

Small RNAs: miRNA and siRNA

Ian M Clark, PhD
Cellular Protease Group,
Biomedical Research Centre,
University of East Anglia,
Norwich, NR4 7TJ.
United Kingdom.
(email: i.clark@uea.ac.uk)

Tamas Dalmay

Every saga has a beginning...

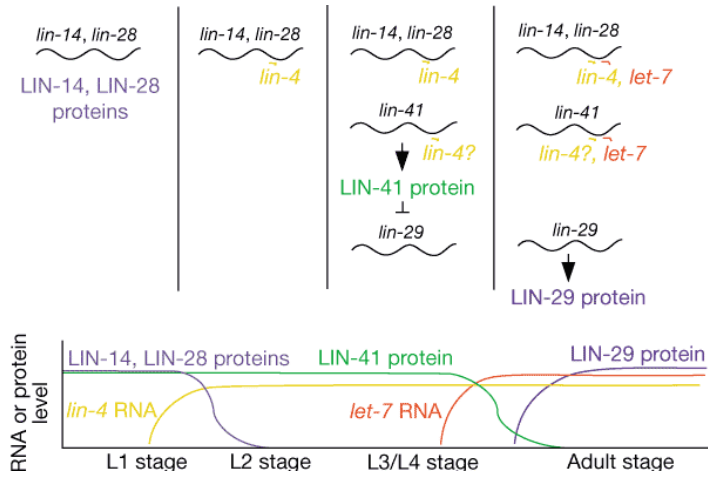
lin-4
was cloned
by genetic mapping
from *C.elegans* in 1993.

It regulates developmental timing
by blocking the expression of heterochronin
genes without reducing the level of the mRNAs.
lin-4 is not a protein-coding gene, but encodes
for

a 21 nucleotide RNA transcript

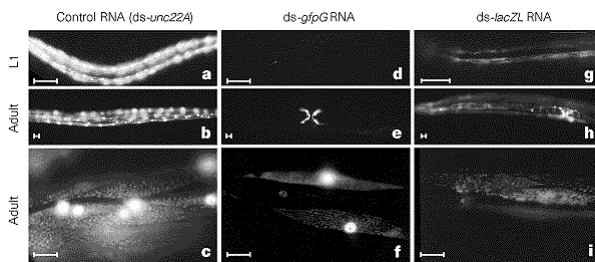
let-7, a similar gene, was cloned in 2000

lin-4 and let-7 downregulate the expression of heterochronin genes



from Reinhart et al
Nature, 2000

The discovery of 'gene silencing'



Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Nature. 1998 391(6669):806-11.



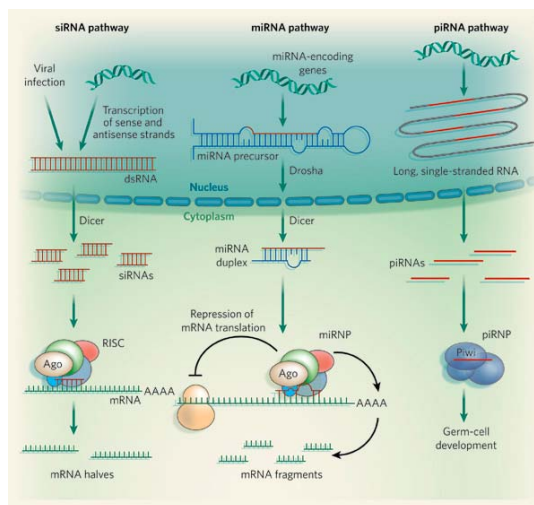
Grohans H & Filipowicz W
Nature (2008) 451, 414-416

FUNCT. PLANT BIOL.29, 1501-1506 (2002); PLANT J.13, 401-409 (1998); PLANT CELL2, 279-289 (1990)

