



Roles of MicroRNAs in Cancer

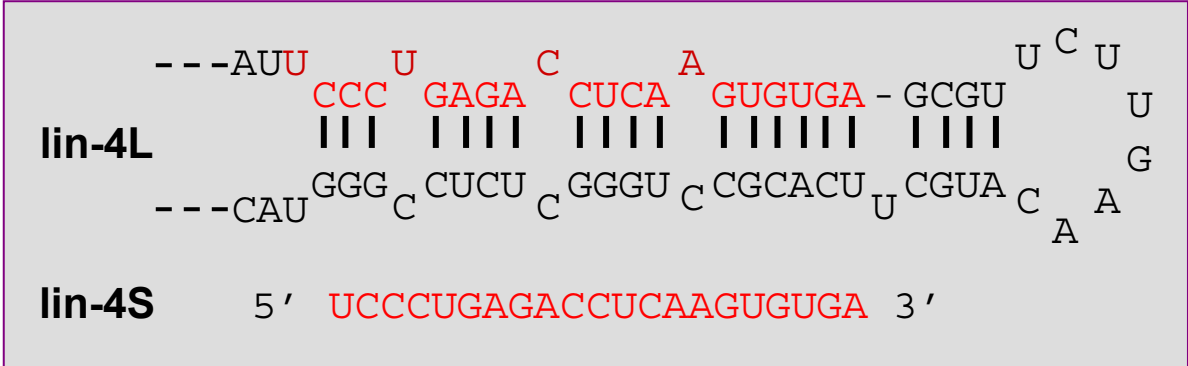
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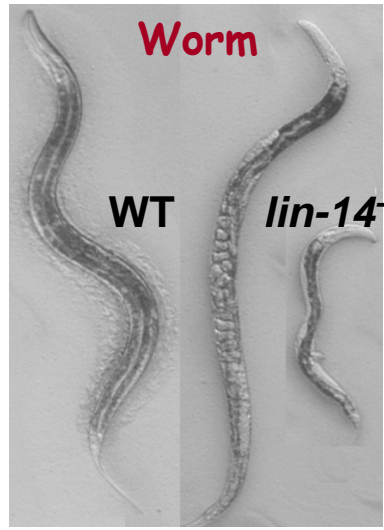
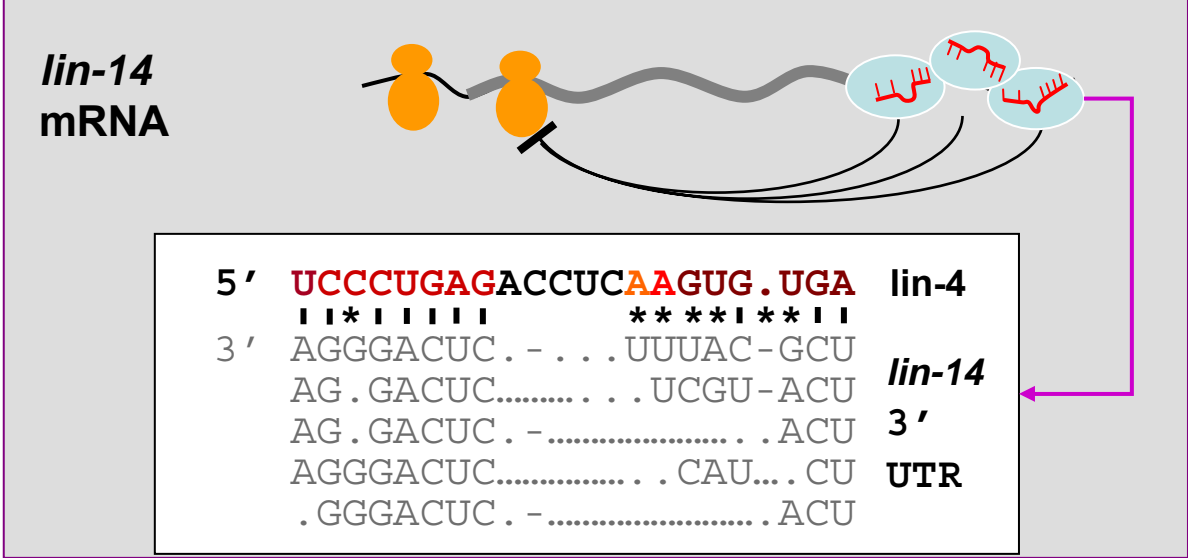
Content

- **Introduction to microRNAs**
 - What are microRNAs?
 - What's new about microRNA research?
 - General approaches used in miRNA research
 - AB's miRNA research tools
- **How are microRNAs linked to cancer?**
 - Early observations
 - MicroRNA oncogenes
 - MicroRNA tumor suppressors
- **Role of miR-34s in p53 tumor suppressor network**

Discovery of microRNAs: Tiny *lin-4* story



Dr. Victor Ambros
Professor
UMass Medical School

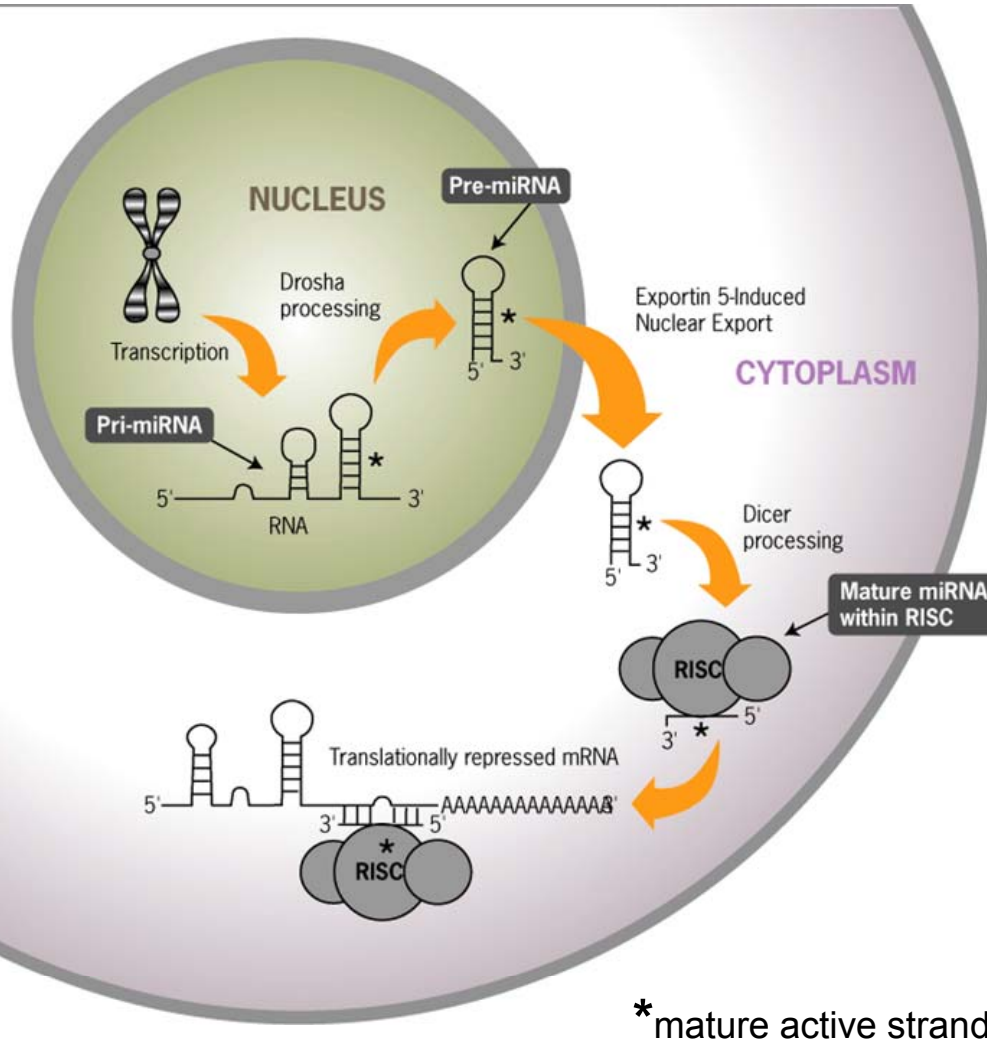


Lee et al. 1993; Wightman et al., 1993; Olson and Ambros, 1999

Definition of MicroRNAs

- ~ 21(18-25) nts in length
- Single-stranded
- Endogenous noncoding RNA molecules
- Processed from stem-loop precursors by RNase III enzymes Drosha and Dicer
- Highly conserved
- Target mRNAs largely for translational repression

Micro RNAs, Macro Significance



Biogenesis:

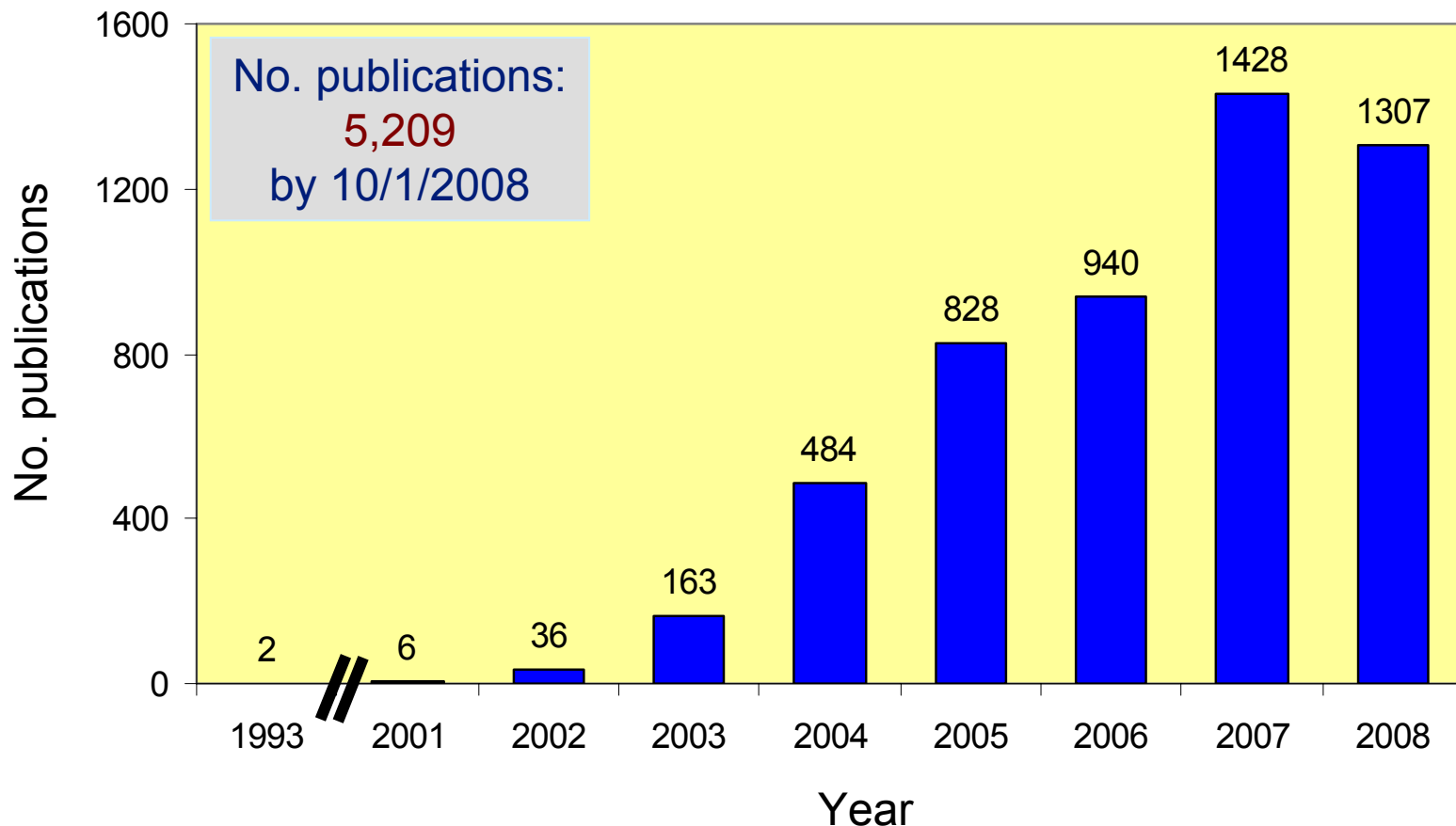
- Initial transcript, pri-miRs by Pol II from the genome
- Cleaved by Drosha into ~60 nt pre-miRs
- To ~22 nt mature miRNA by Dicer
- Post-transcriptional repression (or activation)

Functions:

- Development
 - Timing
 - Hematopoiesis
 - Neuron differentiation
 - Cell death and proliferation
- Diseases
 - Cancers
 - Diabetes
- Epigenetics (RNA/protein, RNA/DNA interaction)

What's new about miRNA research in 2007-8?

- MicroRNA publications increased very rapidly



What's new about miRNA research in 2007-8?

- MicroRNA publications increased significantly
- **How many miRNAs are there in human?**
 - **By 2003:** 145 genes/ 152 mature miRNAs (Sanger Release 3.0)
 - **By 2004:** 192 genes/ 207 mature miRNAs (Sanger Release 5.1)
 - **By 2005:** 281 genes/ 319 mature miRNAs (Sanger Release 7.1)
 - **By 2006:** 411 genes/ 455 mature miRNAs (Sanger Release 9.0)
 - **By 2007:** 541 genes/ 733 mature miRNAs (Sanger Release 10.1)
 - **By 2008:** 695 genes/ 866 mature miRNAs (Sanger Release 12.0)
 - **Total number of miRNA genes predicted:**
 - ~1,000 (Landgraf et al. 2007, Tuschl Lab)
 - >25,000 (Miranda et al. 2006, IBM Bioinformatics Group)

What's new about miRNA research in 2007-8?

- MicroRNA publications increased significantly
- How many miRNAs are there in human?
- **What regulates miRNAs** (He et al. 2007; Diederichs & Harber 2007; Ruby et al. 2007; Viswanathan et al. 2008)?
- **New role of miRNAs as tumor suppressors** (He et al. 2007; Mayr et al. 2007) and **tumor invasion and metastasis** (Ma et al. 2008)
- **MicroRNAs regulate stem cell self-renewal and differentiation** (Wang et al. 2007; Park et al. 2007; Yu et al. 2007; Yi et al. 2008; Tay et al. 2008)
- **MicroRNAs function in immune systems** (Li et al. 2007; Rodriguez et al. 2007; Thai et al. 2007)
- **Viral miRNAs regulate (HIV or HSV-1) replication** (Triboulet et al. 2007; Umbach et al. 2008)
- **MicroRNAs can target 3' UTR, 5' UTR and coding regions** (Lytle et al. 2007; Easow et al. 2007; Tay et al. 2008)

Selective Blockade of MicroRNA Processing by Lin28

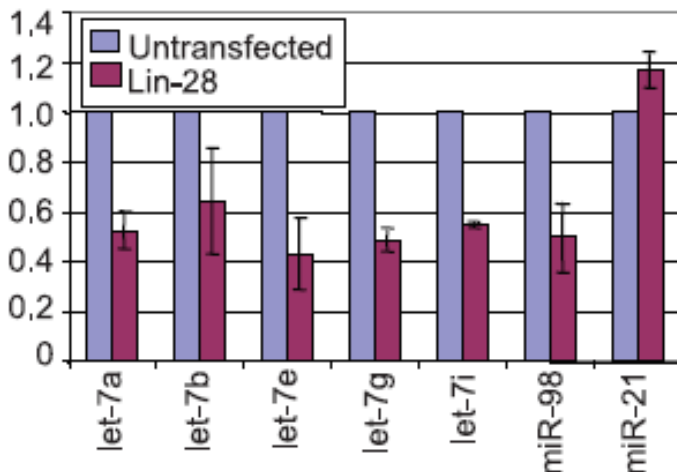
Science
320, 97-100 (2008)

Srinivas R. Viswanathan,¹ George Q. Daley,^{1,2*} Richard I. Gregory^{1*}

MicroRNAs (miRNAs) play critical roles in development, and been observed in human malignancies. Recent evidence su primary miRNA transcripts (pri-miRNAs) is blocked posttrar embryonal carcinoma cells, and primary tumors. Here we s regulated RNA binding protein, selectively blocks the proce cells. Using in vitro and in vivo studies, we found that Lin28 Microprocessor-mediated cleavage of pri-let-7 miRNAs. Our regulator of miRNA biogenesis and suggest that Lin28 may mediated differentiation in stem cells and in certain cance

Key findings:

1. Lin28, a developmentally regulated RNA binding protein, selectively blocks the processing of pri-let-7 in ES cells.
2. Lin28 is a negative regulator of miRNA biogenesis.
3. Together with Nanog, Oct-4 and Sox 2, Lin28 can reprogram human fibroblasts to pluripotent cells. The result suggests that modulating miRNA processing may contribute to the reprogramming of somatic cells to an embryonic state.



General approaches of microRNA research - 1

1. What miRNA genes to start with

1.1 Select known miRNAs from Sanger's miRBase

1.2 "Discover" novel miRNAs by *in silico* prediction

- Assumptions:

- Presence of hairpin structure
- Phylogenetic conservation
- Thermodynamic stability of hairpins
- Genomic location, sequence and structure similarity to known miRNAs

- Web servers: miRscan (Lim et al. 2003); ProMiR (Nam et al. 2005); BayesMiRNAfind (Yousef et al. 2006); Vir-Mir db (Li et al. 2008)

1.3 Discover novel miRNAs by cloning and sequencing

- RNA samples:

- Enriched vs. total RNA; Normal (untreated) vs. patients (treated)
- Caution: It is important to have relatively "pure" cell type

- Methods:

- Small RNA cloning followed by Sanger sequencing/NextGen sequencing
- Follow-up validation using Northern, qPCR and microarray

General approaches of microRNA research – 2

2. How to identify miRNAs of interest

2.1 Differentially expressed miRNAs by using qPCR and microarray

- Normal vs. disease
- Treated vs. untreated
- Wild type vs. knockout (KO)

2.2 miRNAs that are predicted to target a mRNA at 3' UTR based on conserved seed sequence matches

- Some evidence suggested that miRNA could target 5' UTR or coding region

2.3 Direct IP of Ago/miRNA/mRNA complex (Karginov et al. 2007)

General approaches of microRNA research – 3

3. What are the targets of a miRNA

3.1 Gain- or loss-of functions

- miRNA mimics
- miRNA inhibitors
- Mouse KOs

3.2 Luciferase reporter assays

- With wild-type vs. mutant miRNA binding sites

3.3 Expression analysis of punitive target(s) in the presence (enhanced) or absence (reduced) of miRNAs of interest

- mRNA(s)
- Protein(s)

3.4 Phenotypic evaluation (proliferation etc.)

General approaches of microRNA research – 4

4. How a miRNA gene is regulated?

4.1 Discover a punitive miRNA regulator by using gain- or loss-function

- Over-expression or reduced expression of a gene like transcriptional factor
- KOs of a gene

