting the poly(U) polymerase (PUP) TUT4 (also known as Zcchc11 or PUP-2 in worms), which adds multiple uracil resides to the 3′ end of RNA substrates. Polyuridylation of pre-let-7 prevents Dicer processing and induces precursor degradation by an unknown nuclease. **b** | RNA stability is influenced

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miRNA readings

- Carthew and Sontheimer, Origin and mechanism of miRNAs and siRNAs. Cell (2009) 136, 642-655.
- Jacek Krol, Inga Loedige and Witold Filipowicz The widespread regulation of microRNA biogenesis, function and decay. Nature Reviews Genetics 11 2010, 597
- V. Narry Kim, Jinju Han and Mikiko C. Siomi. Biogenesis of small RNAs in animals. Nature Reviews Mol Cell Biol 10 2009, 126