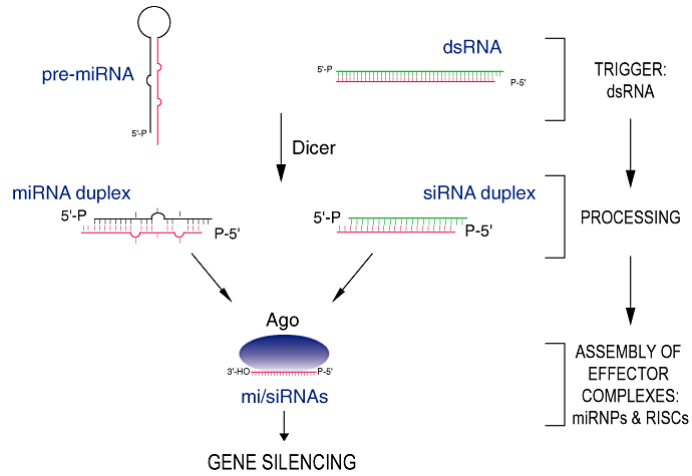
microRNAs

- **biogenesis**
- **identification / measurement**
- **target prediction**
- **target validation**
- **function**

Biogenesis and function of small RNAs

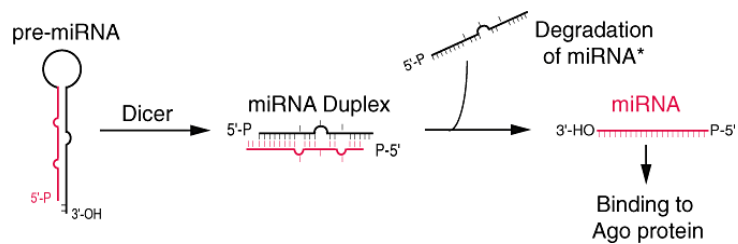


Biogenesis of microRNAs



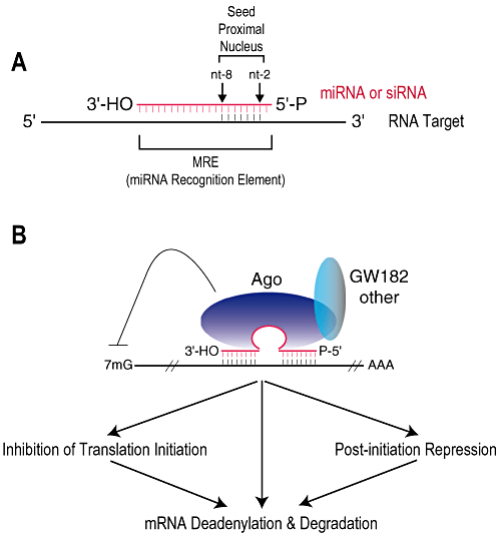
Liu X, Fortin K, Mourelatos Z. MicroRNAs: biogenesis and molecular functions. Brain Pathol. 2008 18(1):113-21.

Biogenesis of microRNAs



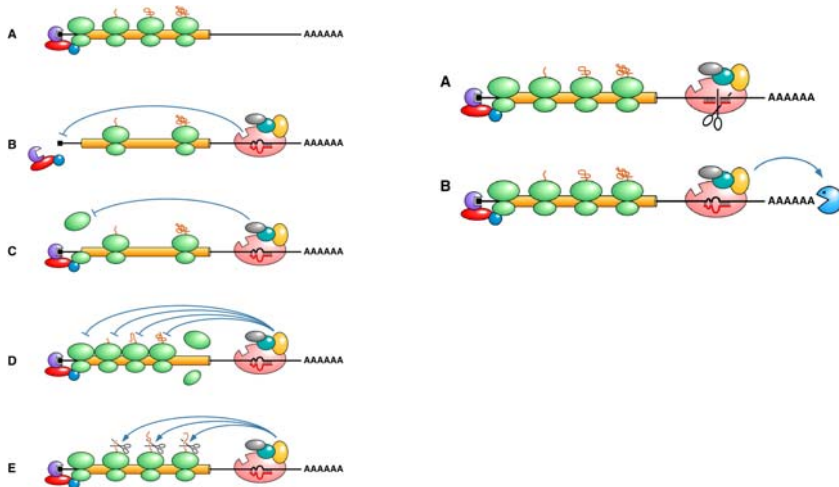
Liu X, Fortin K, Mourelatos Z. MicroRNAs: biogenesis and molecular functions. Brain Pathol. 2008 18(1):113-21.

Function of microRNAs



Liu X, Fortin K, Mourelatos Z. MicroRNAs: biogenesis and molecular functions. Brain Pathol. 2008 18(1):113-21.

