The biology of miRNAs and siRNAs

Bradley S. Cobb
bcobb@rvc.ac.uk

References

He, L. and Hannon, GJ. 2004 MicroRNAs: small RNAs with a big role in gene regulation Nature Reviews Genetics. 5:522-531

Eulalio, A. et al. 2008. Getting to the route of miRNA-mediated gene silencing Cell. 132:9-14.

Li, Q. et al. 2007. miR-181a is an intrinsic modulator of T cell sensitivity and selection. Cell, 129:147-161.

Croce CM. 2009. Causes and consequences of microRNA dysregulation in cancer. Nature Reviews Genetics. 10:704-14.

Outline

miRNA definition and their synthesis

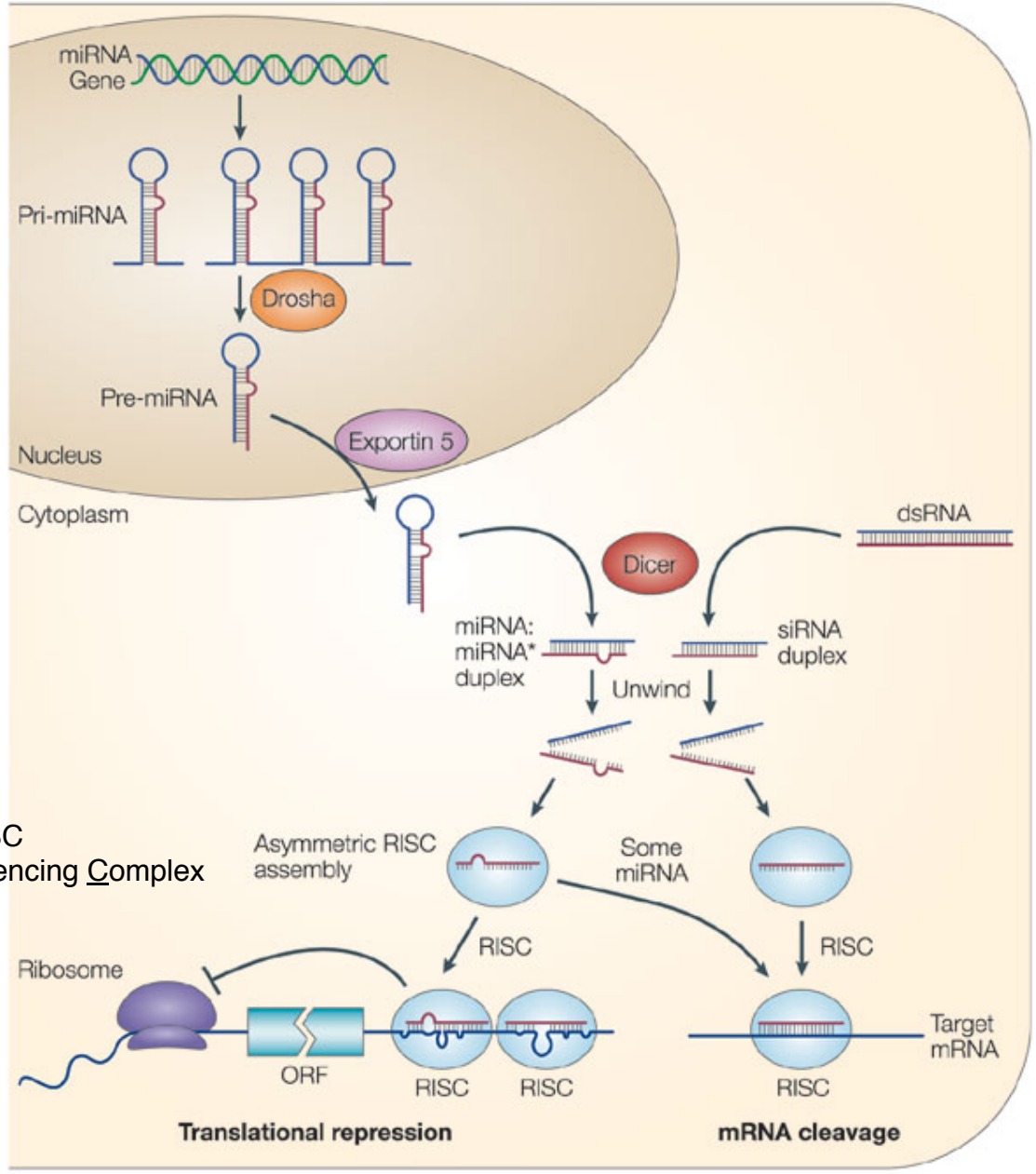
History

Mechanisms of action