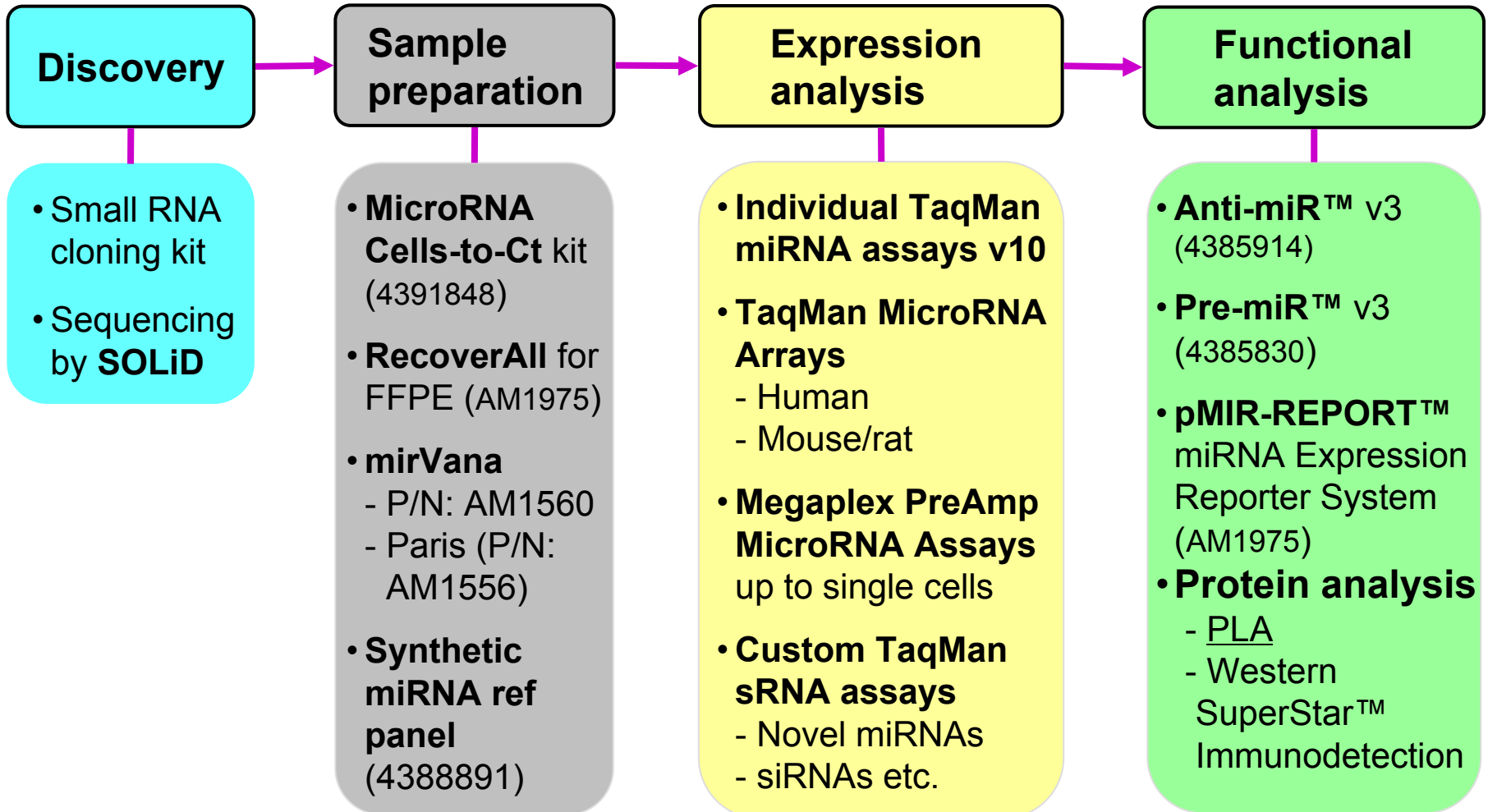
4.2 Identify or predict canonical binding sites in the punitive promoter regions of a miRNA gene

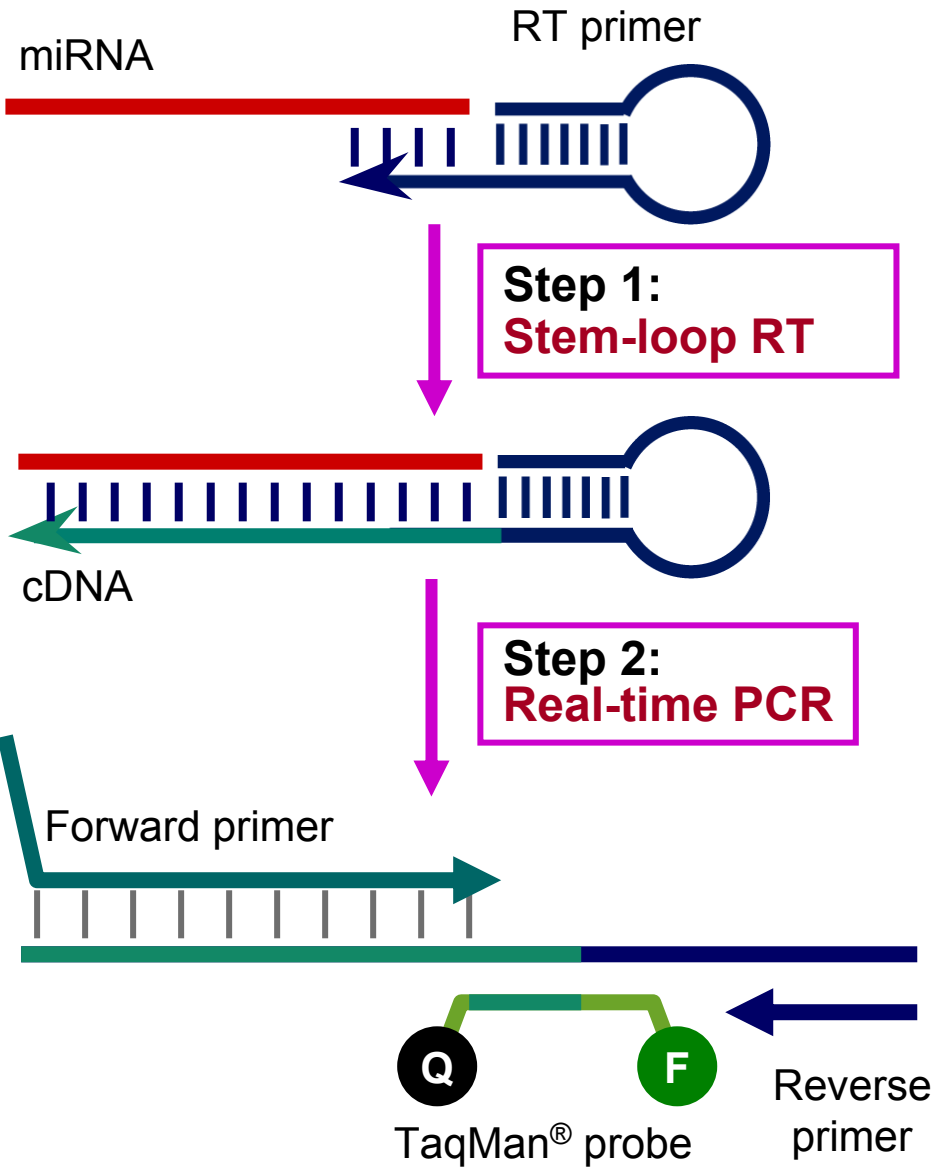
- *In silico* prediction
- ChIP seq or ChIP-on-chip

4.3 ChIP binding assays of miRNA promoters

4.4 Luciferase reporter assay for miRNA promoters

What's new about AB's miRNA tools?

