MICRORNAS

Δήμητρα Ντάφου

Αν. Καθηγήτρια
Τμήμα Βιολογίας, Τομέας Γενετικής, Ανάπτυξης και Μοριακής Βιολογίας
Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης

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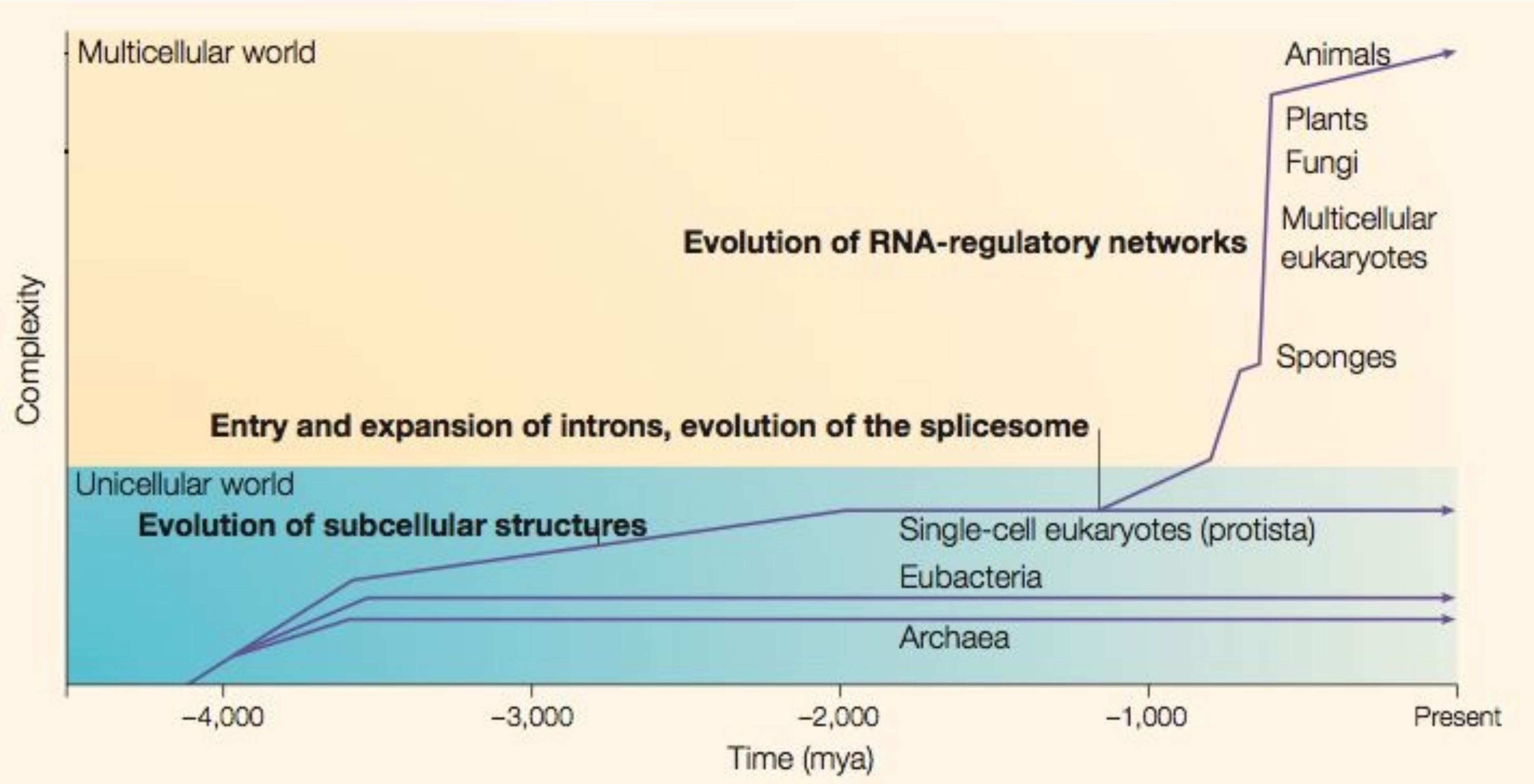
PHASE TWO: INTERPRETATION
I THINK I FOUND A CORNER PIECE.
3 BILLION PIECES
GENOME
THE HUMAN GENOME

A Simplified History of Life on Earth

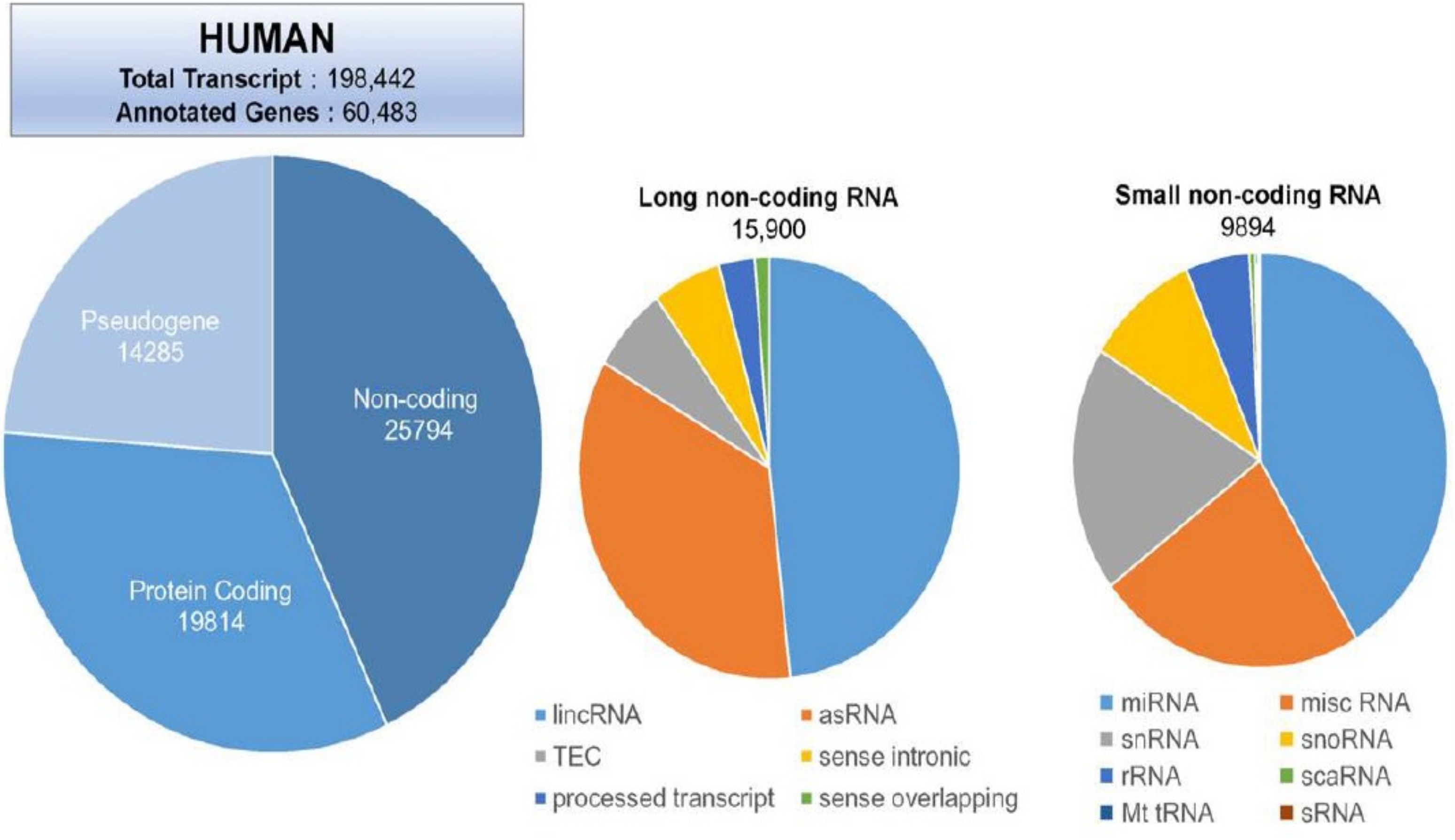


Mattick (2004) Nature Reviews Genetics 5: 316-323.

How is the human genome organised?

- 5% coding and rest of it ***junk*** (repetitive DNA).
- Nuclear and mitochondrial
- You are 99.99% similar to your neighbor

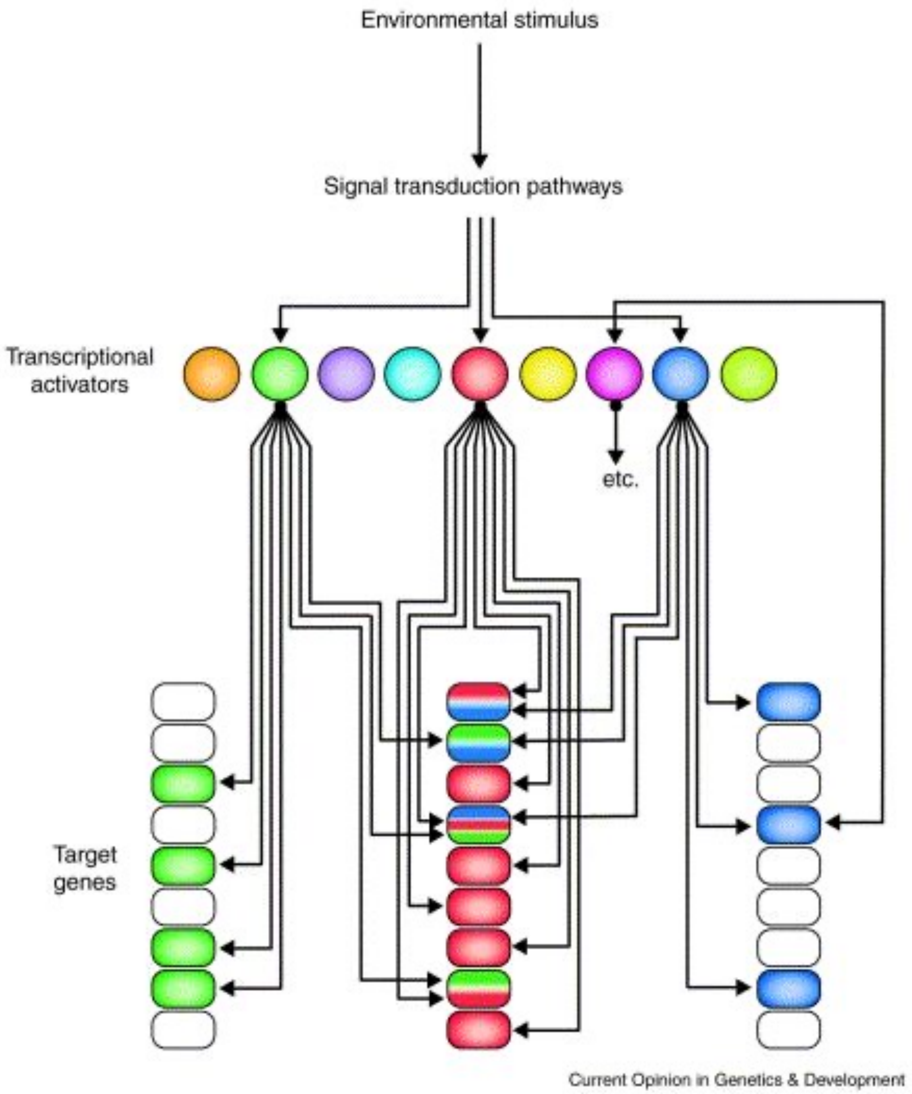
Κατανομή γονιδίων στον άνθρωπο



Gene Regulatory Mechanisms

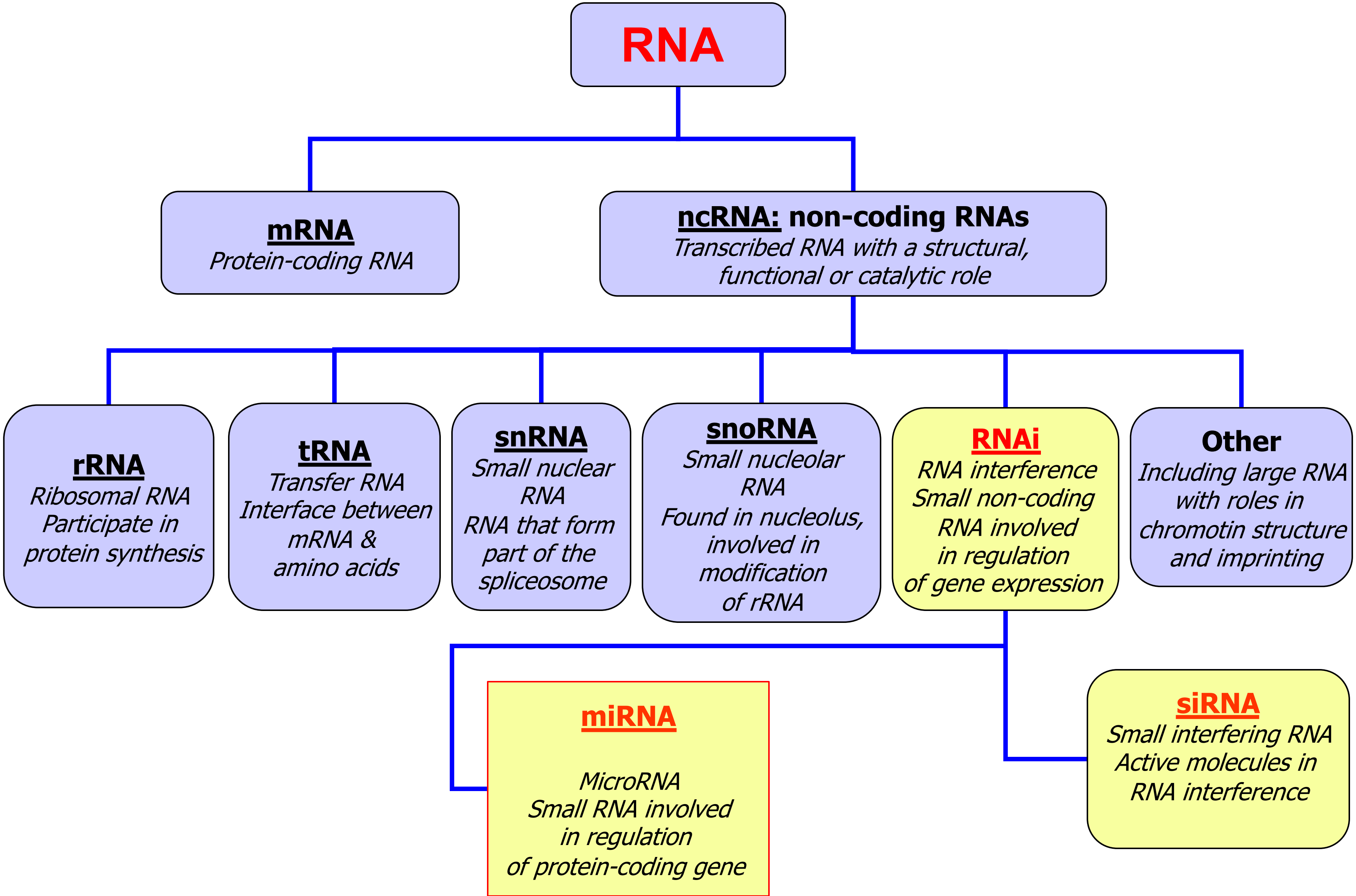
- Transcriptional Mechanisms
  - Type of promoters & RNA polymerase
  - Control of Transcription
    - Constitutive
    - Inducible
    - Repressible
  - Transcription Factors and TFBS
- Translational Mechanisms
  - Micro RNAs (miRNAs and RITS complexes)
    - Translational control
    - mRNA degradation
    - Promoter activation
  - Silencer RNAs (siRNAs & RISC complexes) degrading mRNA
- Epigenetic Mechanisms
  - Chromatin remodeling
  - Histone modifications (acetylation, phosphorylation, methylation …)
  - DNA methylation

Gene Expression Regulatory Network



Non-coding RNA: the formerly known as “junk”

NC-RNAs compose majority of transcription in complex genomes



RNA

mRNA
Protein-coding RNA

ncRNA: non-coding RNAs
Transcribed RNA with a structural, functional or catalytic role

rRNA
Ribosomal RNA
Participate in protein synthesis

tRNA
Transfer RNA
Interface between mRNA & amino acids

snRNA
Small nuclear RNA
RNA that form part of the spliceosome

snoRNA
Small nucleolar RNA
Found in nucleolus, involved in modification of rRNA

RNAi
RNA interference
Small non-coding RNA involved in regulation of gene expression

