Overview of RNAi and microRNAs

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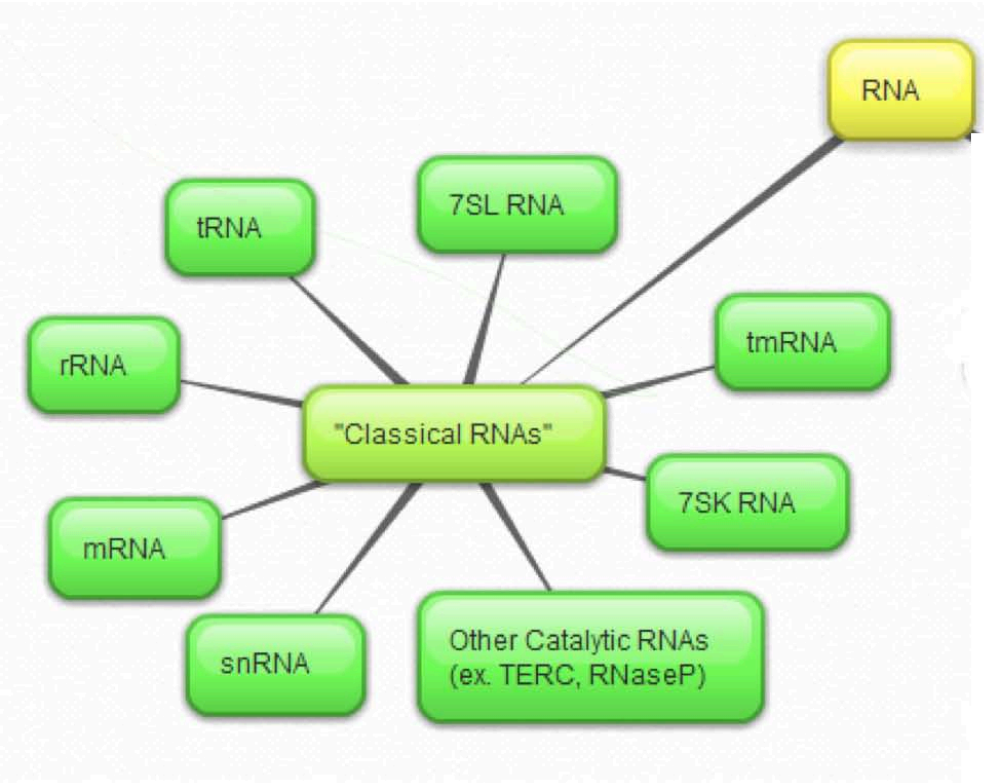
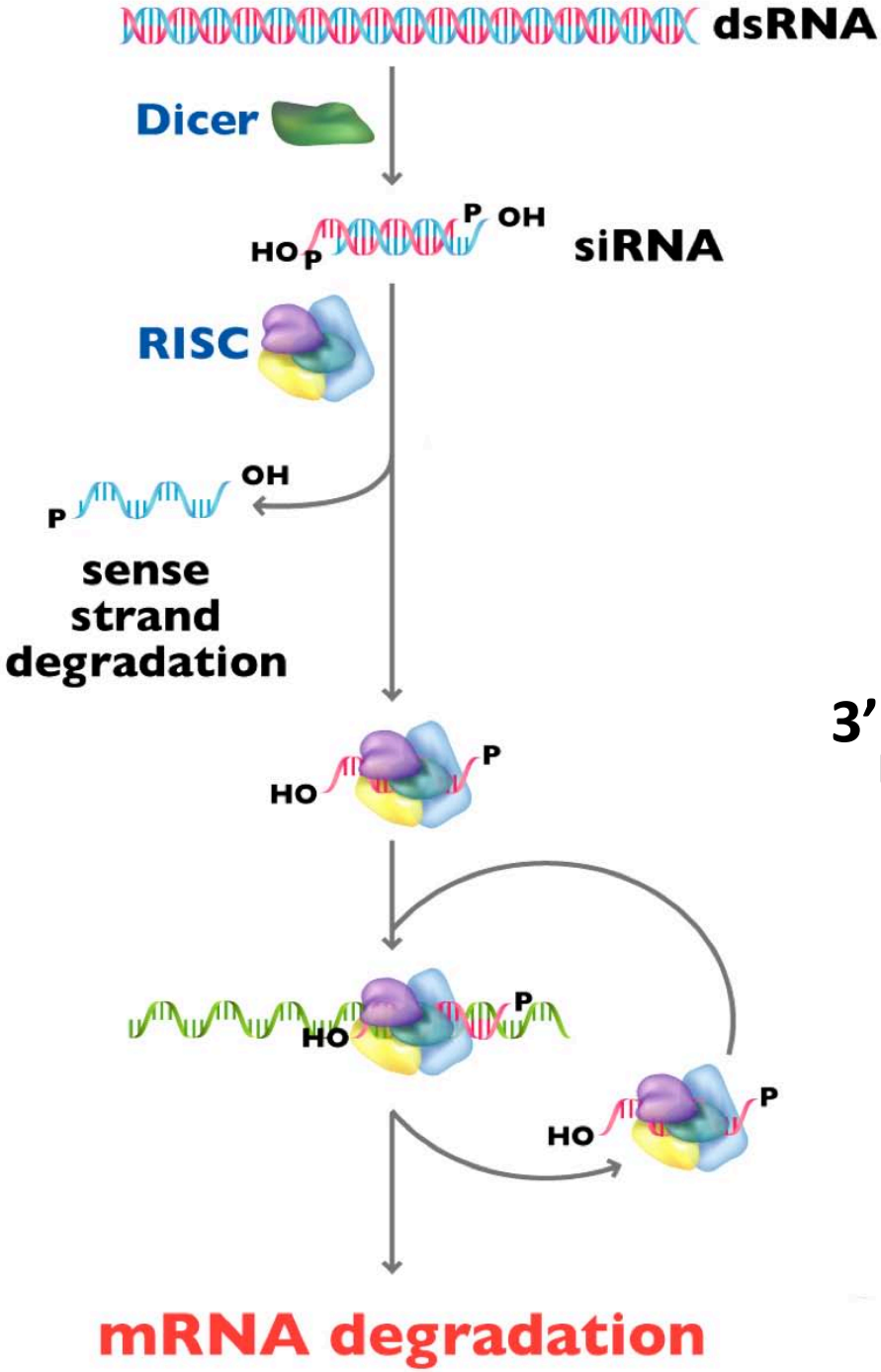
I am a co-founder, Member of the Board of Directors, and Chairman of the SAB of Alnylam, an RNAi company.

