L 5.4

Micro-RNA

miRNA

Micro RNA are a family of small RNA that are transcribed from several locations in genomes.

They have a typical structure, making a stem-loop structure with some mismatches in the stem

MiRBase: http://www.mirbase.org/cgi-bin/browse.pl?org=hsa

Textbook

Cell

Leading Edge
Review

Metazoan MicroRNAs

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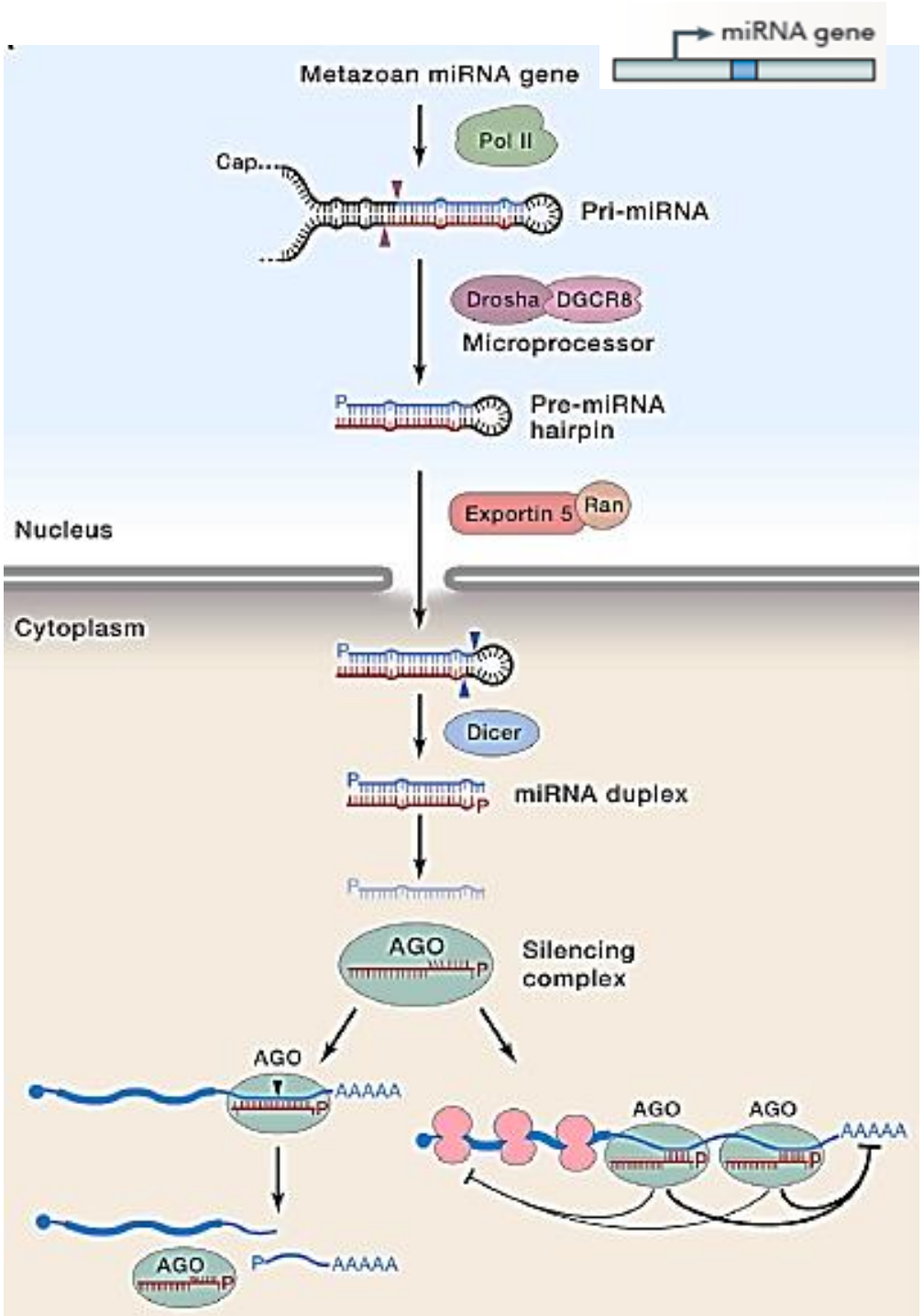
MicroRNAs (miRNAs) are ~22 nt RNAs that direct posttranscriptional repression of mRNA targets in diverse eukaryotic lineages. In humans and other mammals, these small RNAs help sculpt the expression of most mRNAs. This article reviews advances in our understanding of the defining features of metazoan miRNAs and their biogenesis, genomics, and evolution. It then reviews how metazoan miRNAs are regulated, how they recognize and cause repression of their targets, and the biological functions of this repression, with a compilation of knockout phenotypes that shows that important biological functions have been identified for most of the broadly conserved miRNAs of mammals.


miRNA gene
Metazoan miRNA gene
Pol II
Cap
Pri-miRNA
Drosha
DGCR8
Microprocessor
Pre-miRNA
hairpin
Exportin 5
Ran
Nucleus
Cytoplasm
Dicer
miRNA duplex
AGO
Silencing
complex
AGO
AAAAA
AGO
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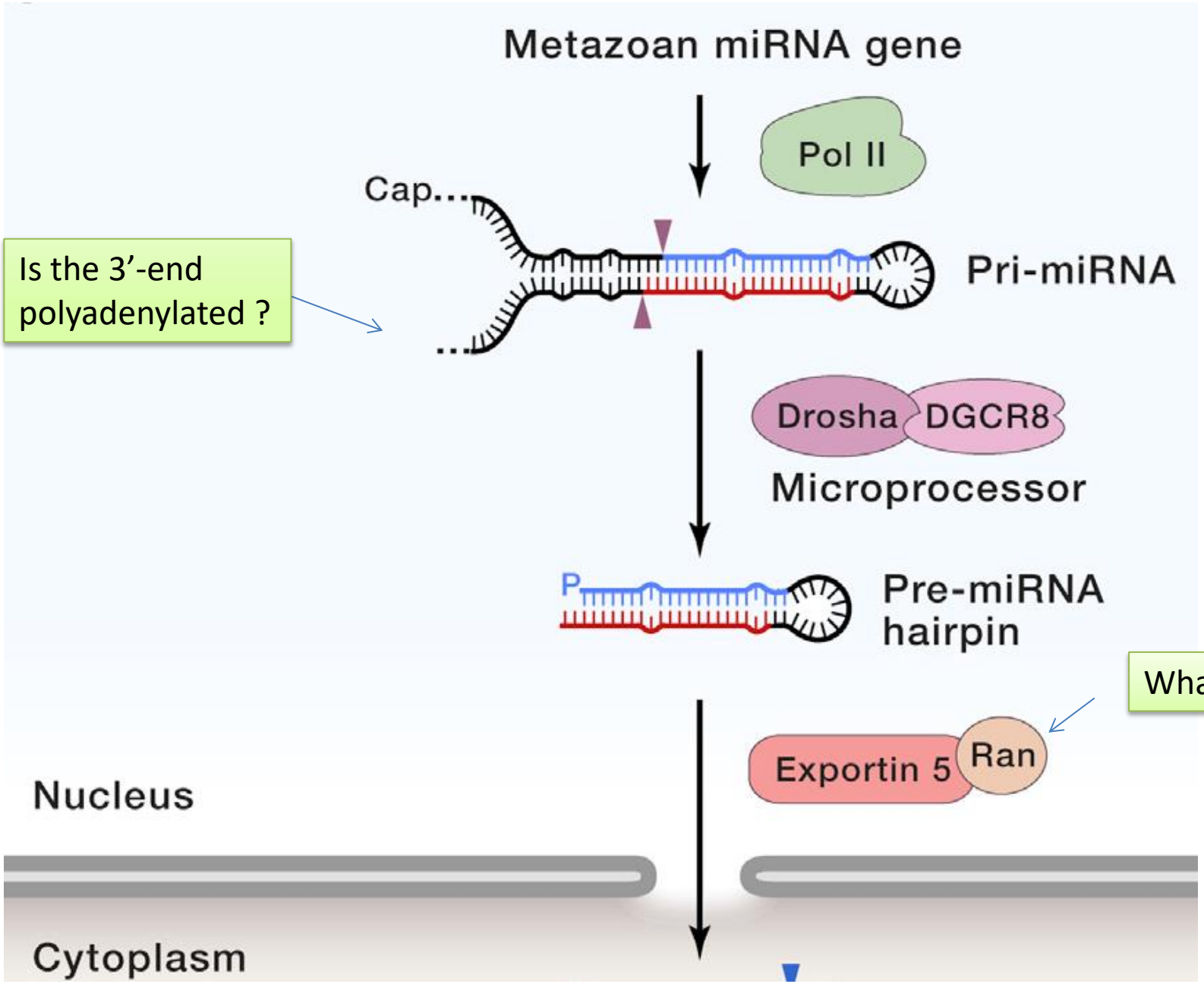
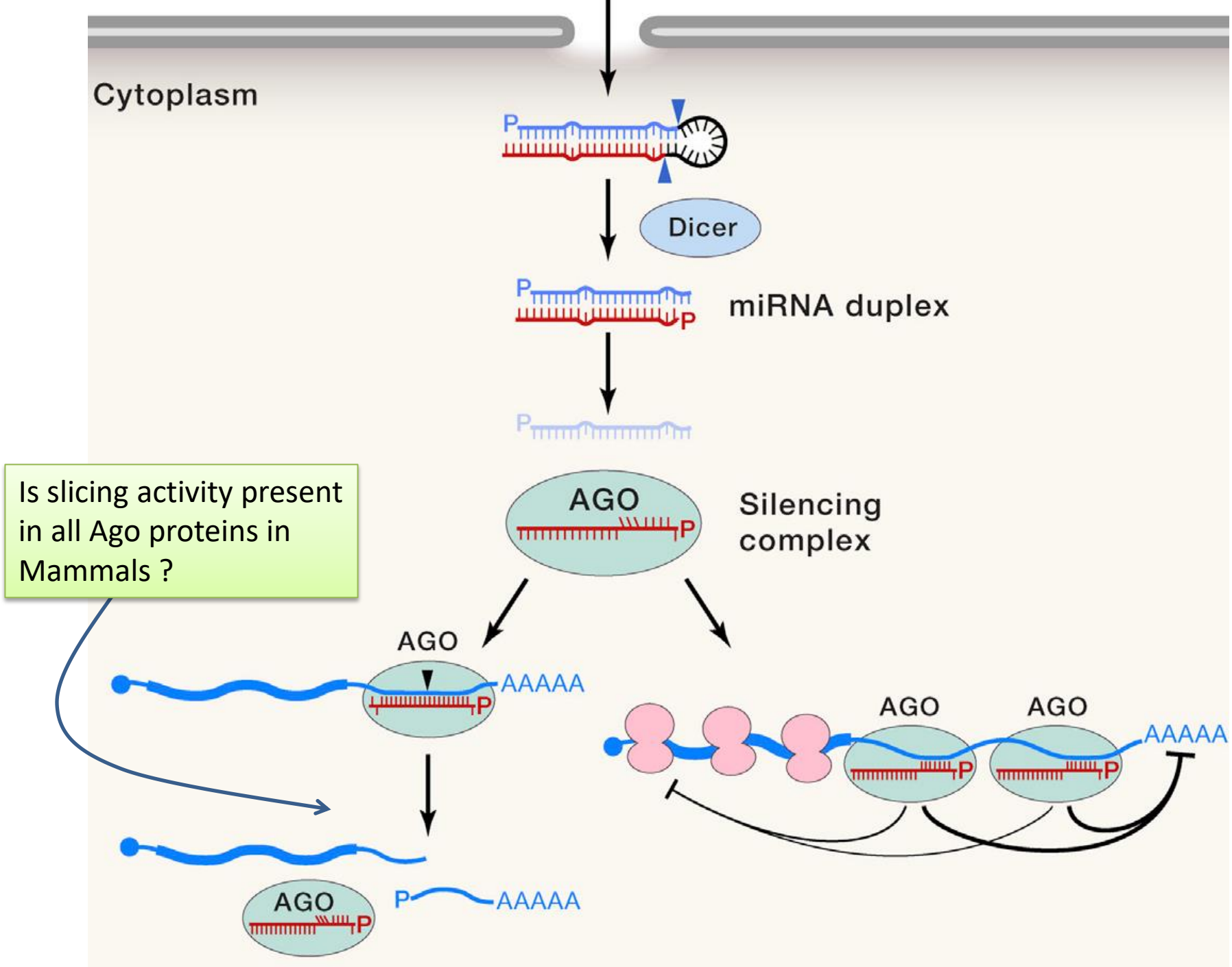
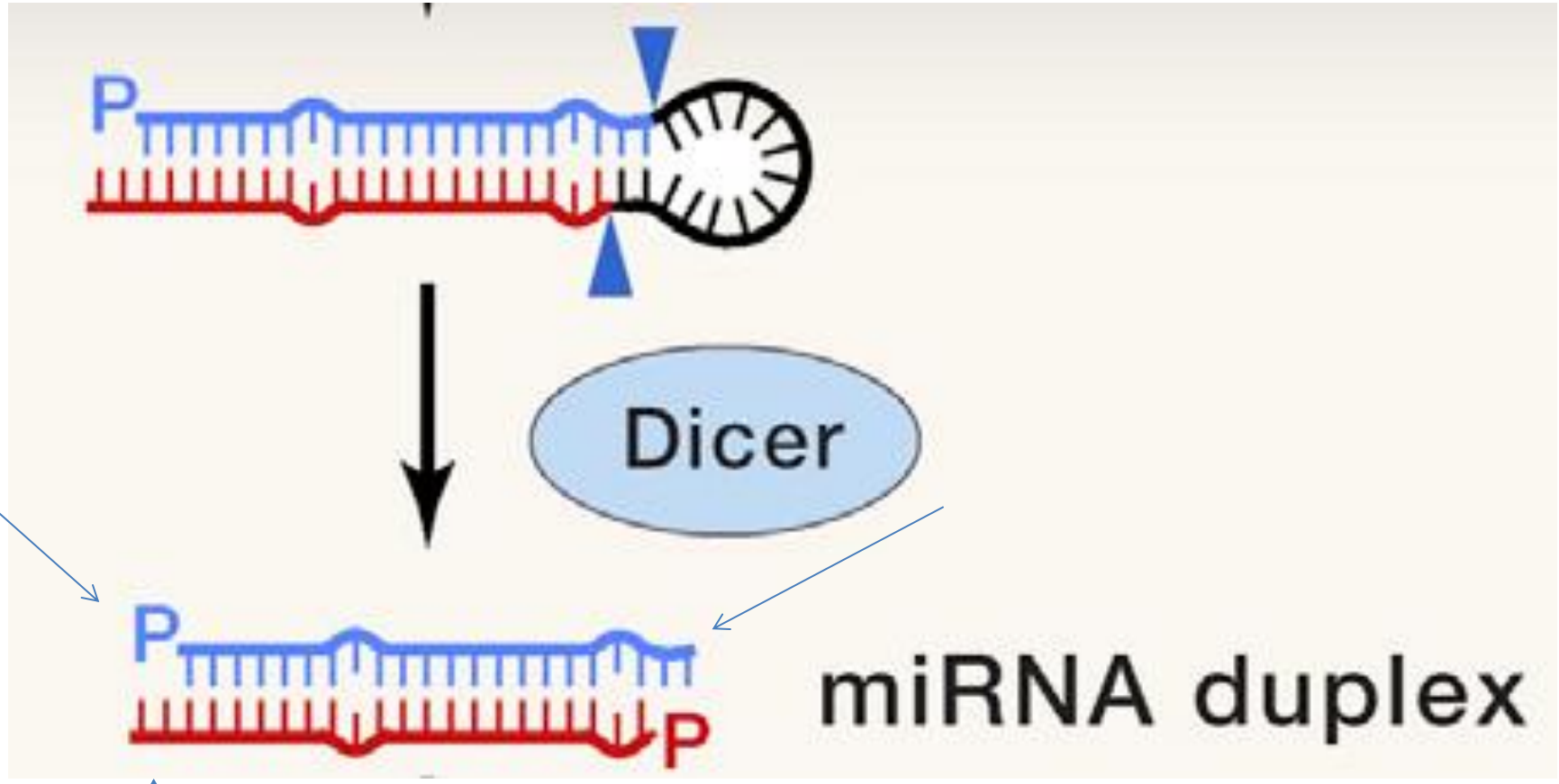
AAAAA
AGO
AAAAA


Metazoan miRNA gene
Pol II
Cap
Pri-miRNA
Is the 3'-end polyadenylated ?
Drosha
DGCR8
Microprocessor
P
Pre-miRNA hairpin
What is Ran ?
Exportin 5
Ran
Nucleus
Cytoplasm


Cytoplasm
P
Dicer
P
P
miRNA duplex
P
AGO
Silencing
complex
Is slicing activity present in all Ago proteins in Mammals ?
AGO
AAAAA
AGO
AGO
AAAAA
AGO
P
AAAAA


P
Dicer
P
P
miRNA duplex

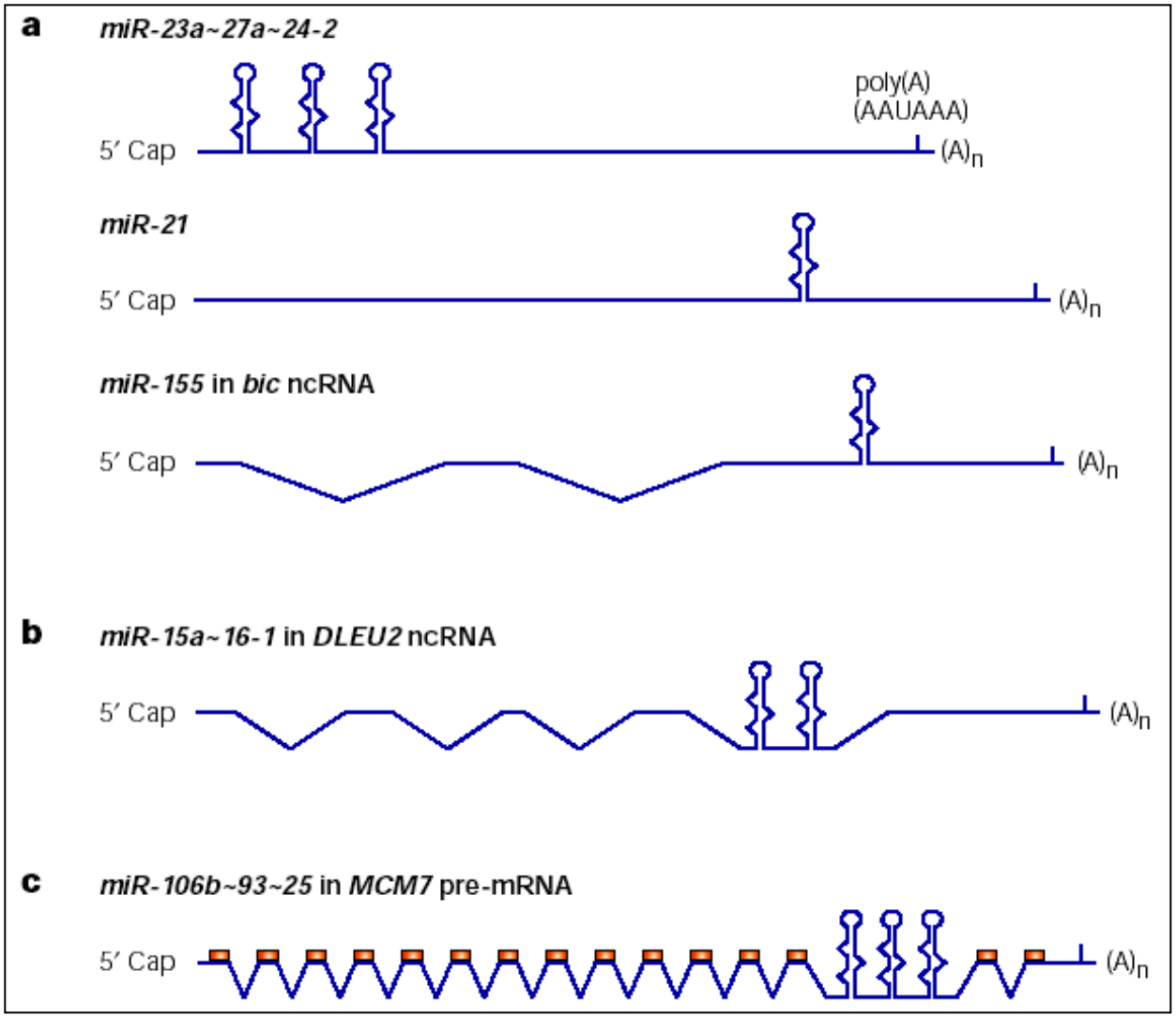


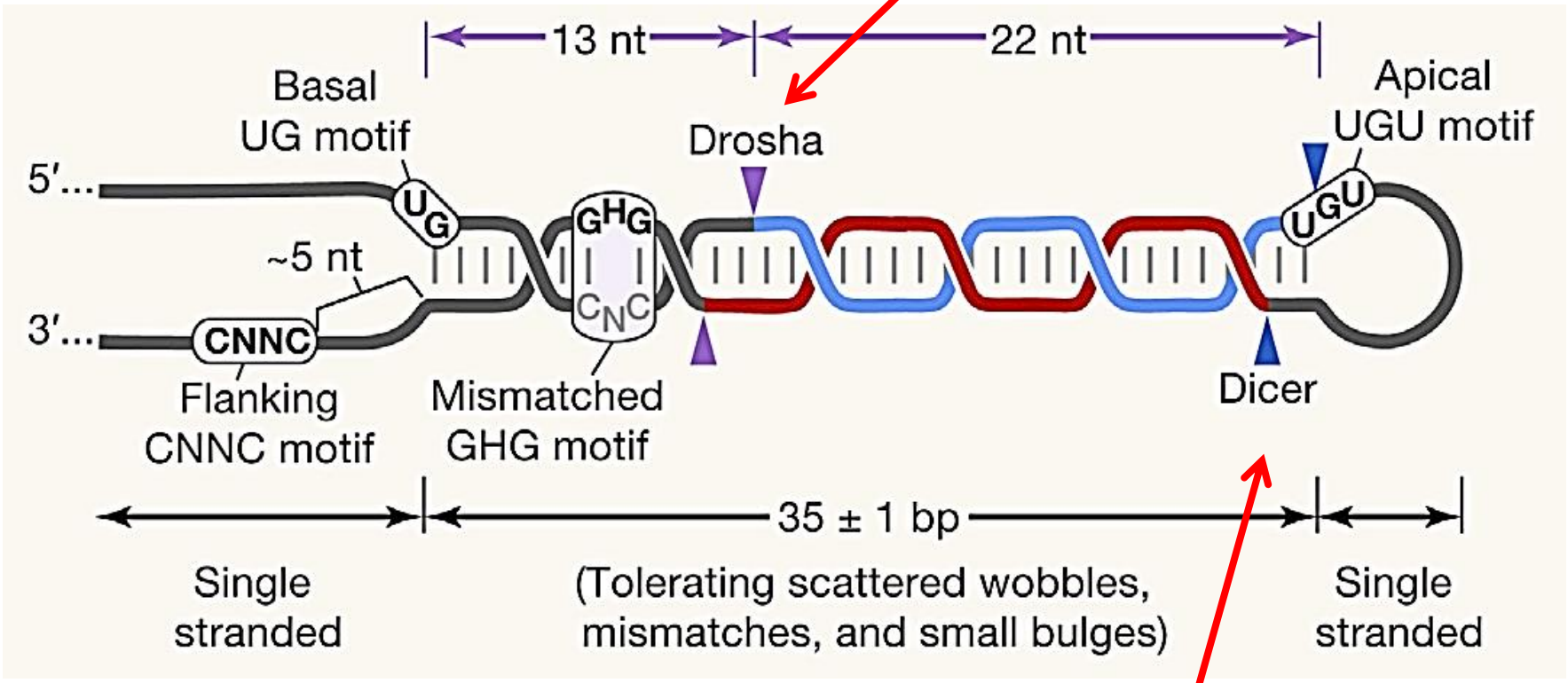
Figure 1 | **The structure of five pri-miRNAs.**

Primary transcripts that encode miRNAs, primiRNAs, contain 5′ cap structures as well as 3′ poly(A) tails. miRNAs can be categorized into three groups according to their genomic locations relative to their positions in an exon or intron.

