Small Regulatory RNAs

A (the) major part of eukaryotic gene regulation is performed by small RNAs

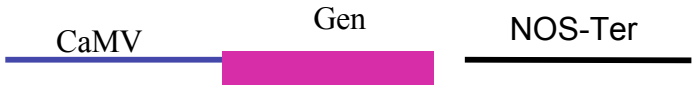
siRNA : useful research and therapeutic tools
miRNAs: major regulators
Emerging RNAs: psnoRNAs
Different pathways to make regulatory RNAs
miRNA/esiRNA pathways
piRNA processing
Mirtrons
miRNA expression is highly regulated

Find the miRNA binding sites for your pet gene
If your pet gene does not have one, use SFRS10

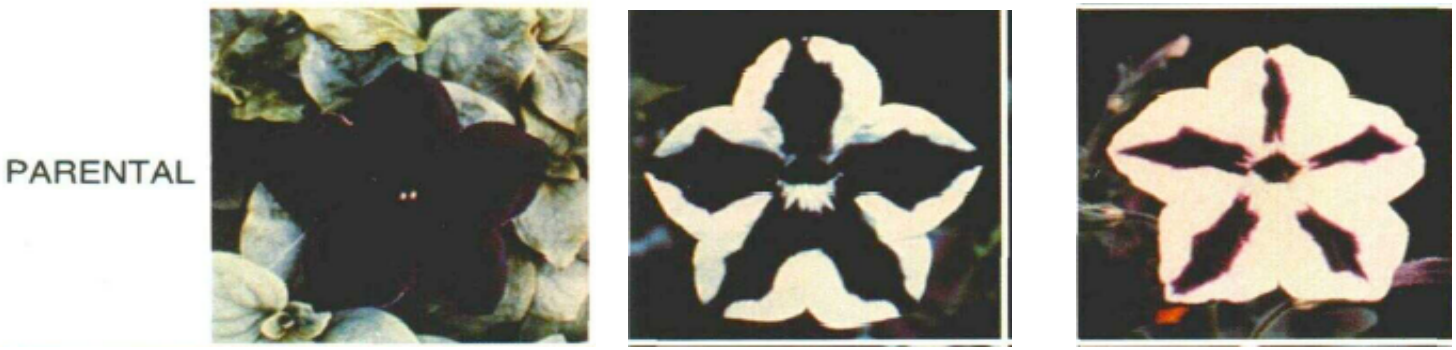
History: Overexpression of a gene leads to its reduction

POST TRANSCRIPTIONAL GENE SILENCING - PTGS

Plants: **Cosuppression** or **Epigenetic gene silencing**:
1990: found that transgenes could suppress the expression of similar endogenous genes, which resulted in loss of function in 10-40% of the transgenic plants.
Exp: Chalcone synthase (anthocyanin synthese pathway) in Petunia



CaMV: Cauliflower Mosaic Virus Promotor
NOS-Ter: Nopalinsynthase Terminator



Both, endogenous and transgene were transcribed but almost no mRNA was detectable, so silencing is on the RNA level ➡ **RNA silencing** (not gene silencing)

RNA forms short duplexes

Short interfering RNA (siRNA)
Micro RNAs (miRNAs)

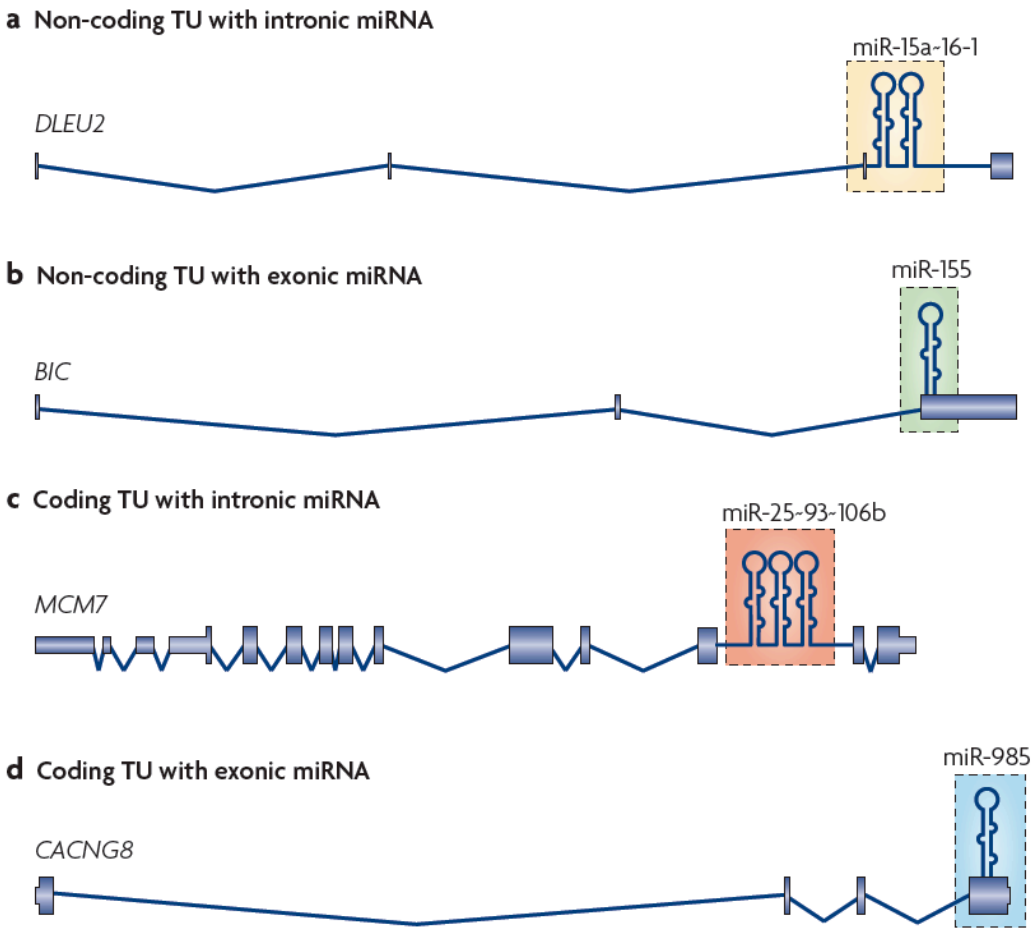
First discovered in plants (post-translational gene silencing = RNA silencing= RNA interference = RNAi)

The transgene expression creates double stranded RNA that are further processed

In mammalian system, the majority of regulatory RNAs are miRNAs
miRNAs are about 22 nt in length
Discovered in 1993 (lin-4) Lee, Feinbaum and Ambros, Cell 843

Why were miRNAs overlooked?

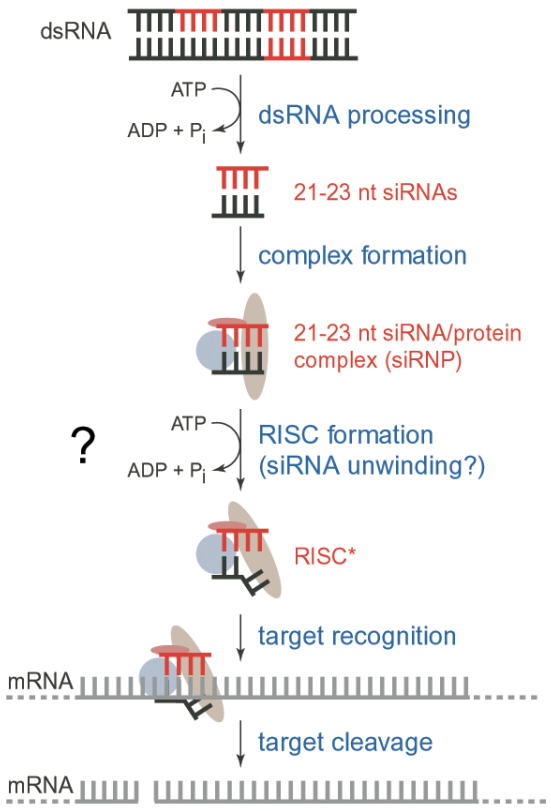
Most miRNAs reside in transcription units



What do the miRNAs have in common?

Kim, Han, Siomi,Nat. Mol Cell Biol, 2009, 126

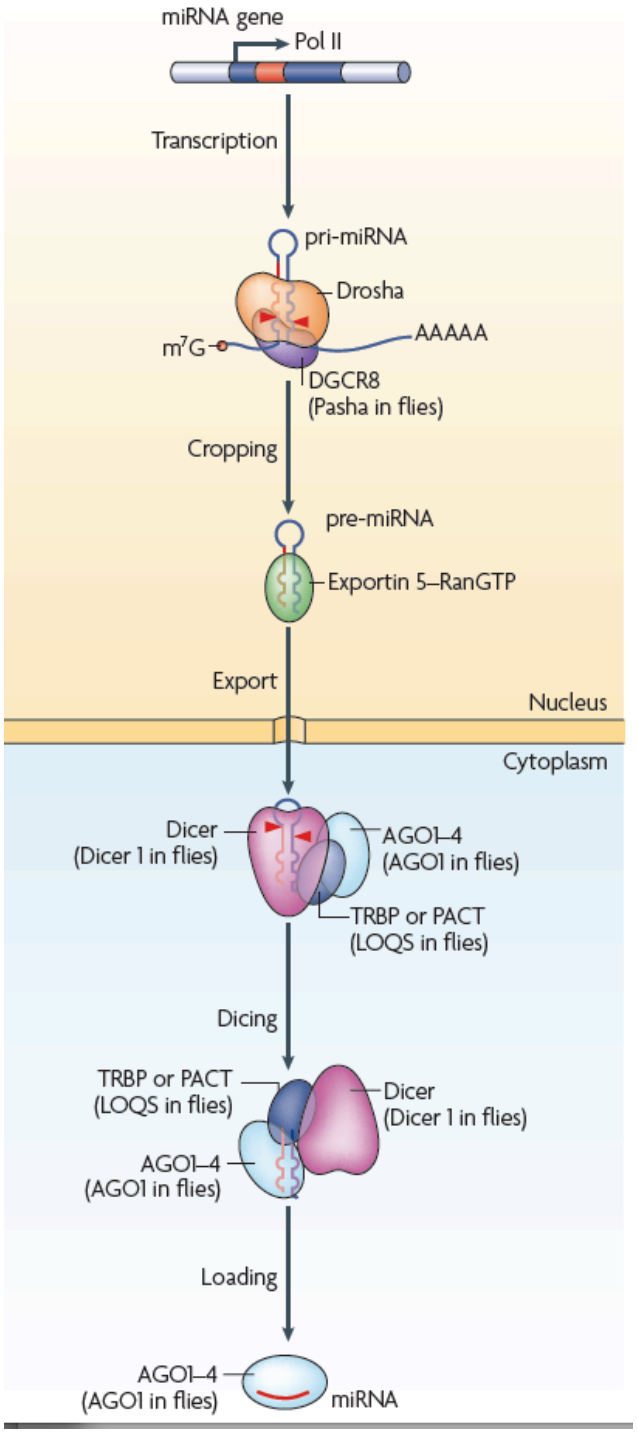
Overview of miRNA and siRNA pathway



RISC: RNA induced silencing complex

Zamore, P.D., Nature structural biology, 8 (2001), 746

miRNAs, siRNAs, piRNAs are bound by proteins that they target to other nucleic acids



Biogenesis of canonical miRNA

RNA with stem-structure (pri-miRNA)

Microprocessor complex: Drosha and DGCR8/pasha

Cleavage by Drosha/DGCR8

Pre-miRNA (ca. 65 nt)

