MicroRNAs and Alzheimer's Disease

Pete Nelson

UNIVERSITY OF KENTUCKY

1. MicroRNA (miRNA) biology: Overview

2. Alzheimer's disease clinical-pathological studies

3. MiRNAs in neurodegenerative diseases
    A. Technical considerations
    B. Work in Alzheimer's disease

Epigenetic mechanisms have special relevance to human CNS

Epigenetic mechanisms have special relevance to human CNS

CNS epigenetics may differ from the (cancer-relevant) “classic” epigenetic mechanisms

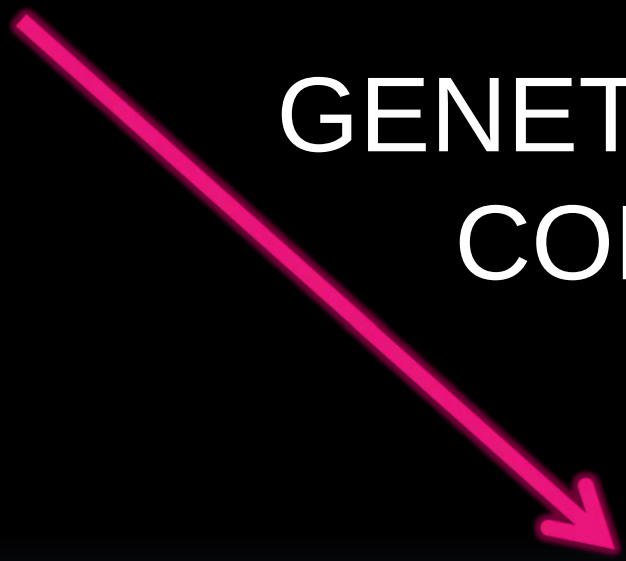
~30,000 protein-coding genes

(for every gene, > 1 billion synapses!)

~100,000,000,000,000 synapses

x ~10,000

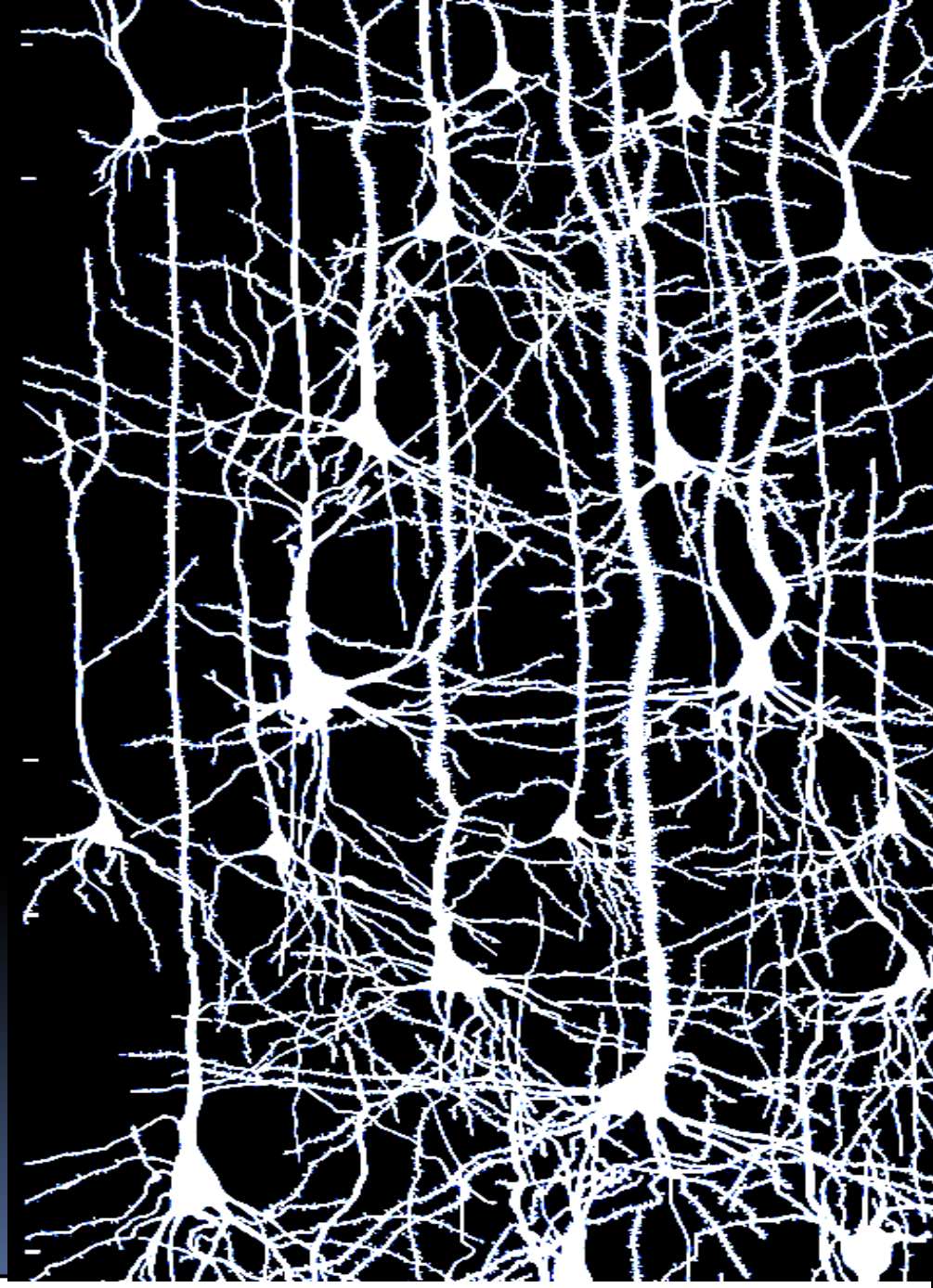
~30,000 protein-coding genes



GENETIC
COMPLEXITY
MULTIPLIERS

~100,000,000,000,000 synapses

Human neurons have special need for localized control of mRNA translation and exquisite sensitivity to the local micro-environments distal to the cell body

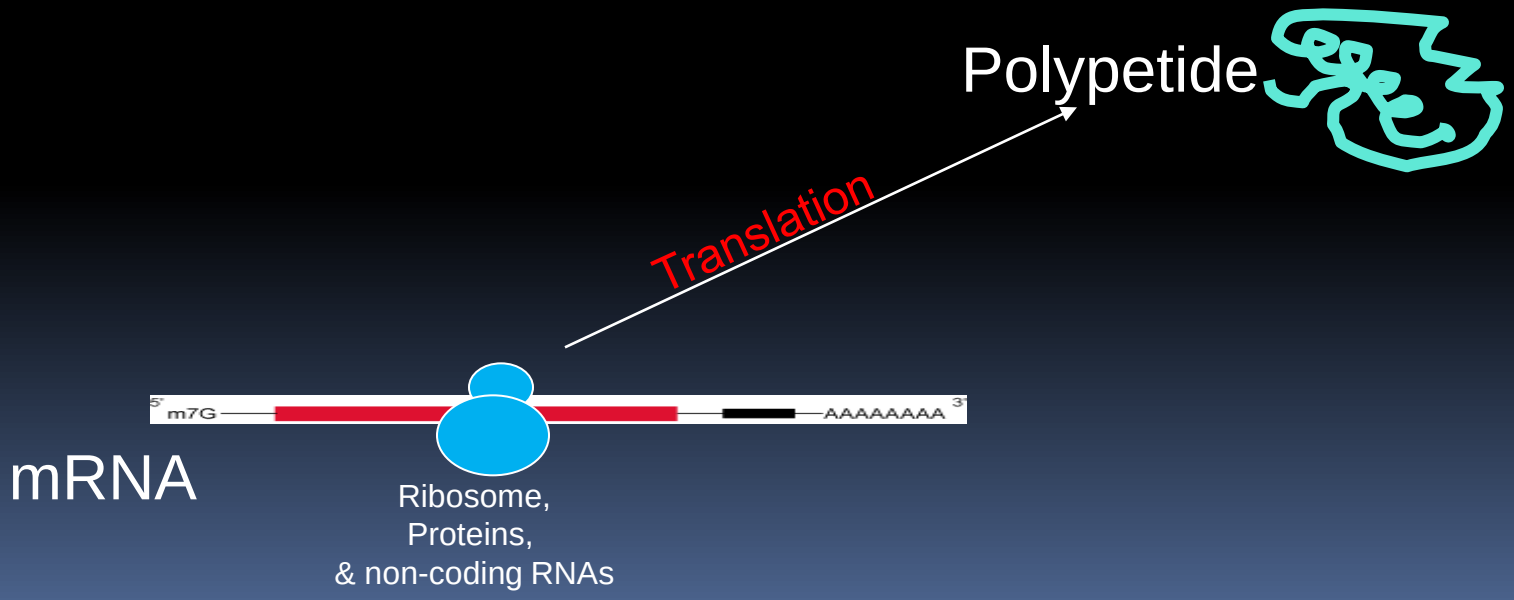


J. LeROY CONEL, Postnatal development of the human cerebral cortex (Volume VIII) 1967

Gene expression regulation:

mRNA is ~5% of total cellular RNA and is poorly correlated with protein levels

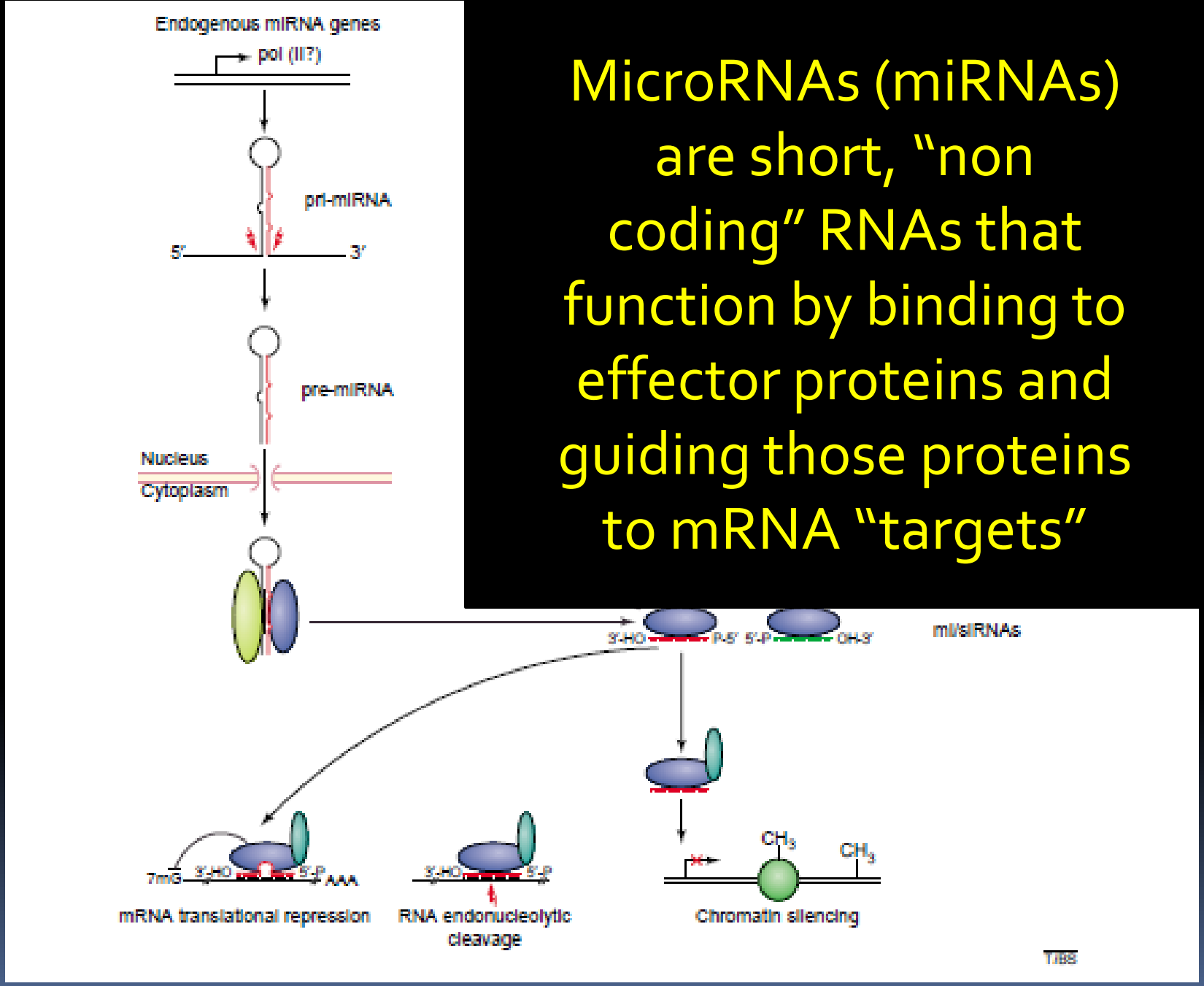
It is increasingly clear that mRNA translation is a key focal point of gene expression regulation



microRNAs (miRNAs)

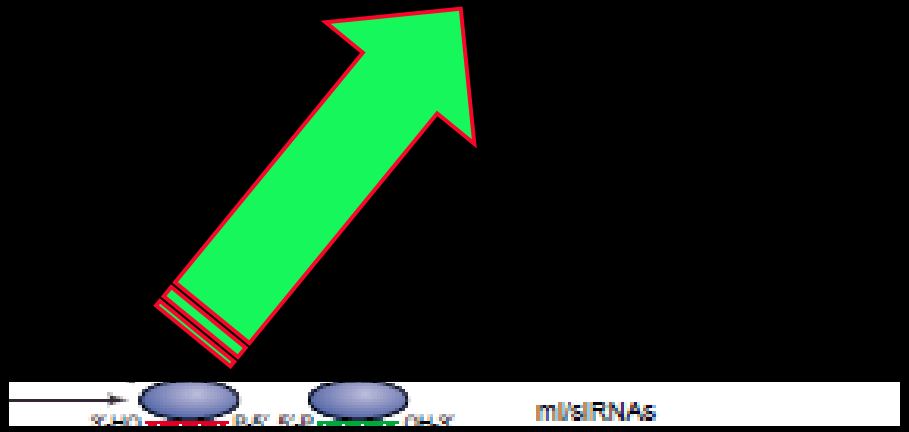
- Small noncoding RNAs (~22 nucleotides)
- Regulate mRNA translation of many/most genes
- Serve many functions in human CNS
- Increase genetic complexity and localized control of mRNA translation

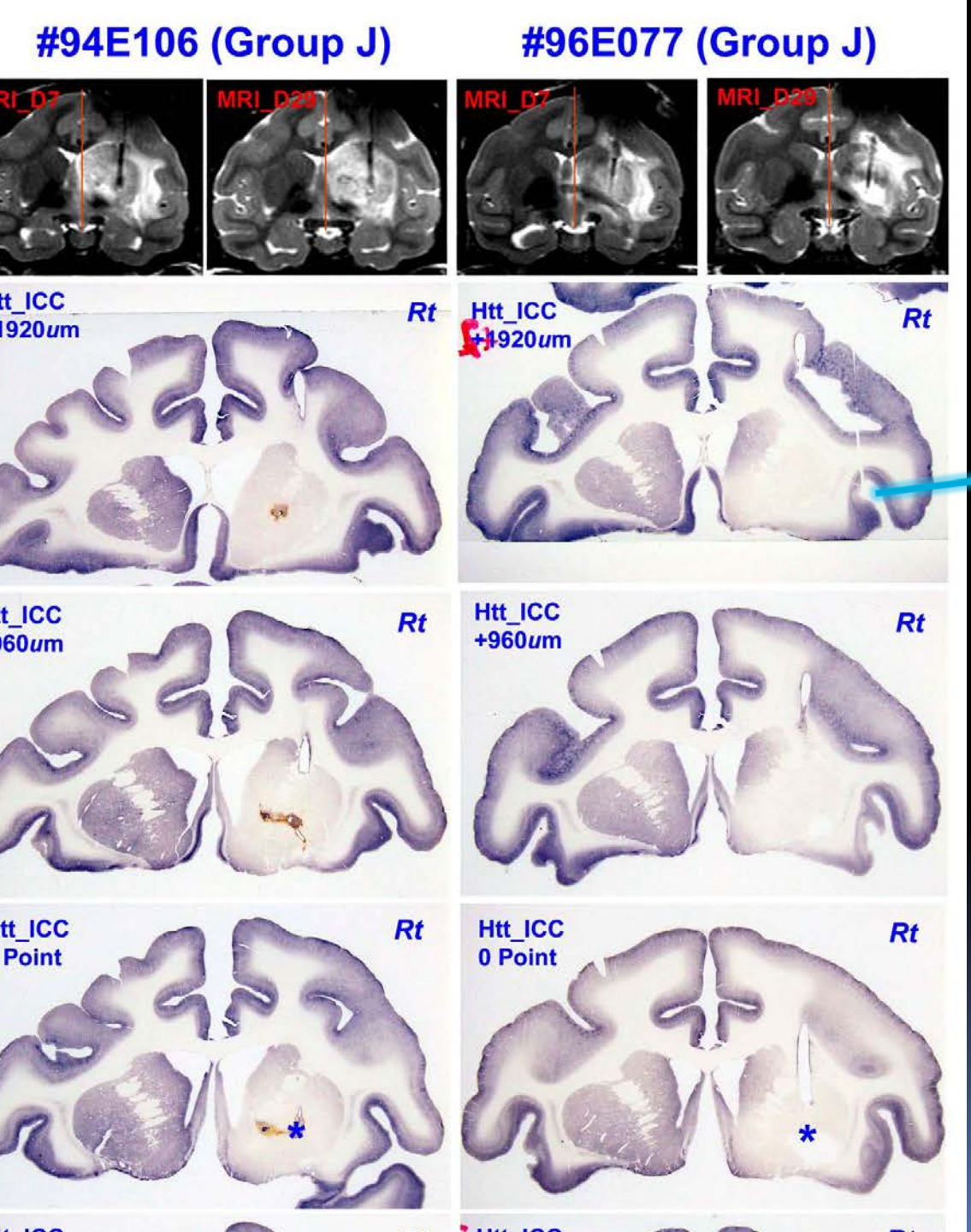
MicroRNAs (miRNAs) are short, "non coding" RNAs that function by binding to effector proteins and guiding those proteins to mRNA "targets"



Nelson P et al, Trends Bioch Sci, 2003

THERAPEUTIC POTENTIAL:
a “loaded allele-killer”





miRNA biology holds promise for therapeutic interventions

Huntingtin knock-down in Rhesus monkeys using infusions of siRNA type agent into the striatum

Much thanks to
Dr. Don Gash
(Alnylam & Medtronic)

