MicroRNAs (I)

Regulación de la expresión génica: Niveles de regulación

La regulación génica puede tomar lugar en cualquiera de los pasos que llevan del DNA (gen) a la proteína

Eucariotas tienen mas posibilidades de regulación que procariotas en los que se regula principalmente el inicio:

- La estructura de la cromatina puede y tiene que ser modificada de diferentes maneras para iniciar la transcripción
- El inicio y los niveles de transcripción se regulan mediante factores de transcripción que se unen a elementos regulatorios en *cis*
- *Splicing*, (adición del *cap* 5') y cola poli(A)
- Proceso del transporte al citoplasma
- Degradación del mRNA: mayor rango de vida media en eucariotas (en procariotas se degradan rápidamente)
- **Regulación post-transcripcional (microRNAs)**
- Se regula la iniciación de la traducción
- Modificaciones postraduccionales pueden cambiar la conformación o llevar a la degradación

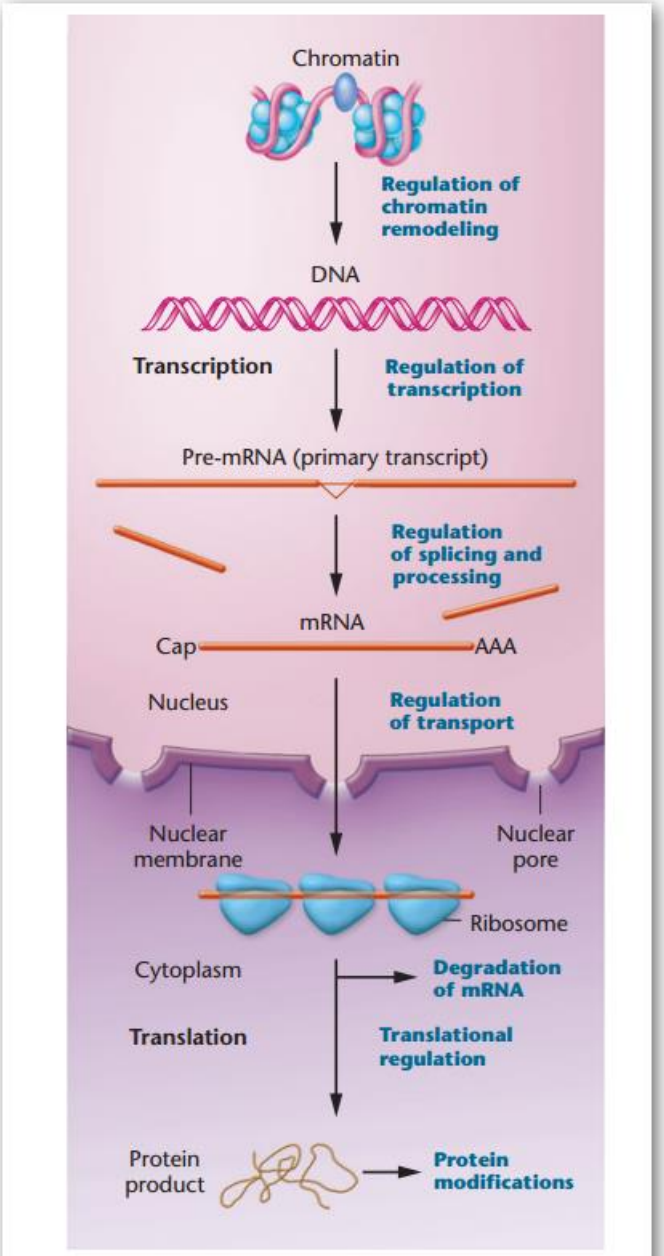
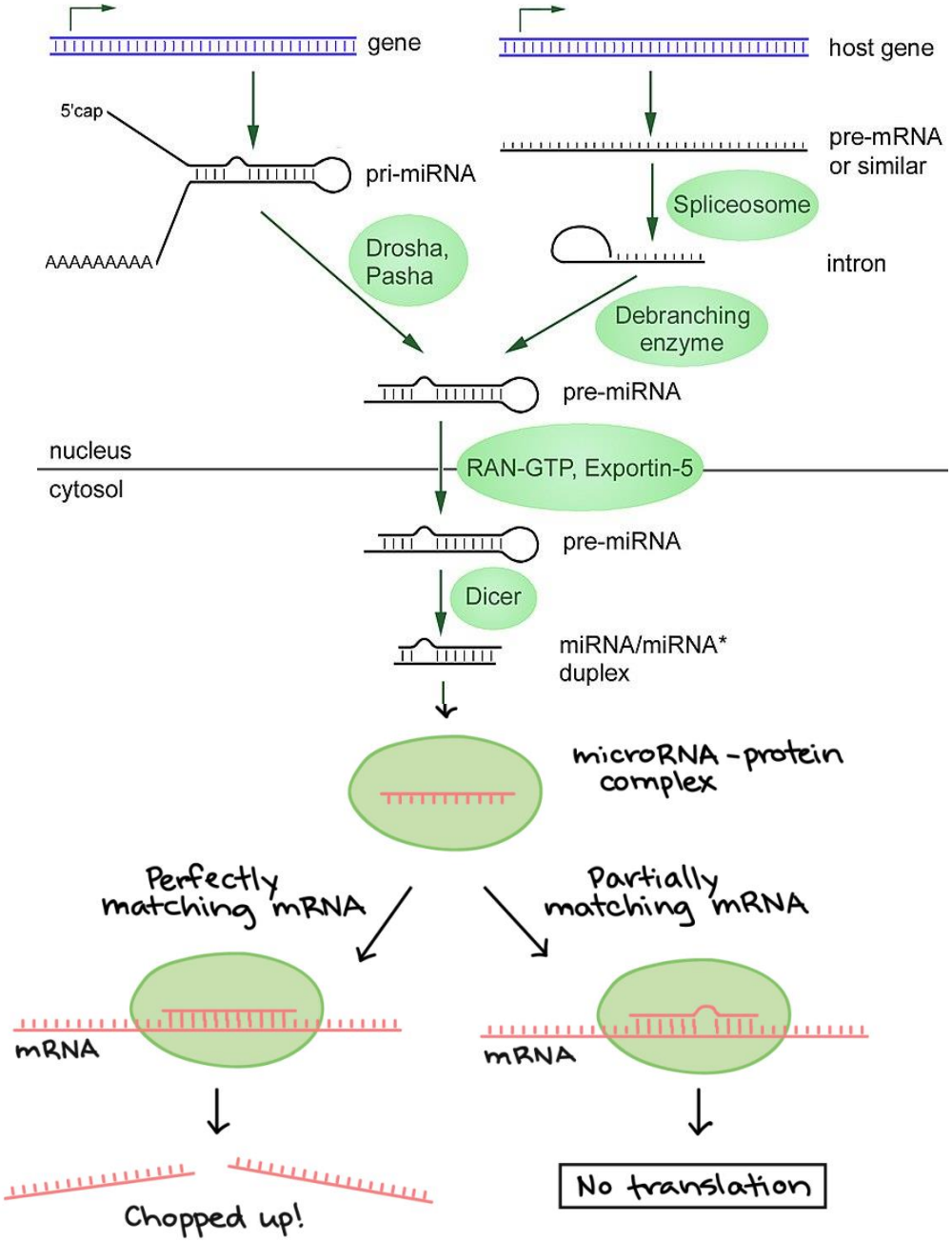


FIGURE 17–1 Regulation can occur at any stage in the expression of genetic material in eukaryotes. All these forms of regulation affect the degree to which a gene is expressed.

Biogenesis

- Most microRNAs are transcribed by Pol-II in the nucleus
- Primary transcripts (pri-microRNA) get cleaved by ribonuclease III enzyme *Drosha*
- The pre-microRNA is transported to the cytoplasm
- pre-microRNAs are cleaved by Dicer → miRNA/miRNA* duplex
- Mature microRNAs have a typical length of 22nt
- microRNAs recognize their target genes by sequence complementarity within a miRNA – protein complex
- Usually only one sequence of the duplex is functional and the other gets degraded



How it all began ...

Cell, Vol. 75, 843–854, December 3, 1993, Copyright © 1993 by Cell Press

The C. elegans Heterochronic Gene *lin-4* Encodes Small RNAs with Antisense Complementarity to *lin-14*

Rosalind C. Lee,*† Rhonda L. Feinbaum,*‡ and Victor Ambros†
Harvard University
Department of Cellular and Developmental Biology
Cambridge, Massachusetts 02138

Ambros and Horvitz, 1987). Animals carrying a *lin-4* loss-of-function (*lf*) mutation, *lin-4(e912)*, display reiterations of early fates at inappropriately late developmental stages; cell lineage patterns normally specific for the L1 are reiterated at later stages, and the animals execute extra larval molts (Chalfie et al., 1981). The consequences of these heterochronic developmental patterns include the ab-

Comparison of the *lin-4* genomic sequence from these four species and site-directed mutagenesis of potential open reading frames indicated that *lin-4* does not encode a protein. Two small *lin-4* transcripts of approximately 22 and 61 nt were identified in C. elegans and found to contain sequences complementary to a repeated sequence element in the 3′ untranslated region (UTR) of *lin-14* mRNA, suggesting that *lin-4* regulates *lin-14* translation via an antisense RNA–RNA interaction.

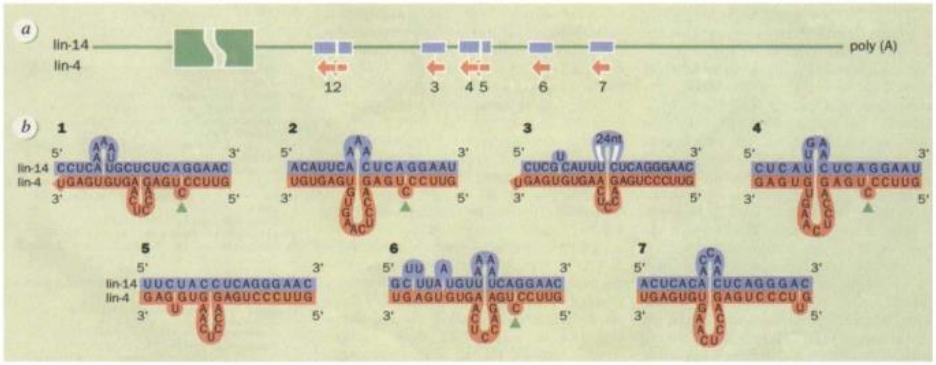
?? ?? ??

News & Views | Published: 06 January 1994

Deviants — or emissaries

Marvin Wickens & Kathy Takayama

Nature **367**, 17–18 (1994) | Download Citation



Proposed base-pairing between *lin-4* RNA and elements in the *lin-14* 3′UTR. *a*, *lin-14* mRNA, with the protein-coding region indicated by a large, broken box. Regions of the *lin-14* 3′UTR which potentially can pair with *lin-4* RNA are in blue, with their relative positions drawn to scale. *lin-4* RNA is in red. *b*, Hypothetical base-pairing schemes; base-pairing has not been demonstrated experimentally (see text). The arrow indicates the C in *lin-4* which, when changed to U, prevents repression of *lin-14* mRNA. The termini of *lin-4* RNA have been deduced using DNA probes, rather than by direct analysis of the RNA, and so are imprecise. (Figure derived from refs 1, 2 and 8.)

How it all began ...