Other
Including large RNA with roles in chromotin structure and imprinting

miRNA

MicroRNA
Small RNA involved in regulation of protein-coding gene

siRNA
Small interfering RNA
Active molecules in RNA interference

Nc-RNAS- a bit of a 'Dark Matter' Function

nc RNA*

small
(20-300 nt)

many more

long
(>200 nt)

microRNAs (~23 nt)	**• decrease mRNA stability/ translation**
Piwi-interacting RNAs (piRNAs, 26-31 nt)	**• gene silencing/ methylation**
Promoter-associated RNAs (small/variable)	**• gene silencing..?**
Long intergenic non-coding RNAs (ncRNAs)	**• chromatin association and gene modulation/ silencing**

RNA interference first discovered in Petunias

Called PTGS, for

“Post Transcriptional Gene Silencing”

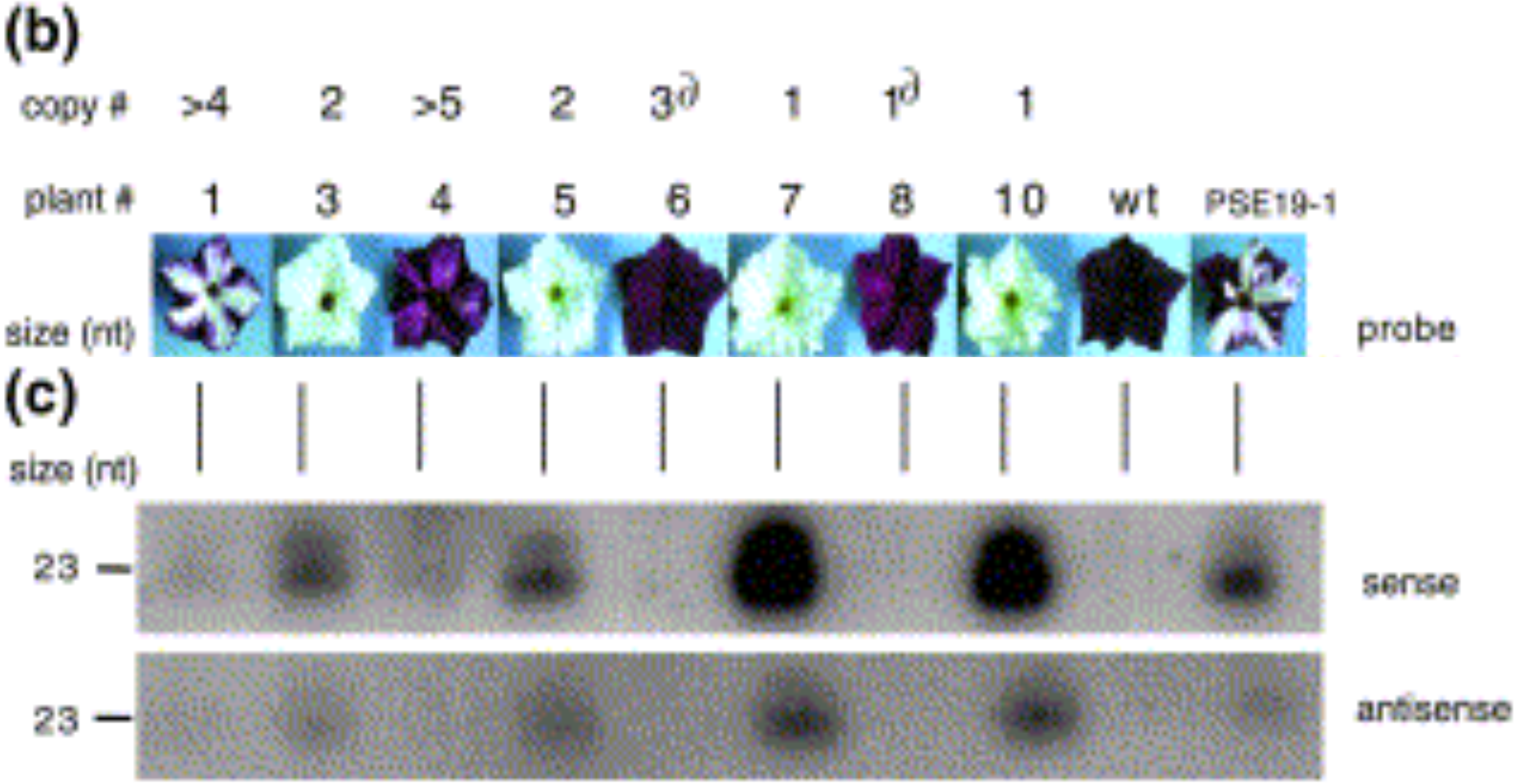
KAREN TWEEDY-HOLMES/CORBIS

s PLANT CELL 2, 279–289 (1990)

Color changes can be induced by RNAi, or PTGS..

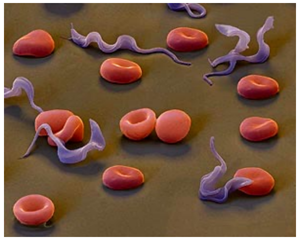
Post transcipt-ional gene silencing

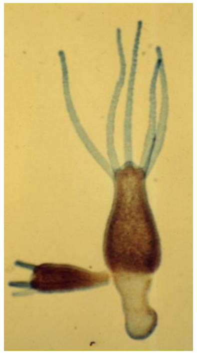
Small (21-23 nts) RNA duplexes, with the same sequence as in the silenced gene, were identified as being responsible for knocking down expression



So what other organisms can do this thing called PTGS?

“Post Transcriptional Gene Silencing”





24 March 2000

Science

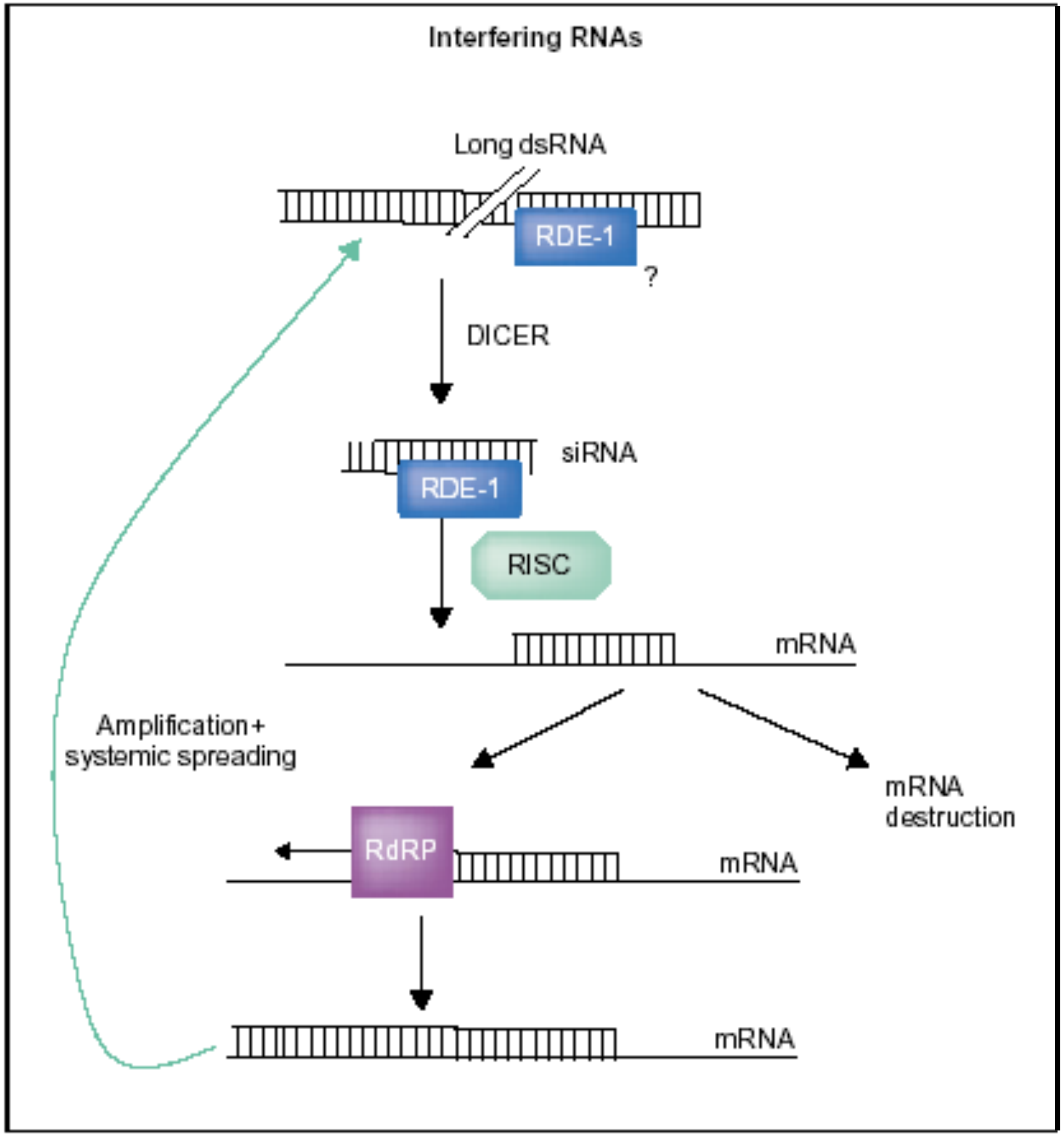
Vol. 287 No. 5461
Pages 2105–2364 $8

The *Drosophila* Genome

AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

***C. Elegans* grow on agar dishes with *E. coli* bacteria as a source of food.**

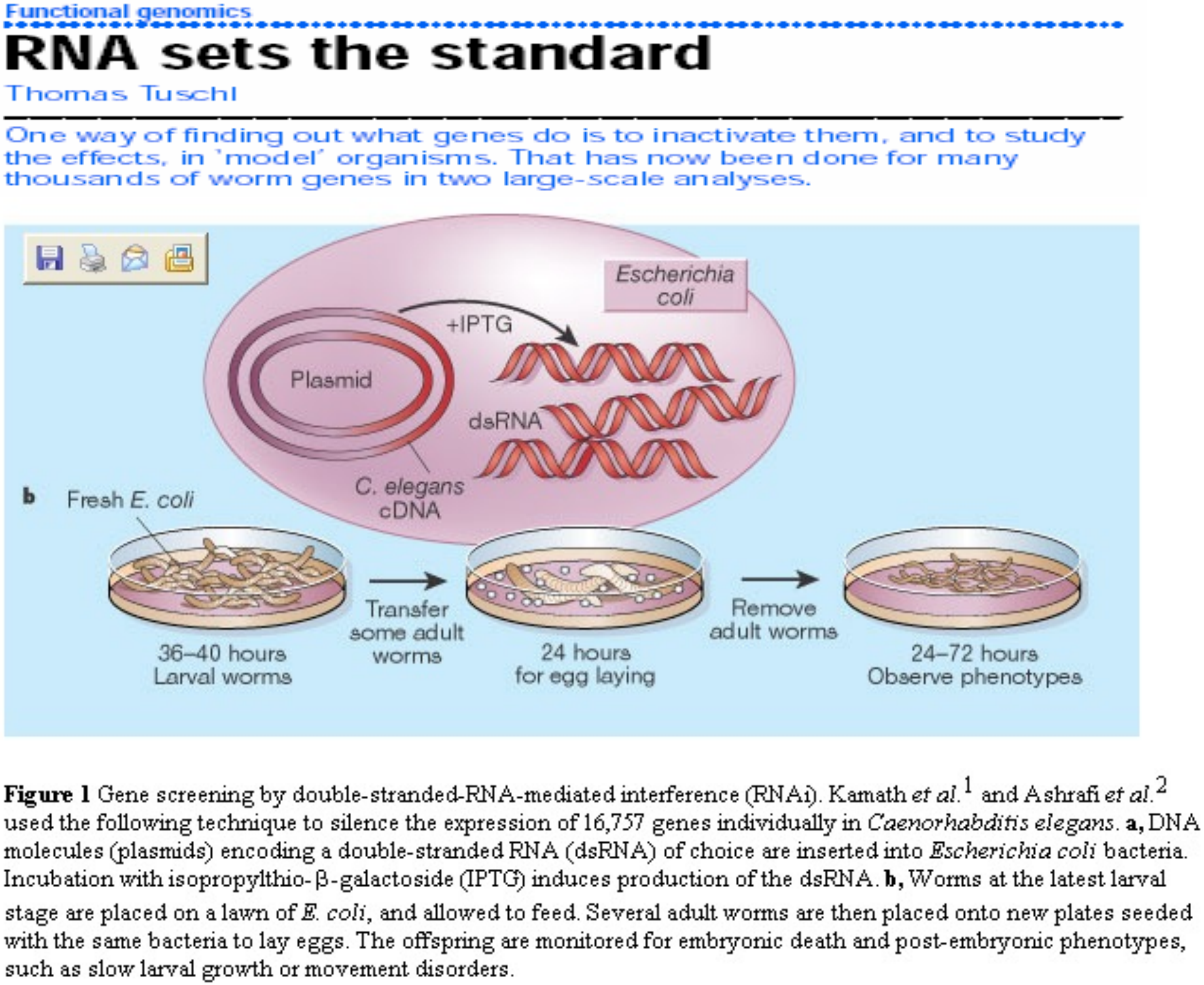
If they eat *E. coli* expressing dsRNA molecules…this creates specific knock-down of gene expression!



In *C. elegans* the siRNA effects can be amplified making the silencing quite stable

This does not appear to happen in mammalian cells

(RISC = RNAi Induced Silencing Complex; RdRP = RNA dependent RNA polymerase)



Functional genomics

RNA sets the standard

Thomas Tuschl

One way of finding out what genes do is to inactivate them, and to study the effects, in 'model' organisms. That has now been done for many thousands of worm genes in two large-scale analyses.

Figure 1 Gene screening by double-stranded-RNA-mediated interference (RNAi). Kamath *et al.*[1] and Ashrafi *et al.*[2] used the following technique to silence the expression of 16,757 genes individually in *Caenorhabditis elegans*. **a,** DNA molecules (plasmids) encoding a double-stranded RNA (dsRNA) of choice are inserted into *Escherichia coli* bacteria. Incubation with isopropylthio-β-galactoside (IPTG) induces production of the dsRNA. **b,** Worms at the latest larval stage are placed on a lawn of *E. coli*, and allowed to feed. Several adult worms are then placed onto new plates seeded with the same bacteria to lay eggs. The offspring are monitored for embryonic death and post-embryonic phenotypes, such as slow larval growth or movement disorders.

http://www.nature.com/cgi-taf/DynaPage.taf?file=/nature/journal/v421/n6920/full/421220a_fs.html&content_filetype=pdf

19,757 genes

16,757 have been inactivated by RNAi

10% display spontaneous phenotype; this 10% is enriched for conserved genes

19,757 genes

16,757 knock down mutants were screened for body fat content

305 knock downs had increased body fat, 112 genes had decreased…

new targets for obesity?

