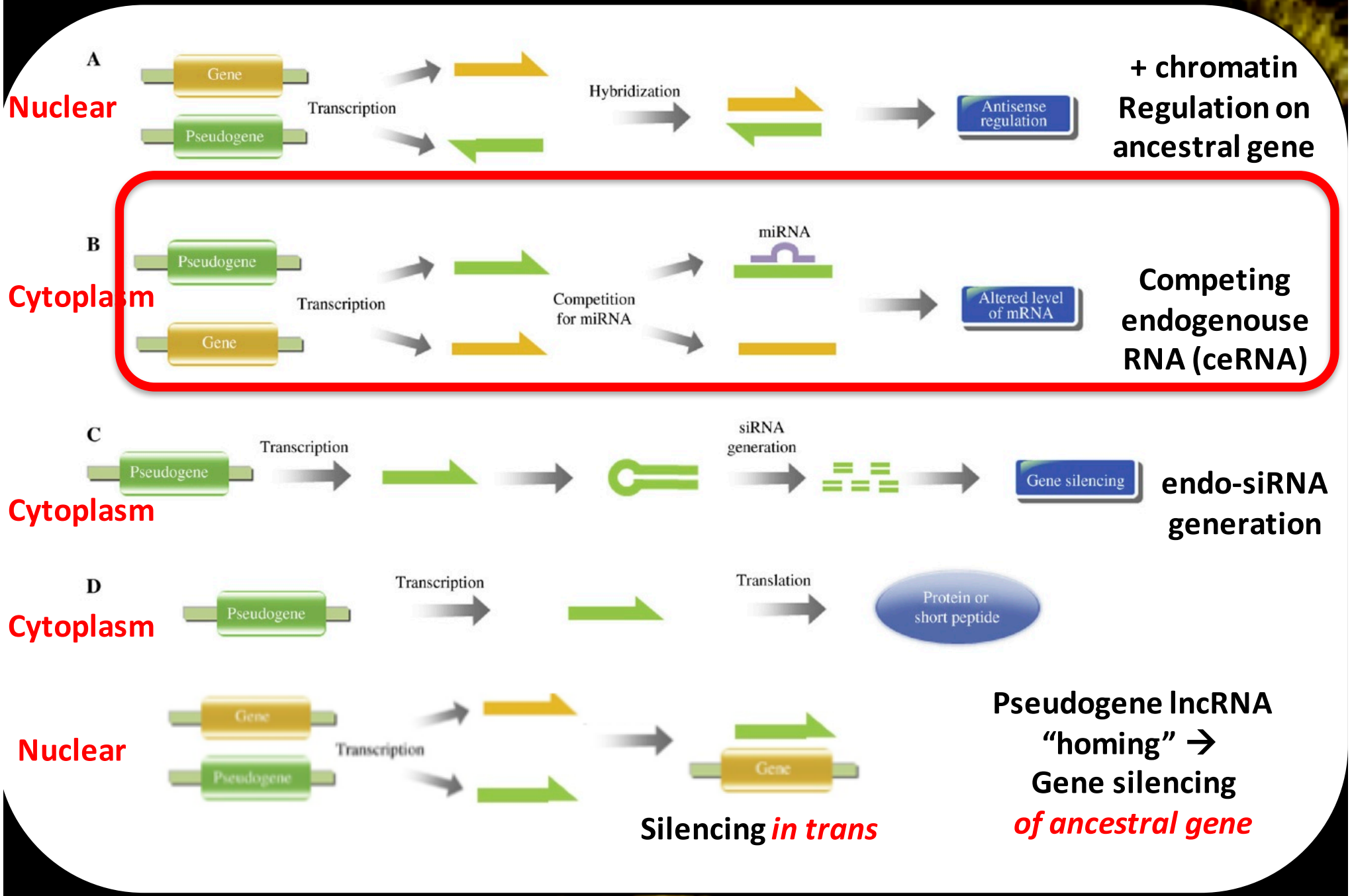
PSEUDOGENE lncRNAs

COMPETING ENDOGENOUS RNAs (ceRNAs)

ceRNAs derived from pseudogenes
PTENP1

seudogenes are powerful regulators of gene expressio



Small ncRNA and gene/chromatin regulation

micro-RNAs = miRNAs

short interfering RNAs = siRNAs

miRNAs and siRNAs are generated by the same machinery

1. Long precursor dsRNA or stem loop RNA **(pri-miRNA)**
   note: pre-miRNA: loop RNA cleaved off by Drosha in nucleus

2. Processing into small RNAs by Dicer (still double-stranded)

   - production of siRNAs
   - pre-miRNA processed to mature miRNAs (21-23 nt)

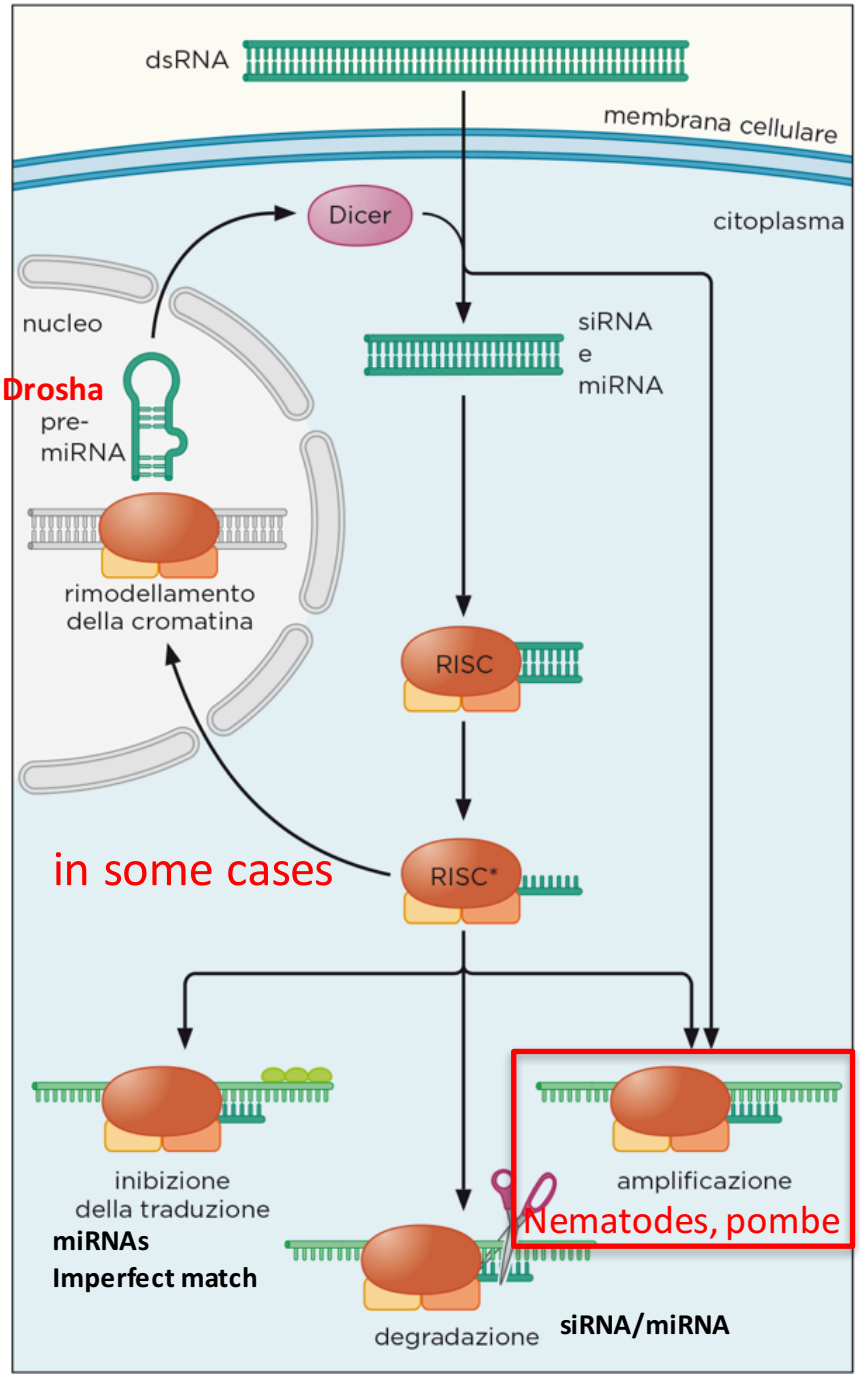
3. Processing by RISC complex
   (RNA induced silencing complex)

4. guide RNA → regulatory RNA
   passenger RNA → will be eliminated

5. 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

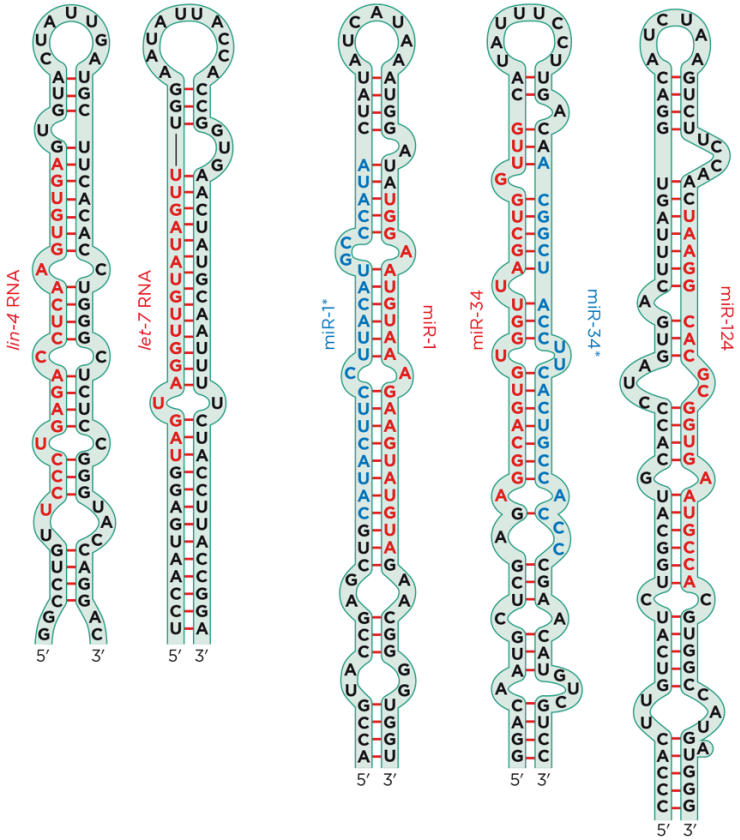
miRNAs: always "trans"-acting on mRNAs
siRNAs: mostly "cis" acting on chromatin (S-pombe)



miRNA dependent regulation of gene expression

One strand generates miRNA

both strand Generate miRNA



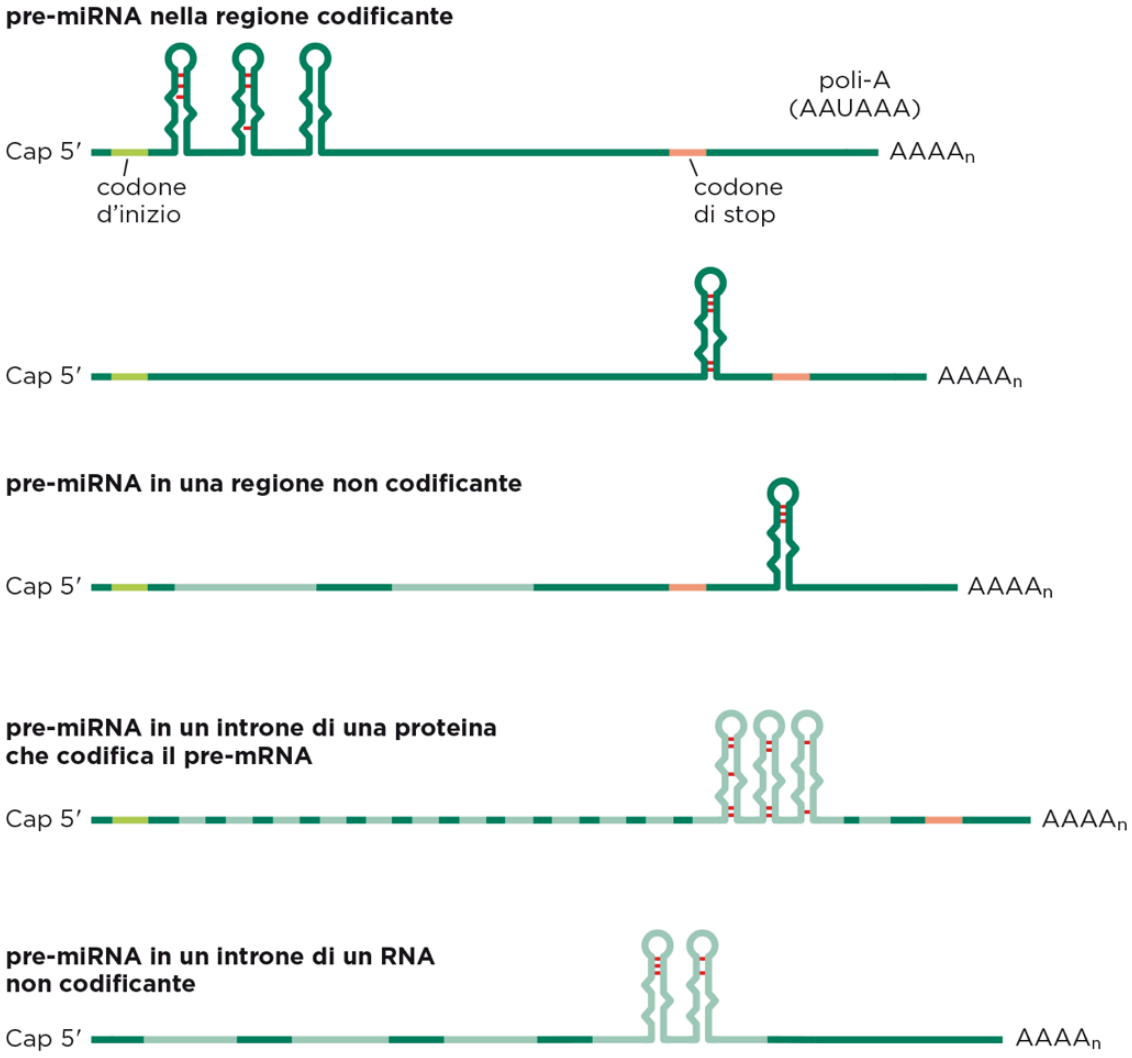
pri-miRNA (primary miRNA

↓ **Drosha**

pre-miRNA (precursor miRNA)