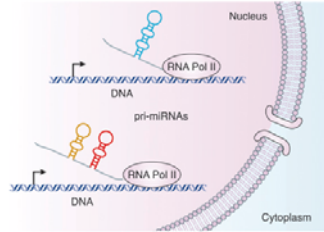
Function of microRNAs



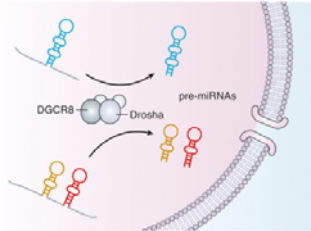
Mol Cell. 2008 29(1):1-7. Let me count the ways: mechanisms of gene regulation by miRNAs and siRNAs. Wu L, Belasco JG.

Biogenesis and function of microRNAs

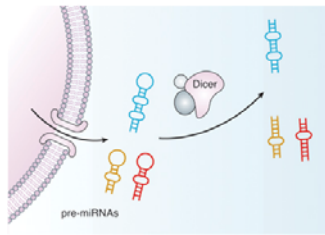
a Pri-miRNAs are transcribed, usually by RNA Polymerase II



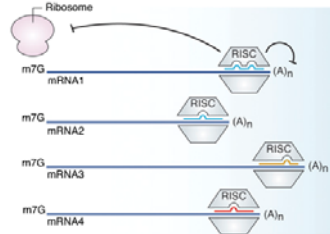
b The 'Microprocessor' complex cleaves the pri-miRNAs to form pre-miRNAs



c The Dicer enzyme complex removes the loop from the pre-miRNA hairpin



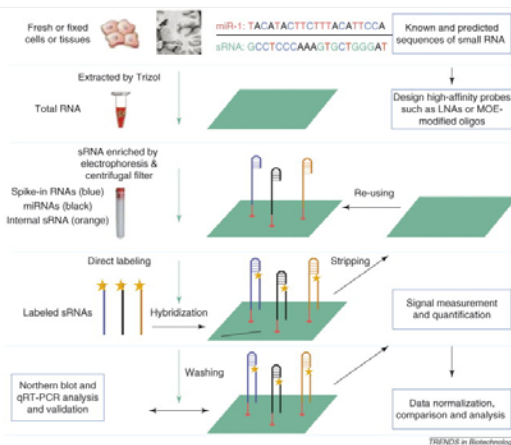
d One strand, the mature microRNA, is incorporated into the RISC protein complex, and guides translational silencing of the target mRNA



Boyd SD Lab Invest (2008) 88, 569-578

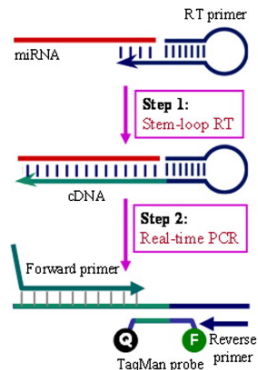
Measurement of microRNAs

Many solid-phase RNA purification methods do not capture small RNAs



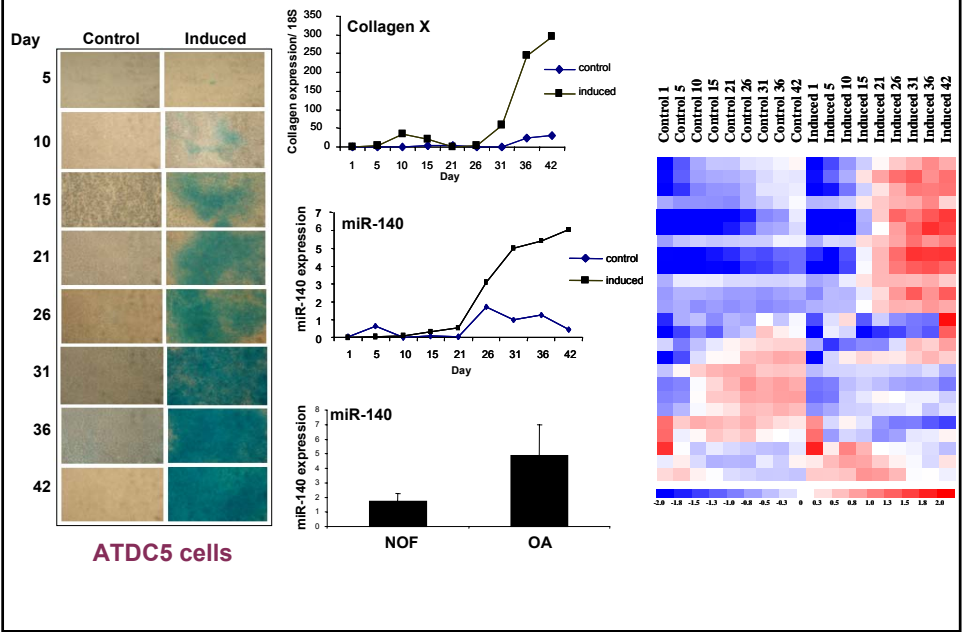
TRENDS in Biotechnology

Yin JQ, Zhao RC, Morris KV. Profiling microRNA expression with microarrays. Trends Biotechnol. 2008 26(2):70-6.