1. MicroRNA (miRNA) biology: BRIEF overview

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   A. Technical considerations
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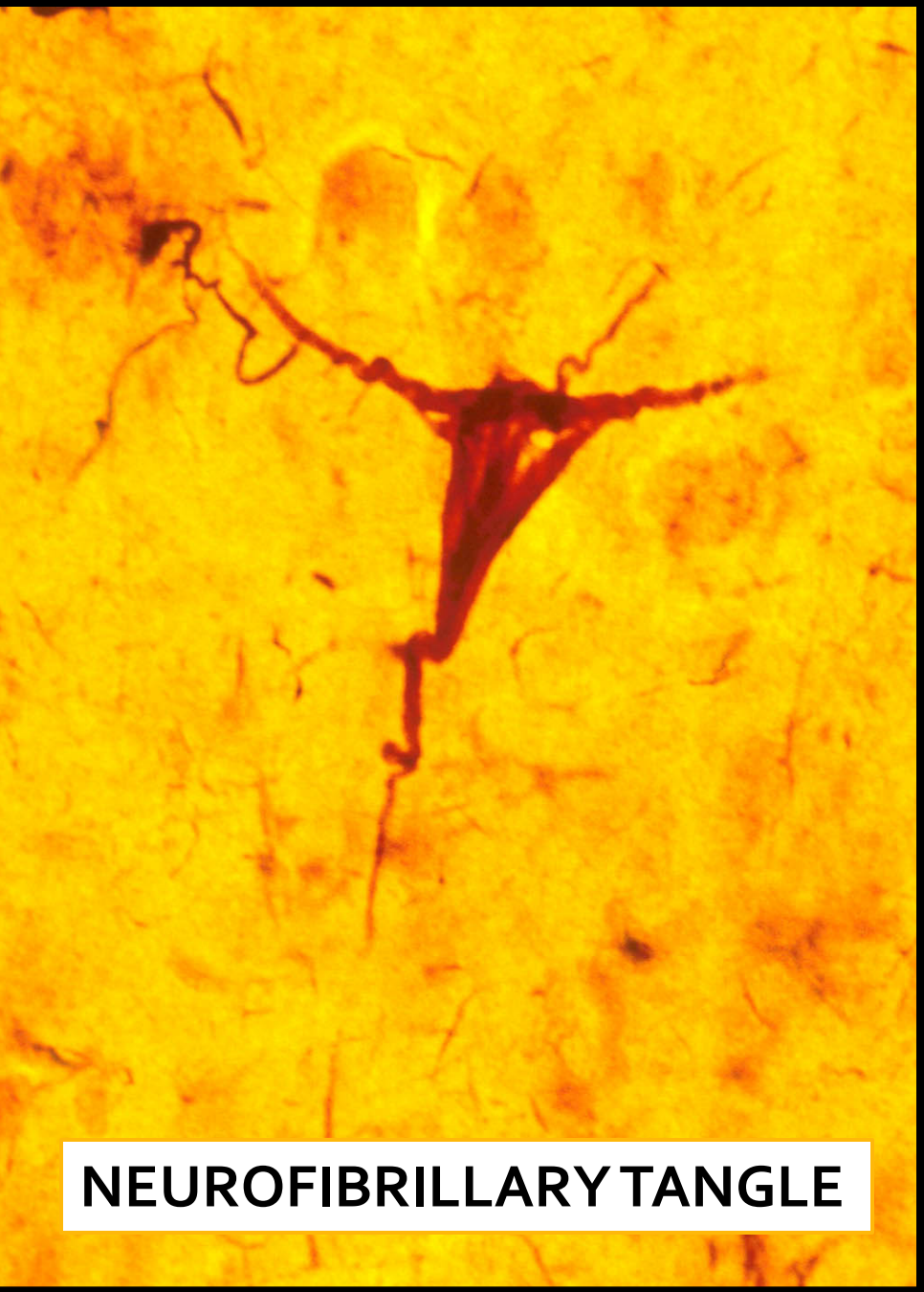
Do miRNAs play a role in Alzheimer's disease ?

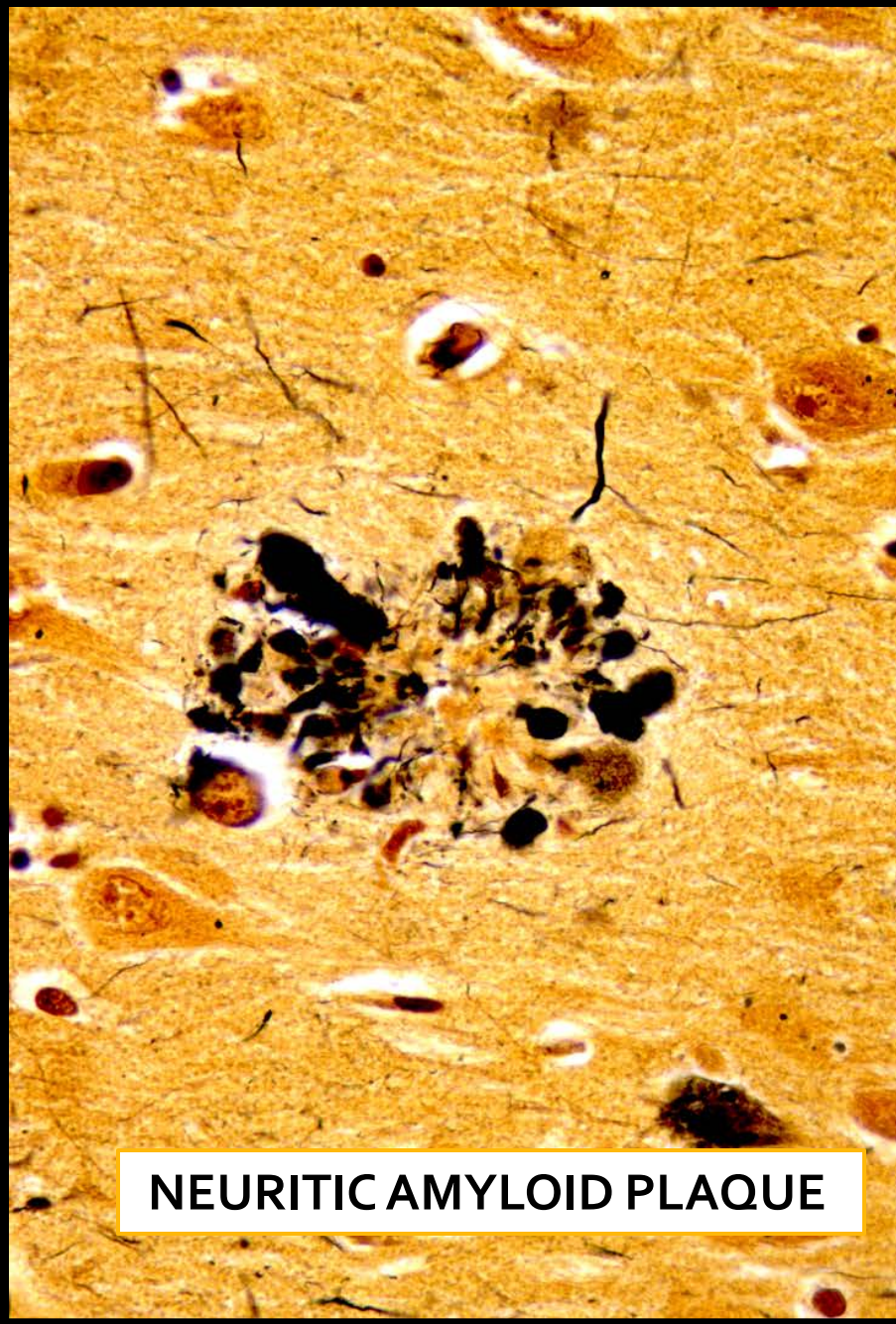
What is Alzheimer's disease ?

What is Alzheimer's disease ?

Clinical	**Cognitive impairment**
Pathological	**Neocortical neurofibrillary tangles**
	Neocortical neuritic amyloid plaques

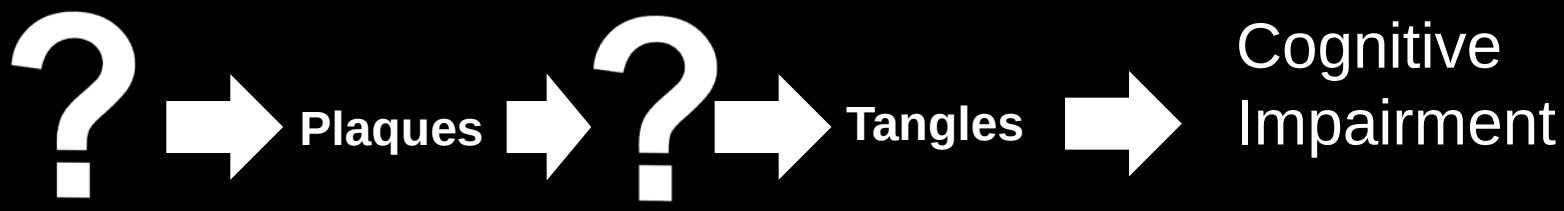


NEUROFIBRILLARY TANGLE



NEURITIC AMYLOID PLAQUE

Clinicopathological studies are compatible with the hypothesis that plaques and tangles contribute to cognitive impairment



1. MicroRNA (miRNA) biology: Overview

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   - A. Technical considerations
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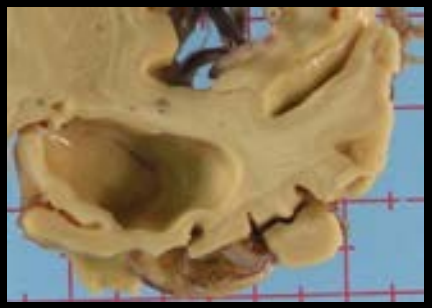
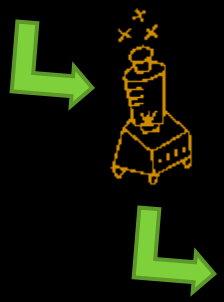
Studying miRNAs in human brain disease: technical notes