2 nt 3' overhang and stem allows export by exportin 5

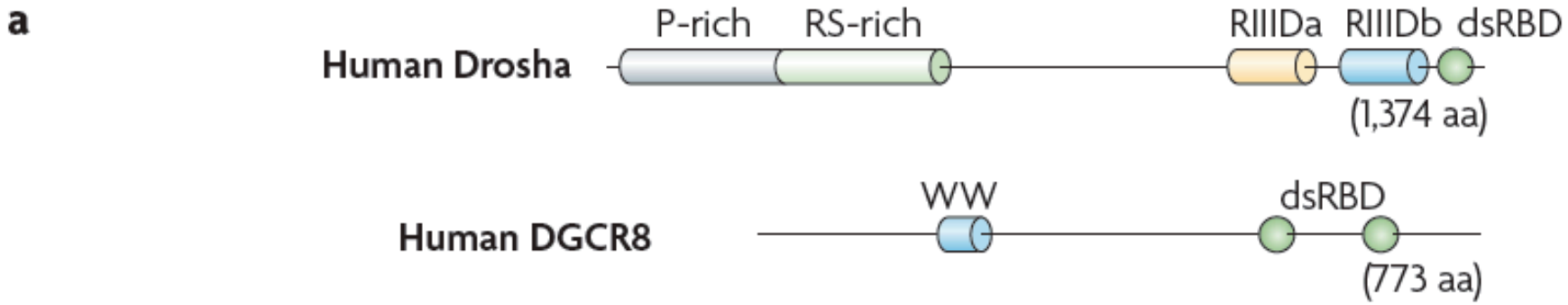
Second processing step by Dicer, cuts 22 nt long fragments

One strand (miRNA)is loaded on argonautes "guide strand", often the one with relatively unstable base pairs at the 5' end
The other strand is degraded (passenger strand)

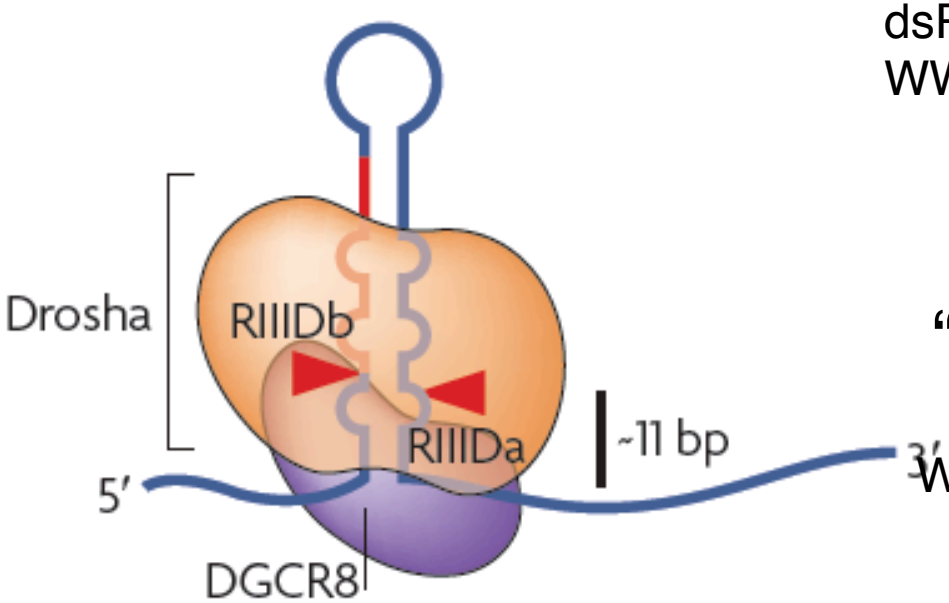
TRBP: TAR RNA binding protein

Kim, Han, Siomi,Nat. Mol Cell Biol, 2009, 126

Domain structure of Drosha



RIIIDa, b: RNAse III domains
dsRBD: double strand RNA binding domain
WW, RS-rich: interaction domains

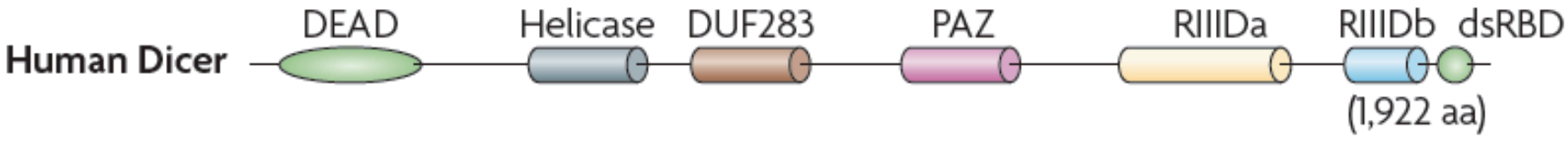


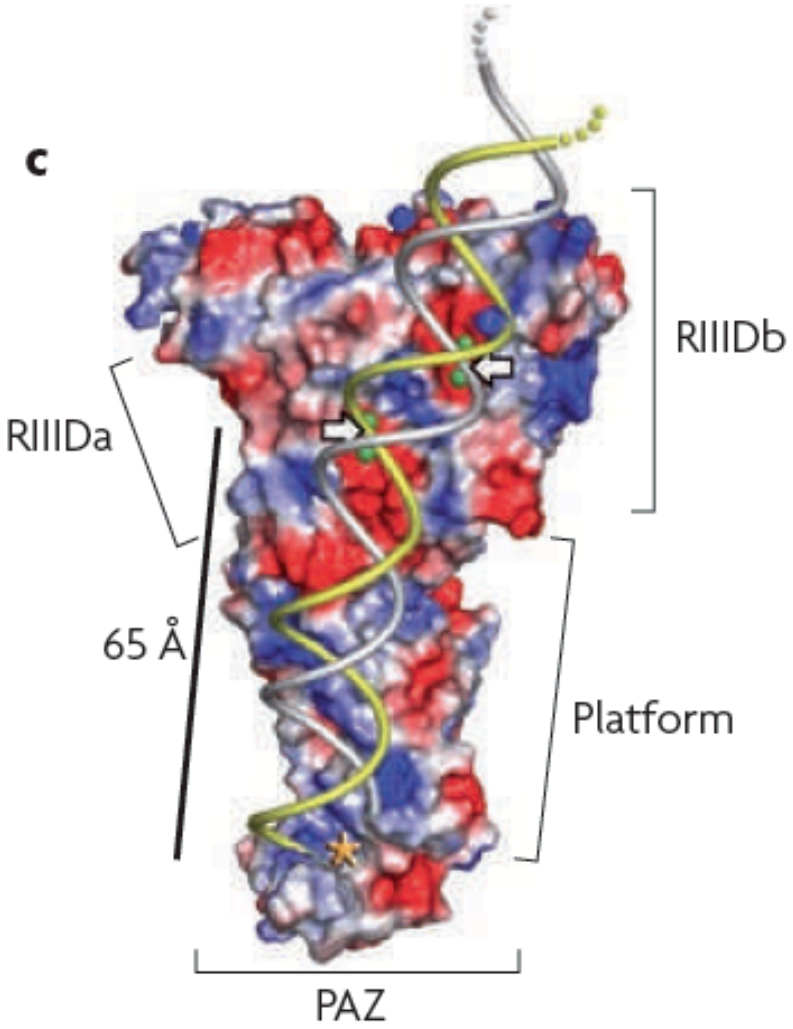
"microprocessor"

WW domain: binds proline rich domains

Kim, Han, Siomi,Nat. Mol Cell Biol, 2009, 126

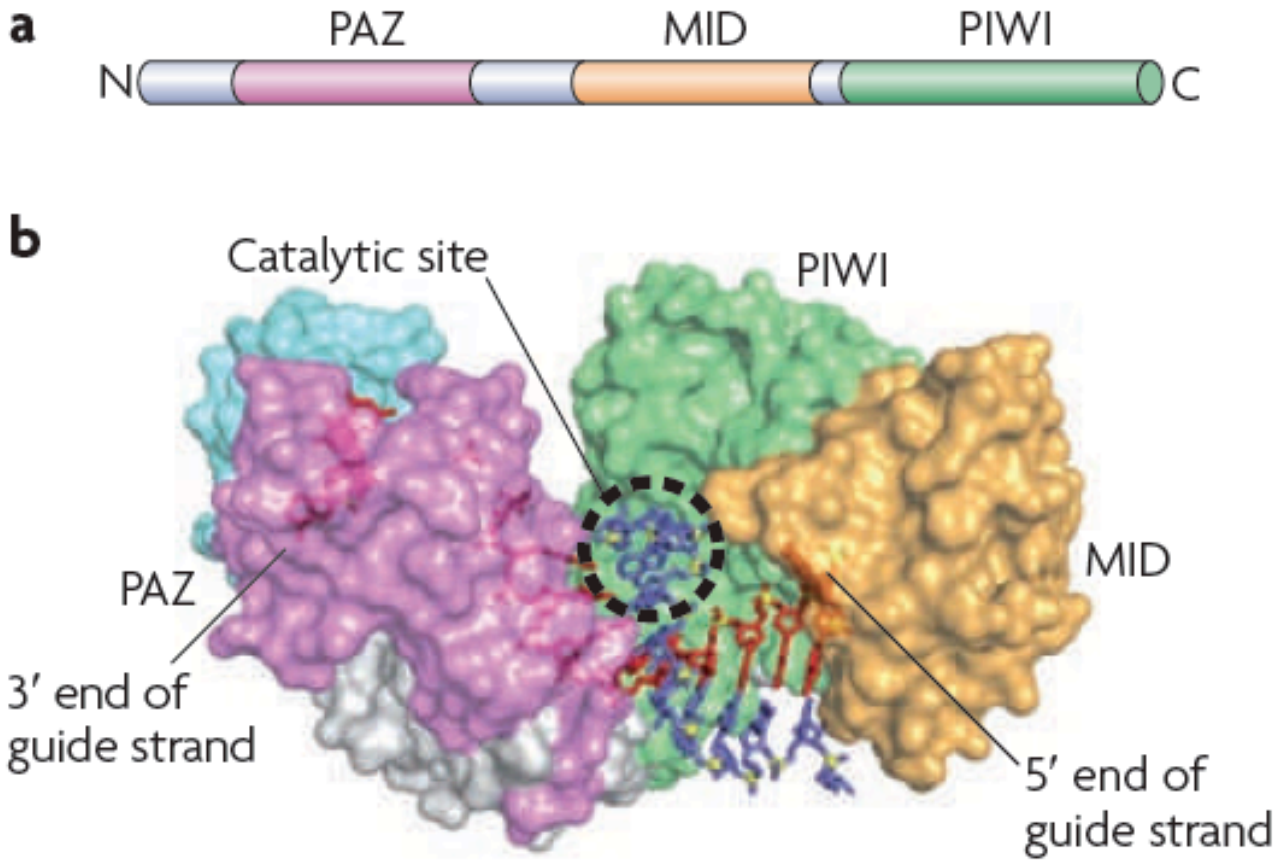
How does Dicer measures RNA length?





