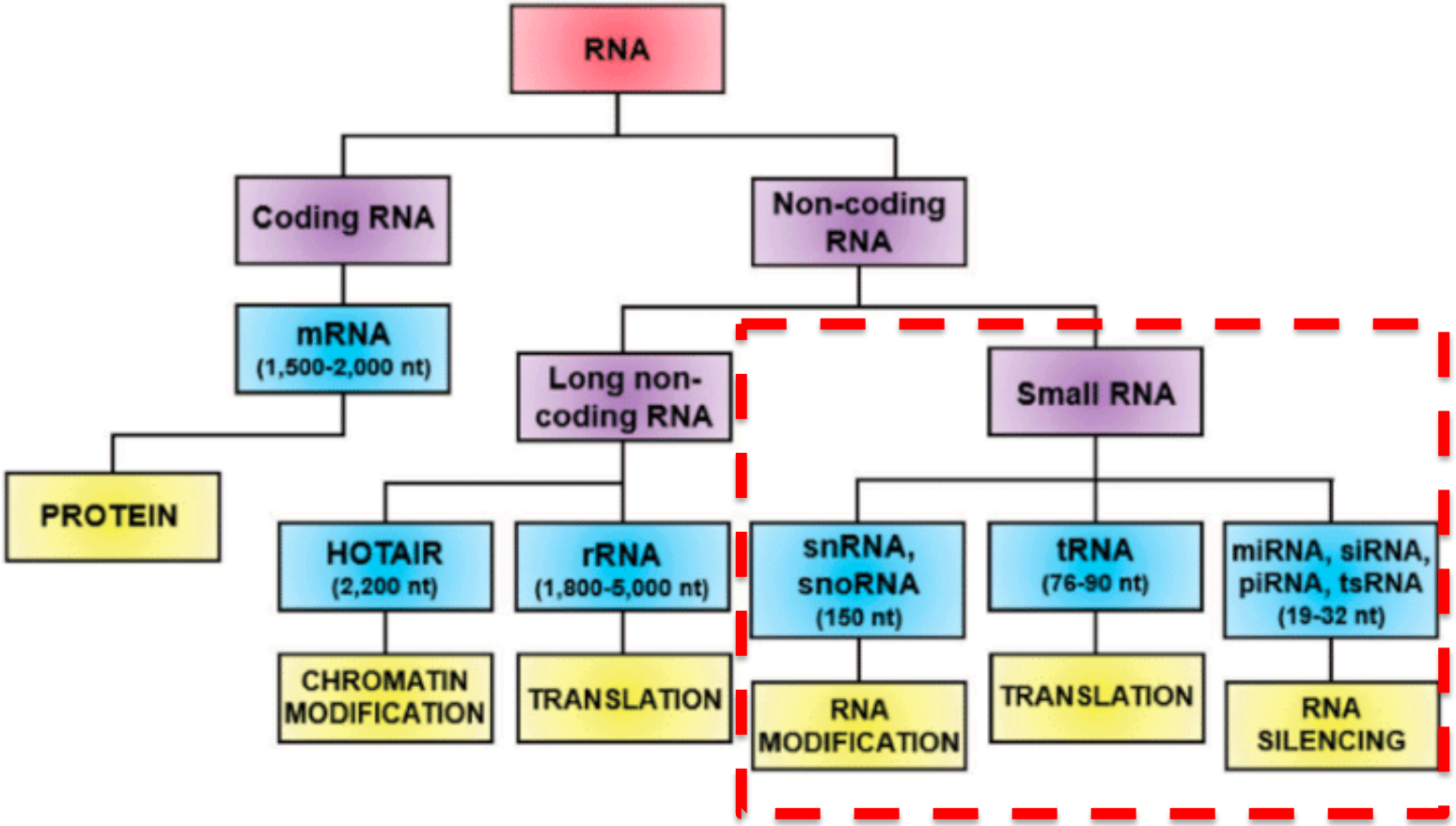
miRNAs AND

COMPETING ENDOGENOUS RNAs (ceRNAs)

Paper: lncRNAs as ceRNAs using PTENP1 as example

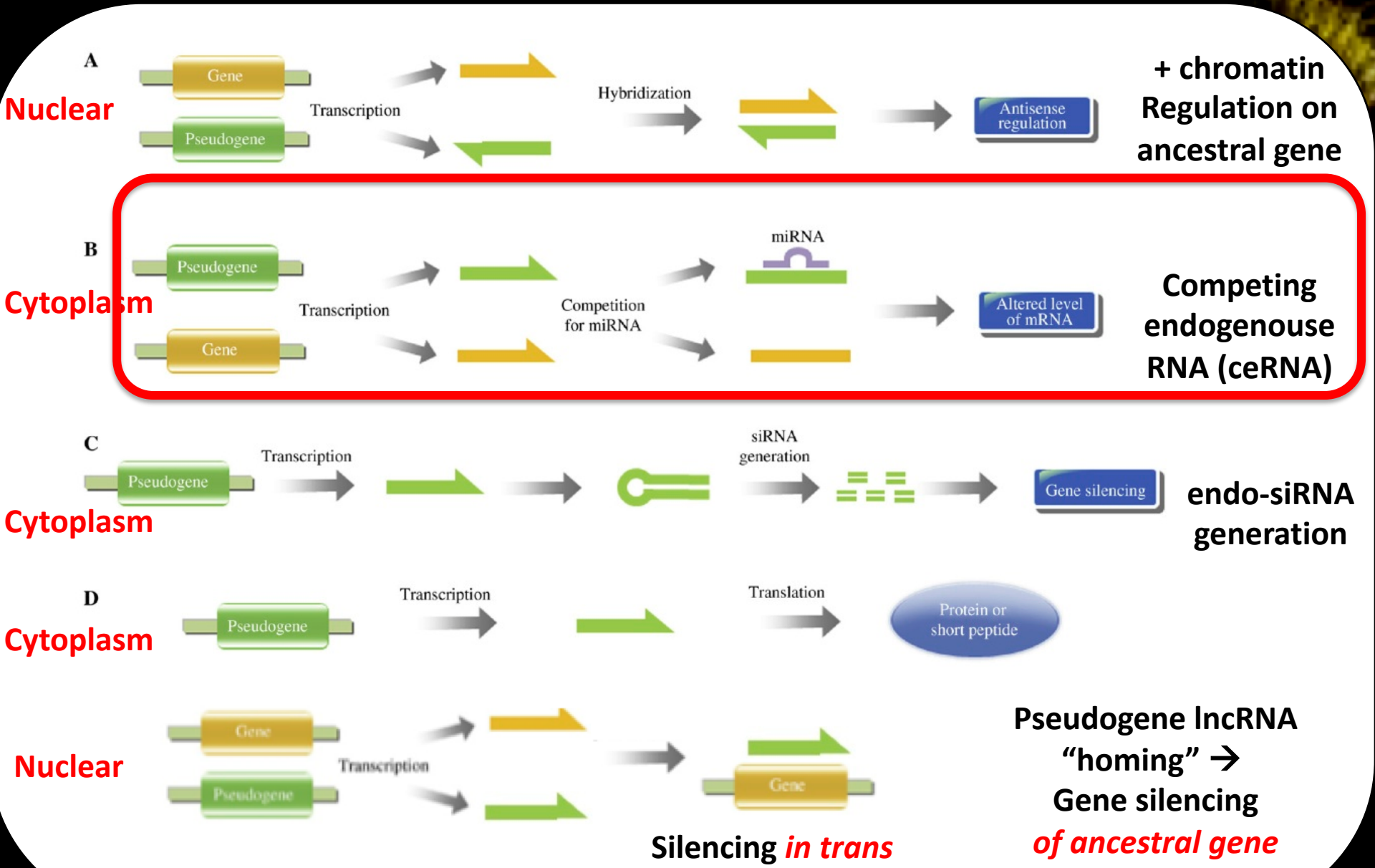
Small ncRNA and gene/chromatin regulation



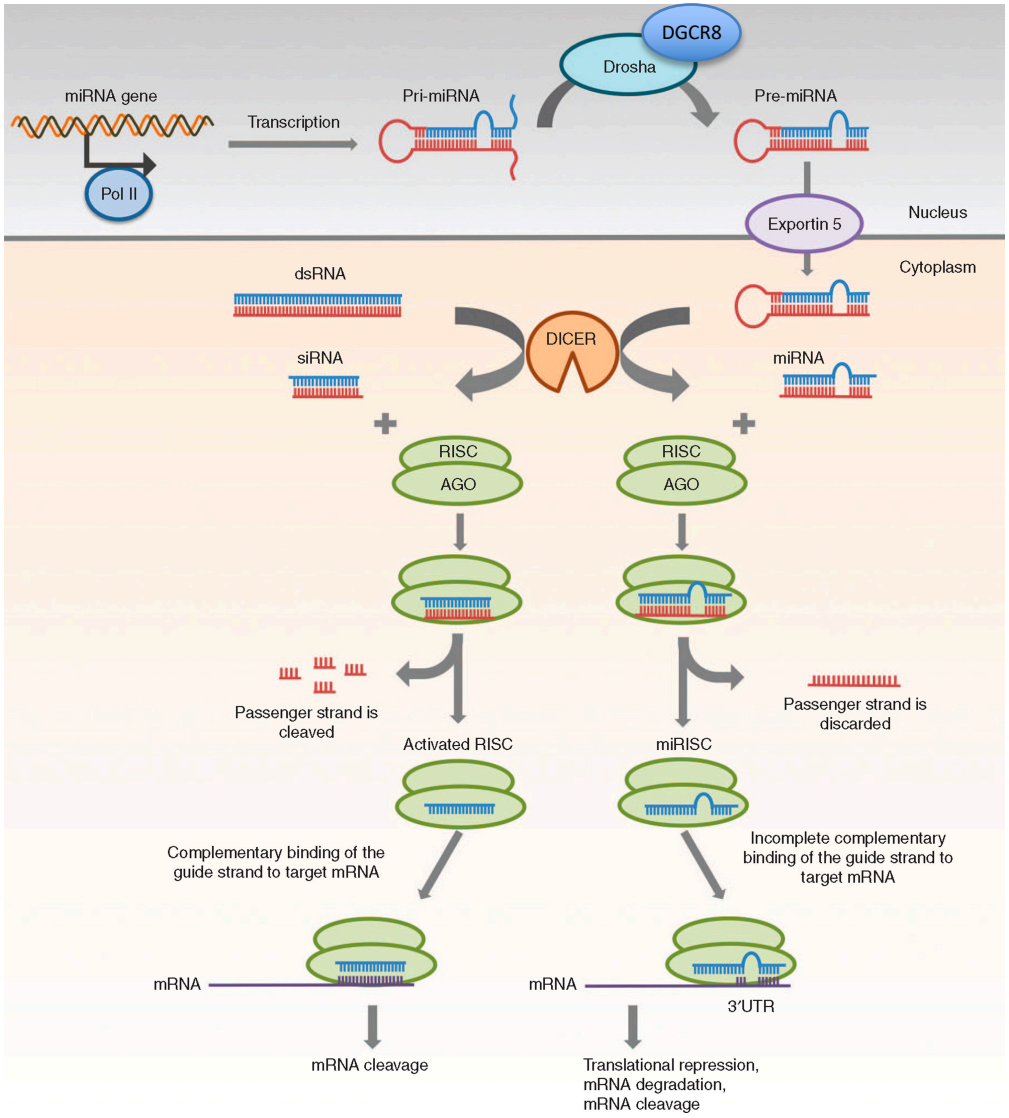
Small RNAs: < 200nucleotides

snoRNA: small nuceolar RNAs Methylation or pseudouridinylation of other RNAs (rRNA, tRNA other small RNA)
snRNAs: localized on Cajal bodies and splicing speckles form snRNPs

seudogenes are powerful regulators of gene expressio



siRNA and miRNA biogenesis and gene regulation



miRNA biogenesis

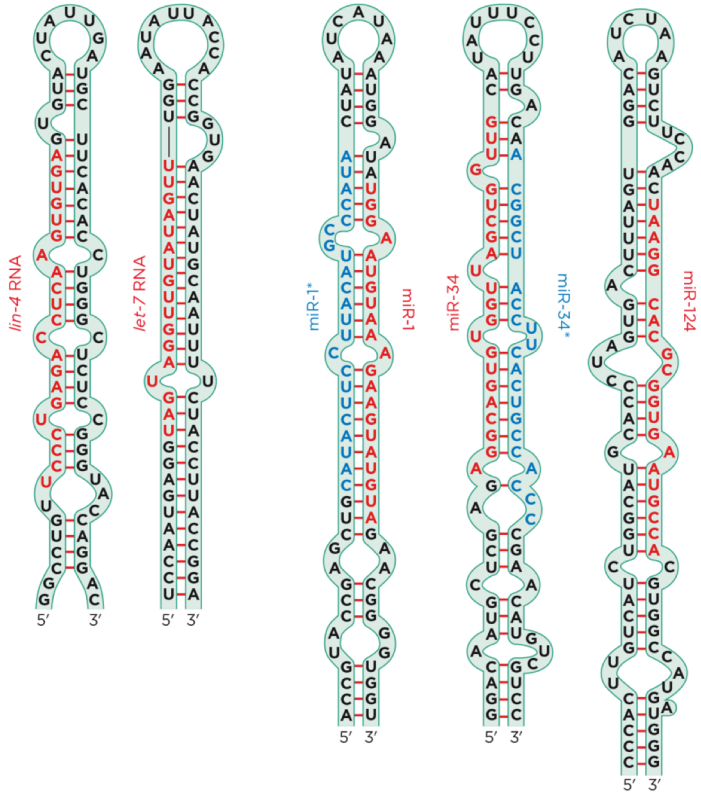
1. Long, unprocessed precursor dsRNA or stem loop RNA **(pri-miRNA**)
2. Processing in the nucleus by the RNaseIII family protein Drosha generates a stem-loop RNA with characteristic length of 65-70 nucleotides. Drosha is in complex with DGCR8 that is important for Drosha activity
3. Exportin 5-RanGTP transports pre-miRNA in ternary complex thought nuclear pore to cytoplasm. RanGAP stimulates GTP; pre-miRNA released from Exportin.
4. RNaseII family enzyme Dicer processes pre-miRNA generating a 20-25 base dsRNA with overhang at the 3'end (2 bases)
5. Transfer of dsRNA to RISC complex (RNA induced silencing complex)
6. Selection of guide RNA → regulatory RNA passenger RNA → will be eliminated
7. RISC complex+guide RNA → regulatory function