NOBEL PRIZE IN PHYSIOLOGY 2006

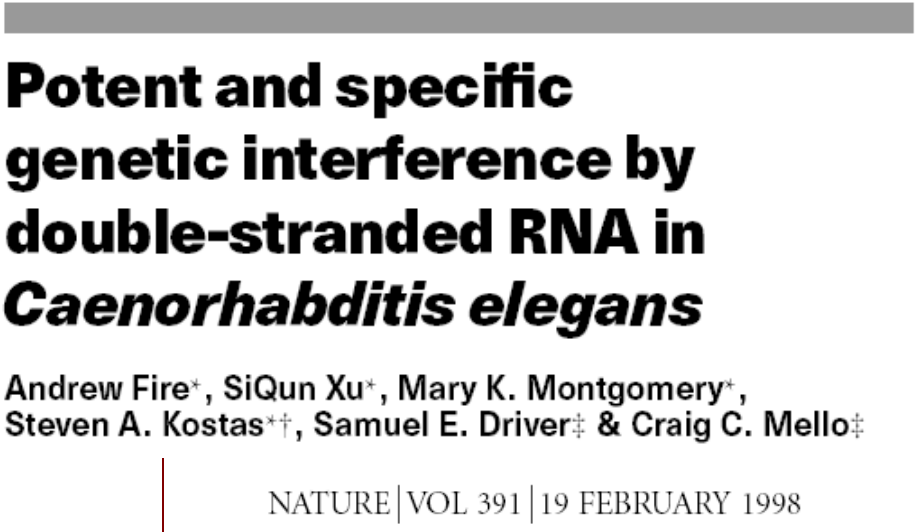
The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference - gene silencing by double-stranded RNA"

Photo: L. Cicero/Stanford

Photo: R. Carlin/UMMAS

Andrew Z. Fire	Craig C. Mello
1/2 of the prize	1/2 of the prize
USA	USA
Stanford University School of Medicine Stanford, CA, USA	University of Massachusetts Medical School Worcester, MA, USA



Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*,
Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

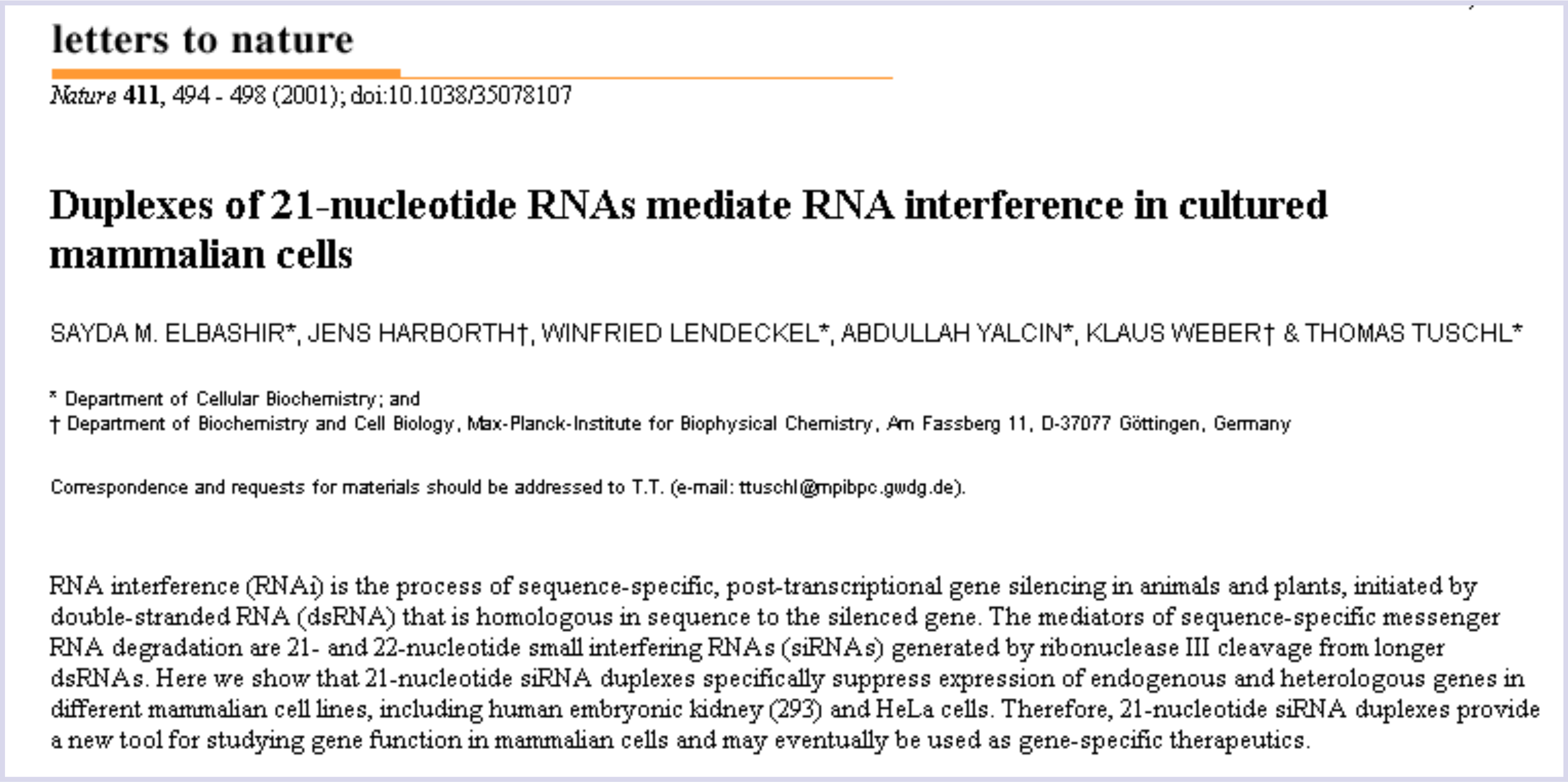
NATURE | VOL 391 | 19 FEBRUARY 1998

Cho WC. MicroRNAs in cancer - from research to therapy. *Biochim Biophys Acta - Rev Cancer* 2010;1805(2):209-217.

C. elegans

So…what about RNAi in mammalian cells…

May 2001…the first report…



letters to nature

Nature **411**, 494 - 498 (2001); doi:10.1038/35078107

Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells

SAYDA M. ELBASHIR*, JENS HARBORTH†, WINFRIED LENDECKEL*, ABDULLAH YALCIN*, KLAUS WEBER† & THOMAS TUSCHL*

* Department of Cellular Biochemistry; and
† Department of Biochemistry and Cell Biology, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11, D-37077 Göttingen, Germany

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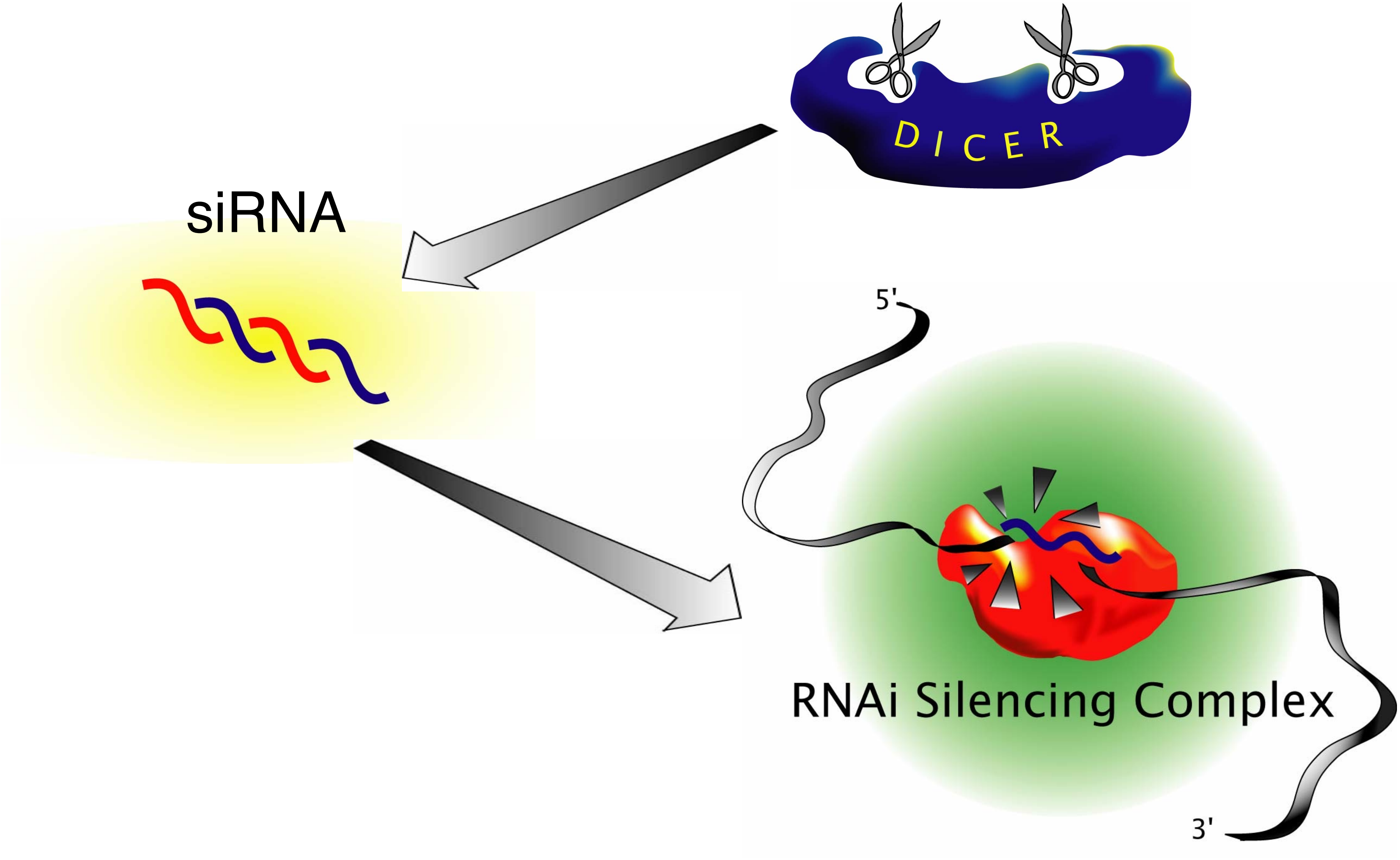
RNA interference (RNAi) is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. The mediators of sequence-specific messenger RNA degradation are 21- and 22-nucleotide small interfering RNAs (siRNAs) generated by ribonuclease III cleavage from longer dsRNAs. Here we show that 21-nucleotide siRNA duplexes specifically suppress expression of endogenous and heterologous genes in different mammalian cell lines, including human embryonic kidney (293) and HeLa cells. Therefore, 21-nucleotide siRNA duplexes provide a new tool for studying gene function in mammalian cells and may eventually be used as gene-specific therapeutics.

How does RNAi work in mammalian cells?

RNAi works post-transcriptionally……..

in key two steps!

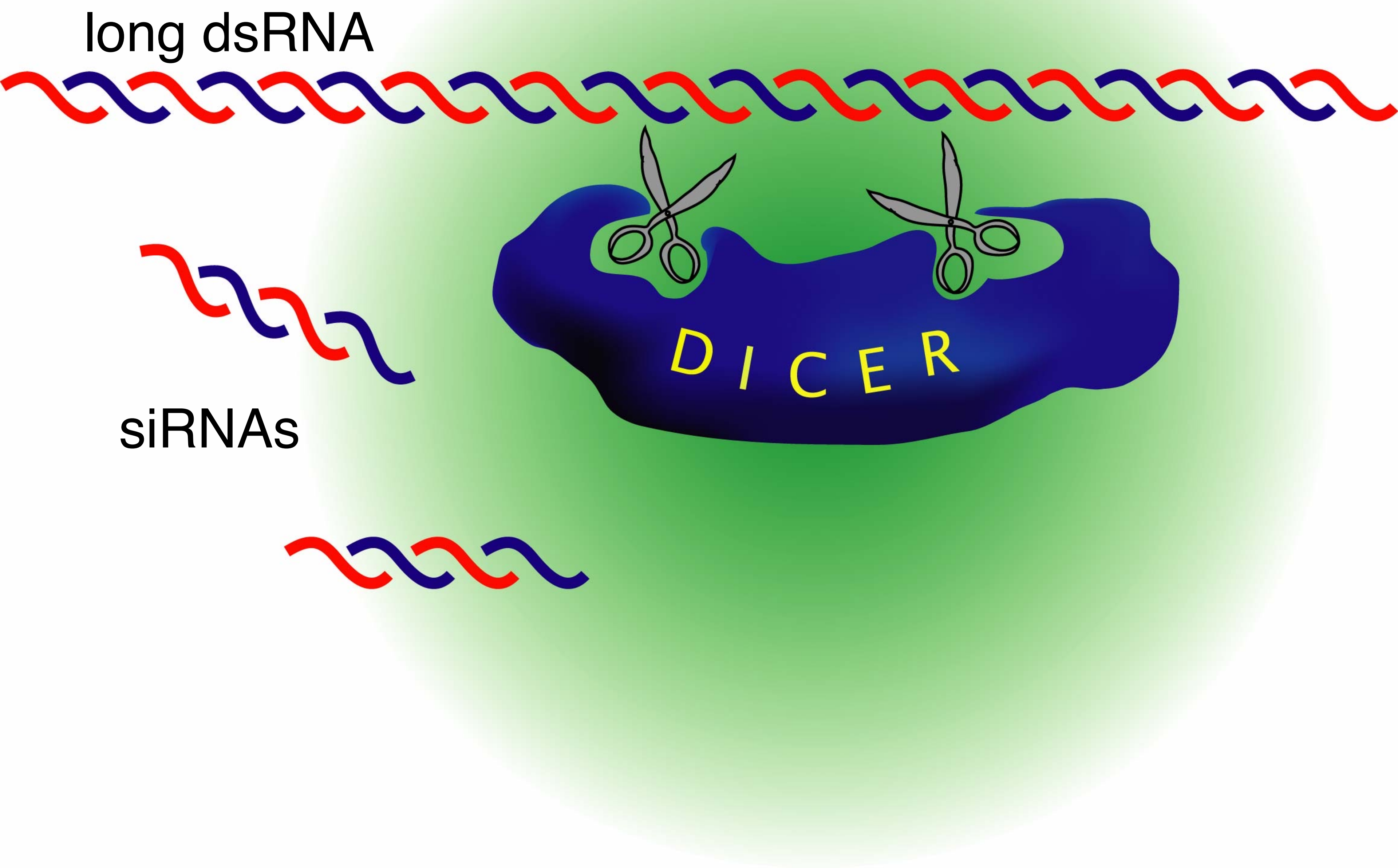
Model for RNAi



siRNA

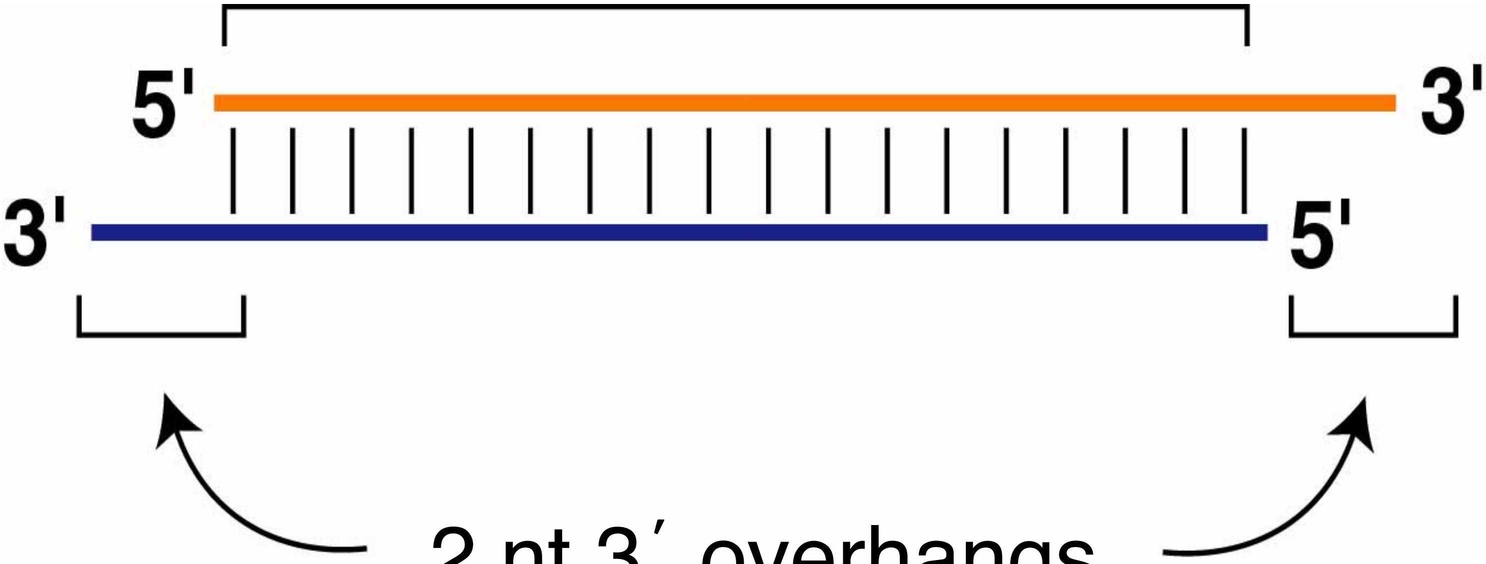
RNAi Silencing Complex

Dicer contains two RNAse III domains



siRNAs have a defined structure

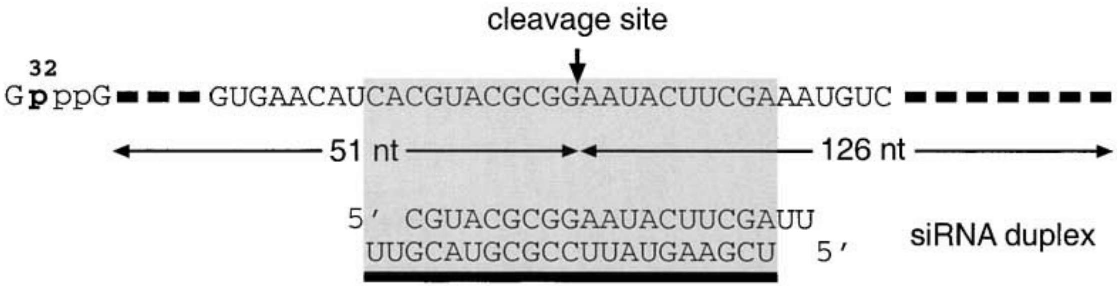
19 nt duplex



2 nt 3′ overhangs

step two:

the antisense strand of the siRNA guides cleavage



cleavage site

G^{32}**p**ppG GUGAACAUCACGUACGCGGAAUACUUCGAAAUGUC

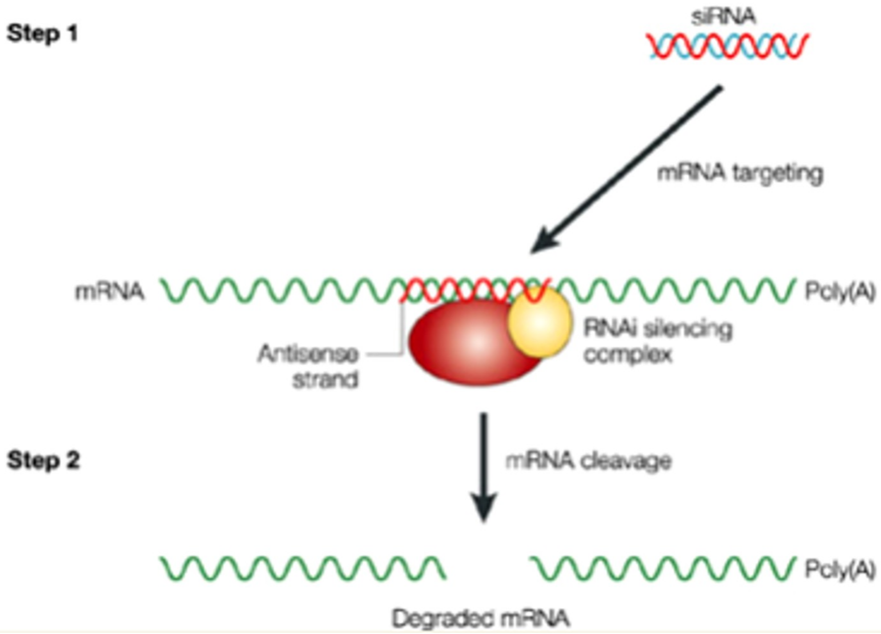
51 nt

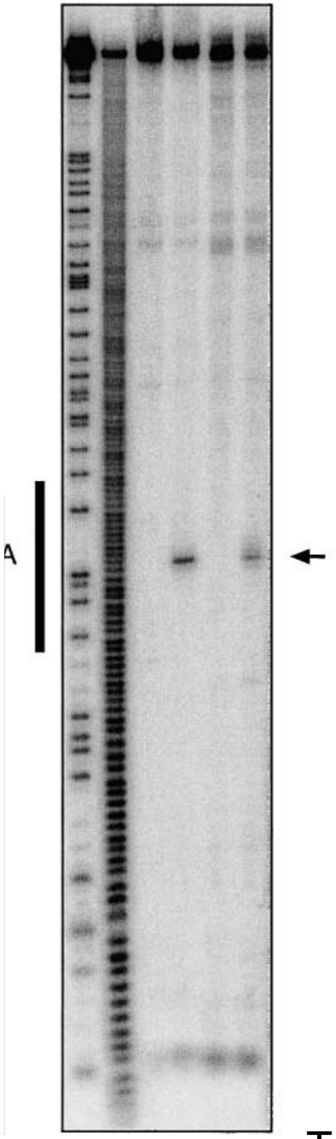
126 nt

5′ CGUACGCGGAAUACUUCGAUU

UUGCAUGCGCCUUAUGAAGCU 5′

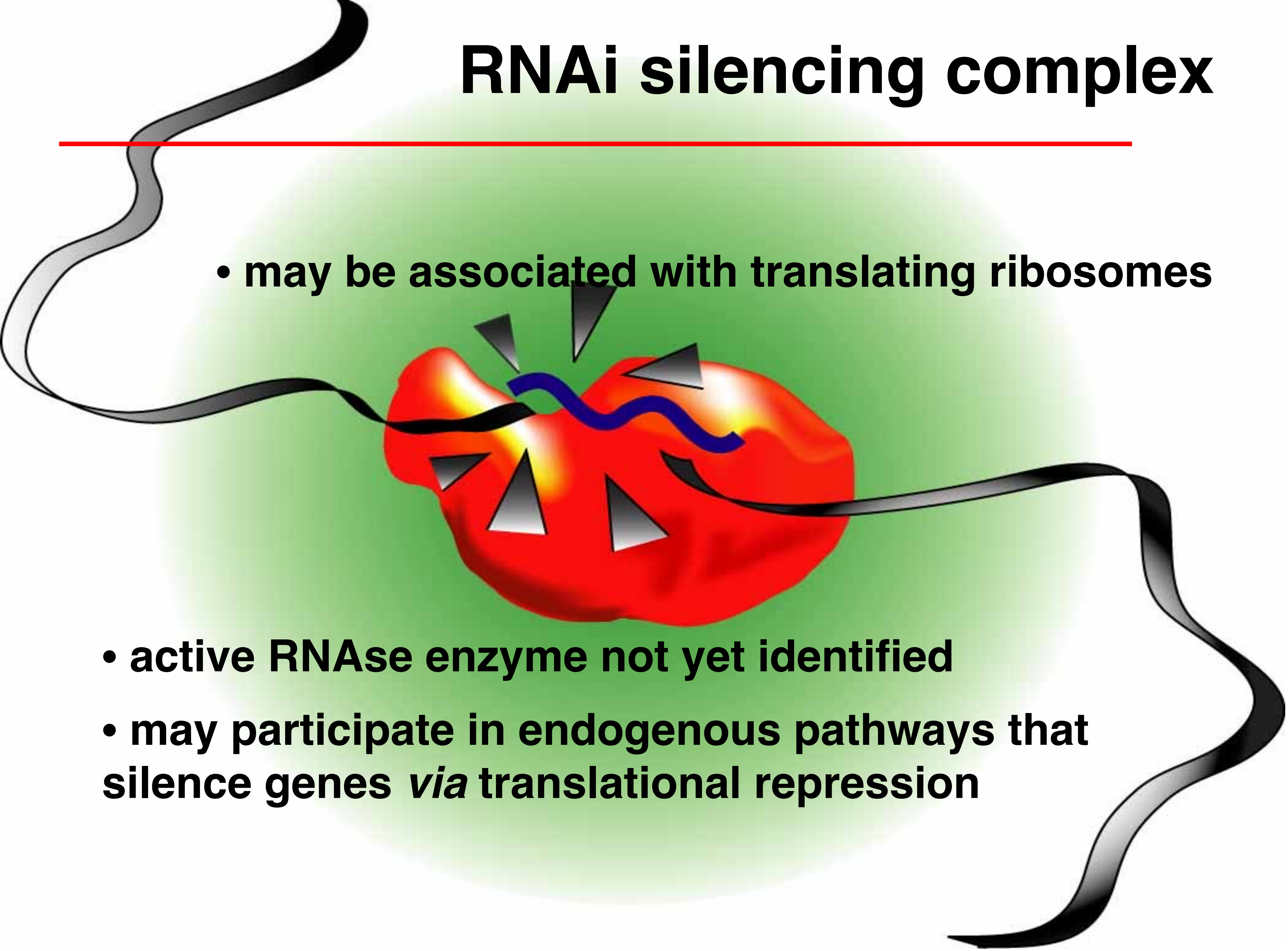
siRNA duplex





Tuschl, 2002

RNAi silencing complex

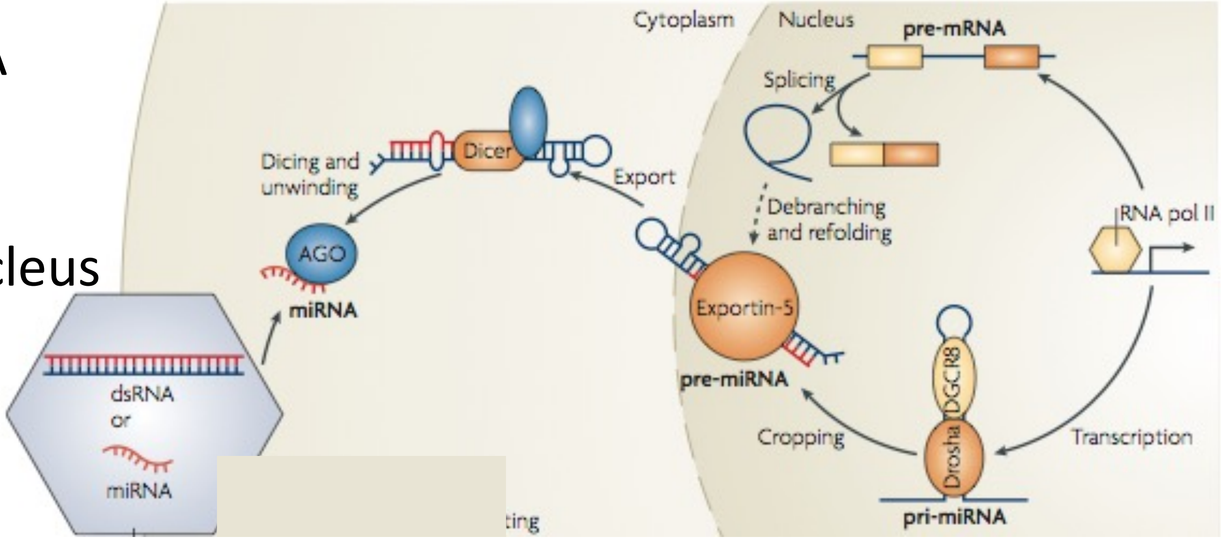


- may be associated with translating ribosomes

- active RNAse enzyme not yet identified
- may participate in endogenous pathways that silence genes *via* translational repression

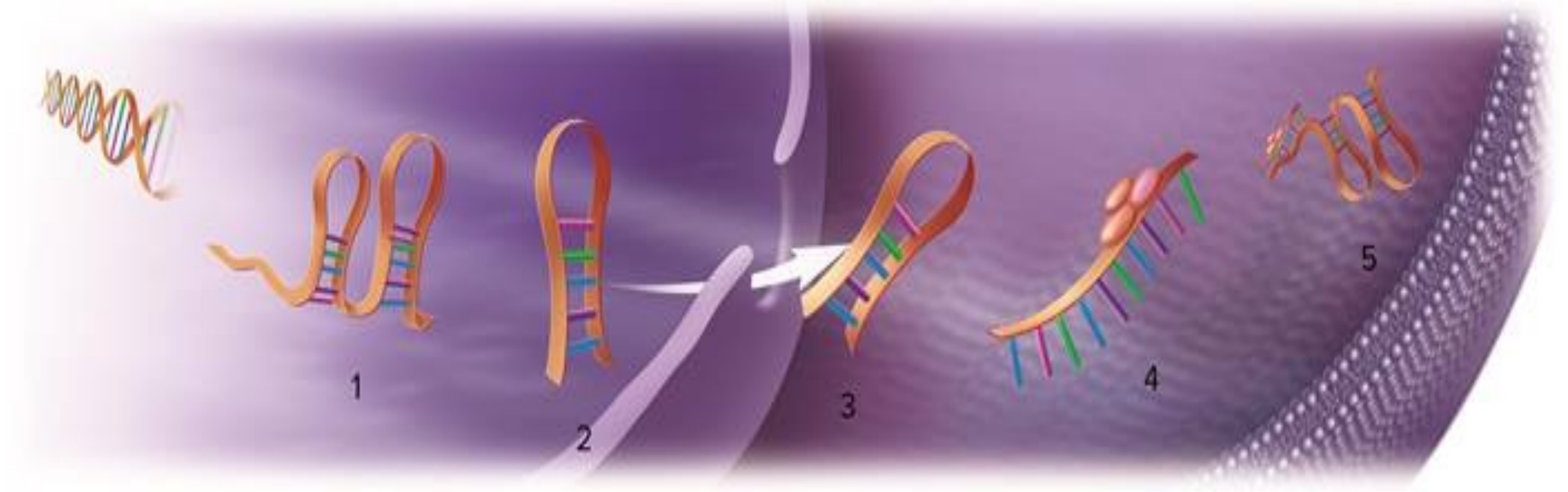
HOW MICRORNAS ARE PRODUCED?

- Mainly produced by RNA polymerase II
- 1st maturation in the nucleus
- 2nd maturation in the cytoplasm
- Active complex: miRISC

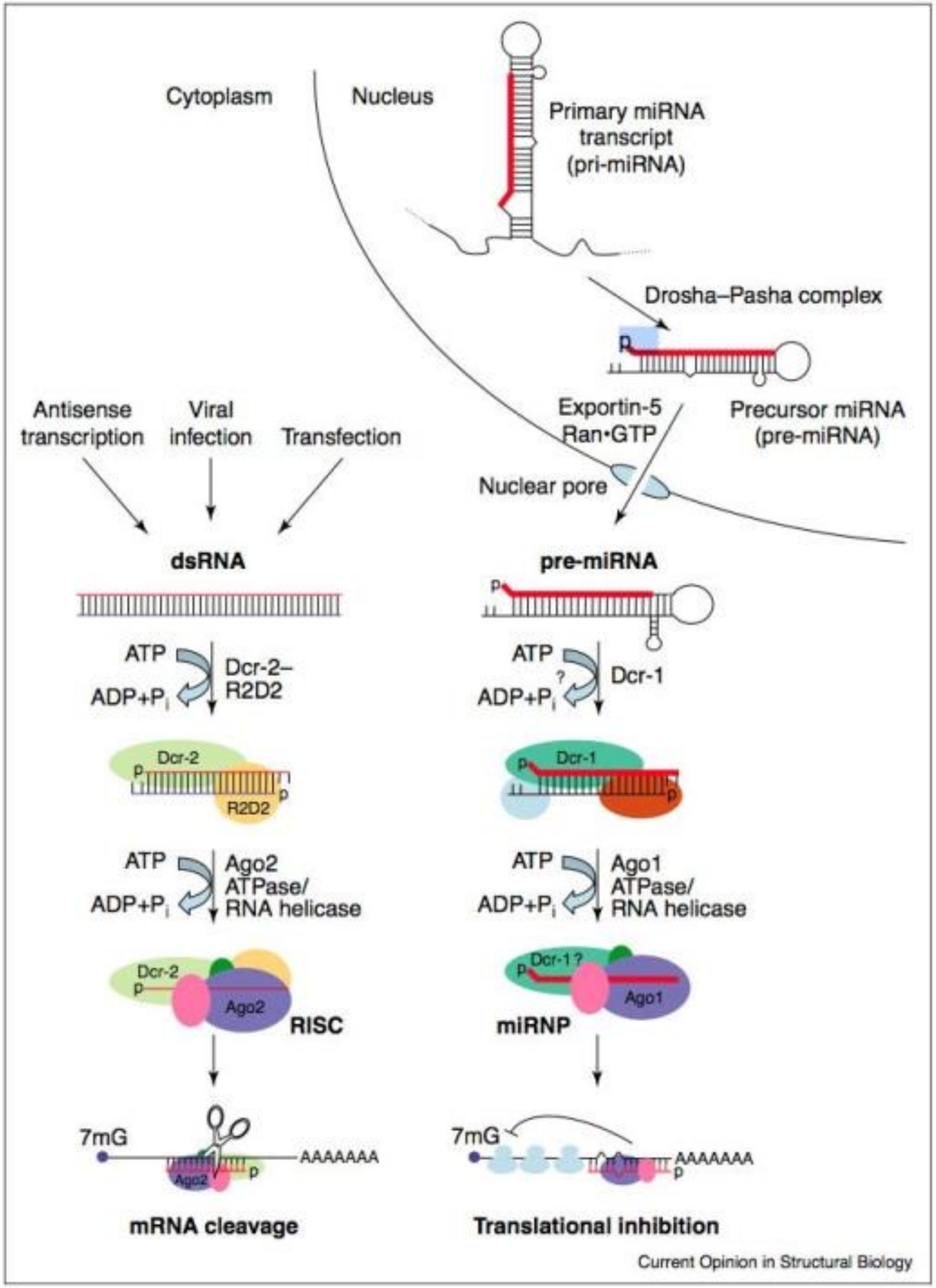


Nature Reviews MCB, 2008

Biogenesis of miRNA



1.The initial gene transcript is called primary miRNA (pri-miRNA)
2.In the cell nucleus, these hairpin-loop molecules are cut to form double-stranded precursor miRNA (pre-miRNA)
3.The pre-miRNA is transported to the cytoplasm. There, it is further cut to form a functional mature miRNA (mature miRNA molecules are about 22 nucleotides long)
4.The mature miRNA first binds with a molecule called the RNA interference silencing complex, or RISC
5.Then the miRNA binds with its target messenger RNA (mRNA), thereby blocking its translation or prompting its degradation