Cell, Vol. 75, 843–854, December 3, 1993, Copyright © 1993 by Cell Press

The C. elegans Heterochronic Gene *lin-4* Encodes Small RNAs with Antisense Complementarity to *lin-14*

Rosalind C. Lee,*† Rhonda L. Feinbaum,*‡ and Victor Ambros†
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?? ↓ ??

News & Views | Published: 06 Ja

Deviants — or

Marvin Wickens & Kathy Takaya

Nature **367**, 17–18 (1994) | Do

Three main principles

1. Not protein coding (non-coding genes) around 22 nt in length
2. Regulate other genes by sequence complementarity
3. The target sites are usually within the 3'UTR region

imentally (see text).
anged to U, prevents
n-4 RNA have been
analysis of the RNA,
1, 2 and 8.)

17

They were emissaries

Conservation of the sequence and temporal expression of *let-7* heterochronic regulatory RNA

Amy E. Pasquinelli*†, Brenda J. Reinhart*†, Frank Slack‡, Mark Q. Martindale§, Mitzi I. Kuroda‖, Betsy Maller‡, David C. Hayward¶, Eldon E. Ball¶, Bernard Degnan#, Peter Müller☆, Jürg Spring☆, Ashok Srinivasan**, Mark Fishman**, John Finnerty††, Joseph Corbo‡‡, Michael Levine‡‡, Patrick Leahy§§, Eric Davidson§§ & Gary Ruvkun*

NATURE | VOL 408 | 2 NOVEMBER 2000 | www.nature.com

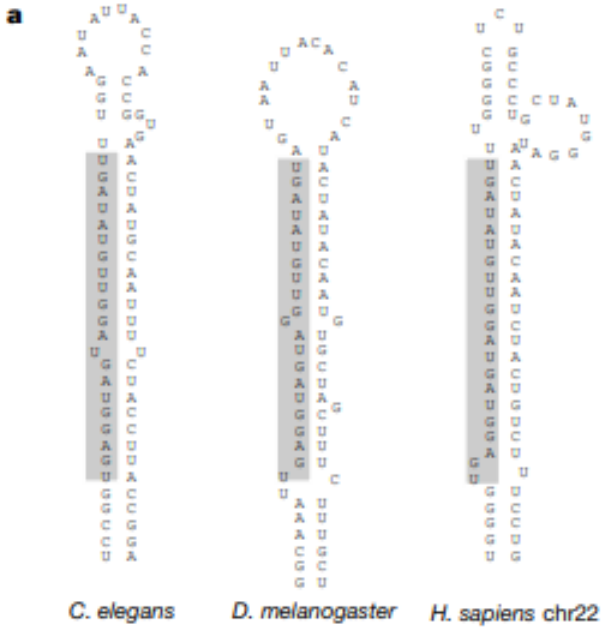

Figure 1 *let-7* gene sequences. **a**, Stem-loop structures of *C. elegans*, *D. melanogaster* and *Homo sapiens let-7* theoretical longer transcripts. The 21-nt *let-7* region is shaded. *let-7* genomic regions from *C. elegans* (Z70203), *D. melanogaster* (AE003659) and *H. sapiens* chromosome 22 (AL049853). The two human *let-7* homologous genes on chromosome 9 are tandemly arranged and separated by 369 base pairs; clustered with the human chromosome 22 *let-7* exact match is an 18/21 match to the *let-7* RNA. **b**, The 3′ UTRs of the *D. melanogaster* (AA3990768) and *Danio rerio lin-41* (AI794385) cDNAs contain *let-7* complementary sites.

- Let-7 is strongly conserved between *C. elegans*, *D. melanogaster* and **H. sapiens**
- Let-7 has complementary sites in 3’UTR

Early 2000 – the show begins

Current Biology
Volume 12, Issue 9, 30 April 2002, Pages 735-739

Brief communication

Identification of Tissue-Specific MicroRNAs from Mouse

Mariana Lagos-Quintana, Reinhard Rauhut, Abdullah Yalcin, Jutta Meyer, Winfried Lendeckel, Thomas Tuschl

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https://doi.org/10.1016/S0960-9822(02)00809-6

- microRNAs exist in most metazoan and plant species
- microRNAs are strongly conserved

Genes Dev. 2002 Jul 1;16(13):1616-26.

MicroRNAs in plants.

Reinhart BJ[1], Weinstein EG, Rhoades MW, Bartel B, Bartel DP.
Author information

Erratum in
Genes Dev 2002 Sep 1;16(17):2313.

Abstract
MicroRNAs (miRNAs) are an extensive class of ~22-nucleotide noncoding RNAs thought to regulate gene expression in metazoans. We find that miRNAs are also present in plants, indicating that this class of noncoding RNA arose early in eukaryotic evolution. In this paper 16 Arabidopsis miRNAs are described, many of which have differential expression patterns in development. Eight are absolutely conserved in the rice genome. The plant miRNA loci potentially encode stem-loop precursors similar to those processed by Dicer (a ribonuclease III) in animals. Mutation of an Arabidopsis Dicer homolog, CARPEL FACTORY, prevents the accumulation of miRNAs, showing that similar mechanisms direct miRNA processing in plants and animals. The previously described roles of CARPEL FACTORY in the development of Arabidopsis embryos, leaves, and floral meristems suggest that the miRNAs could play regulatory roles in the development of plants as well as animals.

REPORT

Identification of Novel Genes Coding for Small Expressed RNAs

Mariana Lagos-Quintana, Reinhard Rauhut, Winfried Lendeckel, Thomas Tuschl*
+ See all authors and affiliations

Science 26 Oct 2001:
Vol. 294, Issue 5543, pp. 853-858
DOI: 10.1126/science.1064921

REPORT

An Abundant Class of Tiny RNAs with Probable Regulatory Roles in *Caenorhabditis elegans*

Nelson C. Lau, Lee P. Lim, Earl G. Weinstein, David P. Bartel*
+ See all authors and affiliations

Science 26 Oct 2001:
Vol. 294, Issue 5543, pp. 858-862
DOI: 10.1126/science.1065062

REPORT

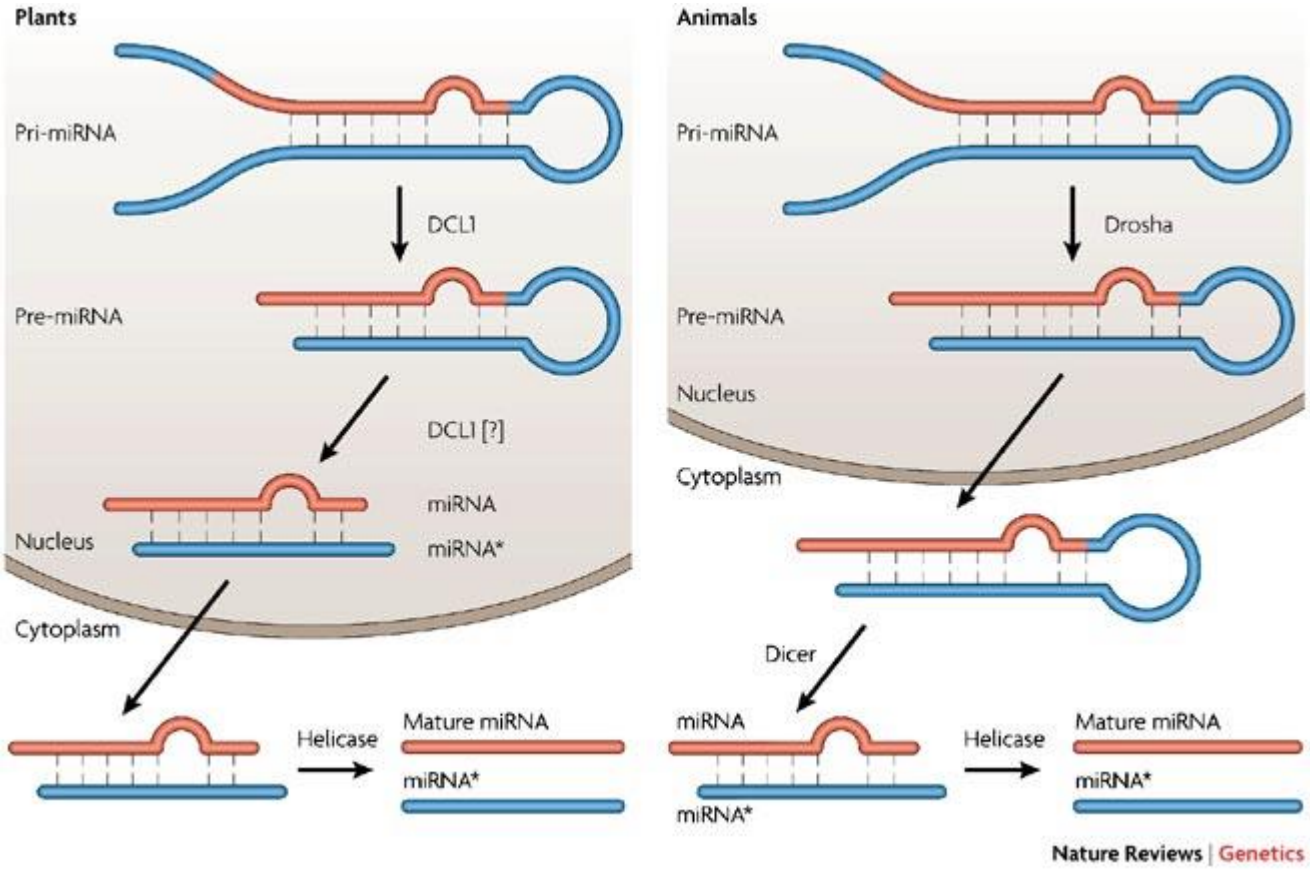
An Extensive Class of Small RNAs in *Caenorhabditis elegans*

Rosalind C. Lee, Victor Ambros*
+ See all authors and affiliations

Science 26 Oct 2001:
Vol. 294, Issue 5543, pp. 862-864
DOI: 10.1126/science.1065329

Comparison between plant and animal microRNAs

- In plants, maturation finishes in the nucleus
- In animals, two different enzymes Drosha and Dicer while in plants only one enzyme cleaves the pri-miRNA/pre-miRNA
- HUA Enhancer (HEN) methylates the mature microRNAs at the 2'-hydroxy termini of both strands



FROM THE FOLLOWING ARTICLE:
The evolution of gene regulation by transcription factors and microRNAs
Kevin Chen & Nikolaus Rajewsky
Nature Reviews Genetics **8**, 93-103 (February 2007)
doi:10.1038/nrg1990