miRNAs in development and cancer

siRNAs as a tool

miRNA and siRNA pathways



RISC
RNA-Induced Silencing Complex

He, L and Hannon, GJ
Nature Reviews Genetics
2004 5:522-531

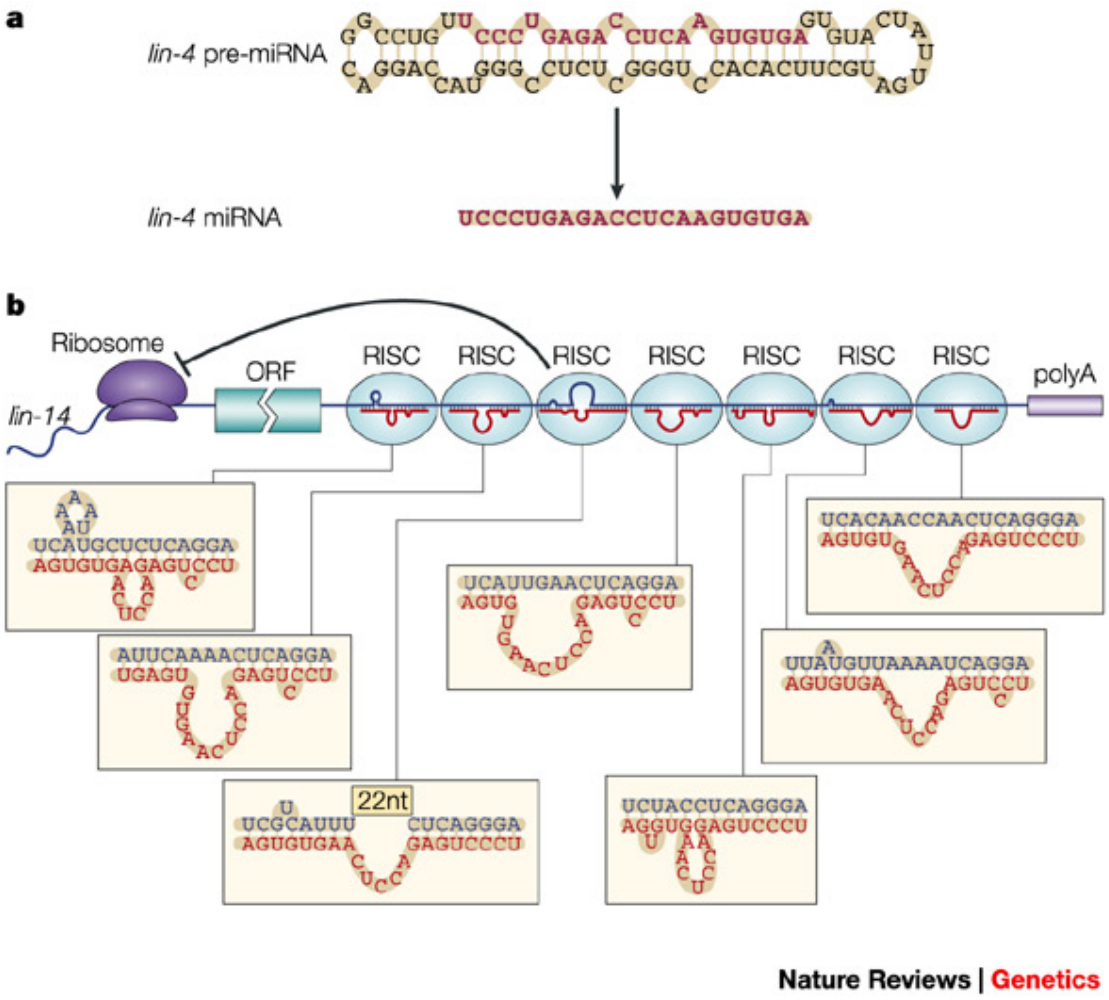
History

Lin 4 regulation of lin 14 in *C. elegans*

Non-protein
Coding gene

Protein coding gene
Important for larval development

Lin 4 regulation of lin 14



He, L and Hannon, GJ
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History

Lin 4 regulation of lin 14 in *C. elegans*

Let 7 evolutionary conserved in metazoans

PTGS in Plants

ds RNA silenced gene expression (RNAi)
Nobel prize in 2006 to Andrew Fire and Craig Mello