Current Opinion in Structural Biology

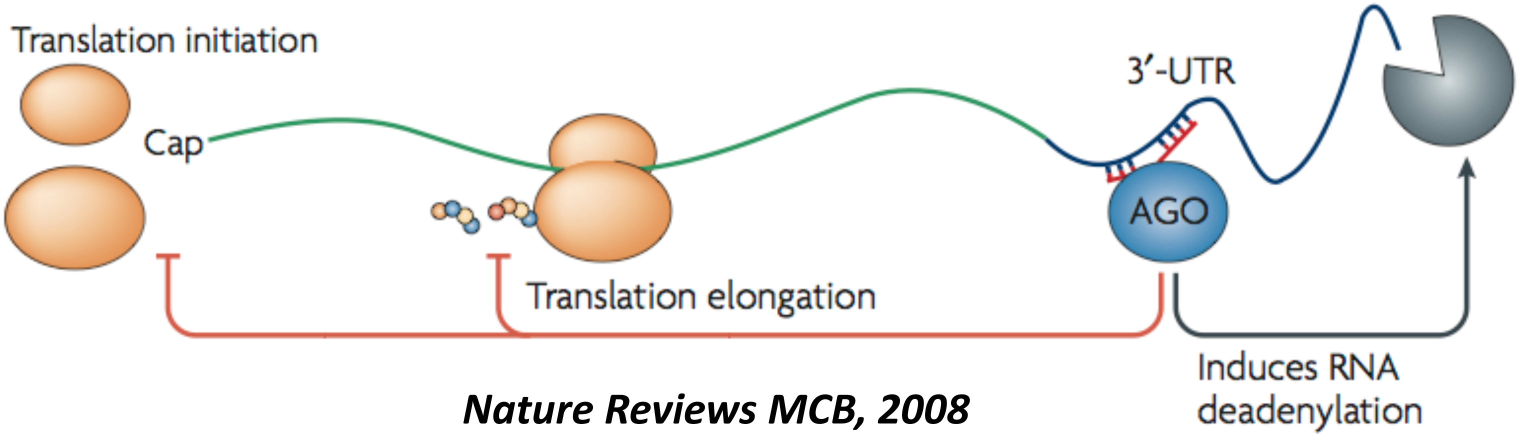
siRNA mediated degradation of mRNA

versus

miRNA mediated inhibition of mRNA translation

How microRNAs work?

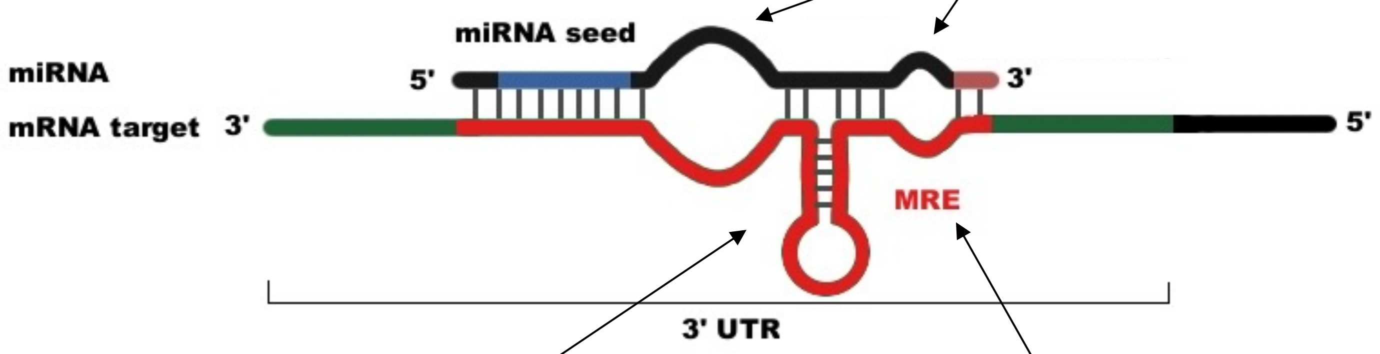
- Partial complementarity with 3’UTR regions (position 2 to 8 critical)
- Cooperative effect
- Abrogate protein synthesis



Nature Reviews MCB, 2008

One microRNA may regulate up to 100 different genes!

miRNA Binding

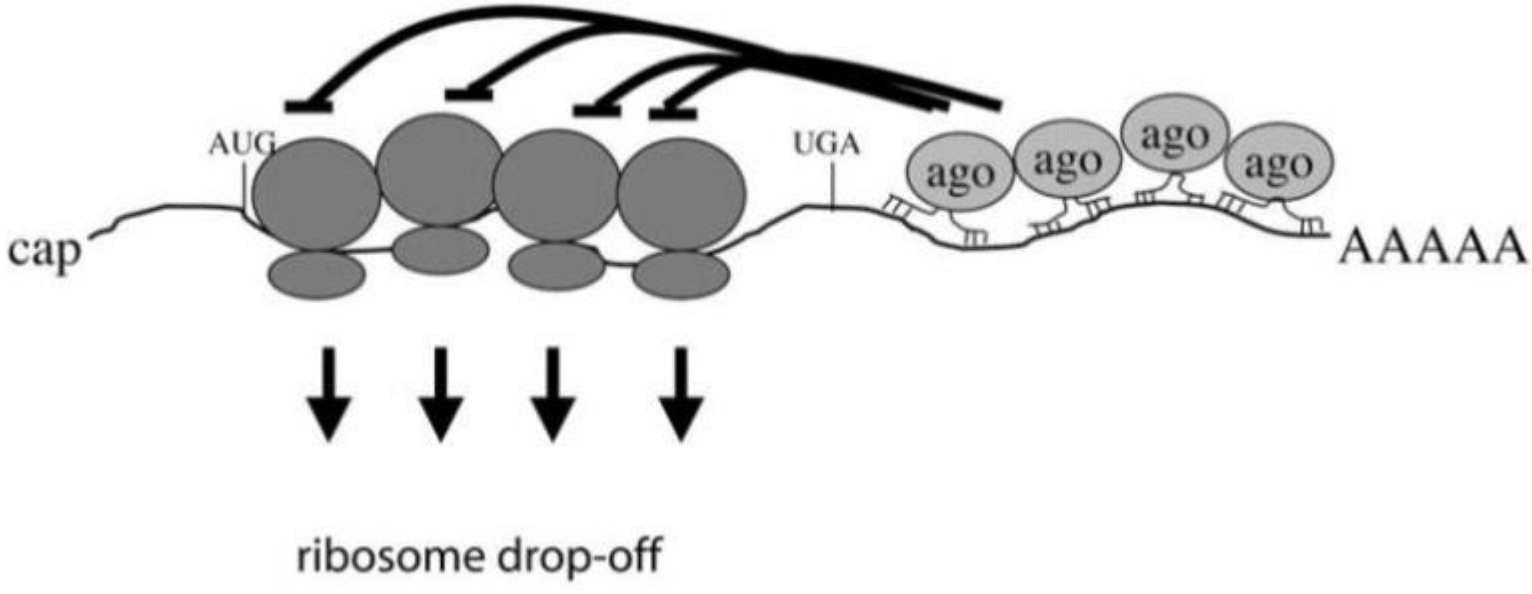


Bulges

Hairpin is more stable
than a simple bulge

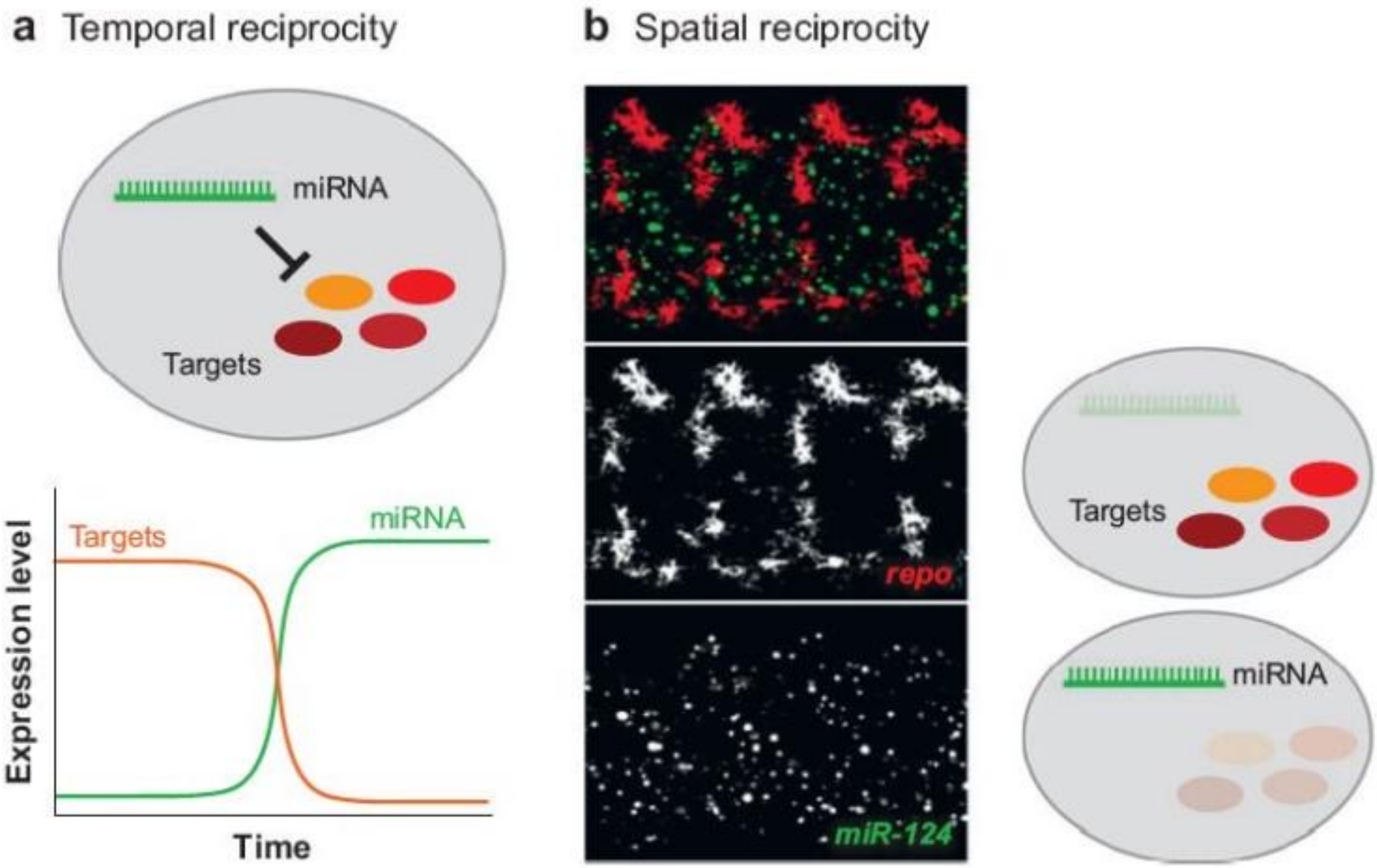
The MRE is known as the “miRNA recognition element.” This is simply the sequence in the target that an miRNA binds to

miRNAs Inhibit Translation by Inducing Ribosome Drop-Off



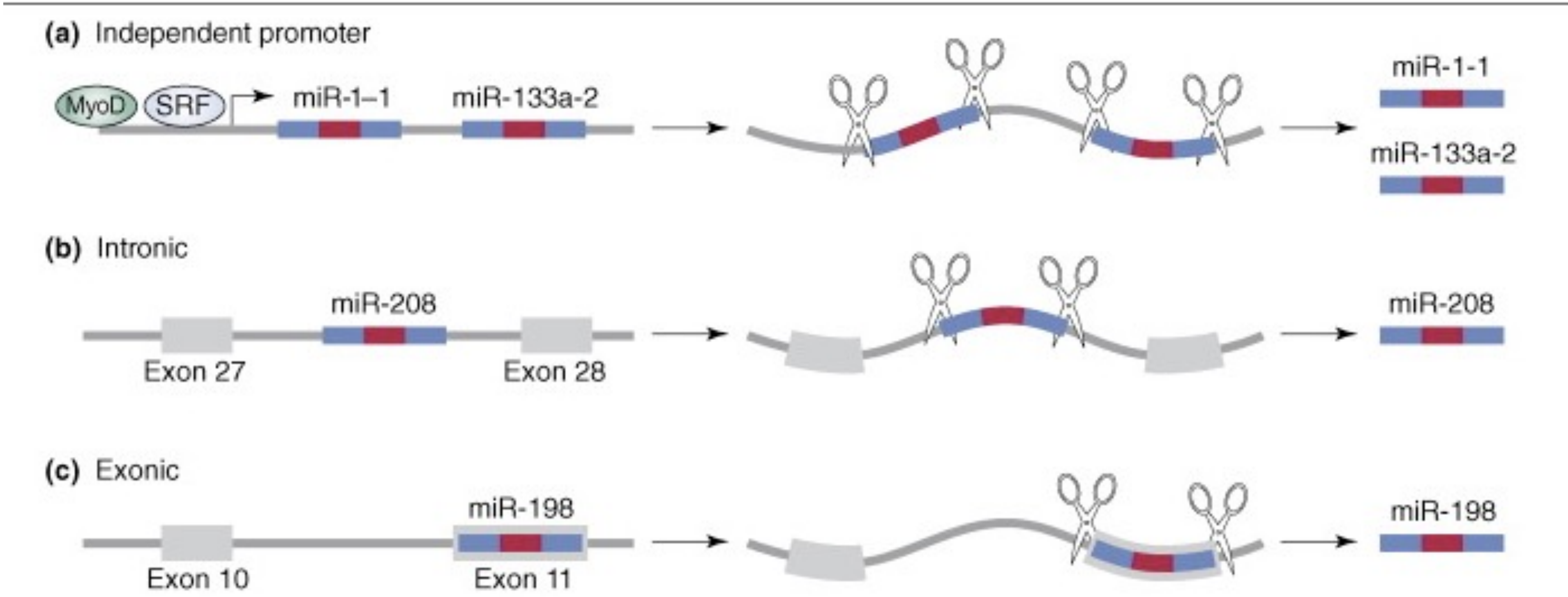
Petersen et al. (2006) Molecular Cell 21: 533-542.

miRNA Expression Results in Temporal and Spatial Reciprocity with Target Expression