↓ **Dicer**

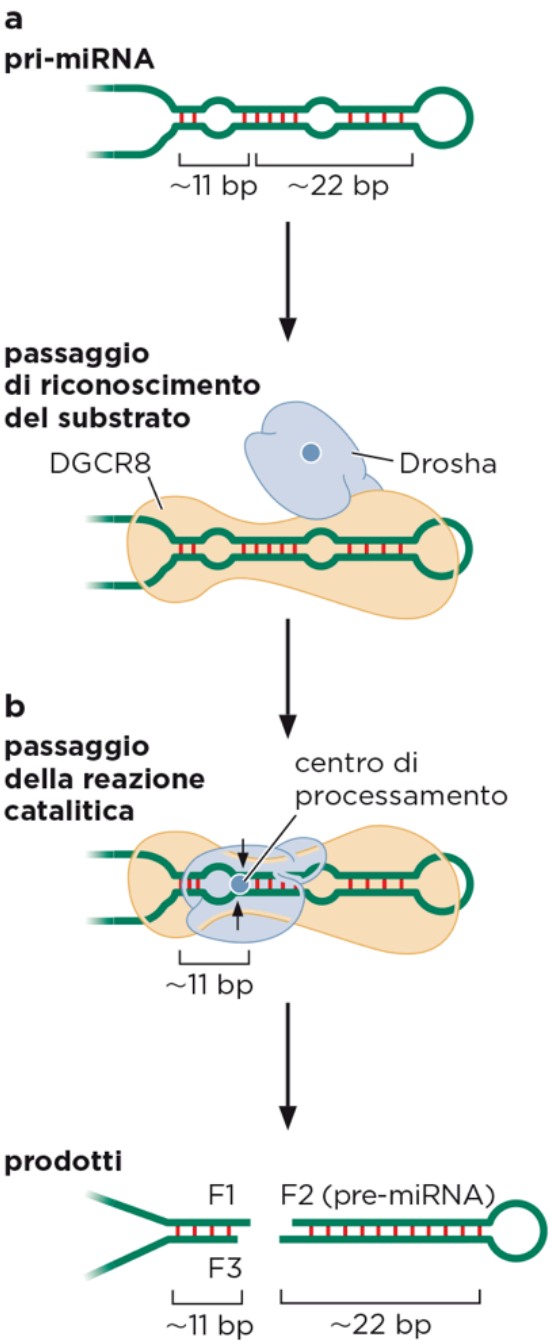
miRNA (mature miRNA)

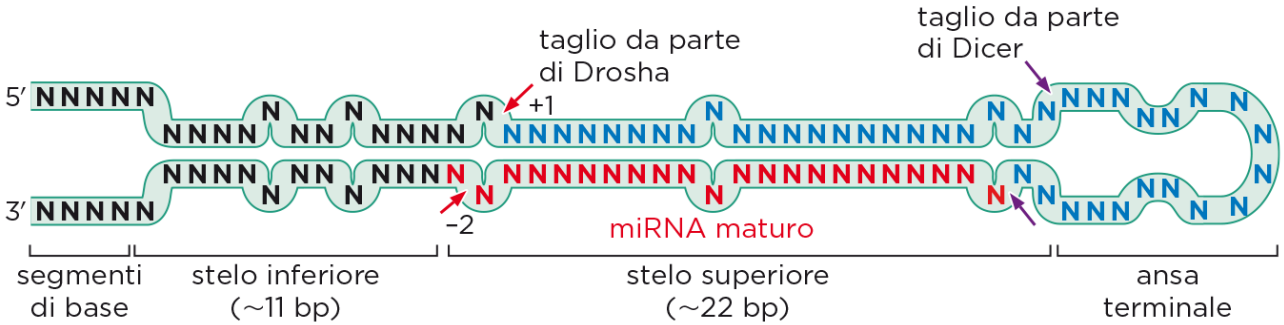


Pre-miRNA in esone di un lncRNA

or separate short gene

miRNA generation - DROSHER





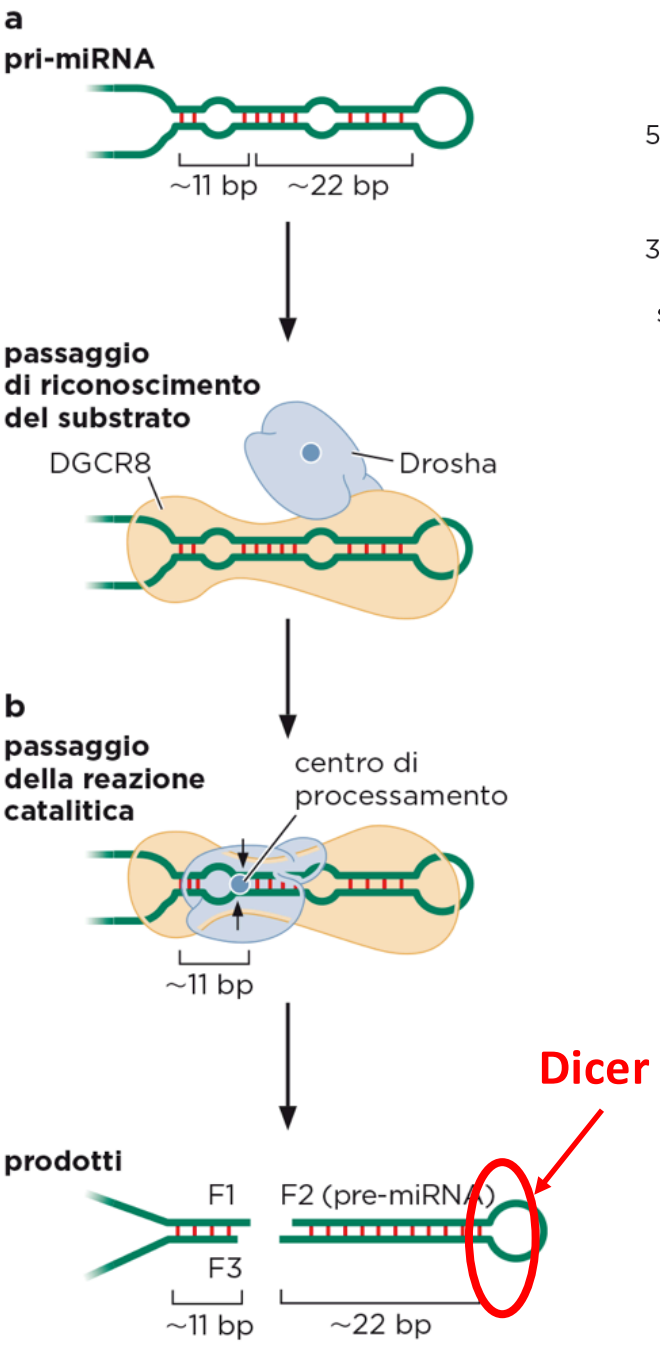
Drosha, Dicer: **Type III RNases**: cut 2 RNA strands in RNA duplex, leave overhang!!

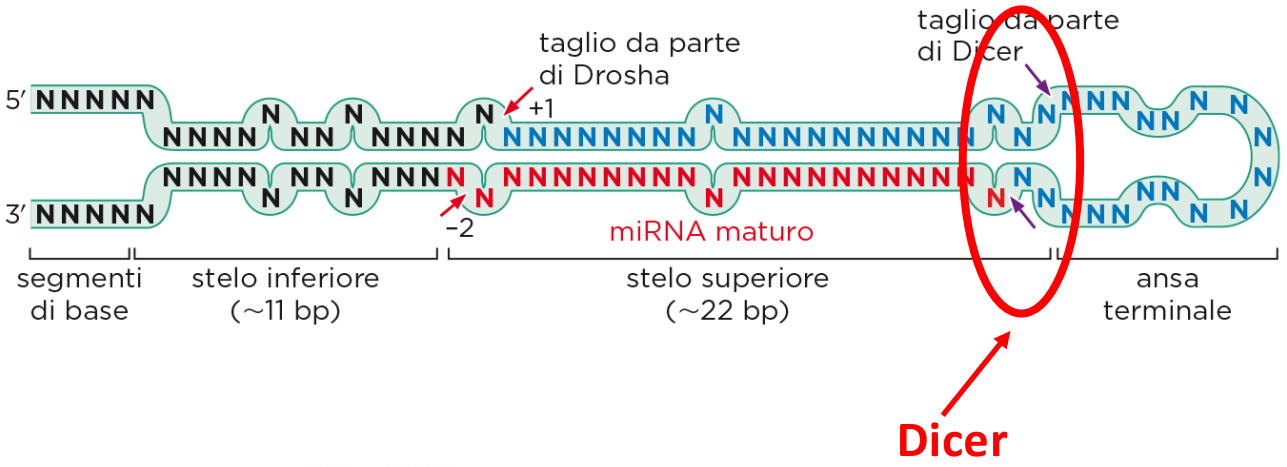
1. Microprocessor (Drosha and DGCR8) generates a 65-70 nt RNA stem loop:

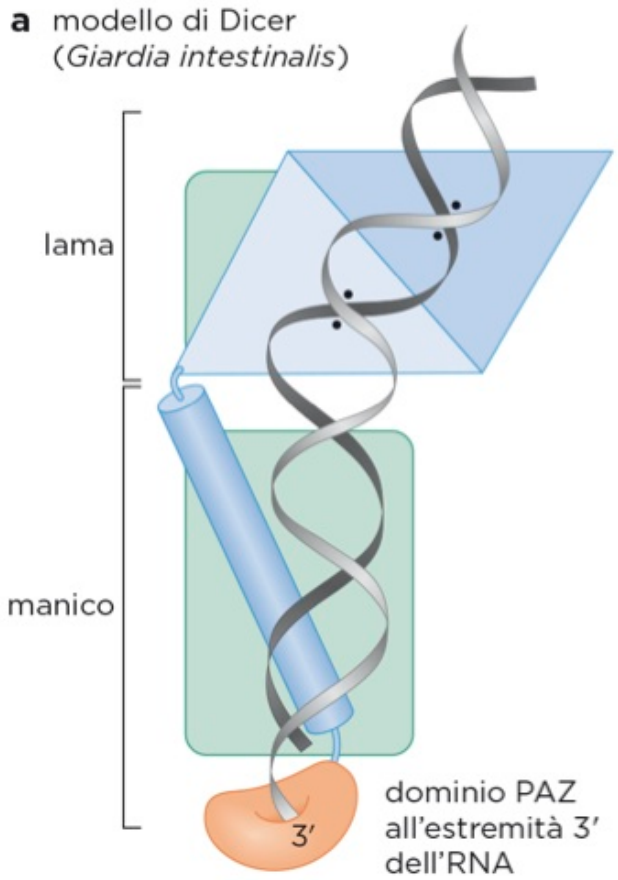
Drosha cuts app. 11 nt after start of dsRNA
5 components: stelo inferiore (11 bp); stelo superiore (22 nt)
ansa terminale; segmenti di base