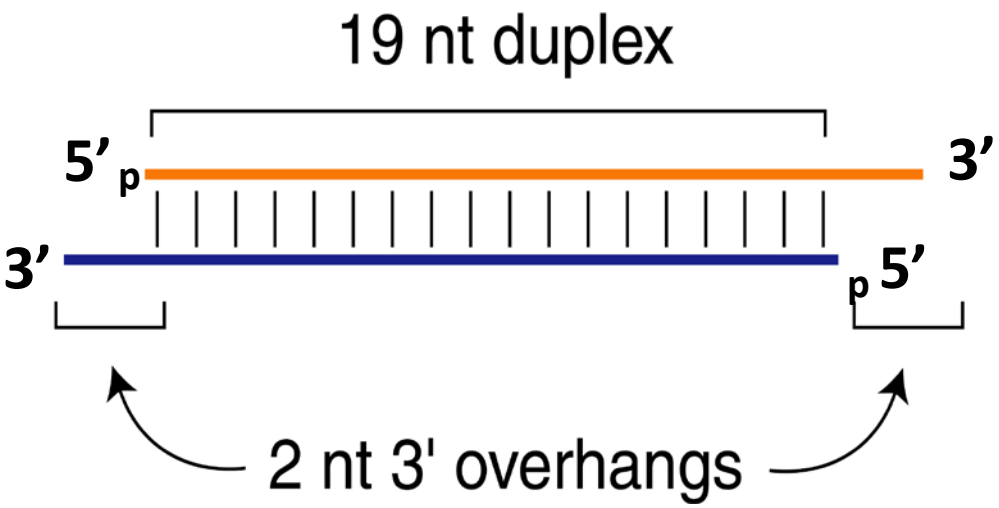
Explosion in world of RNA



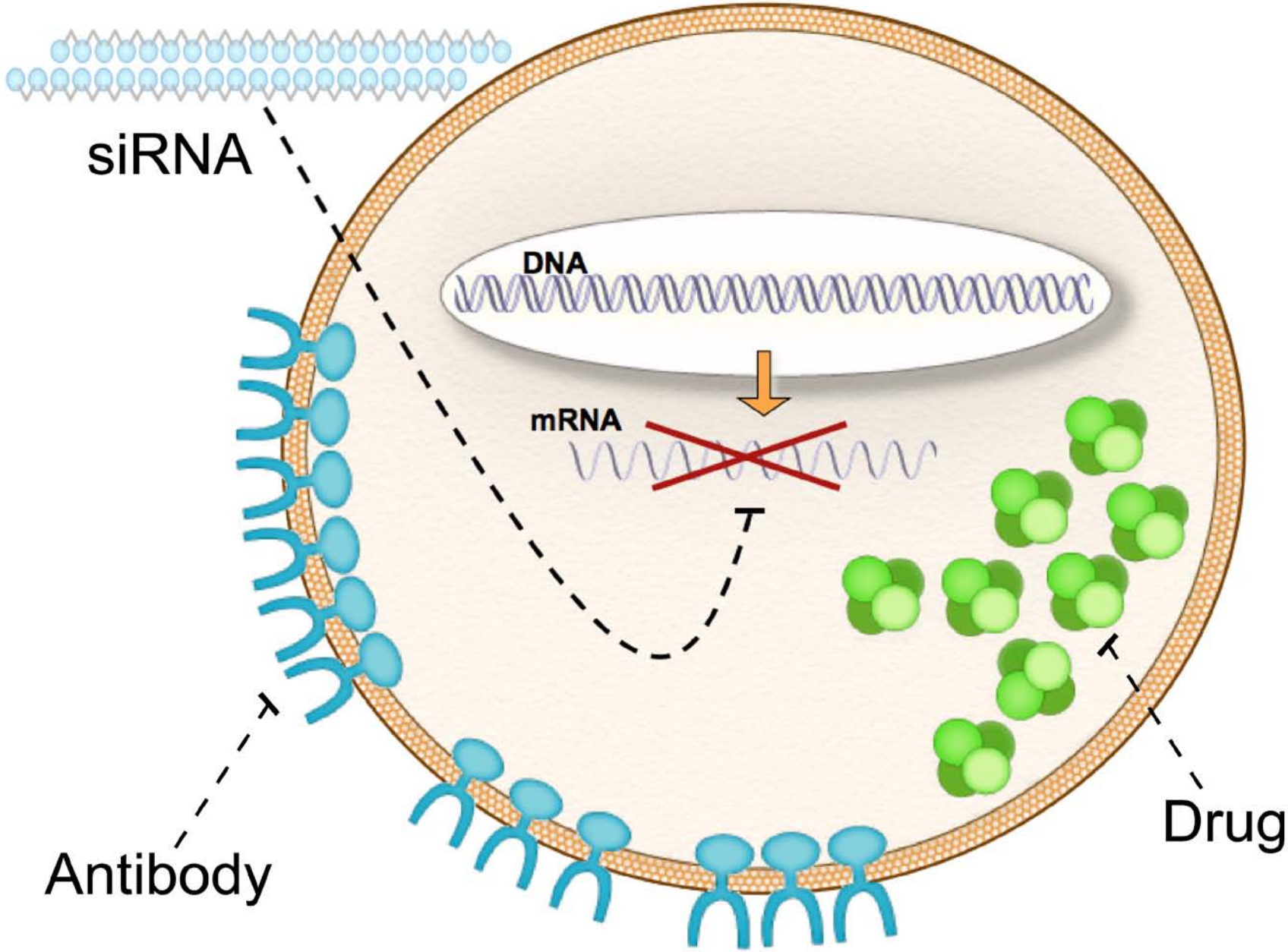
Technology, genomics, and sequencing

Short RNAs silence mRNA degradation





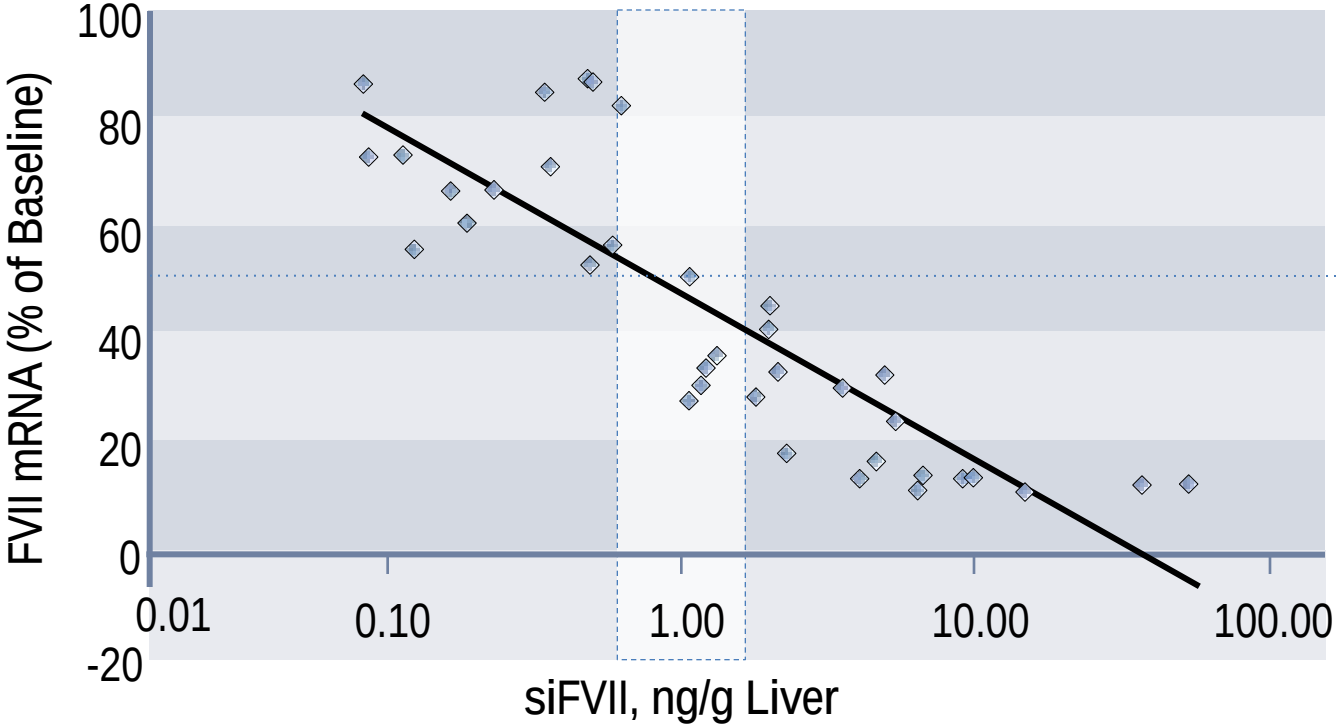
Therapeutic gene silencing



PK/PD Relationship for Liver Silencing with LNPs

Tissue siRNA levels of ~1 ng/g at ED_{50} for mRNA suppression demonstrates potency of natural RNAi pathway *in vivo*

- ☐ Single IV bolus of LNP-siFVII at 0.0625, 0.125 or 0.25 mg/kg
- ☐ Liver mRNA and siRNA levels measured on days 1, 3, 7 and 10 post-dose



Oligo. Ther. Soc., Nov 2009 (Alnylam)