Annu. Rev. Cell Dev. Biol. 2007.23:175-205

Genomic Organization of miRNA Genes

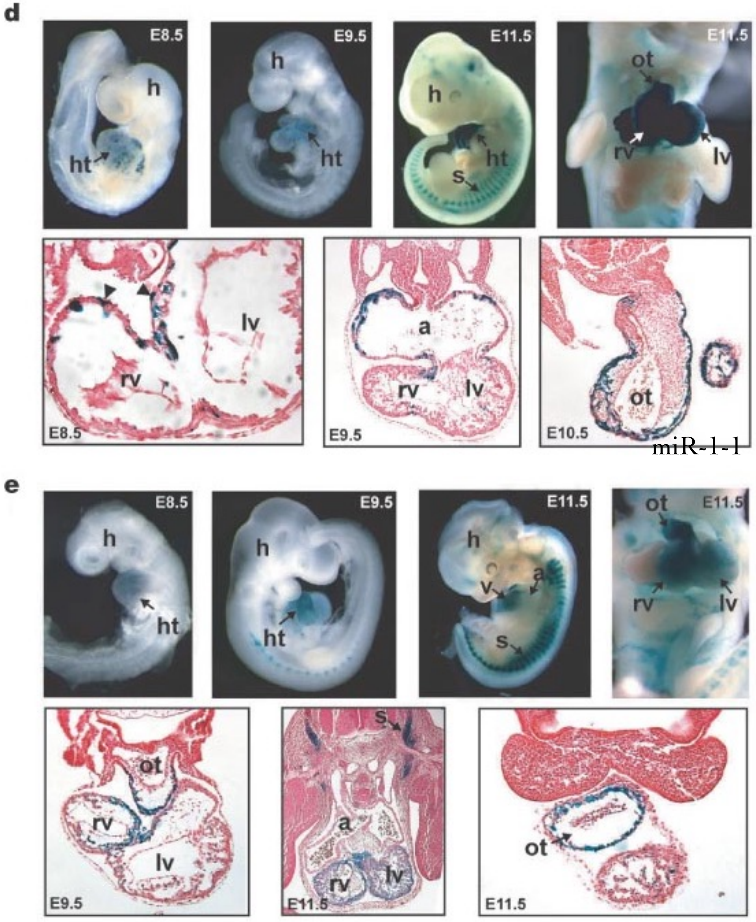


- Intronic miRNAs often in antisense direction, made from own promoter

- Exonic miRNAs - non-coding (or in alternatively spliced exons)

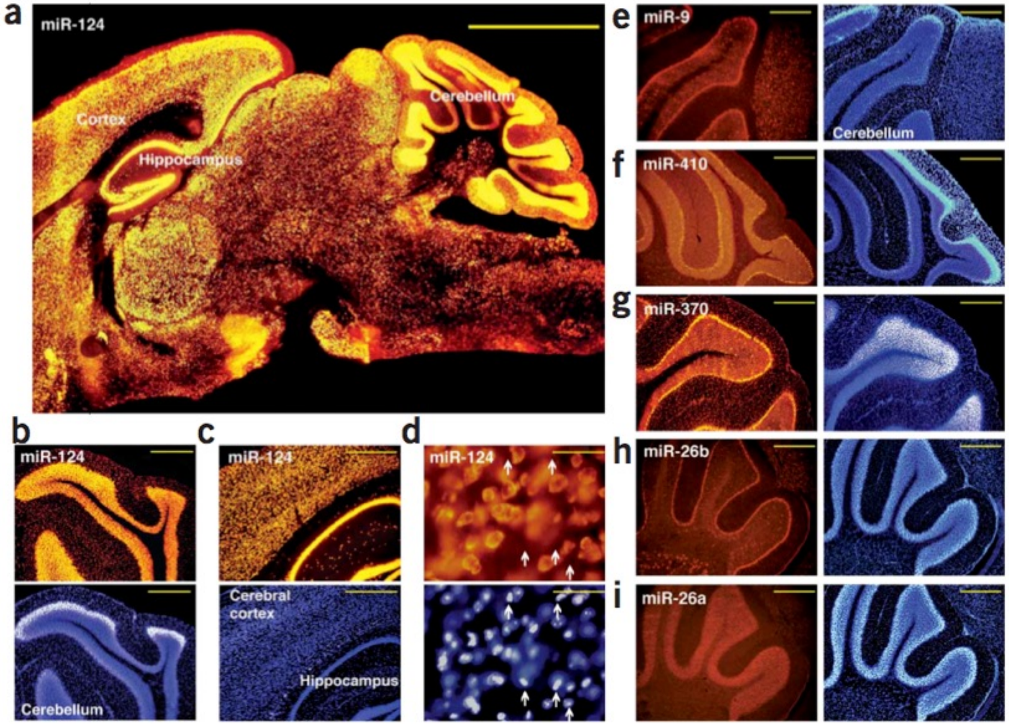
Zhao Y, Srivastava D, TIBS 32:189,2007

Precise expression profile



miR-1-1

miR-1-2

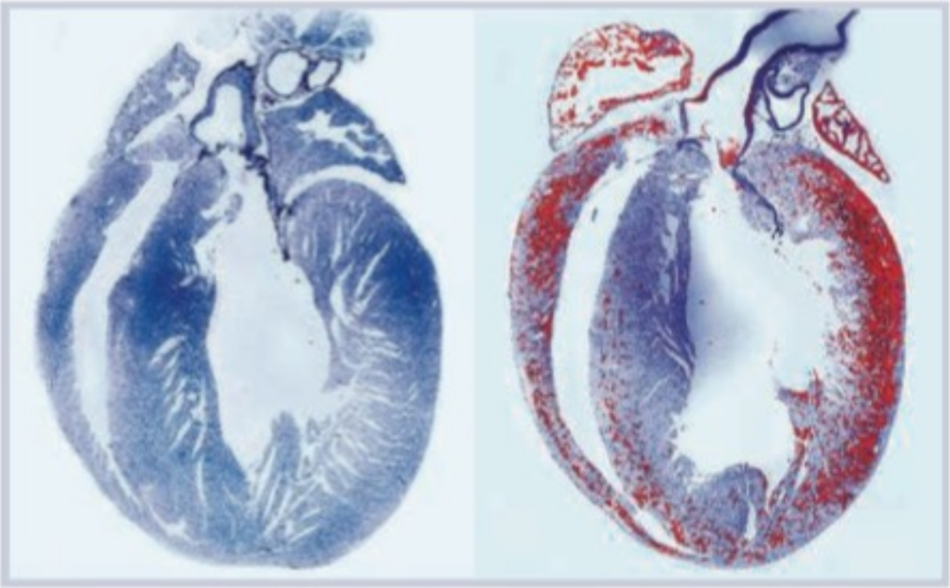


Zhao et al, Nature 2005

Pena et al, Nature Methods 2009

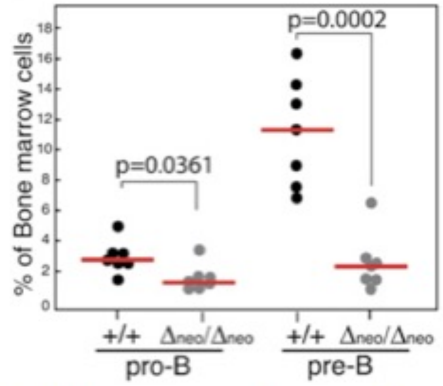
Loss of microRNA leads to fatality

Zhao et al, Cell 2007



Loss of miR-1-2 leads to overproduction of muscle cells

Ventura et al, Cell 2008

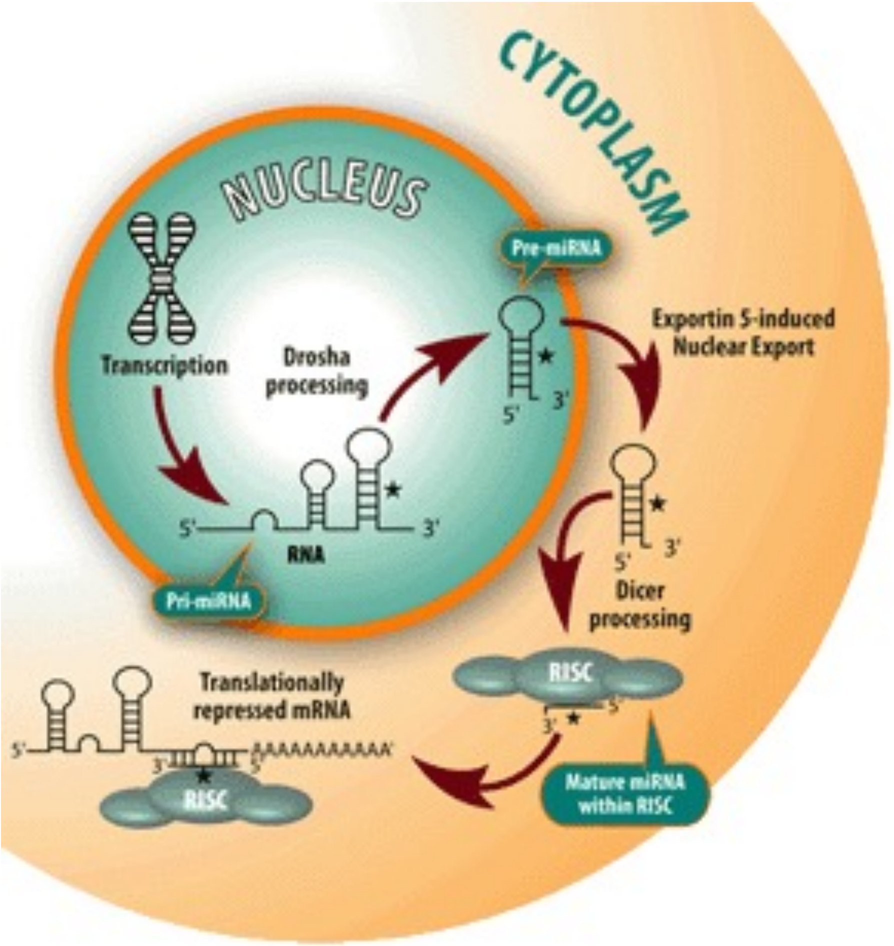


Loss of miR-17-92 cluster is embryonic lethal

Mechanisms linking microRNAs with disease

- **Genomic alterations of microRNAs**
  - Chromosome deletion, amplification, and translocation
  - Single nucleotide polymorphism of miRNA or miRNA targets
- **Alterations on the expression of levels of miRNAs**
  - Transcriptional control: transcription factor, enhancer, repressor
  - Epigenetic modification: DNA methylation, histone acetylation
- **Alteration on the processes of microRNA biogenesis**

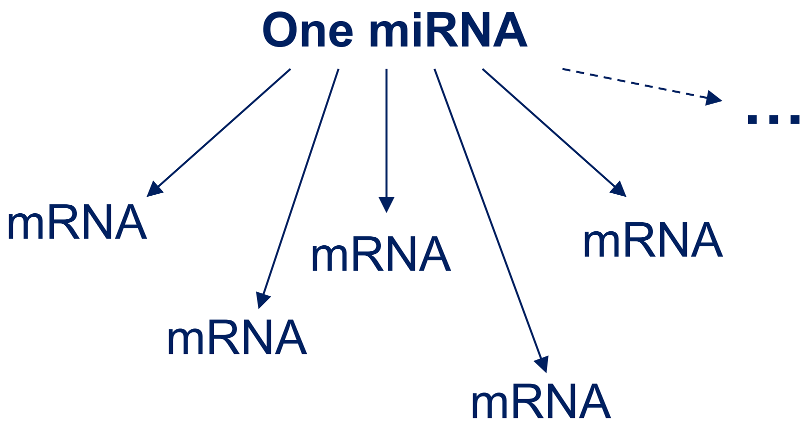
microRNA: a new class of biomarkers



small noncoding RNAs that regulate gene expression by binding complementary sequences of target mRNAs and inducing their degradation or translational repression

Evolutionary conserved

One miRNA has multiple targets


One miRNA

mRNA

mRNA

mRNA

mRNA

mRNA

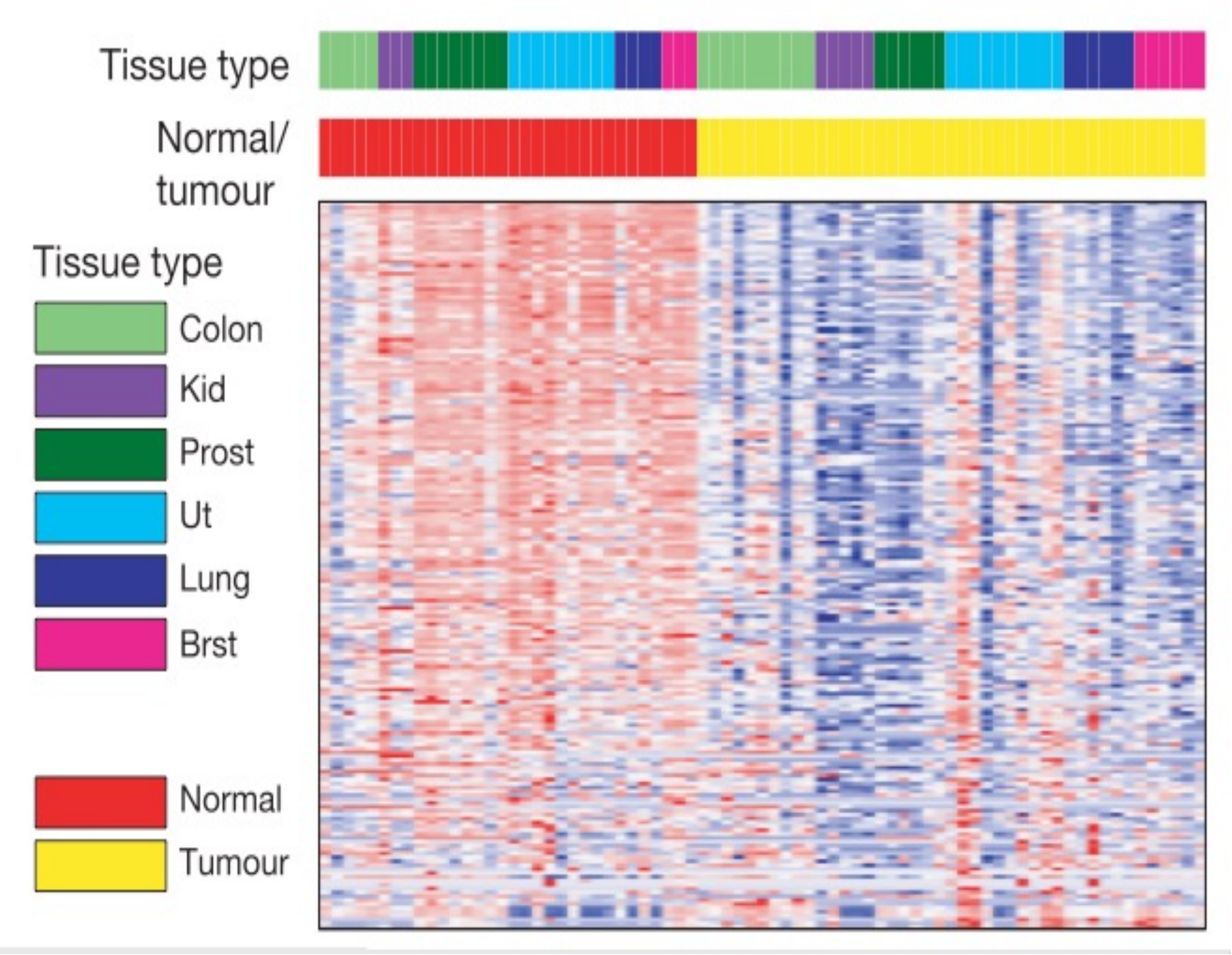
…

microRNAs linked to diseases

- Viral infections
  - *Viruses encodes microRNAs that target viral mRNAs to regulate various stages of the viral life cycle*
  - *Viral microRNAs suppress expression of specific host genes*
  - *Viral infections induce expression of host microRNAs that inhibits expression of cellular genes*
  - *Upon viral infections, host cells express specific microRNA that suppress viral mRNA expression*
- Cardiac, immune, neurological and metabolic disorders