A. RNA degradation = siRNA effect (cutting = "slicing"
B. inhibition of mRNA translation =mRNA effect
C. transfer to nucleus and chromatin regulation = siRNA mediated silencing

miRNA dependent regulation of gene expression

One strand generates miRNA

both strand Generate miRNA



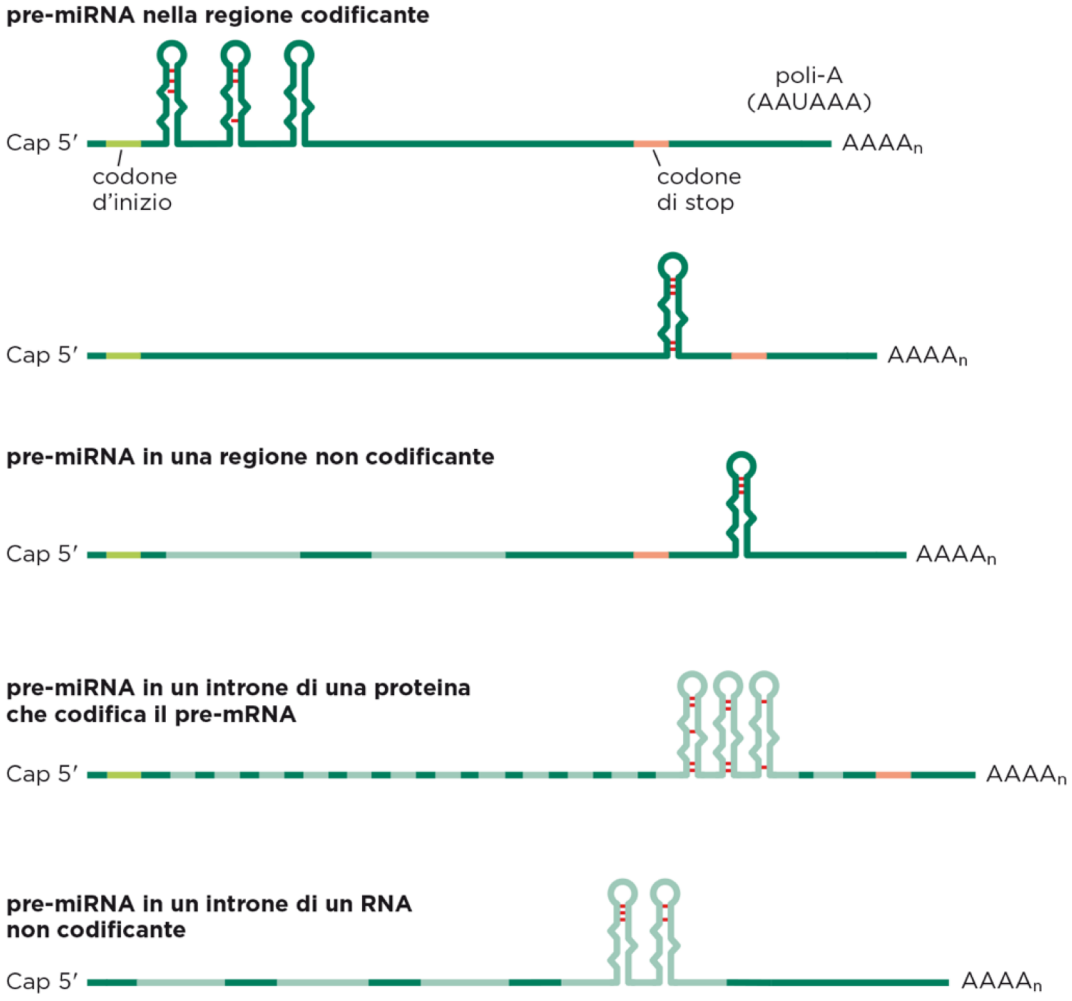
pri-miRNA (primary miRNA

↓ **Drosha**

pre-miRNA (precursor miRNA)

↓ **Dicer**

miRNA (mature miRNA)



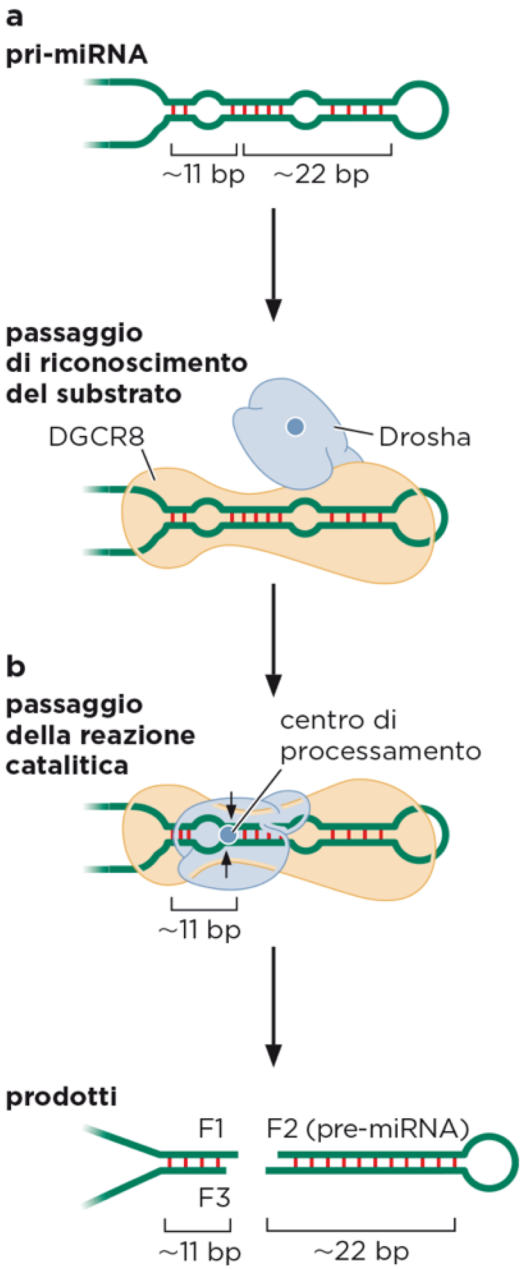
Pre-miRNA encoded by:

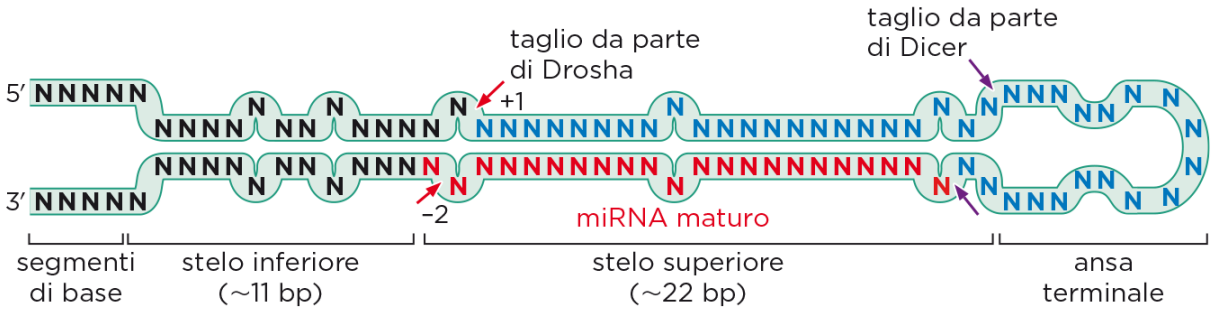
- mRNA genes: INRTONS and few exons

- separate small genes

- Introns and exons of lncRNAs

miRNA generation - DROSHER





Drosha, Dicer form the Micorprocessor comples
cut 2 RNA strands in RNA duplex, leave 2 base 3'overhang!!

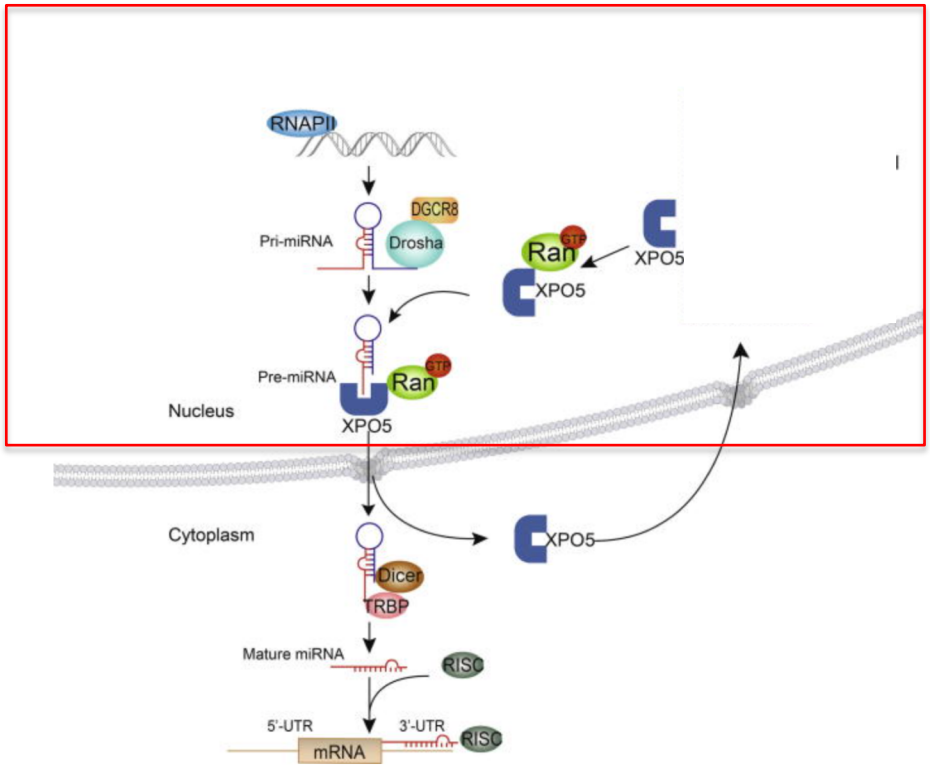
Microprocessor (Drosha and DGCR8) generates a 65-70 nt RNA stem loop:

Drosha cuts app. 11 nt after start of dsRNA region
5 components:

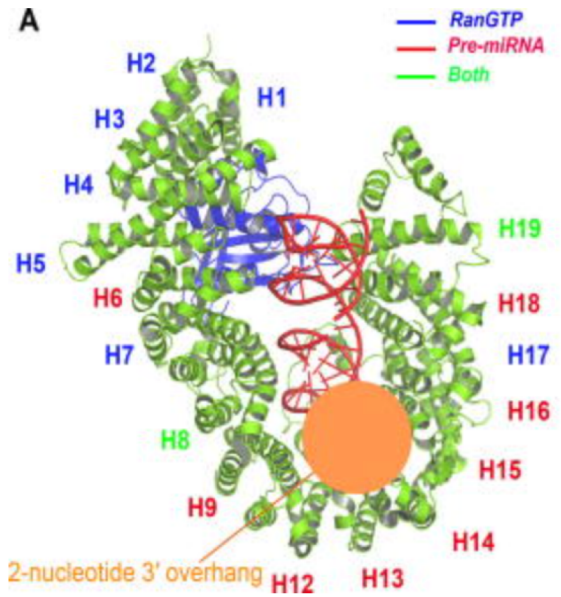
- Lower stem(11 bp);
- Upper stem (22 nt)
- Terminal loop;
- Basal segments of single stranded, unpaired RNA

2. Transfer to cytoplasma
- Via the Exportin 5

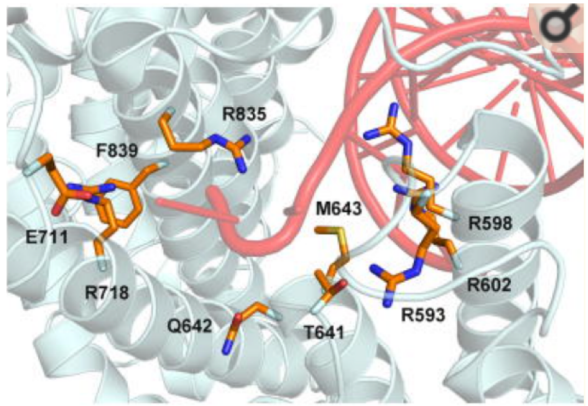
miRNA generation – EXPORTIN-5 (XPO5)



Binding of pre-miRNA by XPO-5 is **not sequence specific**. XPO-5 expected to bind other eventual dsRNA molecules



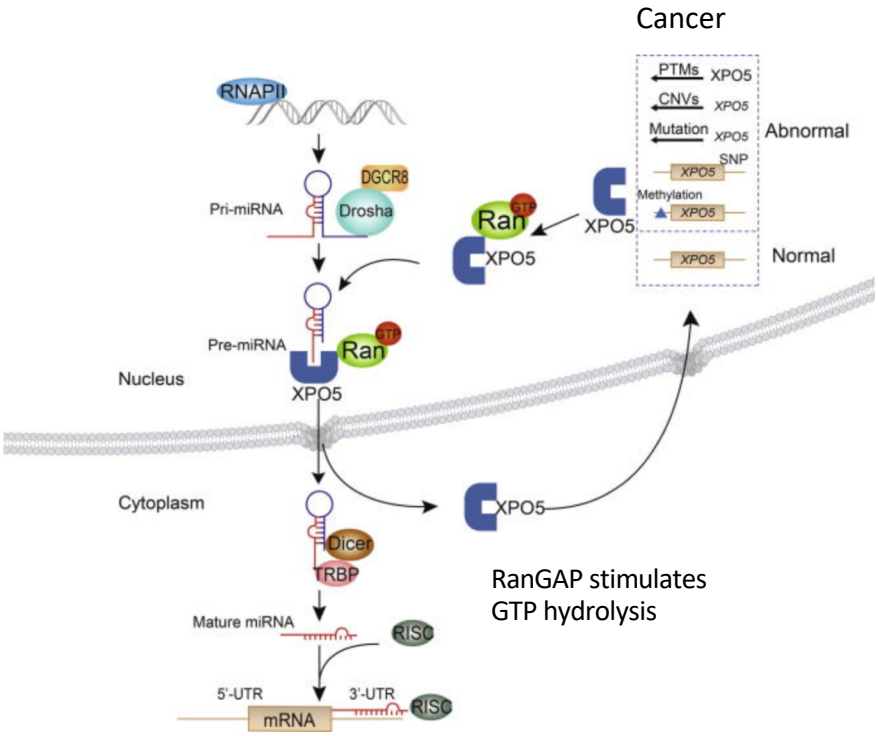
XPO5 recognizes the double-stranded stem structure of the pre-miRNAs via the XPO5 tunnel-like structure comprising HEAT repeats (a tandem repeat protein structural motif composed of two alpha helices linked by a short loop)