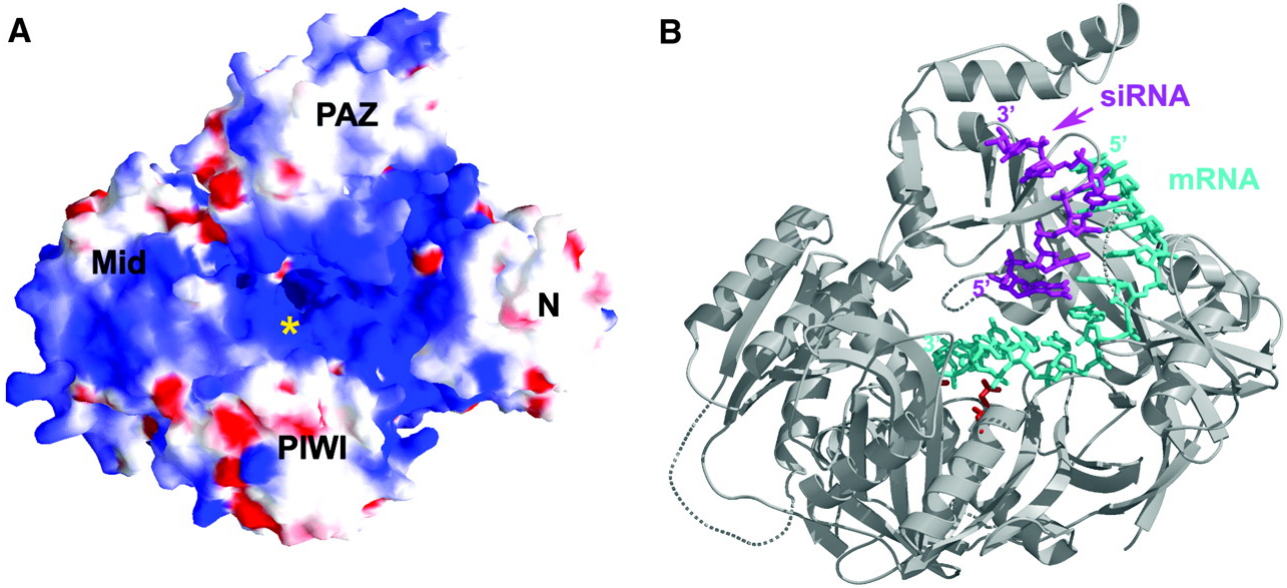
Mechanisms of action

RISC (Argonaute)

siRNAs - Slicer

miRNAs - Translational inhibition

RNAi Slicer



Crystal structure of Argonaute

Song et al, 2004
Science 305:1434

Translational repression by miRNAs

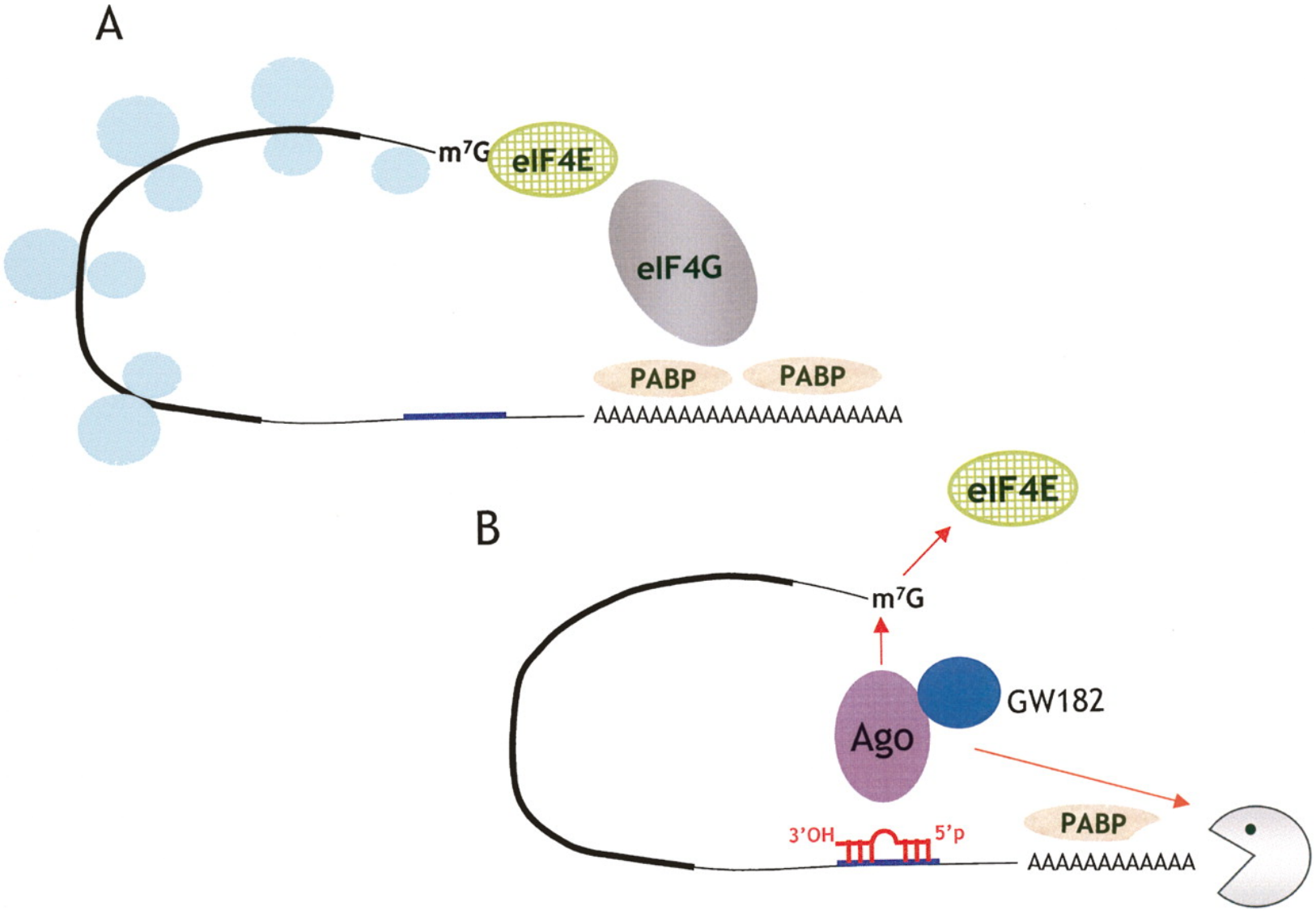
Initiation (Cap binding)