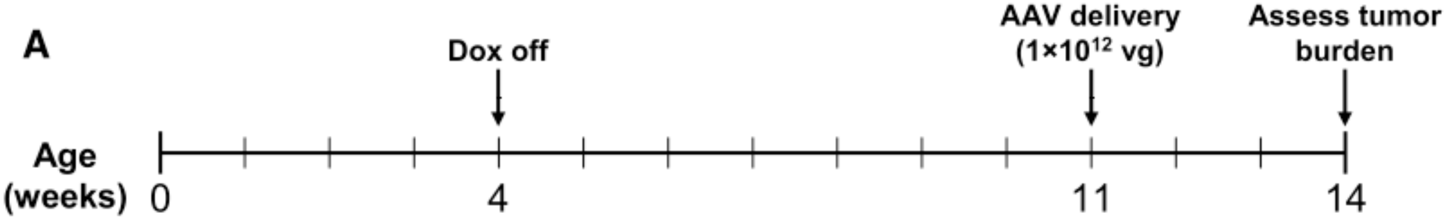
MicroRNAs and Cancer

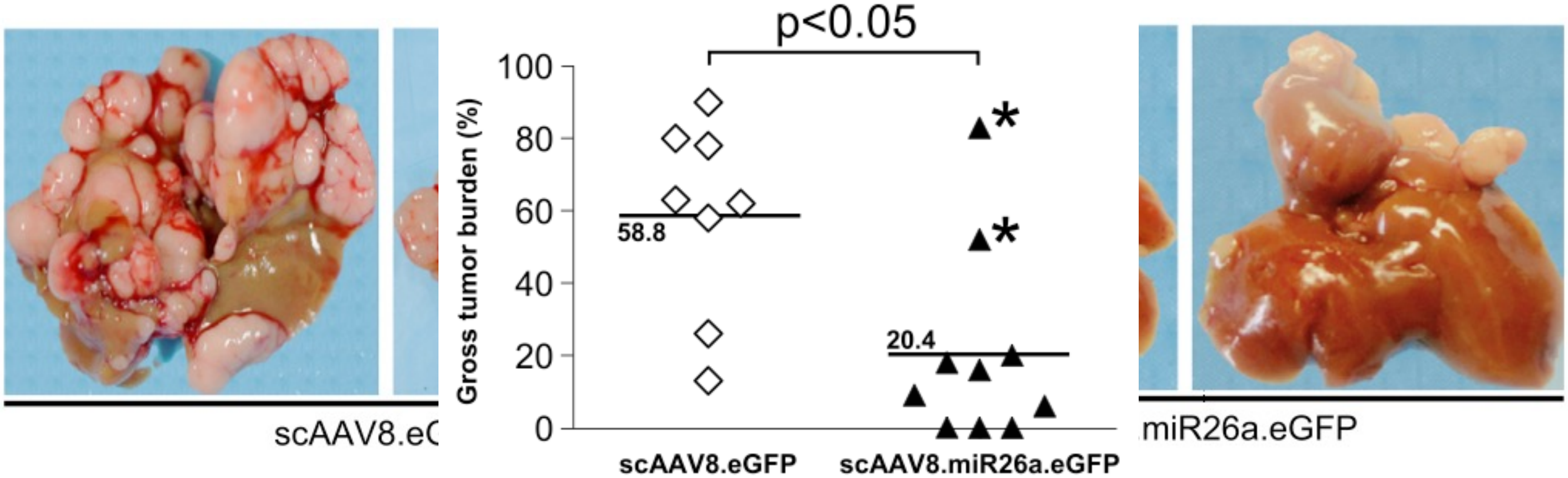
Different expression pro
between healthy and ca
tissue samples



Lu et al, Nature 2005

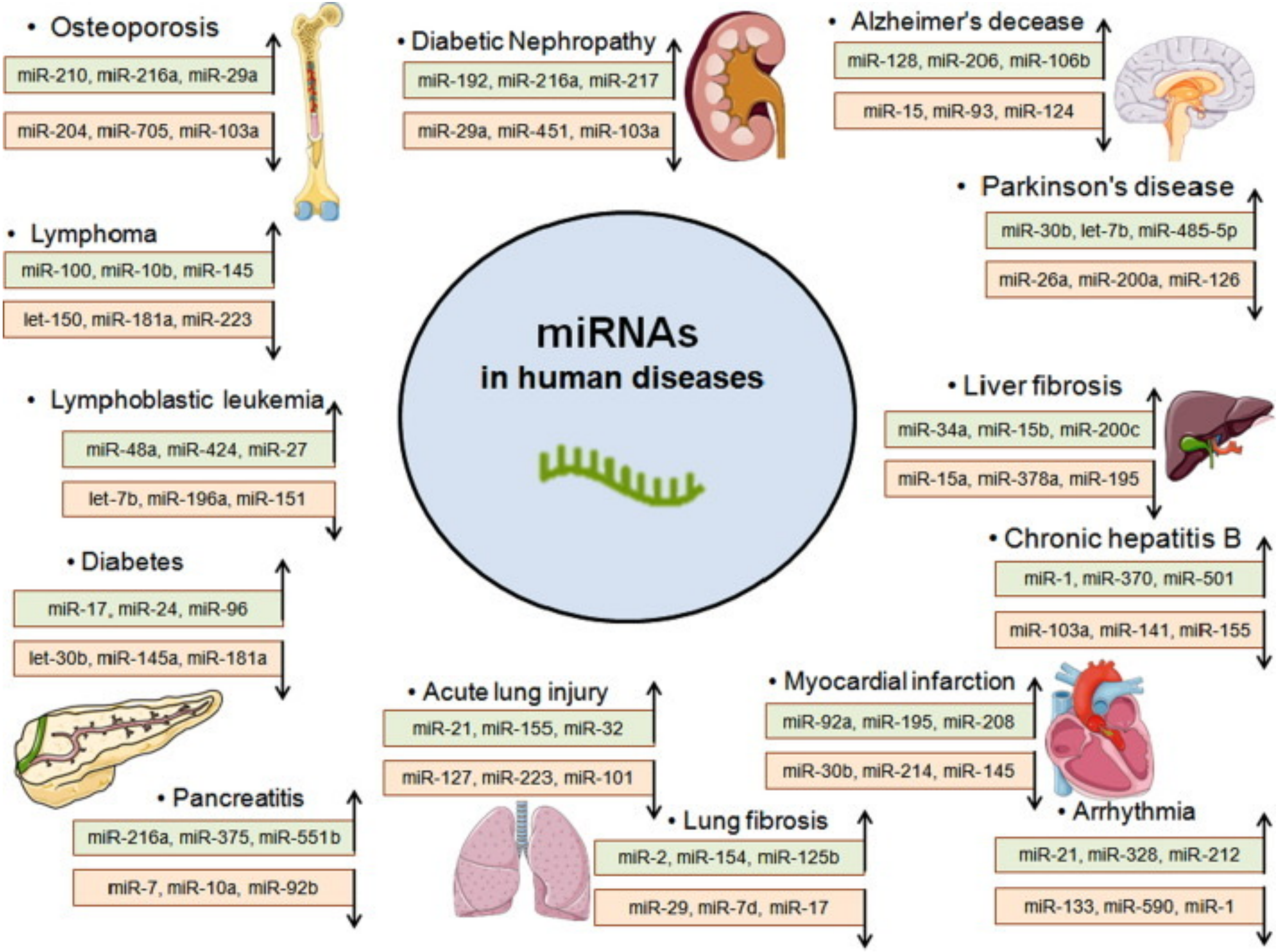
MicroRNAs in cancer therapy

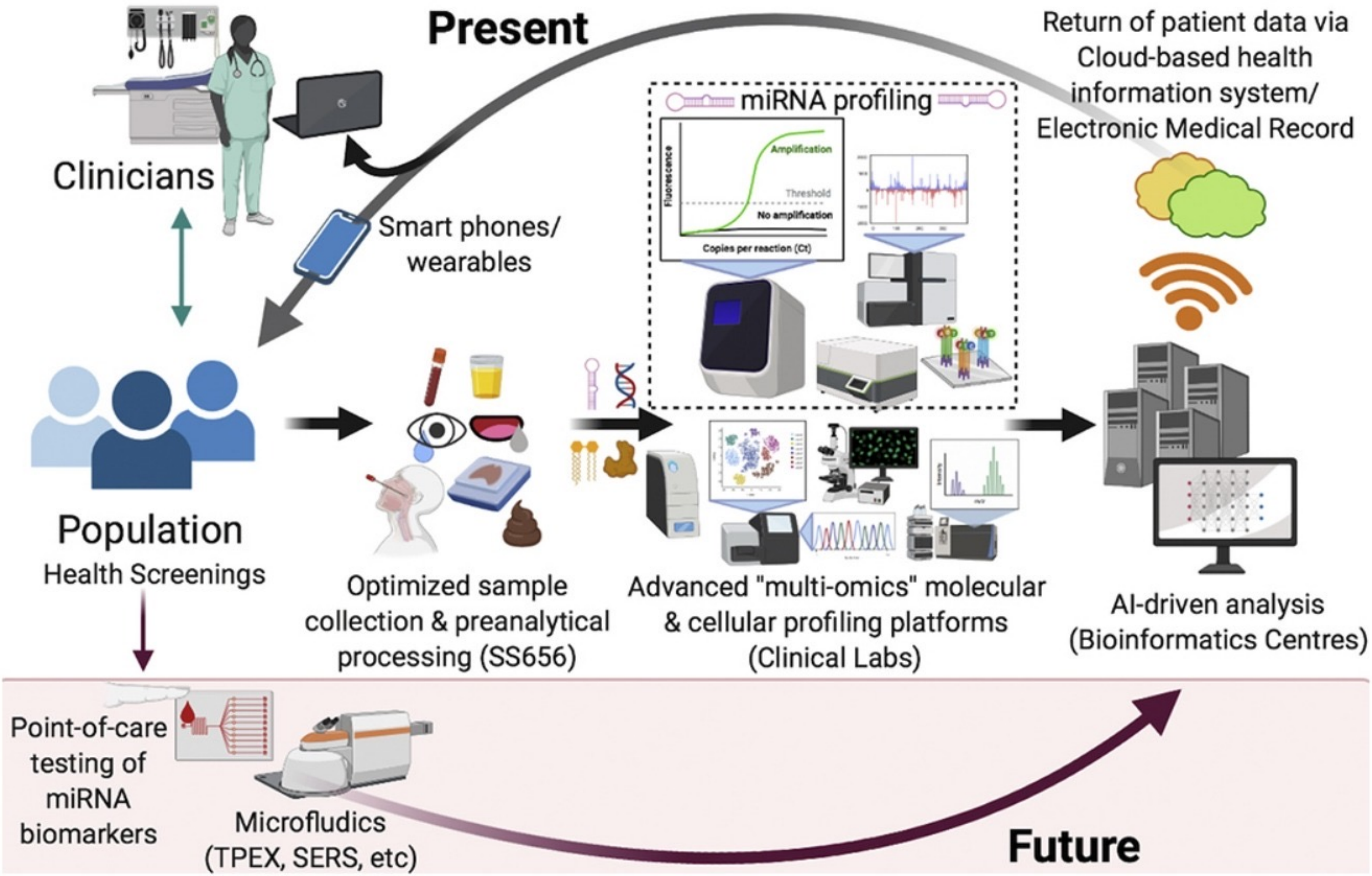




Decrease in tumour mass

Kota et al. Cell, 2009


miRNAs
in human diseases
• Osteoporosis
miR-210, miR-216a, miR-29a
miR-204, miR-705, miR-103a
• Diabetic Nephropathy
miR-192, miR-216a, miR-217
miR-29a, miR-451, miR-103a
• Alzheimer's decease
miR-128, miR-206, miR-106b
miR-15, miR-93, miR-124
• Parkinson's disease
miR-30b, let-7b, miR-485-5p
miR-26a, miR-200a, miR-126
• Lymphoma
miR-100, miR-10b, miR-145
let-150, miR-181a, miR-223
• Lymphoblastic leukemia
miR-48a, miR-424, miR-27
let-7b, miR-196a, miR-151
• Liver fibrosis
miR-34a, miR-15b, miR-200c
miR-15a, miR-378a, miR-195
• Chronic hepatitis B
miR-1, miR-370, miR-501
miR-103a, miR-141, miR-155
• Diabetes
miR-17, miR-24, miR-96
let-30b, miR-145a, miR-181a
• Acute lung injury
miR-21, miR-155, miR-32
miR-127, miR-223, miR-101
• Myocardial infarction
miR-92a, miR-195, miR-208
miR-30b, miR-214, miR-145
• Pancreatitis
miR-216a, miR-375, miR-551b
miR-7, miR-10a, miR-92b
• Lung fibrosis
miR-2, miR-154, miR-125b
miR-29, miR-7d, miR-17
• Arrhythmia
miR-21, miR-328, miR-212
miR-133, miR-590, miR-1


Present
Return of patient data via
Cloud-based health
information system/
Electronic Medical Record
Clinicians
miRNA profiling
Amplification
Fluorescence
Threshold
No amplification
Copies per reaction (Ct)
Smart phones/
wearables
Population
Health Screenings
Optimized sample
collection & preanalytical
processing (SS656)
Advanced "multi-omics" molecular
& cellular profiling platforms
(Clinical Labs)
AI-driven analysis
(Bioinformatics Centres)
Point-of-care
testing of
miRNA
biomarkers
Microfludics
(TPEX, SERS, etc)
Future

Predictive Modelling

- Permit risk stratification.
- Customize treatment

  Less extensive surgery

  Rational drug selection

  Monitoring response to therapy

Predictive or Diagnostic Modelling

Use of one or more biomarkers to determine prognosis or response to treatment beyond usual clinical criteria

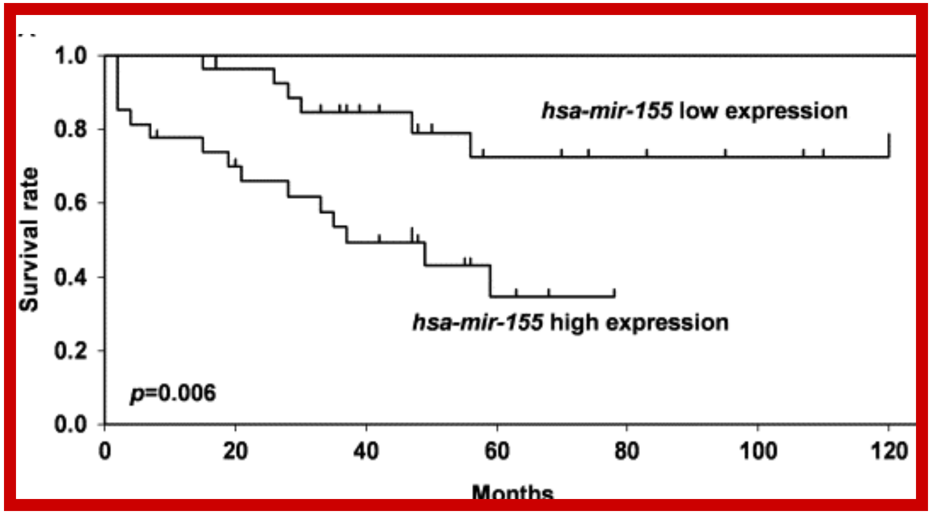
- Tissue based
- Serum or urinary based
- Cellular based

Why are microRNAS such good biomarkers?