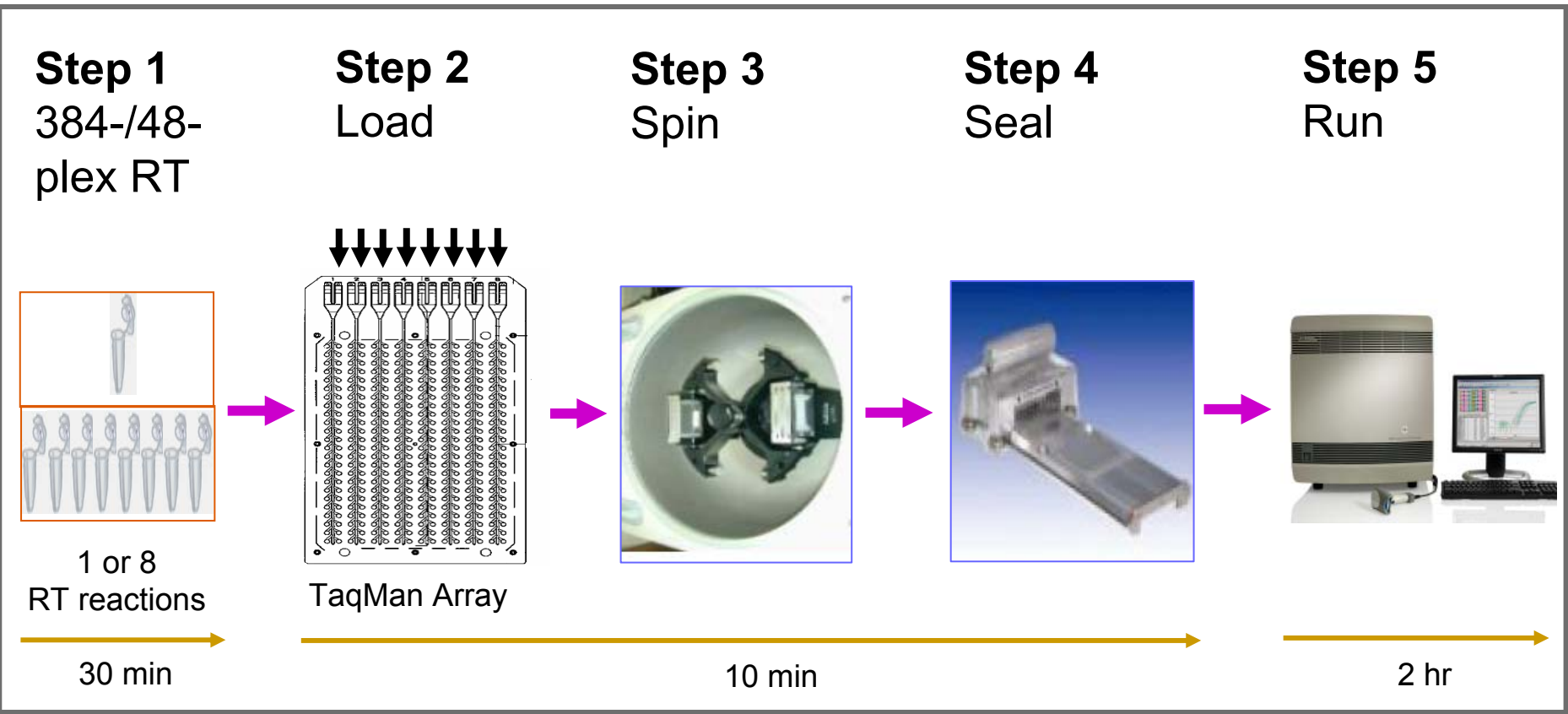




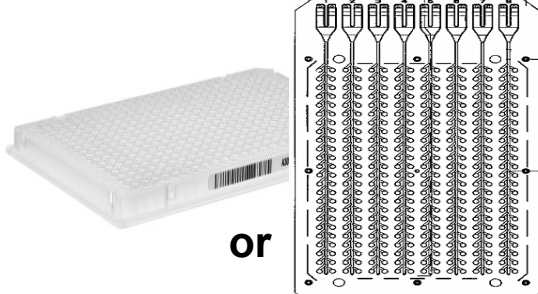
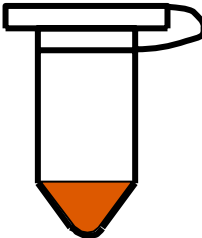
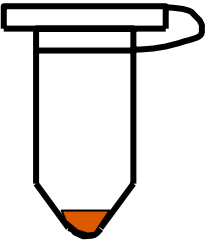
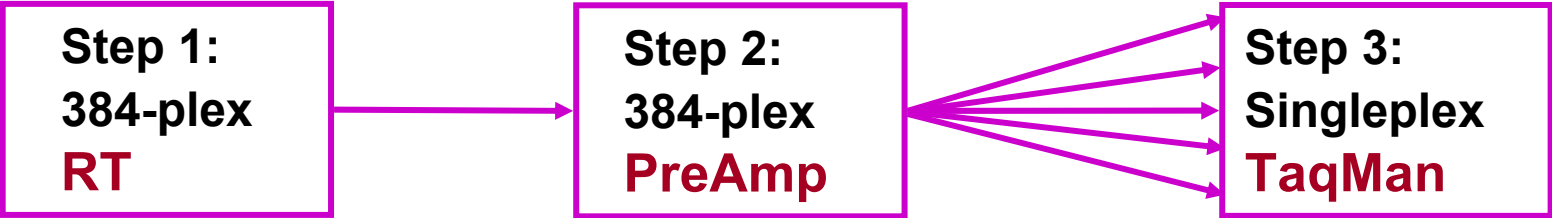
Individual TaqMan[®] miRNA assays

Chen et al. (2005) Nucleic Acid Res. v33: e179

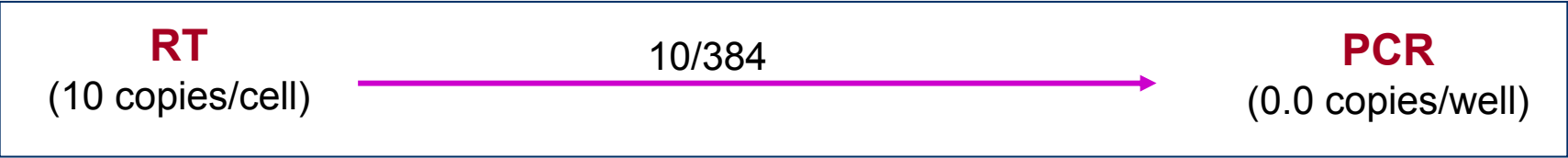
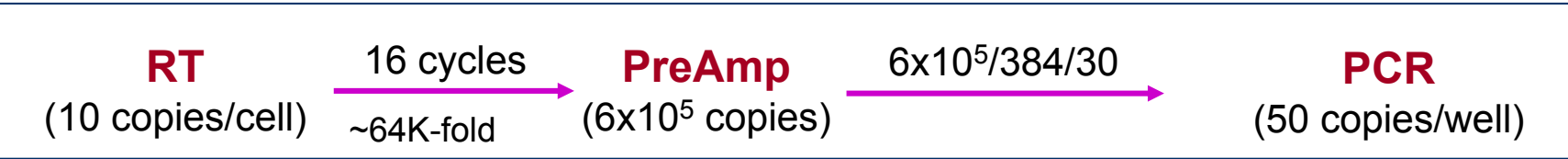
Easy Workflow of TaqMan[®] MicroRNA Arrays



MegaPlex PreAmp TaqMan MicroRNA Assays For Single Cell Gene Expression Profiling



or



How are miRNAs linked to cancer?

- **Early observations**

- First worm & fly miRNAs were shown to control cell proliferation and apoptosis (Lee et al. 1993; Hipfner et al. 2002; Brennecke et al. 2003)
- Many human miRNAs are located in the genome that are commonly amplified or deleted in cancer (Calin et al. 2002, 2004)
- Global down-regulation of miRNA expression in many tumors (Lu et al. 2005)



MicroRNA expression profiles classify human cancers

Jun Lu^{1,4*}, Gad Getz^{1*}, Eric A. Miska^{2*†}, Ezequiel Alvarez-Saavedra², Justin Lamb¹, David Peck¹, Alejandro Sweet-Cordero^{3,4}, Benjamin L. Ebert^{1,4}, Raymond H. Mak^{1,4}, Adolfo A. Ferrando⁴, James R. I. Tyler Jacks^{2,3}, H. Robert Horvitz² & Todd R. Golub^{1,4,6}

Nature 435,
834-838 (2005)

Recent work has revealed the existence of a class of small non-coding RNA species, known as microRNAs (miRNAs), which have critical functions across various biological processes^{1,2}. Here we use a new, bead-based flow cytometric miRNA expression profiling method to present a systematic expression analysis of 217 mammalian miRNAs from 334 samples, including multiple human cancers. The miRNA profiles are surprisingly informative, reflecting the developmental lineage and differentiation state of the tumours. **We observe a general downregulation of miRNAs in tumours compared with normal tissues.** Furthermore, we were able to successfully classify poorly differentiated tumours using miRNA expression profiles, whereas messenger RNA profiles were highly inaccurate when applied to the same samples. These findings highlight the potential of miRNA profiling in cancer diagnosis.

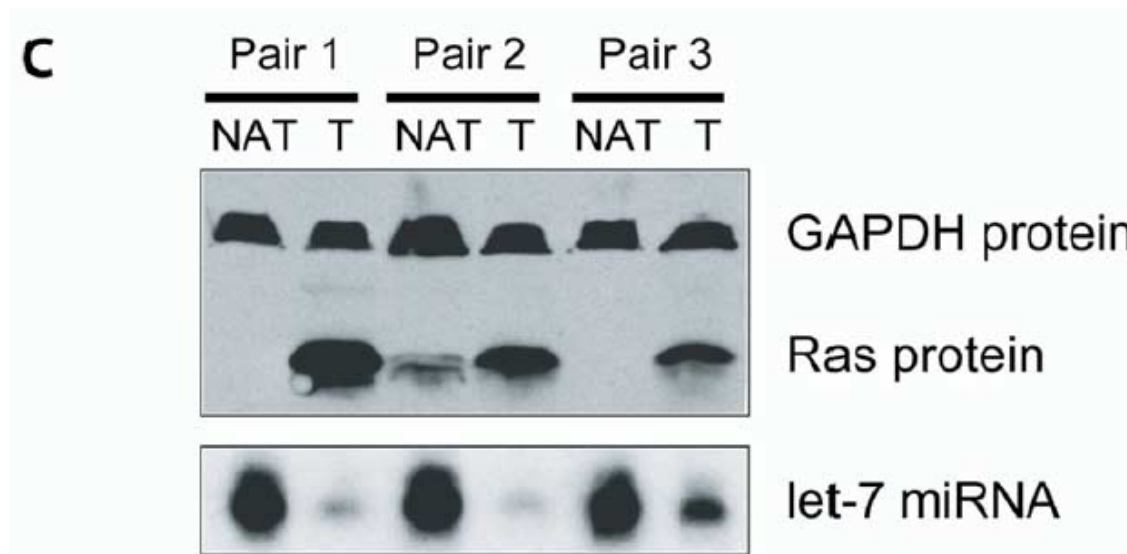
How are miRNAs linked to cancer?

- Early observations
- **MicroRNAs as tumor suppressors**
 - miR-34s are the component of p53 tumor suppressor network (He et al. 2007)
 - miR-15a and miR-16-1 in CLL
 - Common loss of miR-15a and miR-16-1 in CLL, lymphoma, myeloma, and prostate cancer strongly suggest that these two miRNAs act as tumor suppressor genes (Calin et al. 2002)
 - Largely by down-regulating Bcl2 (Cimmino et al. 2005)
 - The let-7 family can negatively regulate Ras
 - Loss or reduction of let-7 in lung cancer leads to RAS over-expression, contributing to tumorigenesis (Johnson et al. 2005)
 - let-7 regulates tumorigenicity of breast cancer cells (Yu et al. 2007)

Cell, Vol. 120, 635–647, March 11, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.cell.2005.01.014

RAS Is Regulated by the *let-7* MicroRNA Family

Johnson et al. (2005) *Cell* 120, 635-647

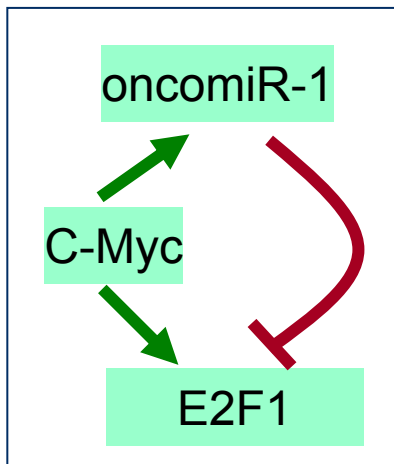


How are miRNAs linked to cancer?