~100 siRNA per cell

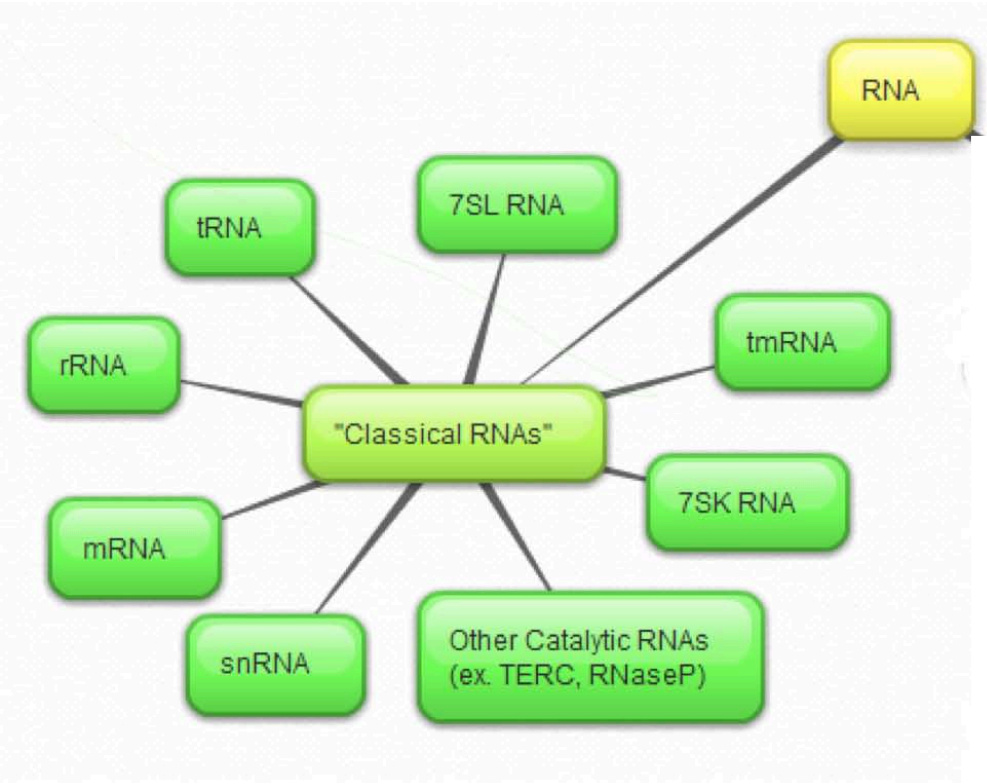
New age of pharmacology

Delivery of siRNA or other macro-molecular agents to specific cells

Steps:

- Target to specific cells
- Uptake of nanoparticle into vesicle
- Release into cytoplasm

Explosion in world of RNA



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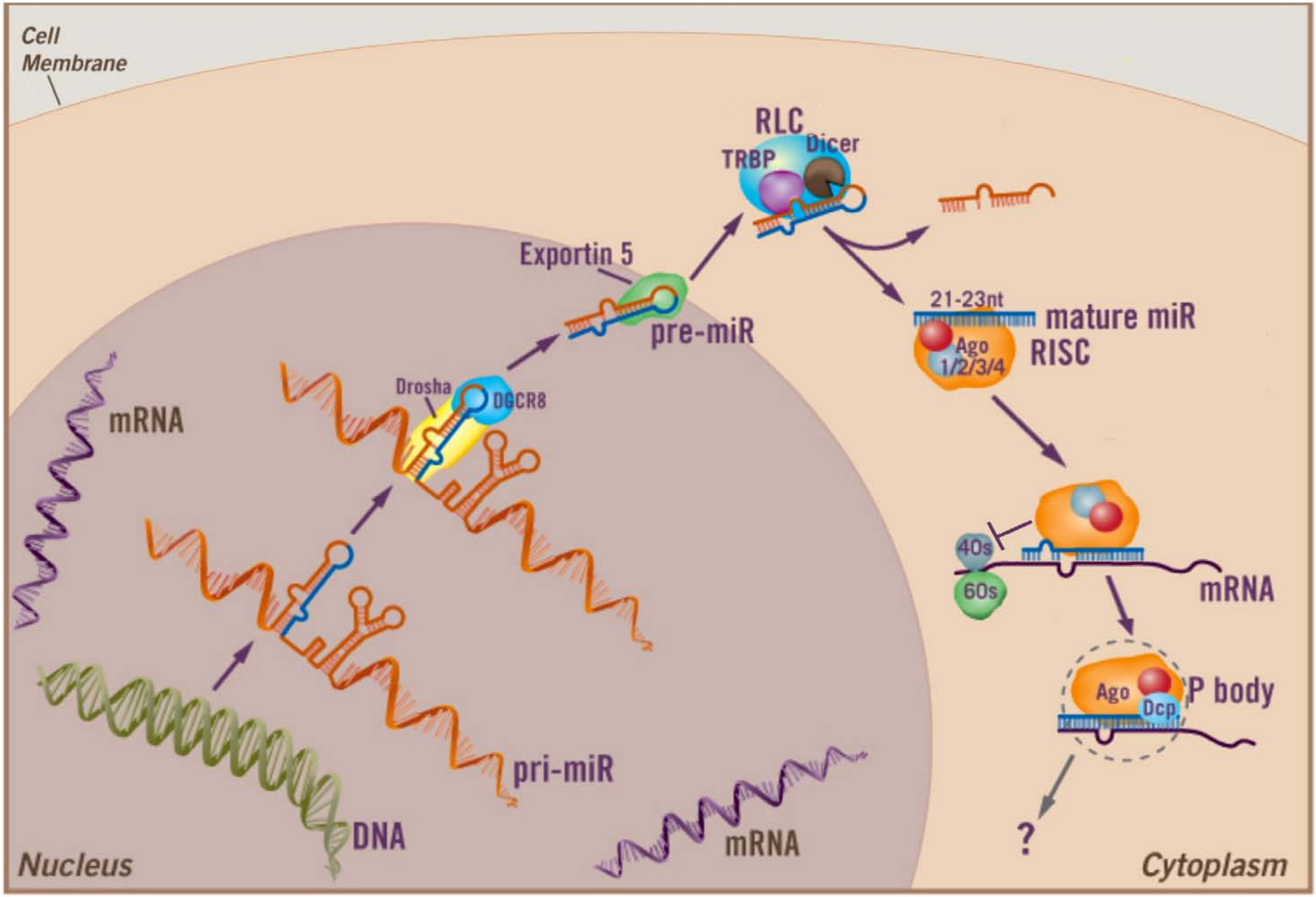
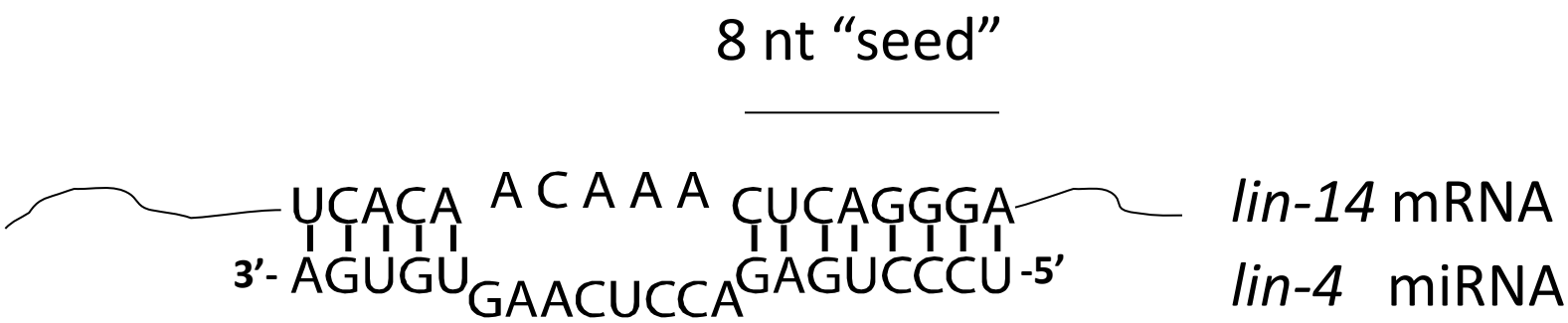
MicroRNA Pathway