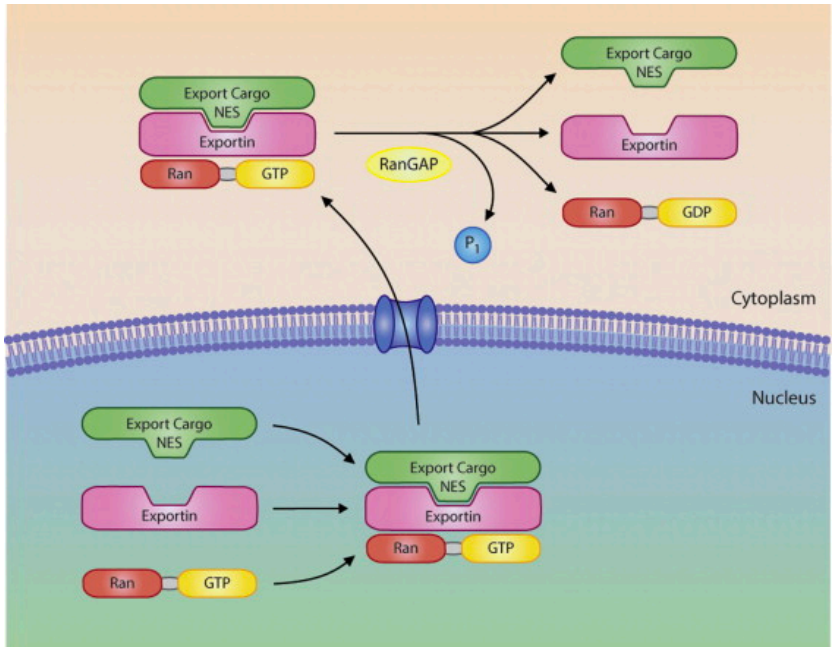
Intermolecular interaction details of the 2-nt 3′overhang structure of pre- miRNA(red) with HEAT repeats 12-15 of XPO5 (grey).

Wu et. al. The Role of Exportin-5 in MicroRNA Biogenesis and Cancer Genomics Proteomics Bioinformatics. 2018.

miRNA generation – EXPORTIN-5 (XPO5)



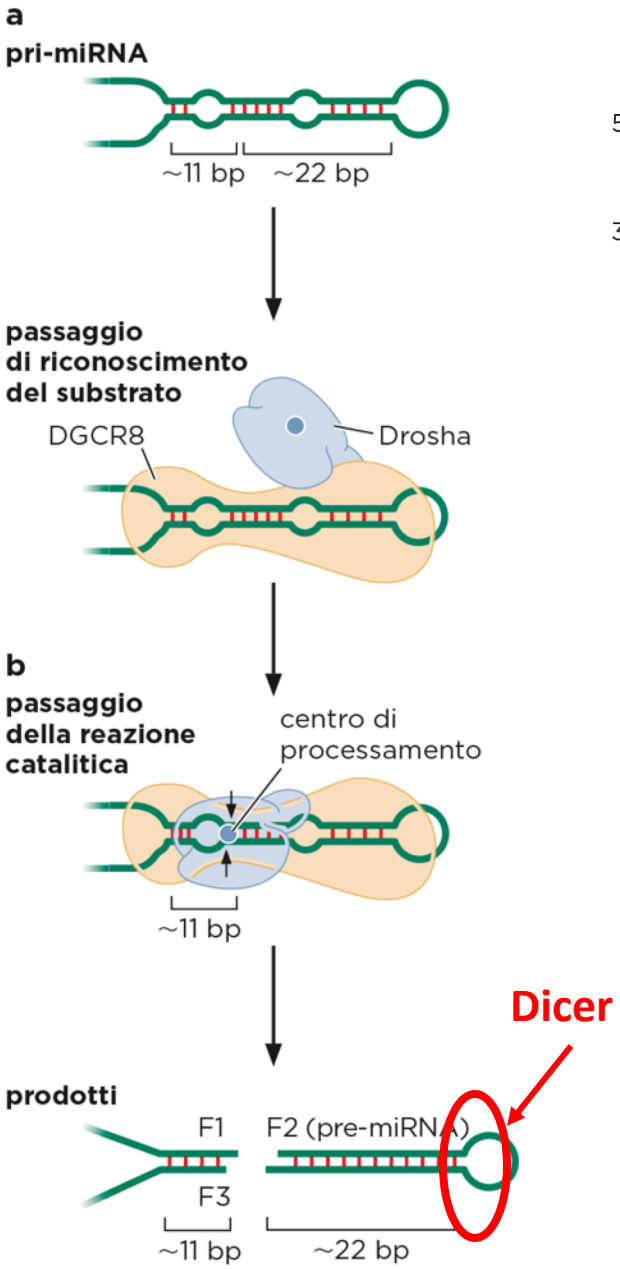
Alterations in XPO-5 can lead to alteration in mature miRNA spectrum. As observed in some cancers

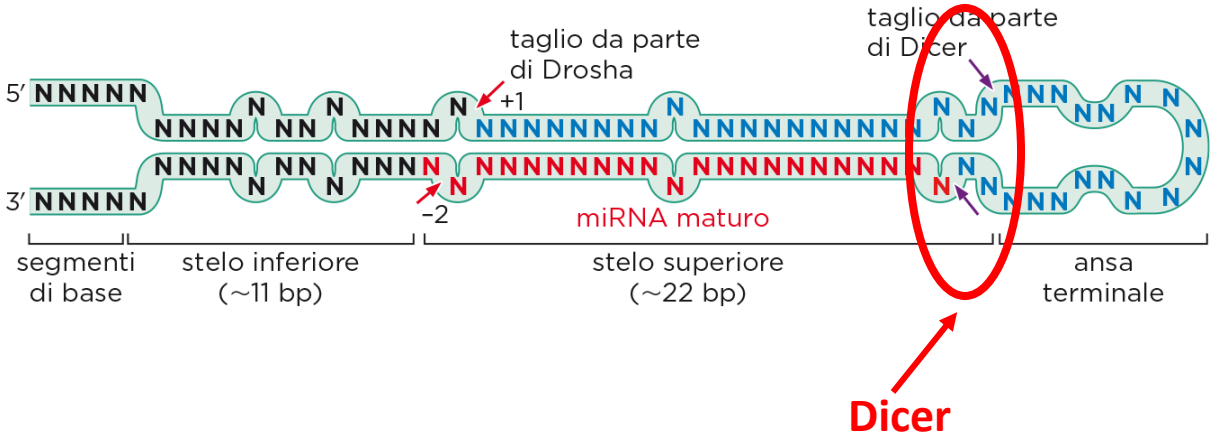


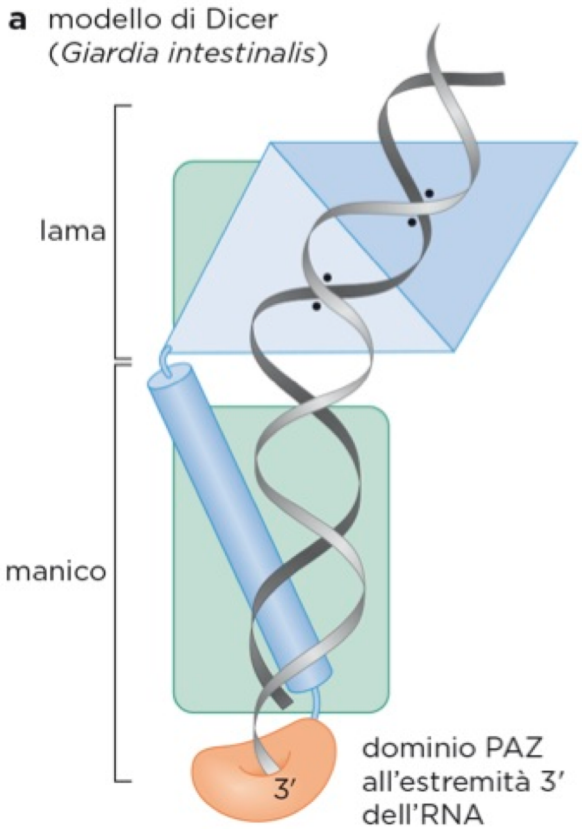
The Ran cycle – Ran exists in a GTP-bound state in the nucleus and a GDP-bound state in the cytoplasm.

Wu et. al. The Role of Exportin-5 in MicroRNA Biogenesis and Cancer Genomics Proteomics Bioinformatics. 2018.

miRNA generation - DICER







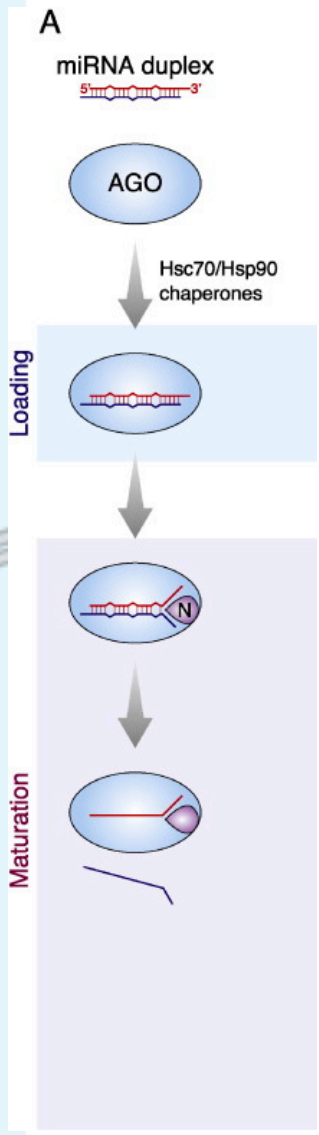
DICER processes all dsRNAs (NOT SEQUENCE SPECIFIC)

2 RNase domains: act on ALL dsRNAs

manico interacts with dsRNA

PAZ interacts with dsRNA

miRNA biogenesis and gene regulation



Assembly of siRNA duplexes into RISC complex

Small RNAs commonly assemble with a member of Argonaute (Ago) family proteins into the effector ncRNP complex called RNA-induced silencing complex (RISC)

Two steps:

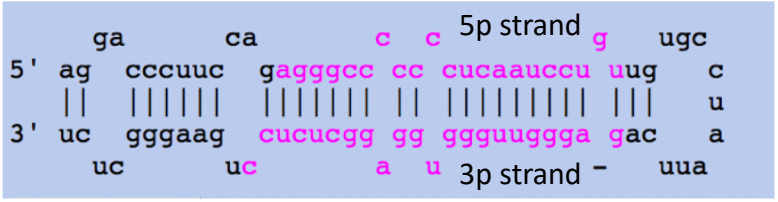
1. *LOADING:*

siRNA duplexes are loaded into AGO proteins by the aid of the Hsc70/Hsp90 chaperone machinery.

2. MATURATION

RISC maturation is initiated by wedging, in which the N domain of Ago subfamily proteins pries open base pairs at the 3' end of the guide strand (paired with the 5' end of passenger strand).
Maturation is completed by passenger ejection, in which passenger strands are ejected from AGO proteins.

IMPORTANT: Only one strand remains in RISC complex
-5p or -3p miRNA strand (orientation given by pri-miRNA)
i.e. miR-296-5p or miR-296-3p



Kobayashi et al. RISC assembly: Coordination between small RNAs and Argonaute proteins. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms 2016

miRNA biogenesis and gene regulation

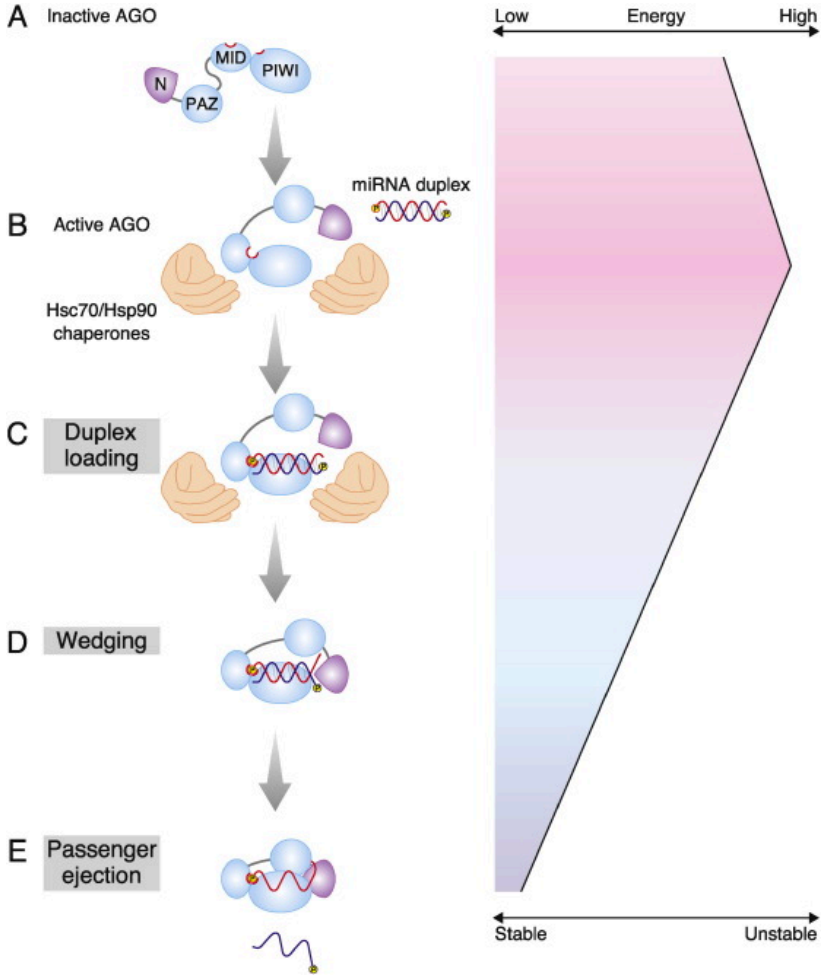
Argonaute proteins represent the core of the RISC complex

~ 22-bp dsRNAs, called miRNA/miRNA* duplexes, are ready to assemble with AGO proteins.