- Early observations
- MicroRNAs as tumor suppressors
- **MicroRNAs as oncogenes:**
 - miR-155
 - miR-155 is processed from BIC RNA (Eis et al. 2005)
 - miR-155 is linked with Myc overexpression and B-cell lymphomas
 - miR-155 is also upregulated in breast, lung, colon, and thyroid cancers (He et al. 2005; Volinia et al. 2006; Costinean et al. 2006)
 - **miR-155 mechanism of action remains unclear!!!**

How are miRNAs linked to cancer?

- MicroRNAs as oncogenes
 - miR-155
 - **oncomiR-1 (miR-17-92 cluster)**
 - It includes 7 miRNAs, miR-17-5p, miR-17-3p, miR-18, miR-19a, miR-19b-1, miR-20, and miR-92-1
 - Located at human 13q31.3 and commonly amplified in human B-cell lymphoma.
 - Overexpression of oncomiR-1 accelerated the c-Myc-induced tumorigenesis in mice (He et al. 2005)
 - c-Myc activates transcription of oncomiR-1 and E2F1 but oncomiR-1 can repress E2F1 translation. cMyc simultaneously activates E2F1 transcription and limits its translation via miRNAs, allowing a tightly controlled proliferative signal (O'Donnell et al. 2005)



How are miRNAs linked to cancer?

- MicroRNAs as oncogenes
 - miR-155
 - oncomiR-1
 - **MicroRNA's role in tumor invasion and metastasis**
(Ma et al. 2007; Budhu et al. 2008; Tavazoie et al. 2008)

Discovery of p53 Tumor Suppressor MicroRNAs - miR-34s

nature

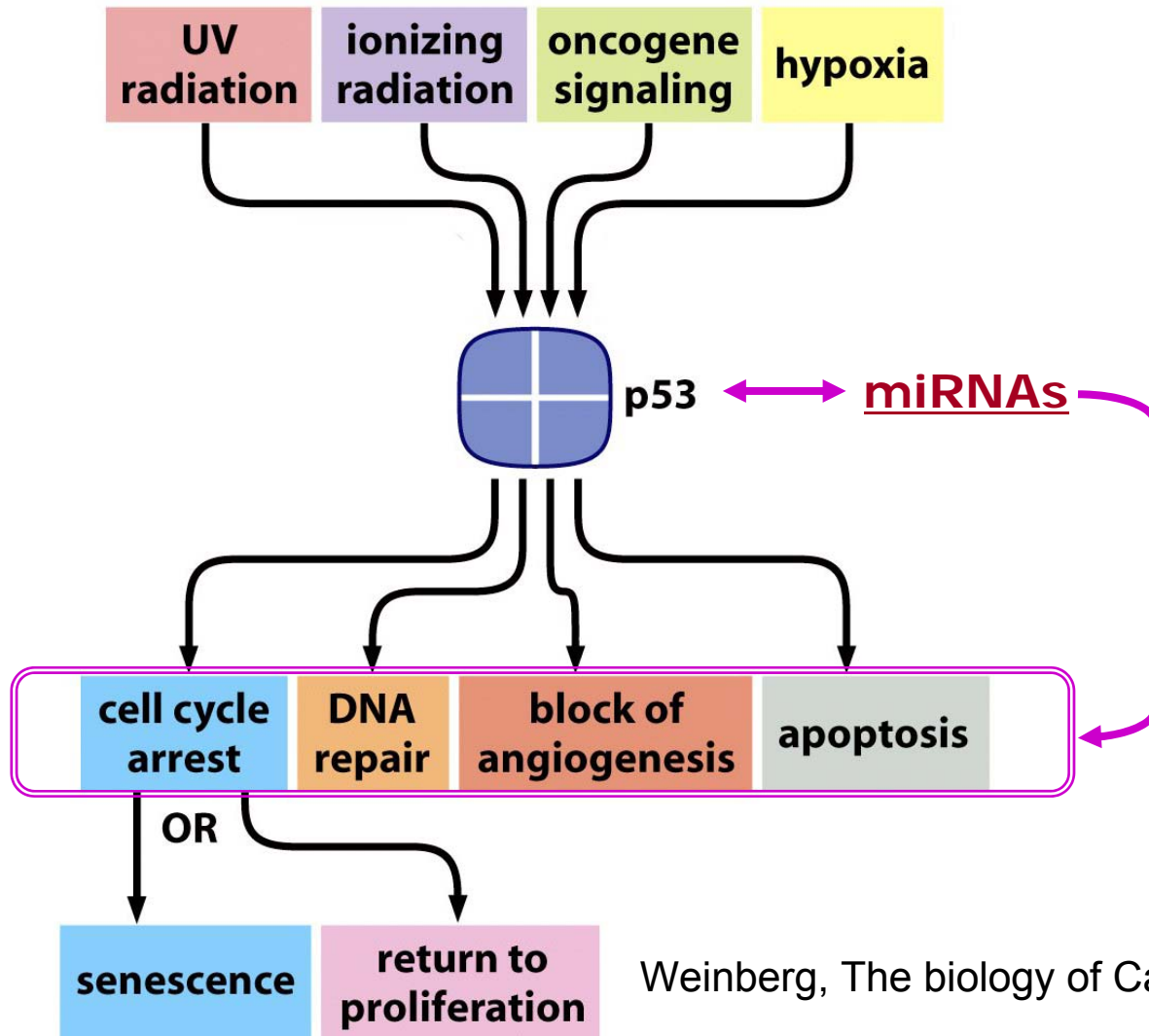
Vol 447|28 June 2007|doi:10.1038/nature05939

LETTERS

A microRNA component of the p53 tumour suppressor network

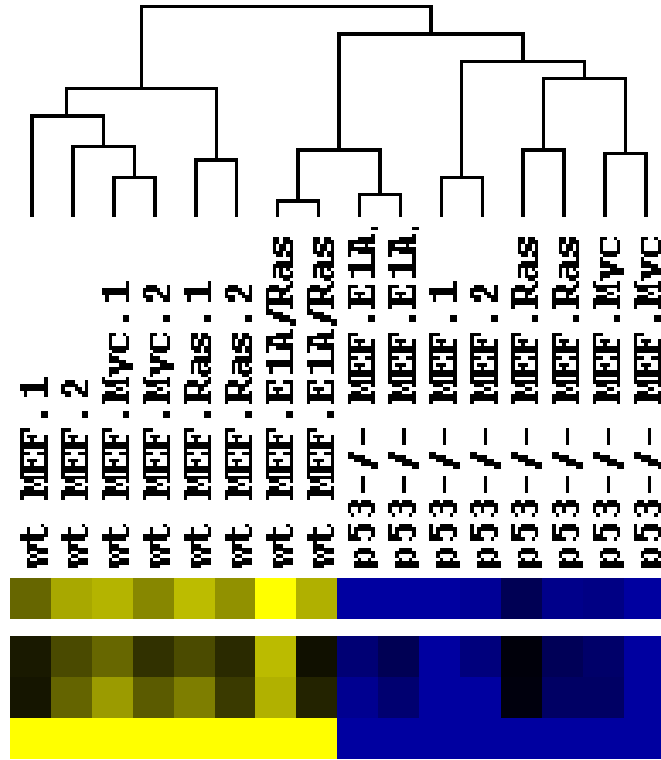
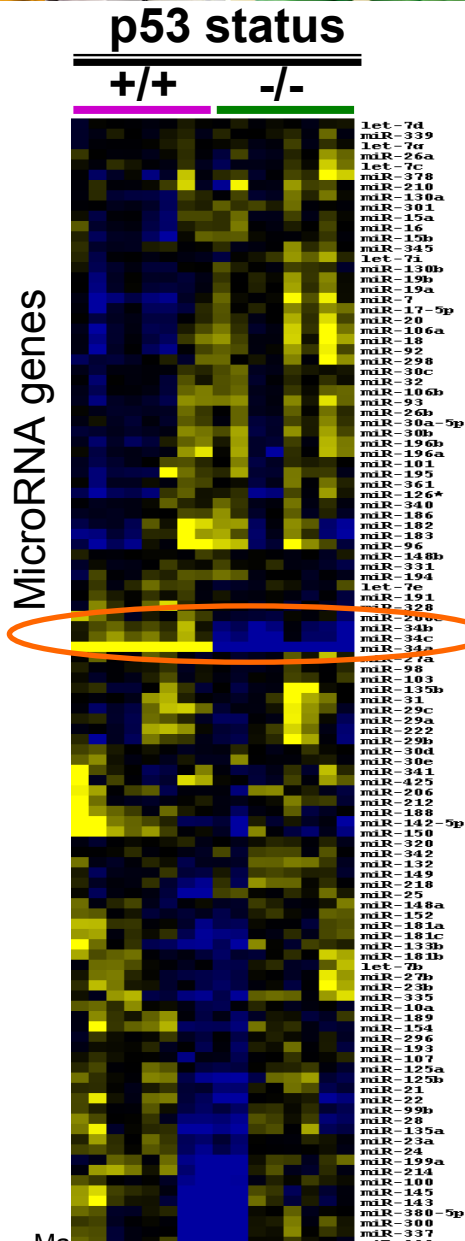
Lin He^{1*}, Xingyue He^{1,2*}, Lee P. Lim³, Elisa de Stanchina^{1†}, Zhenyu Xuan¹, Yu Liang⁴, Wen Xue¹, Lars Zender¹, Jill Magnus³, Dana Ridzon⁴, Aimee L. Jackson³, Peter S. Linsley³, Caifu Chen⁴, Scott W. Lowe¹, Michele A. Cleary³ & Gregory J. Hannon¹