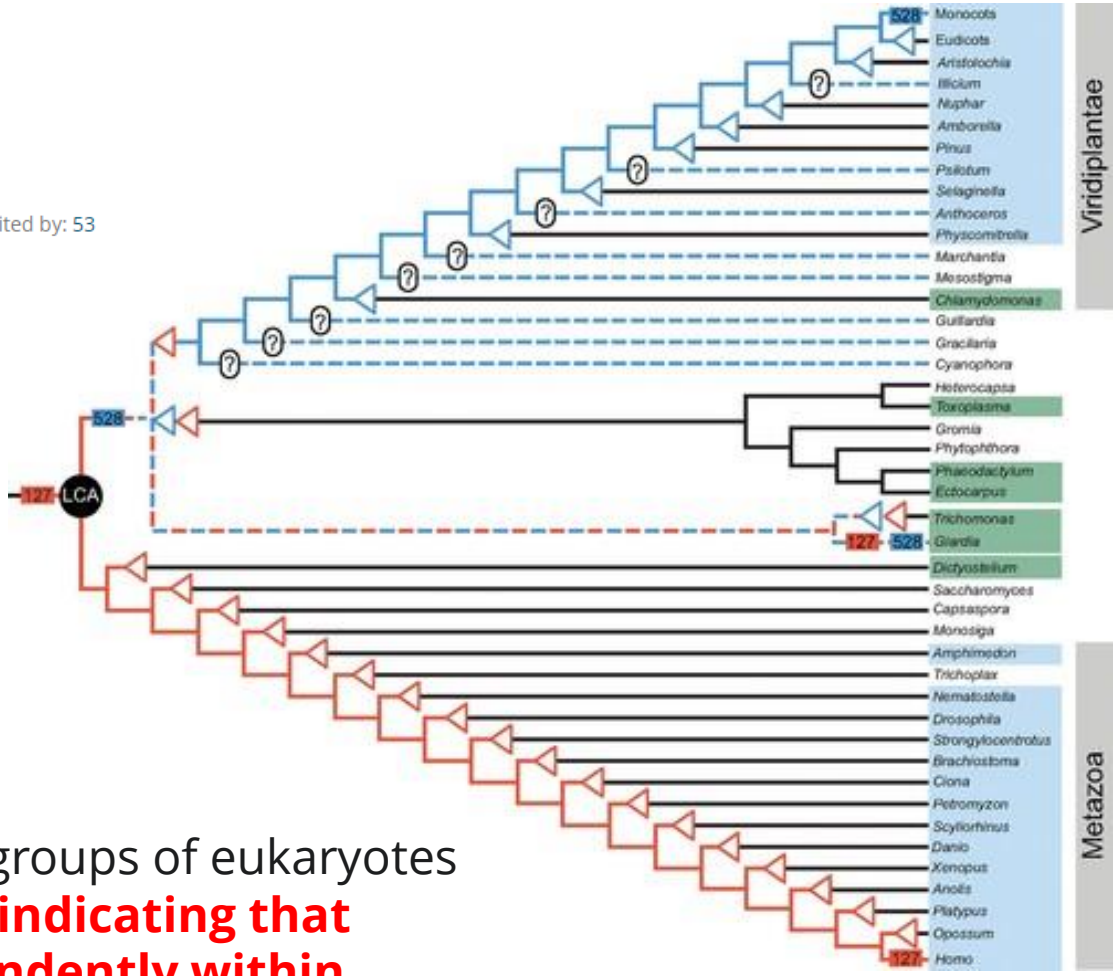
Was the wheel invented twice?

Insights & Perspectives | Free Access

Do miRNAs have a deep evolutionary history?

James E. Tarver, Philip C. J. Donoghue, Kevin J. Peterson

First published: 30 July 2012 | https://doi.org/10.1002/bies.201200055 | Cited by: 53

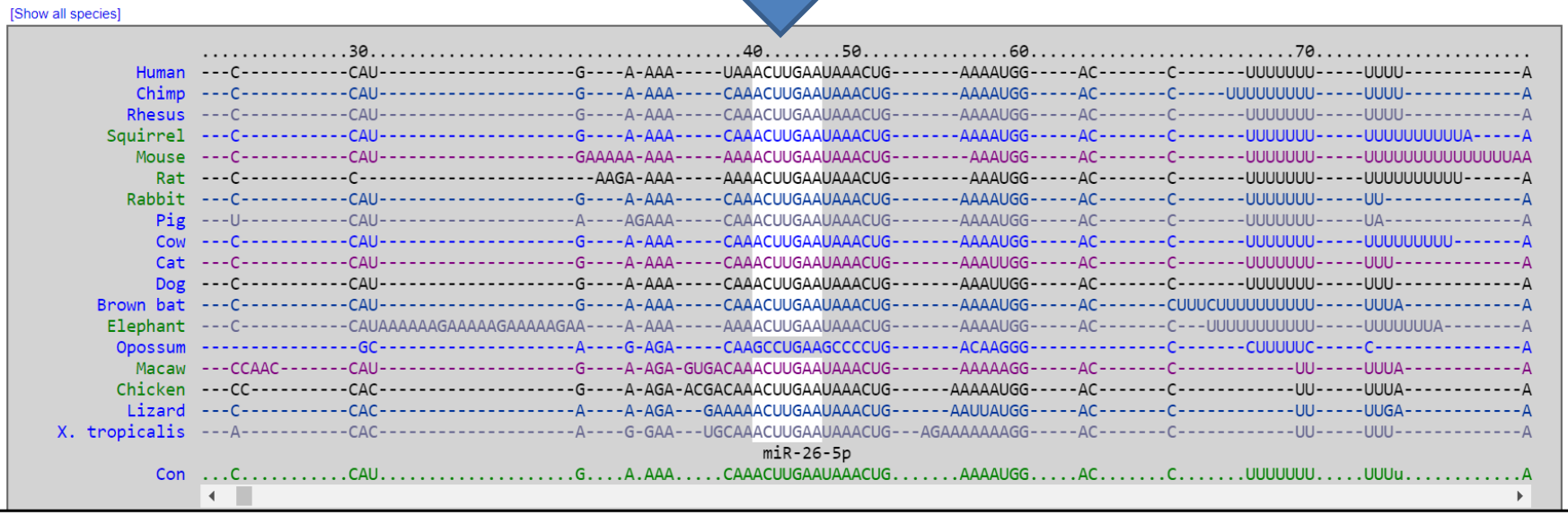


Therefore, at present only five groups of eukaryotes are known to possess miRNAs, **indicating that miRNAs have evolved independently within eukaryotes** through exaptation of their shared inherited RNAi machinery.

Conservation and function

- In total, >45,000 miRNA target sites within human 3′UTRs are conserved above background levels

Conservation of the target site that is complementary to the 5’ end of the microRNA



Conservation and function

- In total, >45,000 miRNA target sites within human 3′UTRs are conserved above background levels
- >60% of human protein-coding genes have been under selective pressure to maintain pairing to miRNAs
- Mammal specific microRNAs have less conserved target sites that microRNAs that have arisen before

➢ Conservation implies function at sequence level
➢ High number of target genes implies that most pathways might be influenced by microRNAs to some degree

Genome Res. 2009 Jan; 19(1): 92–105.
doi: 10.1101/gr.082701.108
PMCID: PMC2612969

Most mammalian mRNAs are conserved targets of microRNAs

Robin C. Friedman,[1,2,3] Kyle Kai-How Farh,[1,2,4] Christopher B. Burge,[1,5] and David P. Bartel[1,2,5]

Author information ► Article notes ► Copyright and License information ►

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2612969/

Reverse genetics: phenotypes produced by microRNAs

Review

Metazoan MicroRNAs

David P. Bartel [1, 2]

Show more

https://doi.org/10.1016/ Cell Volume 173, Issue 1, 22 March 2018, Pages 20-51

- **69 out of 88 miRNA knockout in flies show severe mutant phenotypes**
- **In mice, abnormal phenotypes were observed for 20 highly conserved (bilaterian ancestor) miRNA families**

Table 2. Abnormal Phenotypes Observed in Mice after Knocking Out One or More Members of a Broadly Conserved miRNA Family

miRNA family (# of genes)	miRNA(s) removed	Phenotype
miR-15/16/195/322/497 (7)	miR-15a~16-1	Increased proliferation of B cells; development of lymphoproliferative disorders (Klein et al., 2010); arrested maturation of natural killer cells (Sullivan et al., 2015); increased phagocytosis of macrophages; reduced mortality in bacterial sepsis models (Moon et al., 2014)
	miR-15b~16-2	Development of B cell lymphoproliferative disorders (Lovat et al., 2015)
miR-17/20/93/106 (6)	miR-17, miR-20a	Perinatal lethality, incomplete penetrance; vertebral homeotic transformations and other skeletal defects; reduced body weight; reduced pre-B cells (Han et al., 2015)
miR-18 (2)	miR-18a	Reduced body weight (Han et al., 2015)
miR-19 (3)	miR-19a, miR-19b-1	Perinatal lethality, incomplete penetrance; reduced body weight; reduced tumorigenesis in sensitized background (*Myc*) (Han et al., 2015)
miR-21 (1)	miR-21	Reduced growth and increased apoptosis of eosinophil progenitors (Lu et al., 2013b); altered macrophage polarization with more M2 macrophages and fewer M1 macrophages (Wang et al., 2015); increased bone mass with decreased bone resorption (Hu et al., 2017); altered pathology in >20 disease/injury models

"Indeed, loss-of-function studies disrupting miRNA genes in mice have revealed diverse phenotypes, including defects in the **development** 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, as well as **cellular, physiological, and behavioral defects**. Many of these developmental and physiological defects **affect embryonic or postnatal viability** or cause other severe conditions, such as **epilepsy, deafness, retinal degeneration, infertility, immune disorders, or cancer**. In addition, some miRNA- knockout strains have altered susceptibility to infections, and many have differential responses to mouse models of diseases or injuries."

microRNAs and function

- **Sequence conservation (both of the targets and the microRNA sequences)**
- **Many microRNAs produce phenotypes in knock-down studies**

microRNAs play important roles in many molecular functions and biological processes

How are these functions carried out, how can we classify or define them?

microRNAs and robustness of biological processes

Cell. Author manuscript; available in PMC 2013 Apr 27.
Published in final edited form as:
Cell. 2012 Apr 27; 149(3): 515–524.
doi: 10.1016/j.cell.2012.04.005

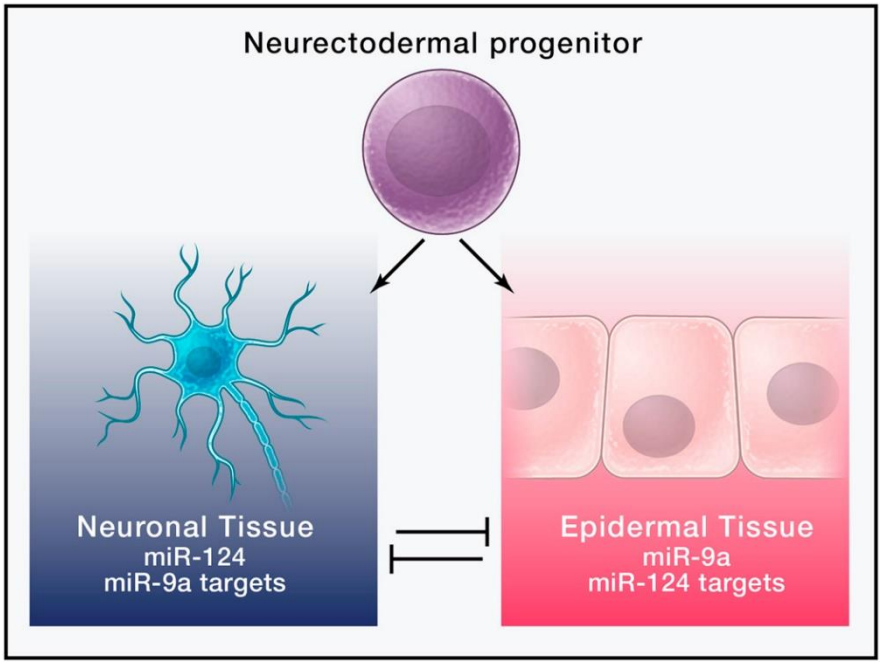
PMCID: PMC3351105
NIHMSID: NIHMS372011
PMID: 22541426

Roles for microRNAs in conferring robustness to biological processes

Margaret S. Ebert[1,2,3] and Phillip A. Sharp[1,2,*]

- Robustness refers to a system's ability to maintain its function in spite of internal or external perturbations
- miRNAs can act to reinforce the transcriptional gene expression program by repressing leaky transcripts.