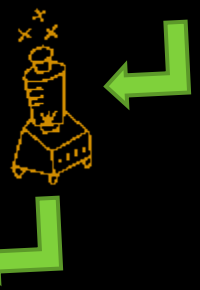
Experimental paradigm:

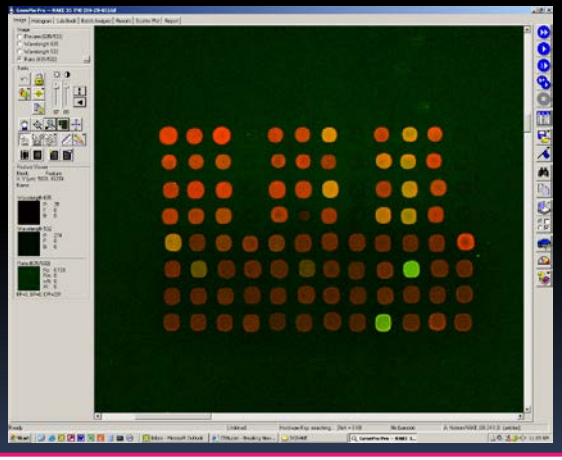
"Control"

"Alzheimer's disease"

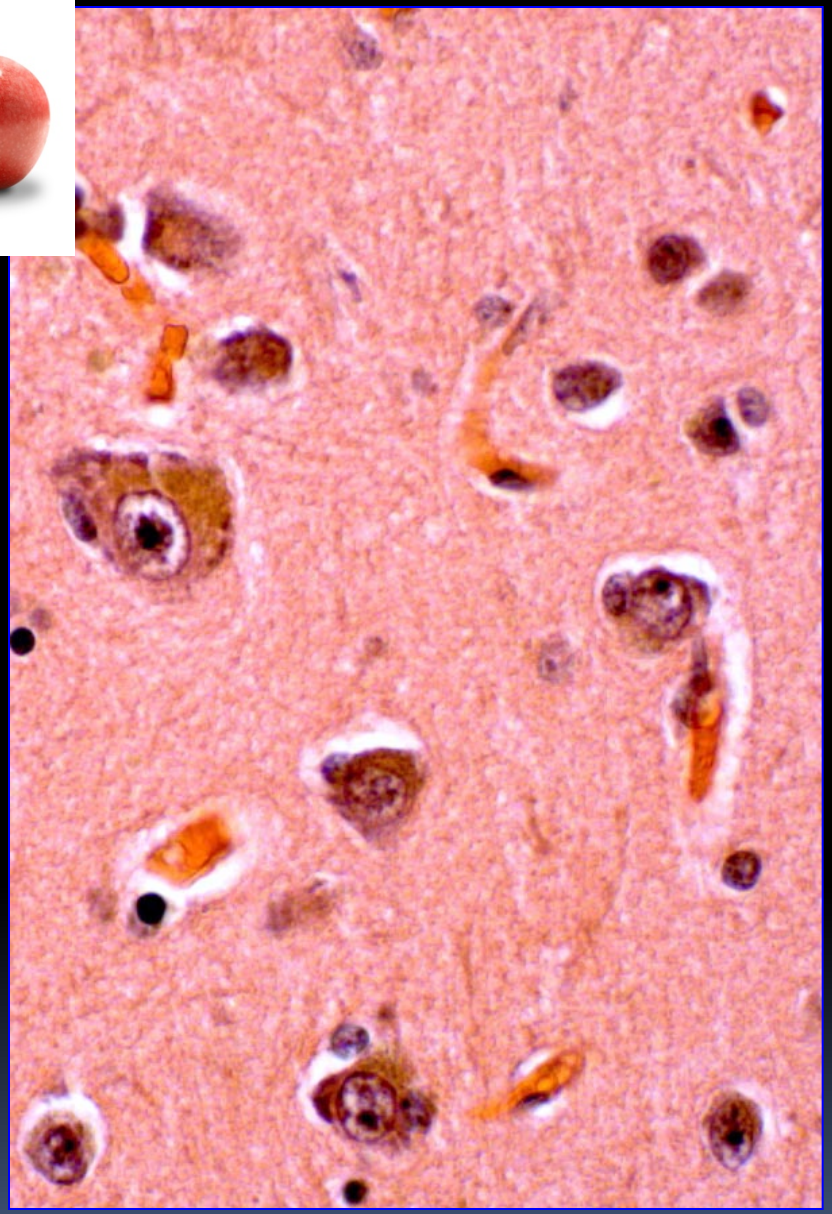


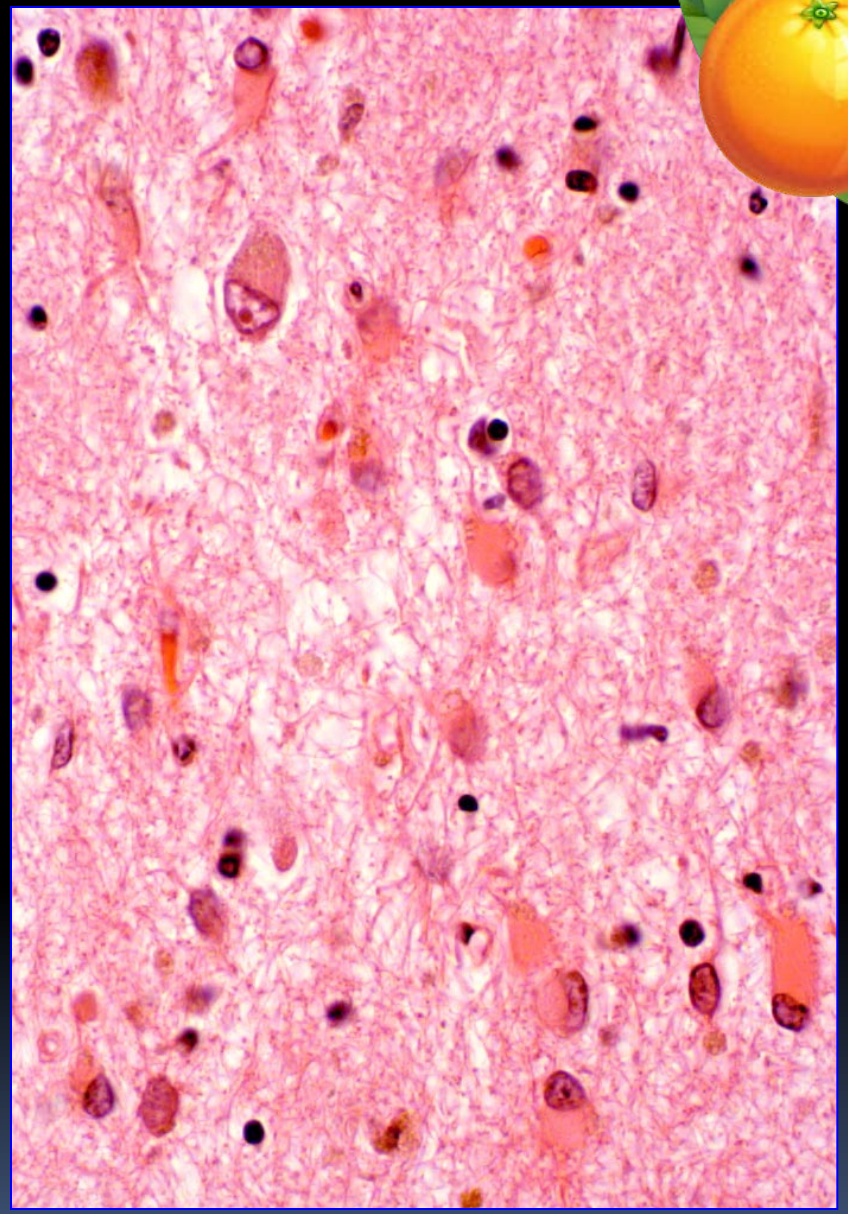






Tissue-level miRNA profiling



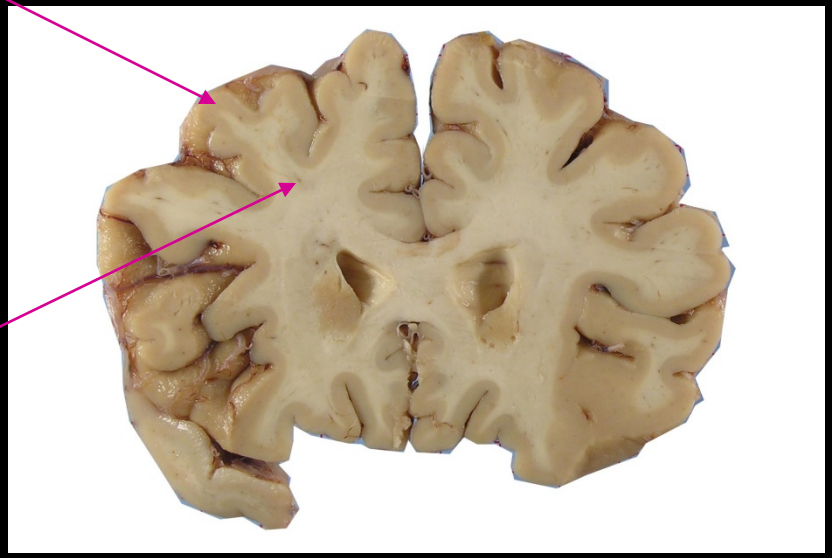


Control cerebral cortex

End-stage AD cerebral cortex

UK
UNIVERSITY OF KENTUCKY

“Gray Matter”; mostly:
-Neurons
-Astrocytes
-Rich vascular



“White Matter”; mostly:
-Oligodendrocytes
-Astrocytes
-Lacks Neurons

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Available online at www.sciencedirect.com
ScienceDirect