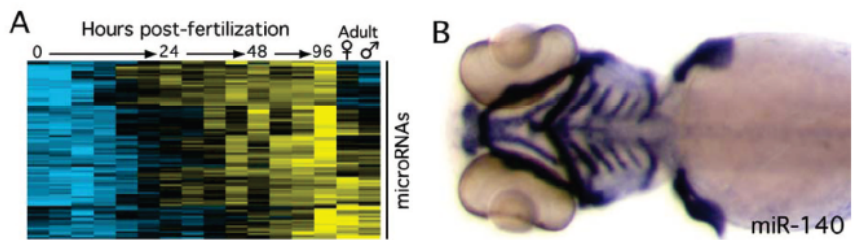


Schmittgen TD et al. Real-time PCR quantification of precursor and mature microRNA. Methods. 2008 44(1):31-8.

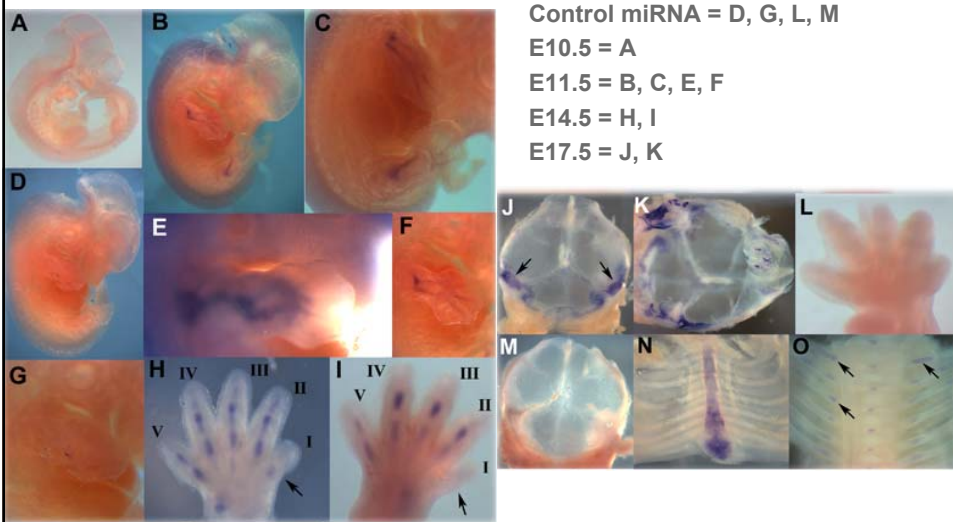
miRNAs and chondrogenesis



miRNA140 is expressed in developing Zebrafish cartilage

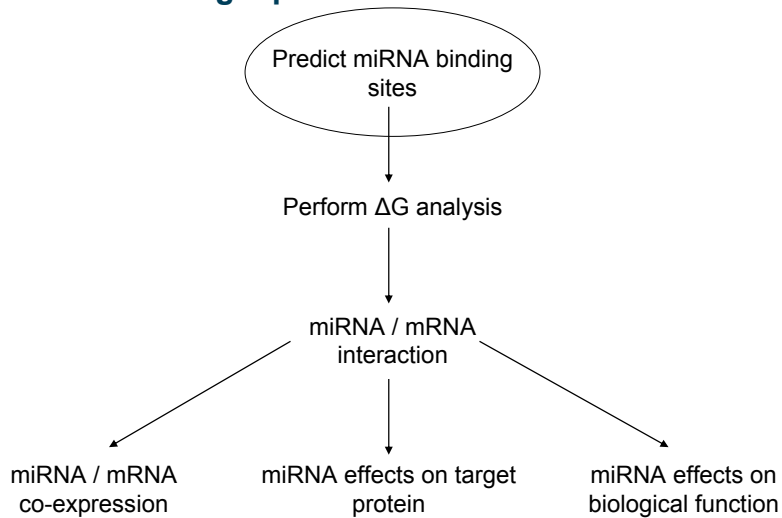


Expression of miR140 in mouse embryonic development In situ hybridisation



Tuddenham et al (2006) FEBS Lett 580:4214

Target prediction and validation



Kuhn DE, Martin MM, Feldman DS, Terry AV Jr, Nuovo GJ, Elton TS.
 Experimental validation of miRNA targets. *Methods*. 2008 44(1):47-54.