First known functions of microRNAs:
"*sharpening developmental transitions by suppressing residual transcripts that were specific to the previous stage*"



Anticorrelated expression of miRNAs and targets in developmental transitions. In the *Drosophila* embryo, neurectodermal progenitors express miR-124 as they differentiate into neurons. Neuronal genes that are induced during this transition tend not to have miR-124 sites, whereas genes expressed in epidermal tissues that are also ectodermal derivatives are enriched for miR-124 sites (Stark et al., 2005). **Thus, expression of miR-124 stabilizes the neuronal transition.** A reciprocal pattern holds for the ectoderm-specific miR-9a.

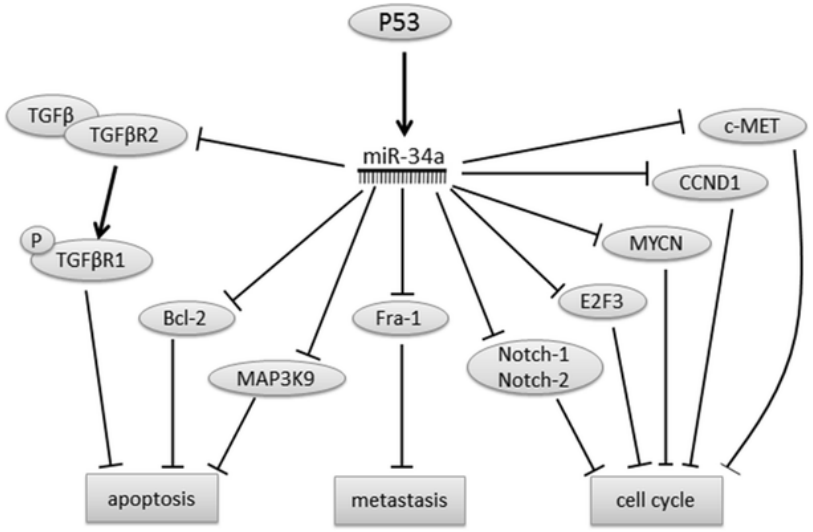
Target recognition

Tumor Biology
April 2015, Volume 36, Issue 4, pp 2481–2490 | Cite as

MicroRNA-34a inhibits the proliferation and promotes the apoptosis of non-small cell lung cancer H1299 cell line by targeting TGFβR2

Authors | Authors and affiliations

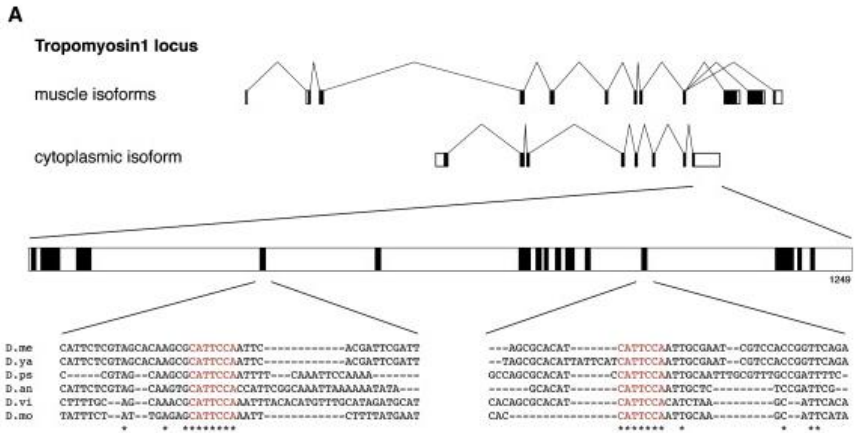
Zhong-Liang Ma, Pin-Pin Hou, Yan-Li Li, De-Tao Wang, Tian-Wei Yuan, Jia-Li Wei, Bo-Tao Zhao, Jia-Tao Lou, Xin-Tai Zhao, Yan Jin ✉, You-Xin Jin ✉



https://www.cell.com/cell/fulltext/S0092-8674(05)01272-9

Animal MicroRNAs Confer Robustness to Gene Expression and Have a Significant Impact on 3′UTR Evolution

Alexander Stark,[1,2,3] Julius Brennecke,[1,2] Natascha Bushati,[1] Robert B. Russell,[1] and Stephen M. Cohen[1,*]
[1]European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany
[2]These authors contributed equally to this work.
[3]Present address: Broad Institute of MIT and Harvard, Cambridge, MA 02141, USA and Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
*Contact: cohen@embl.de
DOI 10.1016/j.cell.2005.11.023

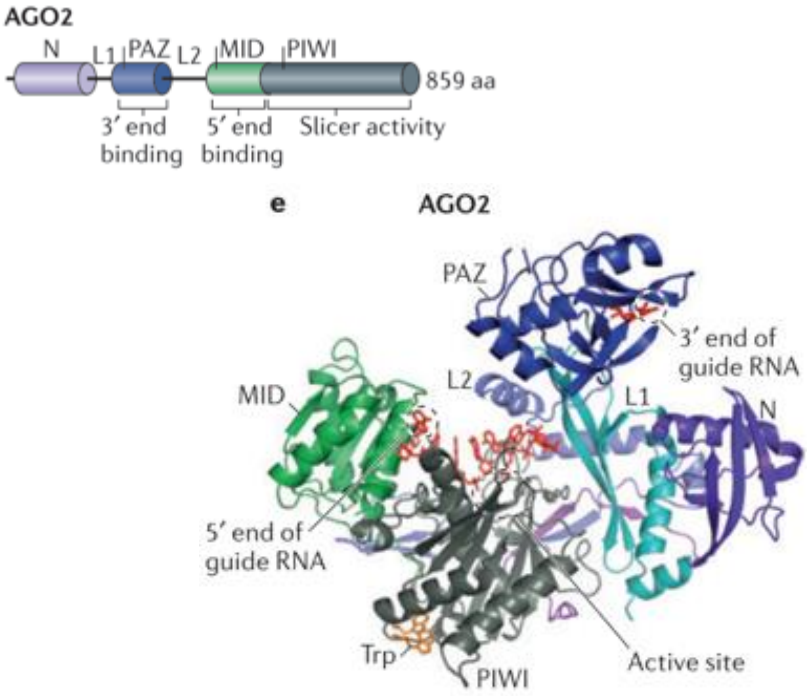


Conferring robustness to gene expression

‘Fine-tuning’ gene expression in complex networks

Both ‘ways’ imply negative regulation of gene expression through sequence complementarity

How are the targets recognized?



- AGO are key proteins
- AGO2 has catalytic activity
- 5′ base of the guide RNA with a preference for U or A binding
- AGO2-deficient mice are embryonic lethal

Review Article | Published: 16 July 2014

Regulation of microRNA biogenesis

Minju Ha & V. Narry Kim

Nature Reviews Molecular Cell Biology **15**, 509–524 (2014) | Download Citation

Metazoan MicroRNAs

David P. Bartel[1,2,*]
[1]Howard Hughes Medical Institute and Whitehead Institute for Biomedical Research, Cambridge, MA 02142, USA
[2]Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
*Correspondence: dbartel@wi.mit.edu
https://doi.org/10.1016/j.cell.2018.03.006

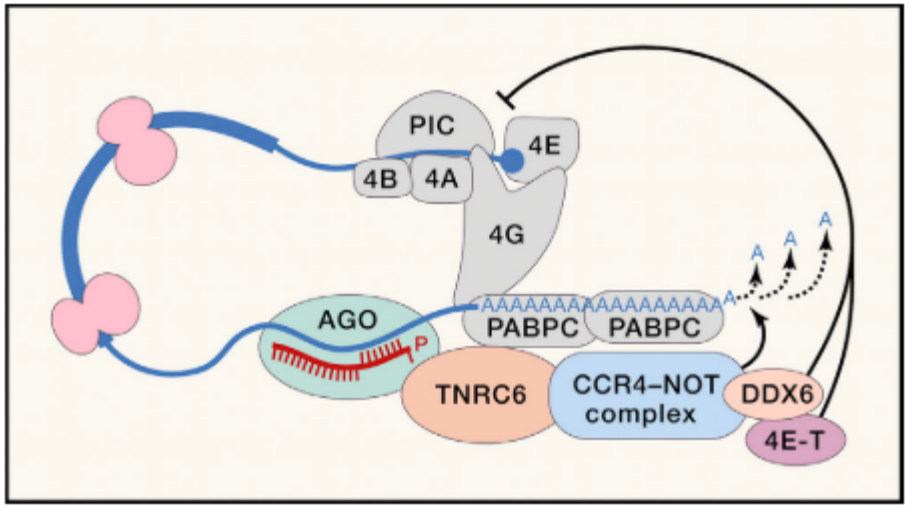


Figure 4. The Dominant Mechanisms of miRNA-Guided Repression in Bilaterian Animals

Guided by the miRNA, the silencing complex associates with the mRNA and recruits TNRC6, which interacts with PABPC and recruits either the PAN2–PAN3 deadenylase complex (not shown) or the CCR4–NOT deadenylase complex, either of which shortens the mRNA poly(A) tail. Alternative downstream consequences of poly(A)-tail shortening, which are not depicted in this figure, consummate this major mode of TNRC6-mediated repression; in early embryos, tail shortening reduces translation initiation with little effect on mRNA stability, whereas in most other developmental contexts, tail shortening hastens decapping and degradation of the mRNA with relatively little effect on translation initiation. Although not through tail shortening, recruitment of TNRC6 can nonetheless repress translation initiation in post-embryonic cells through a parallel mechanism that involves CCR4–NOT-mediated recruitment of DDX6 and 4E-T. This translation initiation normally involves the recruitment of the 43S preinitiation complex (PIC) through the action of initiation factors (4A, 4B, 4E, 4G).

How does the target site look like?