Multiple interation domains:
DEAD/helicase: helicase
PAZ: Piwi argonaute zwille, binds 2nt of 3' overhang
Piwi: P-element induced wimpy testis
RIIIDa, b: RNAse III domains
dsRBD: double strand RNA binding domain

Structure of argonautes

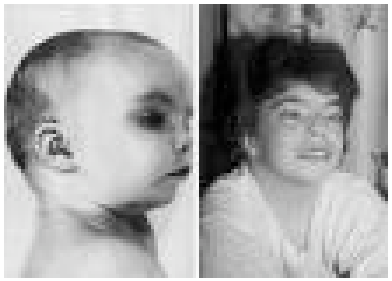


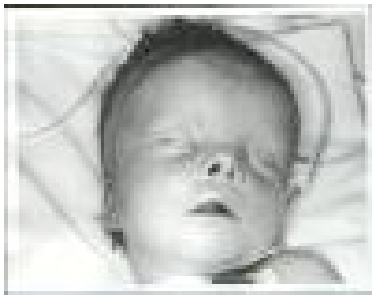
PAZ domain anchors 3’ end
MID domain anchors the 5’ end
PIWI domain: RNAse H similarity, Ago2 has RNAse activity

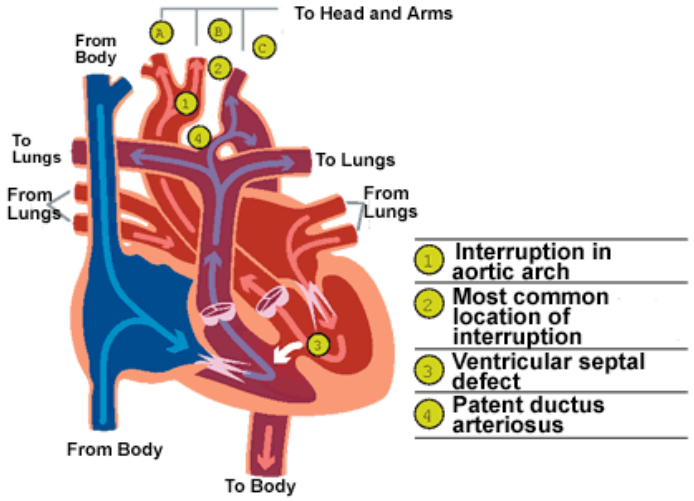
Life without microprocessor

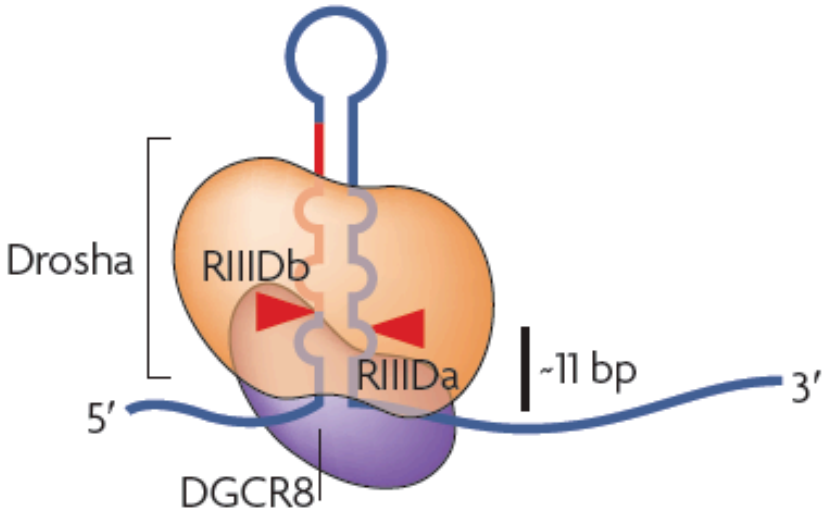
DGCR8= pasha

22q11.2 deletion syndrome, also known as **DiGeorge syndrome (DGS)**, **DiGeorge anomaly, velo-cardio-facial syndrome, Shprintzen syndrome, conotruncal anomaly face syndrome, Strong syndrome, congenital thymic aplasia**, and **thymic hypoplasia**



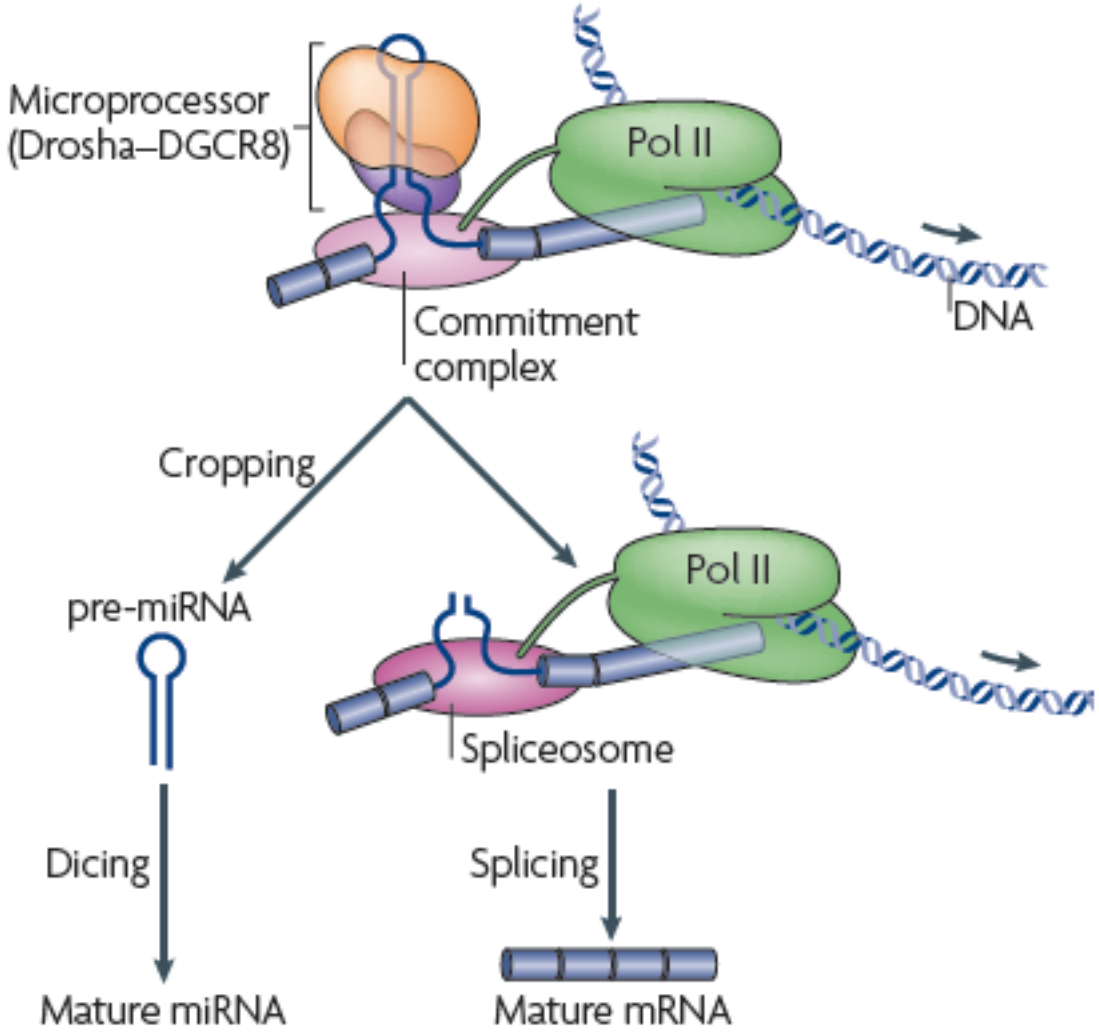






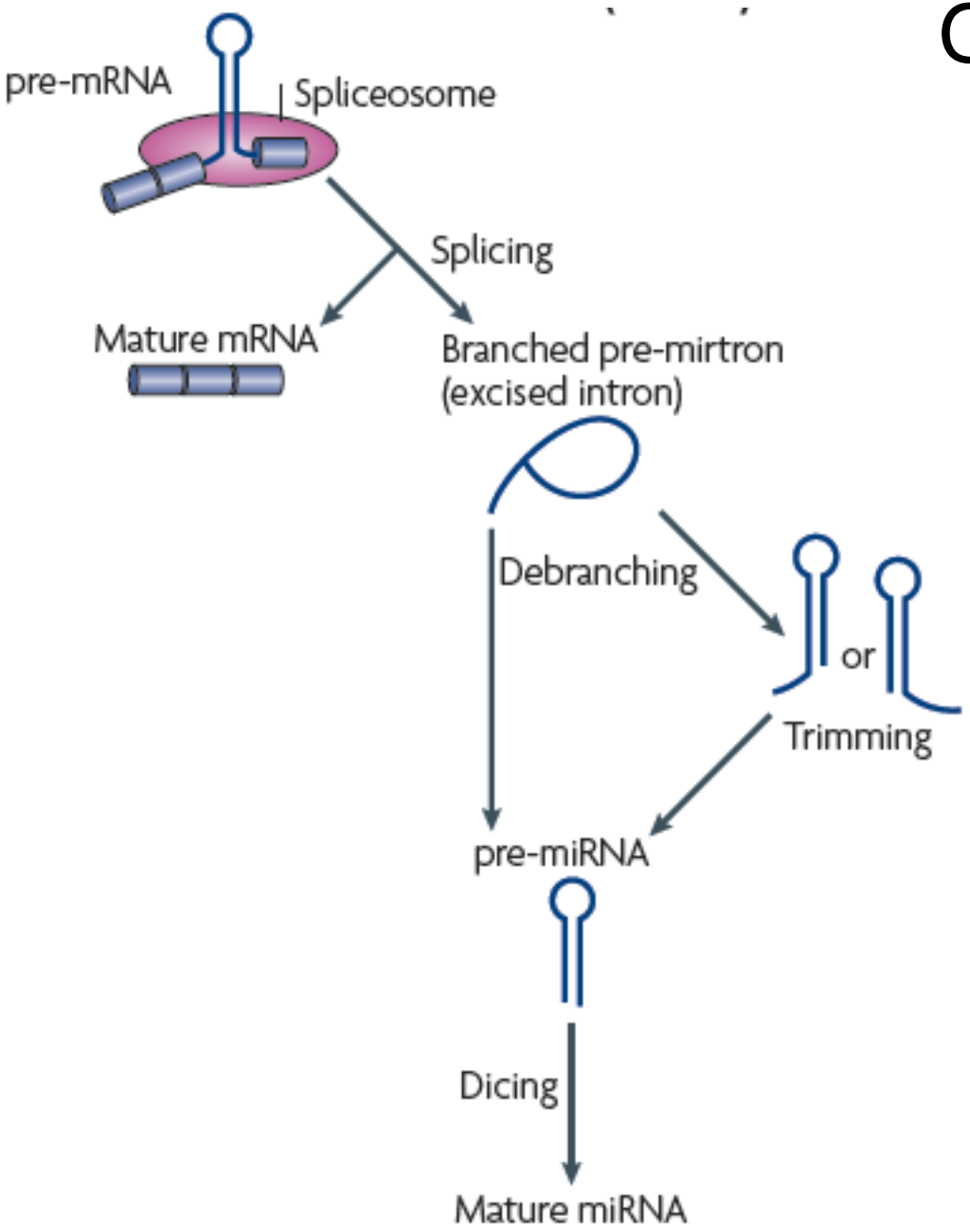
Why do these patients live?