Biochimica et Biophysica Acta xx (2008) xxx–xxx

Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbagrm

Focus on RNA isolation: Obtaining RNA for microRNA (miRNA) expression profiling analyses of neural tissue

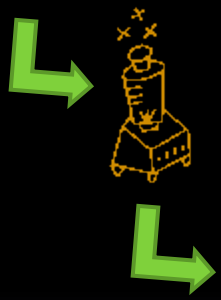
Wang-Xia Wang [a], Bernard R. Wilfred [a], Donald A. Baldwin [b], R. Benjamin Isett [b], Na Ren [c], Arnold Stromberg [c], Peter T. Nelson [a,*]

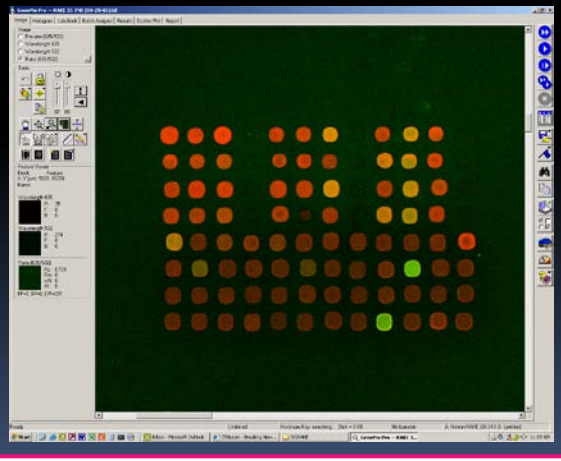
[a] *Sanders-Brown Center on Aging and Department of Pathology, University of Kentucky, USA*
[b] *Department of Pathology and Microarray Core Facility, University of Pennsylvania, USA*
[c] *Department of Statistics, University of Kentucky, USA*

Received 29 November 2007; received in revised form 15 January 2008; accepted 18 January 2008

Acta Neuropathologica, 2011

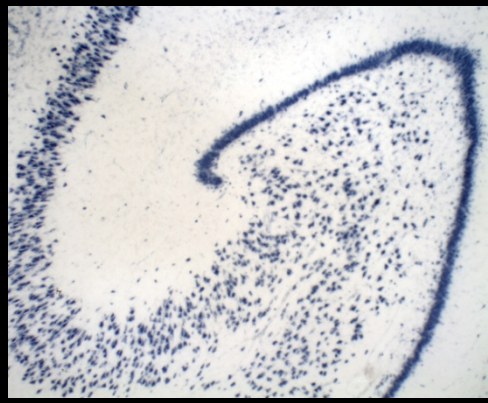
Experimental paradigm:





Tissue-level miRNA profiling

MiRNA in situ hybridization



Should be complemented by cellular-level miRNA profiling

RAKE and LNA-ISH reveal microRNA expression and localization in archival human brain

PETER T. NELSON,[1] DON A. BALDWIN,[1] WIGARD P. KLOOSTERMAN,[2] SAKARI KAUPPINEN,[3] RONALD H.A. PLASTERK,[2] and ZISSIMOS MOURELATOS[1]

[1]Division of Neuropathology and Department of Pathology & Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104, USA
[2]Hubrecht Laboratory, Centre for Biomedical Genetics, Utrecht, The Netherlands
[3]Wilhelm Johannsen Centre for Functional Genome Research, Institute of Medical Biochemistry and Genetics, University of Copenhagen, Denmark

RNA, 2006

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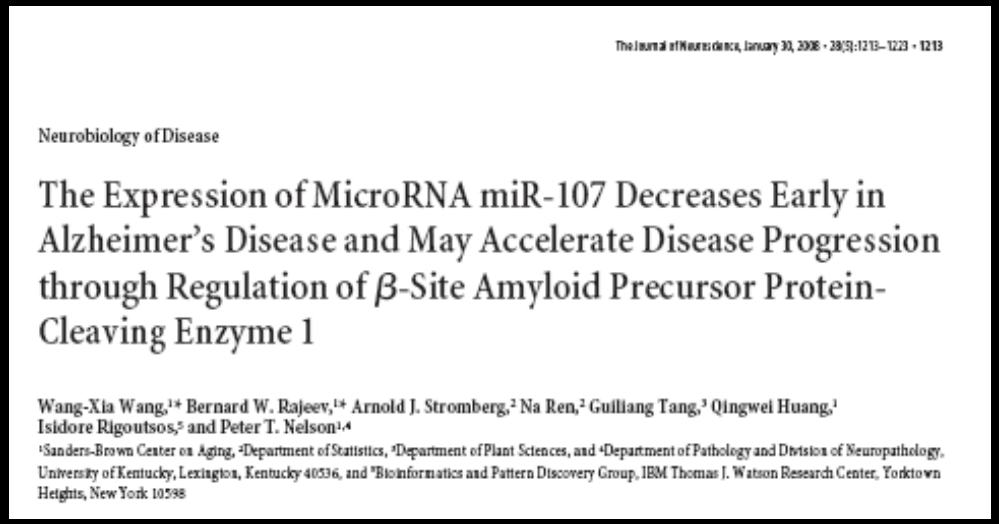
Our Lab's Overall Goal:

Identify all miRNA targets in human brain across a range of pathologies

Our Lab's Overall Goal:
Identify all miRNA targets in human brain across a range of pathologies

My Goal for next 8 minutes:
Convince you that this is a worthy goal by demonstrating an example of a miRNA "story" relevant to AD.

J Neuroscience, 2008



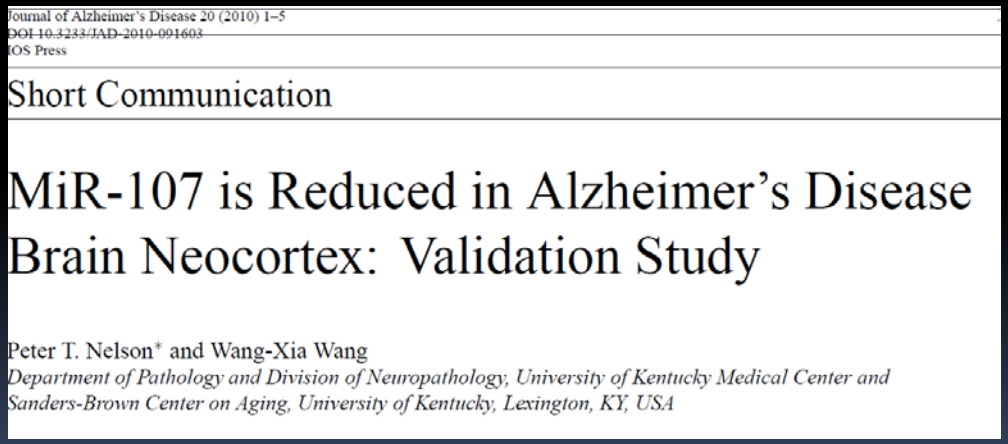
The Journal of Neuroscience, January 30, 2008 • 28(5):1213–1223 • 1213

Neurobiology of Disease

The Expression of MicroRNA miR-107 Decreases Early in Alzheimer's Disease and May Accelerate Disease Progression through Regulation of β-Site Amyloid Precursor Protein-Cleaving Enzyme 1

Wang-Xia Wang,[1]* Bernard W. Rajeev,[1]* Arnold J. Stromberg,[2] Na Ren,[2] Guiliang Tang,[3] Qingwei Huang,[1] Isidore Rigoutsos,[5] and Peter T. Nelson[1,4]

[1]Sanders-Brown Center on Aging, [2]Department of Statistics, [3]Department of Plant Sciences, and [4]Department of Pathology and Division of Neuropathology, University of Kentucky, Lexington, Kentucky 40536, and [5]Bioinformatics and Pattern Discovery Group, IBM Thomas J. Watson Research Center, Yorktown Heights, New York 10598



Journal of Alzheimer's Disease 20 (2010) 1–5
DOI 10.3233/JAD-2010-091603
IOS Press

Short Communication

MiR-107 is Reduced in Alzheimer's Disease Brain Neocortex: Validation Study

Peter T. Nelson* and Wang-Xia Wang
Department of Pathology and Division of Neuropathology, University of Kentucky Medical Center and Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY, USA

J Alzheimer's Disease, 2010

miR-107
is a miRNA that we found to be down-regulated early in AD pathogenesis

Journal of Neurochemistry

JNC

JOURNAL OF NEUROCHEMISTRY | 2011 | 116 | 240–247

doi: 10.1111/j.1471-4159.2010.07097.x

In vivo regulation of amyloid precursor protein neuronal splicing by microRNAs

Pascal Smith,*,† Amelle Al Hashimi,*,† Johanne Girard,*,† Charlotte Delay*,† and Sébastien S. Hébert*,†

*Centre de recherche du CHUQ (CHUL), Axe Neurosciences, Québec, Canada

†Département de psychiatrie et de neurosciences, Faculté de médecine, Université Laval, Québec, Canada