Review Article | Published: 16 July 2014

Regulation of microRNA biogenesis

Minju Ha & V. Narry Kim

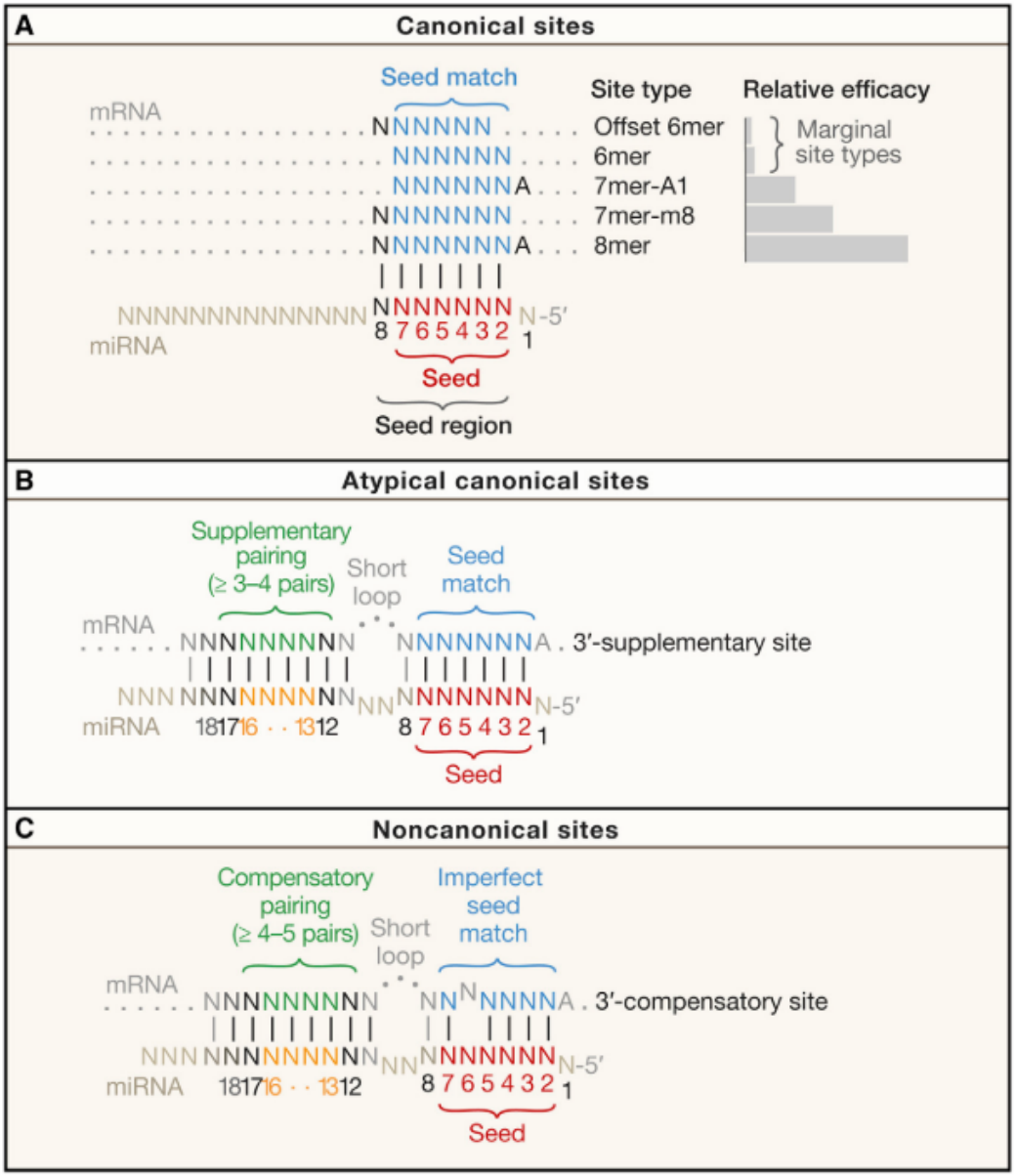
Nature Reviews Molecular Cell Biology **15**, 509–524 (2014) | Download Citation

Canonical sites

- Best studied feature is the frequent existence of a **seed match** region
- Perfect complementarity between microRNA and mRNA from position 2 to 8 of the microRNA

Non-canonical sites

- No seed match
- High compensatory pairing



How can we detect or predict target genes?

Thermodynamic models: Gibbs free energy

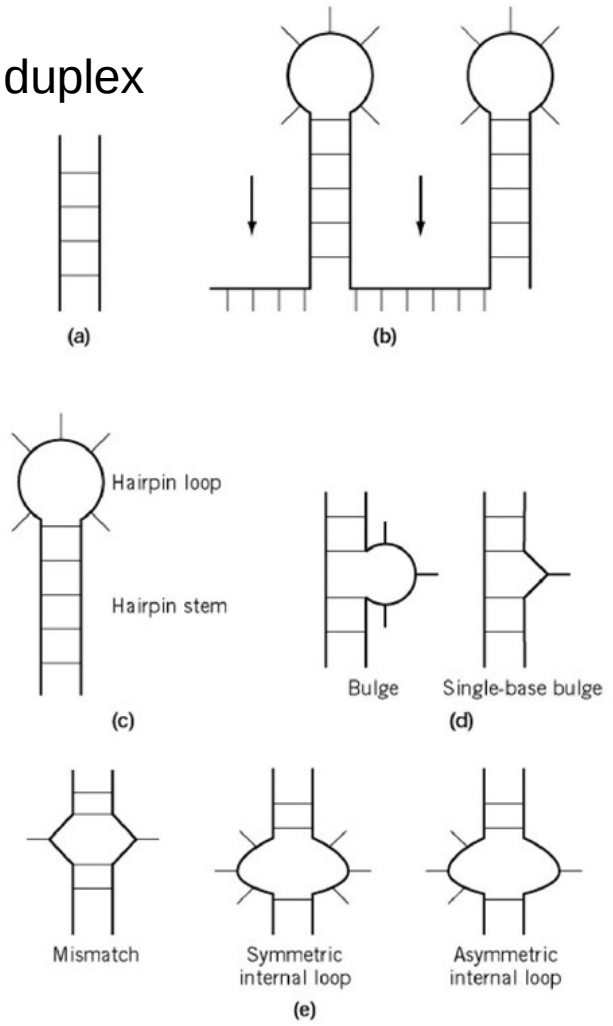
$$\Delta G = \Delta H - T\Delta S$$

Apply computer programs like RNAhybrid to RNA sequences

```
dataset: 1
Target: NM_001198993
length: 1672
MiRNA: hsa-miR-125a-5p
length: 24

mfe: -23.6 kcal/mol
p-value: 1.000000e+00

Position: 1420
target 5'   U    AG      ACCAGCGCCCAGC      C 3'
             UAGG  AGAGGG                GUUAGGG
             GUCC  UUUCCC                UAGUCCC
miRNA  3' AGU    AA      A                    A 5'
```

Seed match from positions 2-8)

Typical secondary structures of RNA molecules



How can we detect or predict target genes?

```
dataset: 1
Target: NM_001198993
length: 1672
MiRNA: hsa-miR-125a-5p
length: 24

mfe: -27.6 kcal/mol
p-value: 1.000000e+00

Position: 1070
target 5' G       GUGUG     CCGUGGUCGGACCCU       CU    U 3'
           CACAGGU      GAGG                 GGUGUC  GGU
           GUGUCCA      UUUC                 CCAUAG  CCA
miRNA  3' A       A                                UC     5'
```

Energetically preferable structures

```
dataset: 1
Target: NM_001198993
length: 1672
MiRNA: hsa-miR-125a-5p
length: 24

mfe: -27.5 kcal/mol
p-value: 1.000000e+00

Position: 868
target 5' G       AGAU       UUC      C 3'
           UC CGGG     GAGGGU    CGGGG
           AG GUCC     UUCCCA    GUCCC
miRNA  3'    U     AAU        UA       A 5'
```

Problems with target predictions:

- Functional target site might not be the one with lowest free energy
- Not all targets have a seed
- Seed complementarity can be due to chance alone
- Many possible combinations between hundreds of microRNAs and thousands of transcripts

→ **Very high number of false positives**

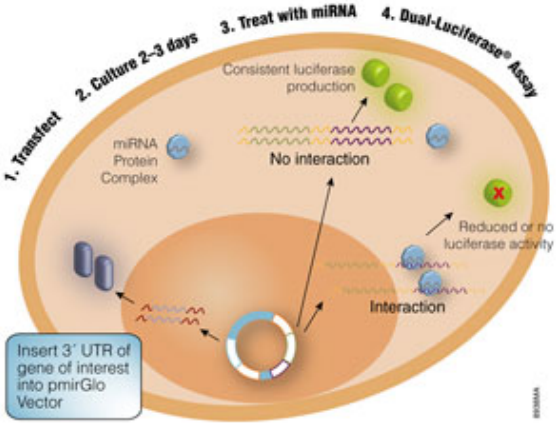
Structure with seed

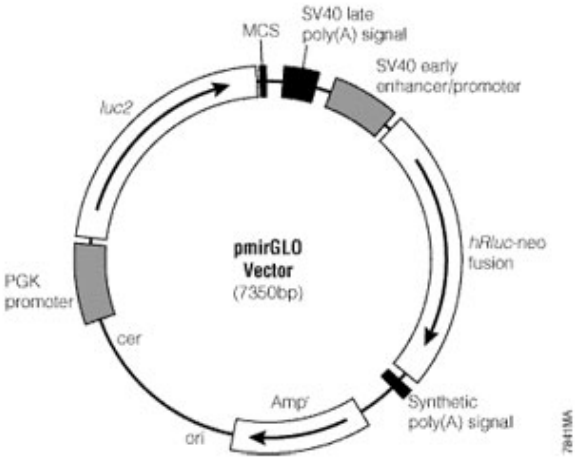
```
dataset: 1
Target: NM_001198993
length: 1672
MiRNA: hsa-miR-125a-5p
length: 24

mfe: -22.2 kcal/mol
p-value: 1.000000e+00

Position: 815
target 5' A   UGUCCUC     ACC        G 3'
           CGC        AGGG    AUUAGGG
           GUG        UCCC    UAGUCCC
miRNA  3' A   UCCAAUU     A          A 5'
```

Golden standard: reporter gene assays

Interaction between one microRNA and one target sequence (3'UTR) can be measured directly

- MicroRNAs (miRNAs) are short RNAs that interact with targets in the 3' UTR of transcripts and result in either mRNA degradation or inhibition of translation.
- Observing miRNA-mediated effects requires a reporter under the control of a weaker promoter so that subtle changes in gene expression can be observed.
- The pmirGLO Dual-Luciferase miRNA Target Expression Vector contains a 3' UTR cloned downstream of the *luc2* firefly luciferase gene under the control of the human phosphoglycerate kinase (PGK) promoter.
  - The PGK is a nonviral universal promoter; can be expressed in yeast, rat, mouse and human cells.
  - The pmirGLO Vector can be used to create Neomycin-resistant stable cell lines.
  - Either the Dual-Luciferase® Reporter Assay System or the Dual-Glo® Luciferase Assay can be used to obtain data.
- The psiCHECK™-2 Vector, designed for target knockdown experiments, has been used to analyze miRNA targets.
  - 3' UTR of interest is cloned into the MCS of psiCHECK™-2 Vector.
  - *Renilla* luciferase under control of the strong SV40 promoter is the reporter.
  - Strong promoter may mask subtle changes in gene expression.