Other ways to make miRNAs



Microprocessor complex comcommitant with splicing and transcription, splicing of a 'cut' intron can occur,
miRNA in a 'host gene'

Kim, Han, Siomi,Nat. Mol Cell Biol, 2009, 126

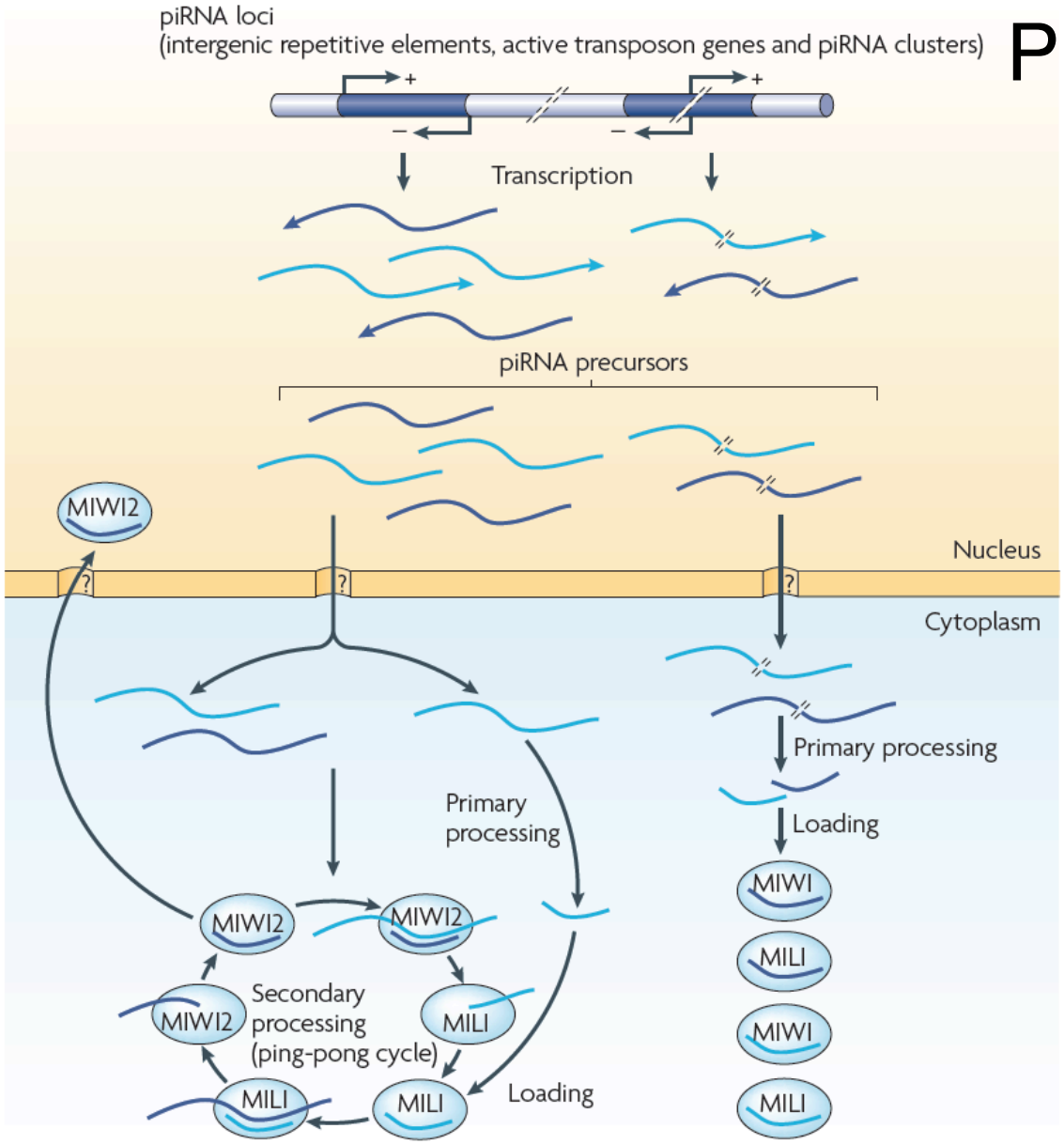


Other ways to make miRNAs: mirtrons

Drosha and DGCR8 independent

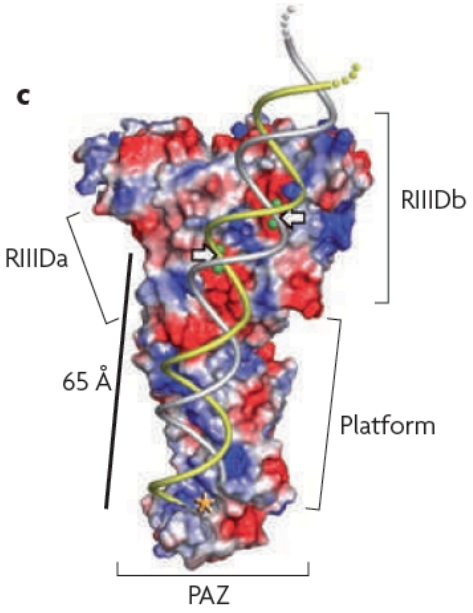
What would do the trimming?

PIWI RNAs (piRNAs)

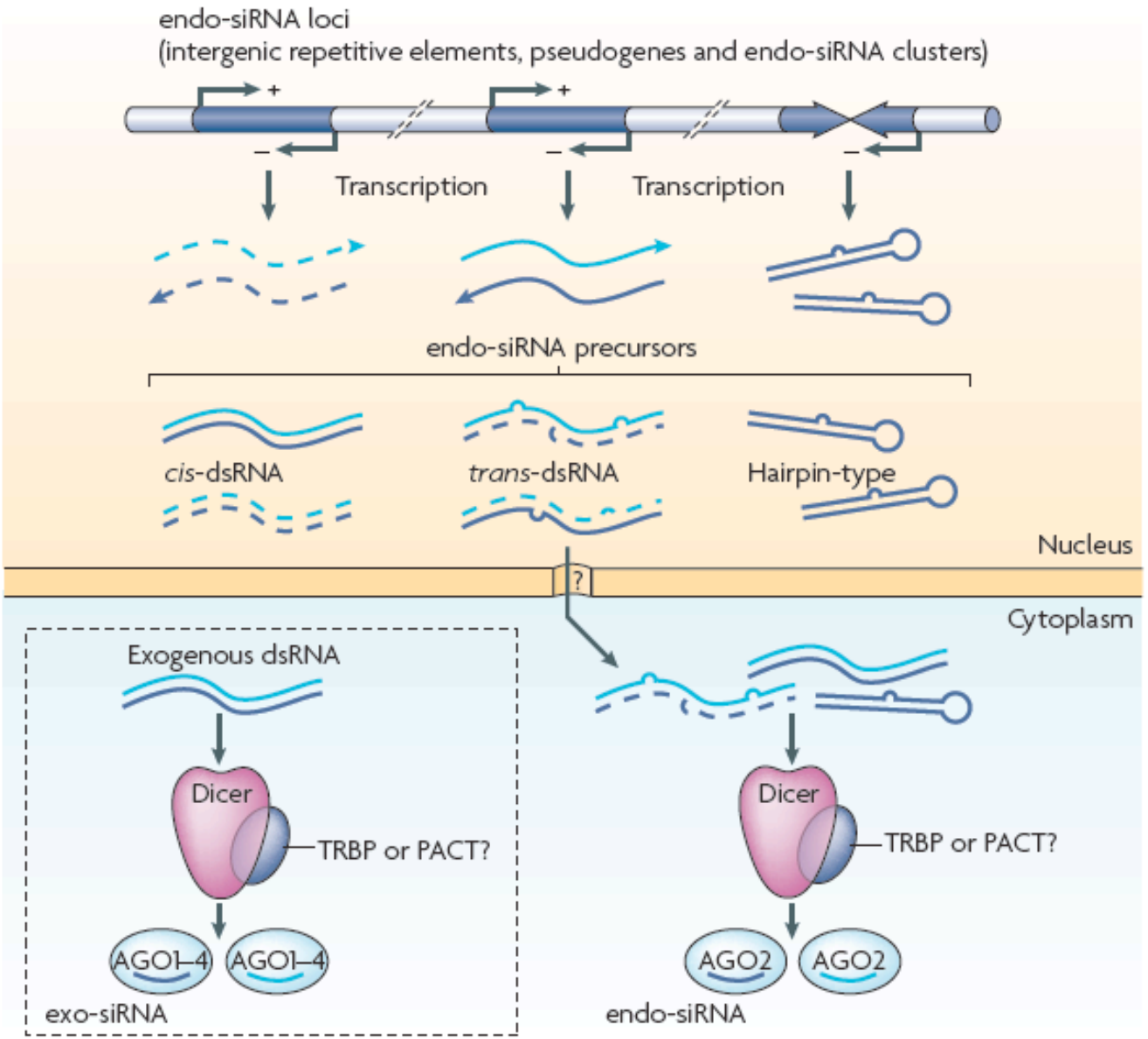


Germ-cell specific RNAs
24-29 nt in length
Earlier in drosophila called repeat associated small interfering RNA (rasiRNA)
Processed by Piwi proteins (mouse MIWI)
MIWI and MILI process RNAs in a 'ping pong mechanism'

PAZ domain common with argonautes and dicer

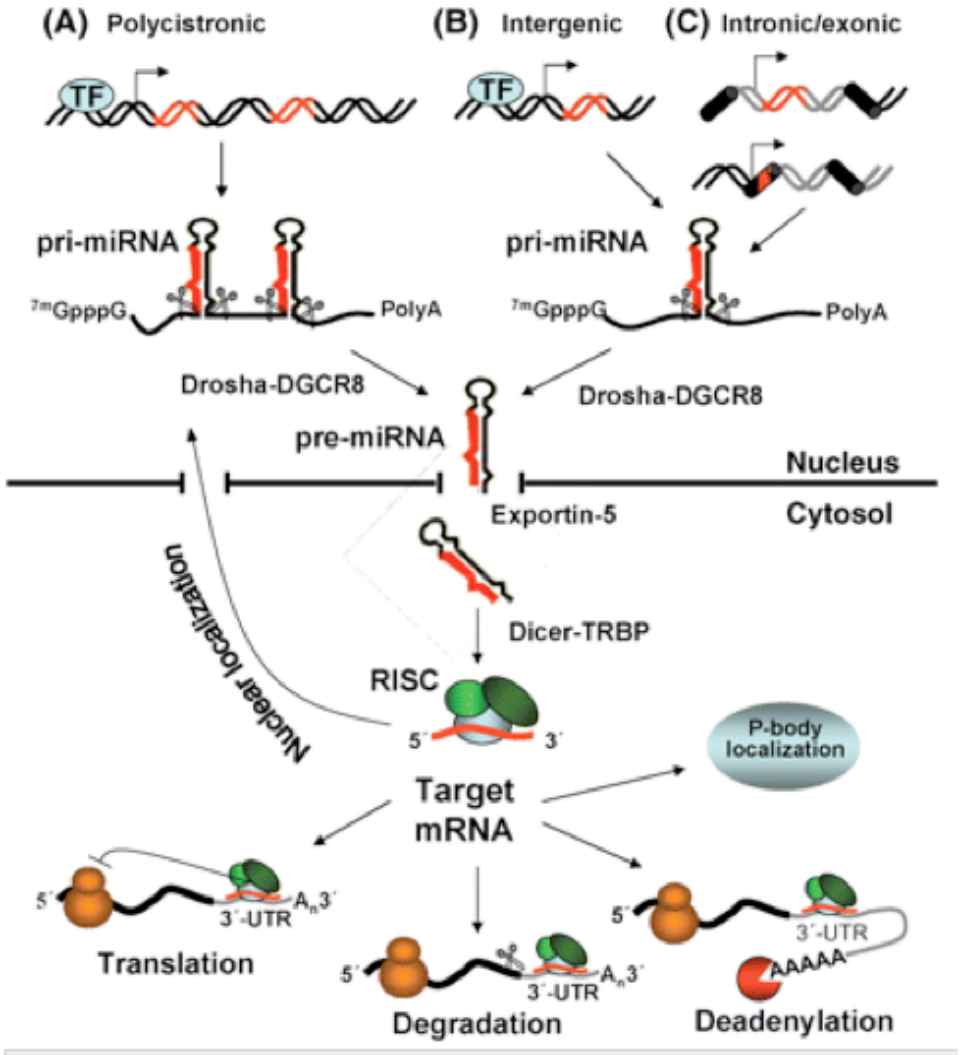


Endo-siRNAs (similar to exon siRNAs)