Proposed anatomy of miRNA/mRNA interactions

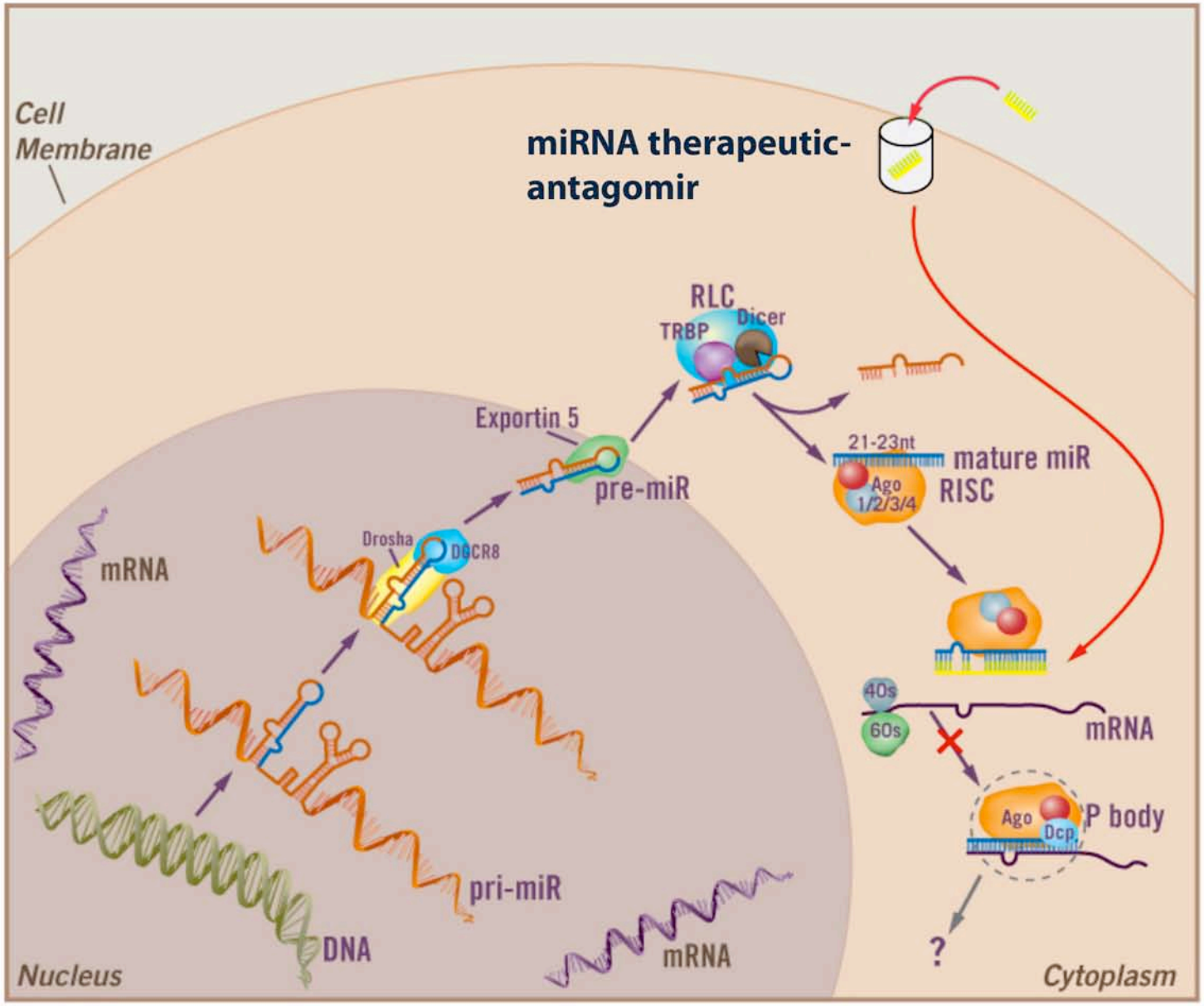


V. Ambros (Lee *et al.* 1993)
L. Ruvkun (Wightman *et al.* 1993)

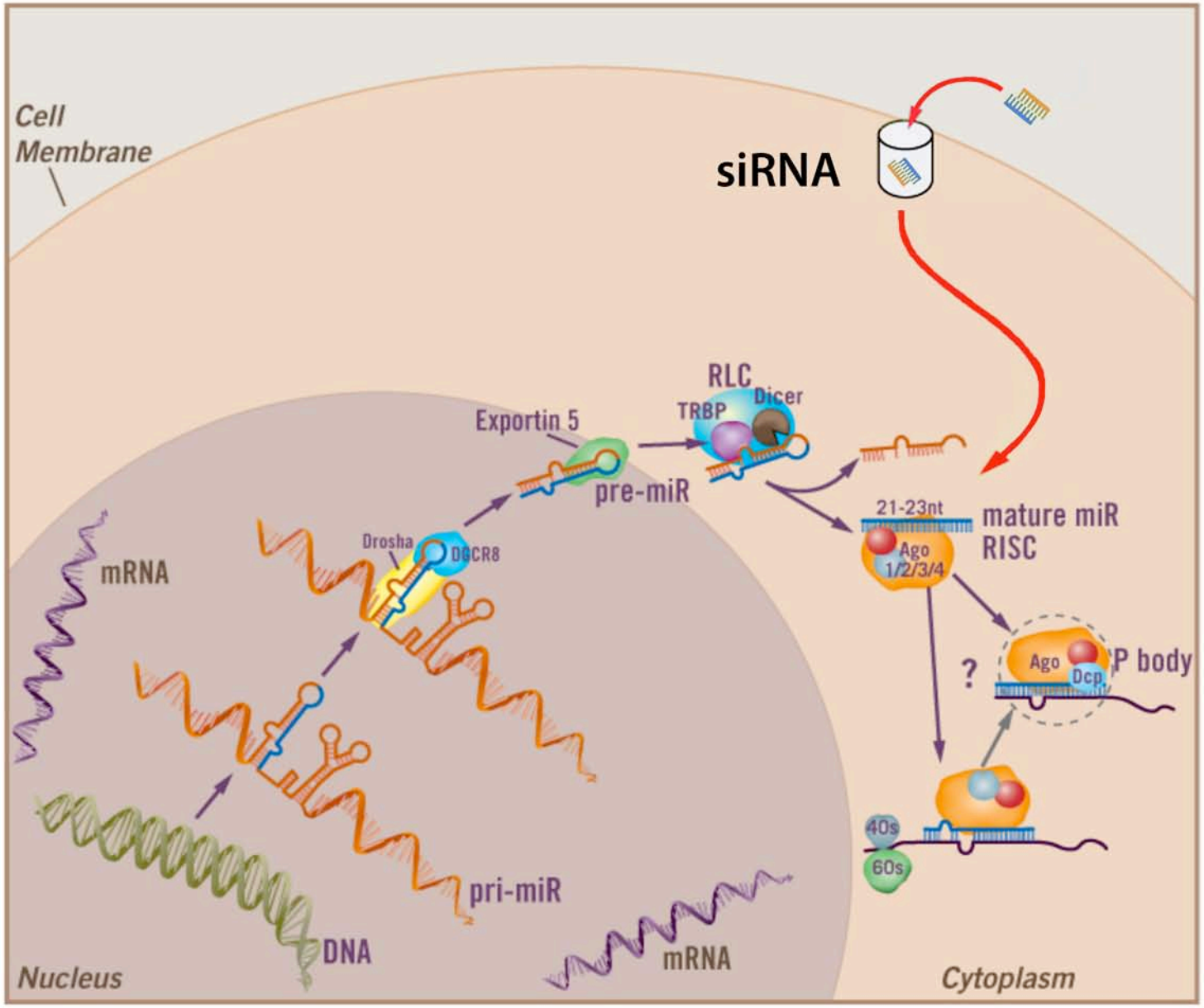
General summary of miRNA activity in mammalian cells

1) Most target sites “seed” interactions in 3’ UTR, some sites in open reading frame
2) Evidence for miRNA targeting through “centered paired” sites 4-14 or 5-15
3) Most protein silencing is less than 1.5 fold, multiple sites up to 10 fold
4) Silencing – most cases mRNA degradation, some examples translation
5) Little evidence of miRNA activity in nucleus