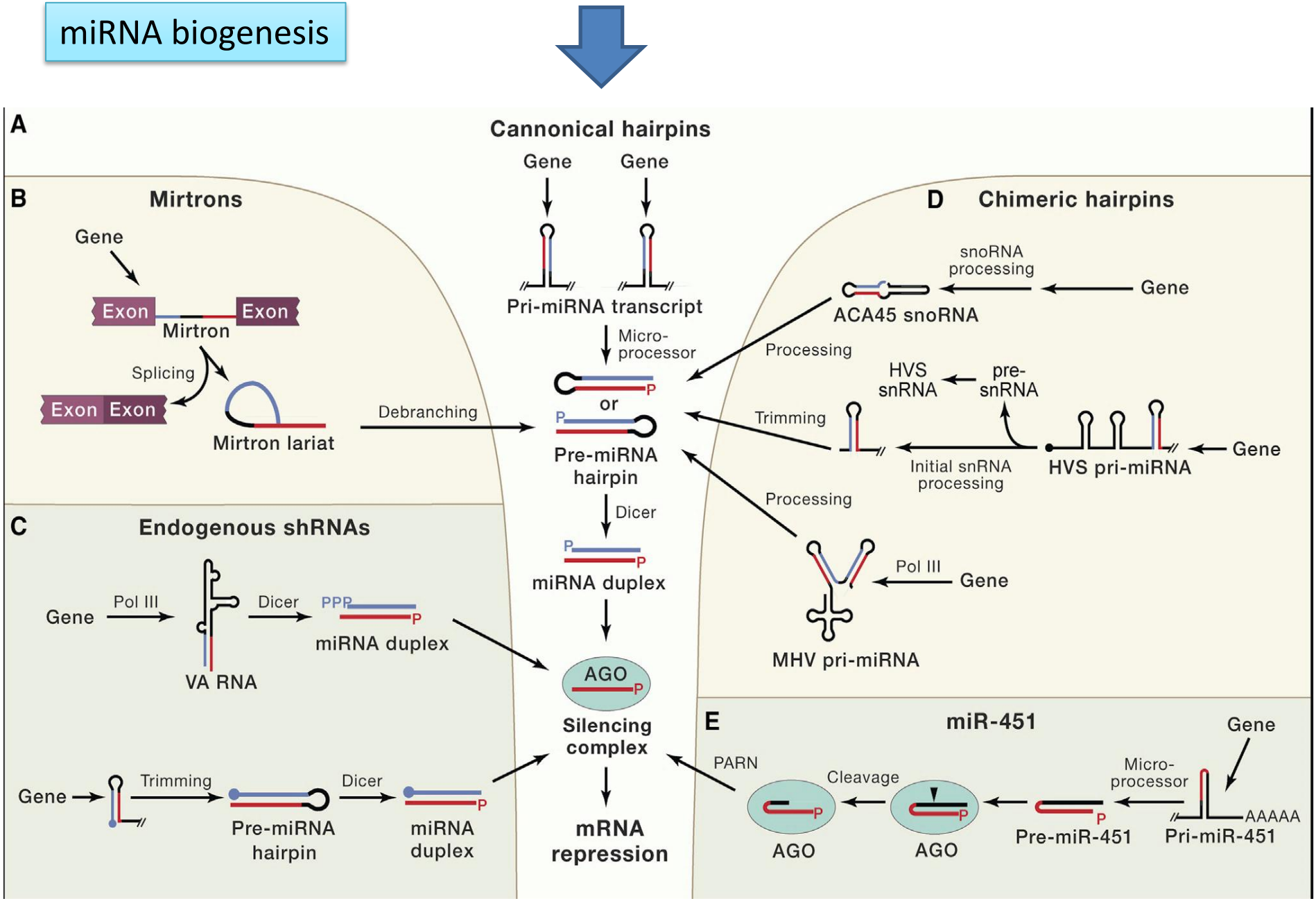
a | Exonic miRNAs in non-coding transcripts such as an *miR-23a~27a~24-2* cluster, *miR-21* and *miR-155*. *miR-155* was found in a previously defined non-coding RNA (ncRNA) gene, *bic*17.

b | Intronic miRNAs in non-coding transcripts. For example, an *miR-15a~16-1* cluster was found in the fourth intron of a previously defined non-coding RNA gene, *DLEU2* (REF. 126). **c** | Intronic miRNAs in protein-coding transcripts. For example, an *miR-106b~93~25* cluster is embedded in the thirteenth intron of DNA replication licensing factor *MCM7* transcript (variant 1, which encodes isoform 1). The mouse *miR-06b~93~25* homologue is also found in the thirteenth intron of the mouse *MCM7* homologue gene15. The hairpins indicate the miRNA stem-loops. Orange boxes indicate the protein-coding region. This figure is not to scale.

Features defining a canonical pre-miRNA



miRNA biogenesis



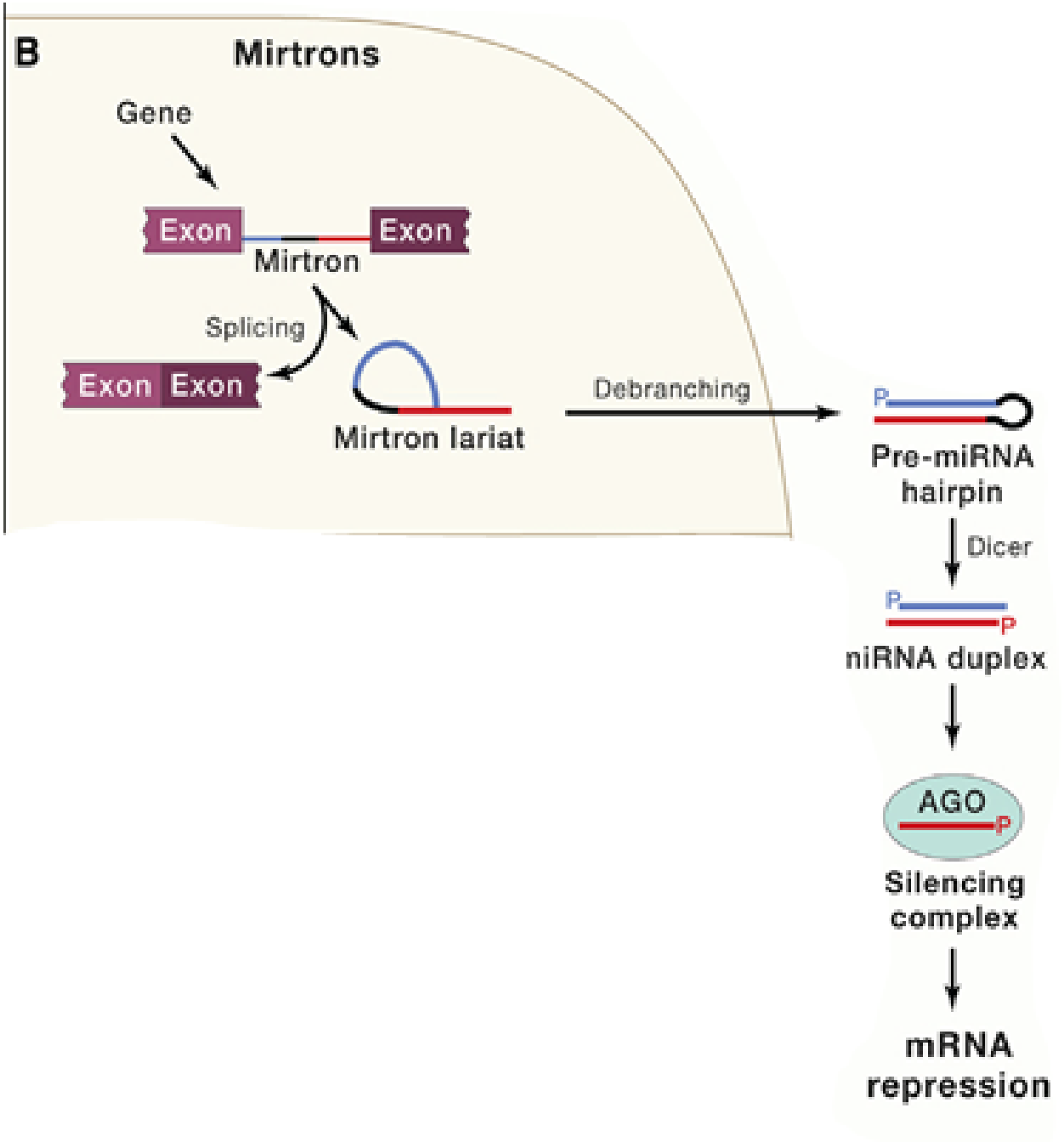
Genomic organization and transcription of miRNA genes

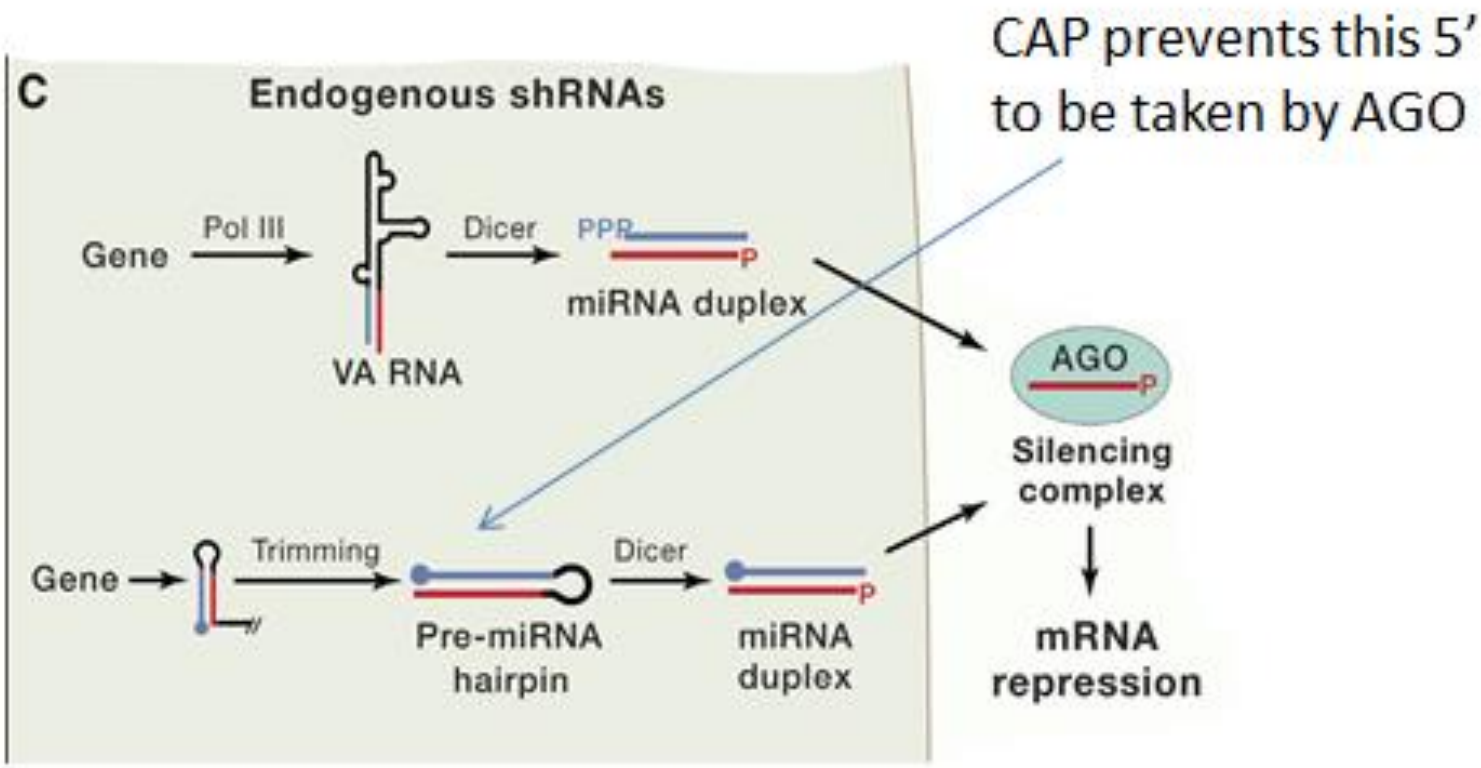
miRNAs are encoded in diverse regions of the genome including both protein coding and non-coding transcription units.

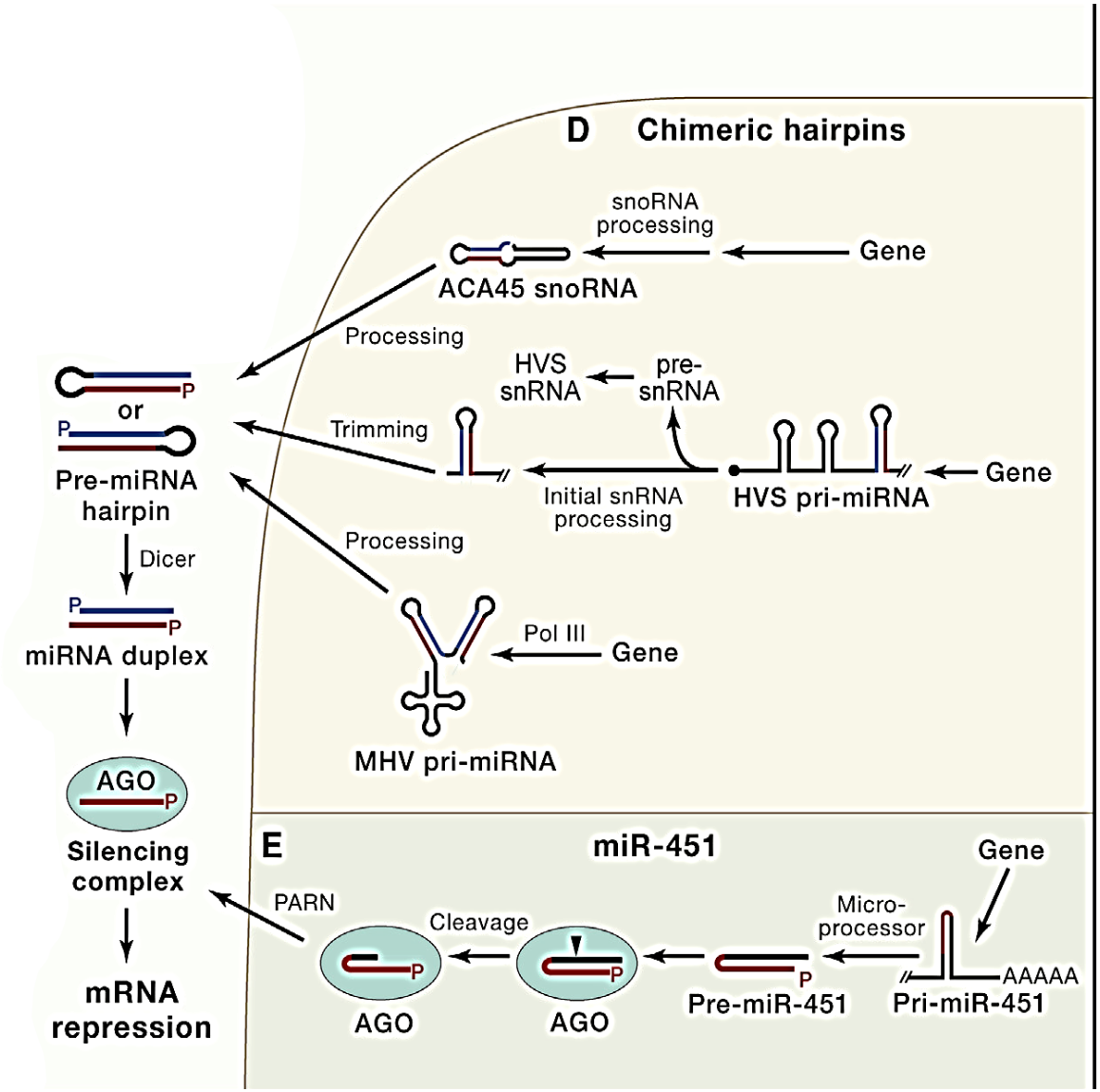
Approximately 50% of miRNAs are derived from noncoding RNA transcripts, while an additional ~40% are located within the introns of protein coding genes.

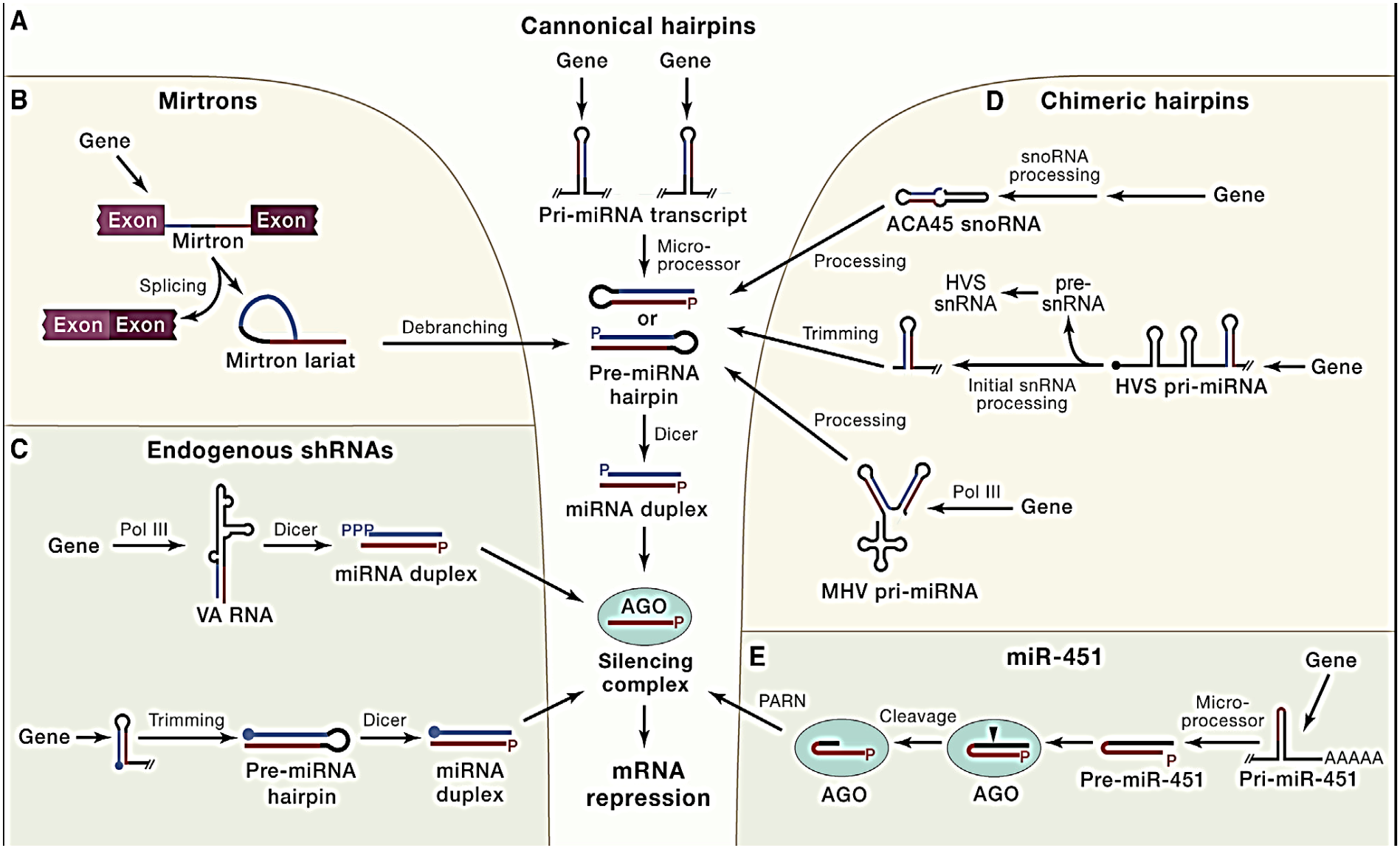
The majority of miRNAs are transcribed by RNA polymerase (RNA pol) II and bear a 7-methyl guanylate cap at the 5' end and poly (A) tail at the 3' end.