Kim, Han, Siomi,Nat. Mol Cell Biol, 2009, 126

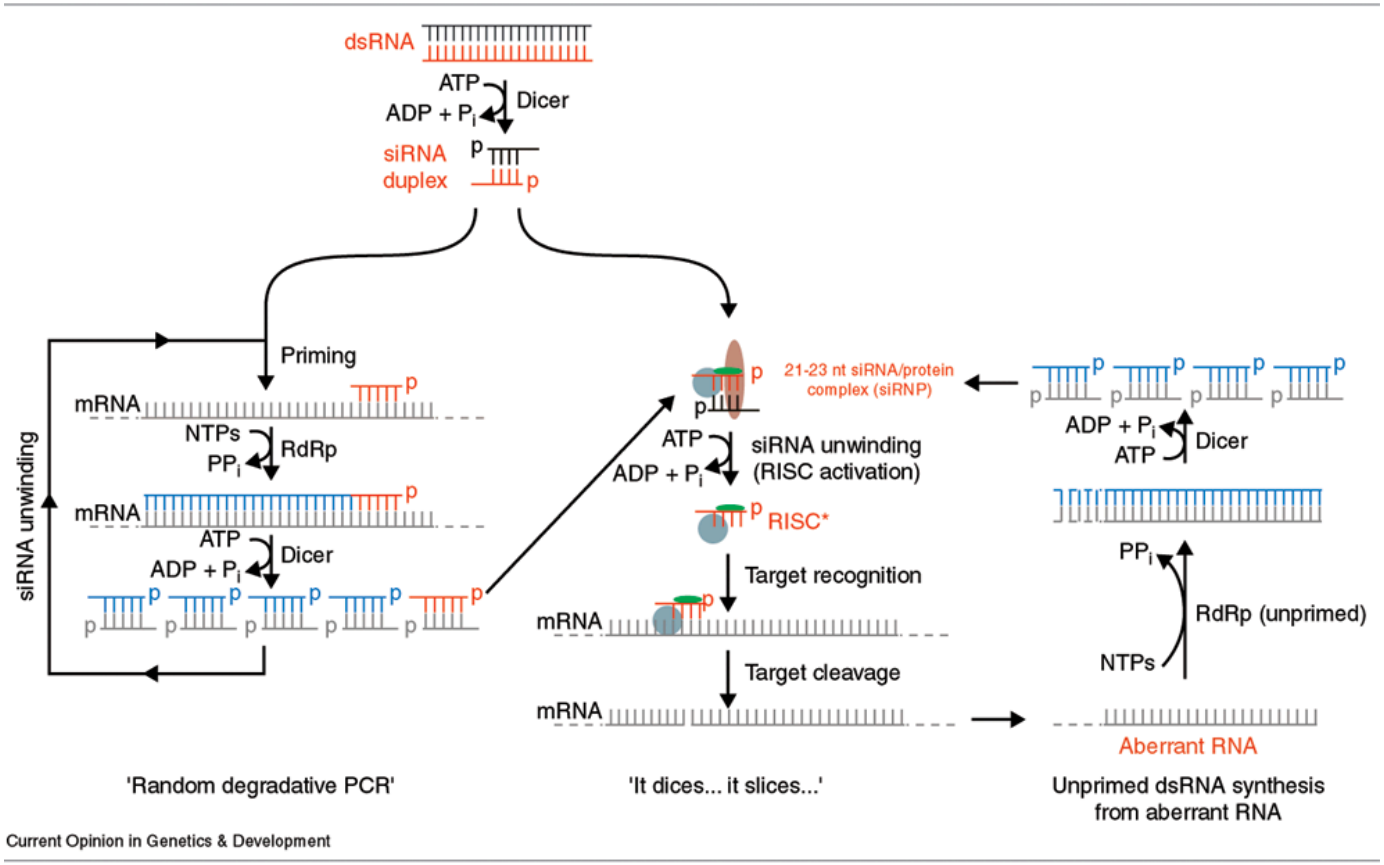
What do miRNAs?



P bodies: processing bodies,
Cytoplasmatic, contain mRNAs, miRNAs and processing enzymes,
Both storage and degradation sites

Drag a protein complex to a target nucleic acid
Translational repression, activation, RNA cleavage, Chromatin organization..

What do siRNAs?



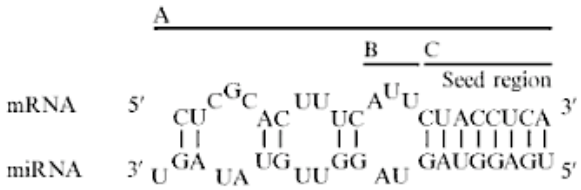
ın integrated model for RNAi and PTGS. In this model, the sequential ction of Dicer (to generate siRNAs) and 'Slicer' (to cleave the target RNA) are considered the primary route for target destruction. Amplification f the siRNAs is postulated to occur by either (or both) 'random legradative PCR' or production of siRNAs from aberrant RNA – that is, the copying of the target RNA or a cleavage product of the target RNA by an RNA-dependent RNA polymerase to generate a dsRNA substrate for Dicer, thereby creating new siRNAs. In the random degradative PCR scheme, the polymerase is envisioned to be primed by an siRNA guide strand. Conversion of aberrant RNA to dsRNA is drawn here unprimed.

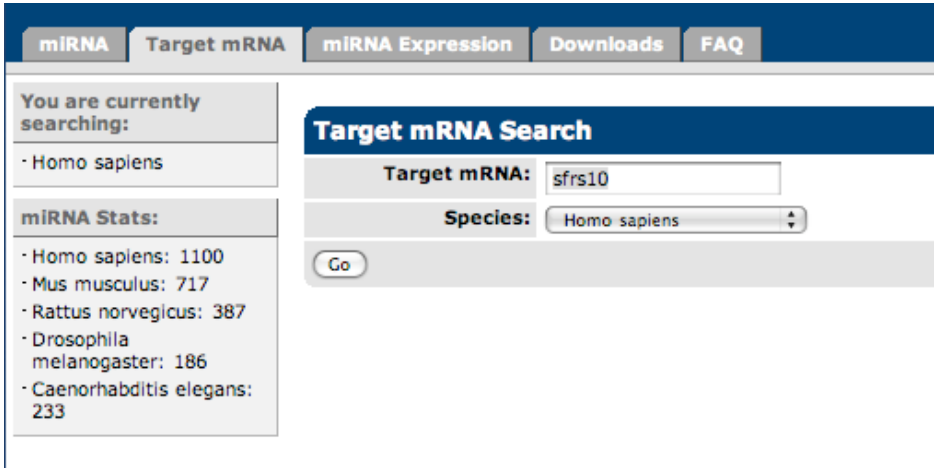
Hutvagner, G. and Zamore, P.D., Current Opinion in Genetics & Development, 12 (2002), 225

Where do you find miRNAs and their targets?

www.miRNA.org

mirbase





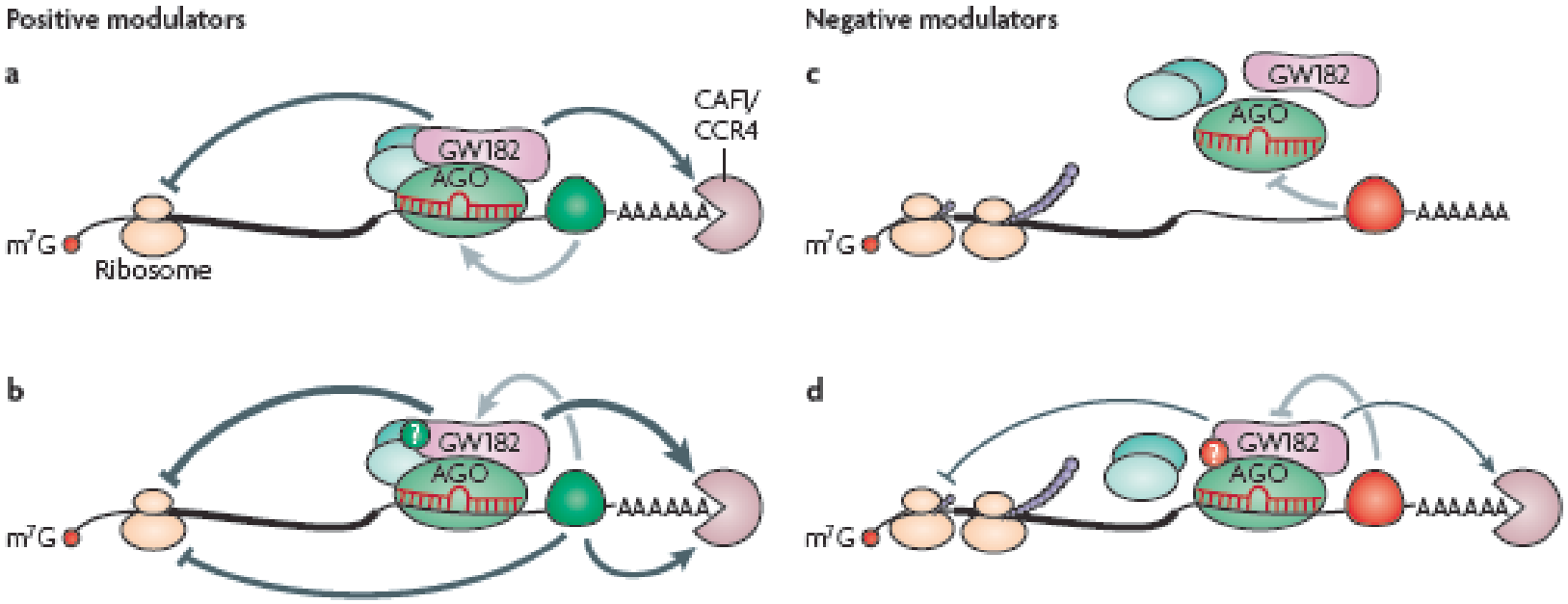
The predictions generally rely on phylogenetic conservation, which is not always correct