Target prediction

Bioinformatic algorithms

miRanda (<http://microrna.sanger.ac.uk>)

TargetScan (<http://www.targetscan.org>)

PicTar (<http://pictar.bio.nyu.edu>)

For miR-140

Max in single prediction: 958 genes

In any two predictions: 37 genes

In all three predictions: 5 genes

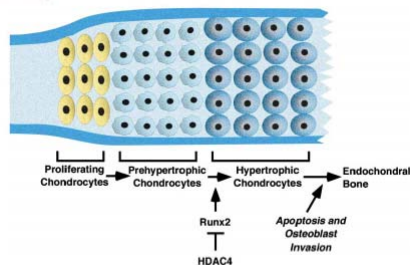
ΔG analysis of approx 70 nucleotides either side of predicted target site (Zhao et al. 2005,7)

e.g. mFold

HDAC4 controls chondrocyte hypertrophy during skeletogenesis

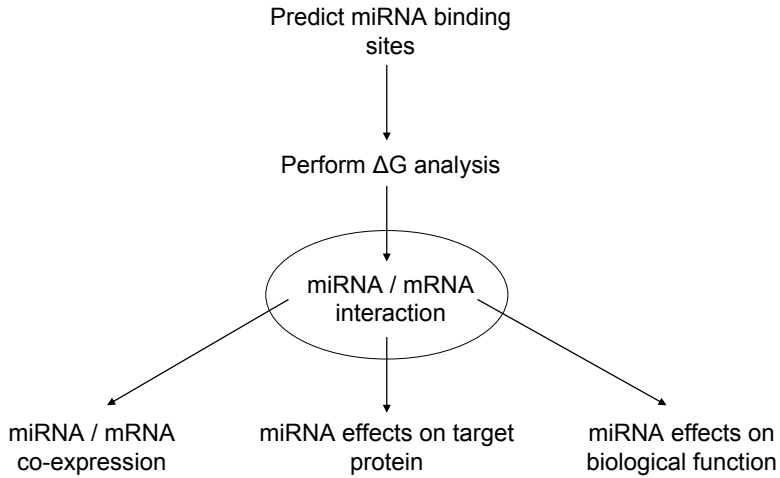


- Premature ossification
- Early onset chondrocyte hypertrophy
- Mimics constitutive Runx2 in chondrocytes