2. Transfer to cytoplasma

miRNA generation - DICER







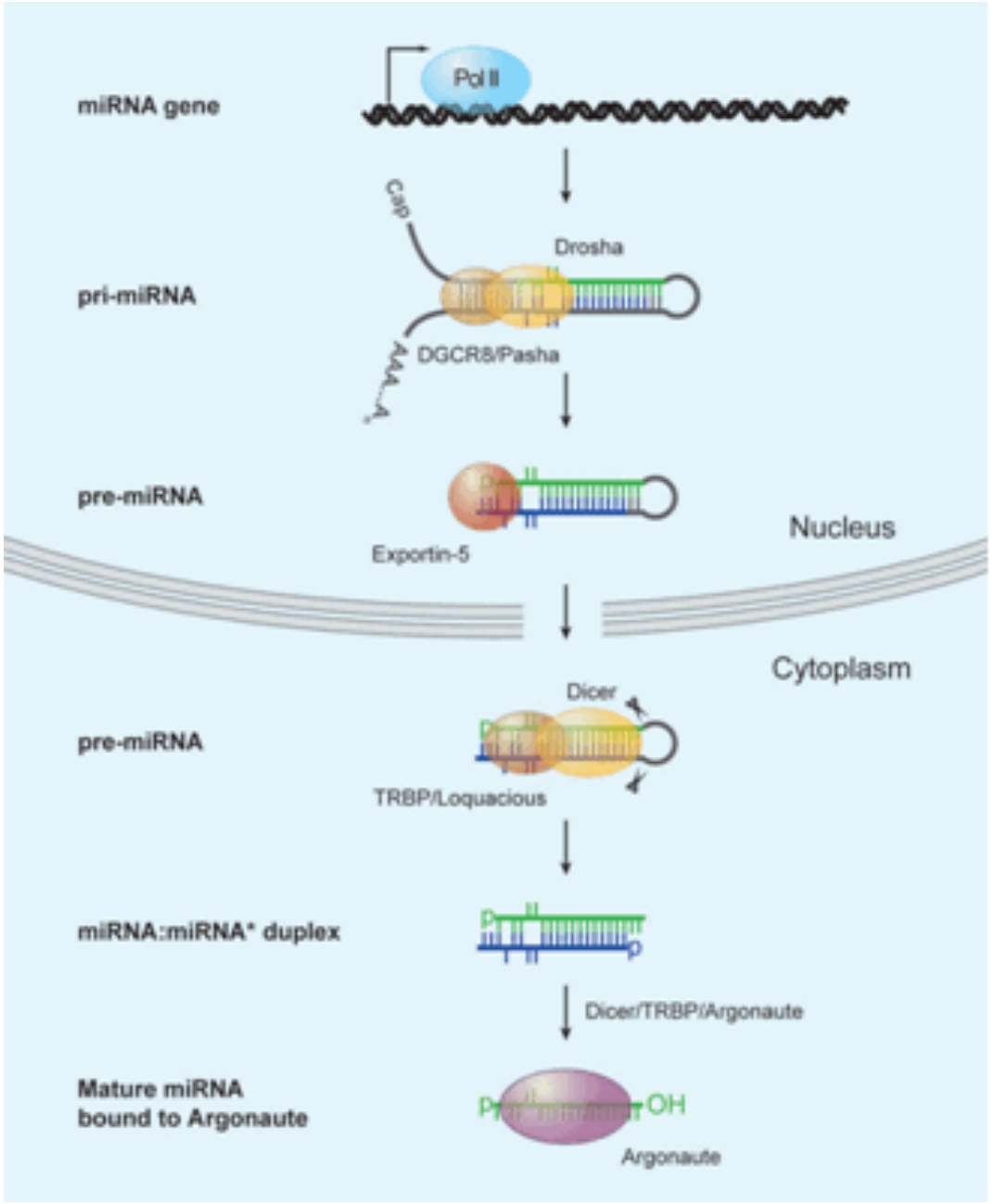
DICER processes all dsRNAs (NOT SEQUENCE SPECIFIC)

2 RNase domains: act on ALL dsRNAs

manico interacts with dsRNA

PAZ interacts with dsRNA

-5p or -3p miRNA
i.e. miR-296-5p
miR-296-3p



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

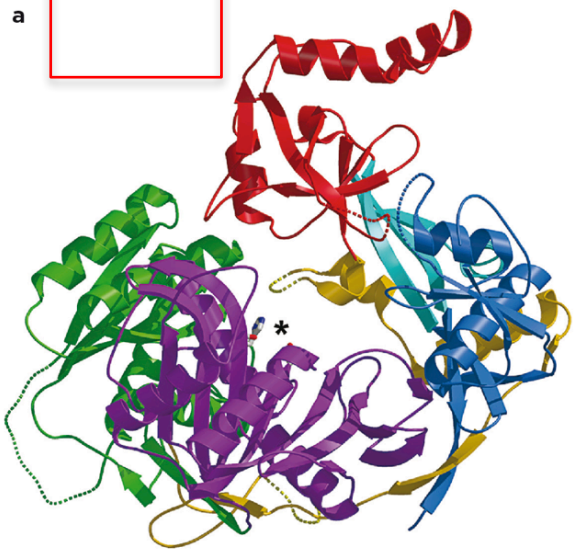
55243 reads, 2.94e+03 reads per million, 62 experiments

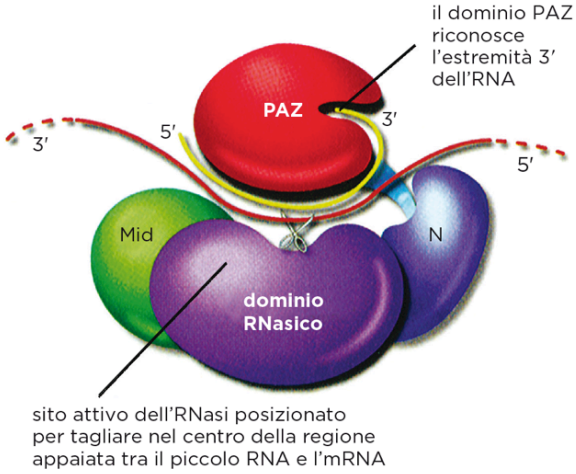
Regulation of gene expression by miRNAs

```
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----GU
```

a b

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





One strand of pre-miRNA is incorporated into the RISC complex (RNA induced Silencing complex) = guide strand
Passenger strand degraded by RISC complex

Base pairing miRNA/siRNA – target RNA
(seed sequence in miRNA is most important for target identification

RNAse domain cleaves target transcript OR translational repression

Regulation of gene expression by miRNAs

Gene regulation

RISC uses the bound guide strand to target complementary 3'-untranslated regions (3'UTR) of mRNA transcripts via Watson-Crick base pairing. RISC can now regulate gene expression of the mRNA transcript in a number of ways.

mRNA degradation

The most understood function of RISC is degrading target mRNA which reduces the levels of transcript available to be translated by ribosomes. There are two main requirements for mRNA degradation to take place:

a near-perfect complementary match between the guide strand and target mRNA sequence, and,
a catalytically active Argonaute protein, called a 'slicer', to cleave the target mRNA.
mRNA degradation is localised in cytoplasmic bodies called P-bodies.

Translational repression

RISC can modulate the loading of ribosome and accessory factors in translation to repress expression of the bound mRNA transcript. Translational repression only requires a partial sequence match between the guide strand and target mRNA.

Translation can be regulated at the initiation step by:
preventing the binding of the eukaryotic translation initiation factor (eIF) to the 5' cap. It has been noted RISC can de-adenylate the 3' poly(A) tail which might contribute to repression via the 5' cap.
preventing the binding of the 60S ribosomal subunit binding to the mRNA can repress translation.
Translation can be regulated at post-initiation steps by:
-promoting premature termination of translation ribosomes, or,
-slowing elongation.
There is still speculation on whether translational repression via initiation and post-initiation is mutually exclusive.

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

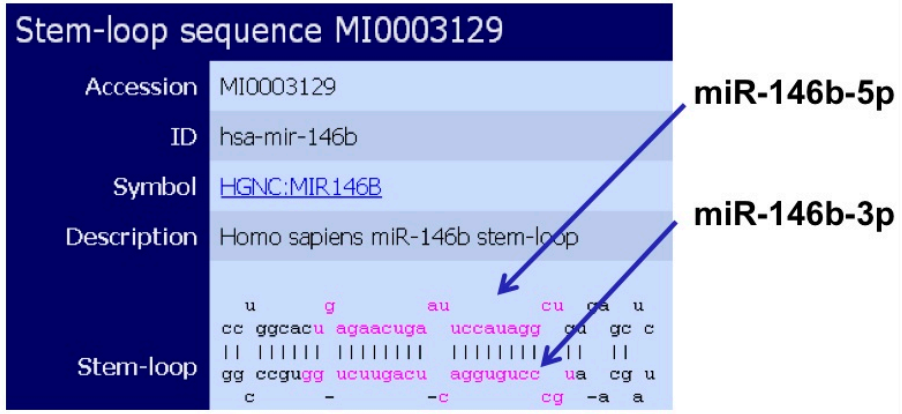
Alleles: all express same mature microRNA

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

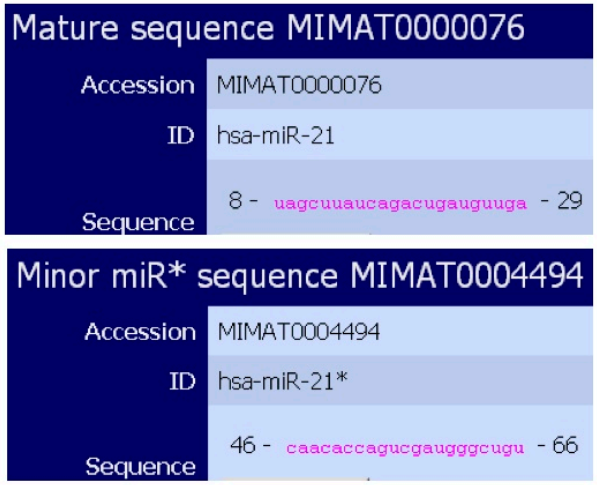
} **Mature miR-7 microRNA expressed**

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]

BACKGRUND 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

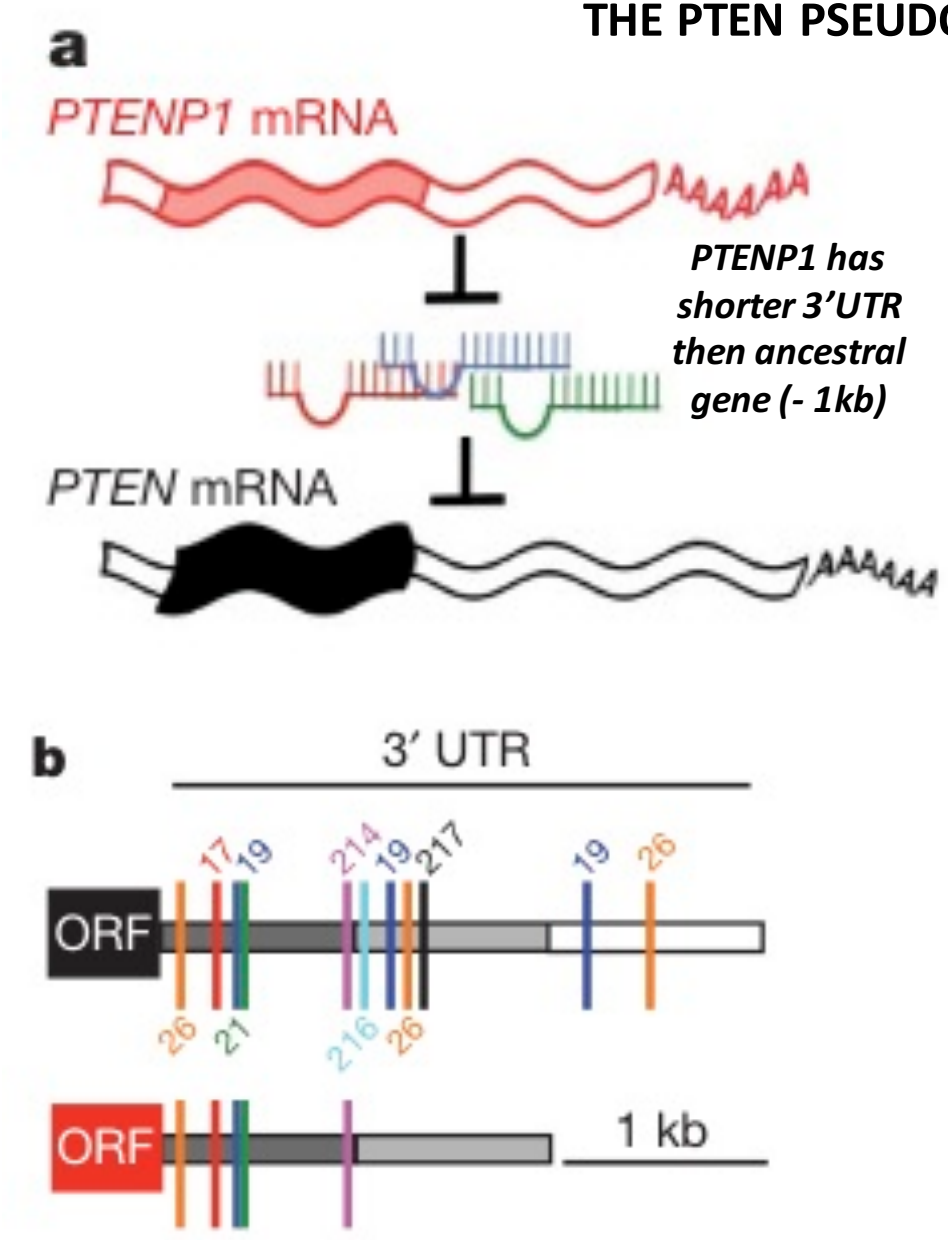
PTEN has generated one processed pseudogene: PTENP1
highly conserved to PTEN

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)