RNA pol III has also been demonstrated to generate the transcripts of a subset of miRNAs.


B
Mirtrons
Gene
Exon
Exon
Mirtron
Splicing
Exon Exon
Mirtron lariat
Debranching
P
Pre-miRNA hairpin
Dicer
P
P
niRNA duplex
AGO
P
Silencing complex
mRNA repression


C
Endogenous shRNAs
Gene
Pol III
VA RNA
Dicer
PPP
P
miRNA duplex
CAP prevents this 5'
to be taken by AGO
AGO
P
Silencing
complex
Gene
Trimming
Pre-miRNA
hairpin
Dicer
P
miRNA
duplex
mRNA
repression


D Chimeric hairpins
snoRNA processing
Gene
ACA45 snoRNA
Processing
HVS snRNA
pre-snRNA
Trimming
Initial snRNA processing
HVS pri-miRNA
Gene
Processing
Pol III
Gene
MHV pri-miRNA
or
Pre-miRNA hairpin
Dicer
miRNA duplex
AGO
Silencing complex
mRNA repression
E
miR-451
Gene
PARN
Cleavage
Micro-processor
AAAAA
AGO
AGO
Pre-miR-451
Pri-miR-451

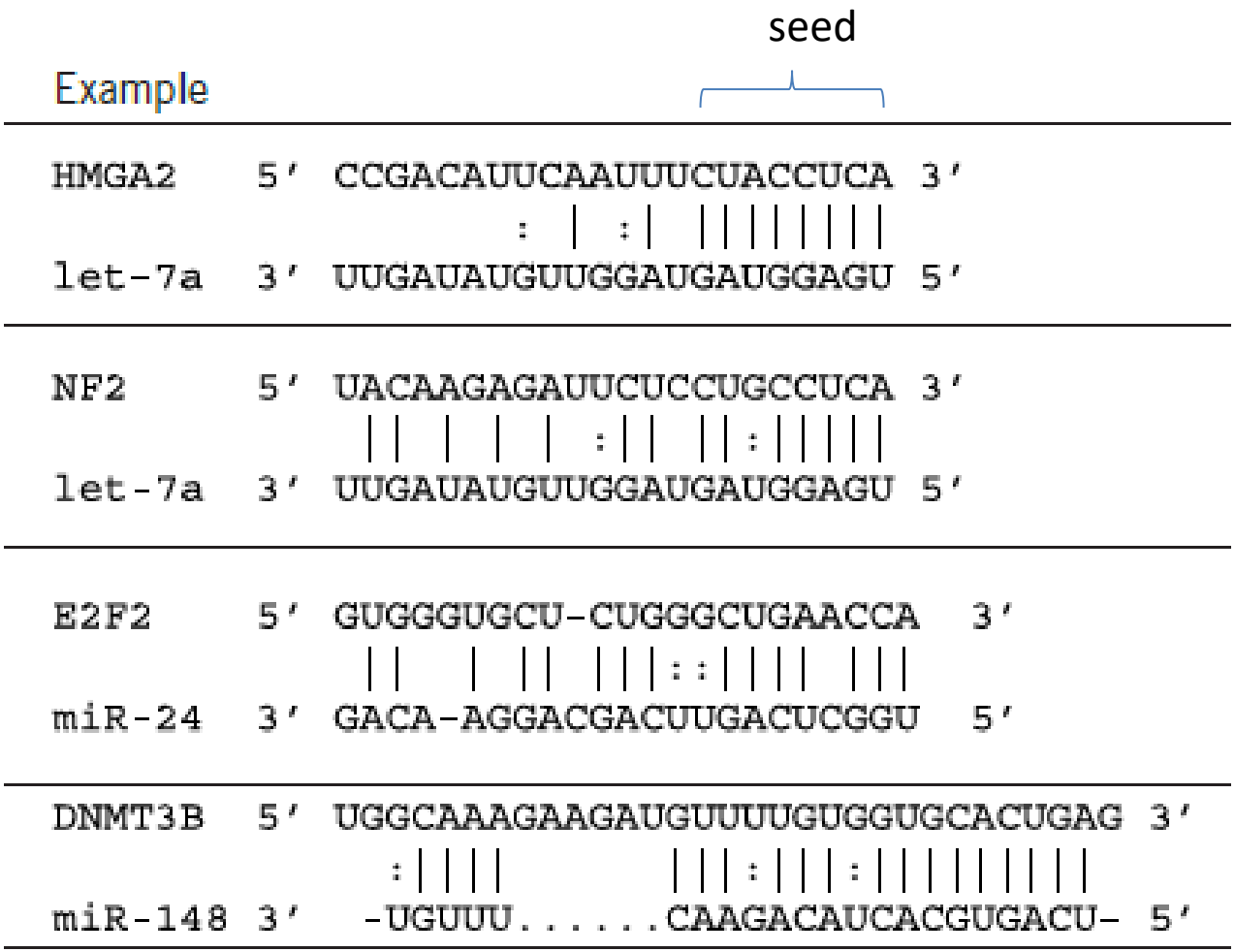

A
Cannonical hairpins
Gene
Gene
Pri-miRNA transcript
Micro-
processor
or
Pre-miRNA
hairpin
Dicer
miRNA duplex
B
Mirtrons
Gene
Exon
Exon
Mirtron
Splicing
Exon Exon
Mirtron lariat
Debranching
C
Endogenous shRNAs
Gene
Pol III
VA RNA
Dicer
miRNA duplex
Gene
Trimming
Pre-miRNA
hairpin
Dicer
miRNA
duplex
AGO
Silencing
complex
mRNA
repression
D
Chimeric hairpins
snoRNA
processing
Gene
ACA45 snoRNA
Processing
HVS
snRNA
pre-
snRNA
Trimming
Initial snRNA
processing
HVS pri-miRNA
Gene
Processing
Pol III
Gene
MHV pri-miRNA
E
miR-451
Gene
Micro-
processor
AAAAA
Pri-miR-451
Pre-miR-451
Cleavage
AGO
PARN
AGO

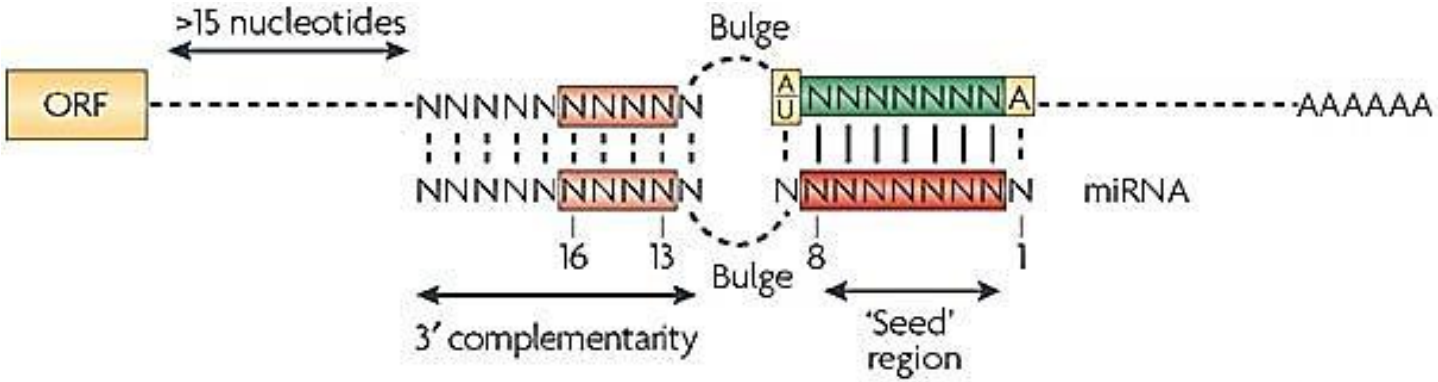
How is the micro-RNA recognized by AGO proteins ?

How do micro-RNAs direct target recognition ?

Examples from first discovered miRNA-mRNA pairs.

The 5’-end sequence of miRNA is the main determinant of targeting:
Continuous Watson-Crick pairing is required here (6-8 nt).
Target sequences mainly in mRNA 3’-UTR.





Filipowich et al., 2008

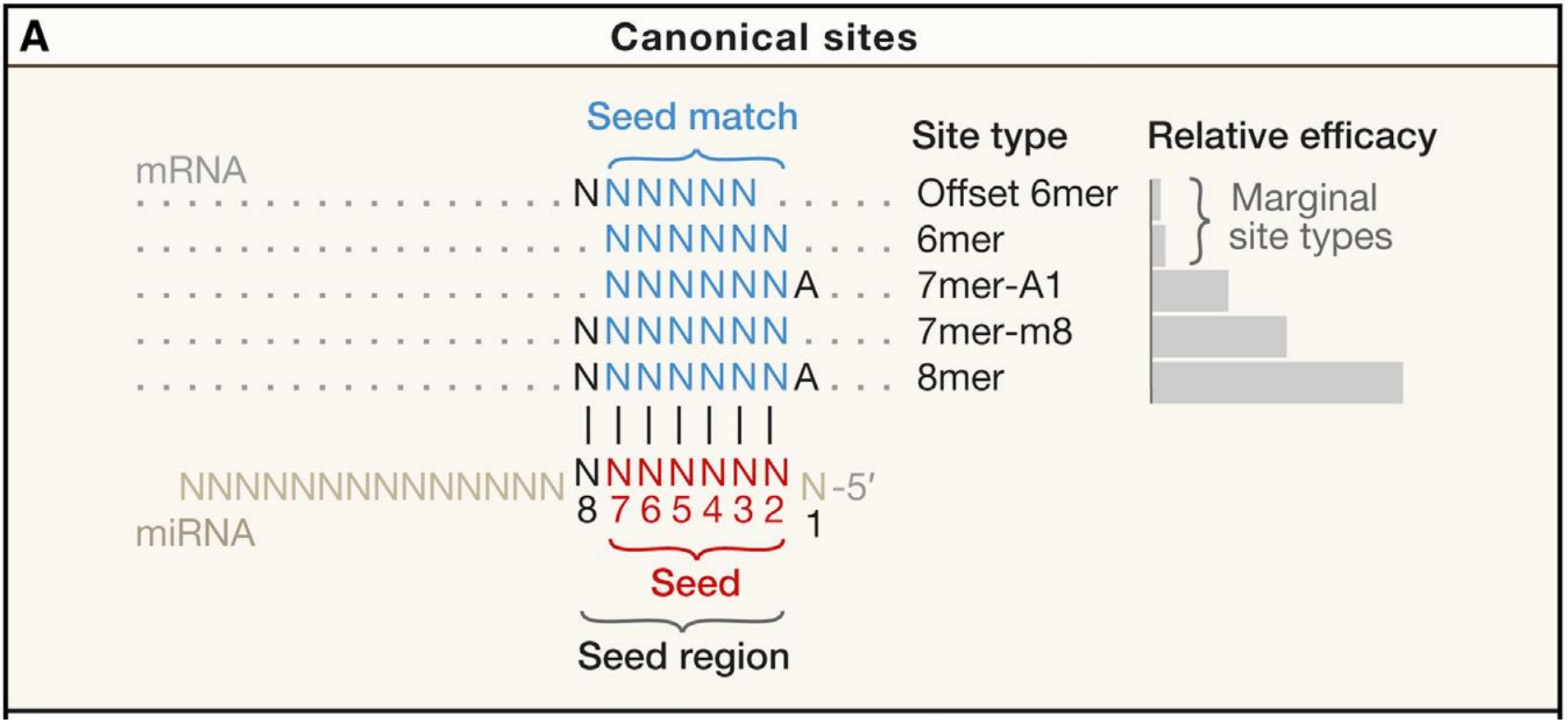
Nature Reviews | Genetics

As a rule of thumb, miRNA recognize their targets using 6-8 nt at their 5'-end, followed by variable loop and some additional complementarity towards 3'-end.

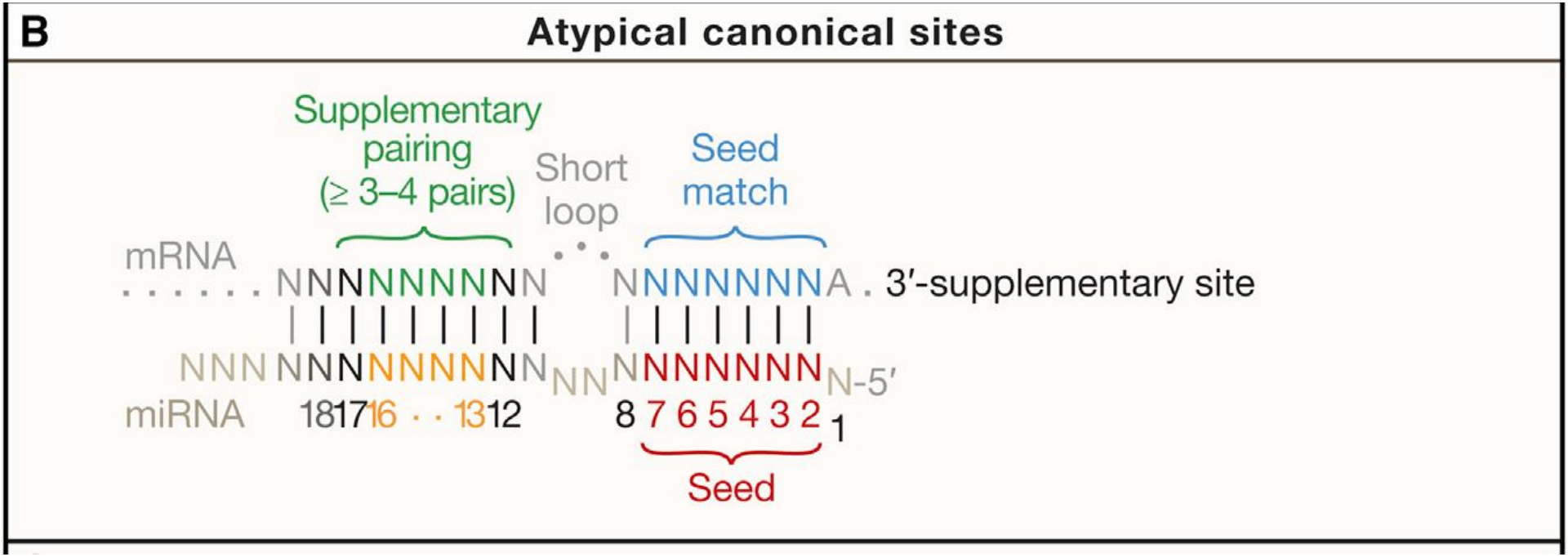
Due to this variable recognition mode, each miRNA recognizes multiple mRNA (or other RNAs) with variable affinity (can calculate from duplex stability).

The union of possible RNA targets of a specific miRNA is called «regulome».

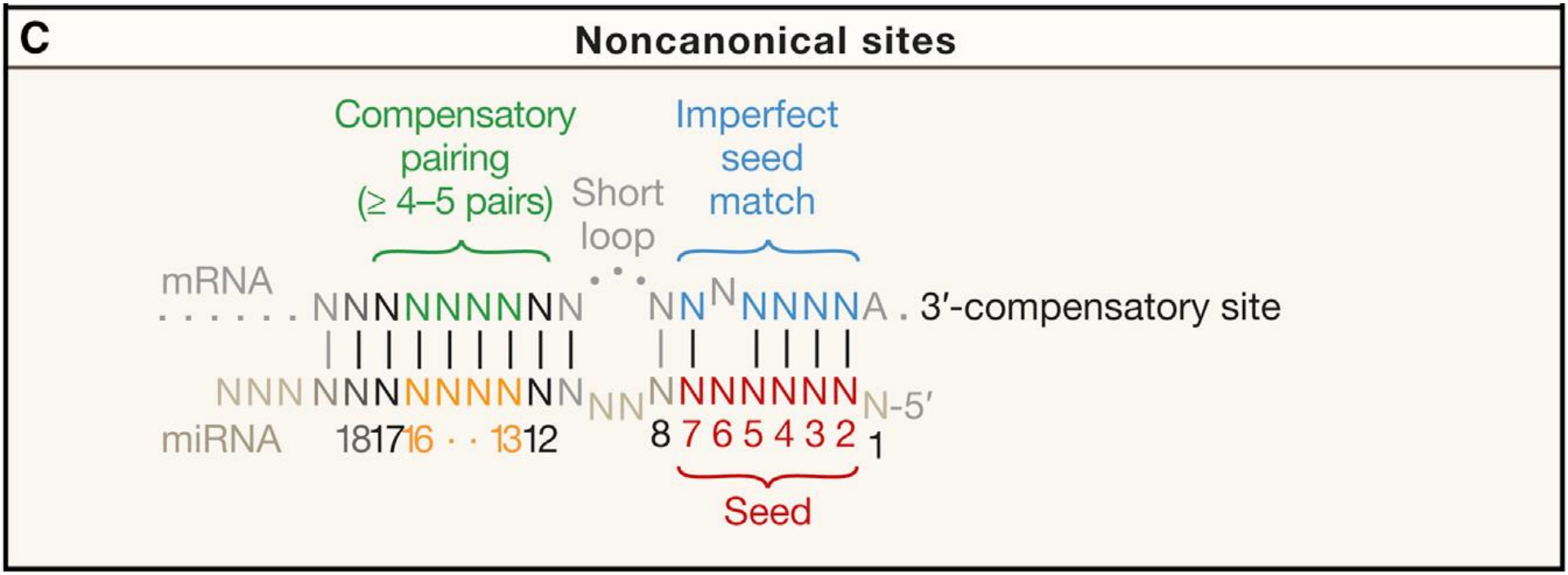
Several algorithms were developed to predict miRNA targets, and are available on websites. However, due to uncertainty of recognition modes, these algorithms work clearly in sub-optimal way.



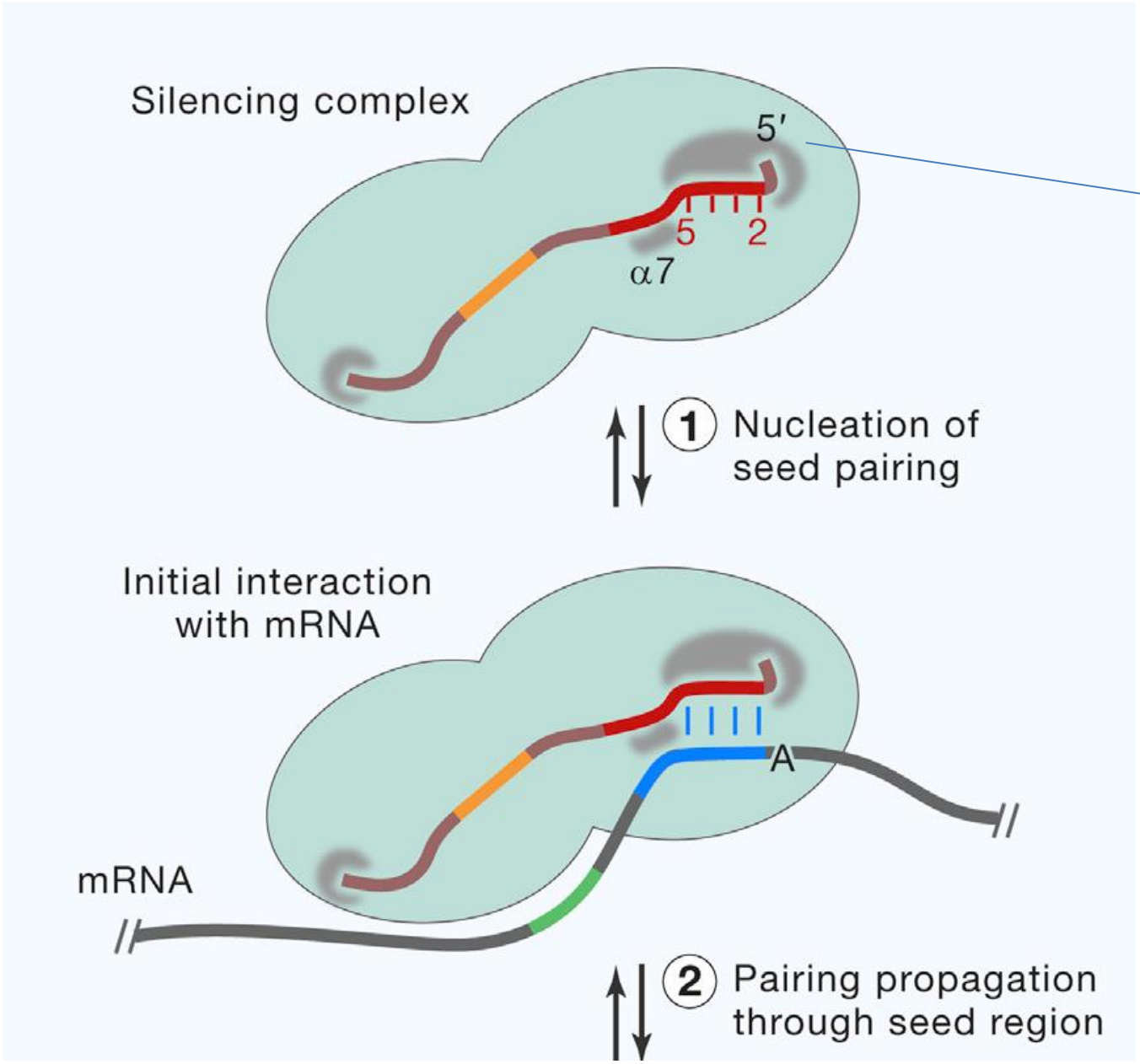
Canonical sites of mammalian miRNAs. These canonical sites each have 6–7 contiguous Watson–Crick pairs (vertical lines) to the seed region of the miRNA (miRNA positions 2–8). Two of these sites also include an A at position 1. Relative site efficacy in mammalian cells is graphed to the right (log scale). The most effective canonical sites are 7–8 nt sites that include a perfect match to the miRNA seed (positions 2–7, red), whereas the 6 nt sites are the least effective.