p53, the guardian of the genome



Weinberg, The biology of Cancer, 2006

MicroRNA expression profile clustered by p53 genotypes



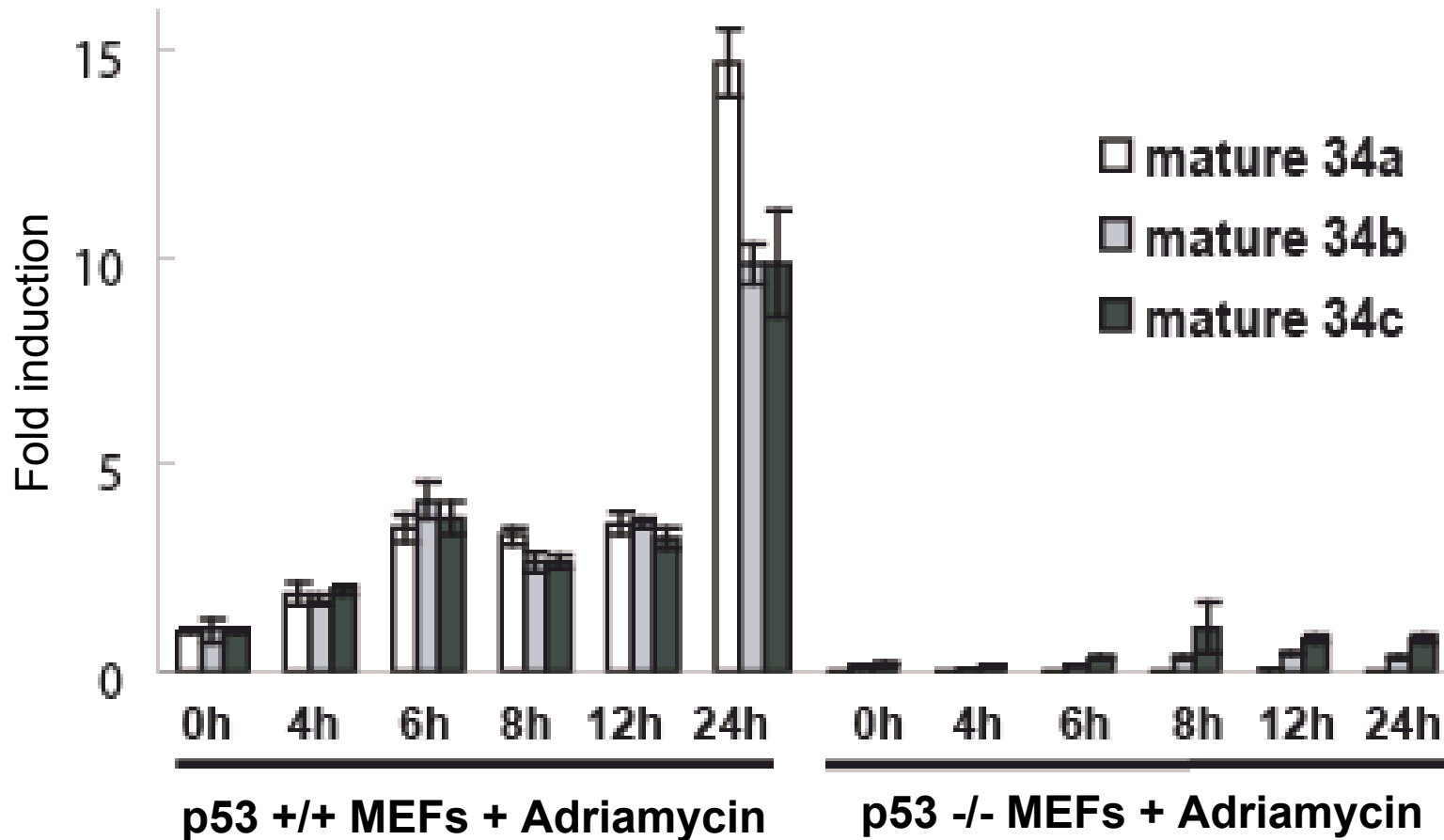
- **Question:**

- Is the induction of miR-34s p53-dependent?

- **To confirm:**

- Activate p53 *in vitro* (DNA damage) and *in vivo* (radiation) to determine whether miR-34 expression directly responds to p53 activation

p53-dependent miR-34 induction *in vitro*



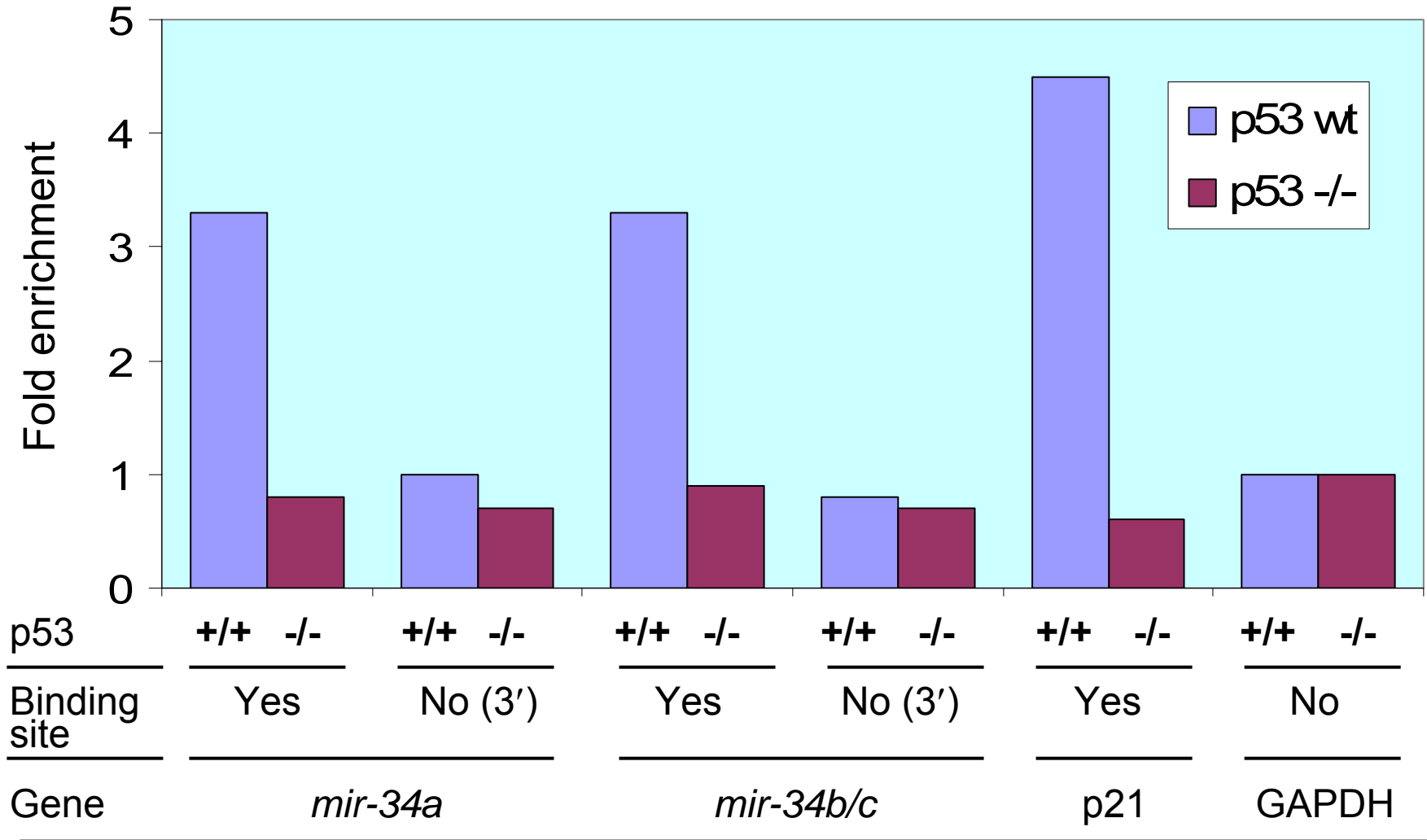
- **Question:**

- Is *mir-34* the direct target of p53?