Seed region

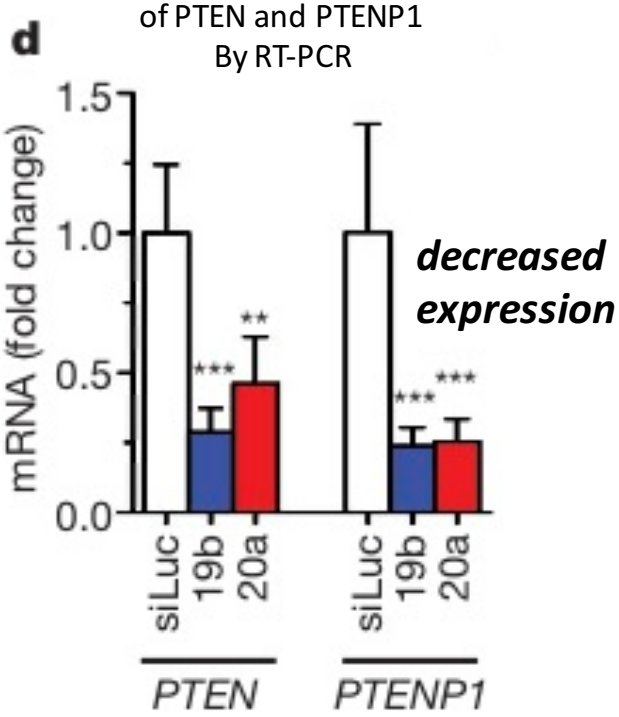
```
5′ GGAUUAAUAAAGAUGGCACUUU   3′ PTEN
   ||| ||  |  ||||||||
3′ GAUGGACGUGAUAUUCGUGAAAU  5′ miR-20a
   ||| ||  |  ||||||||
5′ GGAUUAAUAAAGAUGGCACUUU   3′ PTENP1

5′ UUCACAUCCUACCCCUUUGCAC   3′ PTEN
   ||  |  ||  ||||||||
3′ AGUCAAAACGUACCUAAACGUGU  5′ miR-19b
   ||  |  ||  ||||||||
5′ UUCACAUCAUACCCCUUUGCAC   3′ PTENP1

5′ ACUUGUGGCAACAGAUAAGUU    3′ PTEN
   |      | |||||||||
3′ AGUUGUAGUCAGACUAUUCGAU   5′ miR-21
   |      | |||||||||
5′ ACUUGUGGCAACAGAUAAGUU    3′ PTENP1

5′ ACACCAUGAAAAUAAACUUGAA   3′ PTEN
   |  | ||      ||||||||
3′ UCGGAUAGGACCUAAUGAACUU   5′ miR-26a
   |  | ||      ||||||||
5′ ACACCAUGAAAACAAACUUGAA   3′ PTENP1

5′ UUUCAAUCAUAAUACCUGCUG    3′ PTEN
   ||  |  |  |  ||||||||
3′ UGACGGACAGACACGGACGACA   5′ miR-214
   ||  |  |  |  ||||||||
5′ UUUCAAUCAUA-UACCUGCUG    3′ PTENP1
```

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

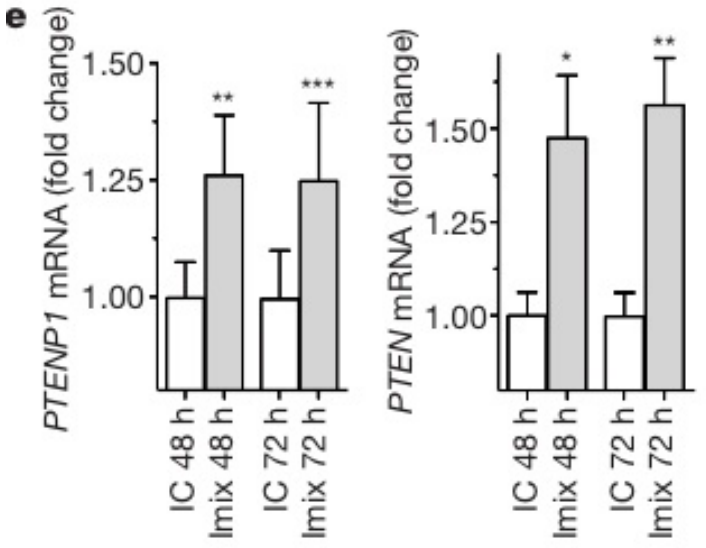
Check expression levels of PTEN and PTENP1 By RT-PCR



decreased expression

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

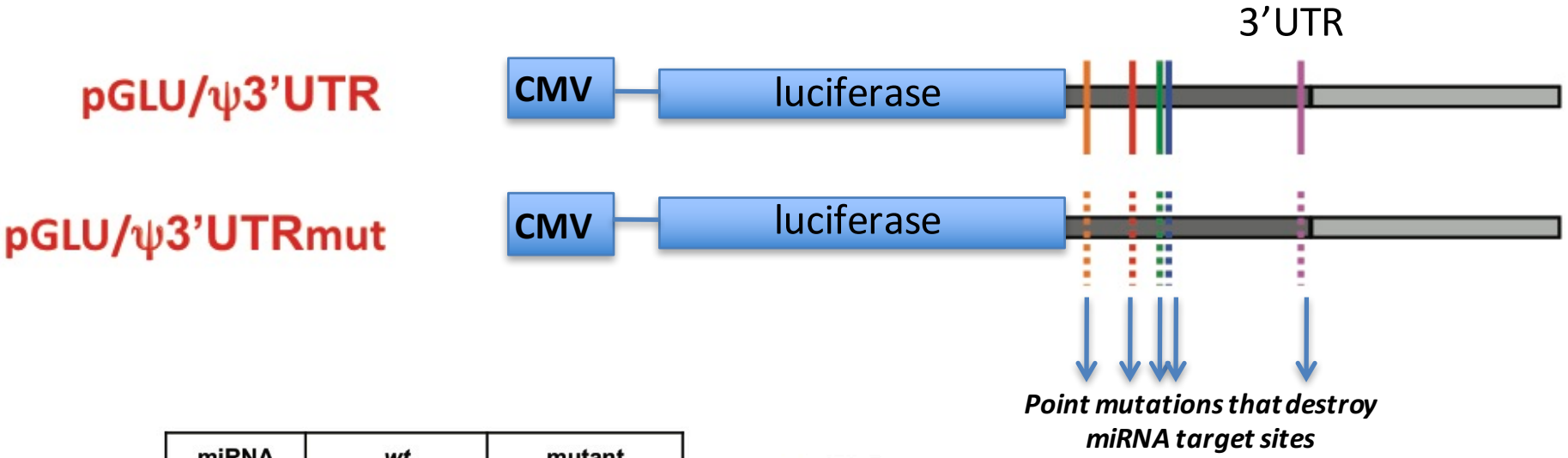
Check expression levels of PTEN and PTENP1 By RT-PCR



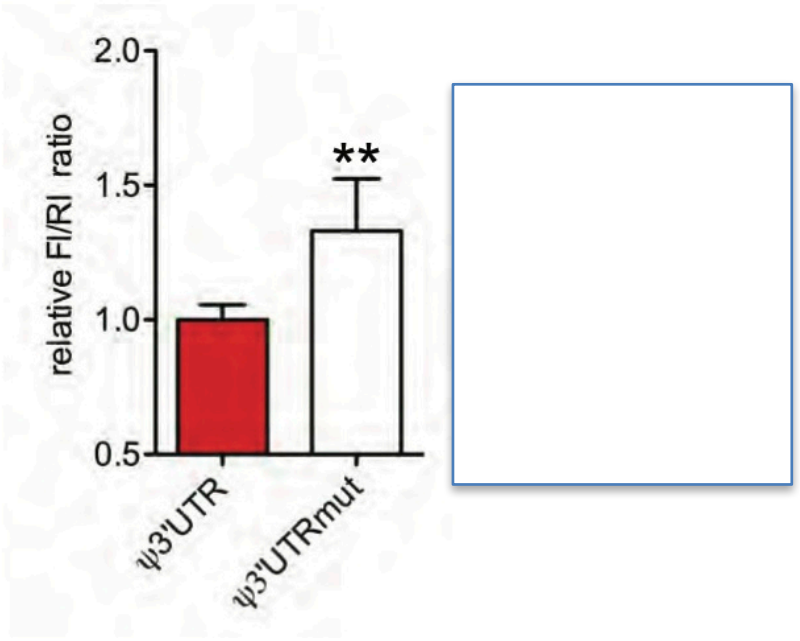
increased expression

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

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

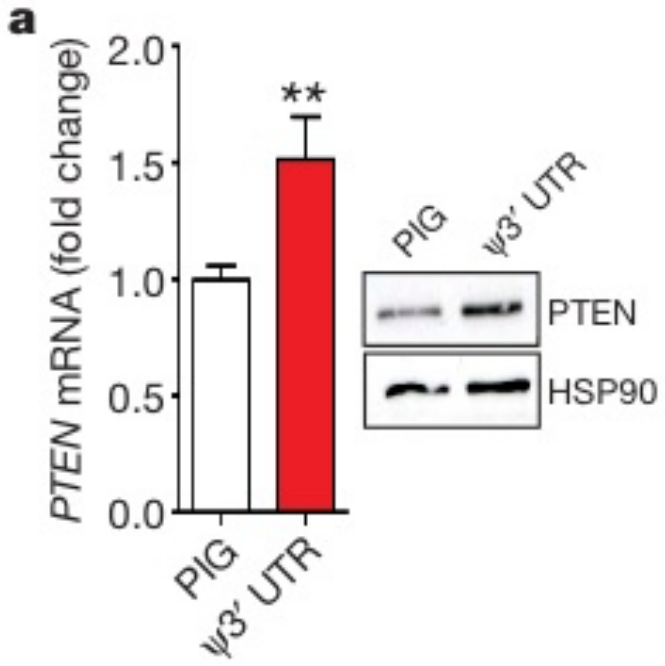


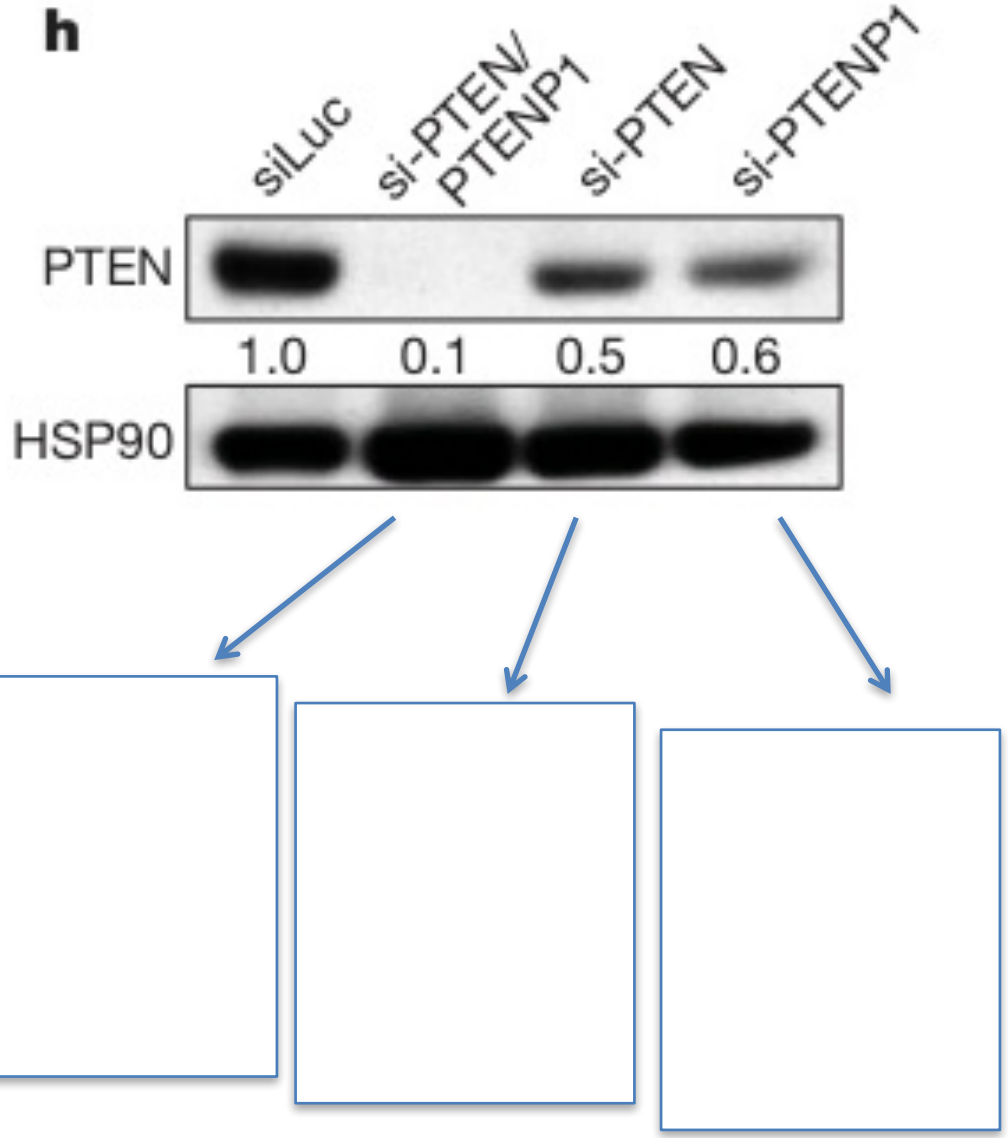
miRNA family	*wt* seed match	mutant seed match
17	GGCACTTT	GcCtCaTa
19	TTTGCAC	aTaGgAg
21	GATAAGTT	GtTtAcTt
26	ACTTGAA	tCaTcAt
214	CCTGCTG	gCaGgTc