The 3’-supplementary site, an atypical type of canonical site.

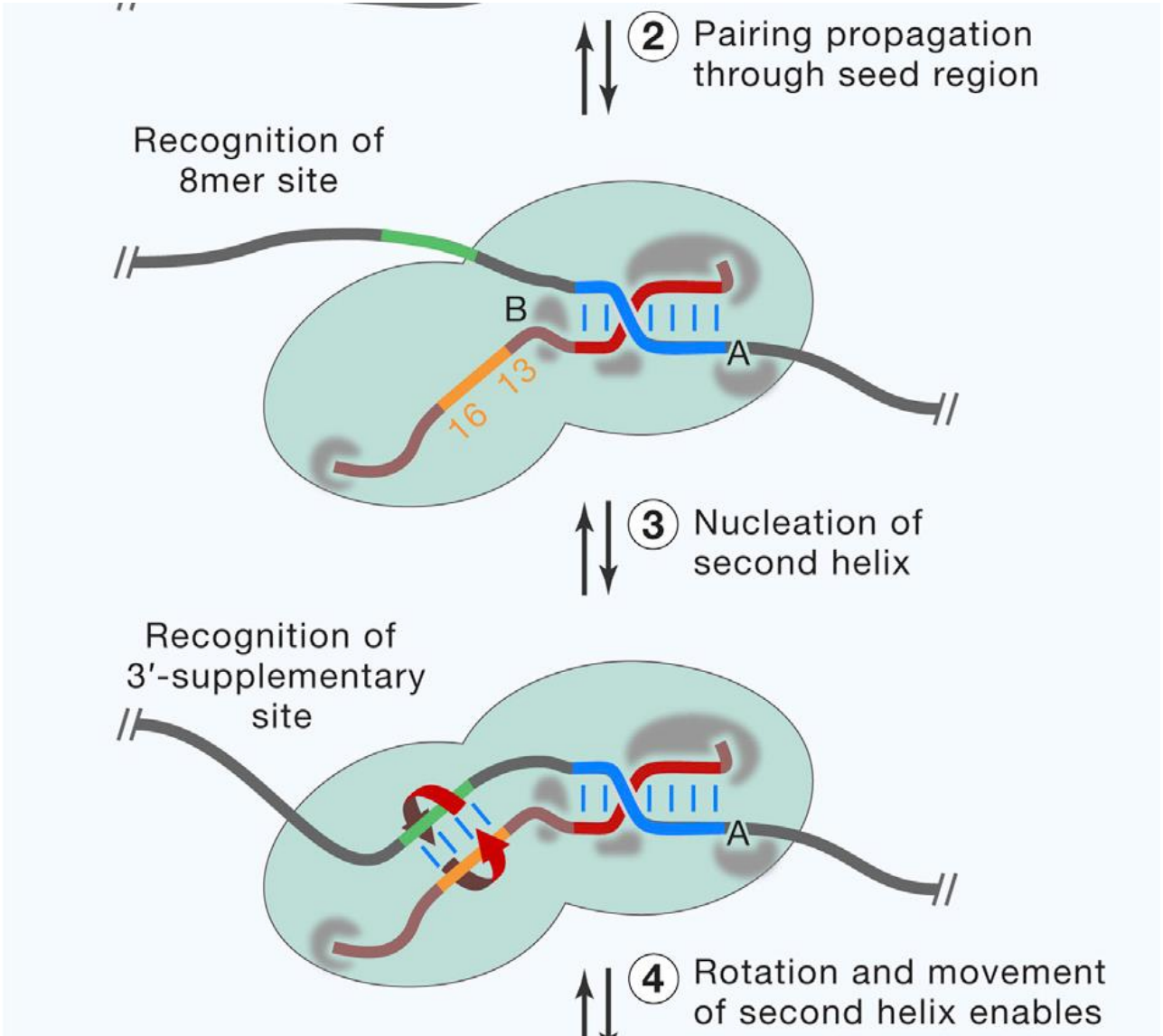


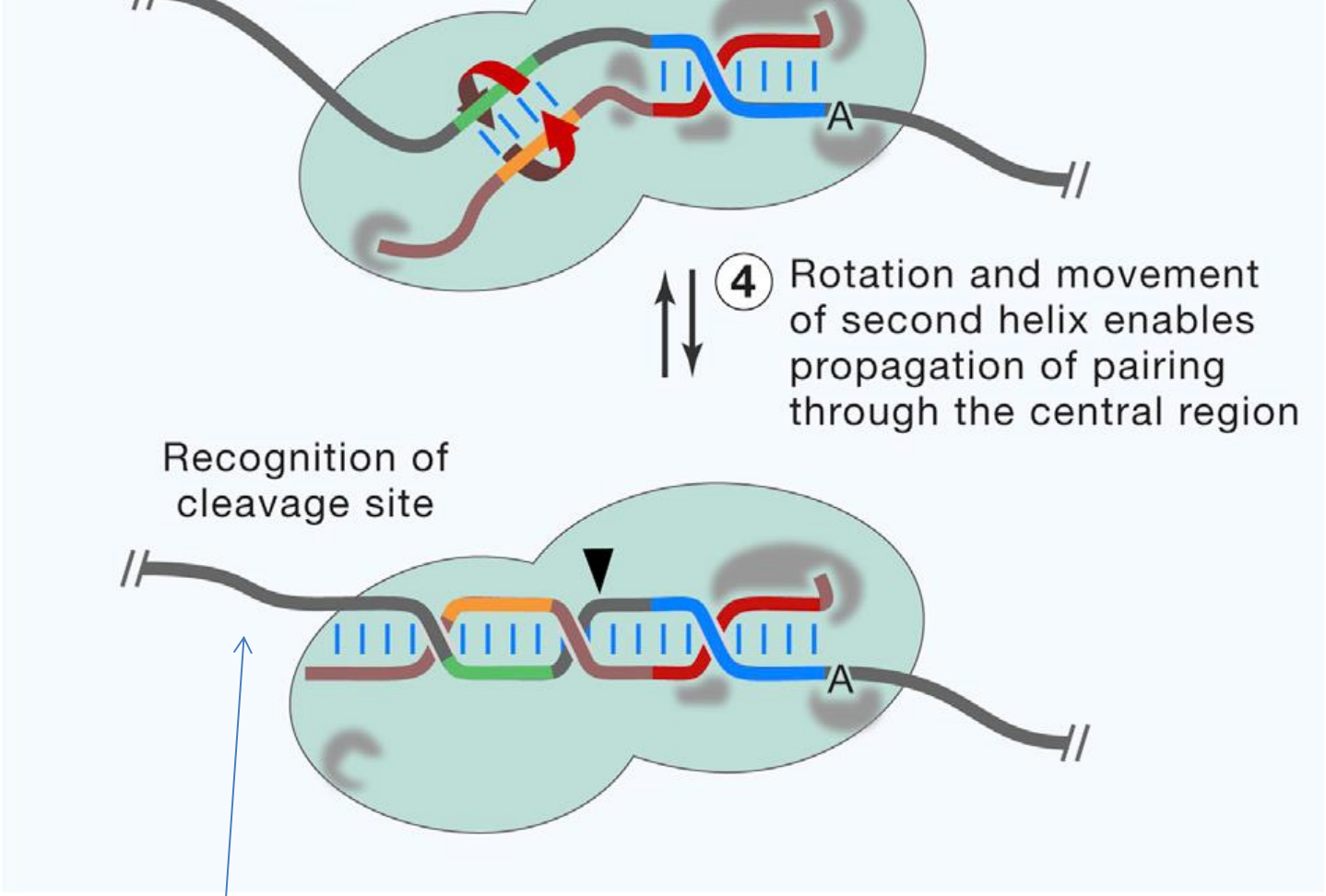
The 3’-compensitory site, a functional type of noncanonical sites



Phospho-required «A» preferred

First nucleotide bound by AGO

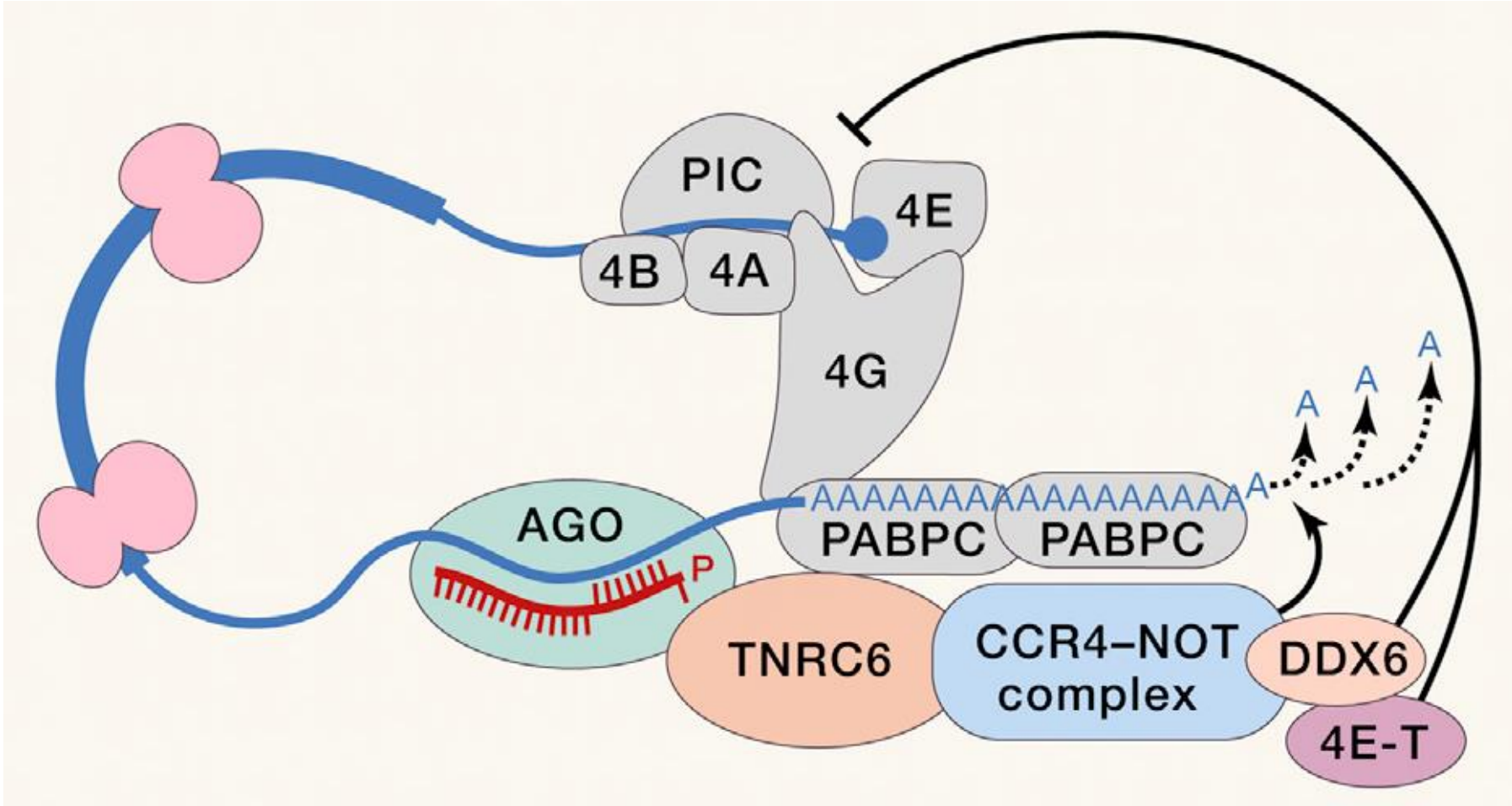

2 Pairing propagation through seed region
Recognition of 8mer site
B
A
16
13
3 Nucleation of second helix
Recognition of 3′-supplementary site
A
4 Rotation and movement of second helix enables


A
4
Rotation and movement of second helix enables propagation of pairing through the central region
Recognition of cleavage site
A

AGO2

How is post-transcriptional silencing performed ?

The Dominant Mechanisms of miRNA-Guided Repression in Bilaterian Animals



Slicing is determined only when maximal pairing between the micro-RNA and target RNA is present (unusual for miRNA)

This is the condition we see with siRNA (natural or laboratory)

500 to 600 verified micro RNAs in Humans. Low % of total predicted.

Some are conserved down to Fish. Conservation very important to predict functional miRNAs.

miRNA divided in Families
– same or similar target recognition.

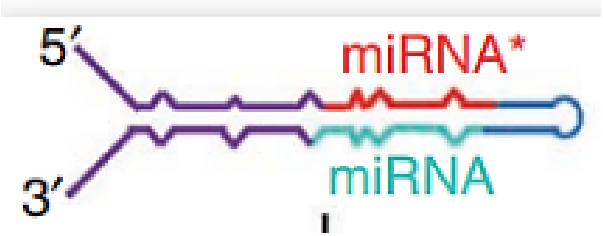
Each miRNA (family) recognizes hundreds of target mRNA (regulon)

The target sets typically show specifically enriched GO terms

miRNA nomenclature

Names/identifiers in database: hsa-mir-121 (first letter=species; numbering: sequential)
Gene: mir-121
Mature miRNA: miR-121

If there are distinct precursors / genes expressing the same miRNA, add suffix number:
hsa-mir-121-1
hsa-mir-121-2
Mature miRNAs with closely related sequences:
hsa-miR-121a
hsa-miR-121b



In some case, two functional miRNAs derive from the two arms of the same precursor.
If mature forms unbalanced quantitatively: miR-56 the major form (*guide*)
miR-56* the minor form (*passenger*)
If no data or no difference: miR-142-5p (from the 5' arm)
miR-142-3p (from the 3' arm)

(from MiRBase database - http://www.mirbase.org/help/nomenclature.shtml

Functions of miRNA

many miRNAs are required for proper development, including that of the skeleton, teeth, brain, eyes, neurons, muscle, heart, lungs, kidneys, vasculature, liver, pancreas, intestine, skin, fat, breast, ovaries, testes, placenta, thymus, and each hematopoietic lineage.

many influence cellular phenomena and functions, including axon sprouting, synapse formation and function, mitotic spindle orientation, polyploidization, ciliogenesis, and diverse functions in various hematopoietic lineages.

many miRNAs influence physiological processes, including cardiac conduction, blood pressure, lipid or cholesterol metabolism, insulin production, pituitary function, mobilization of glycogen, Ab protein degradation, bone resorption, fibrosis, and the overall growth of embryos or pups

many miRNA knockout strains have differential responses to models of neuronal, cardiac, muscular, pulmonary, vascular, endocrinological, hepatic, intestinal, renal, immunological, or metabolic diseases or injuries, and some have altered susceptibility to fungal or bacterial infections or altered propensity to develop tumors in cancer models

Alteration to miRNA expression profile typical of many diseases, primary cancer

Micro RNA have been intensively studied during last ten years.

- ✓ Several miRNAs expressed in each cell
- ✓ Strong tissue- and cell-specificity
- ✓ Developmentally and signal transduction regulated
- ✓ miRNA expression profile severely altered in pathologies
- ✓ Individual miRNA deletion or overexpression: severe effects on cell physiology
- ✓ miRNAs quite stable in serum: possible biomarkers for diseases.

Experimental

Experimental:

Suppression of miRNA can be achieved by antisense technologies, especially using nonhydrolyzable oligos (anti-miR).

Over-expression: minigenes expressing pri-miRNA or pre-miRNA in constitutive or inducible vectors. Also synthetic miRNA mimics in transient transfection.

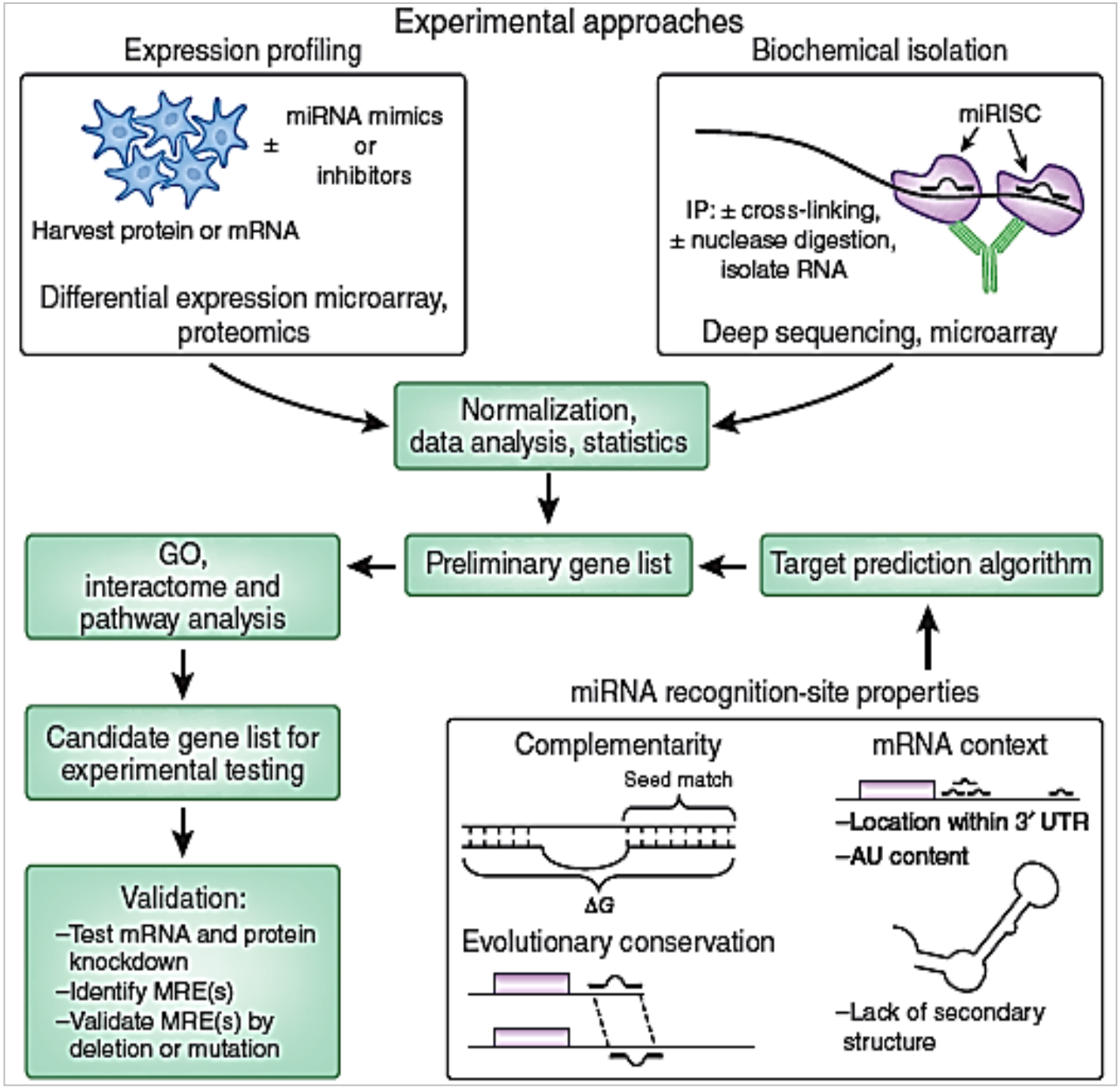
Expression analysis:

RT-PCR methods available (problem: they are very short!)