Degradation (deadenylation)

Elongation (Term. Trans. - Ribosome fall off)

Localization (P bodies)

Translational repression

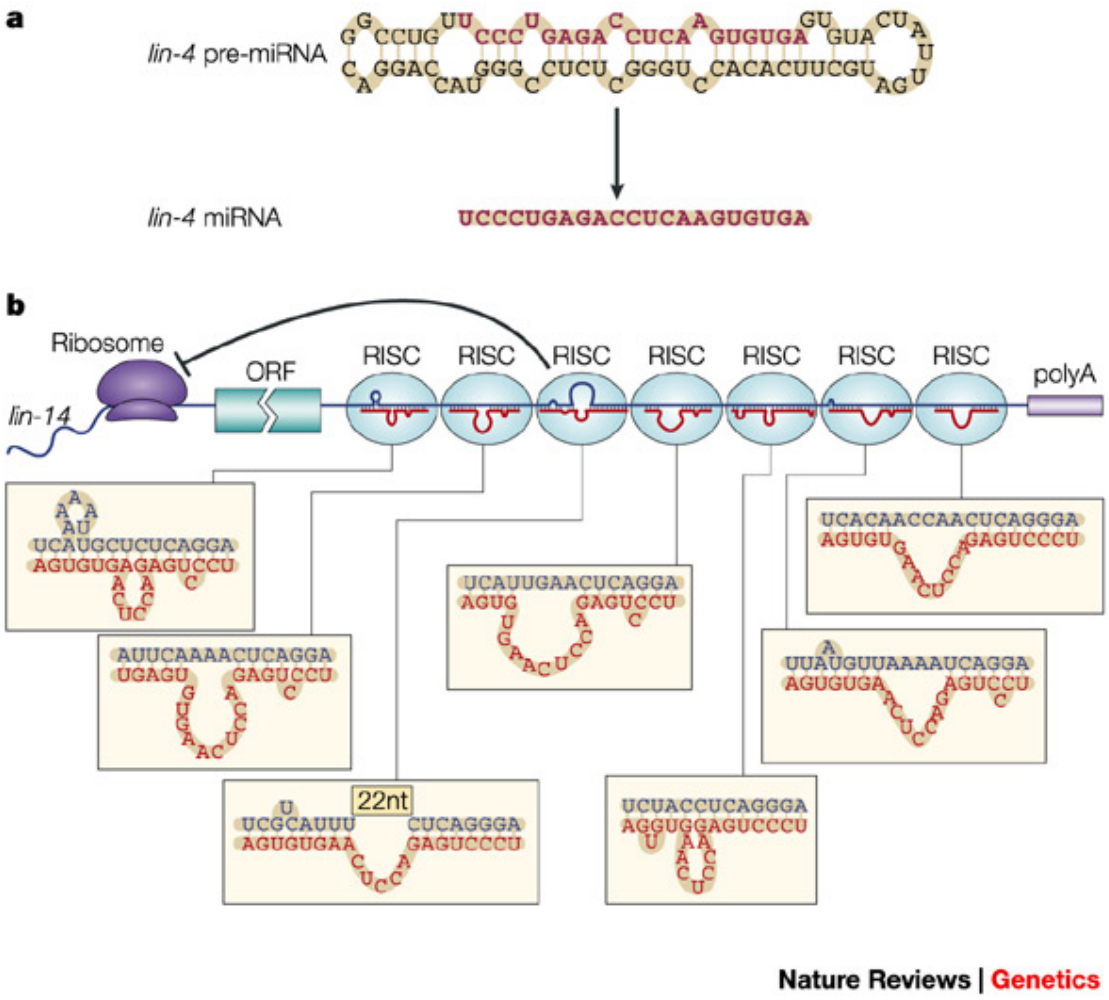


Standart and Jackson 2007, 21:1975-1982

miRNAs regulating development

How to identify miRNA regulatory pathways?