The 3'UTR of PTENP1 sequesters miRNAs

PTENP1 CONTROLS THE EXPRESSION OF PTEN

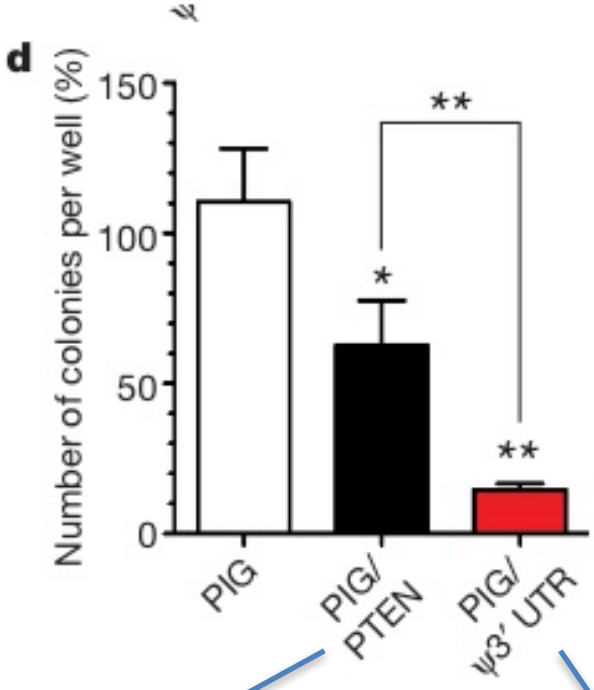


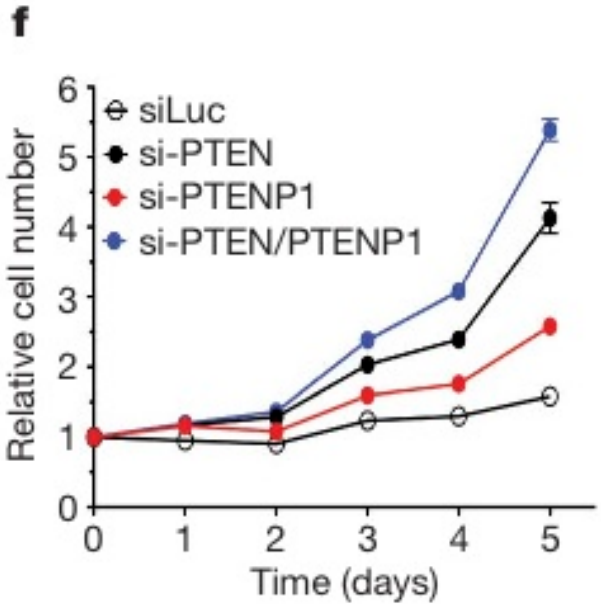


PTENP1 enhances tumorsuppression by PTEN

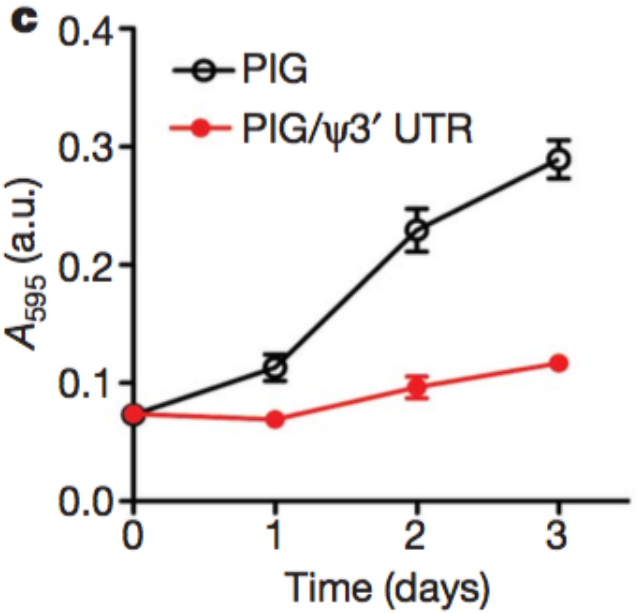
Colony forming assay:
Plate cells at low density
count numbers of colonies
formed by seeded cells
(aggressive cancer cell
form many colonies; cell
with active tumorsuppression
form low number of colonies)

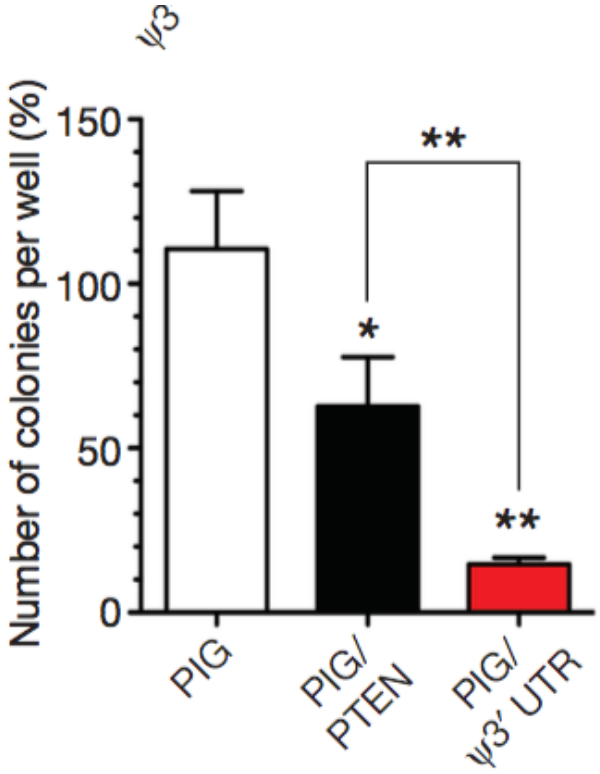
Cumulative cell numbers:
Cancer cells proliferate quickly;
cells with tumorsuppression
proliferate at low rates





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





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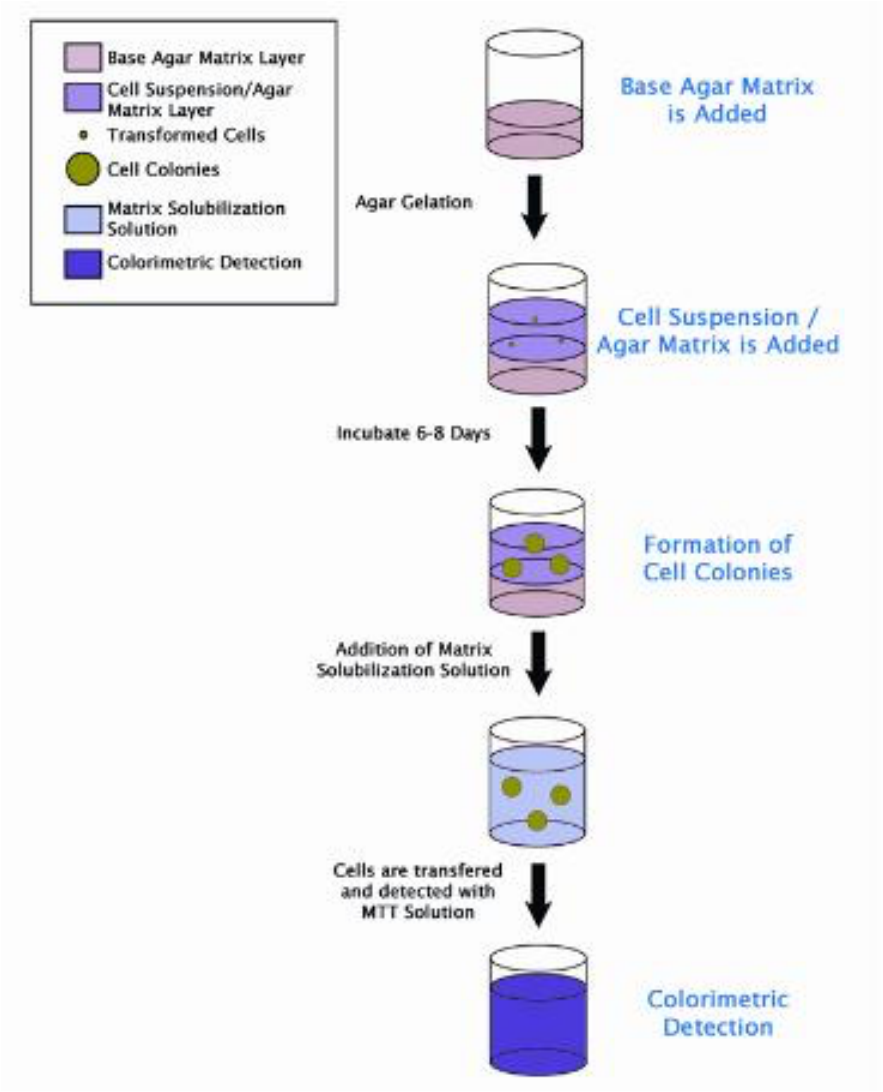
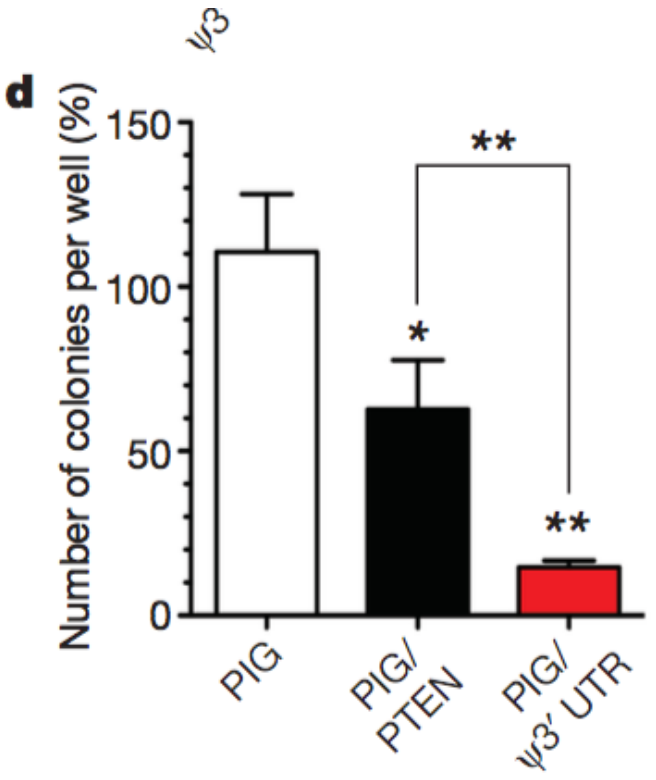
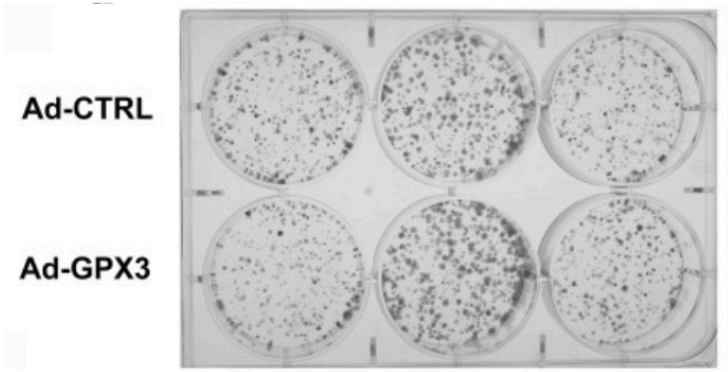
Cell proliferation (normal)

Colony formation in semi-solid medium

Anchorage independent cell proliferation – colony formation assay

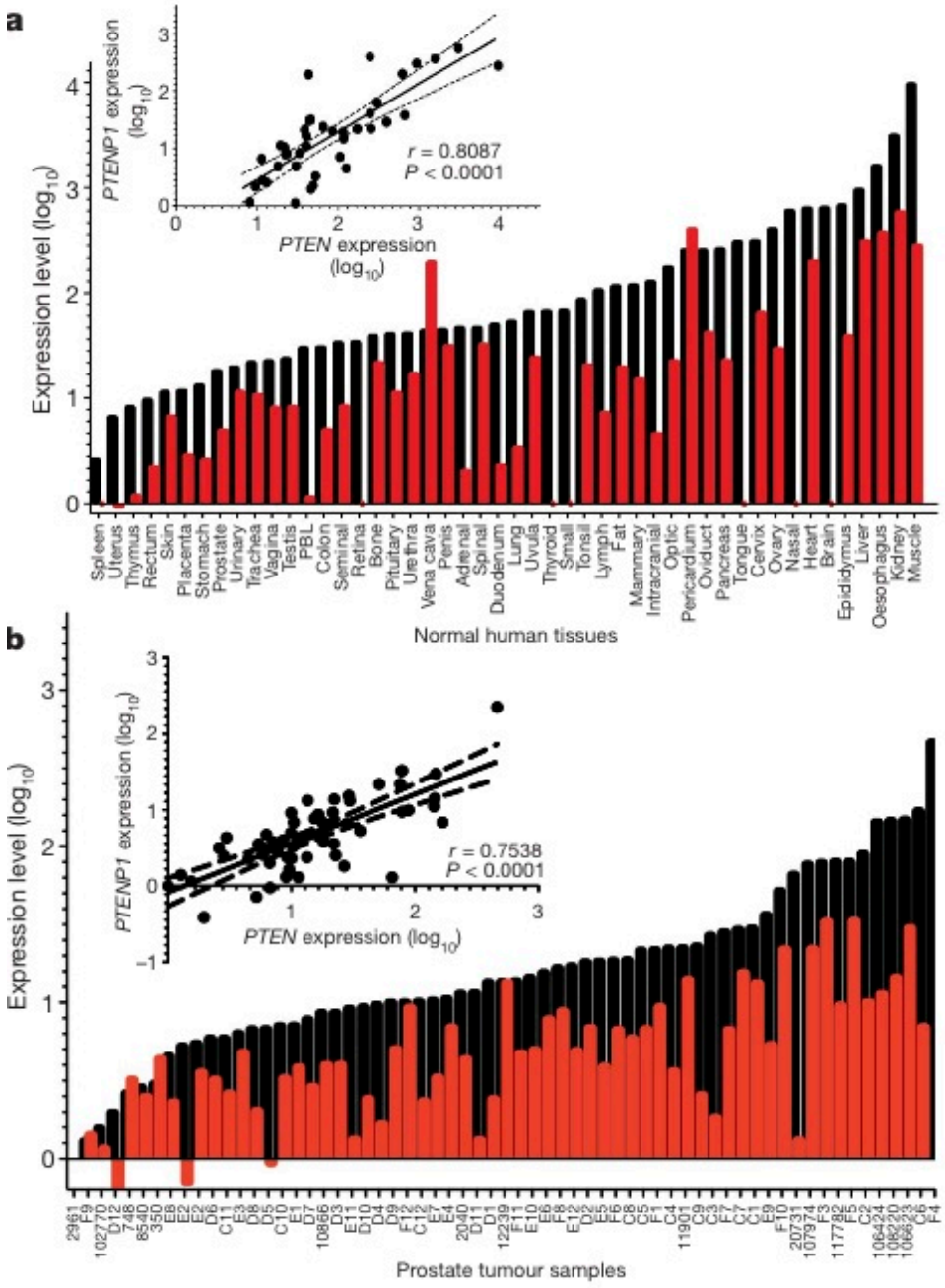






An example

RELEVANCE IN VIVO (HEALTHY AND CANCER)?