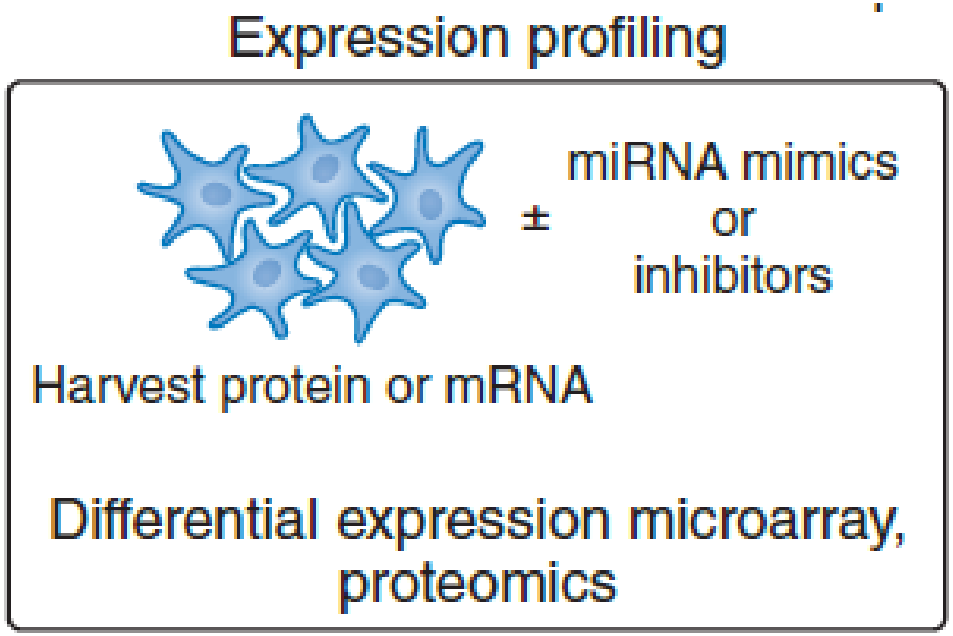
Microarrays carrying probes for all known miRNAs available, also made of LNA oligos instead of normal oligos to increase hybridization specificity

Specific protocol and application for short RNAs NGS.

Wiki Methodology



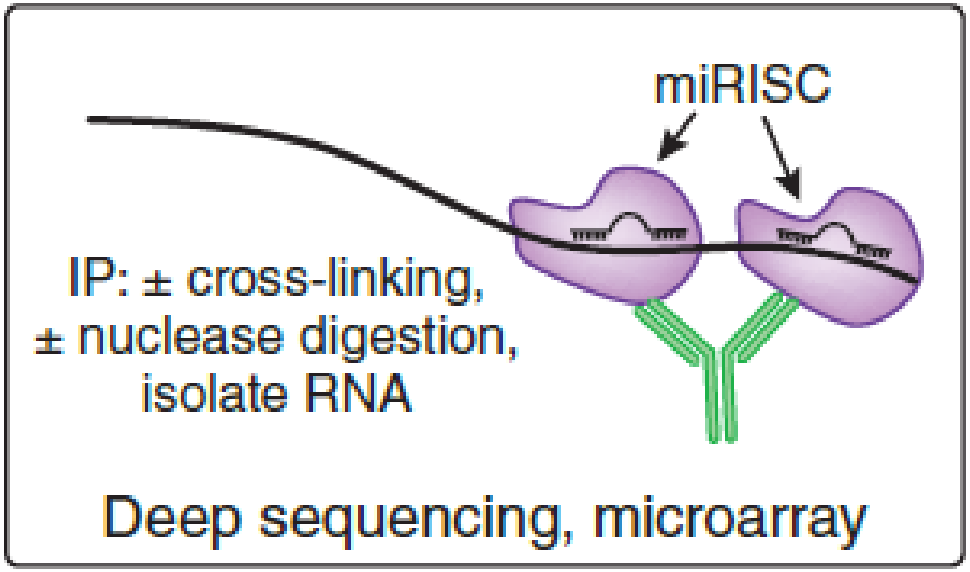
Methods for identifying miRNA targets. Putative target genes can be identified by expression profiling of cells in which the miRNA is overexpressed or antagonized, by biochemical isolation of the miRISC or by target prediction algorithms. These methods generally identify hundreds of candidate genes or more. Bioinformatic analysis of these large candidate gene lists for over-represented Gene Ontology (GO) terms, enriched biological pathways or gene interaction networks can then help researchers to select candidate genes to evaluate experimentally.



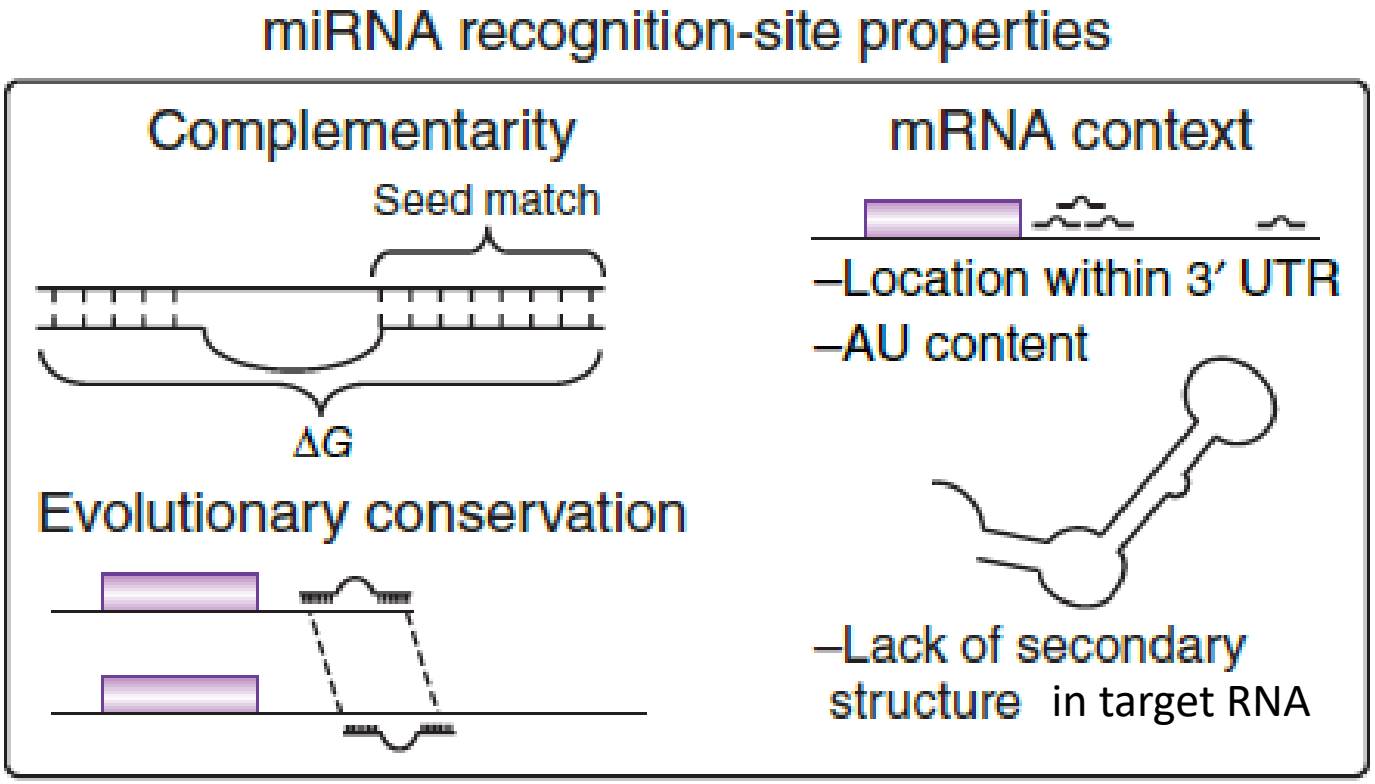
Wiki methodology

«antagomir»
Vectors to express miRNAs
miRNA mimics

Biochemical isolation



Bioinformatics



Number of MRE very important

MicroRNA Targeting Specificity in Mammals: Determinants beyond Seed Pairing

Andrew Grimson,[1,2,4,6] Kyle Kai-How Farh,[1,2,3,4,6] Wendy K. Johnston,[1,2,4] Philip Garrett-Engele,[5] Lee P. Lim,[5,*] and David P. Bartel[1,2,4,*]

[1]Howard Hughes Medical Institute
[2]Department of Biology
[3]Division of Health Sciences and Technology
Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[4]Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge,
[5]Rosetta Inpharmatics, 401 Terry Avenue N, Seattle, WA 98109, USA
[6]These authors contributed equally to this work.
*Correspondence: lee_lim@merck.com (L.P.L.), dbartel@wi.mit.edu (D.P.B.)
DOI 10.1016/j.molcel.2007.06.017

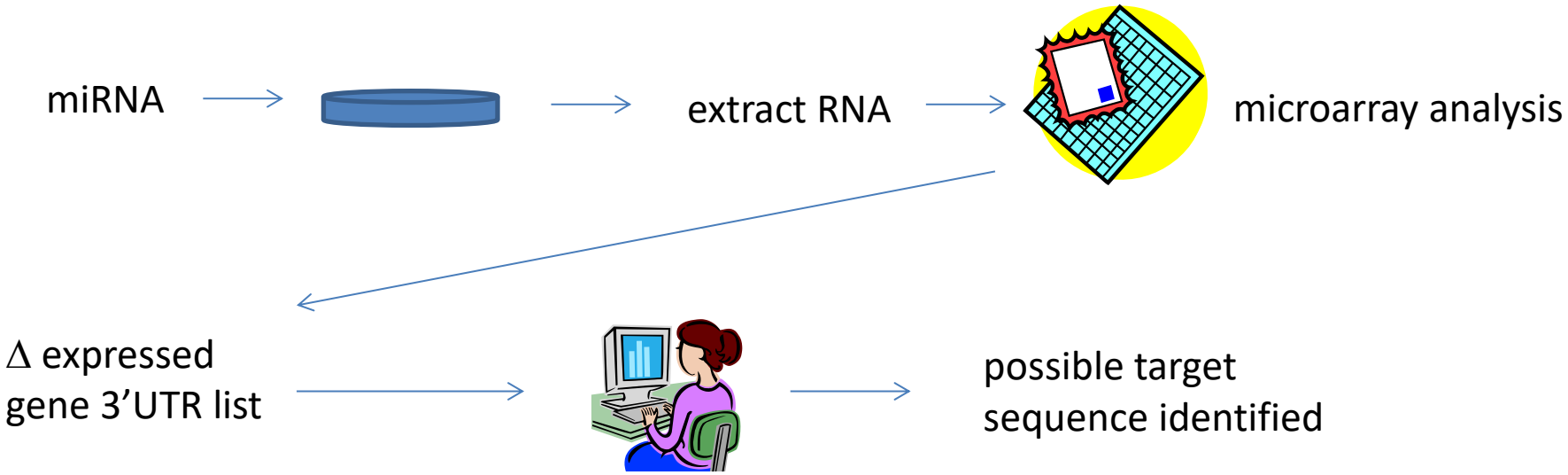
Mammalian microRNAs (miRNAs) pair to 3′UTRs of mRNAs to direct their posttranscriptional repression. Important for target recognition are ~7 nt sites that match the seed region of the miRNA. However, these seed matches are not always sufficient for repression, indicating that other characteristics help specify targeting. By combining computational and experimental approaches, we uncovered five general features of site context that boost site efficacy: AU-rich nucleotide composition near the site, proximity to sites for coexpressed miRNAs (which leads to cooperative action), proximity to residues pairing to miRNA nucleotides 13–16, positioning within the 3′UTR at least 15 nt from the stop codon, and positioning away from the center of long UTRs. A model combining these context determinants quantitatively predicts site performance both for exogenously added miRNAs and for endogenous miRNA-message interactions. Because it predicts site efficacy without recourse to evolutionary conservation, the model also identifies effective nonconserved sites and siRNA off-targets.

HeLa cells transfected with **11 synthetic double-stranded miRNA**

mRNA extracted from cells and analyzed on expression microarrays

Lim, L.P., Lau, N.C., Garrett-Engele, P., Grimson, A., Schelter, J.M., Castle, J., Bartel, D.P., Linsley, P.S., and Johnson, J.M. (2005). Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 433, 769–773.

miRNA targets were then identified in the regulated mRNAs and classified.



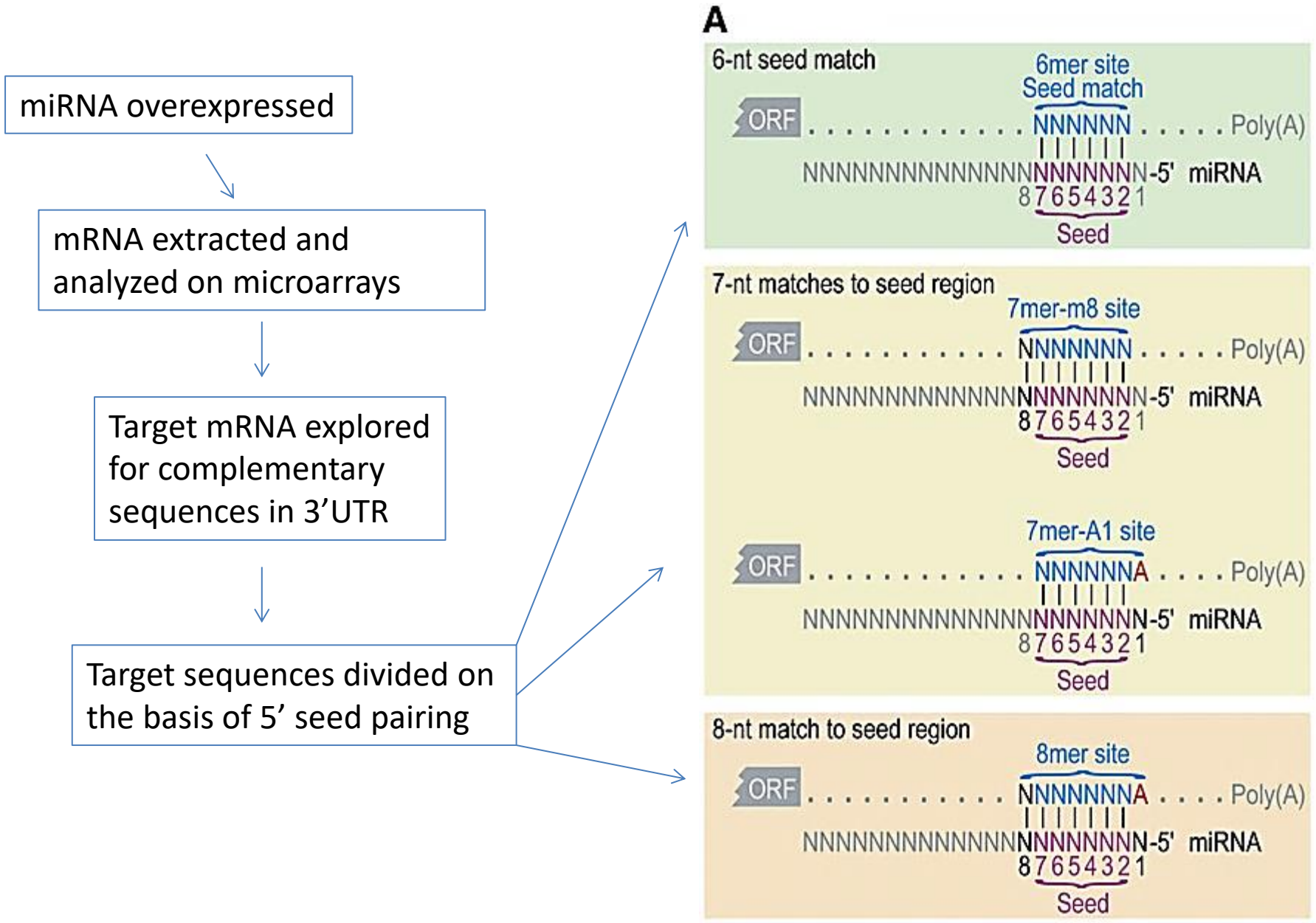
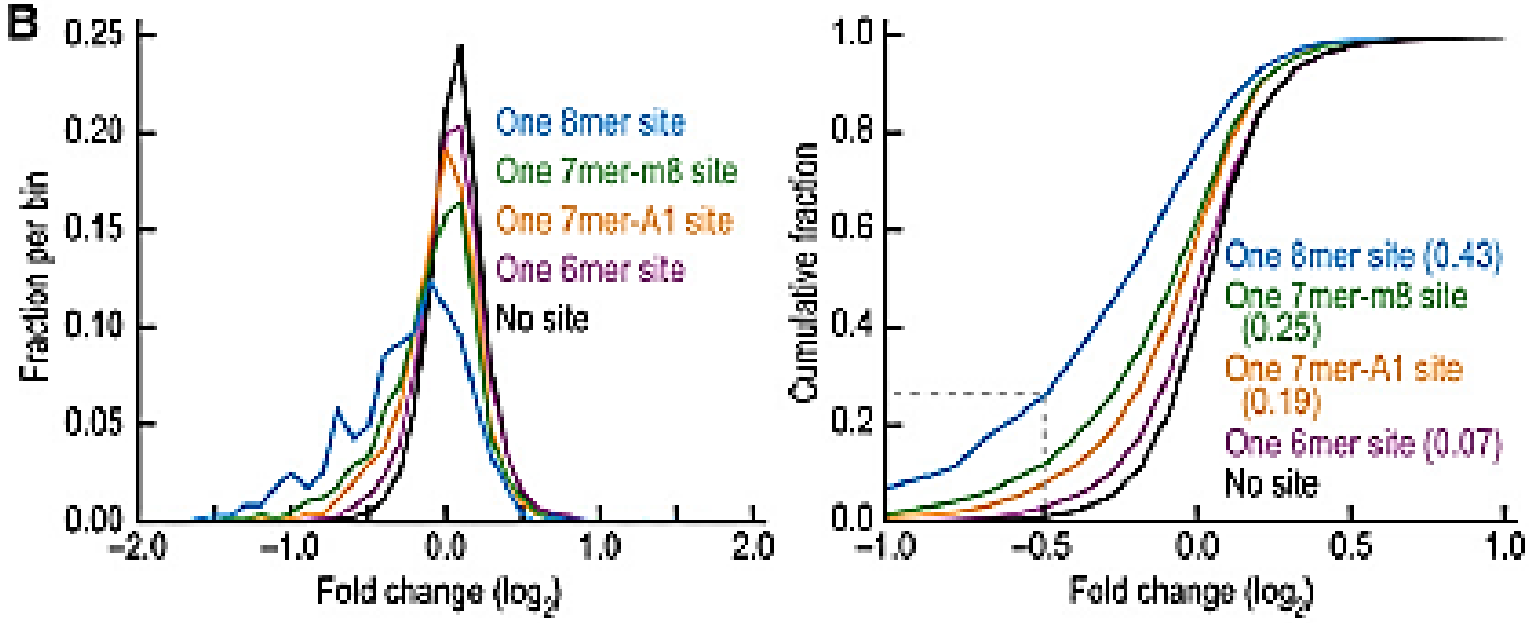


Figure 1. Downregulation of Messages with 6-8Mer Sites (A) Canonical miRNA complementary sites.



(B) Effectiveness of **single canonical sites**. Changes in abundance of mRNAs **after miRNA transfection** were monitored with microarrays. Distributions of changes (0.1 unit bins) for messages containing the indicated single sites in their UTRs are shown (left), together with the cumulative distributions (right). The dashed line in the cumulative distributions indicates that 27% of mRNAs with UTRs containing a single 8mer were downregulated at least 29% (20.5 = 0.71). Results of 11 experiments, each performed in duplicate and each transfecting a duplex for a different miRNA (Table S2), were consolidated. Results shown were an amalgam of the data from all 11 miRNAs; the relative strengths of the different sites were consistent when examining each transfection individually. For the cumulative plots, the minimal fraction of downregulated genes in that distribution is reported (parentheses), based on comparison with the no-site distribution. Repression from UTRs containing an 8mer site was significantly more than that from UTRs with a 7mer-m1 site ($p < 1020$, one-sided K-S test); similar comparisons between UTRs containing a 7mer-m8 site versus a 7mer-A1 site, a 7mer-A1 versus a 6mer, and a 6mer versus no site were also significant ($p < 10\text{-}6$, $p < 10\text{-}20$, and $p < 10\text{-}31$, respectively).