Seed sequence at the 5'end of the miRNA

Predictions need to be tested experimentally

miRNA binding is regulated by other factors

miRNA functions are regulated

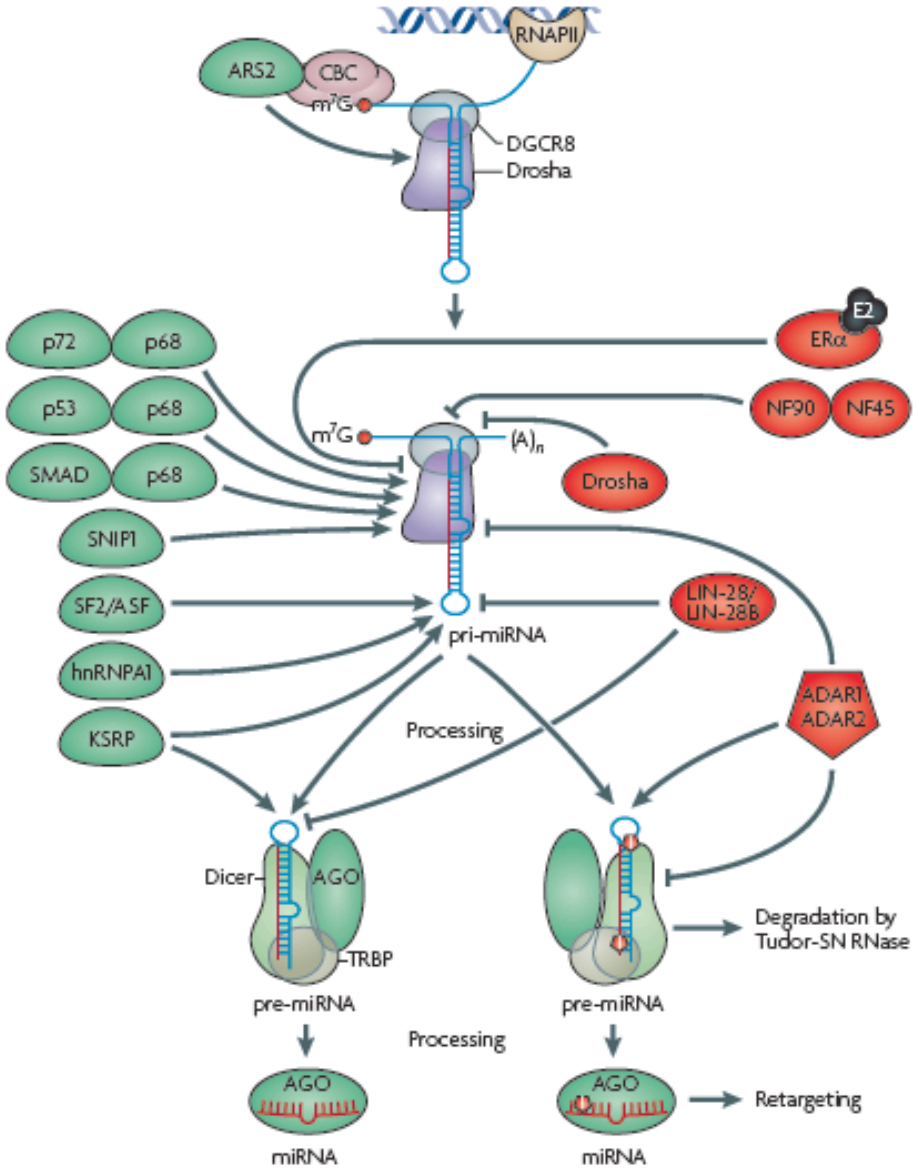


Stabilization of binding
FMRP, phosphorylation?

Antagonizing of binding
HuR, post-translational modification

Krol, Loedige, Filipowicz, Nat. Rev. Genet, 2010. 597

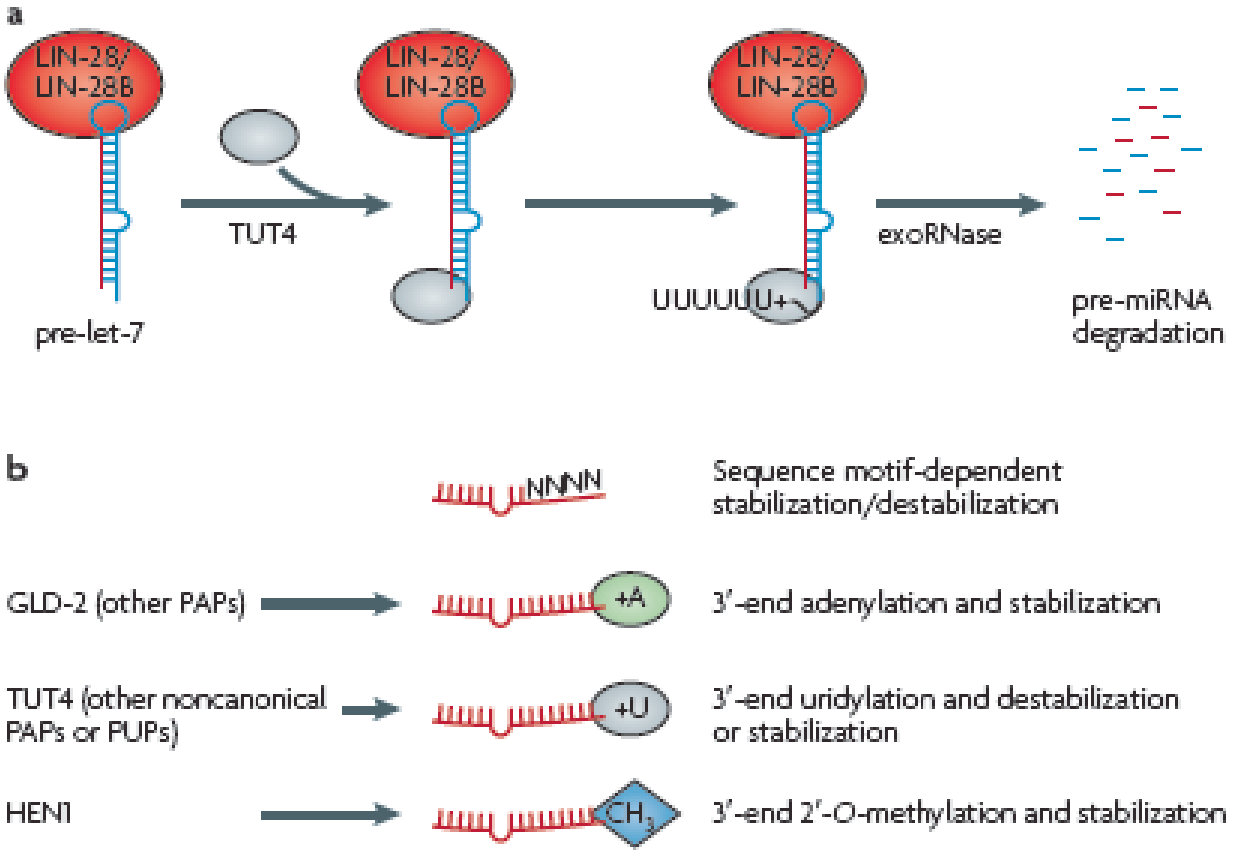
miRNA processing is regulated



Regulate RNA:RNA binding
RNA:protein binding
Helicases
RNA binding proteins

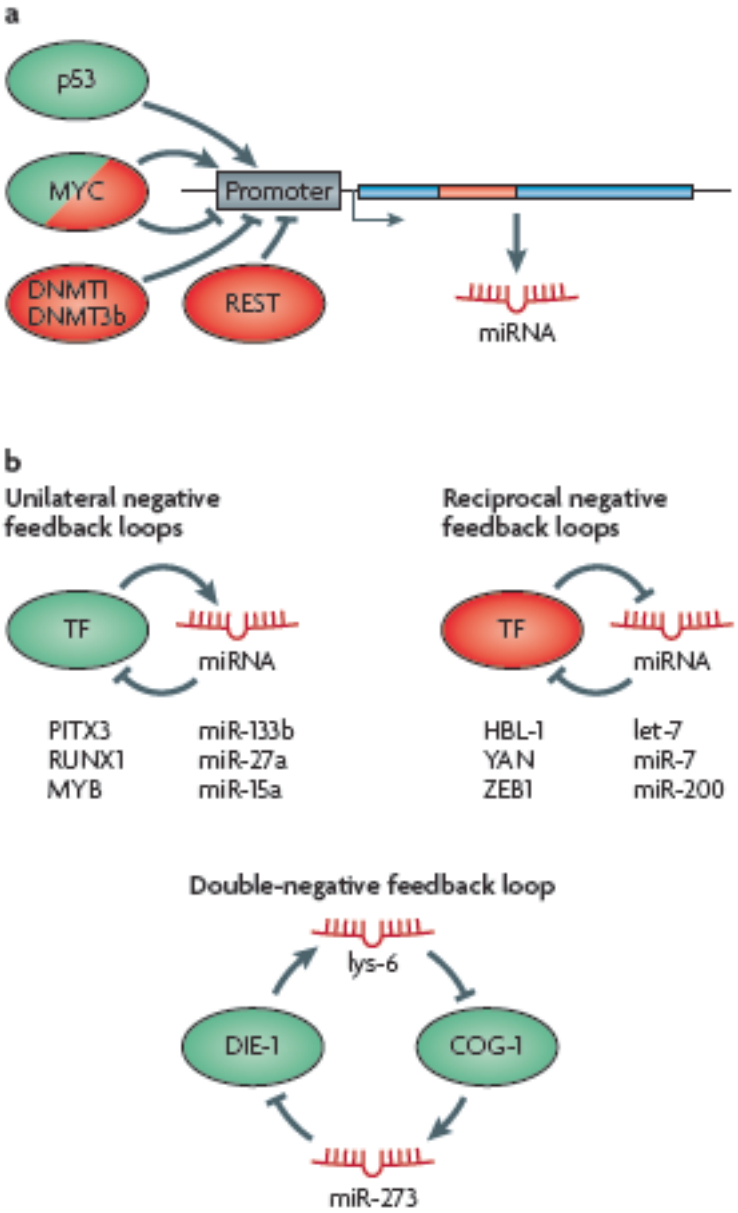
Krol, Loedige, Filipowicz, Nat. Rev. Genet, 2010. 597

miRNAs are modified



Krol, Loedige, Filipowicz, Nat. Rev. Genet, 2010. 597

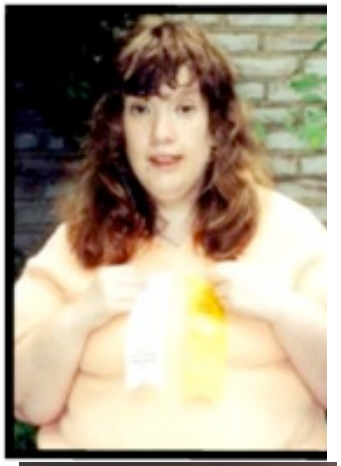
miRNAs form regulatory networks



There are more miRNAs than transcription factors,
Often feedback regulation

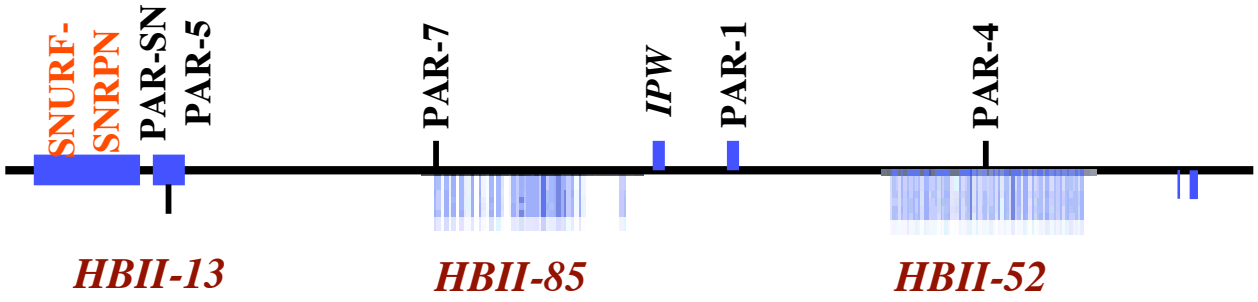
Krol, Loedige, Filipowicz, Nat. Rev. Genet, 2010. 597