Lin 4 regulation of lin 14



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Identifying target genes of miRNAs

Computer algorithms

Reporters

Effects from miRNA miss-expression

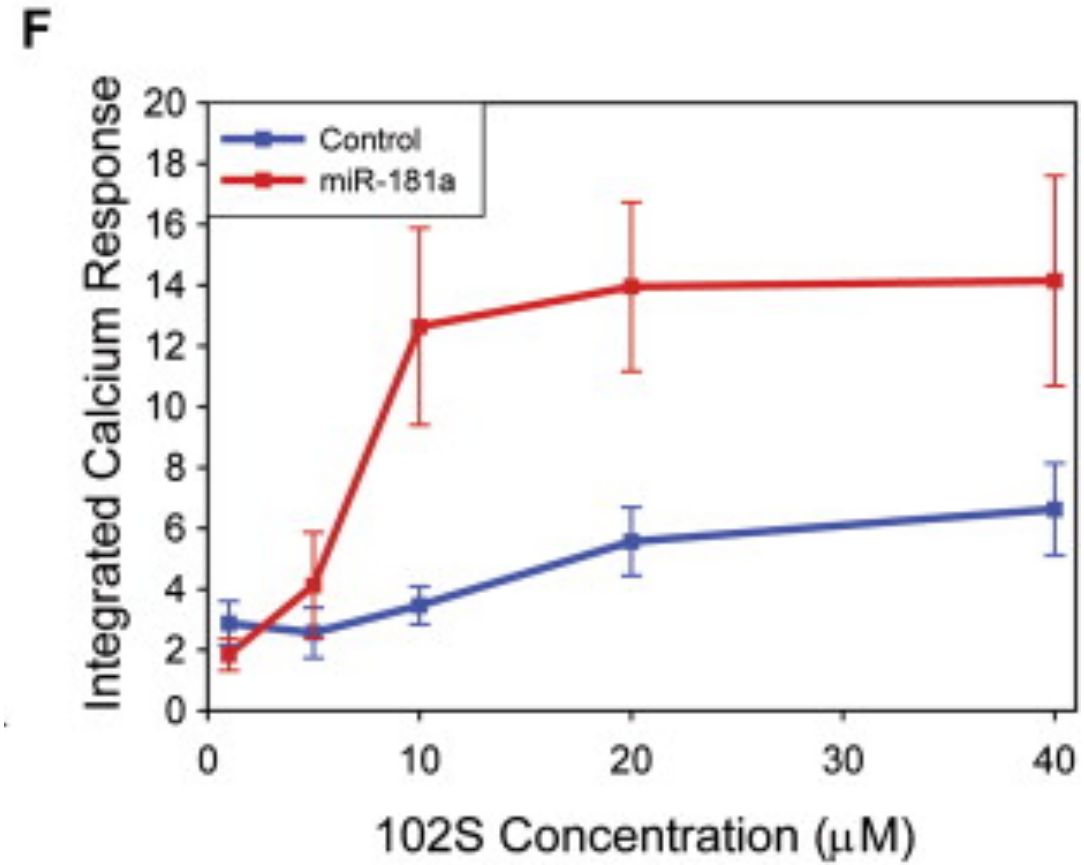
miRNAs in T-cell development

Cell

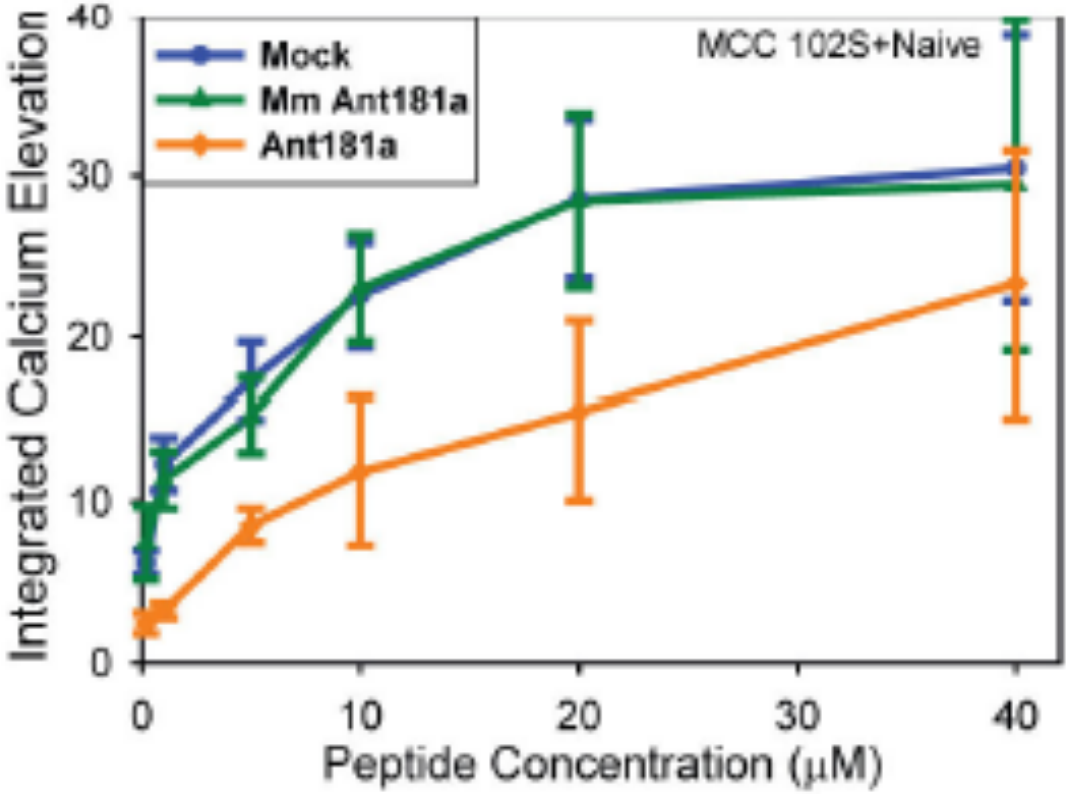
Volume 129, Issue 1, 6 April 2007, Pages 147-161