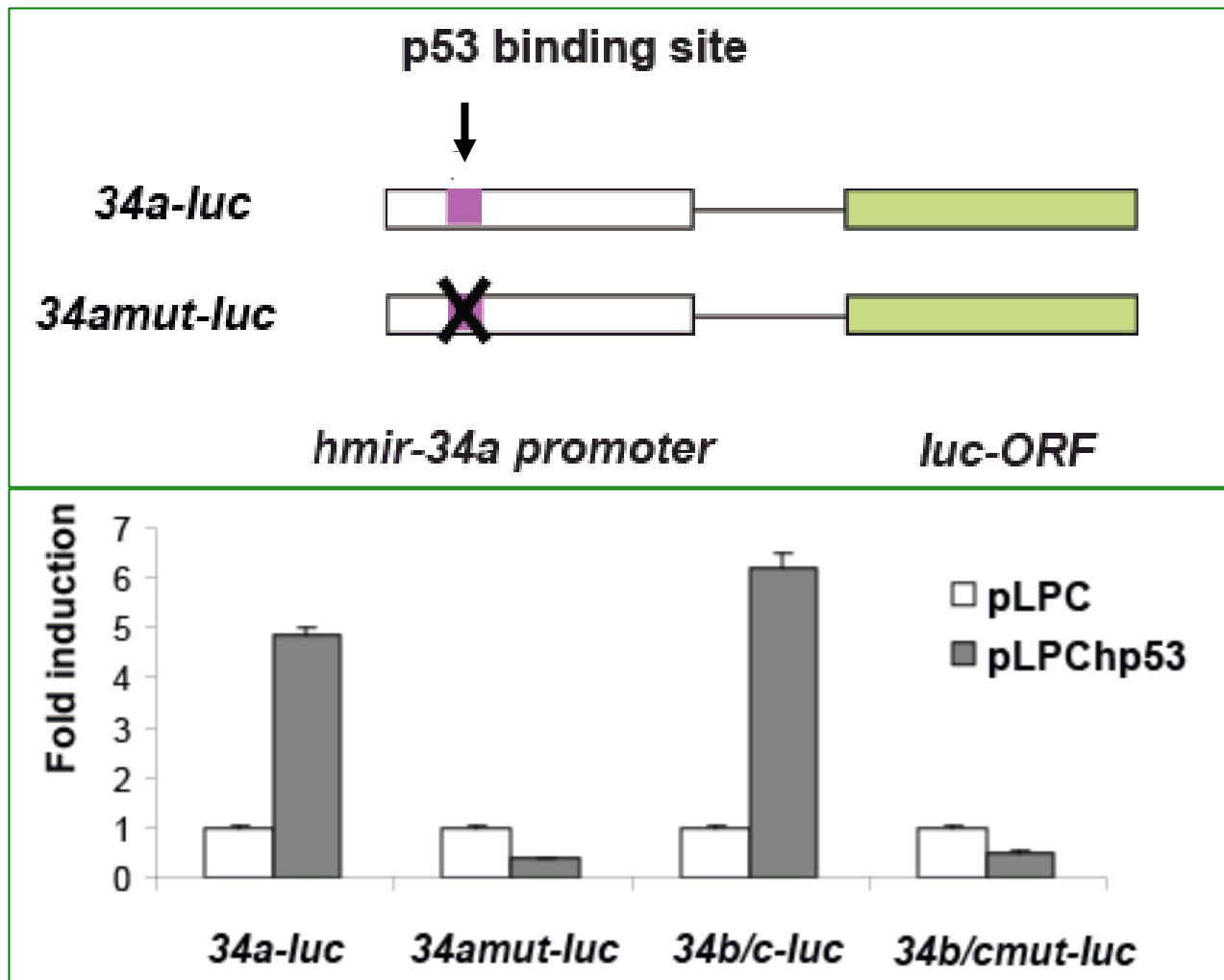
- **To confirm:**

- Canonical p53 binding sites in the punitive promoter regions of human *mir-34a* and *mir-34b/c* were identified (Wei et al. 2006)
- Perform **Chromatin Immunoprecipitation (ChIP)** to confirm whether p53 physically binds to the punitive promoters of *mir-34*.
- Use Luciferase reporter assay to confirm whether p53 binding functionally activates the transcription of *mir-34*?

Direct binding of p53 to *mir-34* confirmed by ChIP



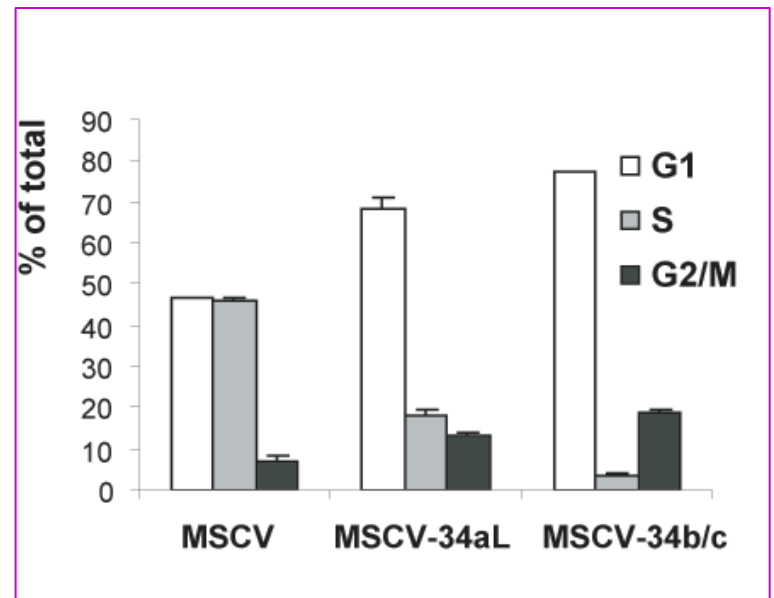
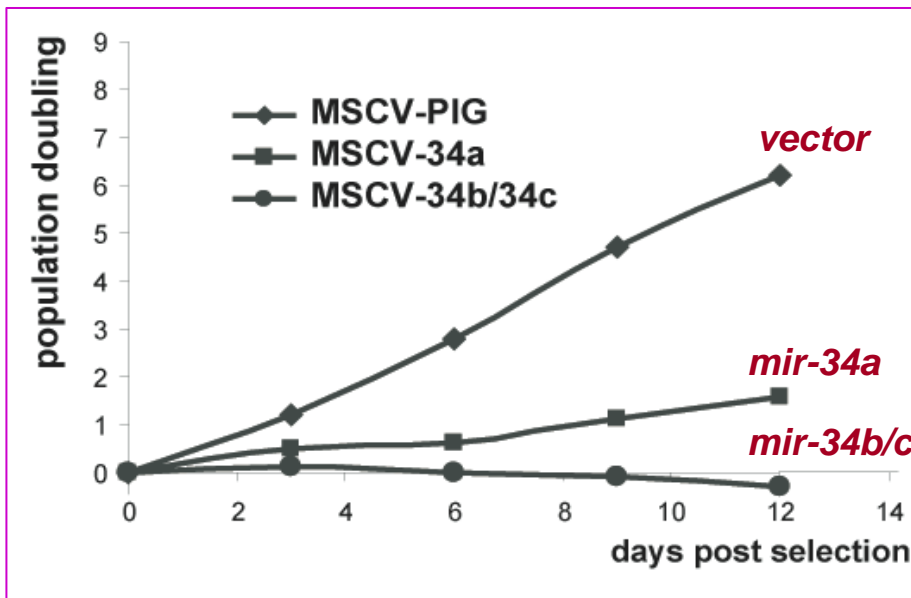
mir-34s are direct transcriptional targets of p53



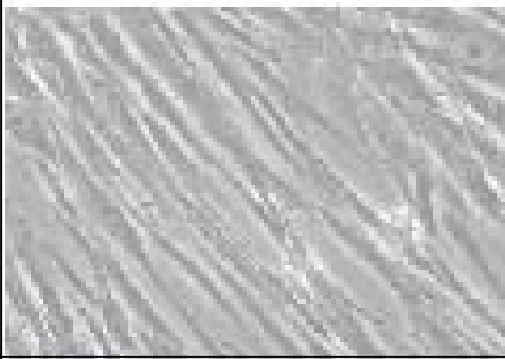




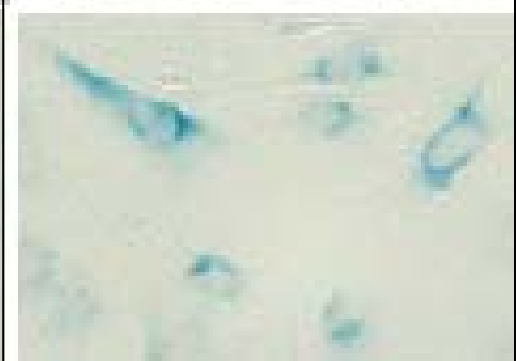
What are the biological consequences by induction of miR-34s? Similar to p53?

- Promote cell cycle arrest and reduce cell proliferation?
- Induce apoptosis or senescence?
- Stop tumor cell grow?

mir-34 inhibits cell proliferation in human fibroblasts



mir-34s induce senescence-like morphological changes

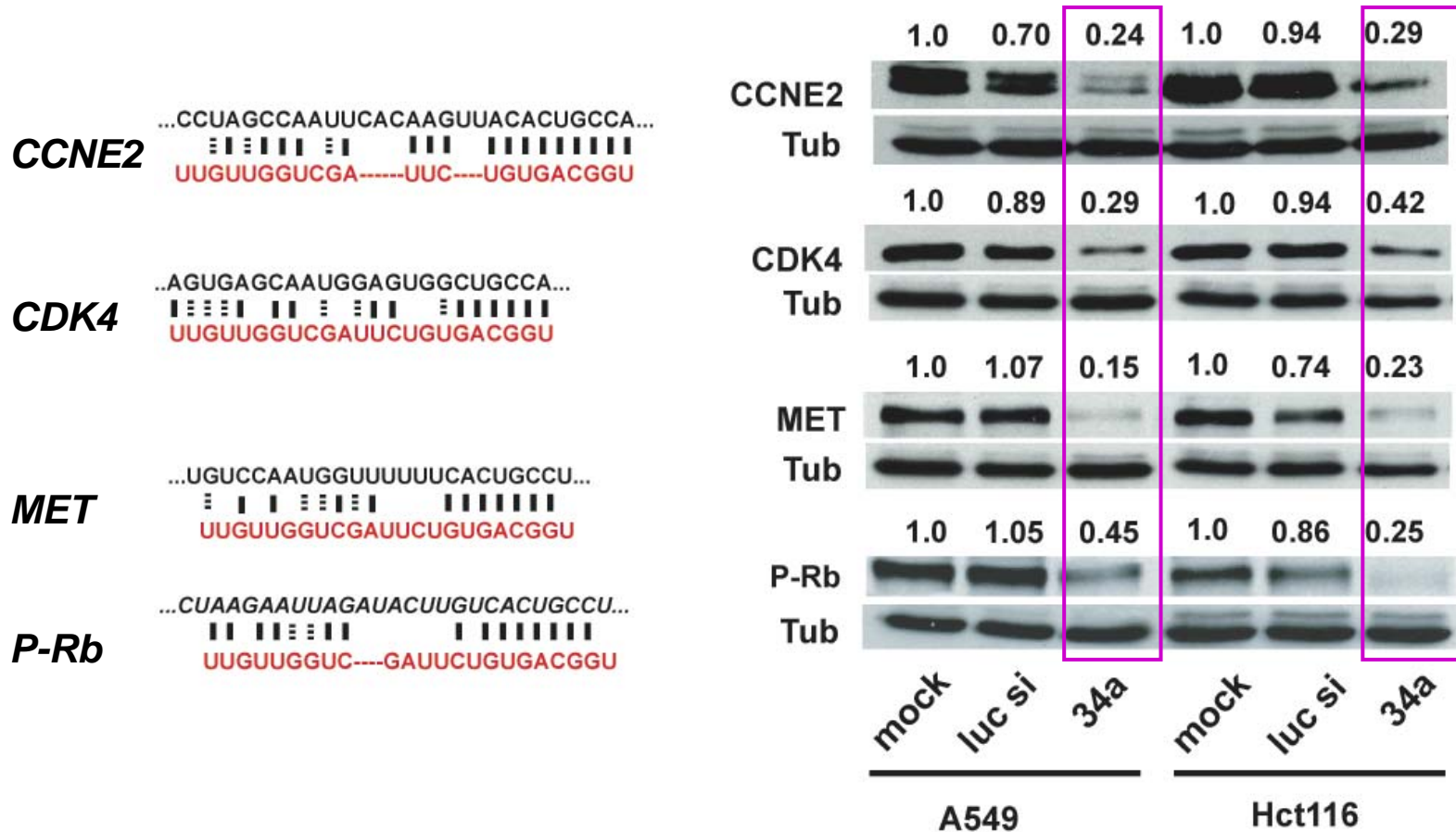
	Vector	miR-34a	miR-34b/34c
Morphology			
SA-β-Gal			

What are targets of miR-34s?