Example of the RNA-Protein immunoprecipitation methods (RIP, CLIP, HITS-CLIP and further methods)

Vol 460|23 July 2009|doi:10.1038/nature08170 nature

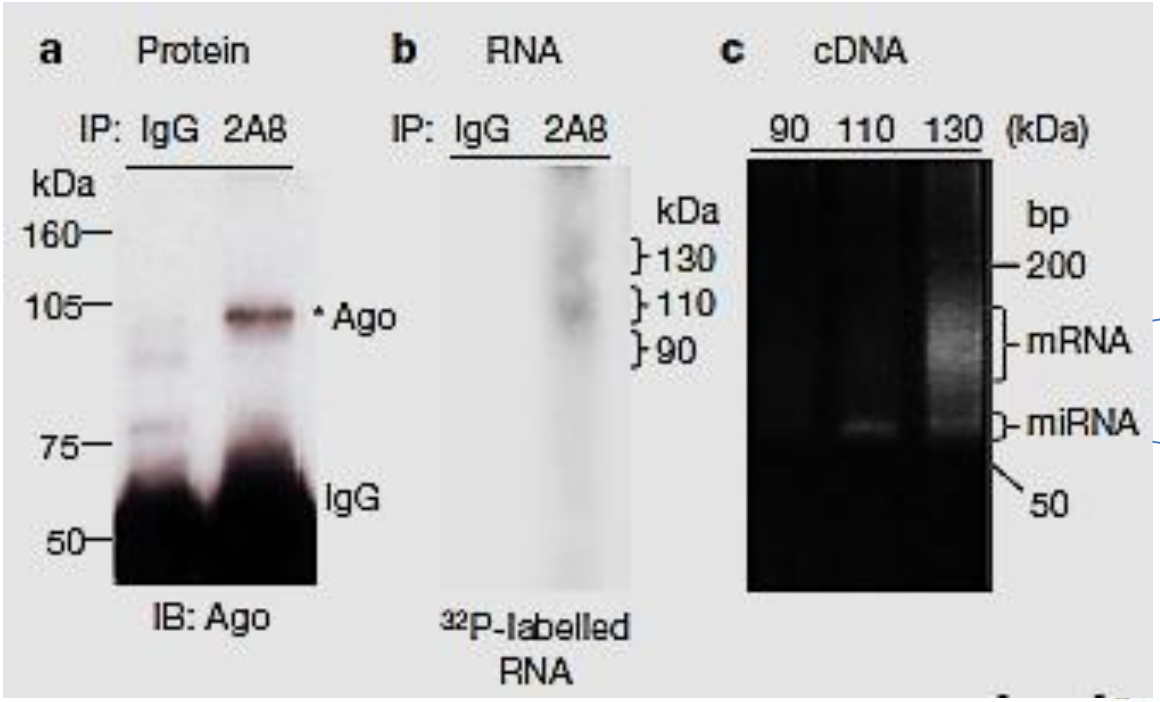
ARTICLES

Argonaute HITS-CLIP decodes microRNA–mRNA interaction maps

Sung Wook Chi[1], Julie B. Zang[1], Aldo Mele[1] & Robert B. Darnell[1]

MicroRNAs (miRNAs) have critical roles in the regulation of gene expression; however, as miRNA activity requires base pairing with only 6–8 nucleotides of messenger RNA, predicting target mRNAs is a major challenge. Recently, high-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation (HITS-CLIP) has identified functional protein–RNA interaction sites. Here we use HITS-CLIP to covalently crosslink native argonaute (Ago, also called Eif2c) protein–RNA complexes in mouse brain. This produced two simultaneous data sets—Ago–miRNA and Ago–mRNA binding sites—that were combined with bioinformatic analysis to identify interaction sites between miRNA and target mRNA. We validated genome-wide interaction maps for miR-124, and generated additional maps for the 20 most abundant miRNAs present in P13 mouse brain. Ago HITS-CLIP provides a general platform for exploring the specificity and range of miRNA action *in vivo*, and identifies precise sequences for targeting clinically relevant miRNA–mRNA interactions.

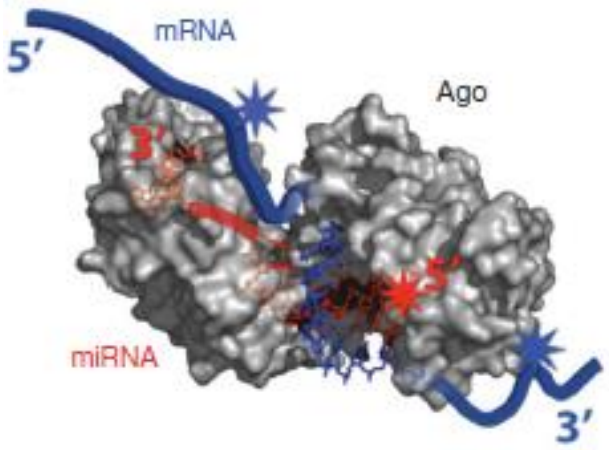
IP using the anti-AGO 2AB antibody reveals different complexes

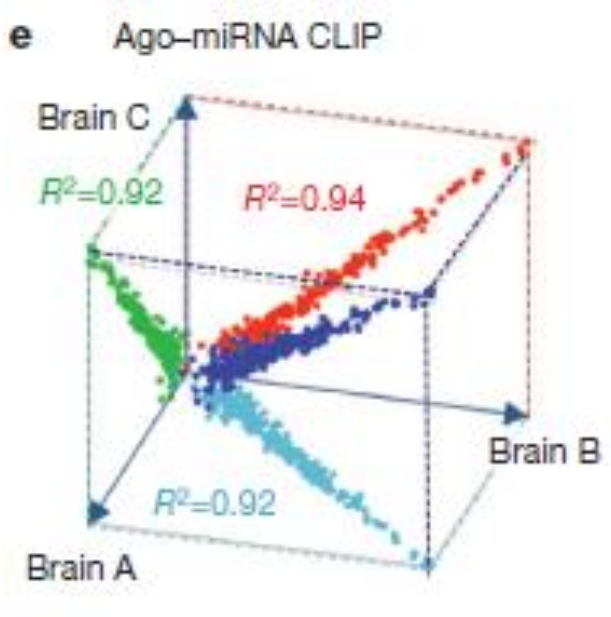


NGS

Short NGS

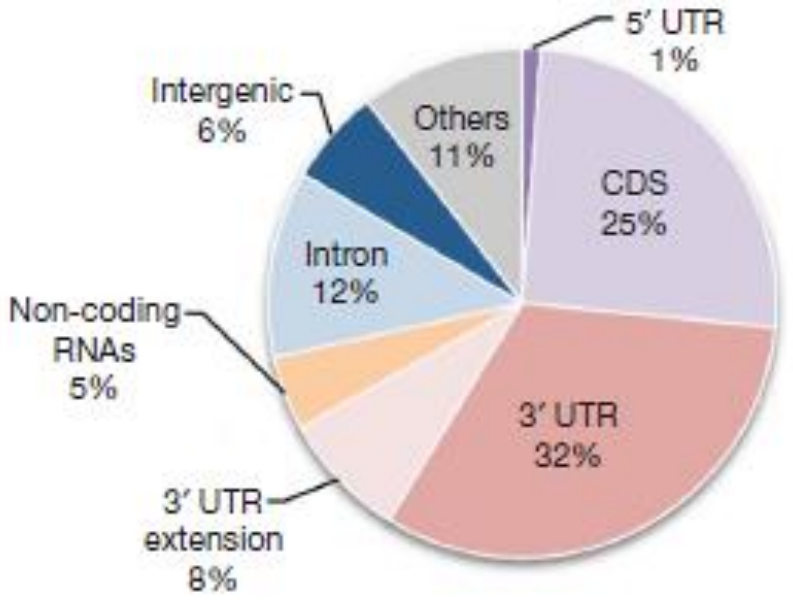
Extract bands, add 36-nt adapters, RT and PCR

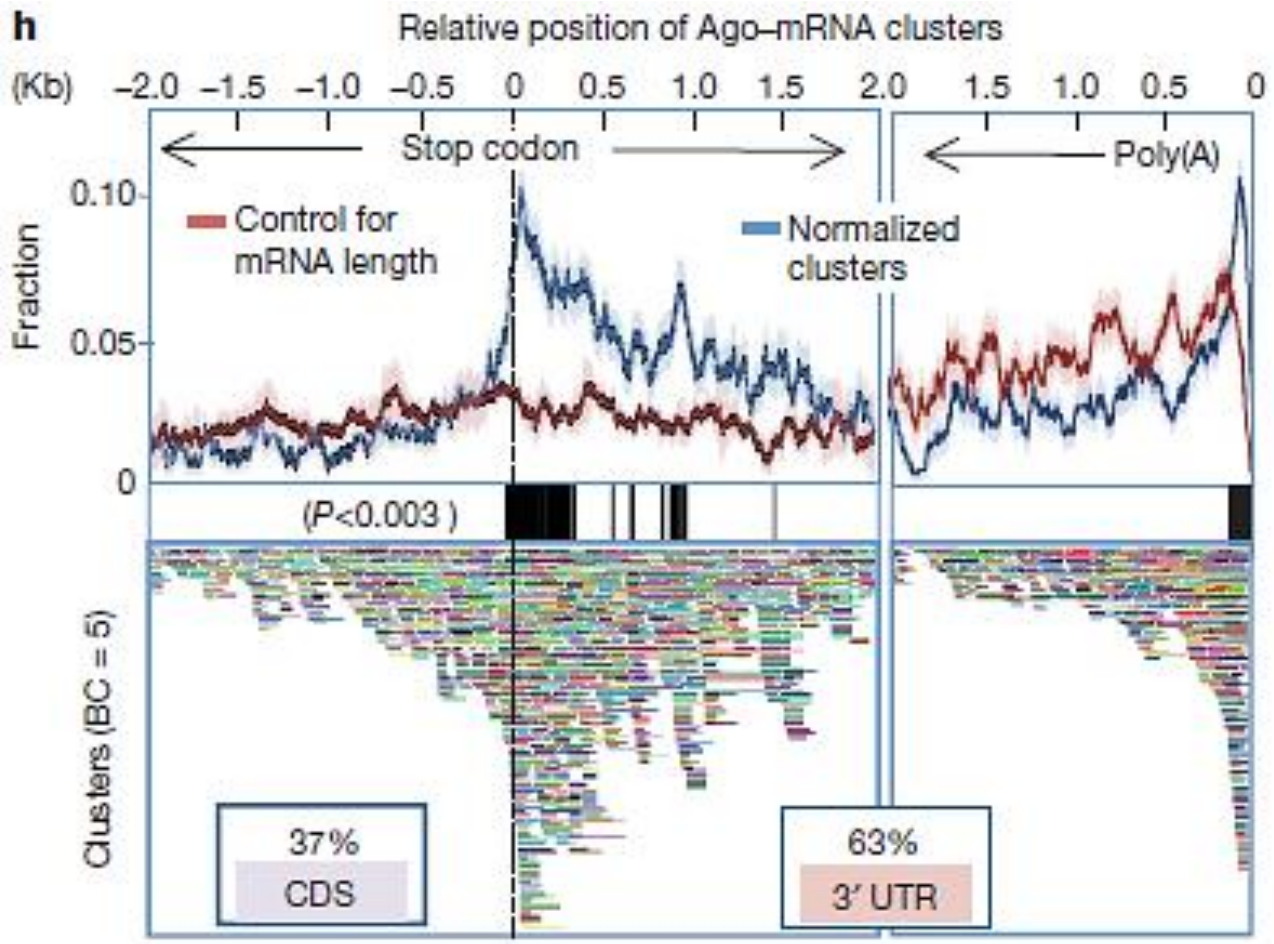




Three replicates from brain

Mapping of targets




h
Relative position of Ago–mRNA clusters
(Kb)
–2.0 –1.5 –1.0 –0.5 0 0.5 1.0 1.5 2.0
1.5 1.0 0.5 0
Stop codon
Poly(A)
Fraction
0.10
0.05
0
Control for mRNA length
Normalized clusters
(P<0.003)
Clusters (BC = 5)
37%
CDS
63%
3′ UTR

Main problems associated with CLIP and derivatives:

1. cross-linking requires intense, short-wavelength irradiation and this yields quite high non-specific results, due to AGO occasional «touch» with flanking mRNA sequences or completely unrelated RNAs.
2. two separate libraries must be generated, and the lists matched bioinformatically (guess level).
3. target (m)RNA sequence protected by AGO footprint is quite long and gives uncertainty to the possible target sequence.

Trying to bypass this problem, researchers developed PAR-CLIP (next slide)

Transcriptome-wide Identification of RNA-Binding Protein and MicroRNA Target Sites by PAR-CLIP

Markus Hafner,[1,5] Markus Landthaler,[1,4,5] Lukas Burger,[2] Mohsen Khorshid,[2] Jean Hausser,[2] Philipp Berninger,[2] Andrea Rothballer,[1] Manuel Ascano, Jr.,[1] Anna-Carina Jungkamp,[1,4] Mathias Munschauer,[1] Alexander Ulrich,[1] Greg S. Wardle,[1] Scott Dewell,[3] Mihaela Zavolan,[2,*] and Thomas Tuschl[1,*]

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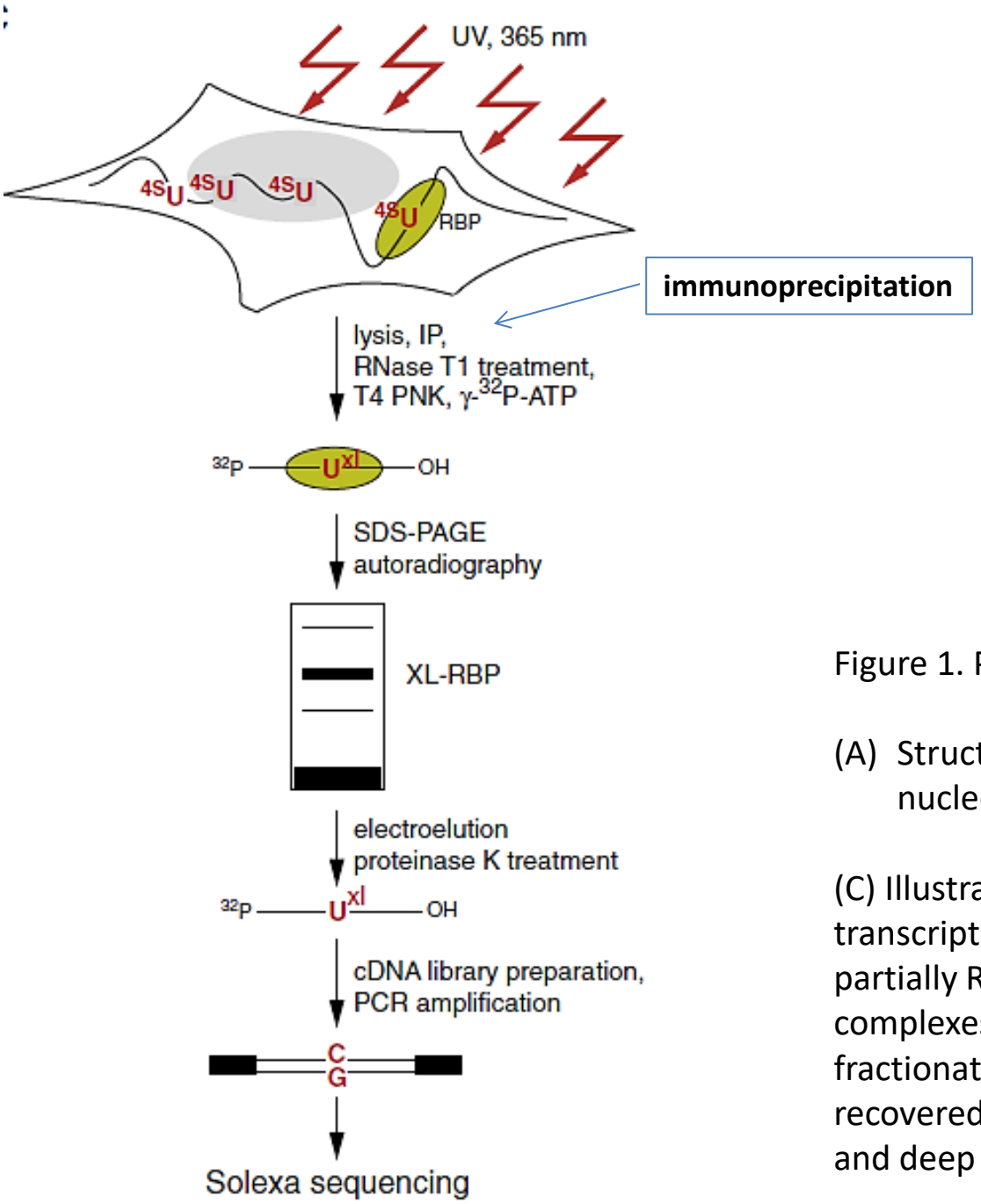
RNA transcripts are subject to posttranscriptional gene regulation involving hundreds of RNA-binding proteins (RBPs) and microRNA-containing ribonucleoprotein complexes (miRNPs) expressed in a cell-type dependent fashion. We developed a cell-based crosslinking approach to determine at high resolution and transcriptome-wide the binding sites of cellular RBPs and miRNPs. The crosslinked sites are revealed by thymidine to cytidine transitions in the cDNAs prepared from immunopurified RNPs of 4-thiouridine-treated cells. We determined the binding sites and regulatory consequences for several intensely studied RBPs and miRNPs, including PUM2, QKI, IGF2BP1-3, AGO/EIF2C1-4 and TNRC6A-C. Our study revealed that these factors bind thousands of sites containing defined sequence motifs and have distinct preferences for exonic versus intronic or coding versus untranslated transcript regions. The precise mapping of binding sites across the transcriptome will be critical to the interpretation of the rapidly emerging data on genetic variation between individuals and how these variations contribute to complex genetic diseases.



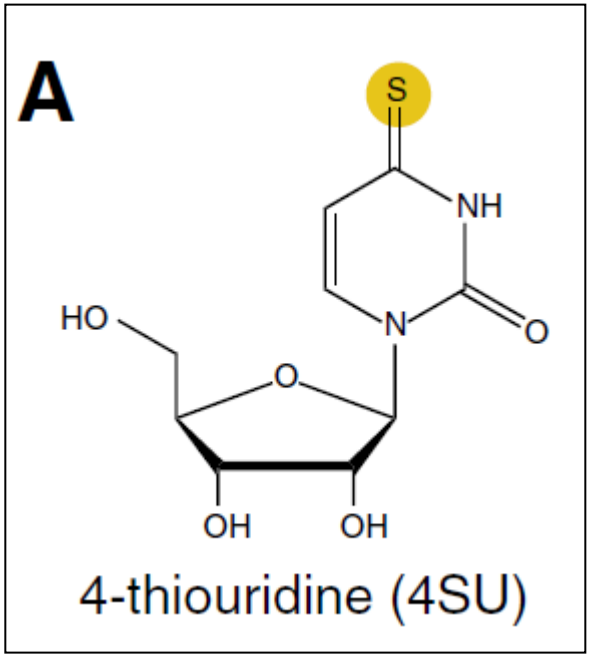
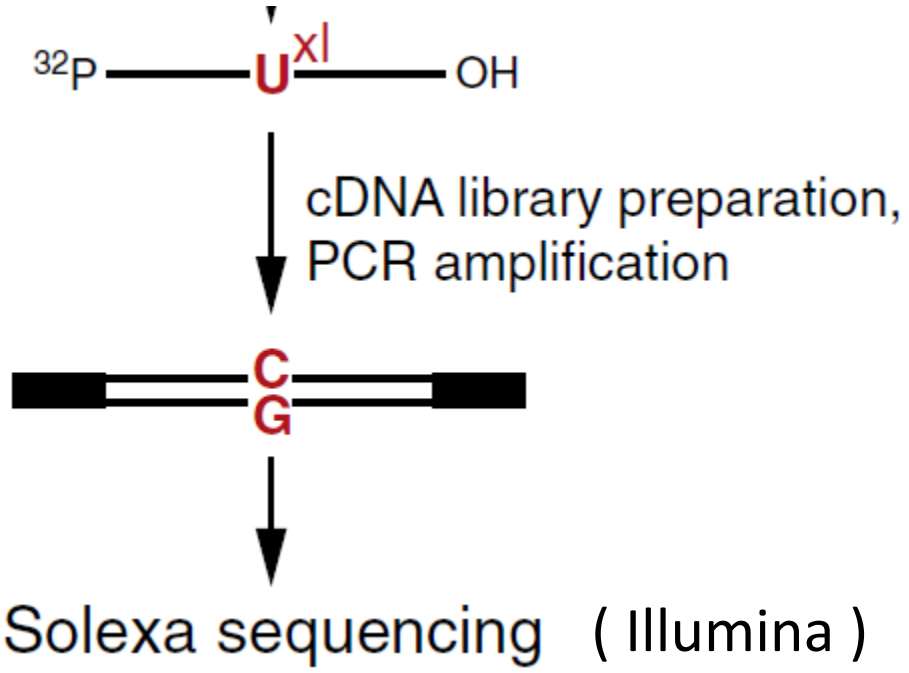


Figure 1. PAR-CLIP Methodology.

(A) Structure of photoactivatable nucleosides.

(C) Illustration of PAR-CLIP. 4SU-labeled transcripts were crosslinked to RBPs and partially RNase-digested RNA-protein complexes were immunopurified and size-fractionated. RNA molecules were recovered and converted into a cDNA library and deep sequenced.



The «U» that was directly cross-linked to te RBP is identified since it is converted to «C» and consequently the targets are univocally identified.

Major problems for these approaches is that they are indirect, i.e. they are based on the generation of **separate libraries**, one for miRNAs and the other for targets (mRNA, lncRNAs, etc). Matching is always based on complementarity searches.