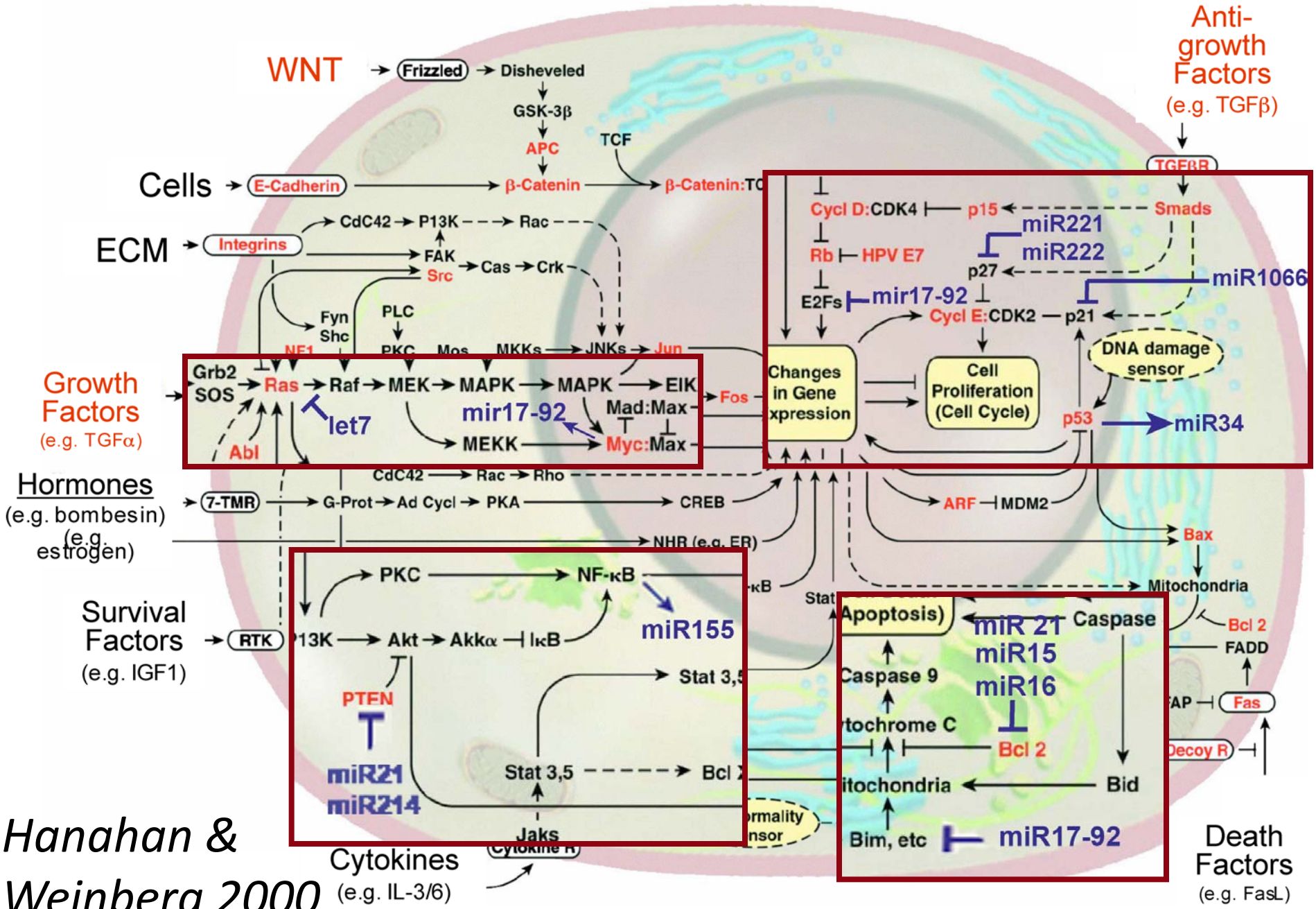
Therapeutic inhibition of microRNA



Therapeutic increase in microRNA



microRNA regulation in cancer network



Hanahan & Weinberg 2000

microRNAs involved in cancer

Oncogene

microRNA		Tumor
miR-155	→	CLL, lung
miR-17-92	→	Lung, AML
miR-21	→	Pancreatic, CLL

Tumor suppressor

microRNA		Tumor
miR-15a, miR-16-1	⊣	CLL, prostate
miR-29b-1, miR29-a, miR-29b-2, miR-29c	⊣	AML, breast
miR-34a, miR-34b, miR-34c	⊣	Colon, lung
miR26-a	⊣	Liver
Let-7a-2	⊣	Lung, colon

Garzon *et al.* 2010
Nature Reviews 9:775

DICER1 is frequently deleted in various human cancers

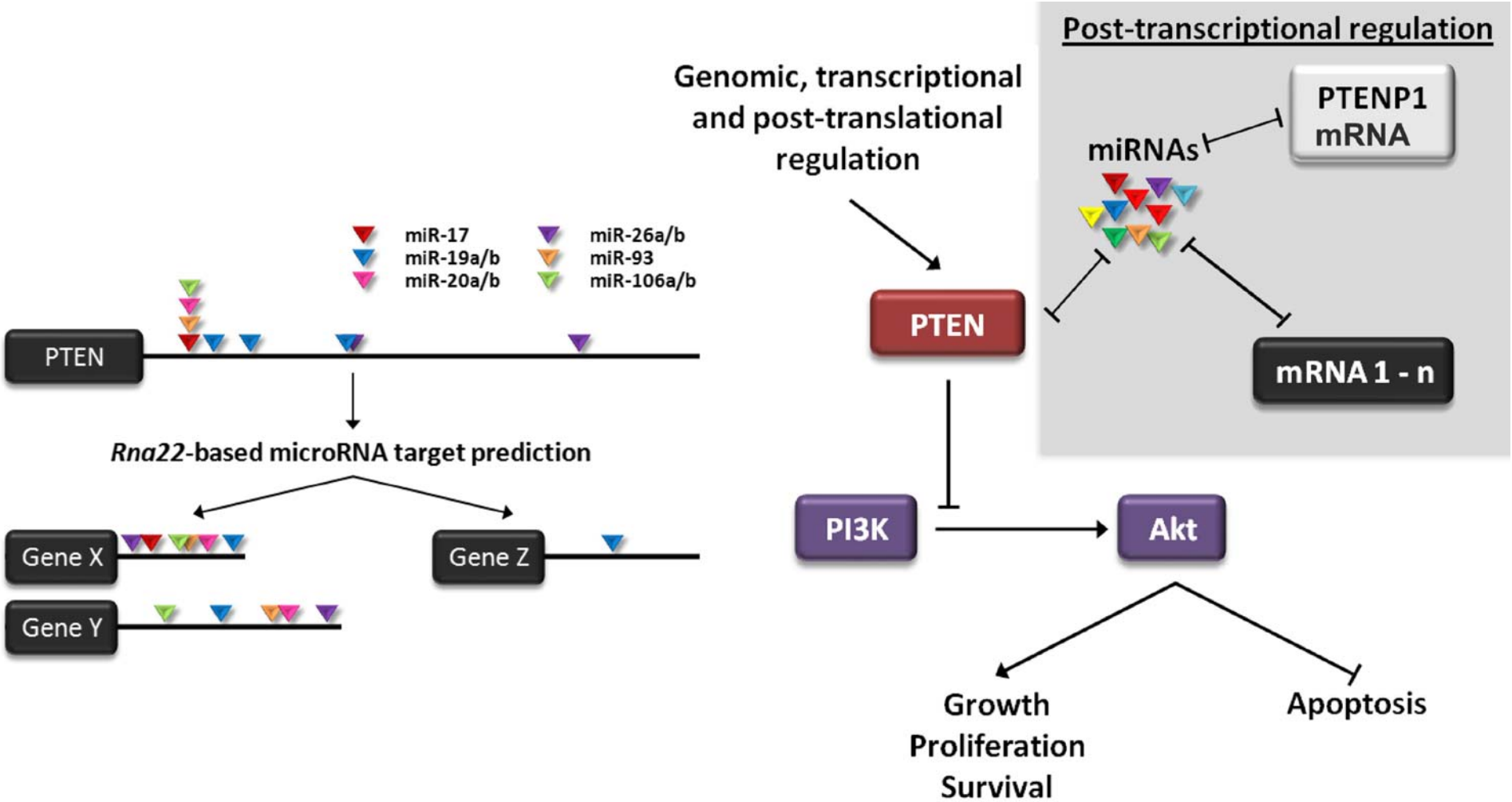
Tumor type	Fraction of tumors with DICER1 hemizygous loss
Breast	17/45
Kidney	13/21
Large intestine	14/39
Liver	3/9
Lung	37/149
Ovary	6/22
Pancreas	6/16
Stomach	9/21