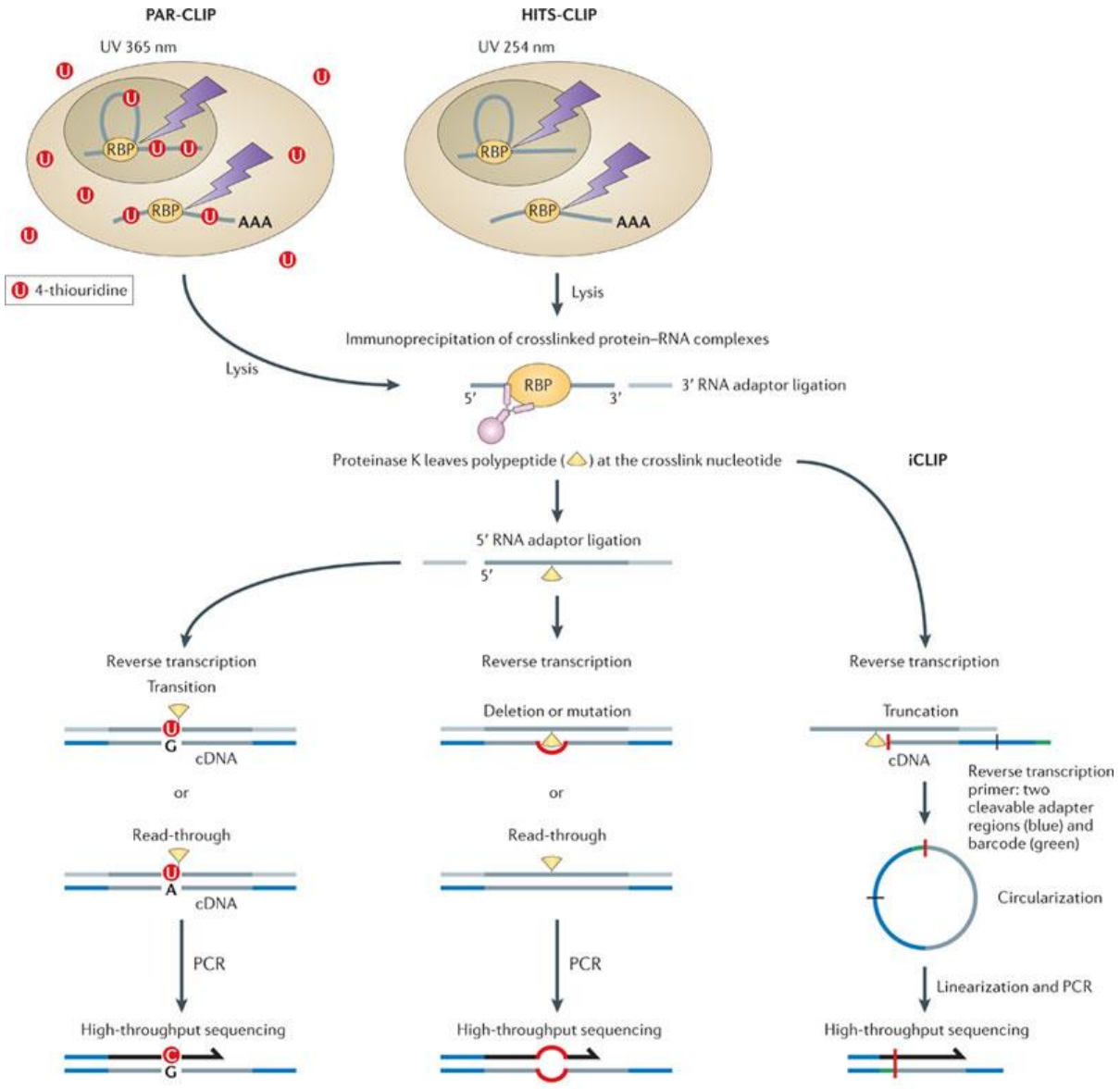


The pmirGLO Vector (Cat. # E1330) has a multiple cloning site downstream of the *luc2* stop codon for insertion of the 3'UTR of interest.

http://www.promega.es/products/pm/applications-of-reporter-gene-assays/analyzing-mirna-targets/

RIP-sequencing

- Cross-linking of RNA y proteins
- Immunoprecipitation for the protein of interest (such as AGO)
- Purify RNA
- Generation of cDNA library
- NGS sequencing and bioinformatics analysis



Progress

Nature Reviews Genetics **13**, 77-83 (February 2012) | doi:10.1038/nrg3141
Corrected online: 31 January 2012

There is an Erratum (1 March 2012) associated with this article.

ARTICLE SERIES: Applications of next-generation sequencing

Protein–RNA interactions: new genomic technologies and perspectives

Julian König[1], Kathi Zarnack[2], Nicholas M. Luscombe[1,2,3] & Jernej Ule[1] About the authors

TarBase

Nucleic Acids Res. 2018 Jan 4; 46(Database issue): D239–D245.
Published online 2017 Nov 16. doi: 10.1093/nar/gkx1141
PMCID: PMC5753203
PMID: 29156006

DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA–gene interactions

Dimitra Karagkouni,[✉] Maria D Paraskevopoulou, Serafeim Chatzopoulos, Ioannis S Vlachos, Spyros Tastsoglou, Ilias Kanellos, Dimitris Papadimitriou, Ioannis Kavakiotis, Sofia Maniou, Giorgos Skoufos, Thanasis Vergoulis, Theodore Dalamagas,[✉] and Artemis G Hatzigeorgiou[✉]

TarBase v.8

Please cite:
Dimitra Karagkouni, Maria D. Paraskevopoulou, Serafeim Chatzopoulos, Ioannis S. Vlachos, Spyros Tastsoglou, Ilias Kanellos, Dimitris Papadimitriou, Ioannis Kavakiotis, Sofia Maniou, Giorgos Skoufos, Thanasis Vergoulis, Theodore Dalamagas, Artemis G. Hatzigeorgiou; DIANA-TarBase v8: a decade-long collection of experimentally supported miRNA–gene interactions, Nucleic Acids Research, gkx1141, https://doi.org/10.1093/nar/gkx1141

Bulk download:
We have updated the bulk download module, which simplifies the download process! Please find the new online form by following this **link**

miRNAs

Genes

Total Entries: 1080276 Interactions: 665843 Cell Types: 516 Tissues: 85
Publications: 1208 Low-yield Methods: 15 High-throughput Methods: 19

Strength and scope of repression

Article | Published: 30 July 2008

Widespread changes in protein synthesis induced by microRNAs

Matthias Selbach ✉, Björn Schwanhäusser, Nadine Thierfelder, Zhuo Fang, Raya Khanin & Nikolaus Rajewsky ✉

Nature **455**, 58–63 (04 September 2008) | Download Citation ⭳

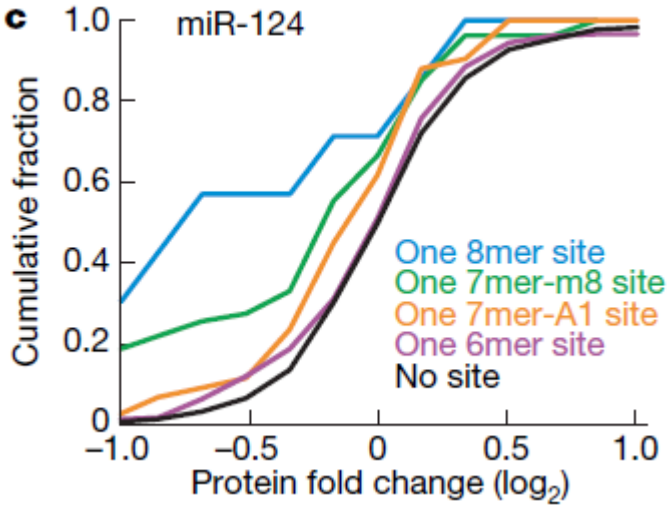
Article | Published: 30 July 2008

The impact of microRNAs on protein output

Daehyun Baek, Judit Villén, Chanseok Shin, Fernando D. Camargo, Steven P. Gygi ✉ & David P. Bartel ✉

Nature **455**, 64–71 (04 September 2008) | Download Citation ⭳

- Hundreds of genes are regulated but normally only to modest degrees
- Targeting is primary through seed matches
- Translationally repressed by more than a third also displayed detectable mRNA destabilization
- For higher repressed targets, mRNA destabilization usually comprised the major component of repression



- Most microRNA/mRNA interactions to fine-tune the protein levels

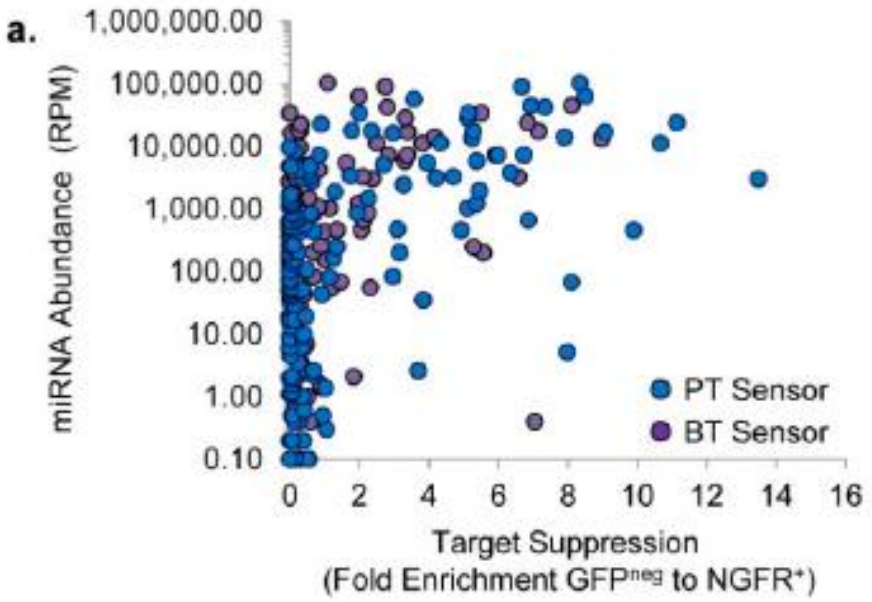
Strength and scope of repression

- Only the most abundant microRNAs within a cell mediate significant target suppression
- Over 60% of detected microRNAs had no discernible activity, indicating that the functional ‘miRNome’ of a cell is considerably smaller than currently inferred from profiling studies

Published in final edited form as:
Nat Methods. ; 9(8): 840–846. doi:10.1038/nmeth.2078.

High-throughput assessment of microRNA activity and function using microRNA sensor and decoy libraries

Gavriel Mullokandov[1,4], **Alessia Baccarini**[1,4], **Albert Ruzo**[1,4], **Anitha D. Jayaprakash**[1], **Navpreet Tung**[1], **Benjamin Israelow**[2], **Matthew J. Evans**[2], **Ravi Sachidanandam**[1], and **Brian D. Brown**[1,3]

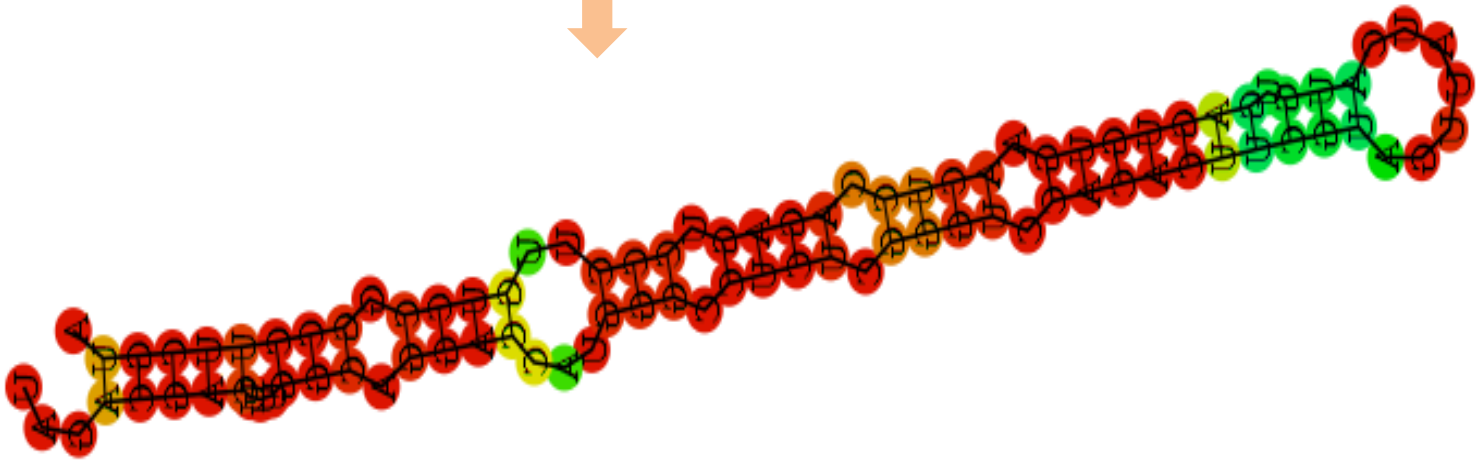


How can we detect or predict microRNA genes?