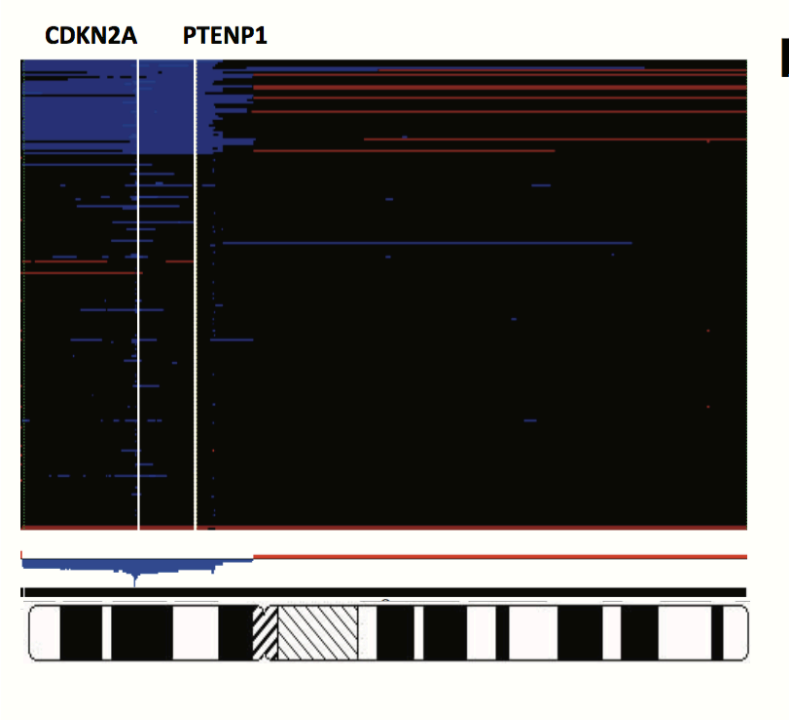


PTEN mRNA expression
Positively correlates with
PTENP1 expression:

= cells with good tumorsuppression
By PTEN expression also
Increase PTENP1 expression
→Increased sponging of miRNAs
→Even more PTEN

RELEVANCE IN HUMAN CANCER????

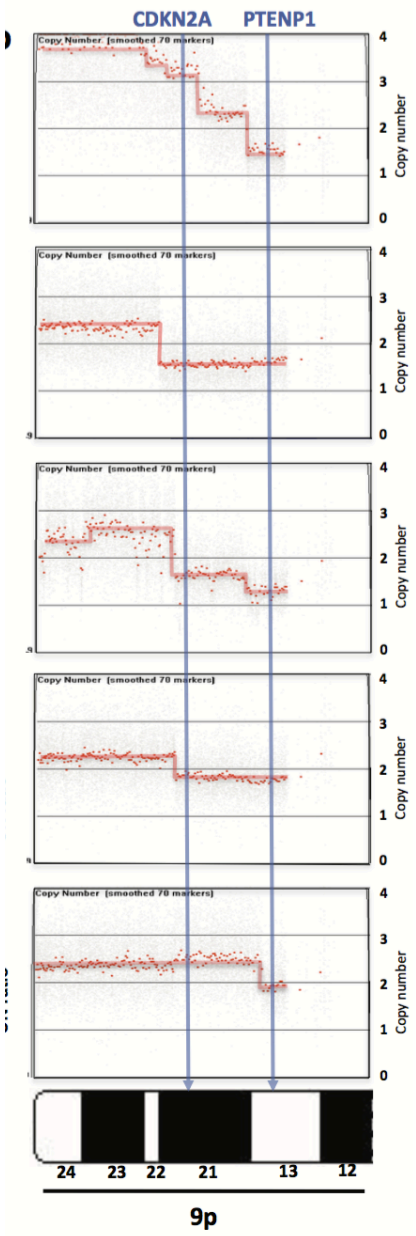
ACUTE LYMPHOBLASTIC LEUKEMIA



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

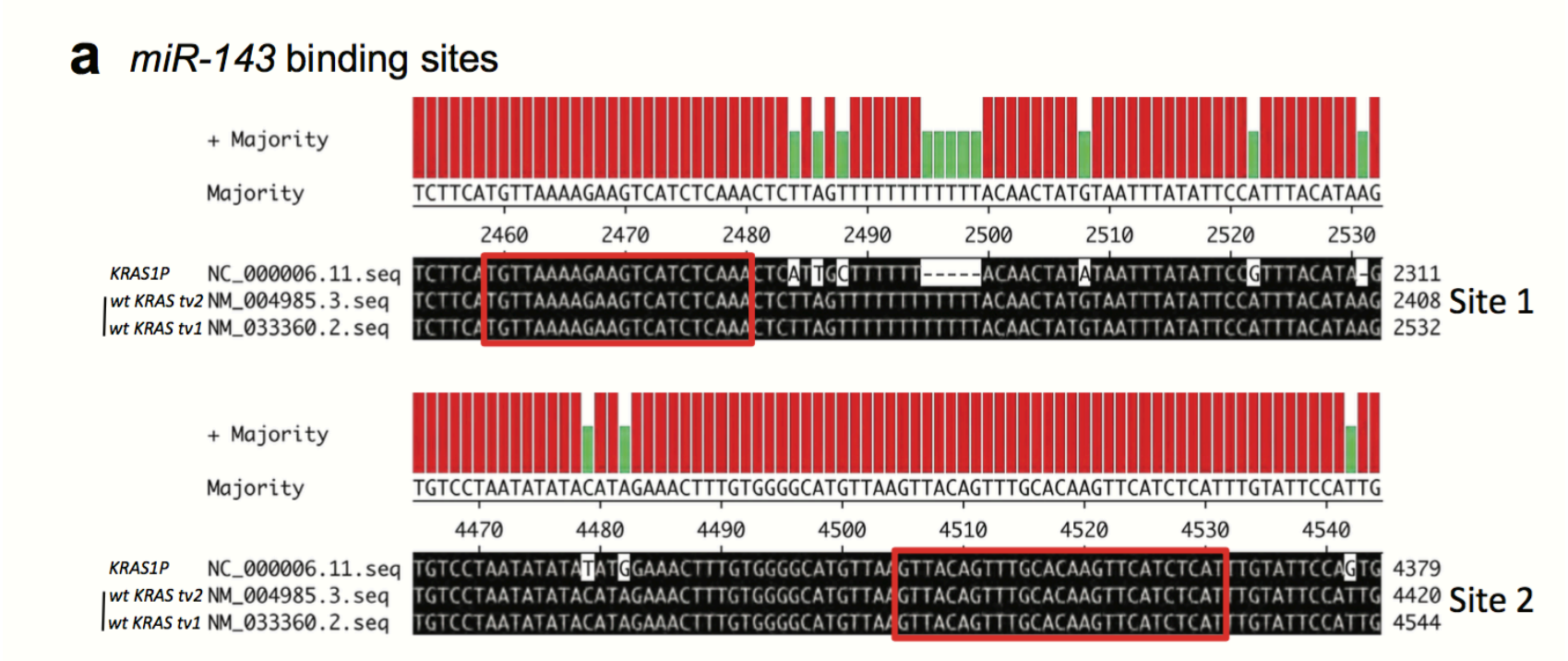


Red line: interesting genes with Copy number alteration

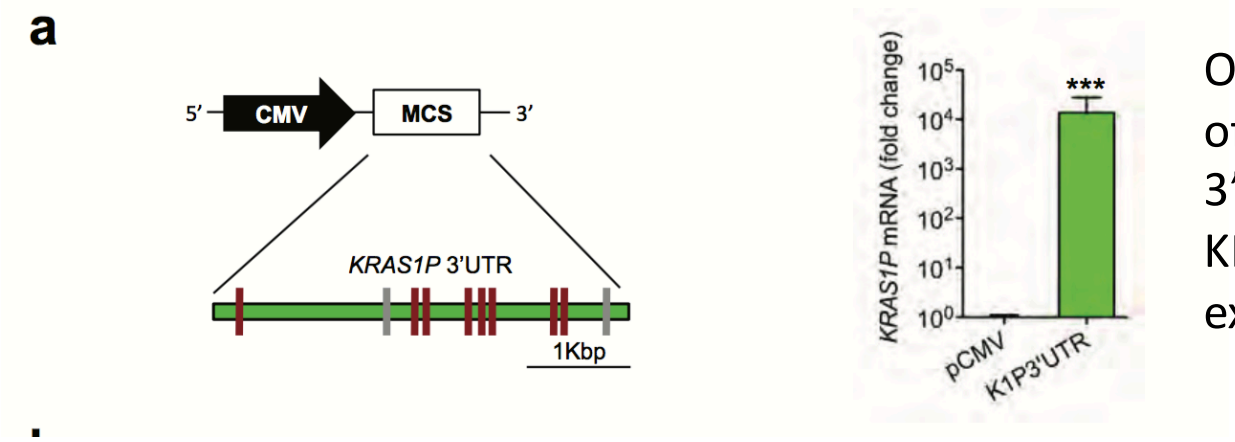
b. Examples of five specific breast cancer patient samples demonstrating losses at the *PTENP1* locus. The graphs were generated using Partek Genomics Suite. X-axis represents chromosome 9p position and Y-axis represents copy number. The red lines highlight regions of gene loss. **c.**

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]

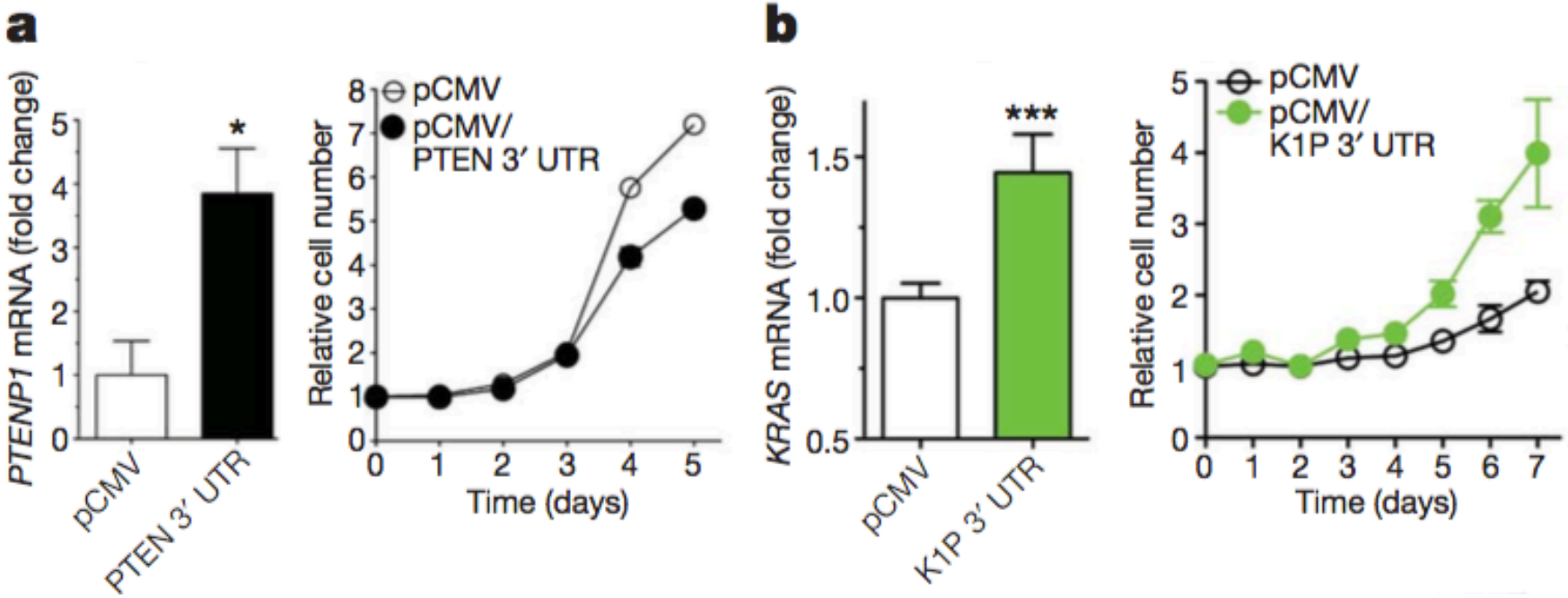
SAME HOLDS TRUE FOR OTHER CANCER RELEVANT GENE: KRAS, KRAS-P1 and miRNAs