Kumar *et al.* 2009
Genes and Development 23:2700

miRNA regulation in a network - cancer

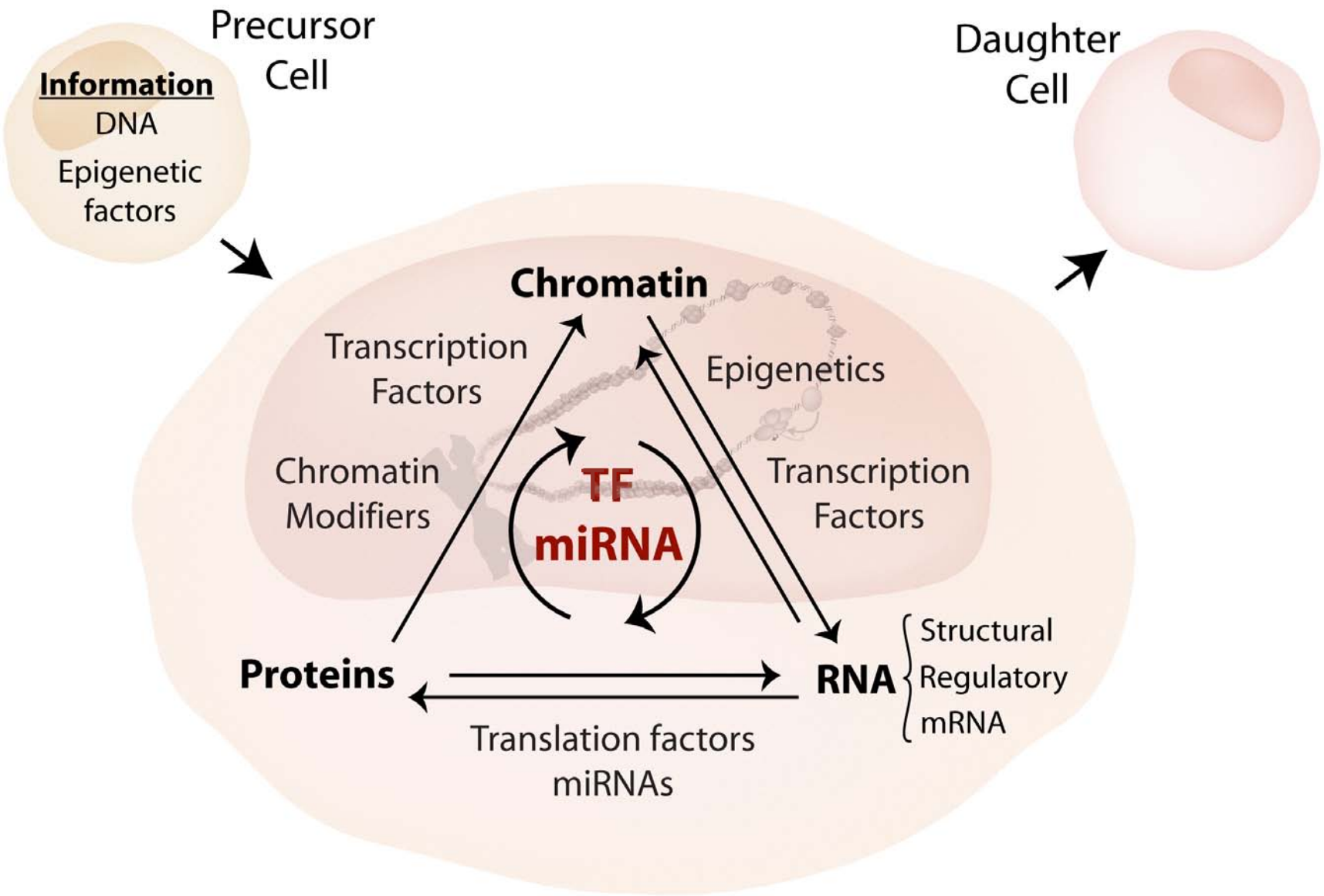
1) Reduction of miRNA control is common in cancer.
2) miRNAs bind to the network of mRNA targets in cells.
3) Could an increased expression of endogenous mRNA targets dilute miRNA regulation of a specific target?

mRNAs compete with PTENP1 mRNA for regulatory miRNAs and promote tumor growth



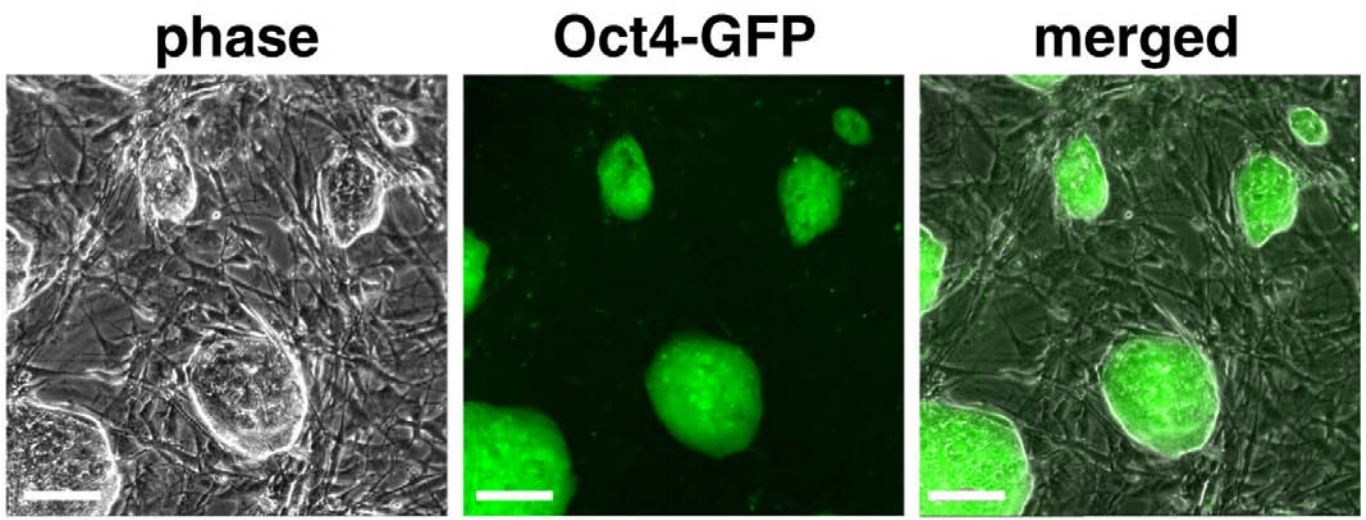
Tay *et al.* 2011 *Cell* 147:344 (Pandolfi)