For this reason, Darnell's group developed a strategy to ligate miRNAs and targets and sequence them together, called CLEAR-CLIP

nature COMMUNICATIONS

ARTICLE

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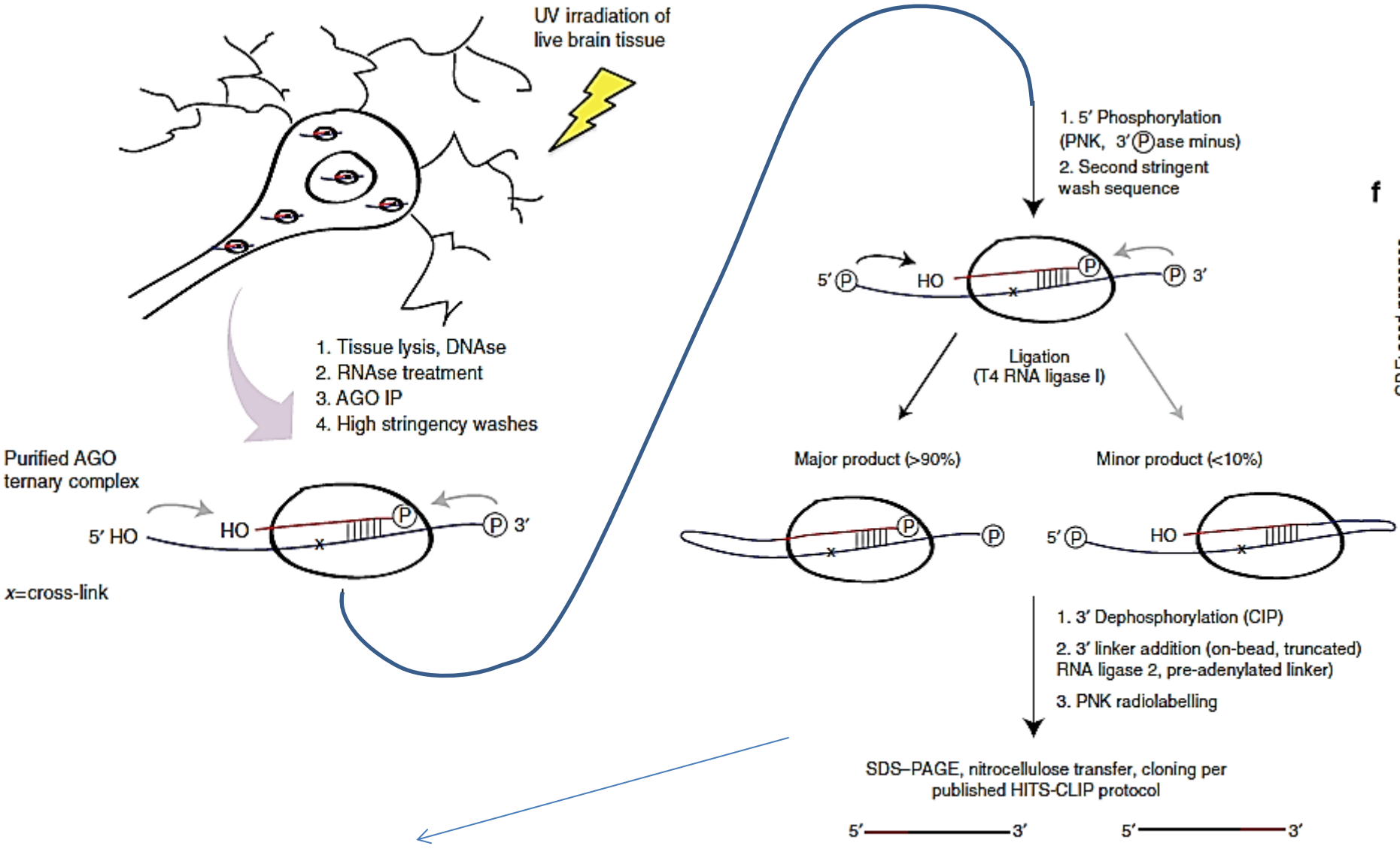
DOI: 10.1038/ncomms9864 OPEN

miRNA–target chimeras reveal miRNA 3′-end pairing as a major determinant of Argonaute target specificity

Michael J. Moore[1], Troels K.H. Scheel[2,3,4], Joseph M. Luna[1,2], Christopher Y. Park[1,5], John J. Fak[1], Eiko Nishiuchi[2], Charles M. Rice[2] & Robert B. Darnell[1,5]

Here we report a modified AGO HITS-CLIP strategy termed CLEAR (covalent ligation of endogenous Argonaute-bound RNAs)-CLIP, which enriches miRNAs ligated to their endogenous mRNA targets. CLEAR-CLIP mapped ca. 130,000 endogenous miRNA–target interactions in mouse brain and B40,000 in human hepatoma cells. Motif and structural analysis define expanded pairing rules for over 200 mammalian miRNAs. Most interactions combine seed-based pairing with distinct, miRNA-specific patterns of auxiliary pairing. At some regulatory sites, this specificity confers distinct silencing functions to miRNA family members with shared seed sequences but divergent 30-ends. This work provides a means for explicit biochemical identification of miRNA sites in vivo, leading to the discovery that miRNA 3’-end pairing is a general determinant of AGO binding specificity.



One library for NGS with joint target-miRNA

How is expression of micro-RNA regulated ?

miRNA and regulatory networks

Regulation of miRNA expression: Transcription

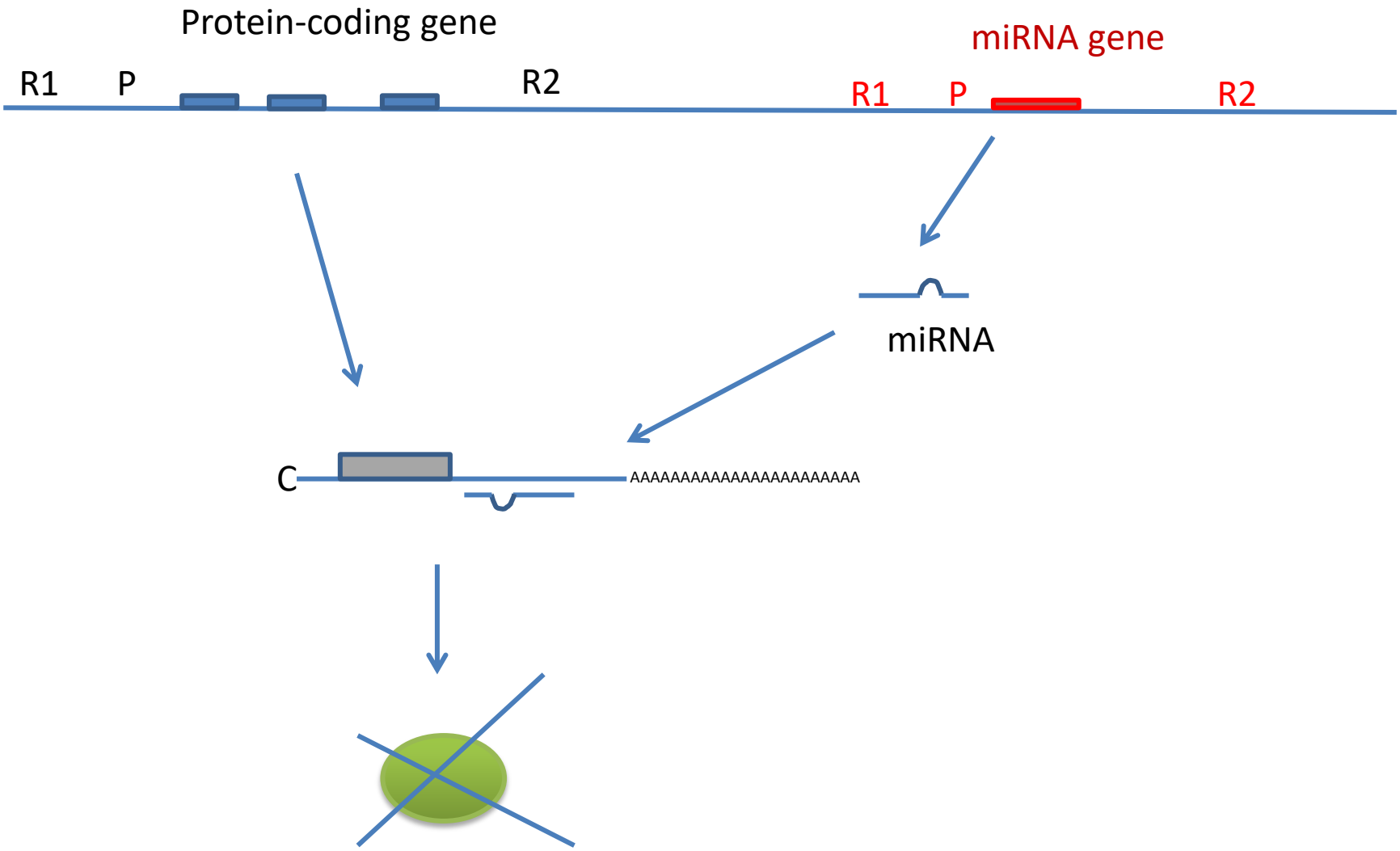
A recent large scale mapping of 175 human miRNA promoters through nucleosome positioning and chromatin immunoprecipitationon-genomic DNA microarray chip (or ChIP-onchip) analysis suggests that

the promoter structure of miRNA genes, including the relative frequencies of CpG islands, TATA box, TFIIB recognition, initiator elements, and histone modifications, is indistinguishable between the promoters of miRNA and mRNA.

Furthermore, DNA binding factors that regulate miRNA transcription largely overlap with those that control protein coding genes.

As a further proof, the expression of specific miRNA is also controlled by signal transduction pathways, in analogy to protein-coding genes.

A two-component regulatory circuit



The American Journal of Pathology, Vol. 176, No. 5, May 2010

DOI: 10.2353/ajpath.2010.090837

Biomarkers, Genomics, Proteomics, and Gene Regulation

Estrogen Receptor α Controls a Gene Network in Luminal-Like Breast Cancer Cells Comprising Multiple Transcription Factors and MicroRNAs

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Oli M.V. Grober,* Ornella Paris,* Lorenzo Ferraro,*
Maria Ravo,* Roberta Tarallo,* Shujun Luo,†
Gary P. Schroth,† Martin Seifert,‡
Christian Zinser,‡ Maria Luisa Chiusano,§
Alessandra Traini,§ Michele De Bortoli,¶
and Alessandro Weisz*‖

Luminal-like breast tumor cells express estrogen receptor α (ERα), a member of the nuclear receptor family of ligand-activated transcription factors that controls their proliferation, survival, and functional status. To identify the molecular determinants of this hormone-responsive tumor phenotype, a comprehensive genome-wide analysis was performed in estrogen stimulated MCF-7 and ZR-75.1 cells by integrating time-course mRNA expression profiling with global mapping of genomic ERα binding sites by chromatin immunoprecipitation coupled to massively parallel sequencing, microRNA expression profiling, and *in silico* analysis of transcription units and receptor binding regions identified. All 1270 genes that were found to respond to 17β-estradiol in both cell lines cluster in 33 highly concordant groups, each of which showed defined kinetics of RNA changes. This hormone-responsive gene set includes several direct targets of ERα and is organized in a gene regulation cascade, stemming from ligand-activated receptor and reaching a large number of downstream targets via AP-2γ, B-cell activating transcription factor, E2F1 and 2, E74-like factor 3, GTF2IRD1, hairy and enhancer of split homologue-1, MYB, SMAD3, RARα, and RXRα transcription factors. MicroRNAs are also integral components of this gene regulation network because miR-107, miR-424, miR-570, miR-618, and miR-760 are regulated by 17β-estradiol along with other microRNAs that can target a significant number of transcripts belonging to one or more estrogen-responsive gene clusters. *(Am J Pathol 2010, 176:2113–2130; DOI:*

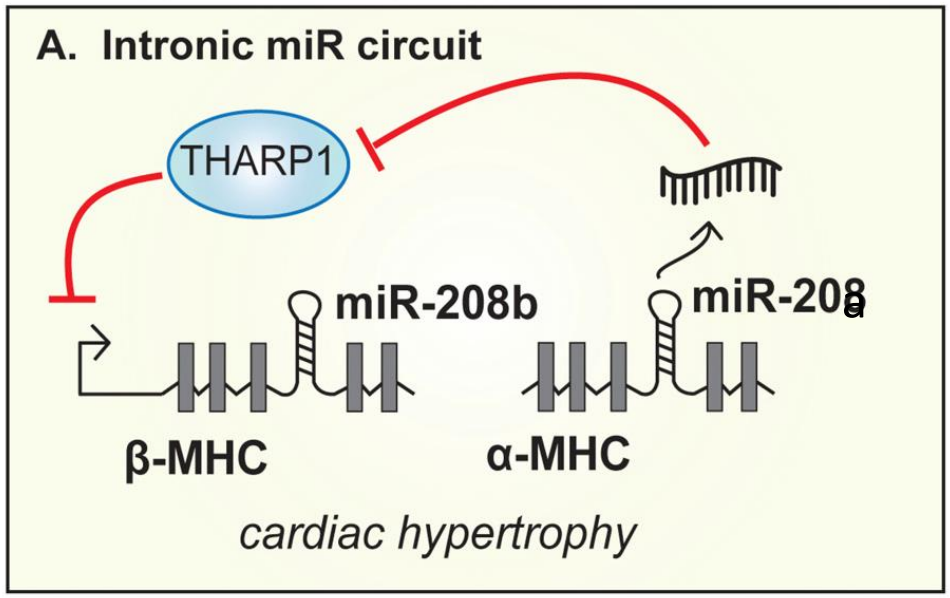
Additional regulations by various signalling pathways were also noticed on various steps of miRNA biogenesis

Controlling the activity of Drosha, Dicer, Exportin 5, components of the RISC complex.

In addition, some miRNA get edited ! (By the adenosine deaminase enzyme: A→I)

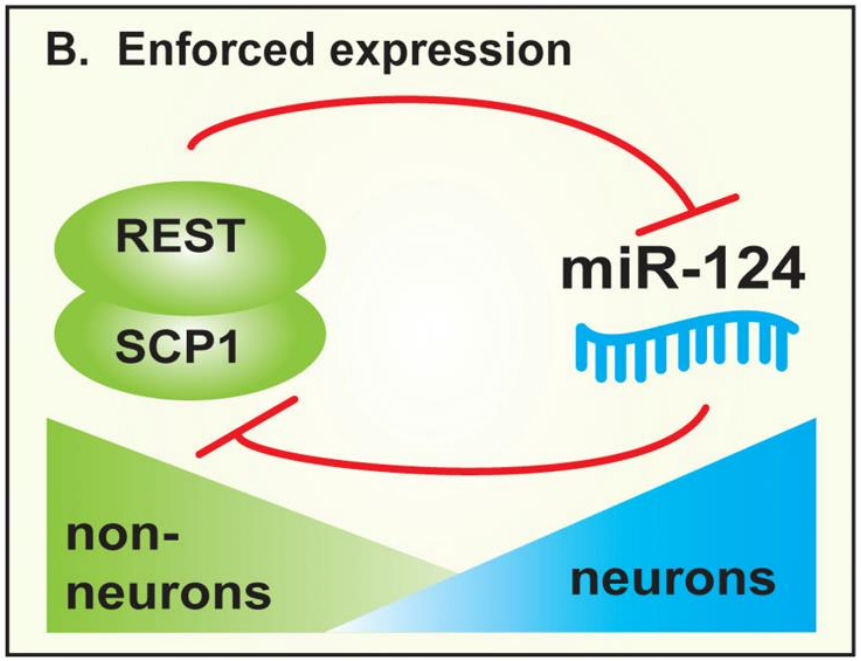
The majority of regulatory pathways are shared

miRNA regulatory circuits



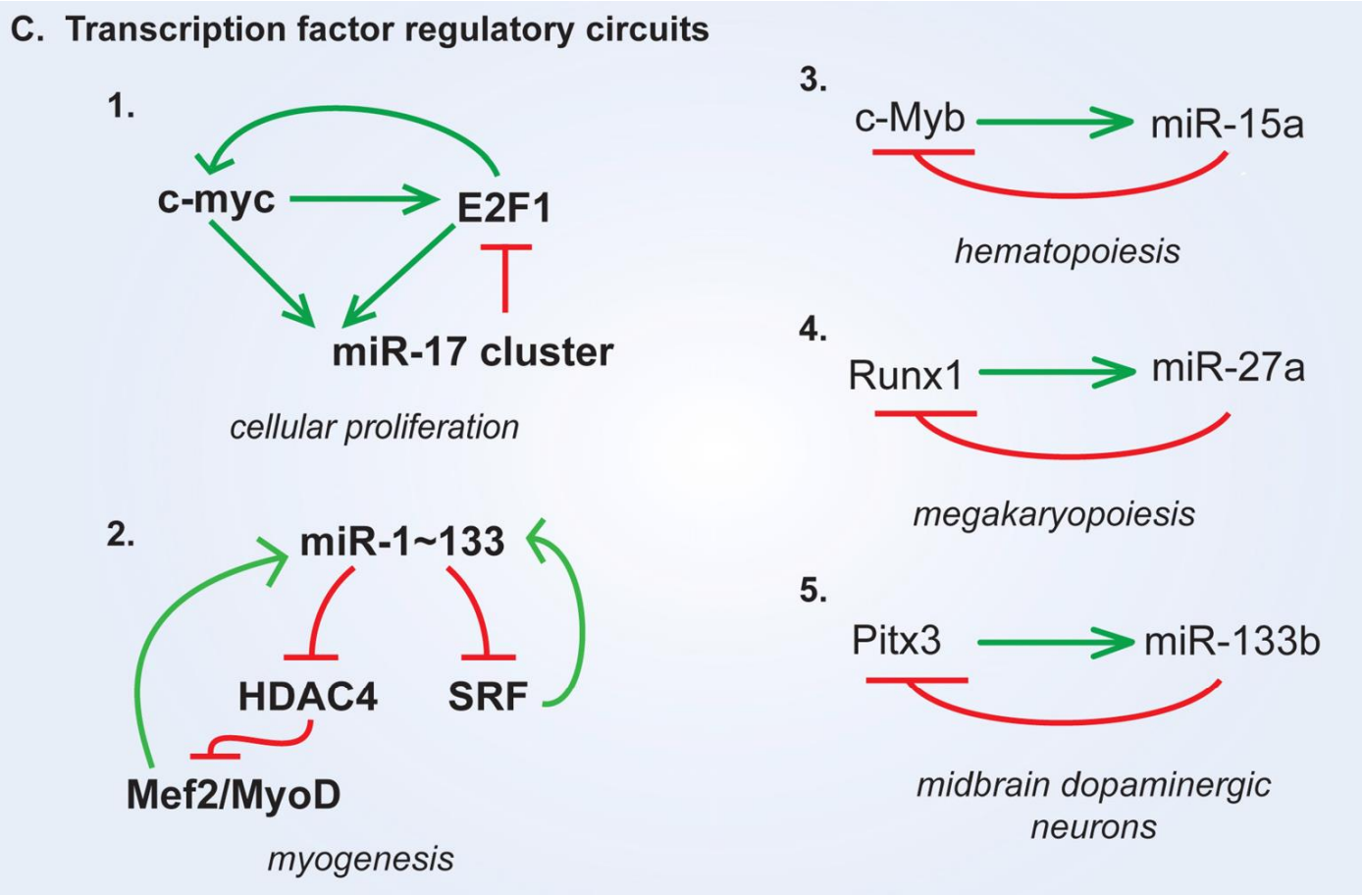
The cardiac specific miR-208 family is encoded within the introns of myosin heavy chain (MHC) genes. miR-208a targets THARP1, and will reduce its level, thus increasing expression of β-MHC.

This is an auto-boosting circuit that is found altered in cardiac hypertrophy.



The expression of miR-124 is negatively regulated by the binding of the RE1 silencing transcription (REST) factor to the promoter in non-neuronal cells

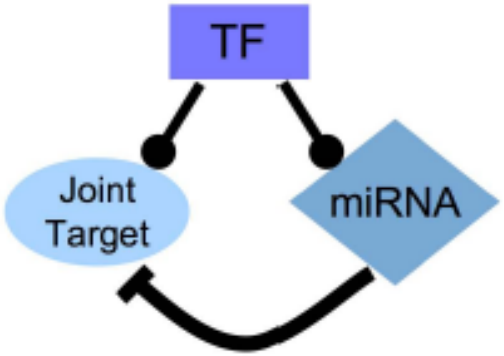
In neurons, miR-124 represses translation of SCP1 that is one component of the REST complex.

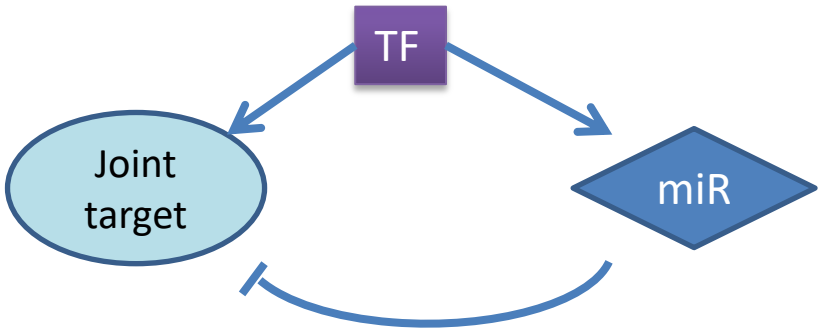


Examples of feed-back regulation of microRNA transcription through the repression of transcription factors.

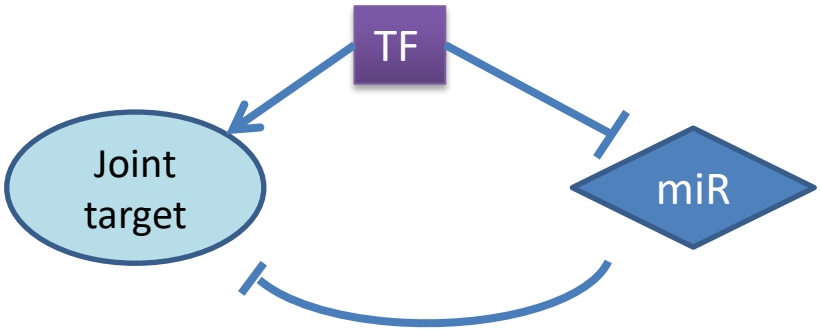
mRNAs that encode for Transcription Factors are very often controlled by miRNA.