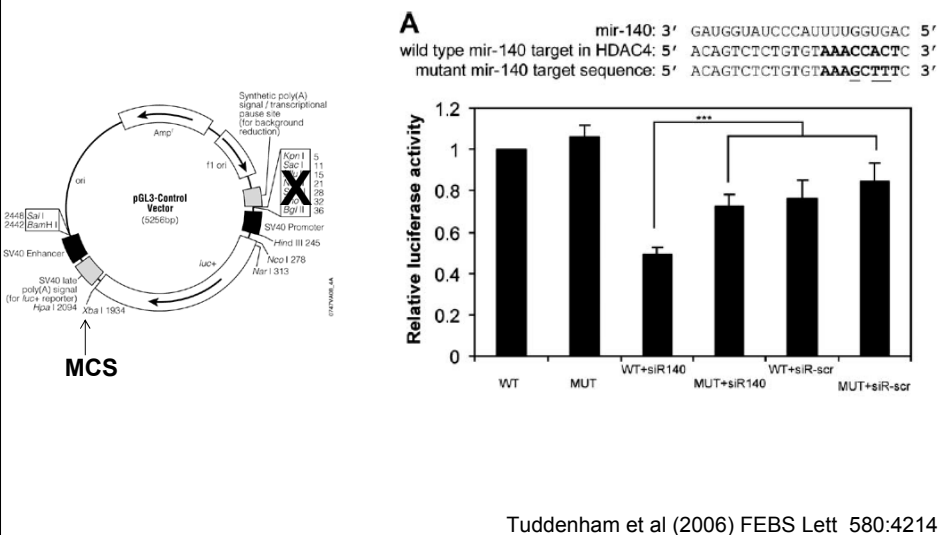
Vega et al (2004) Cell 119:555

Target prediction and validation

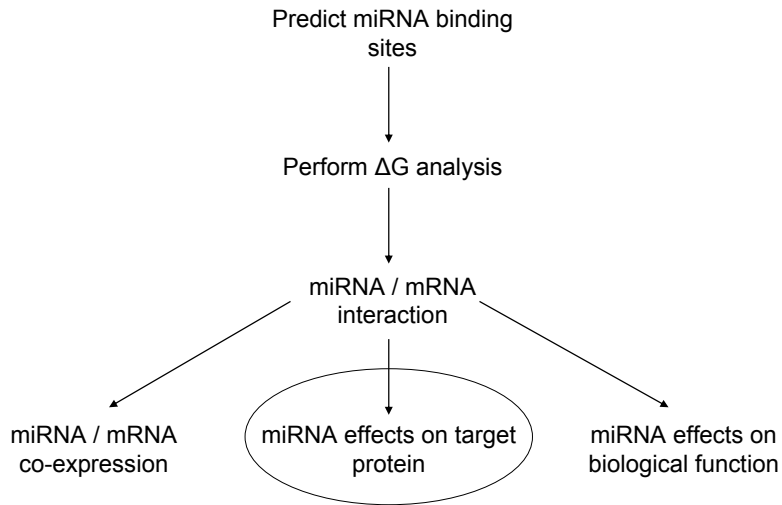


Kuhn DE, Martin MM, Feldman DS, Terry AV Jr, Nuovo GJ, Elton TS.
 Experimental validation of miRNA targets. *Methods*. 2008 44(1):47-54.

HDAC4 is targeted by miR140 Luciferase reporter

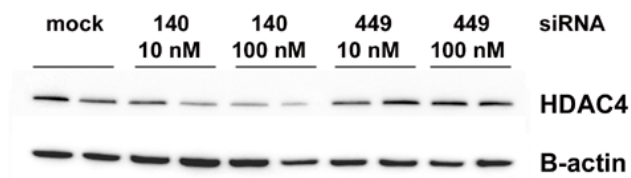


Target prediction and validation



Kuhn DE, Martin MM, Feldman DS, Terry AV Jr, Nuovo GJ, Elton TS.
Experimental validation of miRNA targets. *Methods*. 2008 44(1):47-54.

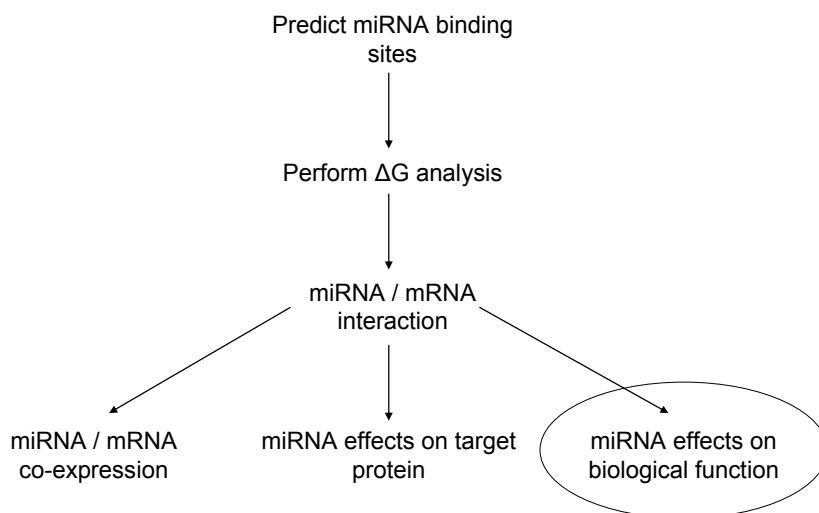
HDAC4 is targeted by miR140 Western blot



Experimental target identification

- **microarray**
 - presumes mRNA level altered
 - miRNA mimic vs. antagomir treated cells?
- **proteomics**
 - e.g. 2D-DIGE, iTRAQ
- **co-immunoprecipitation**
 - antibodies against Ago proteins co-IP bound mRNAs (Easow et al. 2007, Beitzinger et al. 2007)