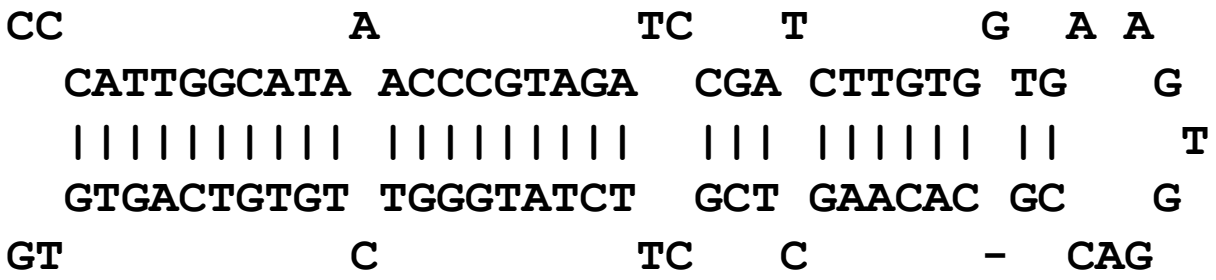
>cel-lin-4 MI0000002
AUGCUUCCGGCCUGUUCCCUGAGACCUCAAGUGUGAGUGUACUAUUGA
UGCUUCACACCUGGGCUCUCCGGGUACCAGGACGGUUUGAGCAGAU

RNAfold

RNAfold WebServer



Detecting new microRNA genes: pre-NGS



Prediction based in compositional and structural features

- Minimum Free Energy
- Number of bindings
- Loop Length
- Number of bulges, bulbs
- Compositional features (G+C content, dinucleotide frequencies)

In the human genome exist approx. 11 million hairpin structures
→ High rate of false positive predictions

microRNAs and robustness of biological processes

Output of a region of 100kb with default parameters → 42 novel miRNA genes

CID miRNA
(Computational Identification of micro RNA)

An miRNA scanning Tool by:
Center for Computational Biology and Bioinformatics
Jawaharlal Nehru University
New Delhi 110067
E-mails: alok0200@mail.jnu.ac.in, st0645@students.jnu.ac.in
Telephone: +91-11-26717630

CIDmiRNA scan is through.

Total precursor miRNAs found: **42**.

FASTA format (sequences only) FASTA format (structures only) FASTA format (both sequences and structures)

S.No.	Position	Length	Normalised Grammar Score	Structural Score
1	1919	76	-0.607686	25
	GAGAATGCAAGGCATTTGGGAGCTGTGTAGCCAGAATCTGGACTTTTAATAGGTCACCAAATGCTCTTGCTTCCTC			
	aau - - g gu-- - g gag gcaag gcauuugg ga cugu ag cca a ||| |||| |||||||| || |||| || ||| a cuc cguuc cguaaacc cu gaua uc ggu u cuu u a g auuu a c			
2	3037	62	-0.569458	41
	GCATGCATGTGTGTGTGTGTGTGTGTGTGTGTGTGCATGCATGCATACATGTTGGGATGC			
	---- ug gu gcau gcaugugugugugugug u g |||| ||||||||||||||| | cgua uguacauacguacguacgu g u gggu gu ug			
3	3684	94	-0.603886	25
	GTTATTGACTTACATTTCCTGGAGAATAAGAGCACTATAGGCATGAAGACATTTTCTTTTCTCTTGTGCATTTTGCCCAGGAAAGAATAATAAC			
	ac aca a aau a uau cau c guuauug uu uuuccugg g aag gcac agg gaaga a ||||||| || ||||||| | ||| |||| ||| |||| u caauaau aa aaaggacc c uuu cgug ucu uuucu u -- g-- - gu- a u-- cu- u			
4	4953	62	-0.564125	53
	GTATATGCACACATGTGTGTGTATCTATGTGTATCTATGTGTATGCATGTGTGTGTGTGT			
	g ug uc u uauaugcacacaugugug ugua ua g |||||||||||||||| |||| || gugugugugguacguau guau au u u gu cu g			
5	6095	98	-0.580363	82
	GTGTATGCGTGTACTCATGTGTGTGTGTGCACACGCTTACGTGCACGTGTGTGTGTGCGTGTGTGTGTGTGTGTGTGTGTGTGTGTACAC			
	ug u ug ug ug u ug u guguaugcg uac caug ug ug cacacgc uacg cacg g ||||||||| ||| |||| || || ||||||| |||| |||| cacaugugu gug gugu gu gu gugugug gugc gugu u gu u gu gu gu u gu g			

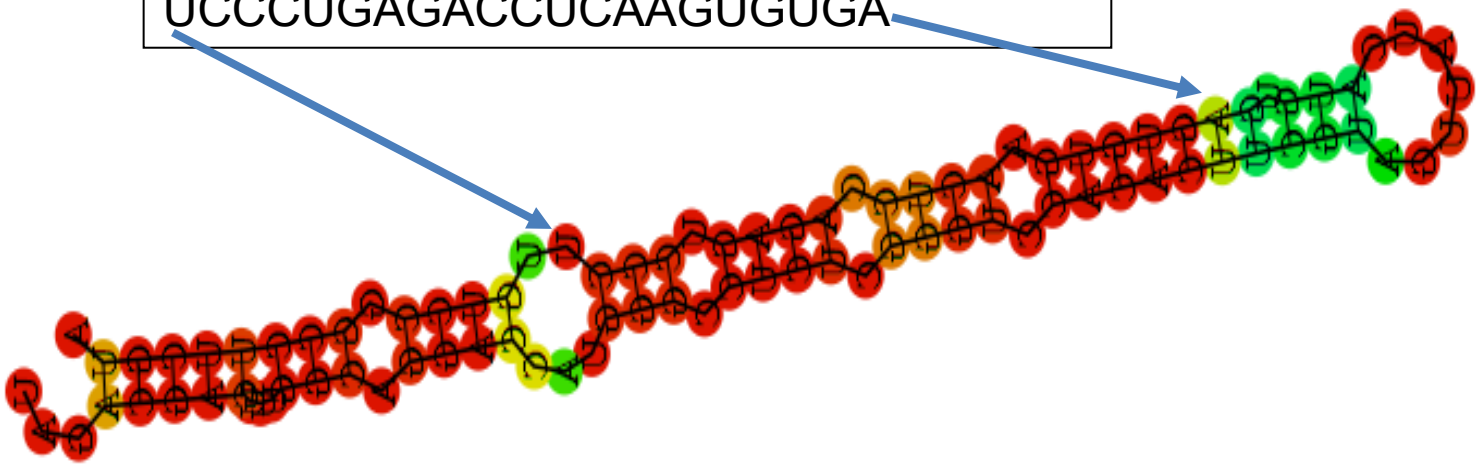
High number of false positive predictions → new methods are needed

How can we detect or predict microRNA genes?

>cel-lin-4 MI0000002
AUGCUUCCGGCCUGUUCCCUGAGACCUCAAGUGUGAGUGUACUAUUGA
UGCUUCACACCUGGGCUCUCCGGGUACCAGGACGGUUUGAGCAGAU

RNAfold

>cel-lin-4-5p MIMAT0000002
UCCCUGAGACCUCAAGUGUGA



Novel microRNAs through high-throughput sequencing

- Drosha/Dicer (DCL in plants) processing patterns can be detected
- Both mature microRNAs (both arms) are represented in the sample?
- 5' end of the mature microRNA shows less fluctuation
- Virtually all reads are organized in one or two clusters

miRBase: the widely used reference database

ABSTRACT

Go to:

The miRNA Registry provides a service for the assignment of miRNA gene names prior to publication. A comprehensive and searchable database of published miRNA sequences is accessible via a web interface (http://www.sanger.ac.uk/Software/Rfam/mirna/), and all sequence and annotation data are freely available for download. Release 2.0 of the database contains 506 miRNA entries from six organisms.

Nucleic Acids Res. 2004 Jan 1; 32(Database issue): D109–D111.
doi: 10.1093/nar/gkh023
PMCID: PMC308757
PMID: 14681370

The microRNA Registry

Sam Griffiths-Jones*

▸ Author information ▸ Article notes ▸ Copyright and License information Disclaimer

- Most used reference database
- During many years the only existing database

miRBase: from microRNA sequences to function

Ana Kozomara, Maria Birgaoanu, Sam Griffiths-Jones

Nucleic Acids Research, Volume 47, Issue D1, 8 January 2019, Pages D155–D162,
https://doi.org/10.1093/nar/gky1141
Published: 13 November 2018 **Article history** ▾