2 Families:

AGO subfamily (e.g., Ago1 and Ago2 in flies and Ago1, Ago2, Ago3 and Ago4 in mammals): binds to miRNAs and siRNAs

PIWI subfamily (e.g., Piwi, Aub and Ago3 in flies; Miwi, Mili and Miwi2 in mice) that binds to piRNAs.



Argonaute (ago) proteins consist of four domains: N, PAZ, MID and PIWI.

MID and PIWI domains: at their intepface, the phosphate group and the base moiety at the 5’ end of the guide small RNA strand is strongly anchored

PAZ domain harbors a pocket that can bind the 3’ end of the guide strand.

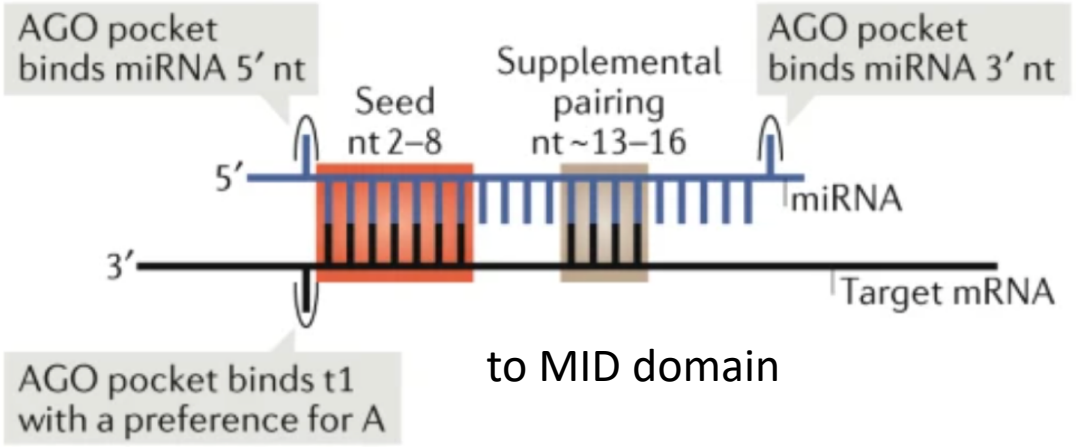
PIWI domain adopts a fold similar to the endoribonuclease RNase H, and binds dsRNA without cleavage.

The N domain plays an important role in separation of the two RNA strands after duplex loading and positions the catalytic PIWI domain correctly for target RNA cleavage.

Strand selection controlled by thermpdynamic asymetry: in general, the strand harboring thermodynamically less stable base pairing at its 5’ end selectively functions as the guide strand.

miRNA biogenesis and gene regulation

miRNA effector Function by Ago1-4



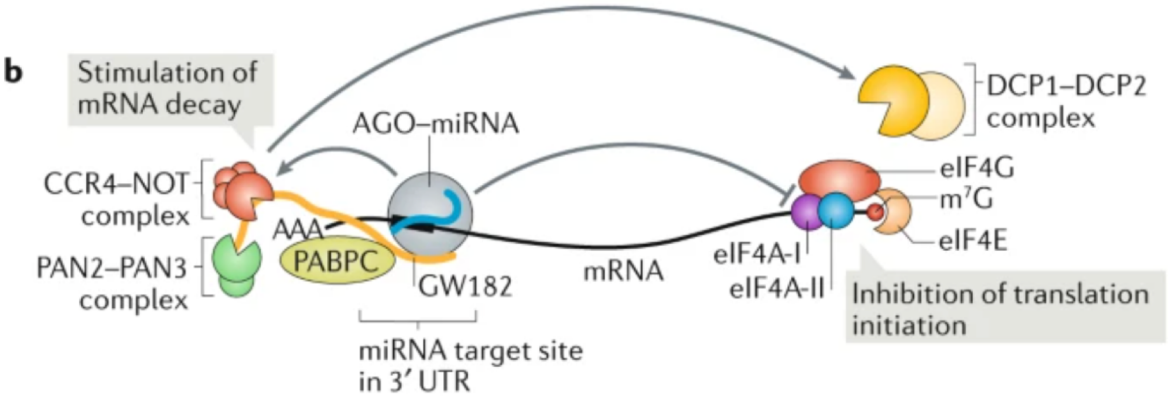
Imperfect miRNA – target site interaction
(normal miRNA situation)

to MID domain

target RNA decay

- Interactions with glycine-tryptophan protein of 182 kDa (GW182) proteins. GW182 binds polyadenylate-binding protein (PABPC) and the deadenylation complexes poly(A)-nuclease deadenylation complex subunit 2 (PAN2)–PAN3 and carbon catabolite repressor protein 4 (CCR4)–NOT leading to **de-adenylation**. Deadenylation is followed by **5′ mRNA decapping** by the complex mRNA-decapping enzyme subunit 1 (DCP1)–DCP2 and 5′–3′ **mRNA degradation**

suppression of translation



- release of eukaryotic initiation factor 4 A-I (eIF4A-I) and eIF4A-II. **Block of initiation of translation**

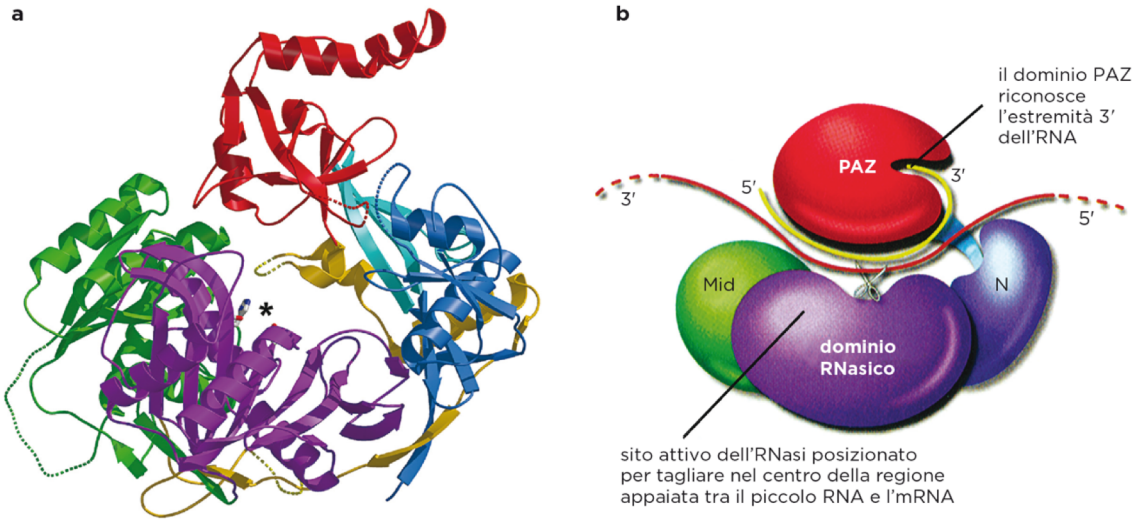
Gebert et al. Regulation of microRNA function in animals. Nat.Rev.Mol.Cell.Biol.2018

Conservation of miRNA target site interaction

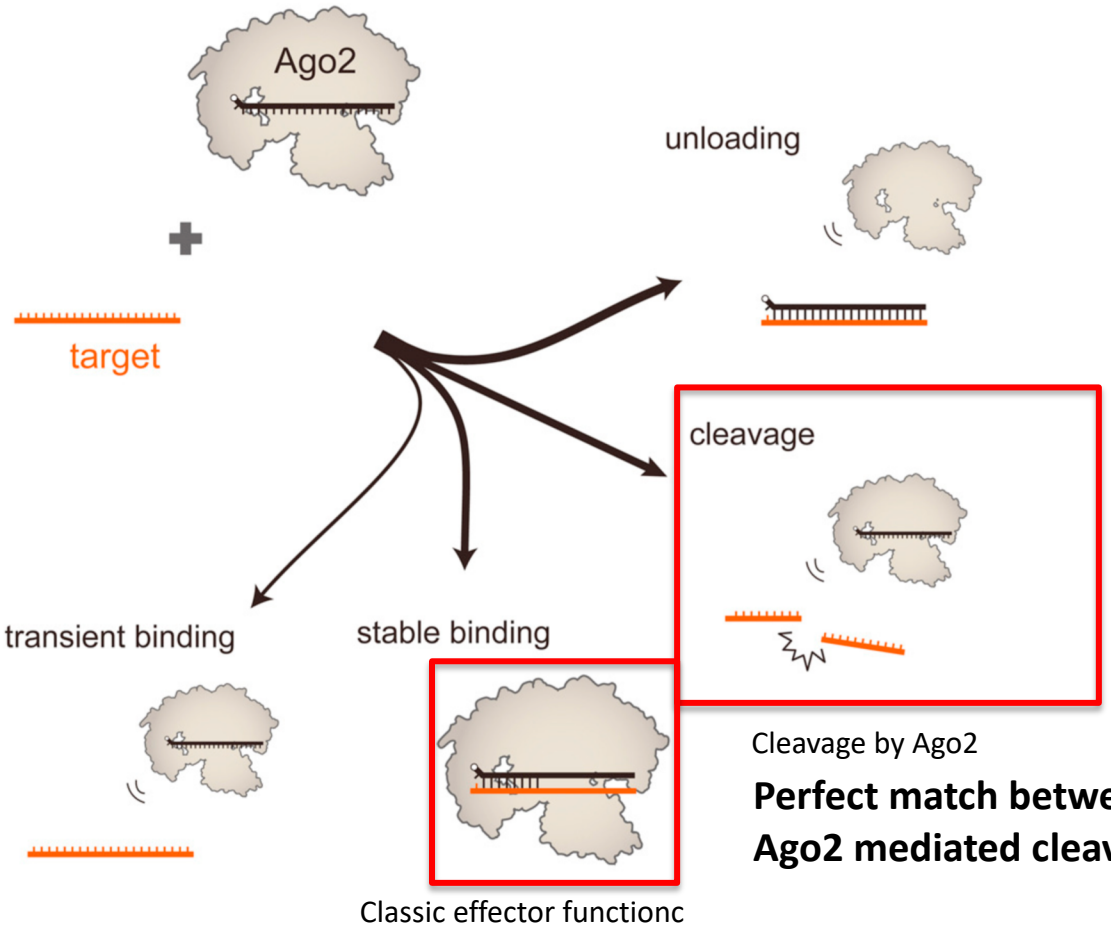
```
hsa-miR155          3′-UGGGGAUAGUG-----CUAAUCGUAAUU-5′
                       |   ||  |:::      |||||||
Human          5′GUAAUUUAAAACUUUUGU-----UUAAAGCAUUAC--------AGUAU----------3′
Chimpanzee     5′GUAAUUUAAAACUUUUGU-----UUAAAGCAUUAC--------AGUAU----------3′
Cow            5′------CAAAA-UUCUGU-----UUAAAGCAUUAC--------AACAU----------3′
Rabbit         5′--------AAAUUUUGGU-----UUAAAGCAUUAU--------UU------------3′

Mouse          5′AUGAUAAAGCAUUAUGGUGGUGGUGGGGCAGUGAGGAGGGGGAAGAGAAAGAGAGUUU-3′
Rat            5′AAGAUGAAGCAUUAUUGU----GUGUGUGUGUGAG--------AGA-----------3′
```

Seed sequence: pos 2-8 in miRNA (5' → 3')