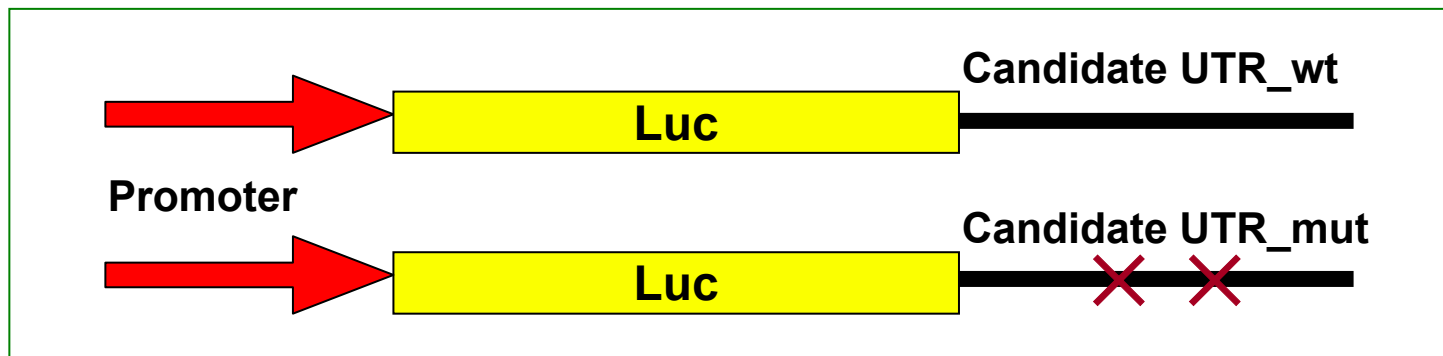
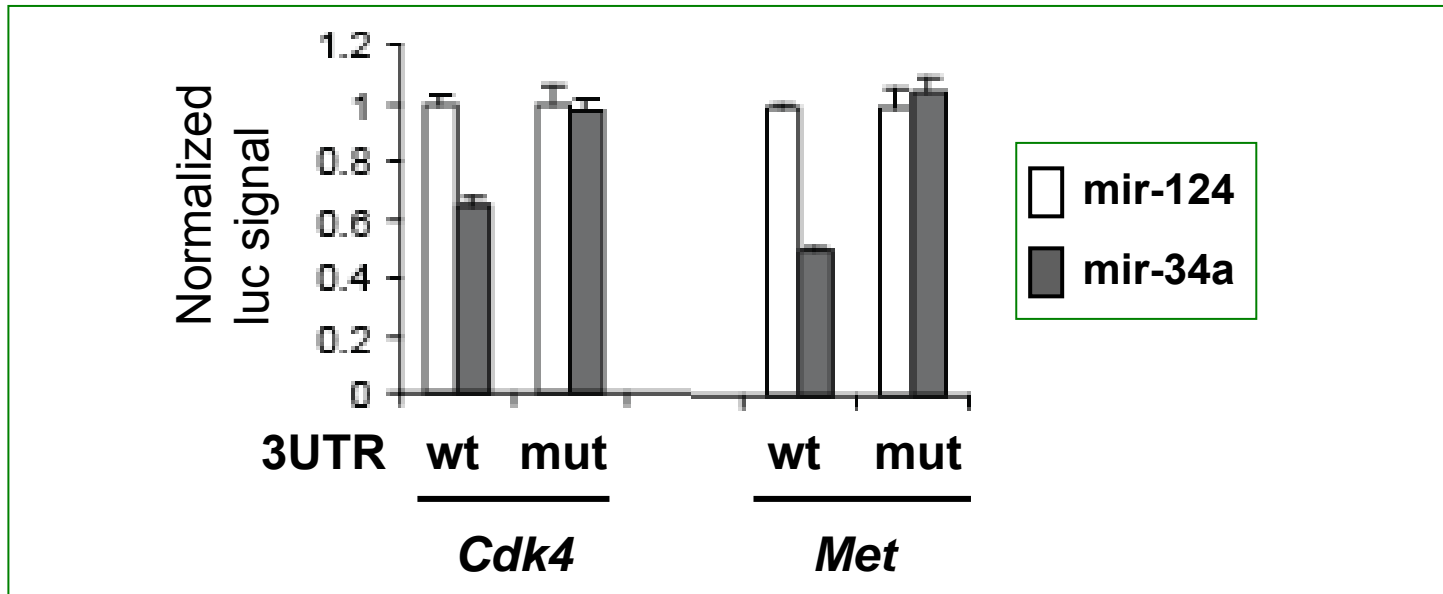
- **Strategy**

- Genome-wide mRNA expression profiling to identify genes which are significantly repressed in miR-34 over-expressed cells
 - Check whether miR-34 binding sites are enriched at the 3' UTR of these repressed genes
 - Look for genes related to cell cycles or previously known to be regulated by p53
- Four top candidate genes are chosen for further analysis
 - CDK4, CCNE2, MET, and P-Rb

Translational repression of miR-34 candidate targets

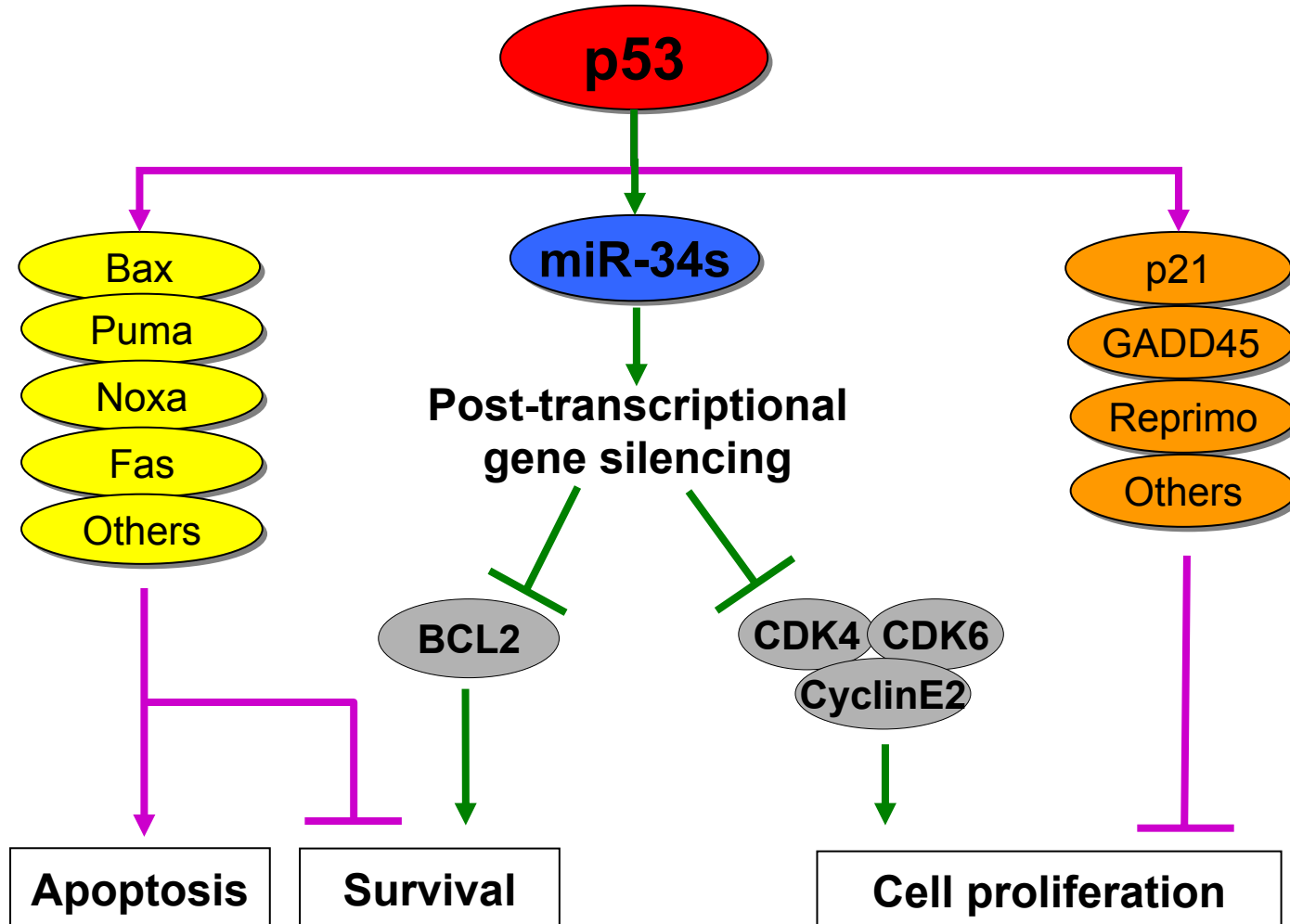


Reporter assays for candidate *mir-34* targets



New hypothesis for miR-34 function

He et al. (2007) Nature Reviews Cancer



● Acknowledgements

Assays R&D Team, Applied Biosystems

Drs. **Greg Hannon** and **Lin He**, Cold Spring Harbor Laboratory, NY

● Notes

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