This suggests a model where feed-forward and feed-back control loops exist.





uncoherent



coherent

Friard et al. BMC Bioinformatics 2010, 11:435
http://www.biomedcentral.com/1471-2105/11/435

BMC Bioinformatics

DATABASE Open Access

CircuitsDB: a database of mixed microRNA/transcription factor feed-forward regulatory circuits in human and mouse

Olivier Friard[1], Angela Re[2], Daniela Taverna[1,3,4], Michele De Bortoli[1,3], Davide Corá[1,5*]

Abstract

Background: Transcription Factors (TFs) and microRNAs (miRNAs) are key players for gene expression regulation in higher eukaryotes. In the last years, a large amount of bioinformatic studies were devoted to the elucidation of transcriptional and post-transcriptional (mostly miRNA-mediated) regulatory interactions, but little is known about the interplay between them.

Description: Here we describe a dynamic web-accessible database, `CircuitsDB`, supporting a genome-wide transcriptional and post-transcriptional regulatory network integration, for the human and mouse genomes, based on a bioinformatic sequence-analysis approach. In particular, `CircuitsDB` is currently focused on the study of mixed miRNA/TF Feed-Forward regulatory Loops (FFLs), i.e. elementary circuits in which a master TF regulates an miRNA and together with it a set of Joint Target protein-coding genes. The database was constructed using an ab-initio oligo analysis procedure for the identification of the transcriptional and post-transcriptional interactions. Several external sources of information were then pooled together to obtain the functional annotation of the proposed interactions. Results for human and mouse genomes are presented in an integrated web tool, that allows users to explore the circuits, investigate their sequence and functional properties and thus suggest possible biological experiments.

Conclusions: We present `CircuitsDB`, a web-server devoted to the study of human and mouse mixed miRNA/TF Feed-Forward regulatory circuits, freely available at: http://biocluster.di.unito.it/circuits/

miRNAs target protein-coding genes

But not only…..

In several experiments miRNA/**lncRNAs** were evidenced

Why should miRNA target noncoding RNA ?

Will miRNA/lncRNA result in de-stabilization of lncRNAs ?
Or in miRNA degradation ?

GENCODE

Version 28 (November 2017 freeze, GRCh38) - Ensembl 92

General stats

Total No of Genes	58381	Total No of Transcripts	203835
Protein-coding genes	19901	Protein-coding transcripts	82335
Long non-coding RNA genes	15779	- full length protein-coding:	56541
Small non-coding RNA genes	7569	- partial length protein-coding:	25794
Pseudogenes	14723	Nonsense mediated decay transcripts	14889
- processed pseudogenes:	10693	Long non-coding RNA loci transcripts	28468
- unprocessed pseudogenes:	3519		
- unitary pseudogenes:	218		
- polymorphic pseudogenes:	38		
- pseudogenes:	18		

H. Sapiens, statistics

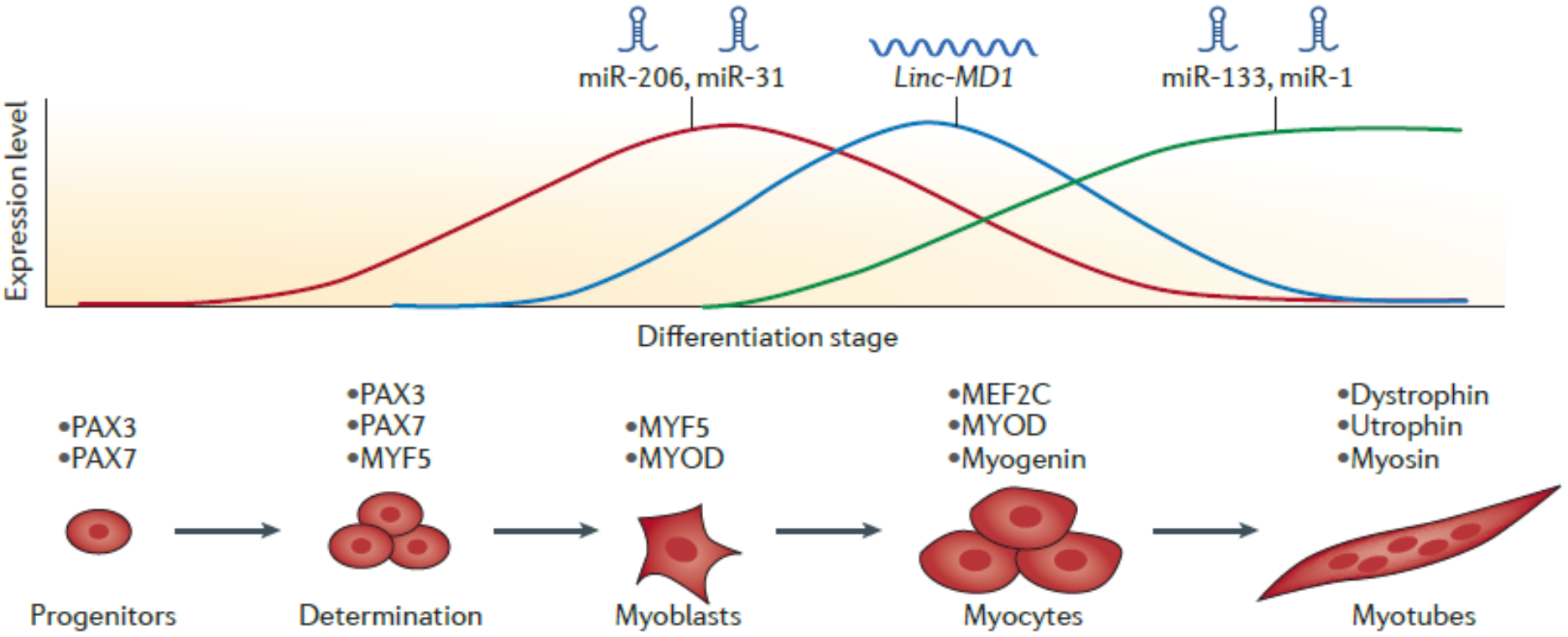
Another layer of regulation.....

A Long Noncoding RNA Controls Muscle Differentiation by Functioning as a Competing Endogenous RNA

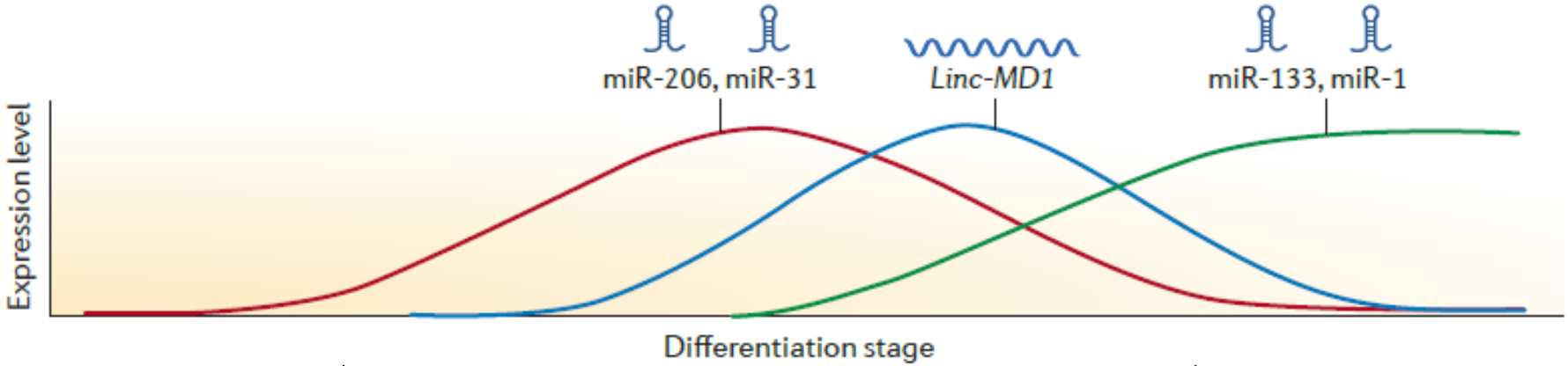
Marcella Cesana,[1,6] Davide Cacchiarelli,[1,6] Ivano Legnini,[1] Tiziana Santini,[1] Olga Sthandier,[1] Mauro Chinappi,[2] Anna Tramontano,[2,3,4] and Irene Bozzoni[1,3,4,5,*]
[1]Department of Biology and Biotechnology "Charles Darwin"
[2]Department of Physics
[3]Institut Pasteur Fondazione Cenci-Bolognetti
[4]Center for Life Nano Science @Sapienza, Istituto Italiano di Tecnologi
[5]IBPM of Consiglio Nazionale delle Ricerche (CNR)
Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy
[6]These authors contributed equally to the work
*Correspondence: irene.bozzoni@uniroma1.it

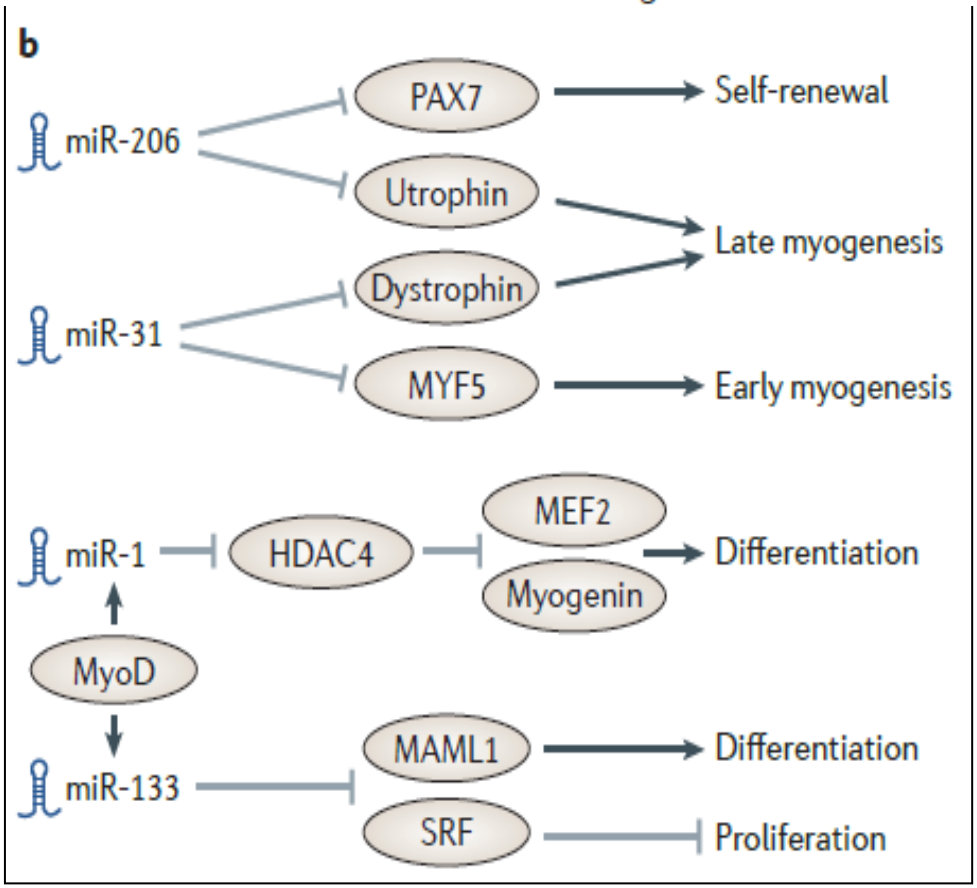
Recently, a new regulatory circuitry has been identified in which RNAs can crosstalk with each other by competing for shared microRNAs. Such competing endogenous RNAs (ceRNAs) regulate the distribution of miRNA molecules on their targets and thereby impose an additional level of post-transcriptional regulation. Here we identify a muscle-specific long noncoding RNA, linc-MD1, which governs the time of muscle differentiation by acting as a ceRNA in mouse and human myoblasts. Downregulation or overexpression of linc-MD1 correlate with retardation or anticipation of the muscle differentiation program, respectively. We show that linc-MD1 "sponges" miR-133 and miR-135 to regulate the expression of MAML1 and MEF2C, transcription factors that activate muscle-specific gene expression. Finally, we demonstrate that linc-MD1 exerts the same control over differentiation timing in human myoblasts, and that its levels are strongly reduced in Duchenne muscle cells. We conclude that the ceRNA network plays an important role in muscle differentiation.

Cell *147*, 358–369, October 14, 2011



Fatica & Bozzoni 2014


miR-206, miR-31
Linc-MD1
miR-133, miR-1
Expression level
Differentiation stage


b
PAX7
Self-renewal
miR-206
Utrophin
Late myogenesis
Dystrophin
miR-31
MYF5
Early myogenesis
MEF2
miR-1
HDAC4
Differentiation
Myogenin
MyoD
MAML1
Differentiation
miR-133
SRF
Proliferation

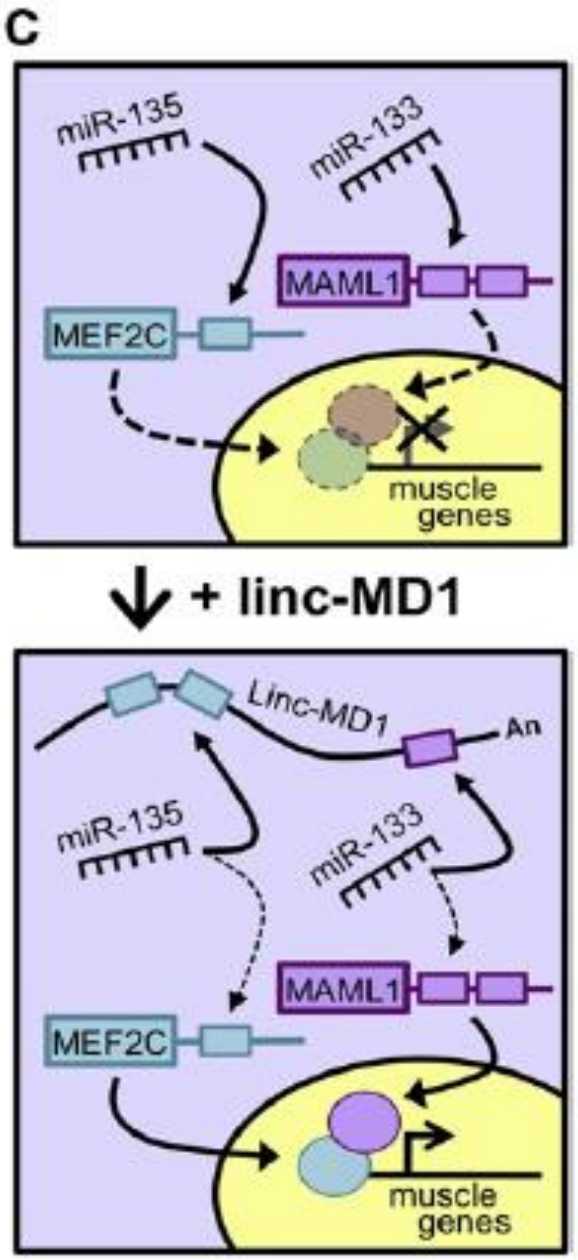
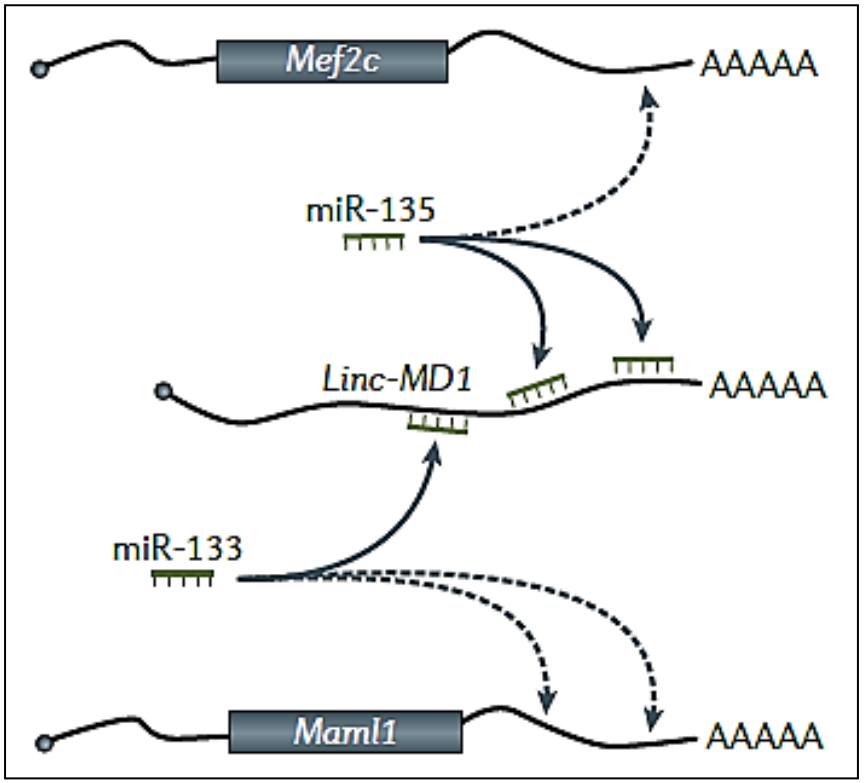
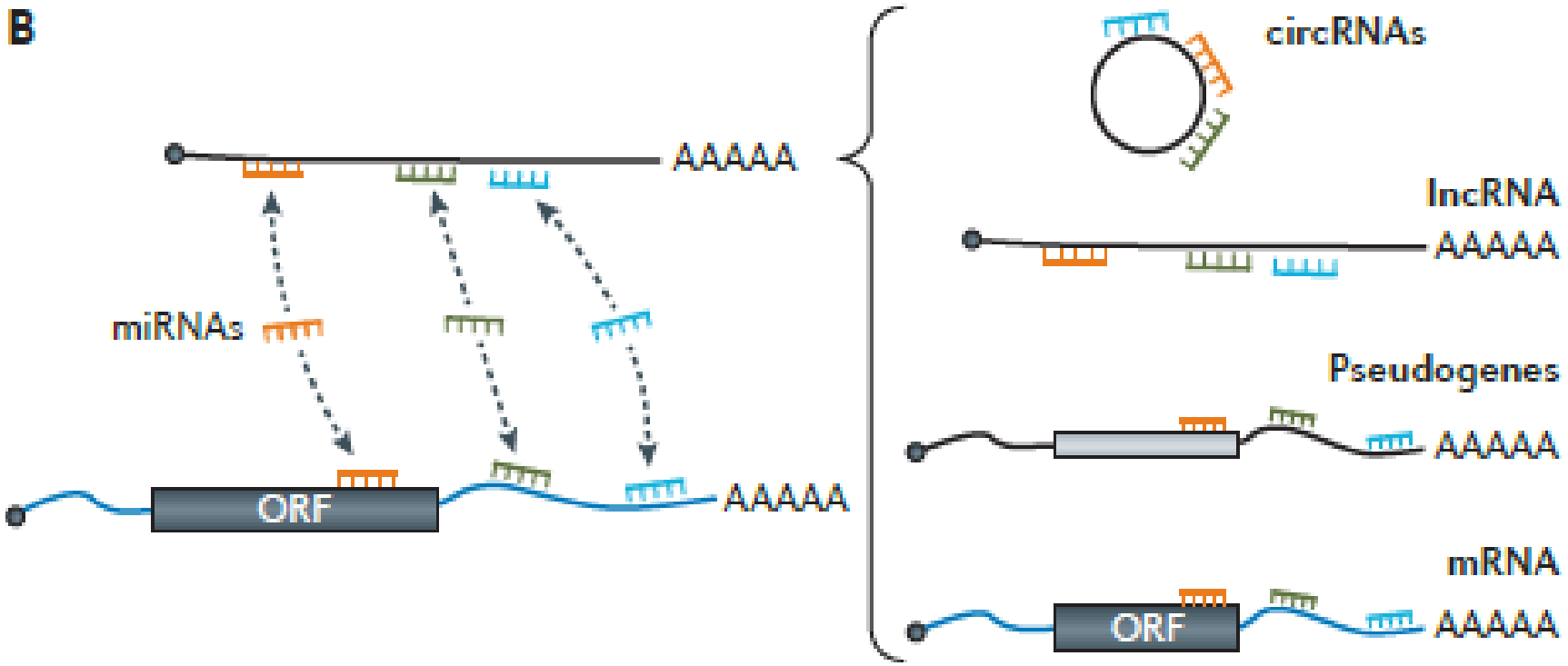


Figure 7. linc-MD1 Is Conserved in Humans, and It Improves Differentiation of Duchenne Myoblasts

(C) Schematic representation of the circuitry linking linc-MD1, miR-135, miR-133, and muscle differentiation. (Cesana et al., 2011)



ceRNAs (competing endogenous RNA)



Base pairing is the mode of action of competing endogenous RNAs. In this case, however, the complementarity is between microRNAs (miRNAs) and different targets (including circular RNAs (circRNAs), lncRNAs, pseudogene transcripts and mRNAs).

Fatica & Bozzoni 2014

Hypothesis strongly discussed
Fiercely adversed by Bartel, by the way...

Hundreds of targets for each miRNA
miRNA levels in cells can not justify large competing effects unless increase or decreas several order of magnitude.

Think about !