Outside validation: Human study

Journal of Neurochemistry

JNC

JOURNAL OF NEUROCHEMISTRY | 2011 | 116 | 240–247

doi: 10.1111/j.1471-4159.2010.07097.x

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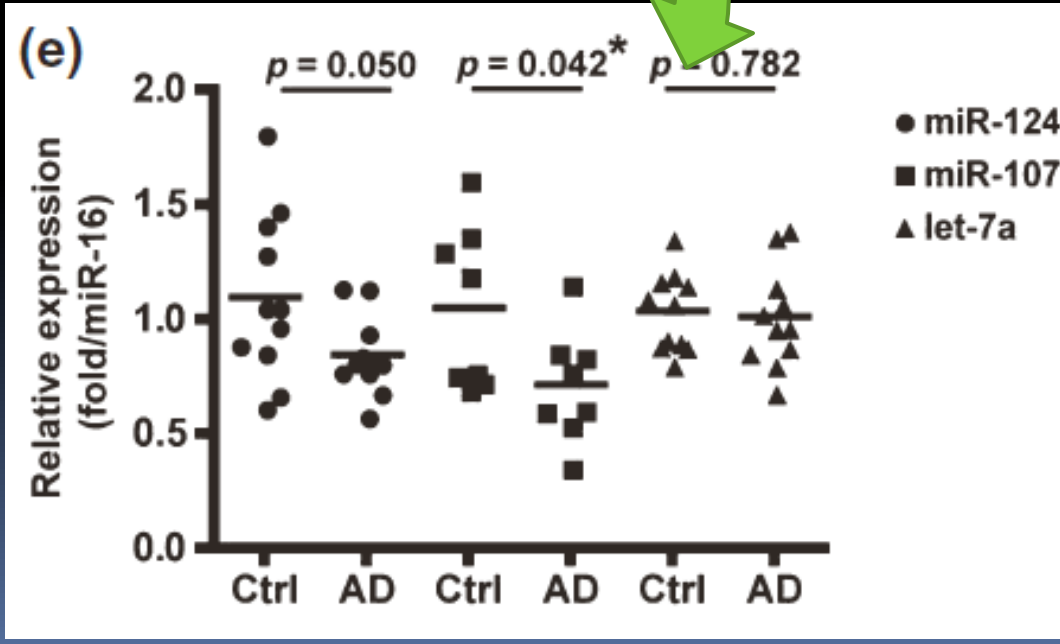
†Département de psychiatrie et de neurosciences, Faculté de médecine, Université Laval, Québec, Canada



OPEN ACCESS Freely available online

PLoS one

MicroRNA-Related Cofilin Abnormality in Alzheimer's Disease

Jiaqi Yao[1], Tom Hennessey[1], Alex Flynt[2], Eric Lai[2], M. Flint Beal[1], Michael T. Lin[1]*

1 Department of Neurology, Weill-Cornell Medical College, New York, New York, United States of America, 2 Department of Developmental Biology, Memorial Sloan Kettering Cancer Center, New York, New York, United States of America

Outside validation: Transgenic mice study

OPEN ACCESS Freely available online

PLoS one

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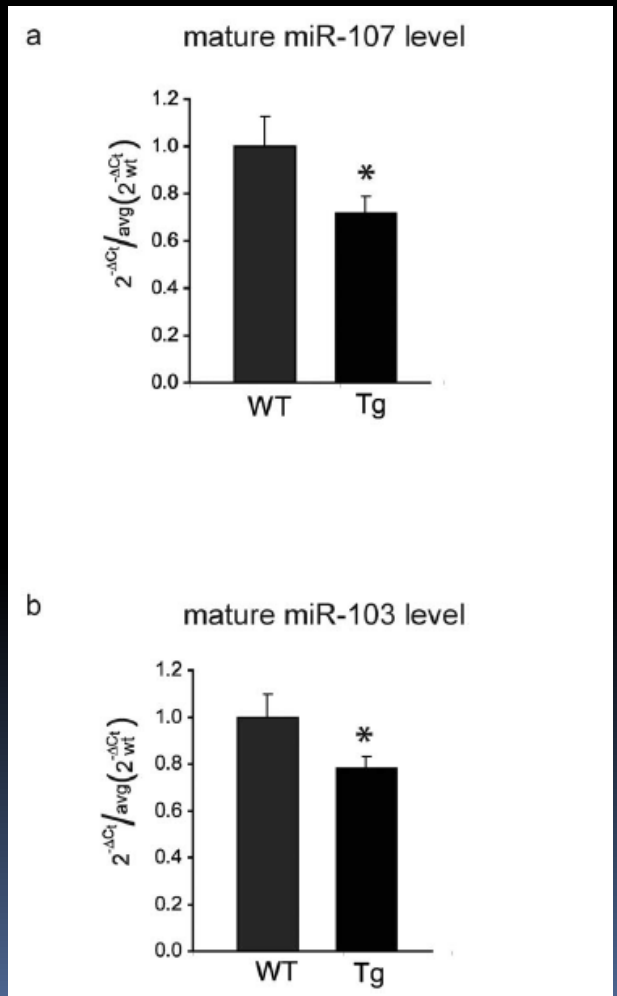
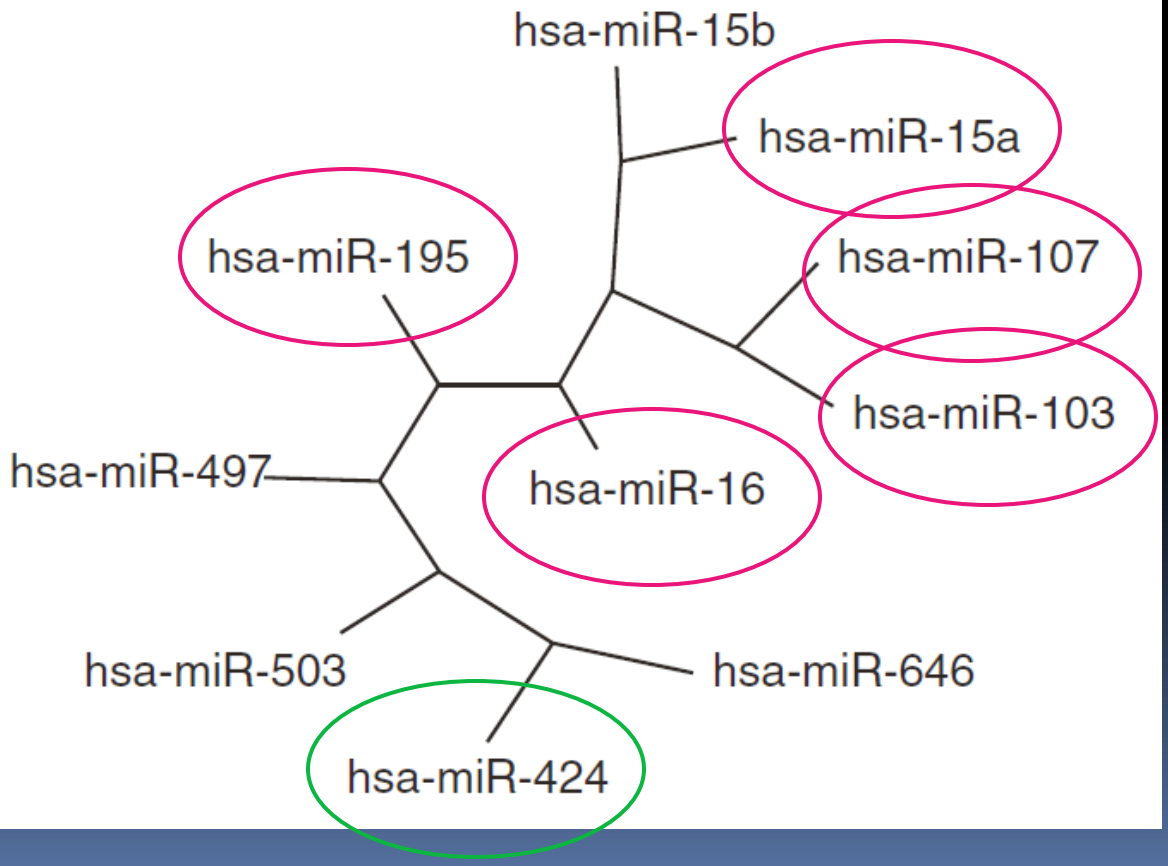


Figure 5. MiR-103 and miR-107 levels are decreased in APP transgenic mouse brains. (a) Levels of mature miR-107 are

	*		#
hsa-miR-107	A G C A G C	A U U G U A C A G G G C U A U C A
hsa-miR-103	A G C A G C	A U U G U A C A G G G C U A U G A
hsa-miR-15a U	A G C A G C	A C A U A A U G G U U U G U G
hsa-miR-15b U	A G C A G C	A C A U C A U G G U U U A C A
hsa-miR-16 U	A G C A G C	A C G U A A A U A U U G G C G
hsa-miR-195 U	A G C A G C	A C A G A A A U A U U G G C C
hsa-miR-497 C	A G C A G C	A C A C U G U G G U U U G U
hsa-miR-503 U	A G C A G C	G G G A A C A G U U C U G C A G
hsa-miR-424 C	A G C A G C	A A U U C A U G U U U U G A A
hsa-miR-646 A	A G C A G C	U G C C U C U G A G G C



miR-15/107 gene group:

Expressed in all human cells

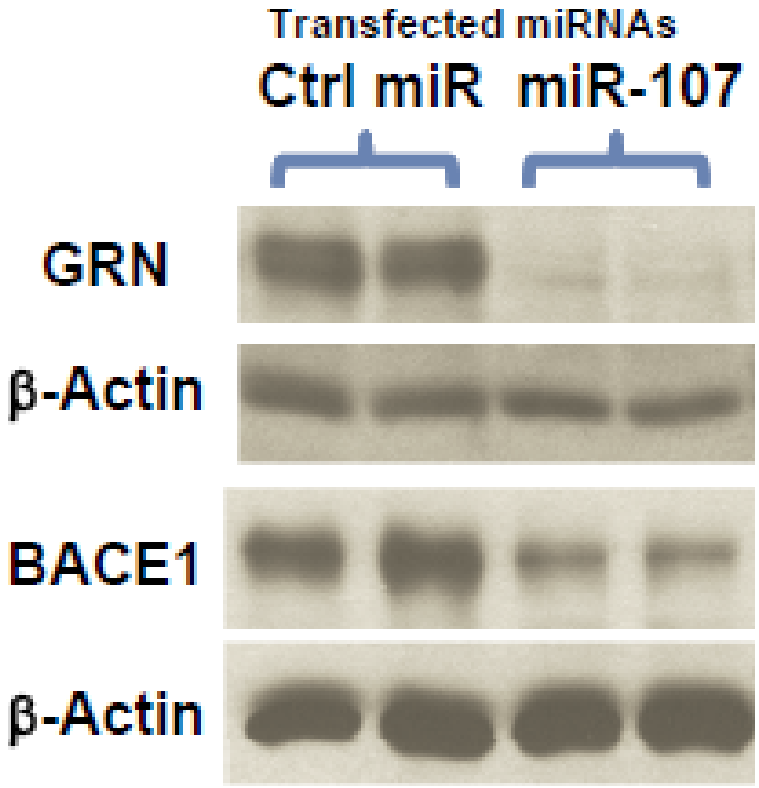
Similar 5' seed motif
--dictates activity

5 different highly-expressed miRNAs downregulated in AD gray matter: strong potential amplification effect

J Mol Biol, 2010
Acta Neuropathol 2011

Because of the studies in human brains, we wanted to know the targets of miR-107

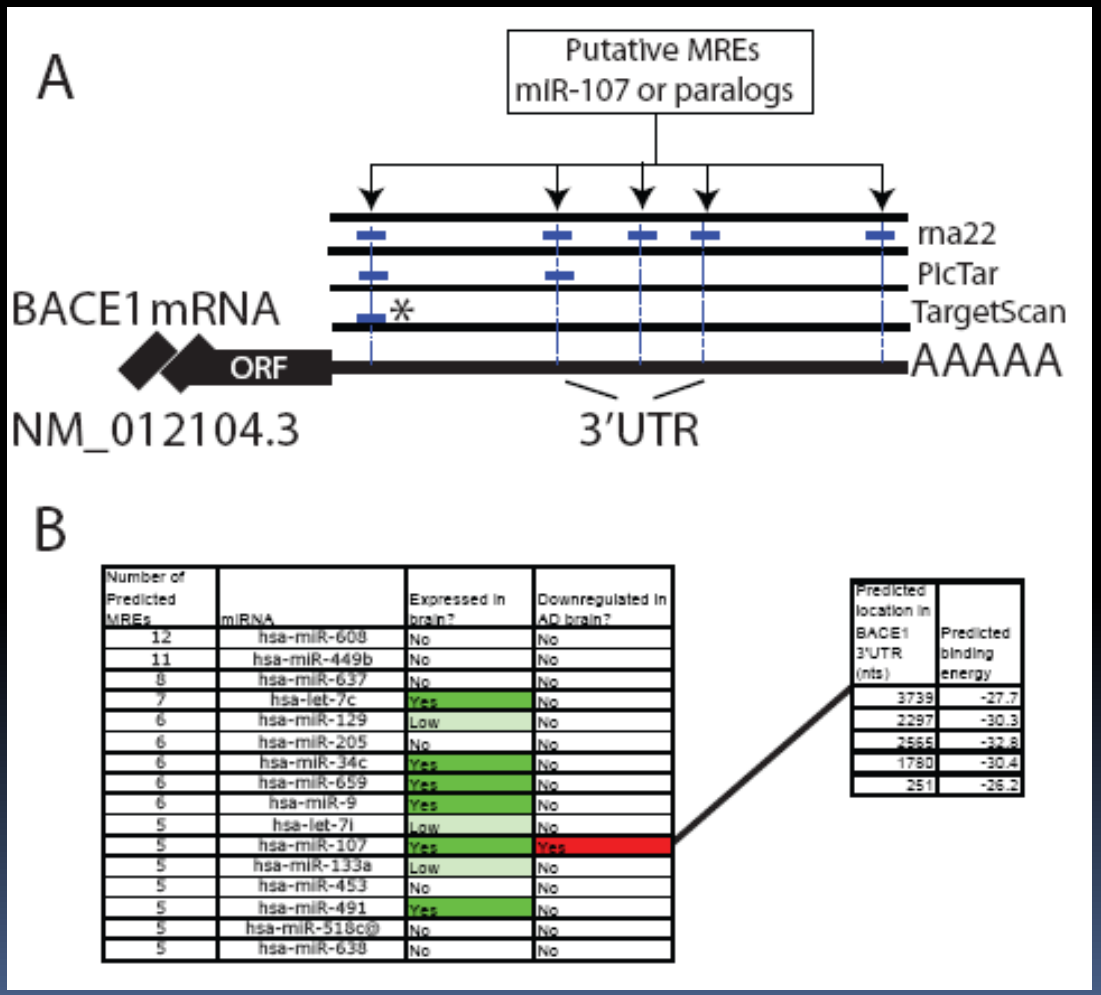
Western blots after miRNA transfections



miR-107 regulates both GRN/PGRN and BACE1

BACE1:

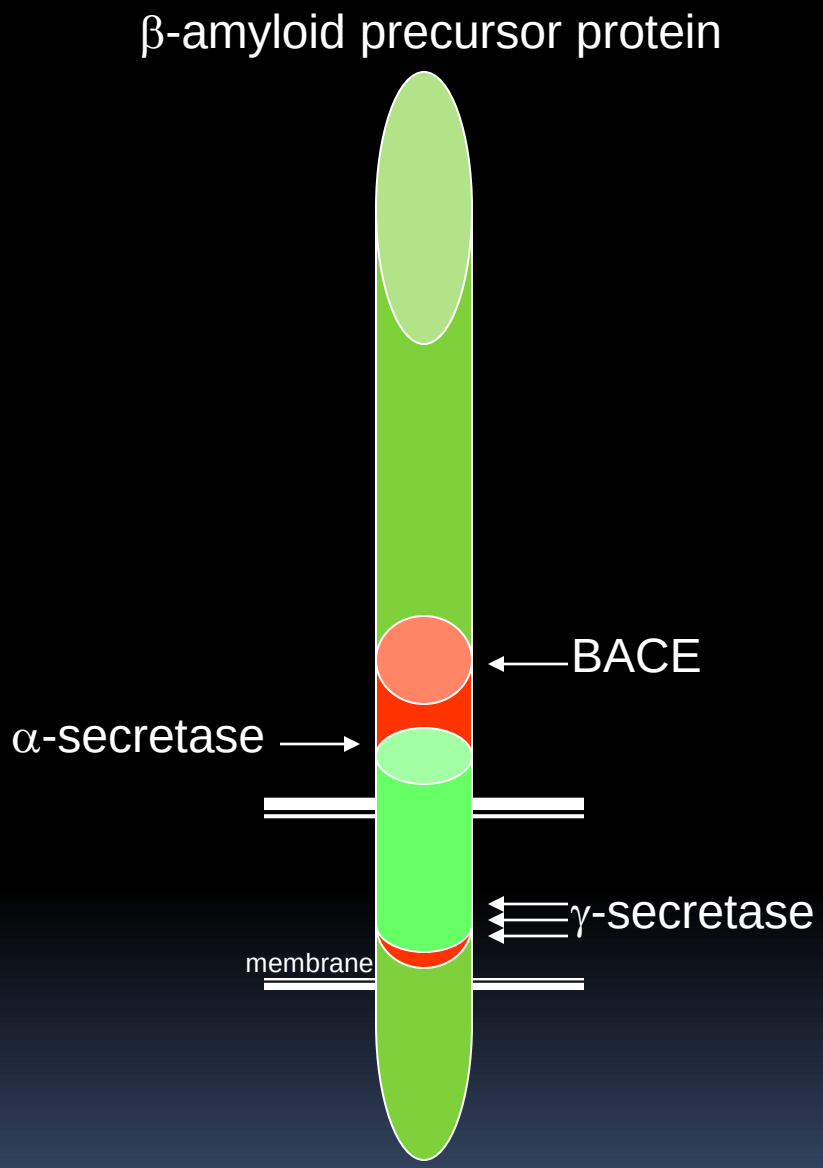
Beta-site amyloid precursor protein cleaving enzyme 1