miR-181a Is an Intrinsic Modulator of T Cell Sensitivity and Selection
Qi-Jing et al

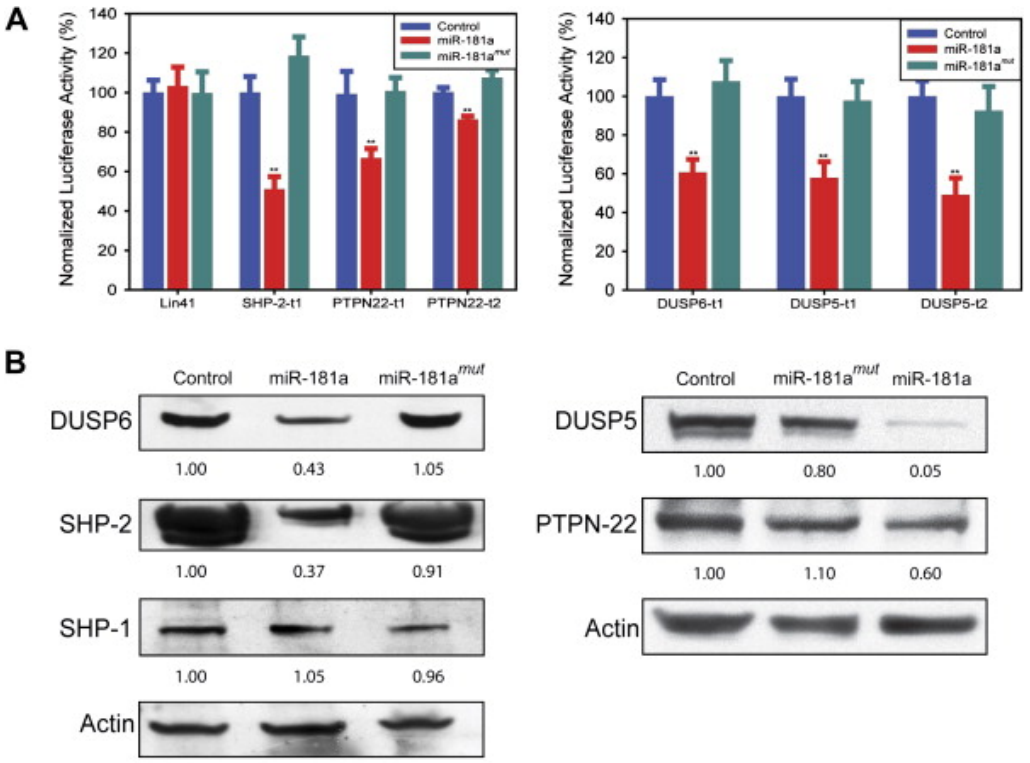
miR-181a enhances T cell signaling



Antagomirs to miR-181a decrease T cell signaling



miR-181a regulates phosphatase genes

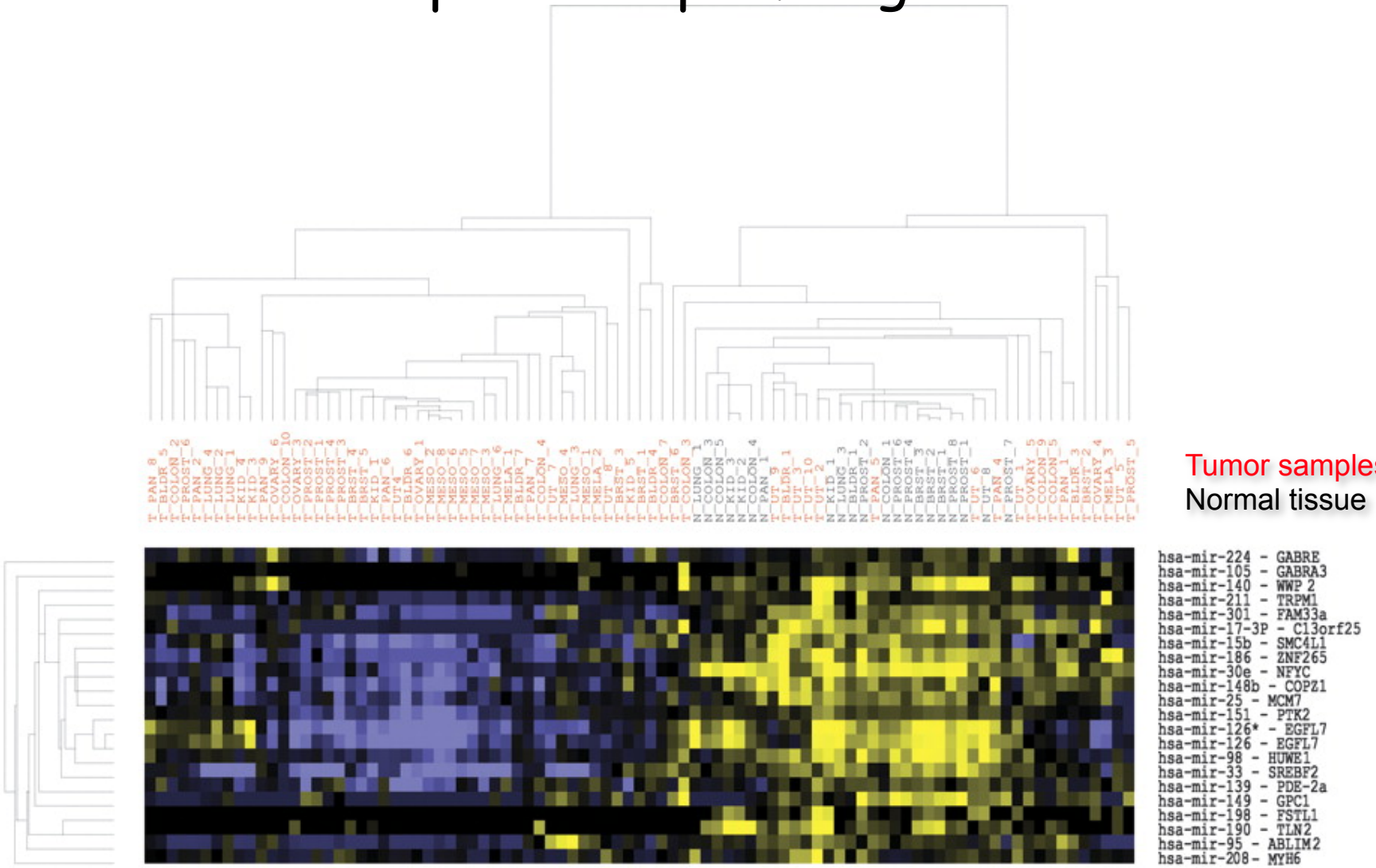


miRNAs in Cancer

Profiling miRNA expression patterns in cancers

miRNAs as tumor suppressors & oncogenes

miRNA expression profiling in Cancer



Low expression High expression

microRNAs tend be lower expressed in tumors

Thomson et. al. 2006 Genes & Dev. 20:2202-2207

Profiling miRNA expression patterns in cancers

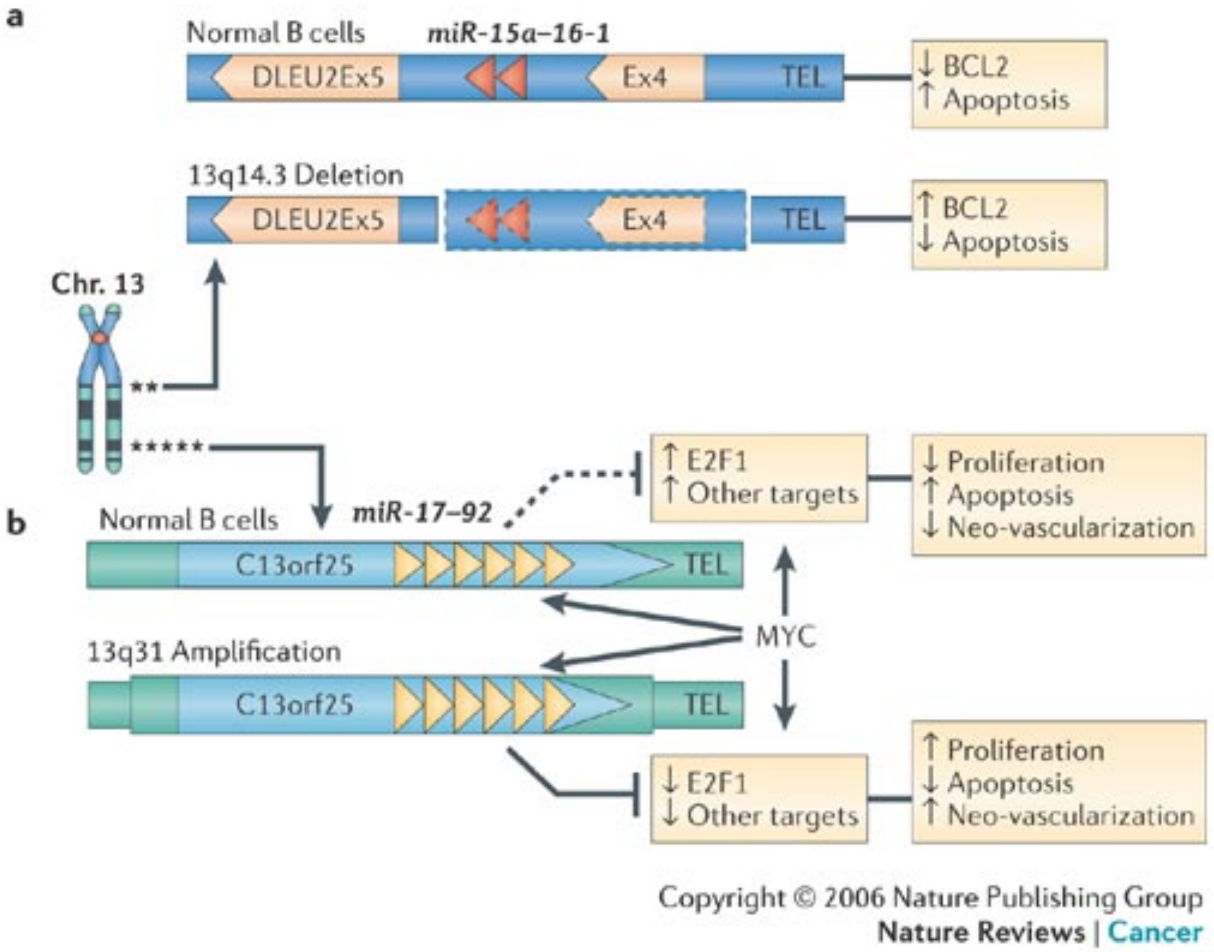
Cancer type*	MiRNA profiling data	Significance	Refs
Chronic lymphocytic leukaemia	A unique signature of 13 genes associated with prognostic factors (ZAP70 and IgVH mutation status) and progression (time from diagnosis to therapy)	MiRNAs as diagnostic markers (the identification of two categories of patients)	49,35
Lung adenocarcinoma	Molecular signatures that differ with tumour histology; miRNA profiles correlated with survival (*miR-155* and *let-7*)	MiRNAs as prognostic and diagnostic markers	53