miRNA effector Function by Ago2

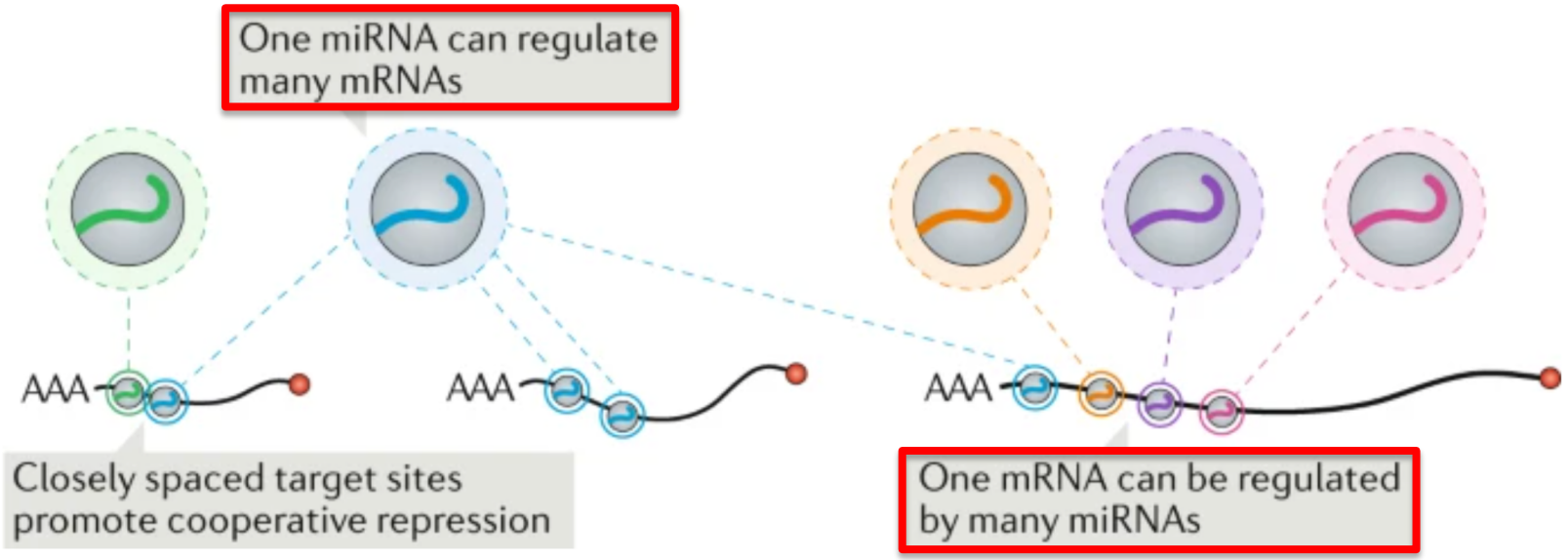


Cleavage by Ago2

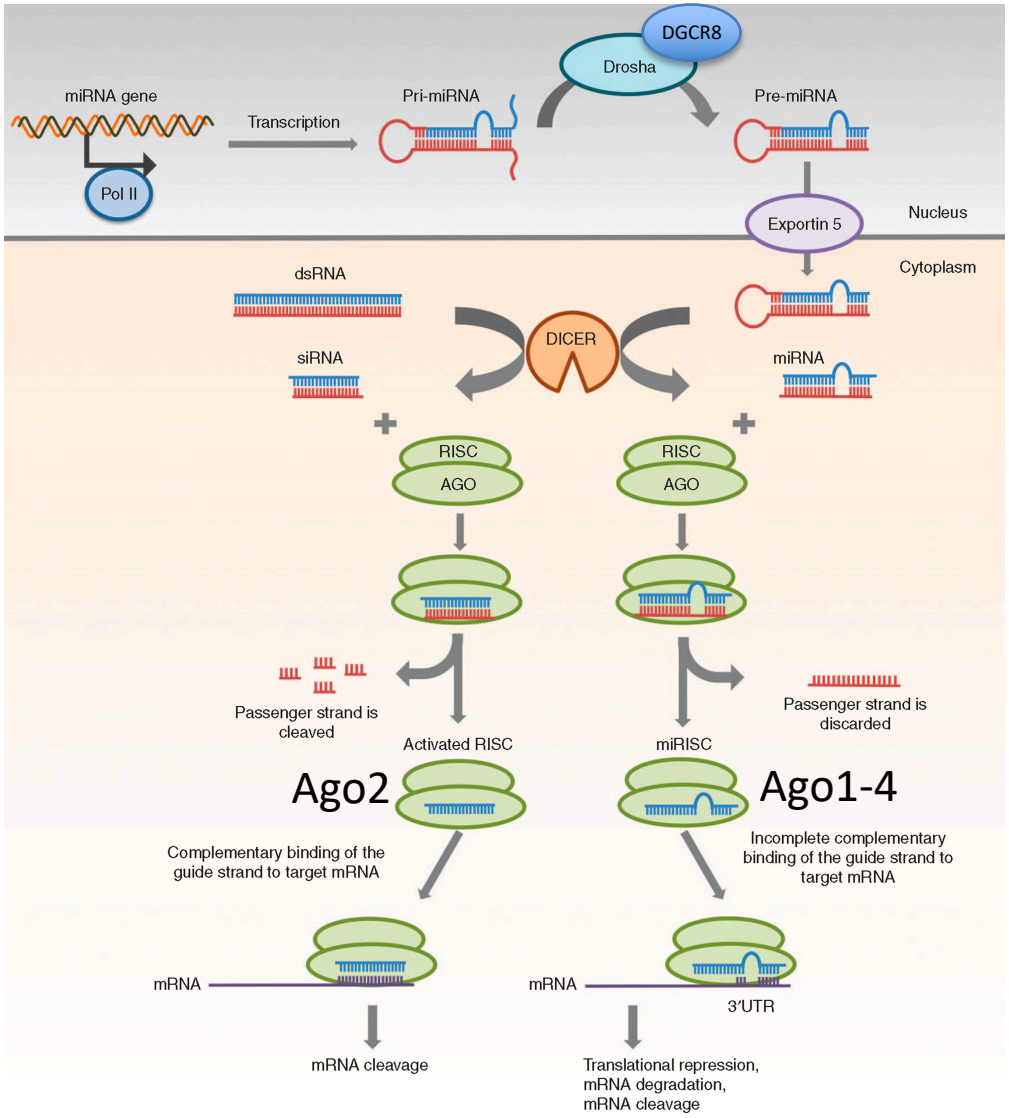
Perfect match between miRNA and target RNA induces Ago2 mediated cleavage = slicing

--> Basis for siRNA mediated knock-down

miRNA biogenesis and gene regulation



miRNA and siRNA biogenesis and gene regulation



siRNA biogenesis

1. Long, unprocessed precursor dsRNA or stem loop in cytplasm
2. RNaseII family enzyme Dicer processes pre-miRNA generating a 20-25 base dsRNA with overhang at the 3'end (2 bases)
3. Transfer of dsRNA to RISC complex (RNA induced silencing complex)
4. Selection of guide RNA → regulatory RNA
   passenger RNA → will be eliminated
7. RISC complex+guide RNA → regulatory function

A. RNA degradation = siRNA effect (cutting = "slicing"
B. transfer to nucleus and chromatin
   regulation = siRNA mediated silencing

Stem-loop sequence hsa-mir-296

Accession	MI0000747
Symbol	HGNC:MIR296
Description	Homo sapiens miR-296 stem-loop
Gene family	MIPF0000159; mir-296

Community annotation

This text is a summary paragraph taken from the Wikipedia entry entitled miR-296. miRBase and Rfam are facilitating community annotation of microRNA families and entries in Wikipedia. Read more ...

miR-296 is a family of microRNA precursors found in mammals, including humans. The ~22 nucleotide mature miRNA sequence is excised from the precursor hairpin by the enzyme Dicer. This sequence then associates with RISC which effects RNA interference. miR-296 has been named an "angiomiR" due to being characterised as a microRNA which regulates angiogenesis, the process of growth and creation of new blood vessels. miR-296 is thought to have a specific role in cancer in promoting tumour angiogenesis. It achieves this by targeting HGS mRNA, reducing its expression in endothelial cells which then results in greater number of VEGF receptors. miR-296 has predicted target sites in the transcription factor NANOG and may also contribute to carcinogenesis by dysregulating p53.

Show Wikipedia entry | View @ Wikipedia | Edit Wikipedia entry

Stem-loop

```
     ga      ca       c  c         g   ugc
5' ag  cccuuc  gagggcc cc cucaauccu uug   c
   ||  ||||||  ||||||| || ||||||||| |||   u
3' uc  gggaag  cucucgg gg ggguuggga gac   a
     uc      uc       a  u         -   uua
```

Get sequence

Deep sequencing

1633 reads, 355 reads per million, 59 experiments

Stem-loop sequence hsa-mir-155

Accession MI0000681

Symbol HGNC:MIR155

Description Homo sapiens miR-155 stem-loop

Gene family MIPF0000157; mir-155

Community annotation

This text is a summary paragraph taken from the Wikipedia entry entitled Mir-155. miRBase and Rfam are facilitating community annotation of microRNA families and entries in Wikipedia. Read more ...

MiR-155 is a microRNA that in humans is encoded by the MIR155 host gene or MIR155HG. MiR-155 plays an important role in various physiological and pathological processes. Exogenous molecular control in vivo of miR-155 expression may inhibit malignant growth, viral infections, and attenuate the progression of cardiovascular diseases.

Show Wikipedia entry | View @ Wikipedia | Edit Wikipedia entry

Stem-loop

```
                 c   a        -u   c
5' cuguuaaugcuaau gug uagggguu  uug c
   ||||||||||||||| ||| ||||||||  |||
3' gacaauuacgauua uac auccucag  aac u
                 -   -        uc   c
```

Get sequence

Deep sequencing

55243 reads, 2.94e+03 reads per million, 62 experiments

MicroRNA Nomenclature

Different miRNA genes that have different location in the genome, but each of them produces a miRNA with identical seqeunce (i.e. hsa-miR-7

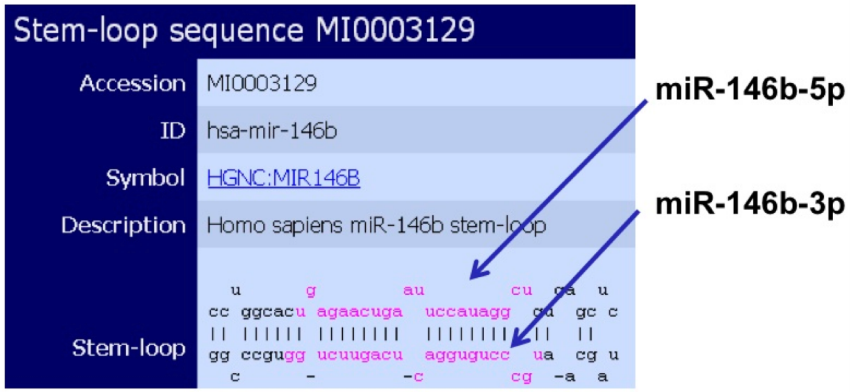
Alleles: all express same mature microRNA

hsa-mir-7-1
hsa-mir-7-2
hsa-mir-7-3

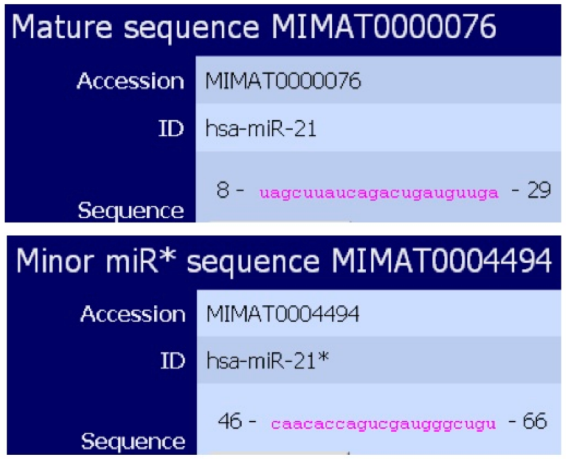
} **Mature miR-7 microRNA expressed**

http://www.mirbase.org

Dual precursors: express two mature microRNAs equally



Star forms: express two mature microRNAs unequally



SBI System Biosciences
Accelerating Discoveries in Functional Genomics

Current nomenclature

Old nomenclature:
* miRNA referes to the strand present at lower levels → thought to be non-functional

Vol 465|24 June 2010|doi:10.1038/nature09144 nature

ARTICLES

A coding-independent function of gene and pseudogene mRNAs regulates tumour biology

Laura Poliseno[1]*†, Leonardo Salmena[1]*, Jiangwen Zhang[2], Brett Carver[3], William J. Haveman[1] & Pier Paolo Pandolfi[1]

BACKGROUND ON PTEN

PTEN: heterozygous mutations: CANCER FORMATION (=haploinsuffcient tumorsuppressor gene)

TARGETING OF PTEN BY miRNAs: reduction of PTEN expression → promotion of tumor formation!!!!

CELLS ARE EXTREMLY SENSITIVE TO SLIGHT CHANGES IN GENE EXPRESSION LEVELS

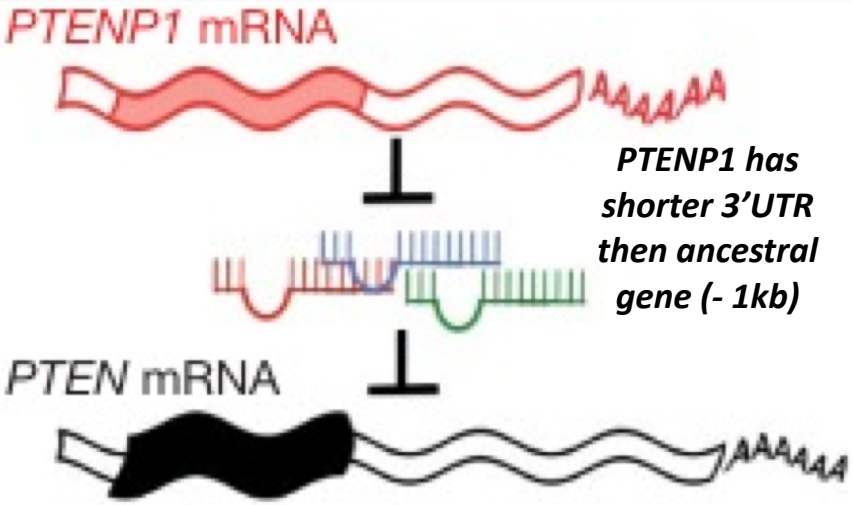
PTEN has generated one processed pseudogene: PTENP1 highly conserved to PTEN

QUESTION: DOES PTENP1 IMPACT ON PTEN EXPRESSION VIA SPONGING miRNAs???

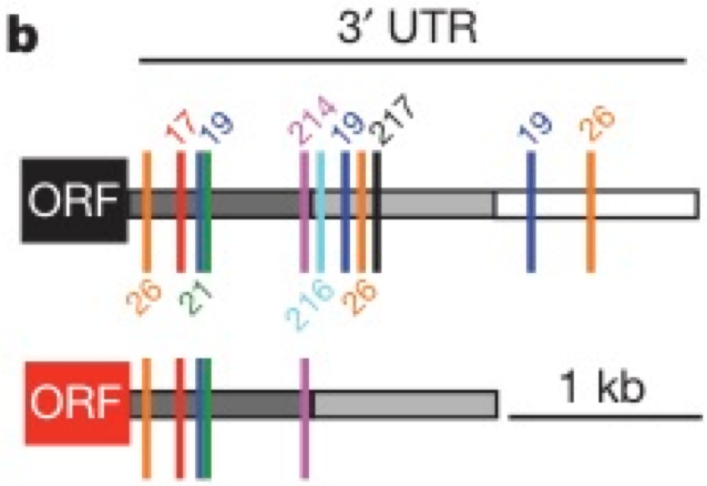
PTEN Wikipedia: Phosphatase and tensin homolog (PTEN) is a protein that, in humans, is encoded by the PTEN gene. Mutations of this gene are a step in the development of many cancers. PTEN orthologs have been identified in most mammals for which complete genome data are available.

This gene was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. The protein encoded by this gene is a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase. It contains a tensin-like domain as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases. Unlike most of the protein tyrosine phosphatases, this protein preferentially dephosphorylates phosphoinositide substrates. It negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate in cells and functions as a tumor suppressor by negatively regulating Akt/PKB signaling pathway.