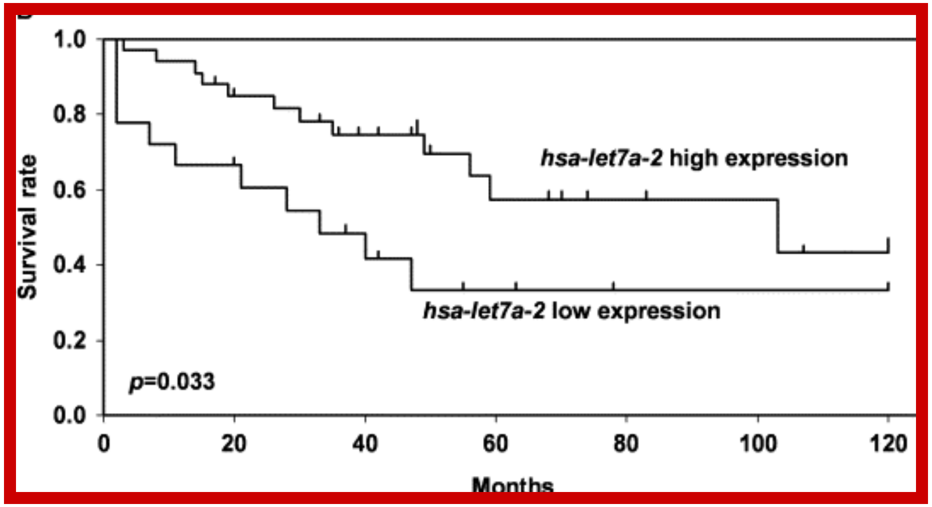
- Extremely stable in fluids as well as on formalin-fixed paraffin-embedded tissue
- Expression profile correlates well between fresh and formalin-fixed paraffin-embedded samples
- Resistant to degradation

Unique microRNA profile in lung cancer diagnosis and prognosis

- A univariate Cox proportional hazard regression model with a global permutation test indicated that expression of the miRNAs *hsa-mir-155* and *hsa-let-7a-2* were related to adenocarcinoma patient outcome

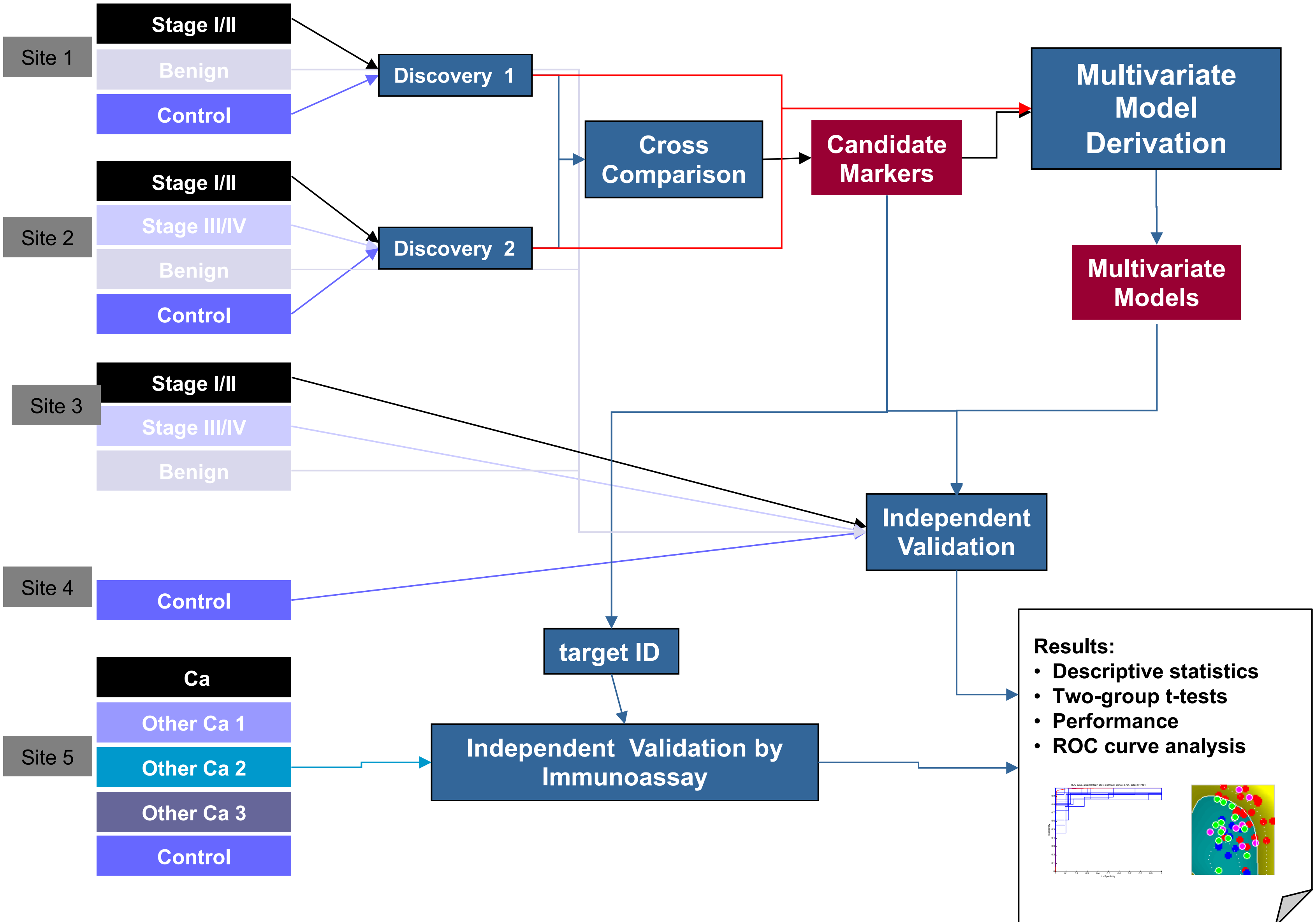


Lung adenocarcinoma patients with either high *hsa-mir-155* or reduced *hsa-let-7a-2* expression had poor survival



Yanaihara N, *et al.* Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell* 2006, 9:189-198.

Study Design for Biomarker Validation



Site 1
- Stage I/II
- Benign
- Control

Site 2
- Stage I/II
- Stage III/IV
- Benign
- Control

Site 3
- Stage I/II
- Stage III/IV
- Benign

Site 4
- Control

Site 5
- Ca
- Other Ca 1
- Other Ca 2
- Other Ca 3
- Control

Discovery 1

Discovery 2

Cross Comparison

Candidate Markers

Multivariate Model Derivation

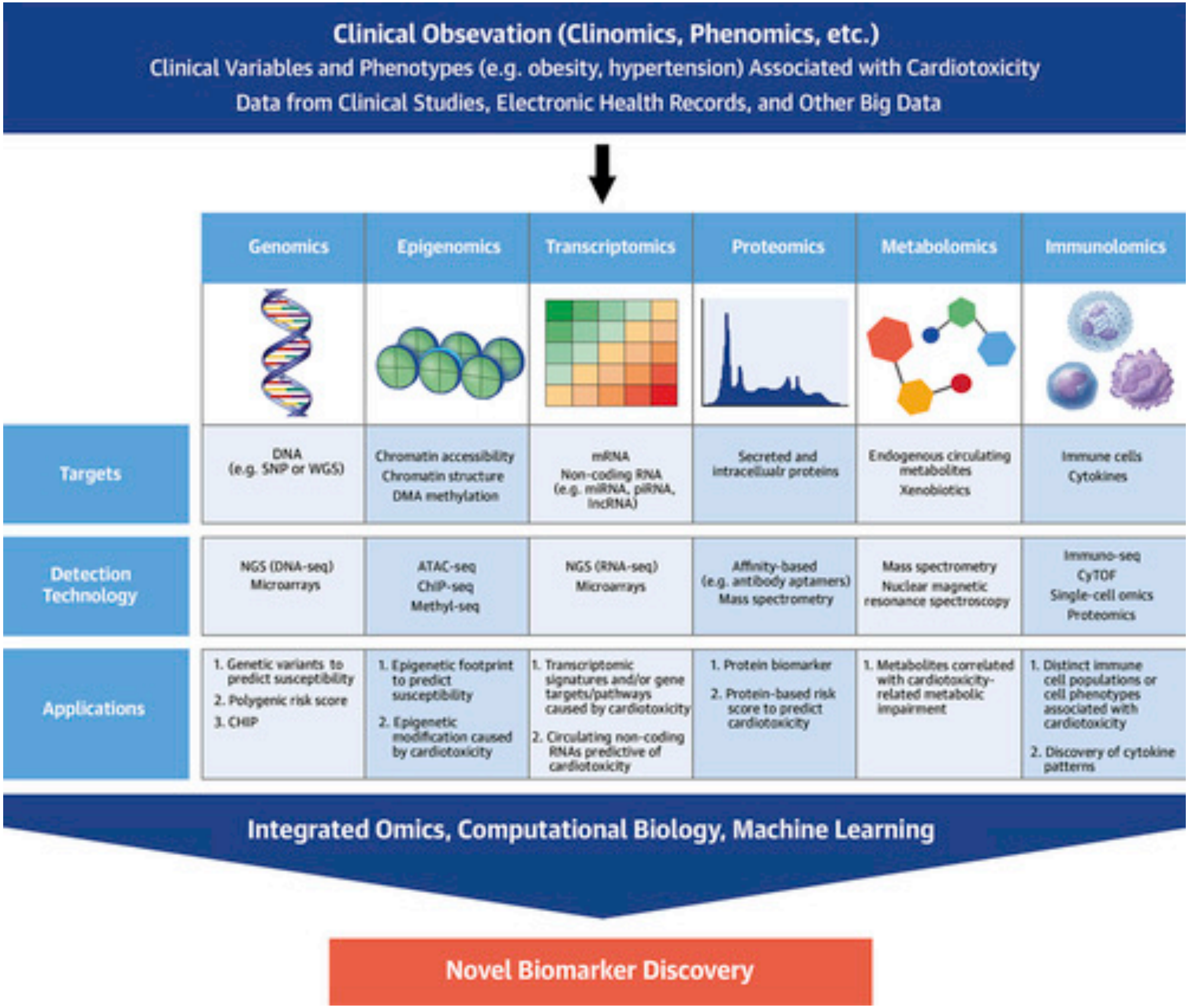
Multivariate Models

Independent Validation

target ID

Independent Validation by Immunoassay

Results:
- Descriptive statistics
- Two-group t-tests
- Performance
- ROC curve analysis



Clinical Obsevation (Clinomics, Phenomics, etc.)

Clinical Variables and Phenotypes (e.g. obesity, hypertension) Associated with Cardiotoxicity

Data from Clinical Studies, Electronic Health Records, and Other Big Data

	Genomics	Epigenomics	Transcriptomics	Proteomics	Metabolomics	Immunolomics
Targets	DNA (e.g. SNP or WGS)	Chromatin accessibility Chromatin structure DMA methylation	mRNA Non-coding RNA (e.g. miRNA, piRNA, lncRNA)	Secreted and intracellualr proteins	Endogenous circulating metabolites Xenobiotics	Immune cells Cytokines
Detection Technology	NGS (DNA-seq) Microarrays	ATAC-seq ChIP-seq Methyl-seq	NGS (RNA-seq) Microarrays	Affinity-based (e.g. antibody aptamers) Mass spectrometry	Mass spectrometry Nuclear magnetic resonance spectroscopy	Immuno-seq CyTOF Single-cell omics Proteomics
Applications	1. Genetic variants to predict susceptibility 2. Polygenic risk score 3. CHIP	1. Epigenetic footprint to predict susceptibility 2. Epigenetic modification caused by cardiotoxicity	1. Transcriptomic signatures and/or gene targets/pathways caused by cardiotoxicity 2. Circulating non-coding RNAs predictive of cardiotoxicity	1. Protein biomarker 2. Protein-based risk score to predict cardiotoxicity	1. Metabolites correlated with cardiotoxicity-related metabolic impairment	1. Distinct immune cell populations or cell phenotypes associated with cardiotoxicity 2. Discovery of cytokine patterns

Integrated Omics, Computational Biology, Machine Learning

Novel Biomarker Discovery

microRNA Biomarker discovery workflow and panel selection options

DISCOVERY PHASE

- Genome wide screening
- Normalization, QC, processing
- Candidate miRNA discovery screen
- Bioinformatics, data analysis

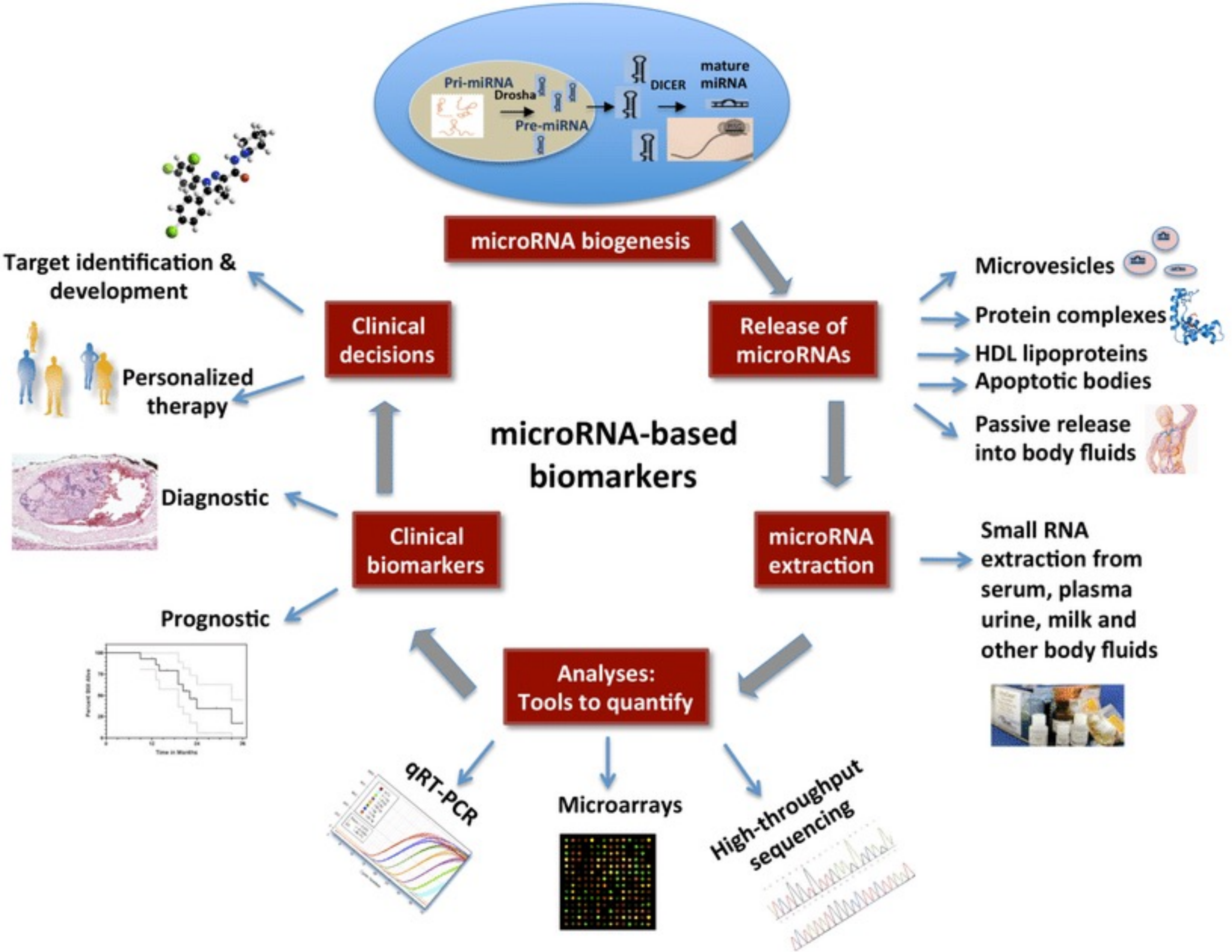
VALIDATION PHASE

- Validation Set miRNA signature

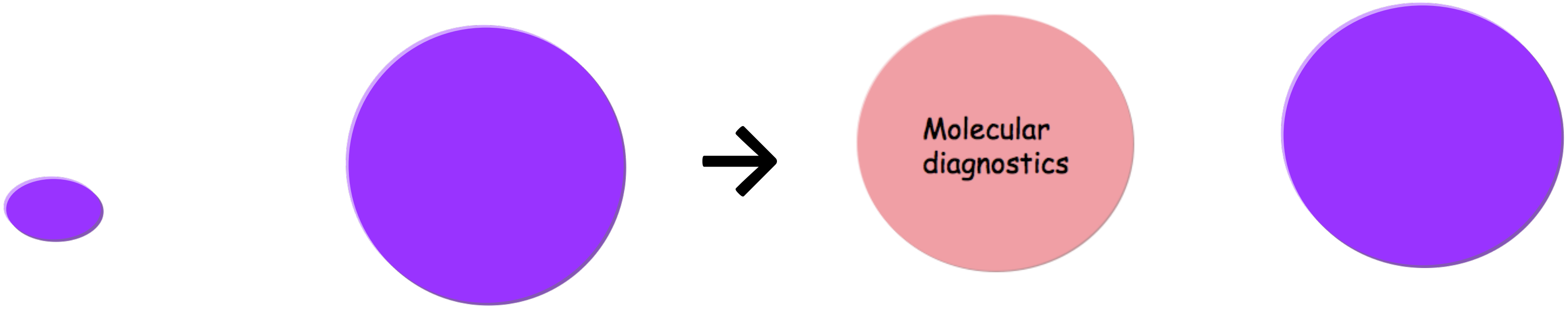
Pre-defined miRNome PCR panels
Human and Mouse&Rat
(Ready-to-Use)

Pre-defined Focus PCR panels
Serum/plasma and Cancer
(Ready-to-Use)

Pick&Mix, custom PCR panels
(Ready-to-Use)


Pri-miRNA
Drosha
Pre-miRNA
DICER
mature miRNA
microRNA biogenesis
Release of microRNAs
Microvesicles
Protein complexes
HDL lipoproteins
Apoptotic bodies
Passive release into body fluids
microRNA extraction
Small RNA extraction from serum, plasma urine, milk and other body fluids
Analyses: Tools to quantify
qRT-PCR
Microarrays
High-throughput sequencing
Clinical biomarkers
Diagnostic
Prognostic
Clinical decisions
Target identification & development
Personalized therapy
microRNA-based biomarkers

MicroRNA profiling Platforms



Conventional cancer treatment:

Diagnosis

Stage, Grade,
IHC

Treatment

Chemotherapy

Personalized cancer treatment:

Molecular
diagnostics

Diagnosis:

Which pathways are active?

Treatment:

Pathway
targeted
therapy