Breast carcinoma	MiRNA expression correlates with specific pathological features	MiRNAs as prognostic markers	50
Endocrine pancreatic tumours	A signature that distinguishes endocrine from acinar tumours; the overexpression of *miR-21* is strongly associated with both a high Ki67 proliferation index and the presence of liver metastases	MiRNAs as diagnostic and prognostic markers	54
Hepatocellular carcinoma	MiRNA expression correlated with differentiation	MiRNAs as prognostic markers	52
Papillary thyroid carcinoma	MiRNA upregulation (for example, *miR-221* and *miR-222*) in tumoral cells and normal cells adjacent to tumours, but not in normal thyroids without cancers	MiRNAs probably involved in cancer initiation	37 114
Glioblastoma	A specific signature compared with normal tissues	MiRNAs as diagnostic markers	51
Human cancers	MiRNA-expression profiles accurately classify cancers; an miRNA classifier classes poorly differentiated samples better than a messenger RNA classifier	MiRNAs as diagnostic markers	41
Human solid cancers	Common signature for distinct types of solid carcinomas	Specific miRNAs are involved in common molecular pathways	47

*Only data from microarray studies reporting results on human primary tumours were included in this table. IgV_H, immunoglobulin heavy-chain variable-region, MiRNA, microRNA. ZAP70, 70 kDa zeta-associated protein.

Calin and Croce *Nature Reviews Cancer* **6**, 857–866 (November 2006) | doi:10.1038/nrc1997

nature REVIEWS CANCER

miRNAs as tumor suppressors & oncogenes



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nature REVIEWS CANCER

siRNAs as a tool

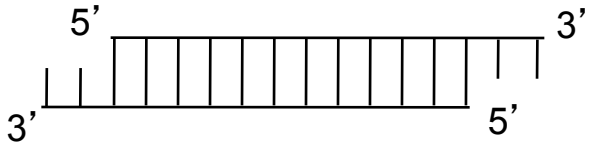
Genetic approach to examine function of a gene

1. Design

2. Delivery

siRNA Design

Choosing a region of a transcript to target



Strand with most unstable 5’ end
is incorporated Into Argonaute

Whitehead siRNA selection program
http://jura.wi.mit.edu/bioc/siRNAext/

Delivery of siRNAs

Synthetic oligonucleotides

Hairpin expression vectors
Use pol III expression cassette