Regulation of alternative splicing by small RNAs: Prader-Willi Syndrom

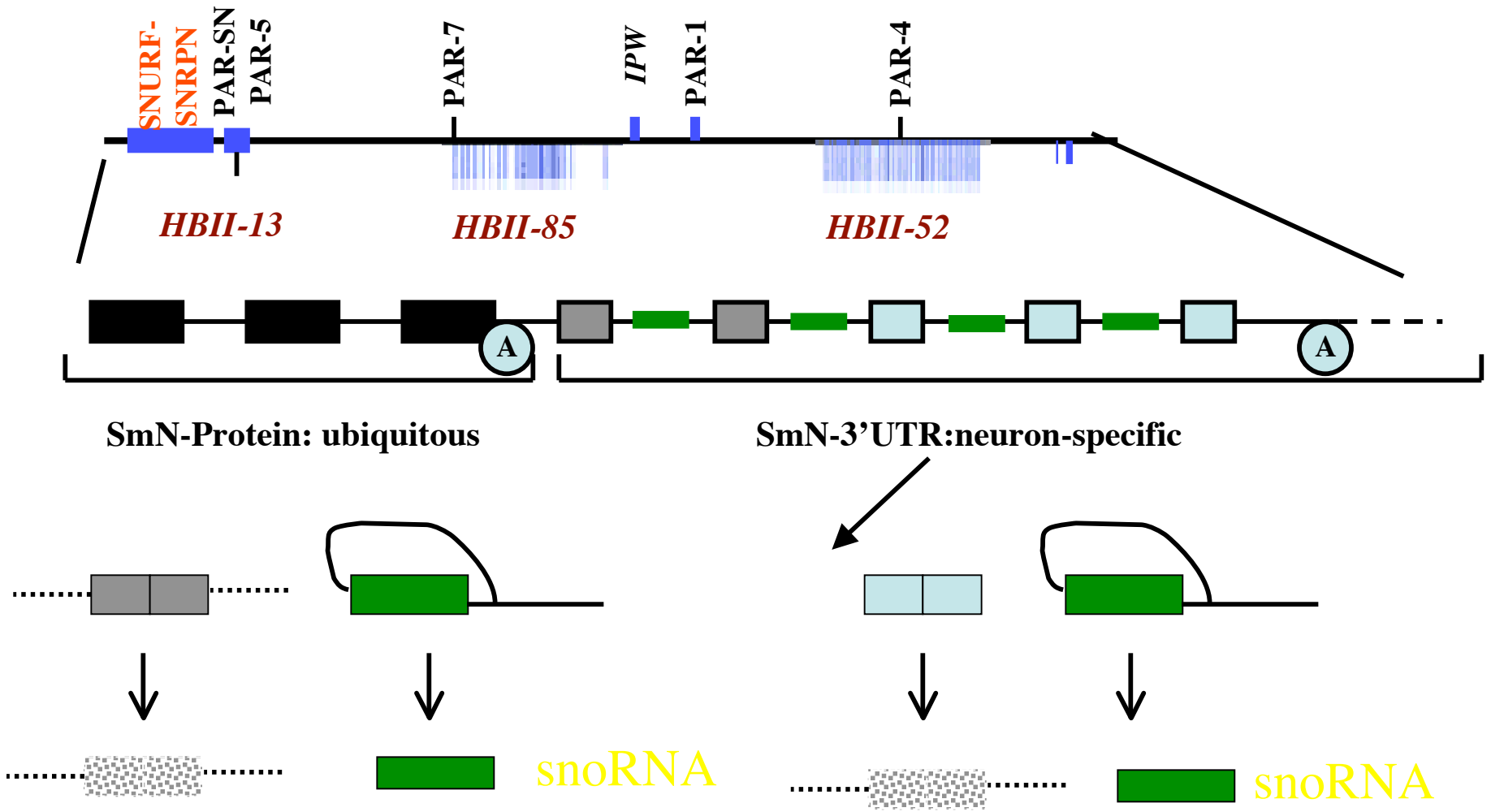


- Inherited disease, 1:15.000
- loss of the paternal allel from a maternally imprinted region on chromosome15
- clinic: pleiotrophic phenotype, obese, mental retardation, temper tantrums, hormone changes, genetic diversity (new mutations)
- Treatment: growth hormone substitution, behavioral therapy, previously SSRIs (selective serotonin reuptake inhibitors)
- Only a few proteins in the deleted gene region (SNRPN, Necdin, MAGEL2), their loss is excluded for most of the disease phenotype
- Contains several clusters of snoRNAs between non-coding exons

La Monstrua Desnuda (Eugenia Martinez Vallejo) 1680, JC de Mirande



HBII-52 is a brain-specific snoRNA missing in children with PWS

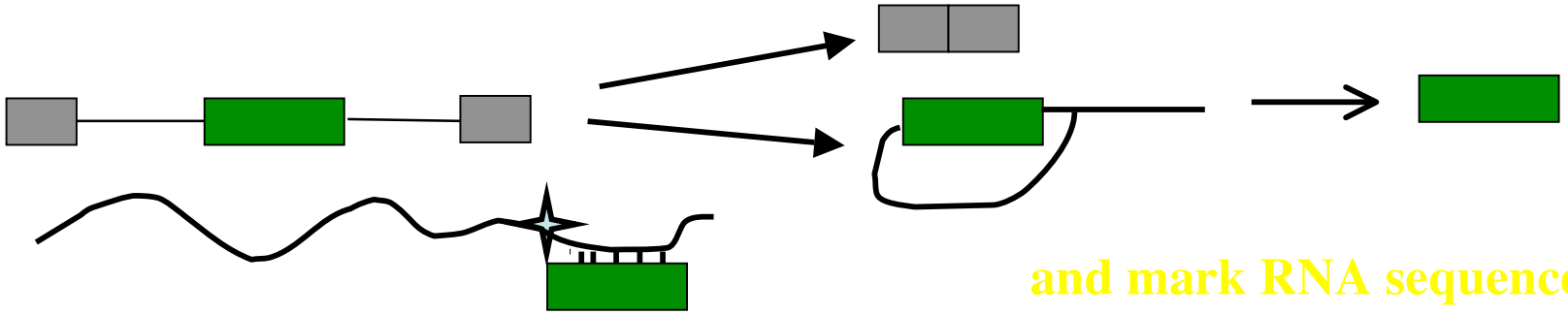


47 copies of nearly identical HBII-52 snoRNAs

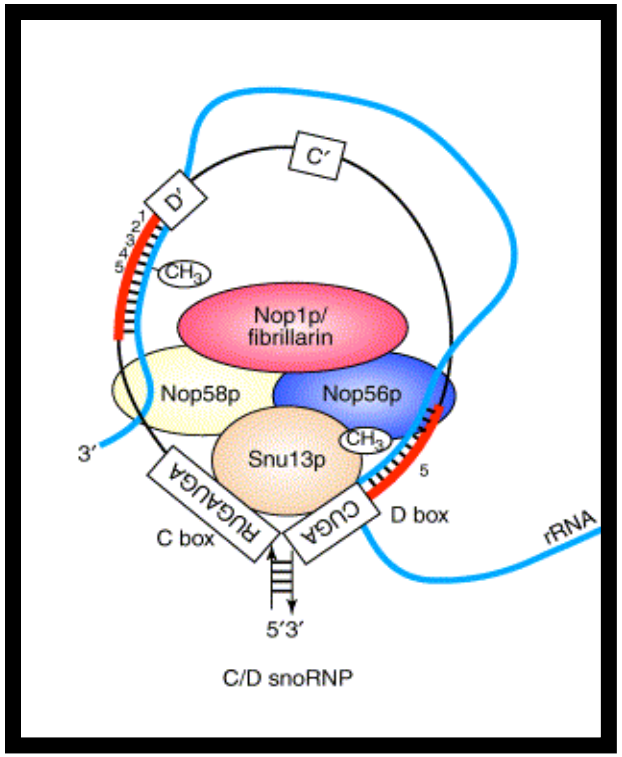
Cavaille, J….., and Hüttenhofer, PNAS, 97:14311