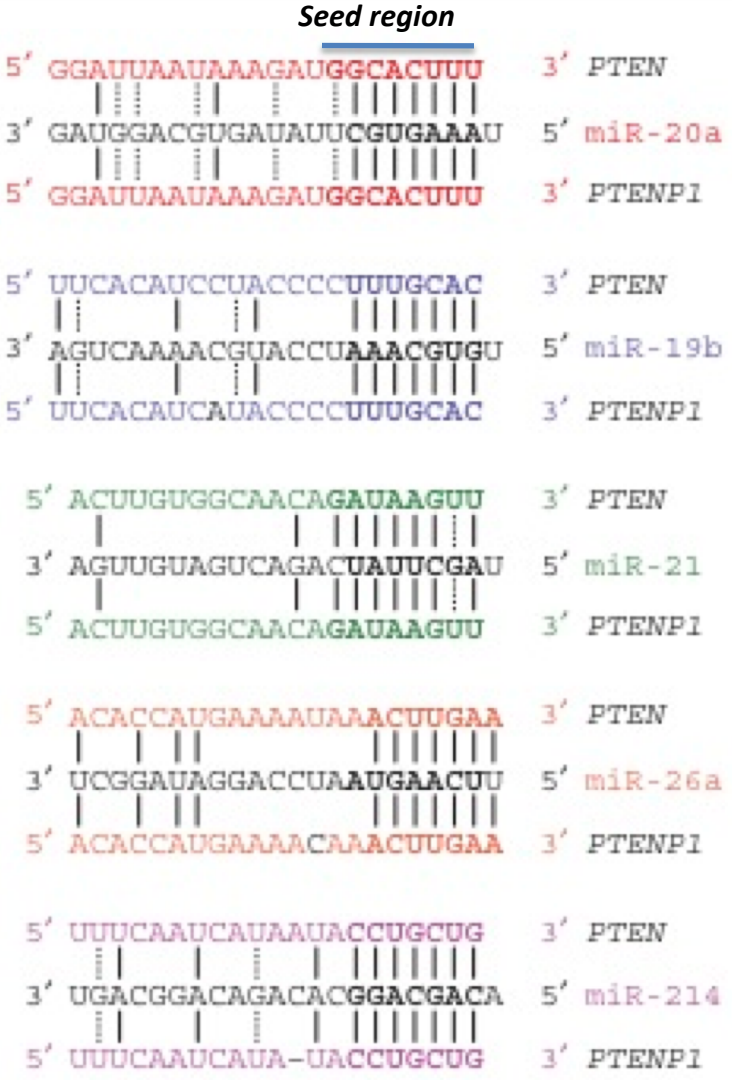
THE PTEN PSEUDOGENE PTENP1



PTENP1 has shorter 3'UTR then ancestral gene (- 1kb)



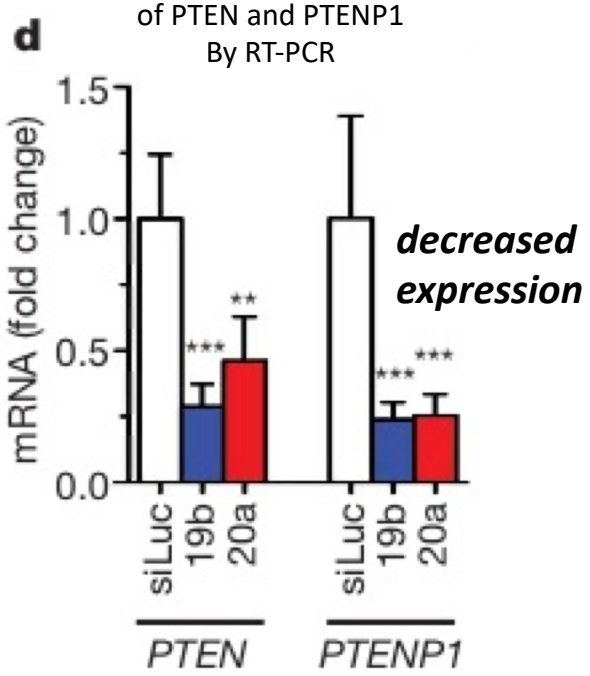
Some target sites of PTEN specific miRNAs are also present in PTENP1



miRNAs target both RNAs: PTEN and PTENP1

Transefection of cells with miRNAs specific for PTEN and PTENP1

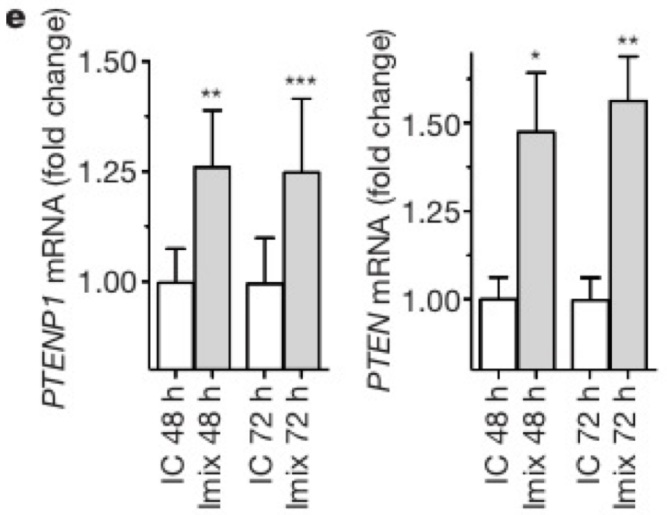
Check expression levels of PTEN and PTENP1 By RT-PCR



decreased expression

Transefection of cells with siRNAs that target miRNAs that are specific for PTEN and PTENP1

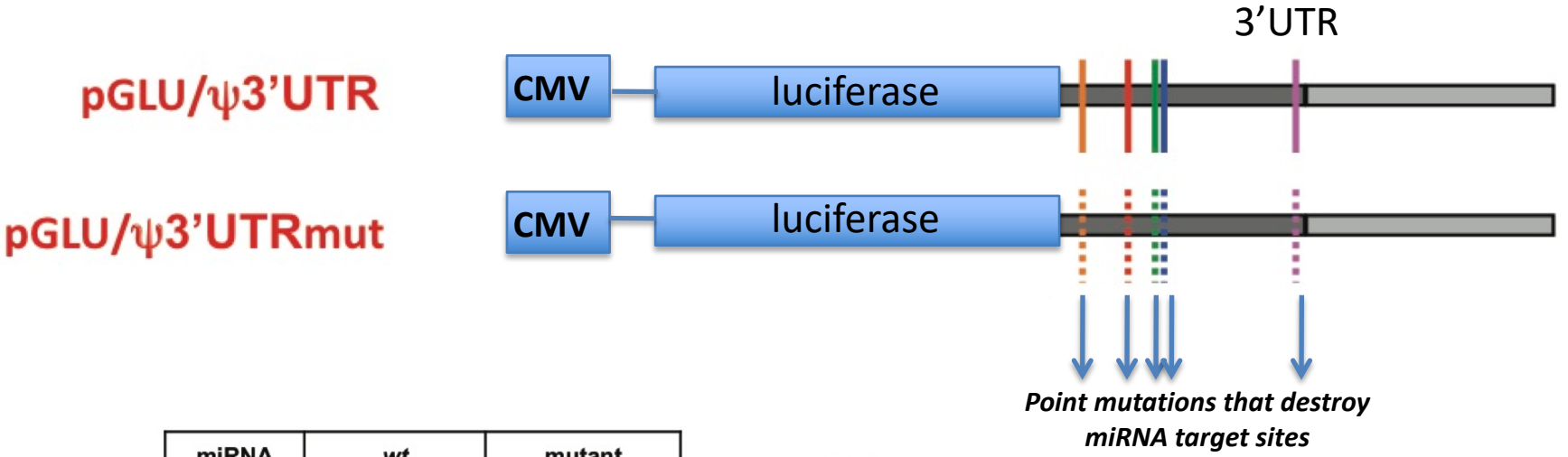
Check expression levels of PTEN and PTENP1 By RT-PCR



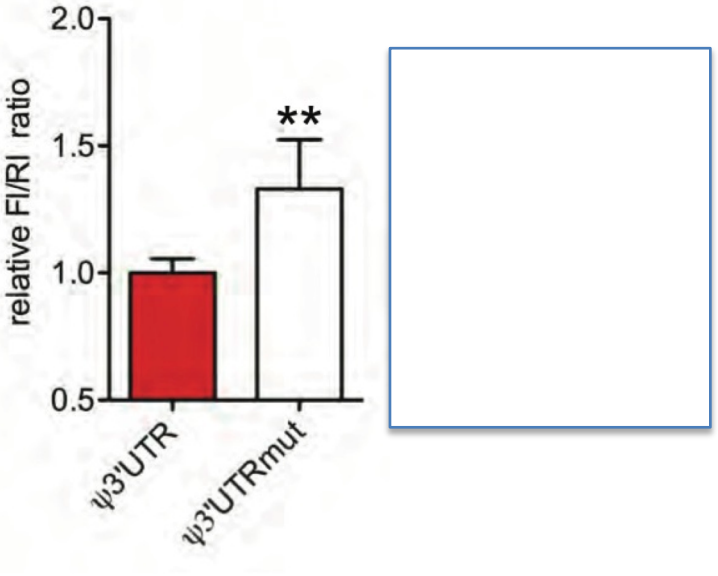
increased expression

miR-19c and miR-20c target both RNAs

DEMONSTRATION OF miRNA – PTENP1_3'UTR INTERACTION USING A LUCIFERASE REPORTER ASSAY

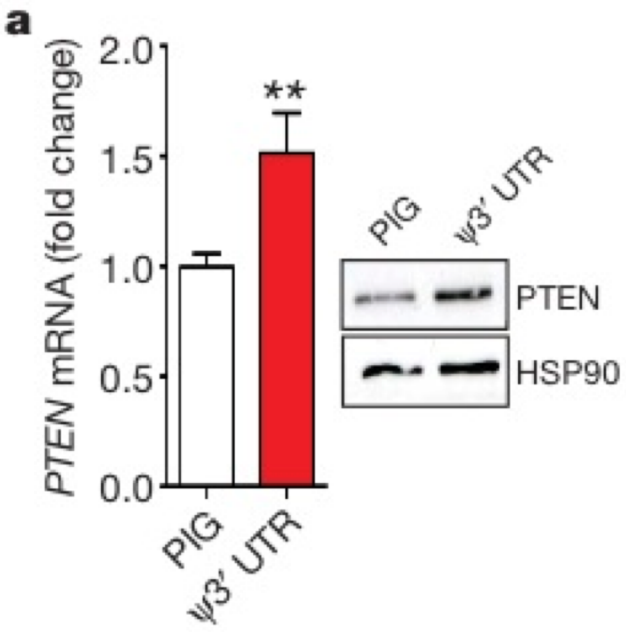


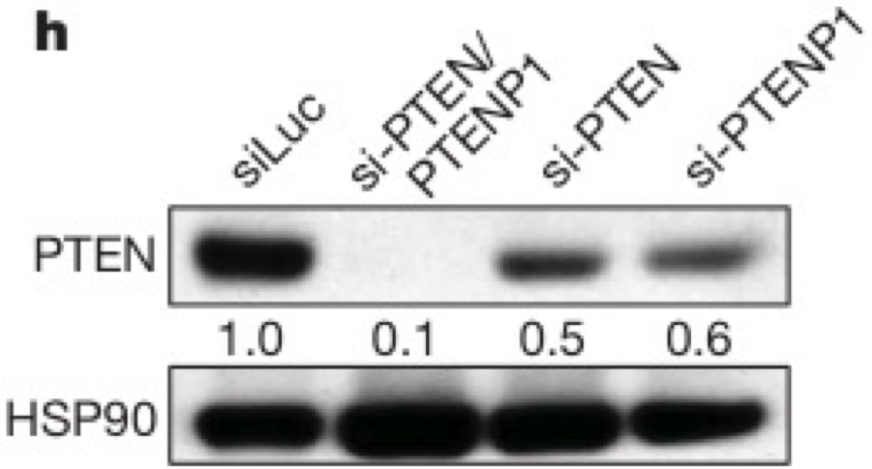
miRNA family	*wt* seed match	mutant seed match
17	GGCACTTT	GcCtCaTa
19	TTTGCAC	aTaGgAg
21	GATAAGTT	GtTtAcTt
26	ACTTGAA	tCaTcAt
214	CCTGCTG	gCaGgTc