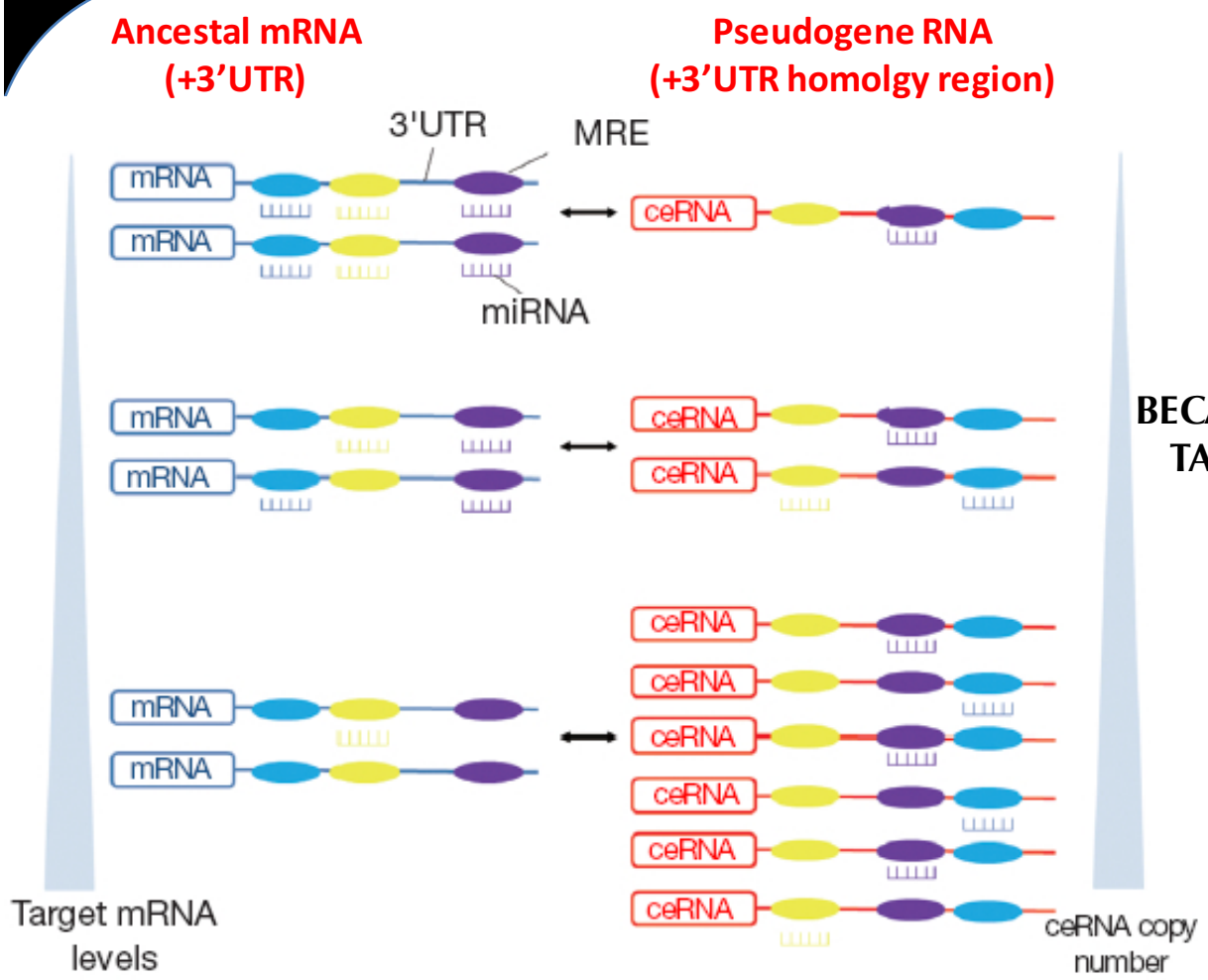
SAME HOLD TRUE FOR OTHER CANCER RELEVANT GENE: KRAS



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

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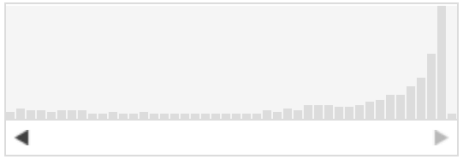
1. Identification and integrated analysis of differentially expressed lncRNAs and circRNAs reveal the potential **ceRNA** networks during PDLSC osteogenic differentiation.
Gu X, Li M, Jin Y, Liu D, Wei F.
BMC Genet. 2017 Dec 2;18(1):100. doi: 10.1186/s12863-017-0569-4.
PMID: 29197342

2. Long noncoding RNA CCAT1 functions as a **ceRNA** to antagonize the effect of miR-410 on the down-regulation of ITPKB in human HCT-116 and HCT-8 cells.
Li B, Shi C, Zhao J, Li B.
Oncotarget. 2017 Oct 7;8(54):92855-92863. doi: 10.18632/oncotarget.21612. eCollection 2017 Nov 3.
PMID: 29190961 **Free Article**

3. Long non-coding RNA UICLM promotes colorectal cancer liver metastasis by acting as a **ceRNA** for microRNA-215 to regulate ZEB2 expression.
Chen DL, Lu YX, Zhang JX, Wei XL, Wang F, Zeng ZL, Pan ZZ, Yuan YF, Wang FH, Pelicano H, Chiao PJ, Huang P, Xie D, Li YH, Ju HQ, Xu RH.
Theranostics. 2017 Oct 17;7(19):4836-4849. doi: 10.7150/thno.20942. eCollection 2017.
PMID: 29187907 **Free Article**

4. Serum Vitamin D Status, Vitamin D Receptor Polymorphism, and Glucose Homeostasis in Healthy Subjects

Results by year



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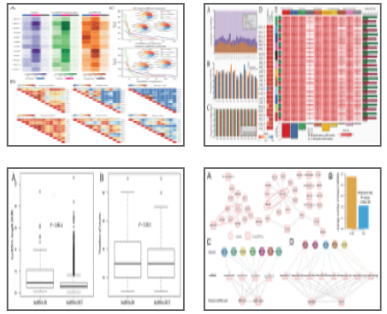
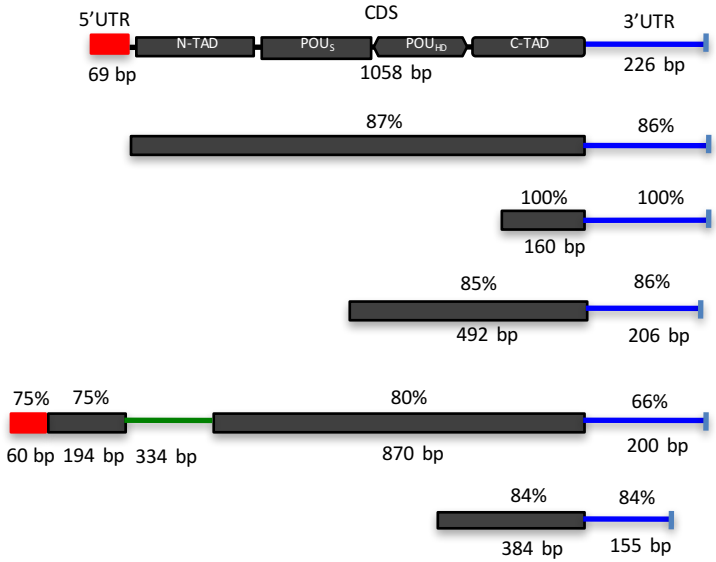
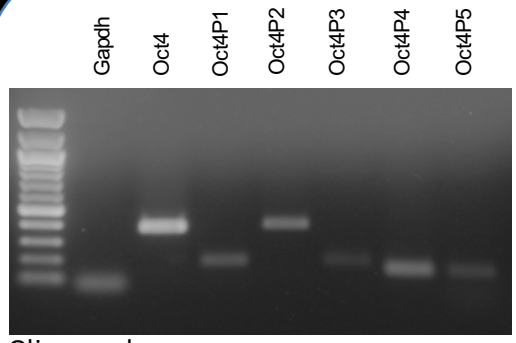
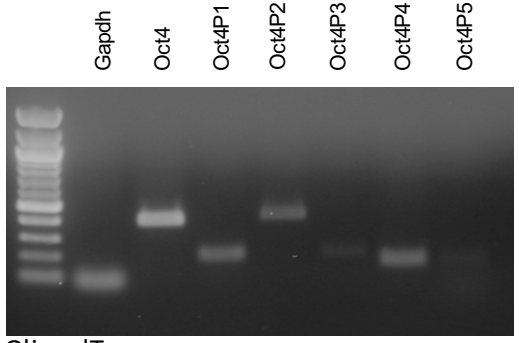


Ancestral OCT4 gave rise to 5 processed pseudogenes that are expressed in mESCs



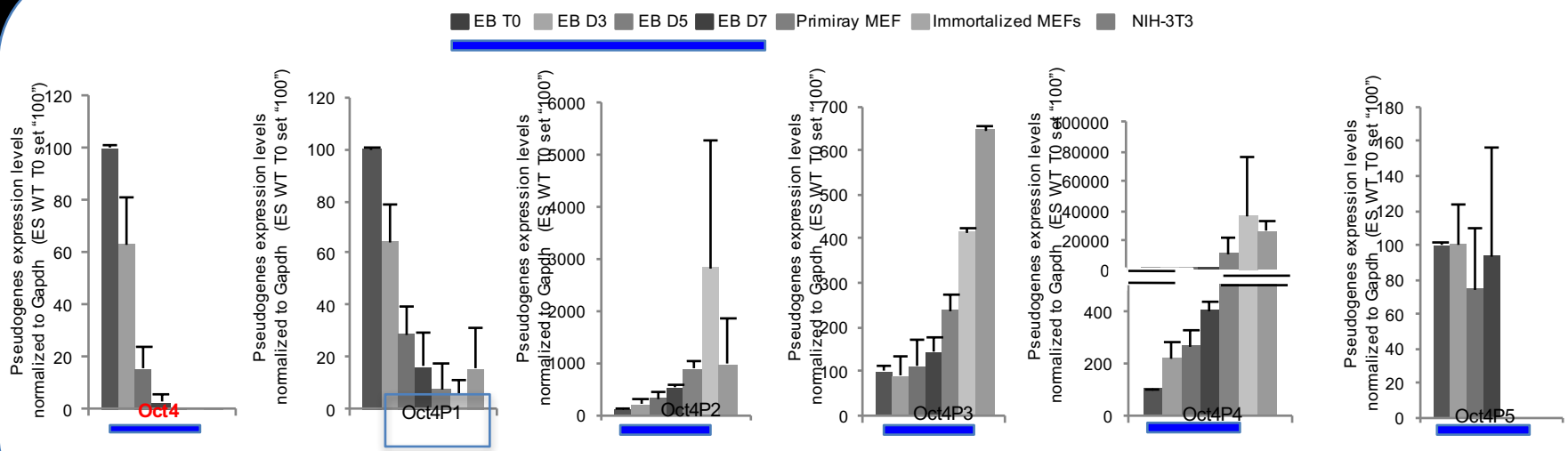


Oligo random

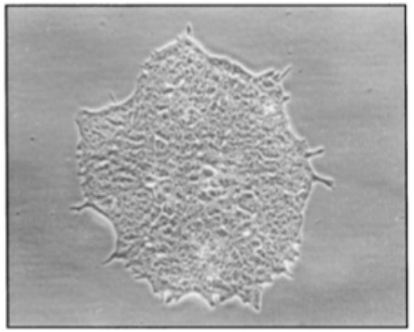


Oligo dT

Oct4 pseudogenes are tightly controlled during the differentiation of mESCs

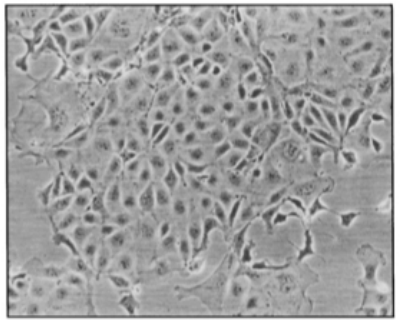


SELF-RENEWAL



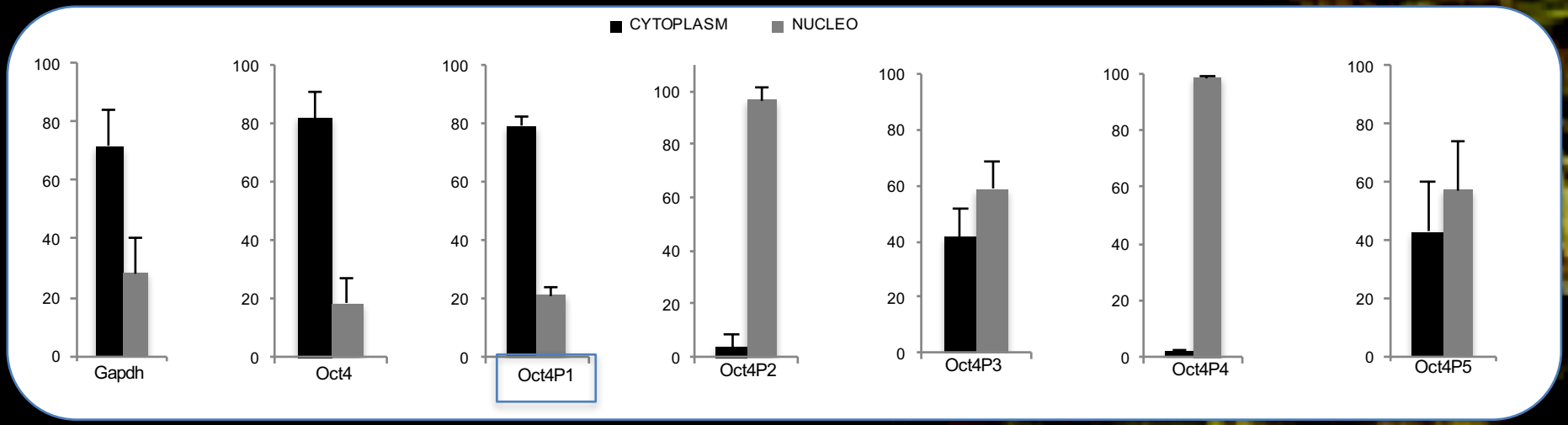
Oct4
Oct4P1 (-10X)