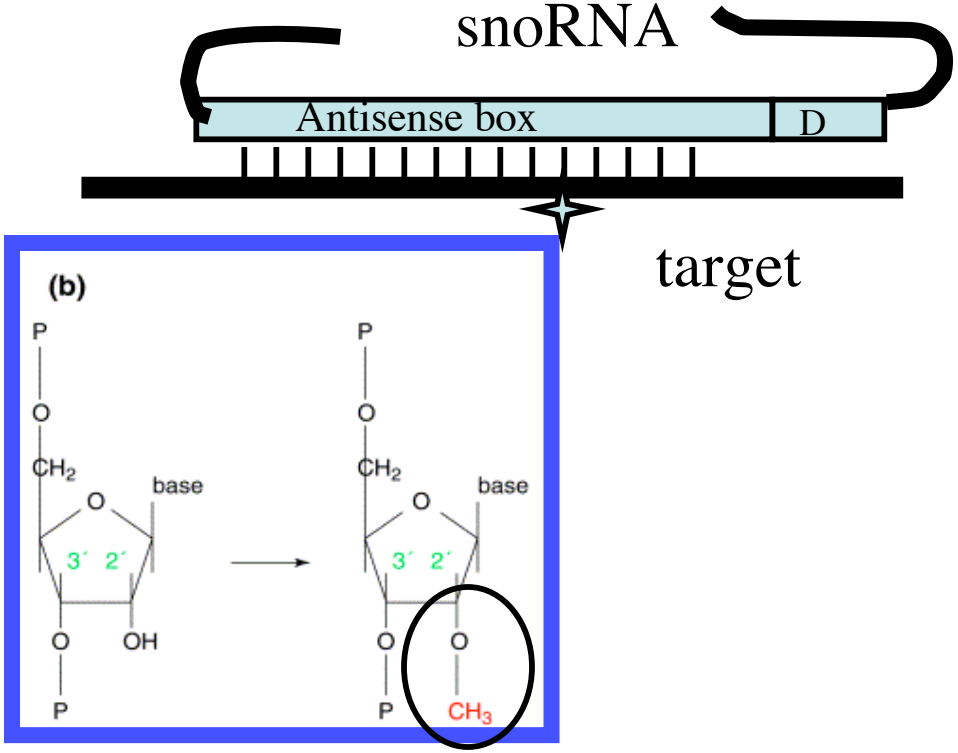
HBII-52 is a C/D-box snoRNA

•snoRNAs are released during splicing,



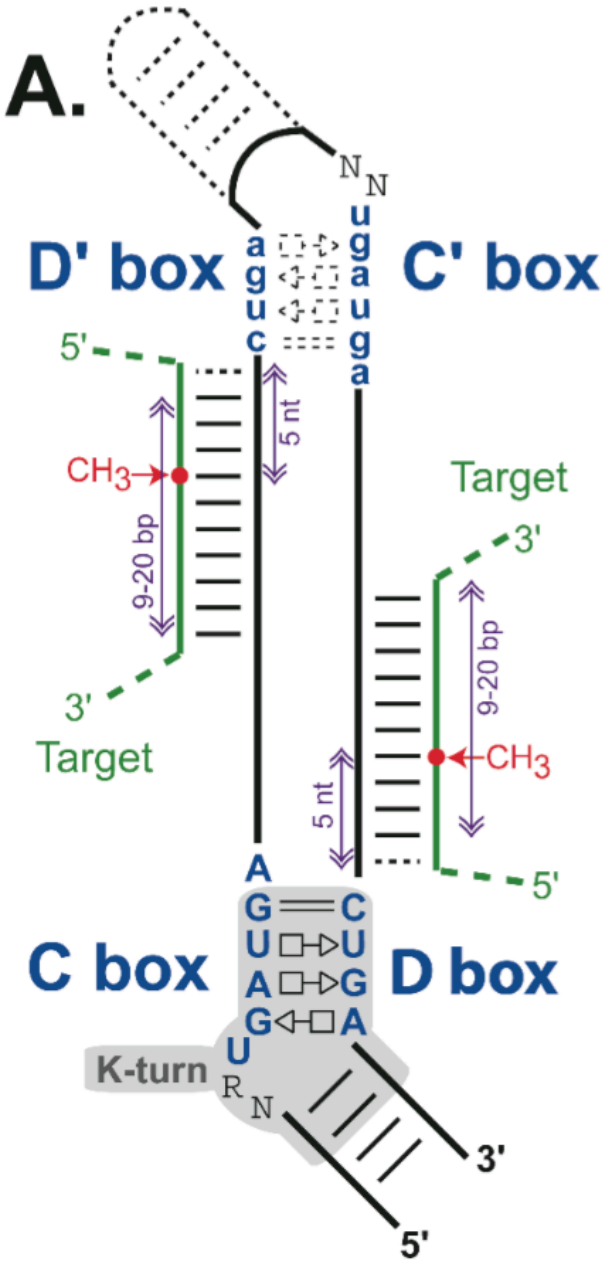
and mark RNA sequences





From: Brown JW et. al., 2003, *Trends in Plant Sciences*

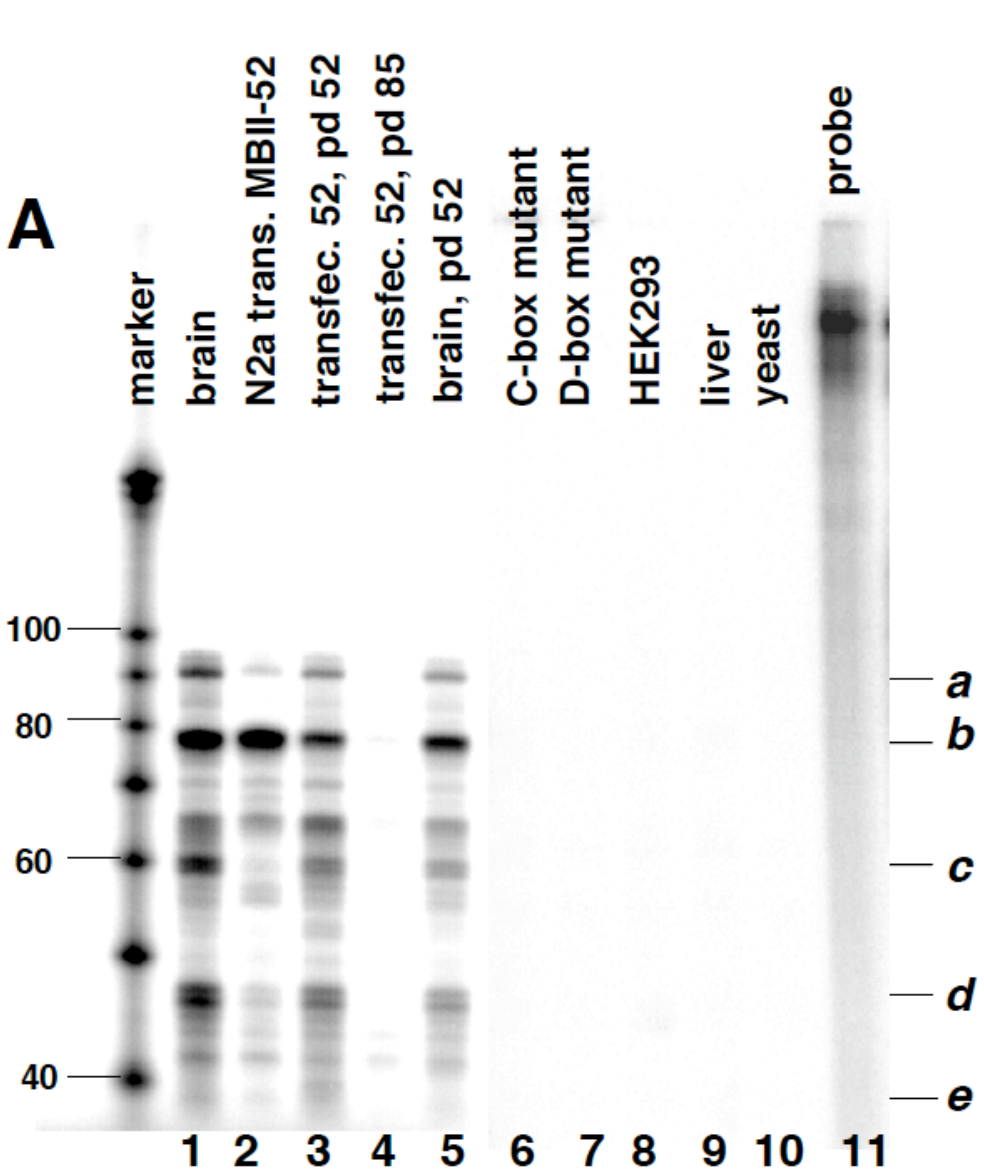
C/D box snoRNA

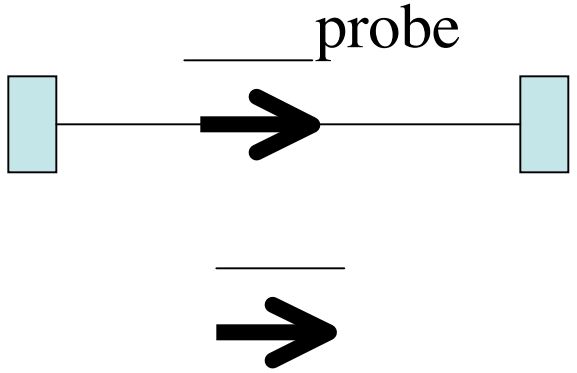


C/D box snoRNAs have folded structures, similar to pri-miRNAs

Role of orphan snoRNAs?

RNAse protection of one snoRNA copy

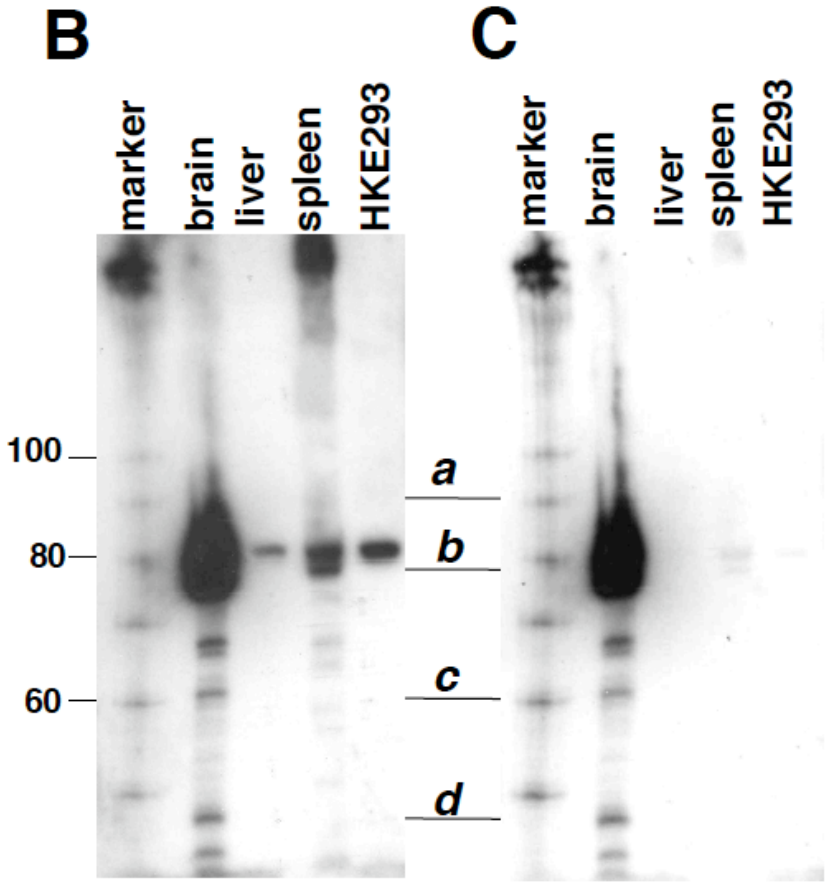




What do you expect?

Kishore et al, HMG, 2010

Northern Blot

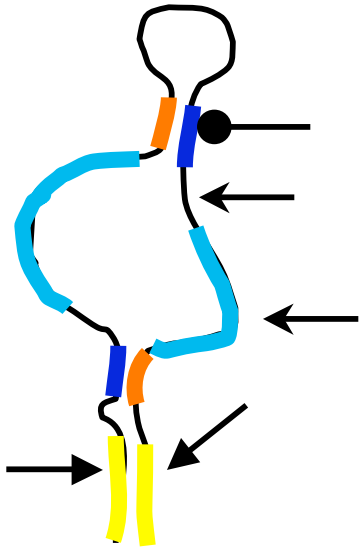


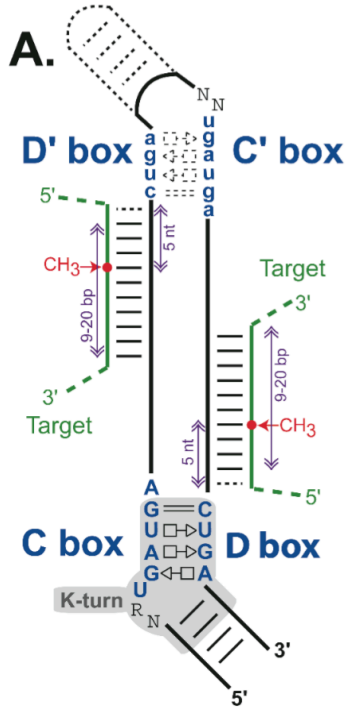
Sequences and summary

D

```
                                Stem    C box          D'box      C' box          Antisense box   D-box Stem
form A (87 nt)          ACTGGGUCAAUGAUGACAACCCAAUGUCAUGAAGAAAGGUGAUGACAUAAAAUUCAUGCUCAAUAGGAUUACGCUGAGGCCCAACCA
form B (73 nt)                AAUGAUGACAACCCAAUGUCAUGAAGAAAGGUGAUGACAUAAAAUUCAUGCUCAAUAGGACUACGCUGAGGCC
form C (60 nt)              GGUCAAUGAUGACAACCCAAUGUCAUGAAGAAAGGUGAUGACAUAAAAUUCAUGCUCAAU
form D (44 nt)            UGGGUCAAUGAUGACAACCCAAUGUCAUGAAGAAAGGUGAUGAC
form E (38 nt)              GGUCAAUGAUGACAACCCAAUGUCAUGAAGAAAGGUGA
form E (37 nt)                   AUGAUGACAACCCAAUGUCAUGAAGAAAGGUGAUGAC
```

SnoRNAs give rise to a new form of 'miRNA like' nuclear regulatory RNAs





Summary

miRNAs are key regulatory of gene expression and function

miRNAs work by targeting other nucleic acids,
And bringing protein complexes to their targets

Networks of miRNA mRNA regulation, in **theory** easier to predict than protein: nucleic acids interaction

Find the miRNA binding sites for your pet gene
If your pet gene does not have one, use SFRS10

www.miRNA.org