The 3'UTR of PTENP1 sequesters miRNAs

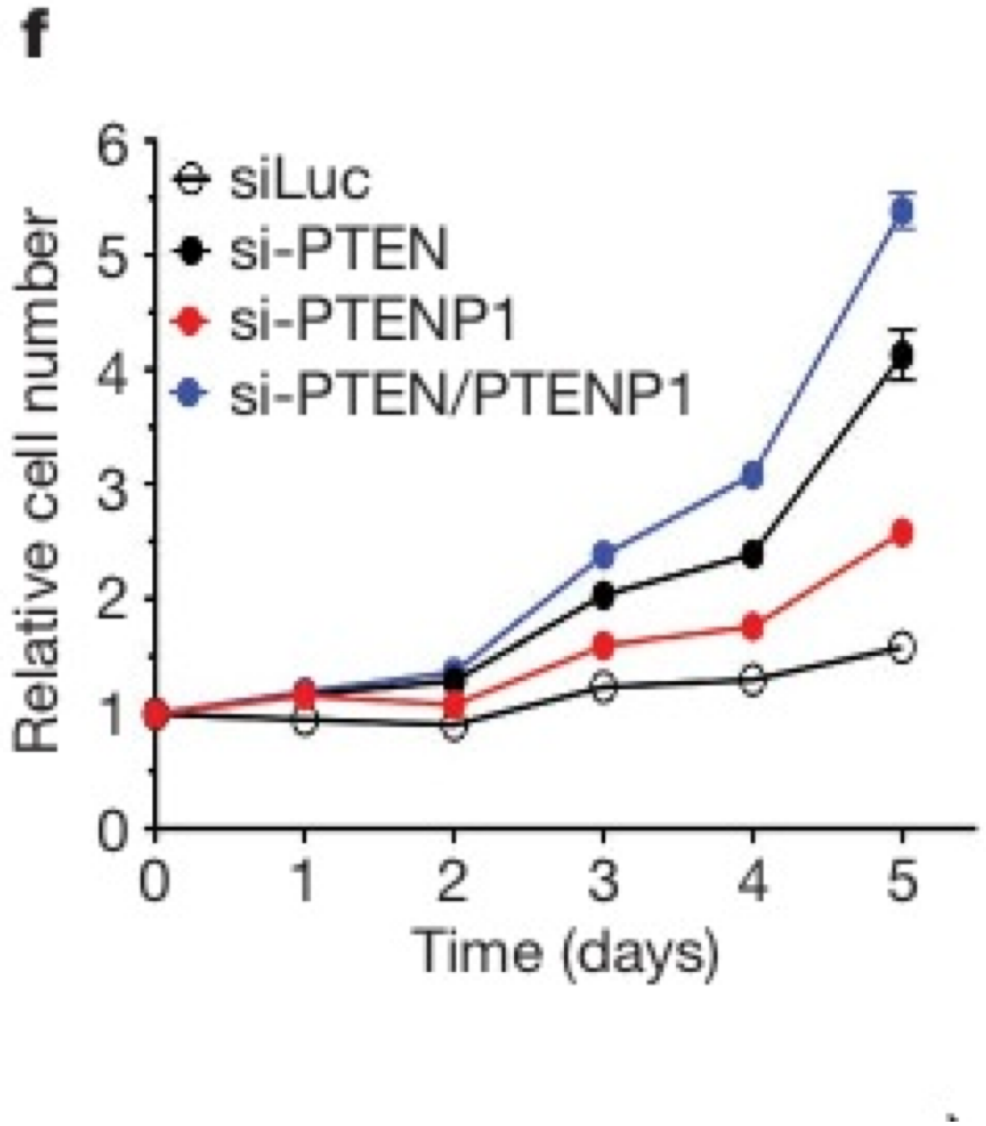
PTENP1 CONTROLS THE EXPRESSION OF PTEN





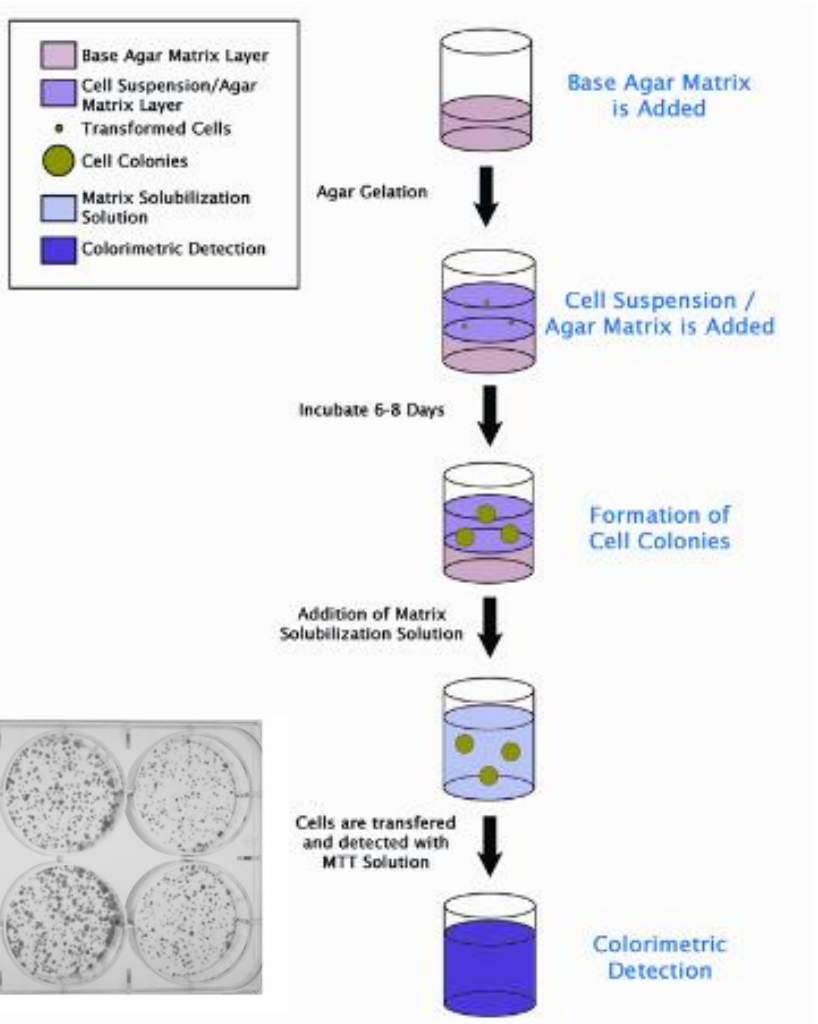
ion

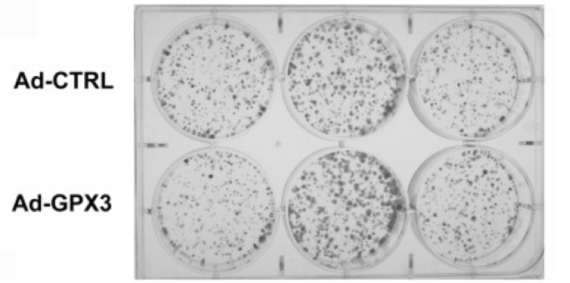
PTENP1 suppresses tumor cell proliferation



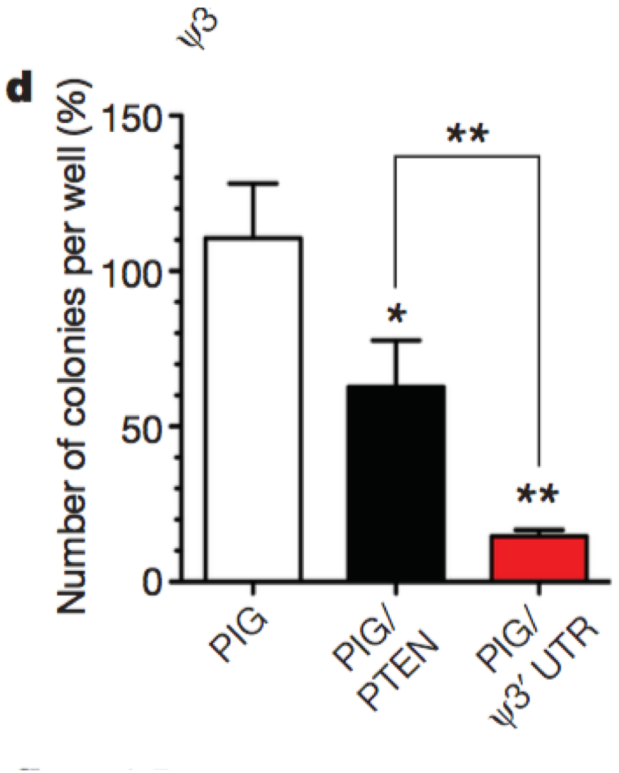
Cumulative cell numbers:
Cancer cells proliferate quickly;
cells with tumorsuppression
proliferate at low rates

Anchorage independent cell proliferation – colony formation assay

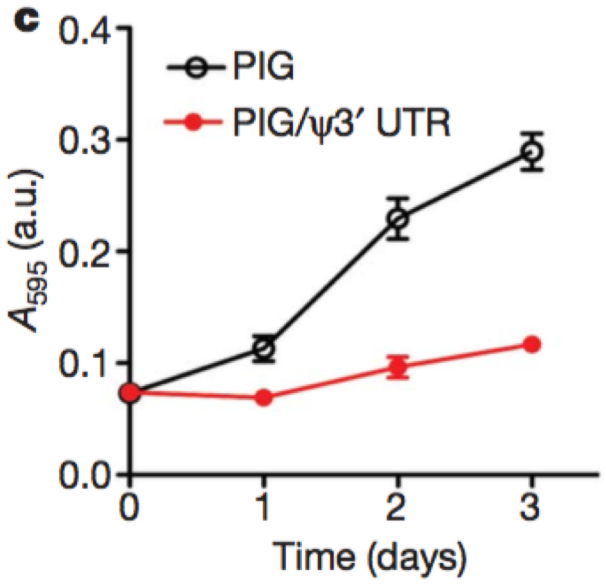




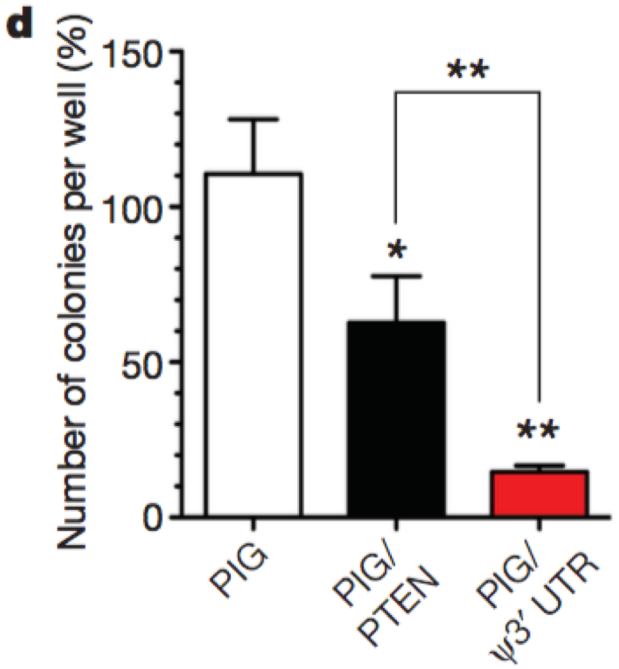
An example



Ectopic expression of PTEP-P1 3'UTR sequence reduces cancer cell proliferation

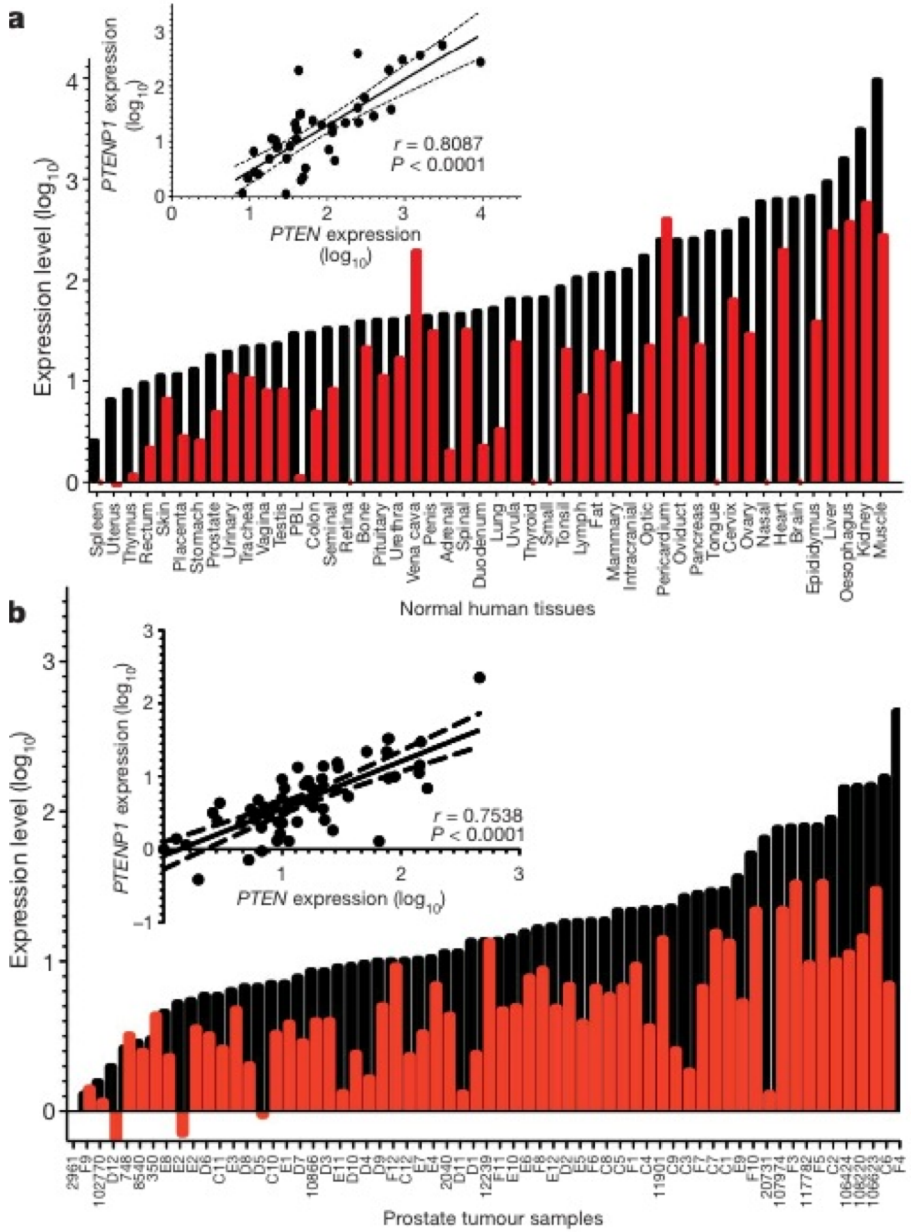


Cell proliferation (normal)



Colony formation in semi-solid medium

RELEVANCE IN VIVO (HEALTHY AND CANCER)?

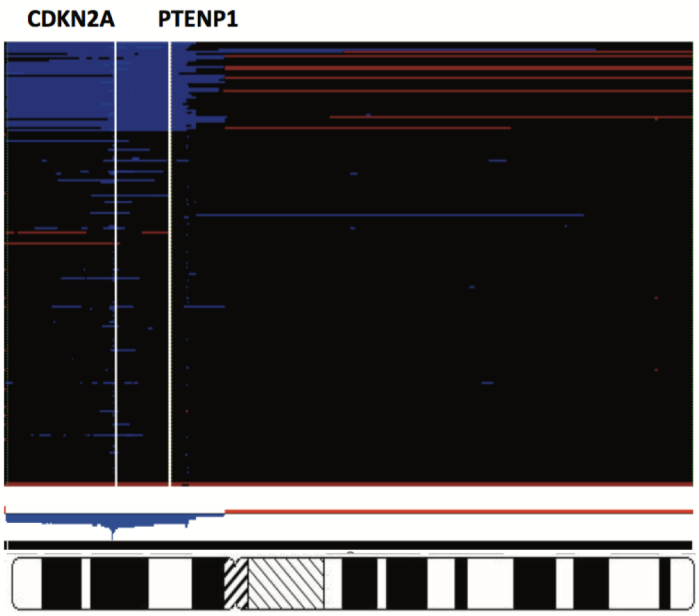


PTEN mRNA expression
Positively correlates with
PTENP1 expression:

Presence of PTENP1 sponges miRNAs
→ increased levels of PTEN

RELEVANCE IN HUMAN CANCER????