Transcription Factors (TFs) and miRNAs are master regulators



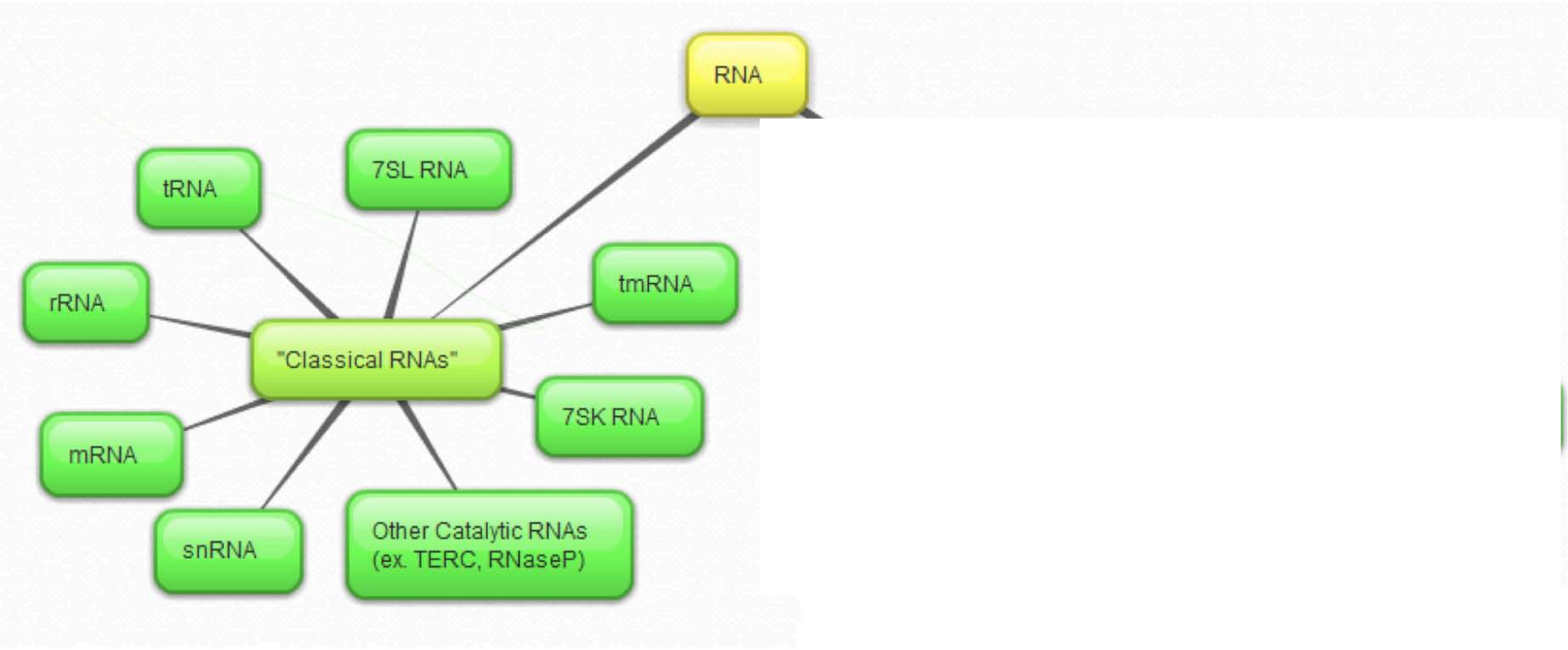
miR302/367 can reprogram mouse fibroblasts to a pluripotent cell phenotype

mmu-miR-302a	UAAGUGCUUCCAUGUUUUGGUGA
mmu-miR-302b	UAAGUGCUUCCAUGUUUUAGUAG
mmu-miR-302c	UAAGUGCUUCCAUGUUUCAGUGG
mmu-miR-302d	UAAGUGCUUCCAUGUUUGAGUGU
mmu-miR-367	GAAUUGCACUUUAGCAAUGGUGA



Anokye-Danso *et al.* (2011) *Cell Stem Cell* 8:376
E. Morrissey, Univ. of Penn.

Explosion in world of RNA



Technology, genomics, and sequencing

piRNAs and germ line

1. piRNAs silence transposable elements by epigenetic mechanisms in Drosophila.

2. The mechanism(s) mediating piRNA silencing has not been established but probably targets nascent RNAs.

3. Endo-siRNAs (21-22 nts) have been identified in mouse oocytes. Some of these contain sequences of pseudogenes indicating copy number dependence.

4. Small piRNA population also present in mouse oocytes.

5. piRNAs silence transposable elements in mouse.

piRNAs and germ line

1. Argonaute family - two clades
   a) Argonaute proteins in somatic and germ cells.
   b) Piwi proteins in germ cell

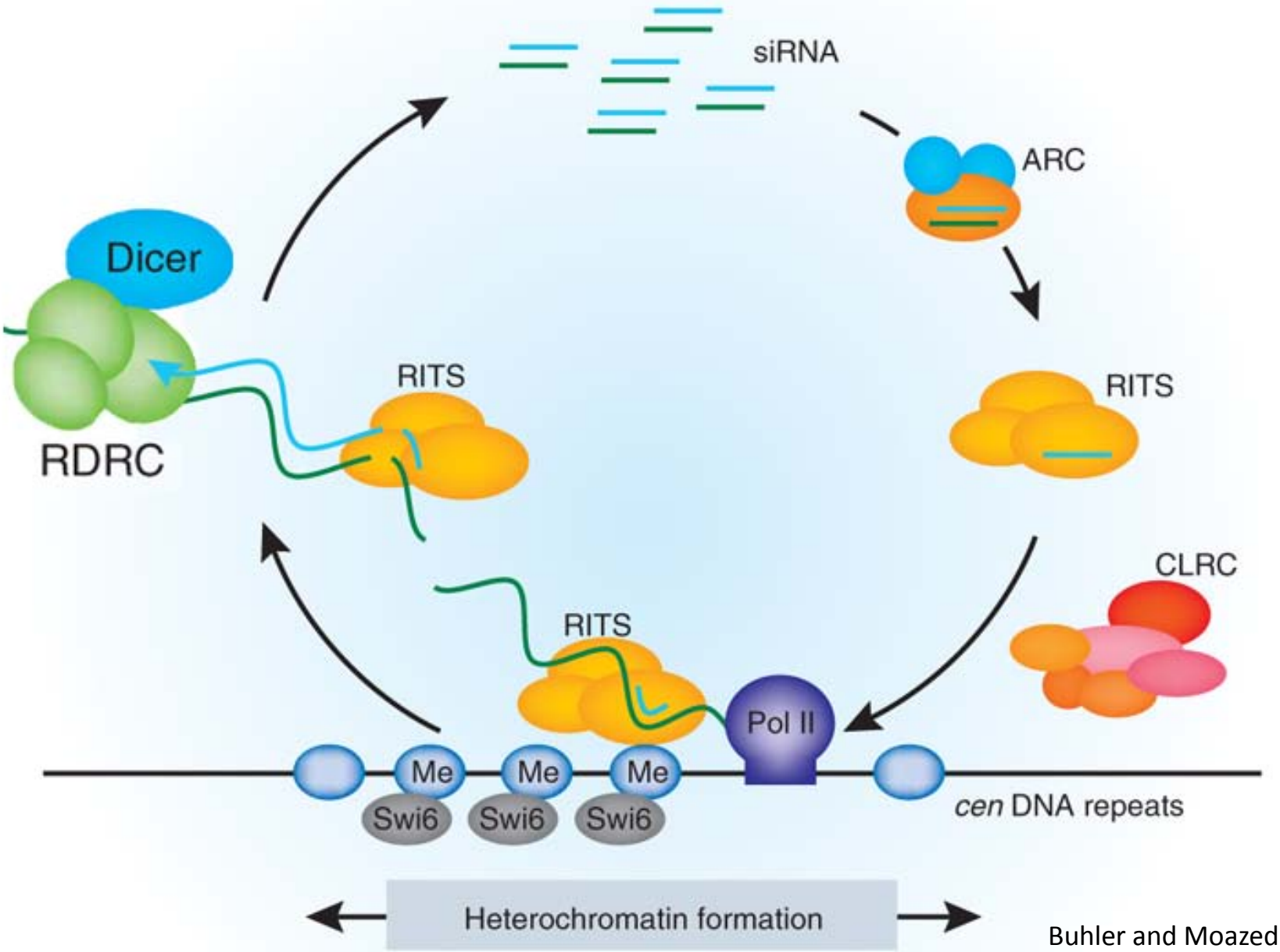
2. Three Piwi-related proteins expressed in mouse germline and associated cells - MIWI, MILI, and MIWI2.