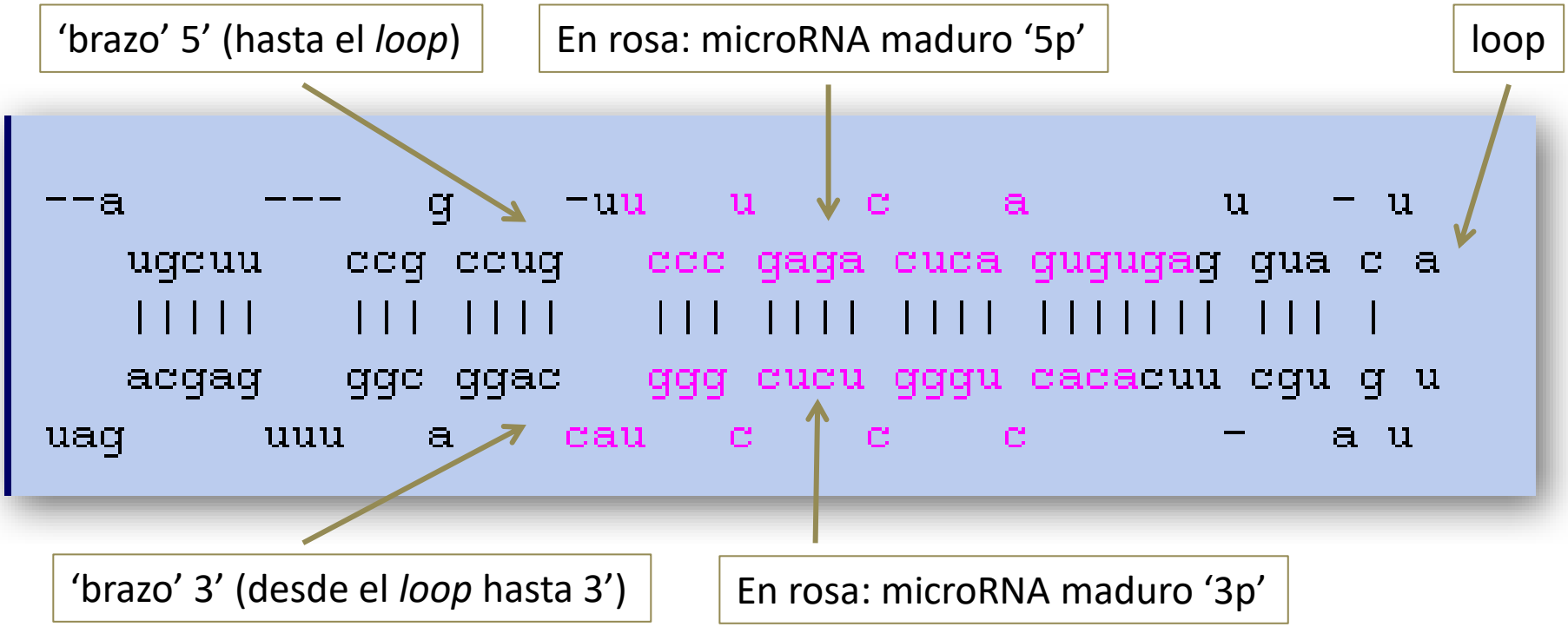
Abstract

miRBase catalogs, names and distributes microRNA gene sequences. The latest release of miRBase (v22) contains microRNA sequences from 271 organisms: 38 589 hairpin precursors and 48 860 mature microRNAs. We describe improvements to the database and website to provide more information about the quality of microRNA gene annotations, and the cellular functions of their products. We have collected 1493 small RNA deep sequencing datasets and mapped a total of 5.5 billion reads to microRNA sequences. The read mapping patterns provide strong support for the validity of between 20% and 65% of microRNA annotations in different well-studied animal genomes, and evidence for the removal of >200 sequences from the database. To improve the availability of microRNA functional information, we are disseminating Gene Ontology terms annotated against miRBase sequences. We have also used a text-mining approach to search for microRNA gene names in the full-text of open access articles. Over 500 000 sentences from 18 542 papers contain microRNA names. We score these sentences for functional information and link them with 12 519 microRNA entries. The sentences themselves, and word clouds built from them, provide effective summaries of the functional information about specific microRNAs. miRBase is publicly and freely available at http://mirbase.org/.

Estructura secundaria

El primer microRNA: lin-4

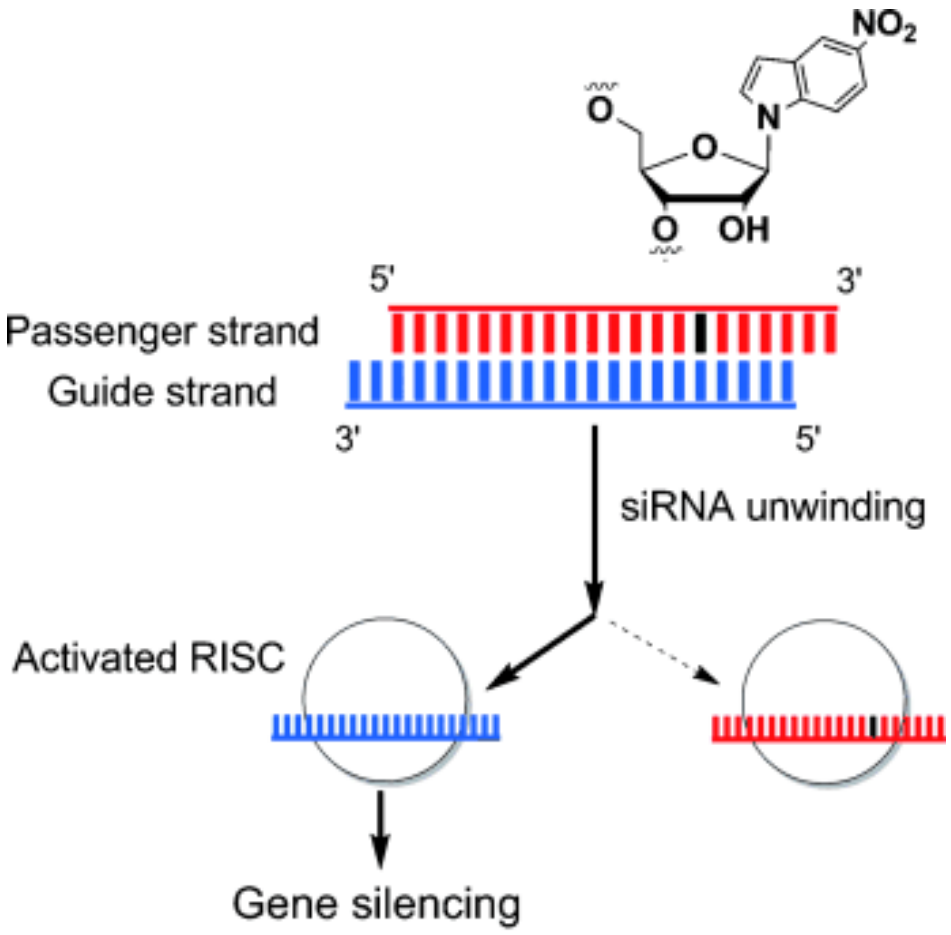
- El pre-microRNA tiene aproximadamente entre 70-100 nt
- La estructura secundaria del pre-microRNA está caracterizada por una 'horquilla'



Nomenclatura

Guide strand: el microRNA maduro funcional (la secuencia que se va a incorporar en RISC)

Passenger strand: la secuencia que normalmente se degrada



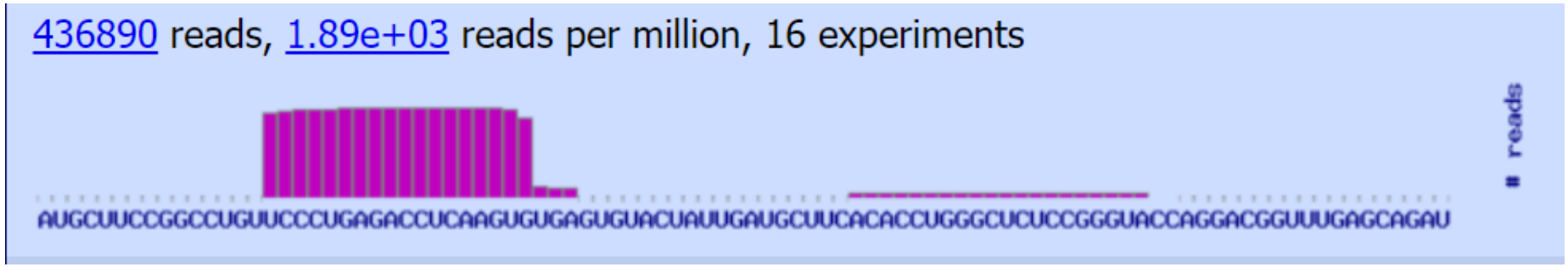
miRBase: nomenclatura

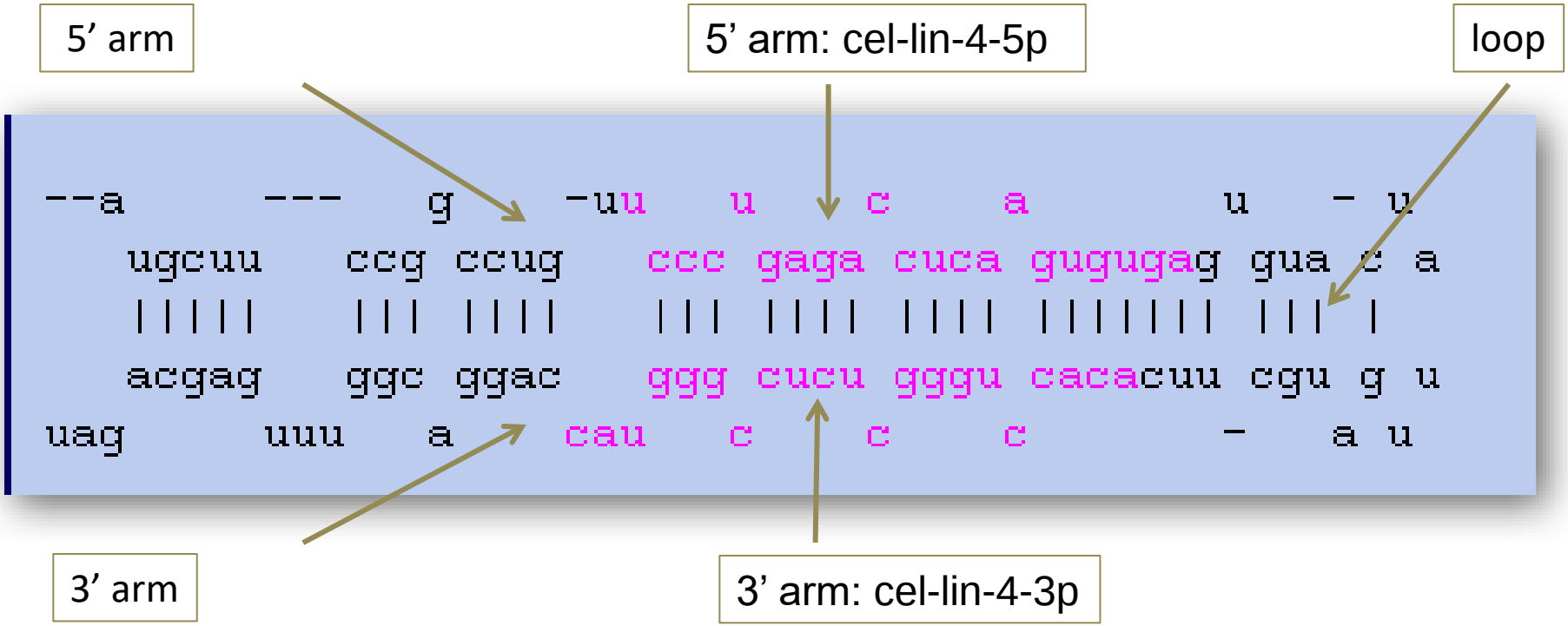
- Los microRNA en miRBase suelen tener nombres compuestos de 4 partes como: mmu-miR-375-5p.
  - Las primeras tres letras indican la especie. Por ejemplo, hsa para humano, mmu para ratón, rno para rata, etc.
  - Un nombre de microRNA en minúscula (hsa-**mir**-22) hace referencia al gen de microRNA o al pre-microRNA. Al microRNA maduro se refiere con '**miR**' (hsa-miR-22-5p)
  - El número que lleva el nombre del microRNA se asigna de forma secuencial.
  - 5p hace referencia al brazo (5p o 3p), 5' en este caso
  - ¡OJO! Antiguamente al microRNA maduro menos frecuente se asignaba un asterisco, por ejemplo hsa-miR-19 (microRNA predominante) y hsa-miR-19*

- Excepciones a estas reglas se mantienen por motivos históricos en las familias let-7 y lin-4
- Mas información acerca de la nomenclatura se puede encontrar en el siguiente articulo: Victor Ambros, Bonnie Bartel, David P. Bartel, Christopher B. Burge, James C. Carrington, Xuemei Chen, Gideon Dreyfuss, Sean R. Eddy, Sam Griffiths-Jones, Mhairi Marshall, Marjori Matzke, Gary Ruvkun, and Thomas Tuschl. **A uniform system for microRNA annotation.** *RNA* 2003 9(3):277-279.

Some nomenclature (miRBase)

cel-lin-4





The Metazoan microRNA compl

By courtesy of
Bastian Fromm