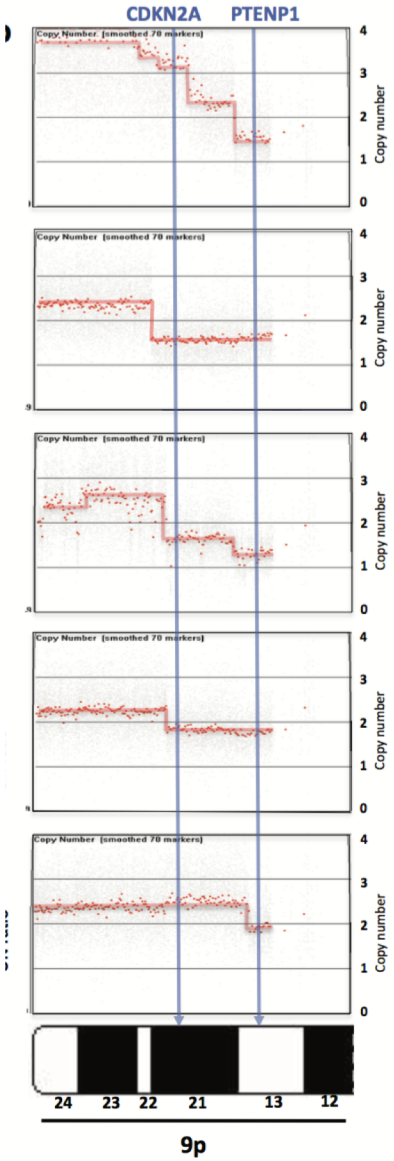
ACUTE LYMPHOBLASTIC LEUKEMIA



colon cancer. a. Non clustered heat map downloaded from the Cancer Workbench website (https://cgwb.nci.nih.gov/cgi-bin/heatmap) displaying the TARGET Acute Lymphoblastic Leukemia (ALL) project CGH database from St. Jude/NCI. Data points have been sorted for loss copy number at the *PTENP1* locus. Red represents copy number gains. Blue represents copy number losses.

Copy number gains (red)
Copy number losses (blue)

BREAST CANCER

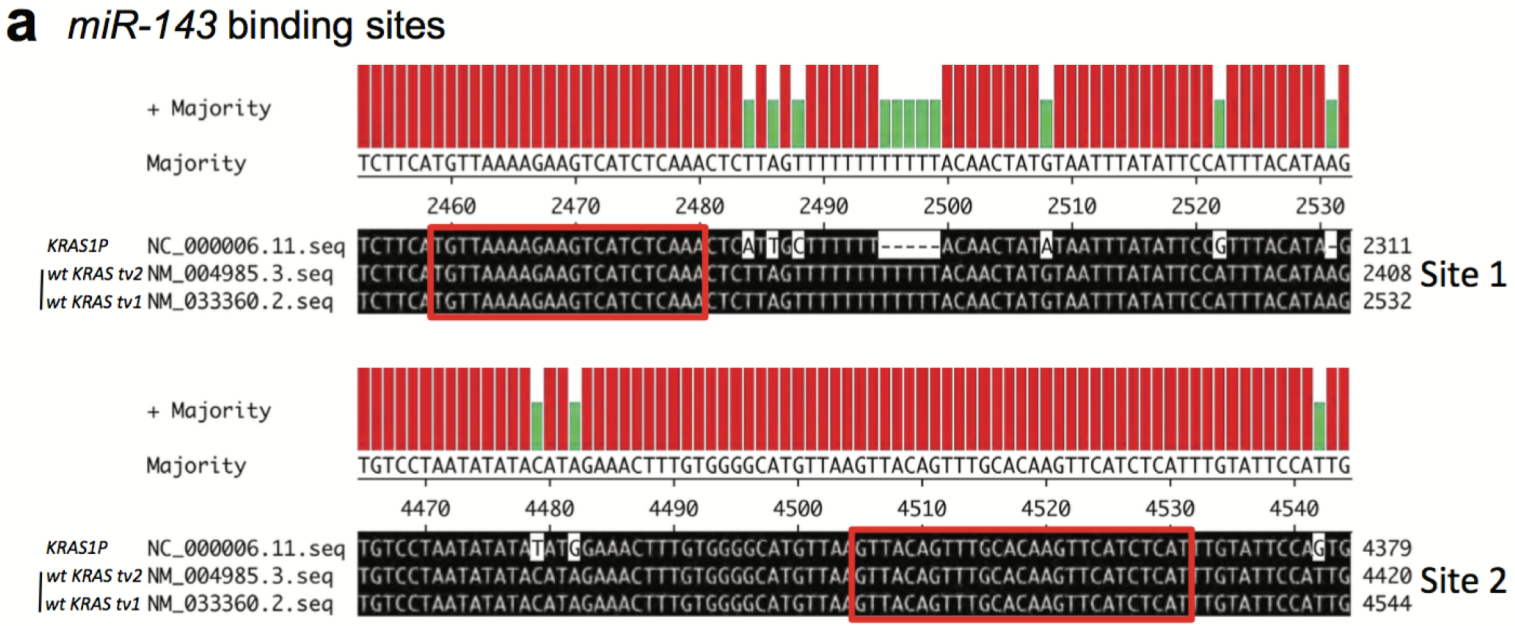


Red line: interesting genes with Copy number alteration

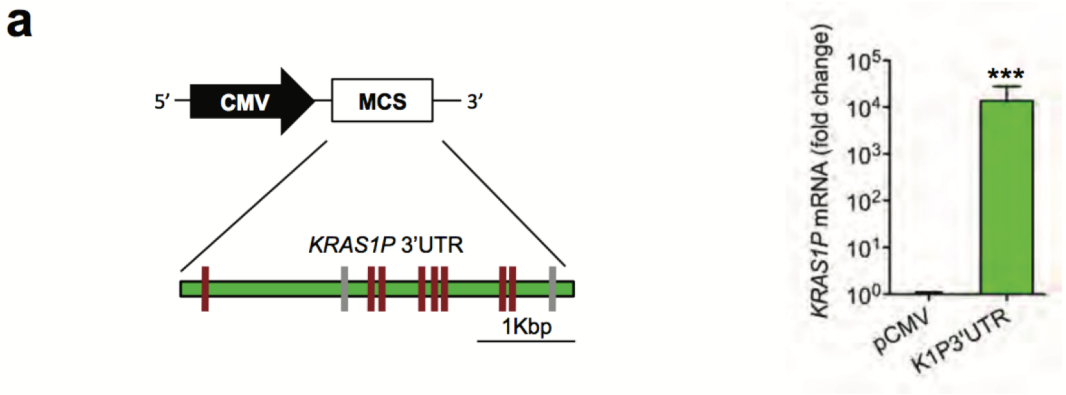
b. Examples of five specific breast cancer patient samples demonstrating losses at the *PTENP1* locus. The graphs were generated using Partek Genomics Suite. X-axis represents chromosome 9p position and Y-axis represents copy number. The red lines highlight regions of gene loss. **c.**

CDKN2A, also known as **cyclin-dependent kinase Inhibitor 2A,** is a gene which in humans is located at chromosome 9, band p21.3.[5] It is ubiquitously expressed in many tissues and cell types.[6] The gene codes for two proteins, including the INK4 family member p16 (or p16INK4a) and p14arf.[7] Both act as tumor suppressors by regulating the cell cycle. p16 inhibits cyclin dependent kinases 4 and 6 (CDK4 and CDK6) and thereby activates the retinoblastoma (Rb) family of proteins, which block traversal from G1 to S-phase. p14ARF (known as p19ARF in the mouse) activates the p53 tumor suppressor. Somatic mutations of CDKN2A are common in the majority of human cancers, with estimates that CDKN2A is the second most commonly inactivated gene in cancer after p53. Germline mutations of CDKN2A are associated with familial melanoma, glioblastoma and pancreatic cancer.[8] The *CDKN2A* gene also contains one of 27 SNPs associated with increased risk of coronary artery disease.[9]

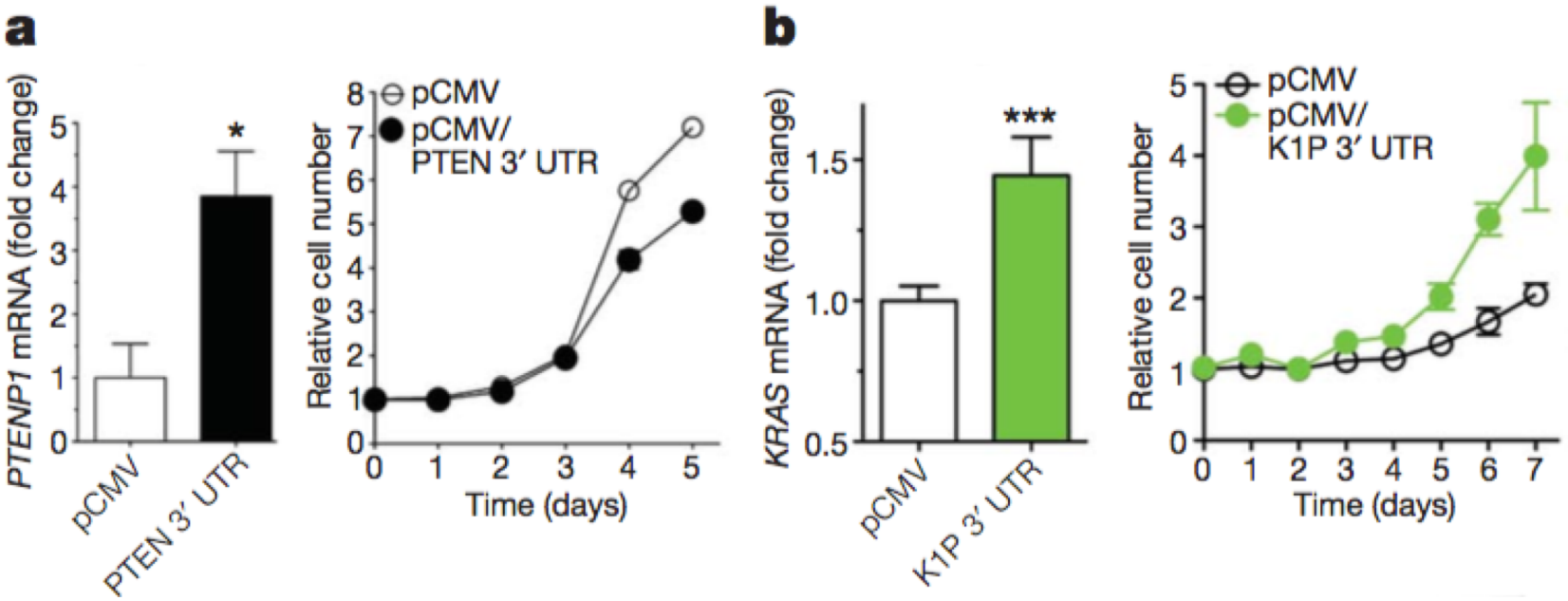
SAME HOLDS TRUE FOR OTHER CANCER RELEVANT GENE: KRAS, KRAS-P1 and miRNAs



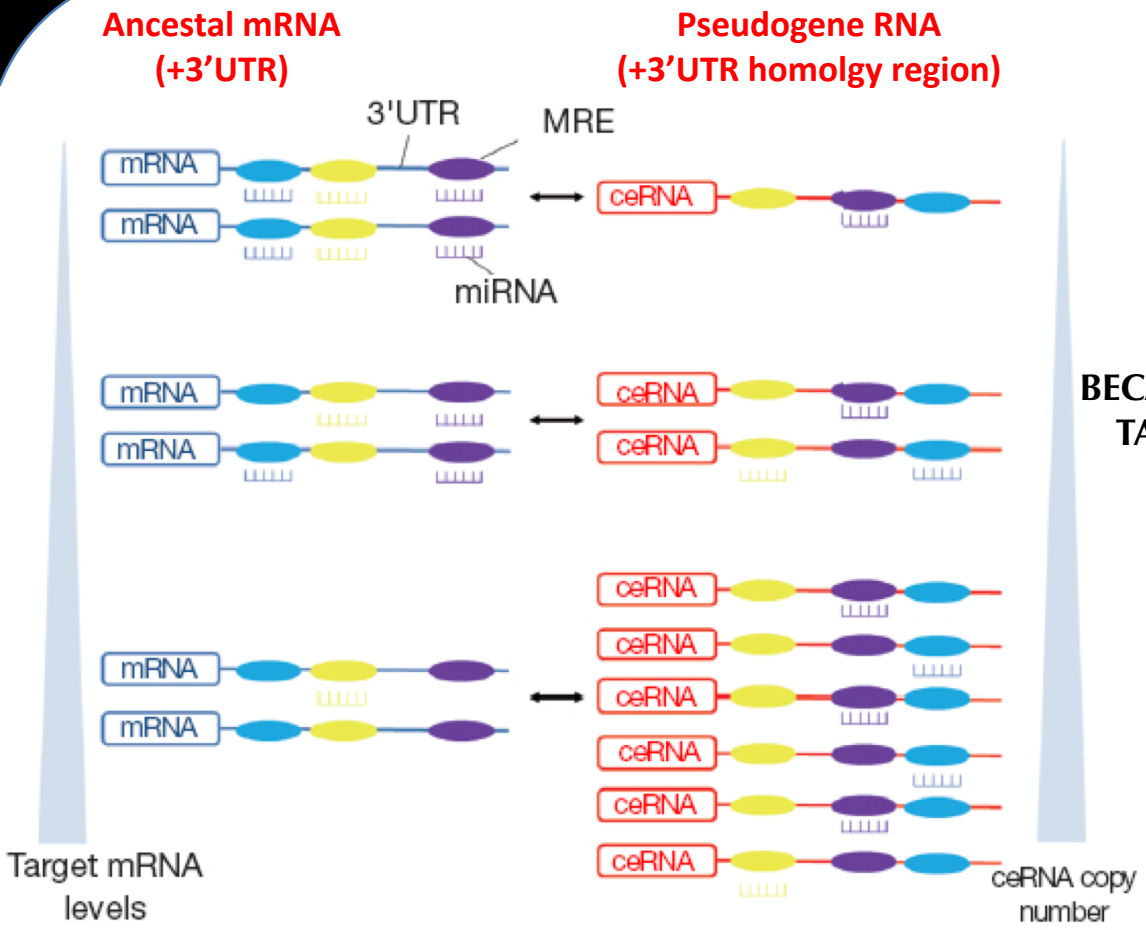
SAME HOLD TRUE FOR OTHER CANCER RELEVANT GENE: KRAS



Overexpression of KRAS1P 3'UTR increases KRAS mRNA expression



Pseudogene sponge miRNAs that target the ancestral gene



The model holds
true for all RNAs
that share a miRNA binding site
=ceRNAs

PSEUDOGENES ARE POTENT
BECAUSE THEY SHARE MORE THEN 1 miRNA
TARGET SITE WITH A CORRESPONDING
mRNA FROM AN ANCESTRAL GENE

Evolution of ncRNAs to fine-tune the expression of ancestral genes

2010: original discovery paper

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