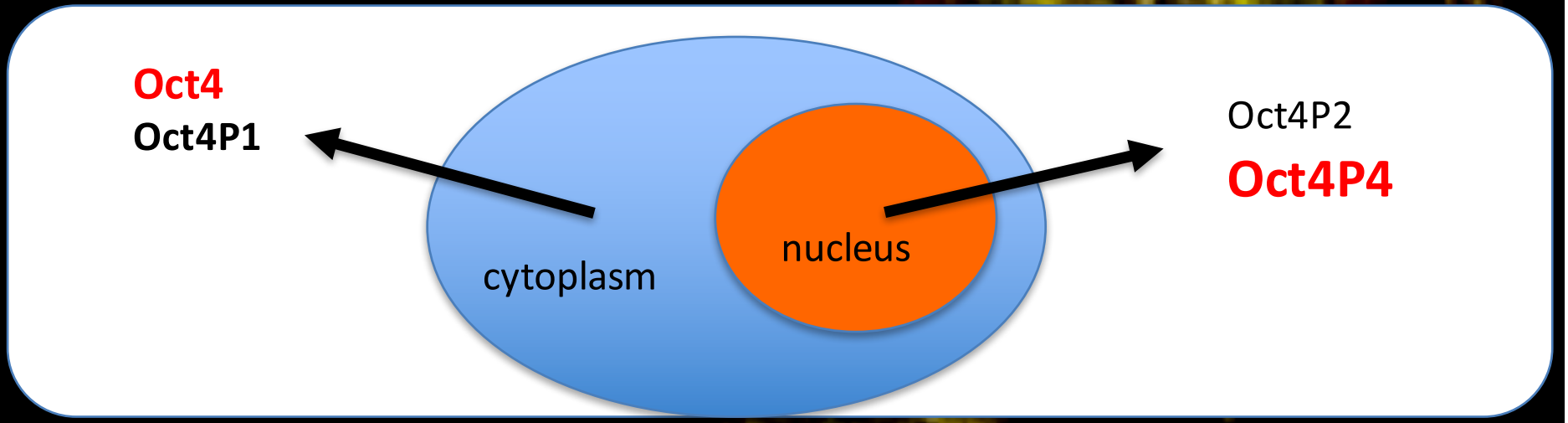
DIFFERENTIATION



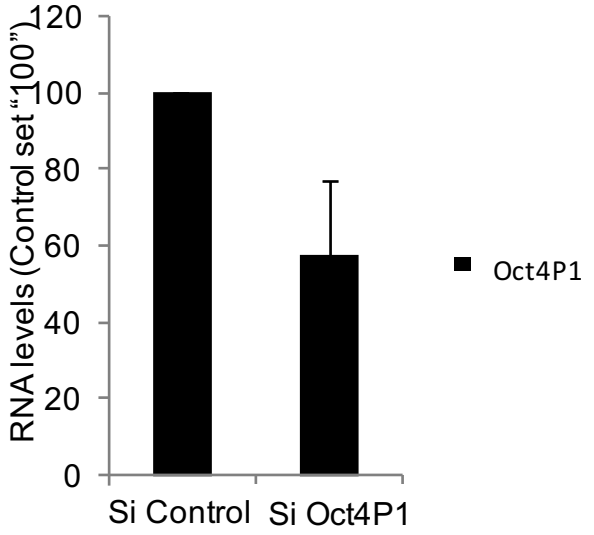
Oct4P2 (+9x)
Oct4P3 (+2x)
Oct4P4 (+4x;
Fiborbl. +200x)

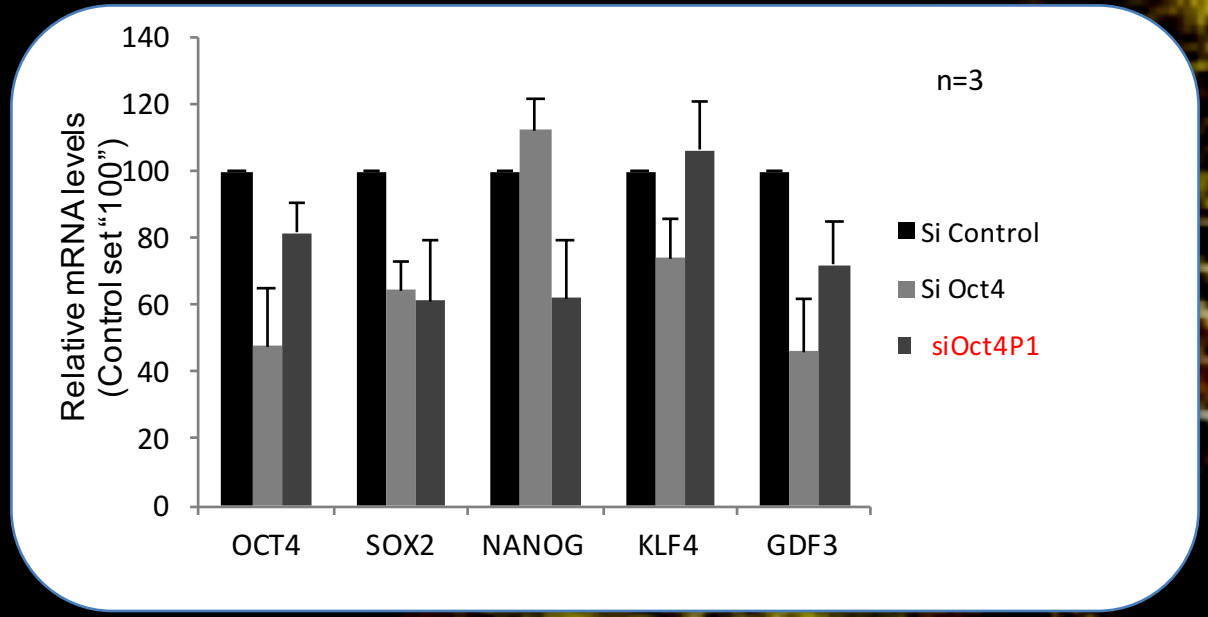
OCT4 pseudogenes are localized to nuceloplasm or cytoplasm

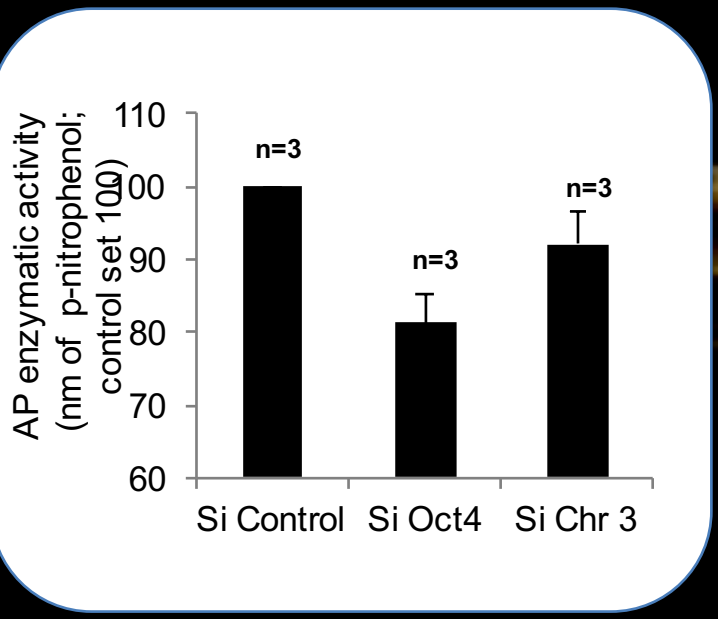


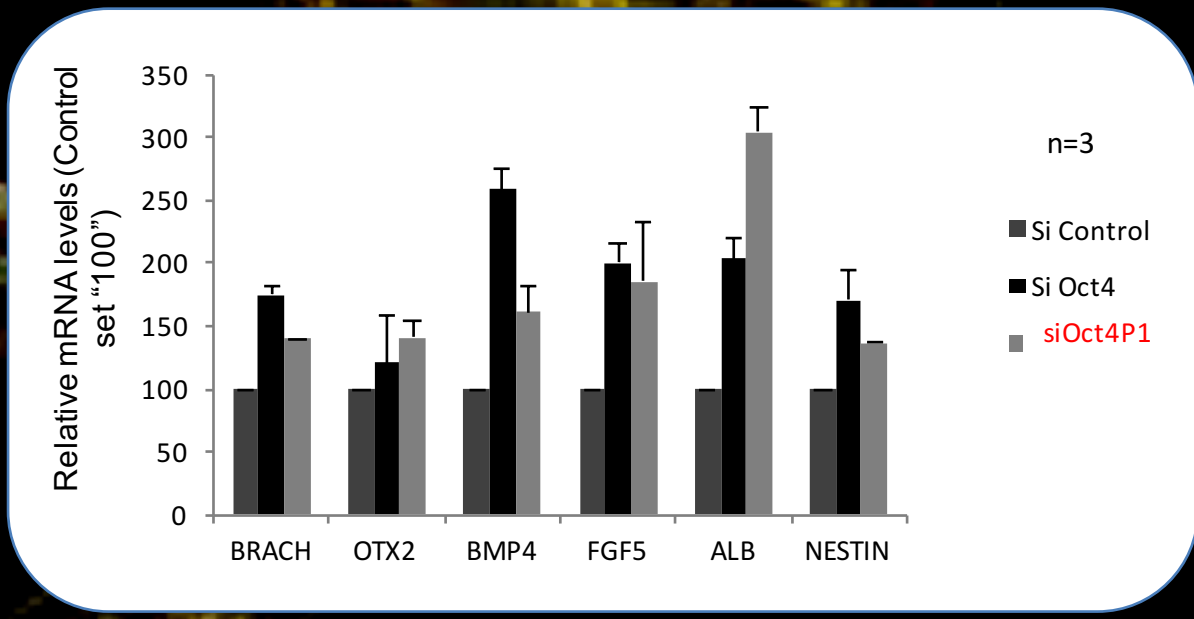


Cytoplasmatic OCT4P1 promotes mESC self-renewal

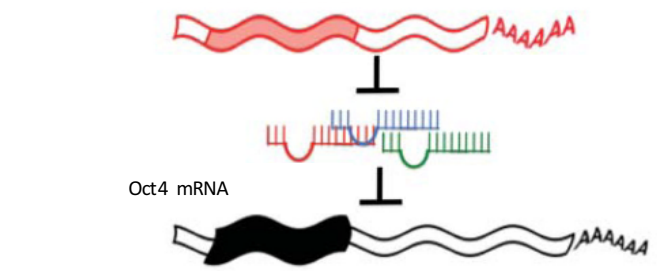


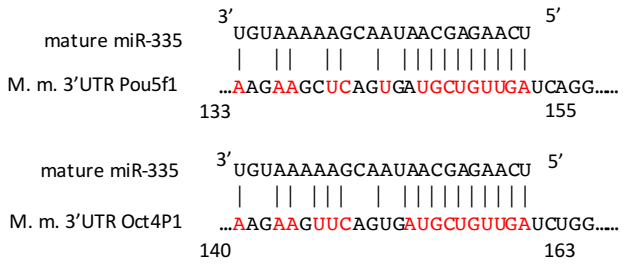




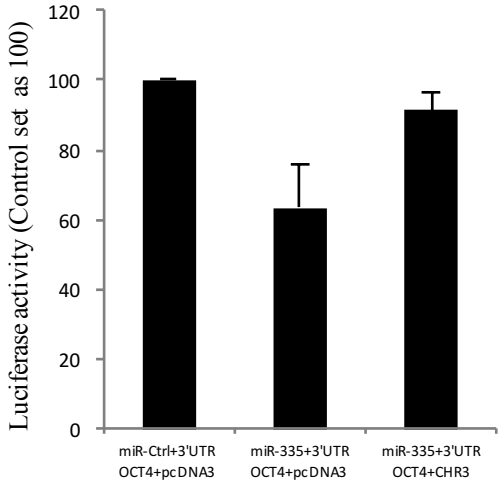


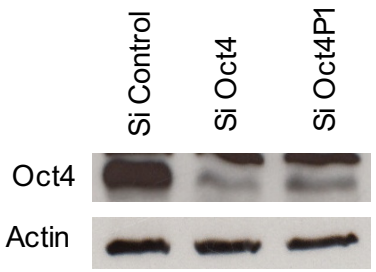
Cytoplasmatic OCT4P1 acts as Oct4/Rb1 ceRNA

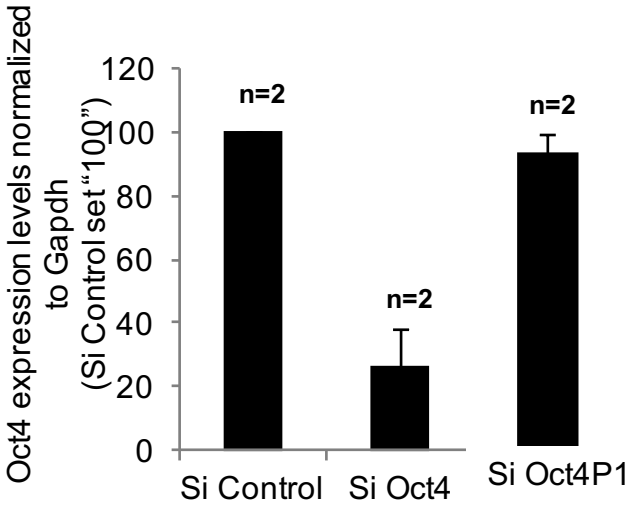




Pou5f1 is the genename for Oct4







EVOLUTION OF PSEUDOGENES OF THE SAME GENE CAN ACQUIRE COMPLETELY DIFFERENT FUNCTIONS

seudogenes are powerful regulators of gene expressio