3. Mutations in MIWI, MILI, and MIWI2 cause defects in male but not female germline.

4. Piwi proteins associated with piRNAs (~30 nts), somewhat enriched in repetitive sequences.

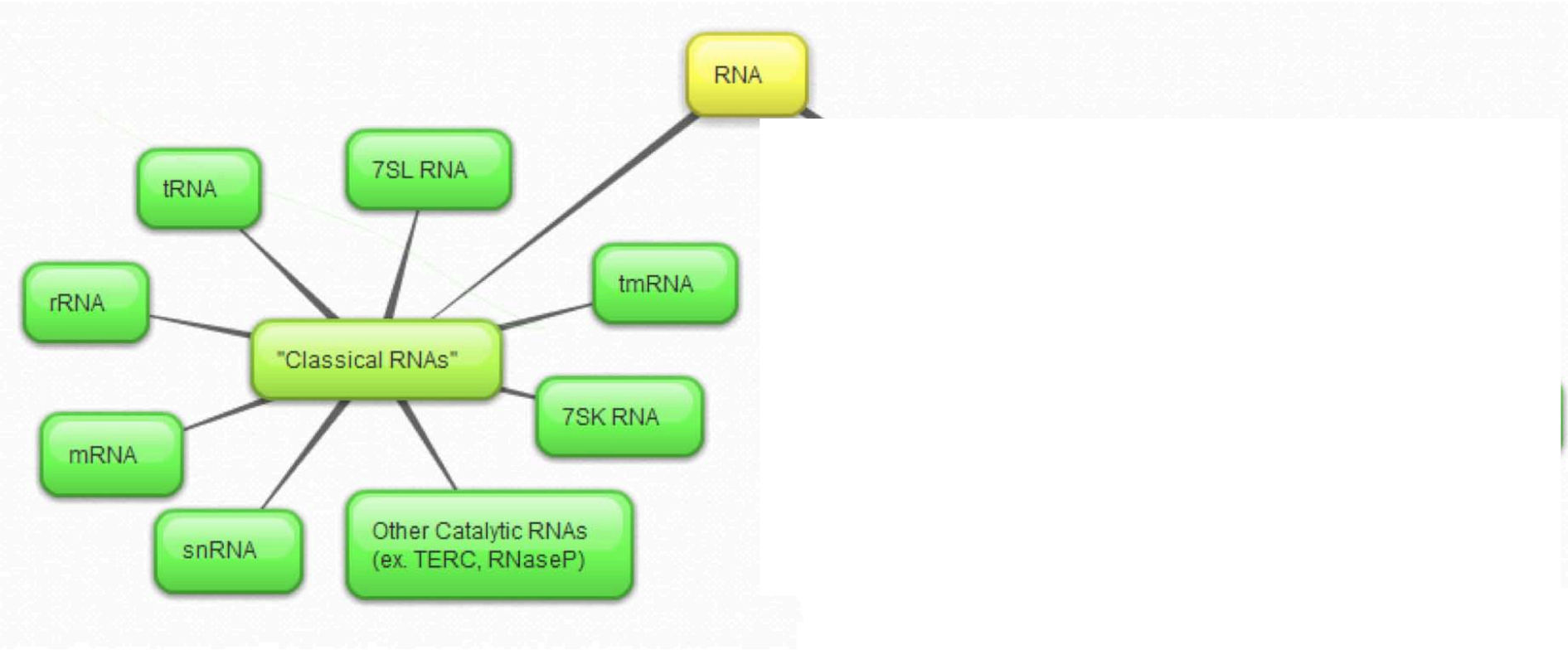
5. piRNAs are produced by Ping-Pong mechanism involving both sense and anti-sense transcription and two Piwi-related proteins.

RNAi-mediated heterochromatin assembly in fission yeast



Buhler and Moazed 2007
Nat Struct Mol Biol 14:1041

Explosion in world of RNA



Technology, genomics, and sequencing

lincRNAs

Proposed ~3,500 large intergenic non-coding RNAs

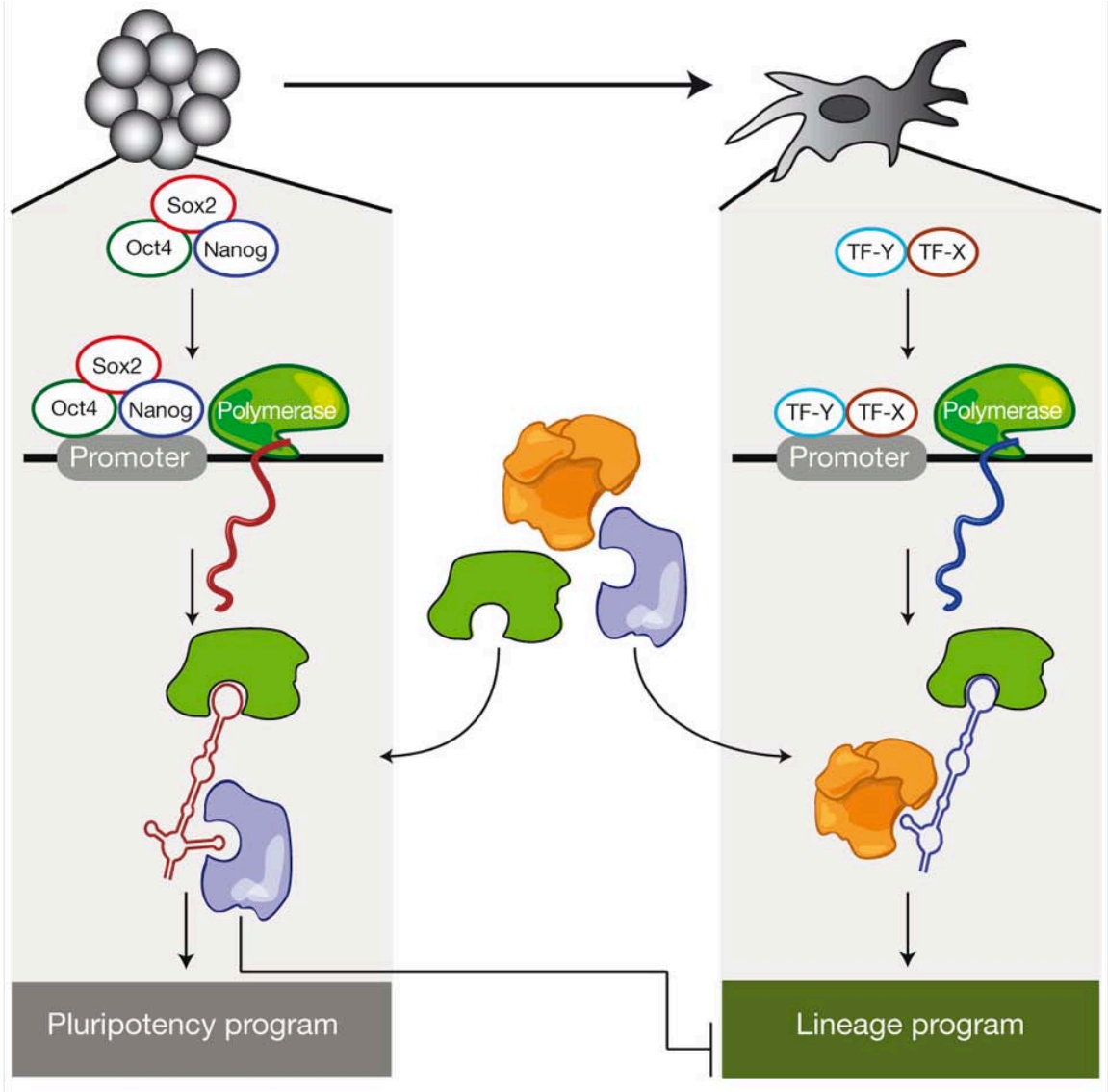
a) Identified by chromatin signatures of active genes
b) Evolutionarily conserved
c) Chromatin associated

Function – act in circuitry controlling pluripotency and differentiation*

a) shRNAs targeting lincRNAs (147) in embryonic stem cells produced a phenotype (137-gene expression), 26 caused changes in pluripotency and 30 induced differentiation.

*Guttman *et al.* (2011) *Nature* 477:295

A model for lincRNA integration in the molecular circuitry of the cell



Guttman *et al.* (2011) *Nature* 477:295

Conclusions

1) Gene silencing through RNAi-related mechanisms offers new therapeutic agents.
2) Mammalian systems use RNA as information transfer agents, catalytic agents, decoding agents, and regulatory agents.
3) RNA-based therapeutics need to be judged as other types of agents, drugs and proteins, in terms of benefits relative to side-effects.

Acknowledgments

Sharp Lab, MIT

Arvind Ravi

Margaret Ebert

Mary Lindstrom

Allan Gurtan

Jesse Zamudio

Ryan Flynn

Albert Almada

Tristan Kooistra

Tyler Jacks

Madhu Kumar

Paul Chang

Sejal Vyas

Christopher Burge

Rudolf Jaenisch

Rick Young

Paul Anderson (HMS)

Nancy Kedersha

Rod Bronson (Tufts)

Alexander van Oudenaarden (MIT)

Shankar Muhherji

KOCH INSTITUTE for Integrative Cancer Research at MIT