Target prediction and validation

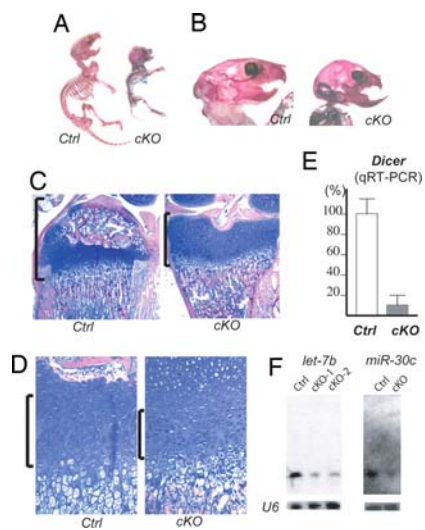


Kuhn DE, Martin MM, Feldman DS, Terry AV Jr, Nuovo GJ, Elton TS.
Experimental validation of miRNA targets. *Methods*. 2008 44(1):47-54.

Functional analyses

- expression of miRNA mimic
 - transient
 - stable
 - in vivo
- expression of miRNA antagonist
- knockout mouse
 - specific miRNA
 - conditional Dicer null

In vivo Col2-Cre:Dicer^{fl/fl}



Kobayashi T. et al. PNAS 2008;105:1949-1954

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PNAS

Control of Stress-Dependent Cardiac Growth and Gene Expression by a MicroRNA

Eva van Rooij,¹ Lillian B. Sutherland,¹ Xiaoxia Qi,¹ James A. Richardson,^{1,2} Joseph Hill,³ Eric N. Olson^{1*}

575 (2007); **316** *Science*

Regulation of the Germinal Center Response by MicroRNA-155

To-Ha Thai,¹ Dinis Pedro Calado,¹ Stefano Casola,² K. Mark Ansel,¹ Changchun Xiao,¹ Yingzi Xue,¹ Andrew Murphy,² David Frenthewey,² David Valenzuela,¹ Jeffery L. Kutok,⁴ Marc Schmidt-Supprjan,¹ Nikolaus Rajewsky,² George Yancopoulos,¹ Anjana Rao,¹ Klaus Rajewsky^{1*}

604 (2007); **316** *Science*

Requirement of *bic/microRNA-155* for Normal Immune Function

Antony Rodriguez,^{1*} Elena Vigorito,^{2*} Simon Clare,¹ Madhuri V. Warren,^{1,3} Philippe Couttet,¹ Dalya R. Soond,² Stijn van Dongen,¹ Russell J. Grocock,¹ Partha P. Das,⁴ Eric A. Miska,⁴ David Vetrie,² Klaus Okkenhaug,² Anton J. Enright,¹ Gordon Dougan,¹ Martin Turner,¹ Allan Bradley¹

608 (2007); **316** *Science*

Dysregulation of Cardiogenesis, Cardiac Conduction, and Cell Cycle in Mice Lacking miRNA-1-2

Yong Zhao,^{1,2,3,4} Joshua F. Ransom,^{1,2,3} Ankang Li,^{5,7} Vasanth Venkathram,^{1,4} Morgan von Dröhlen,¹ Abhis N. Muthi,¹ Takahitoi Tsuchihashi,^{1,5,7} Michael T. McManus,⁶ Robert J. Schwartz,¹ and Deepak Srivastava^{1,2,3,4}

303 (2007); **129** *Cell*

Summary

MicroRNAs (678 human, 472 mouse) are abundant in vertebrate genomes

MicroRNAs are thought to regulate approx. 30% of protein-coding genes

MicroRNAs (RISC) can block translation and/or degrade mRNA

Target prediction algorithms remain unreliable

MicroRNAs may be important therapeutic molecules or targets

Conclusions

The role of miRNAs in many biological processes is unknown

Published data for miRNAs in calcified tissues is sparse

miR-223 in osteoclast differentiation (RAW264.7 cells) – Sugatani and Hruska 2007

miR-26a targets SMAD1 to modulate osteogenic differentiation of human adipose tissue-derived stem cells – Luzi et al 2008

A number of bioengineering materials regulate miRNA expression in osteoblasts – e.g. Palmieri et al 2008