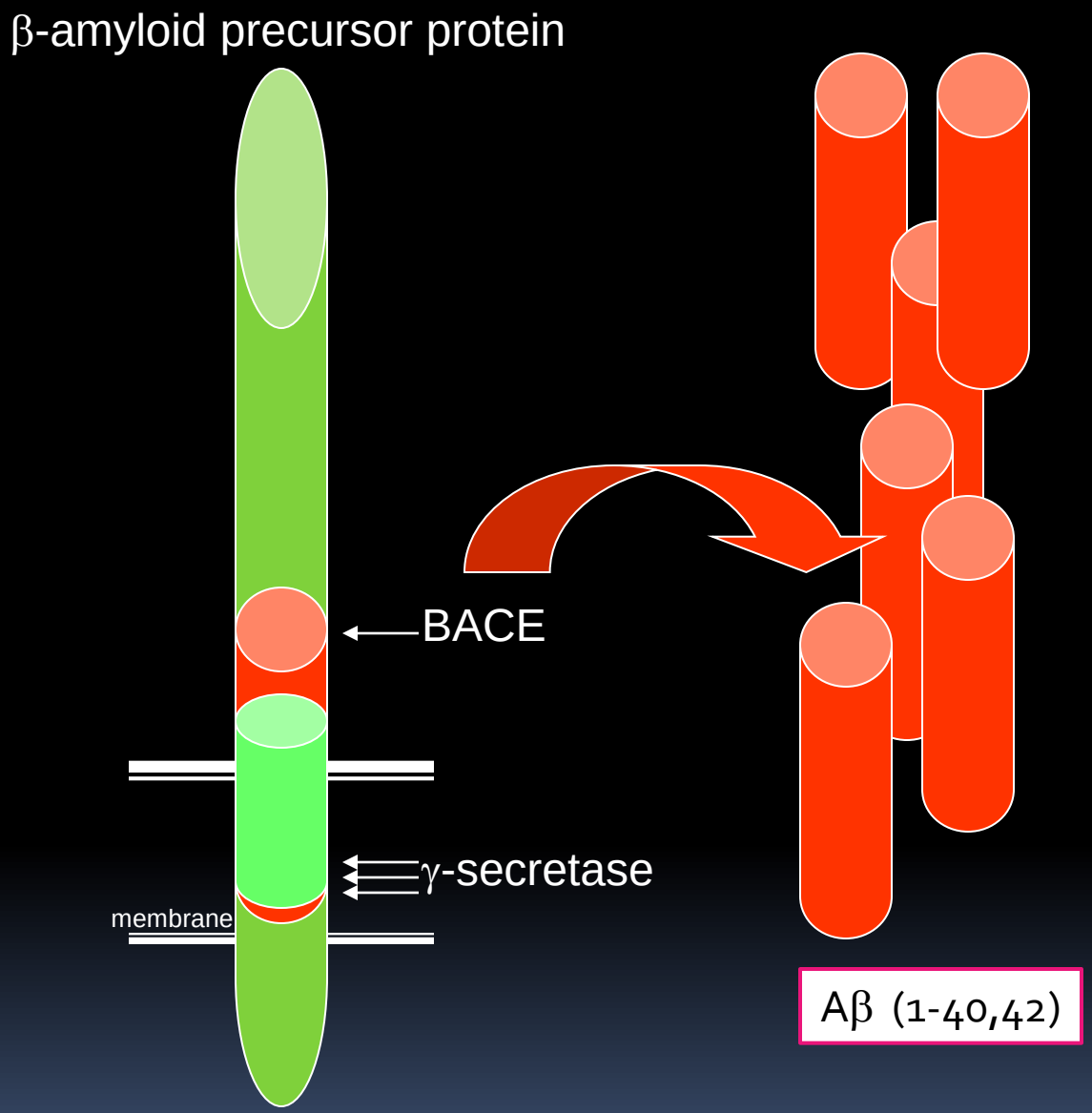


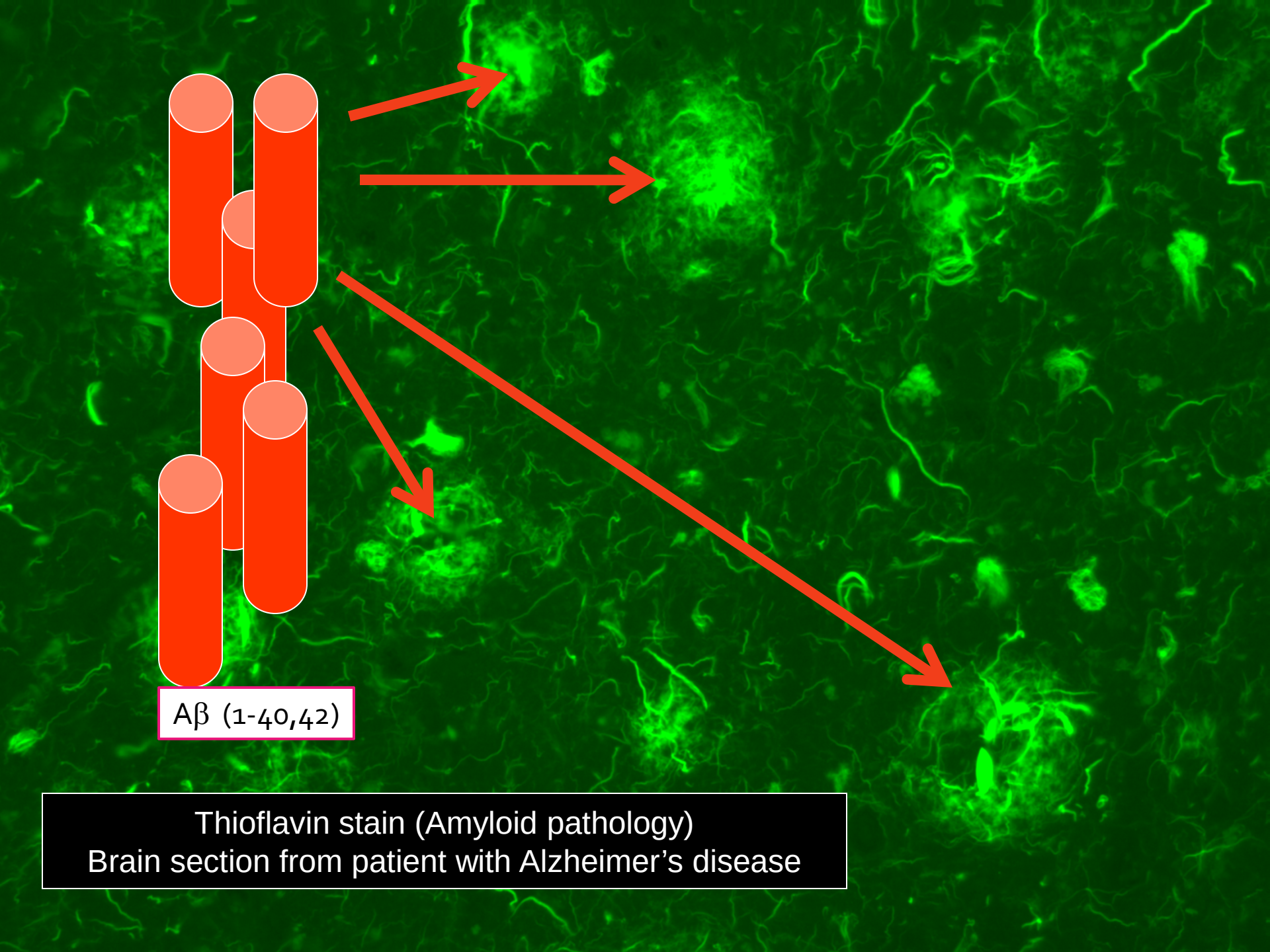
Number of Predicted MREs	miRNA	Expressed in brain?	Downregulated in AD brain?
12	hsa-miR-608	No	No
11	hsa-miR-449b	No	No
8	hsa-miR-637	No	No
7	hsa-let-7c	Yes	No
6	hsa-miR-129	Low	No
6	hsa-miR-205	No	No
6	hsa-miR-34c	Yes	No
6	hsa-miR-659	Yes	No
6	hsa-miR-9	Yes	No
5	hsa-let-7i	Low	No
5	hsa-miR-107	Yes	Yes
5	hsa-miR-133a	Low	No
5	hsa-miR-453	No	No
5	hsa-miR-491	Yes	No
5	hsa-miR-518c@	No	No
5	hsa-miR-638	No	No

Predicted location in BACE1 3'UTR (nts)	Predicted binding energy
3739	-27.7
2297	-30.3
2565	-32.8
1780	-30.4
251	-26.2

W-X Wang et al,
J Neurosci 2008


β-amyloid precursor protein
BACE
α-secretase
γ-secretase
membrane


β-amyloid precursor protein
BACE
γ-secretase
membrane
Aβ (1-40,42)



Thioflavin stain (Amyloid pathology)
Brain section from patient with Alzheimer's disease

BACE1:

Beta-site amyloid precursor protein cleaving enzyme 1

Cleaves APP to produce toxic Abeta(1-40) and/or Abeta(1-42) peptides

Important point:

↓ **BACE1 -- GOOD**

↑ **BACE1 -- BAD**

Ohno, M., E.A. Sametsky, L.H. Younkin, H. Oakley, S.G. Younkin, M. Citron, R. Vassar, and J.F. Disterhoft, BACE1 deficiency rescues memory deficits and cholinergic dysfunction in a mouse model of Alzheimer's disease. Neuron, 2004. 41(1): p. 27-33.

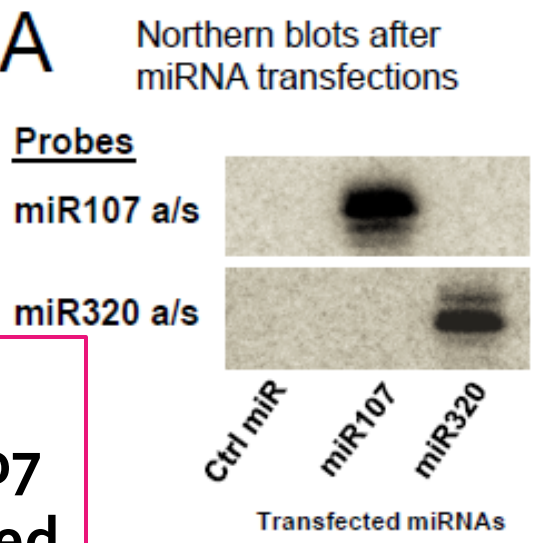
Marambaud, P., N. Chevallier, K. Ancolio, and F. Checler, Post-transcriptional contribution of a cAMP-dependent pathway to the formation of alpha- and beta/gamma-secretases-derived products of beta APP maturation in human cells expressing wild-type and Swedish mutated beta APP. Mol Med, 1998. 4(11): p. 715-23.

Rossner, S., M. Sastre, K. Bourne, and S.F. Lichtenthaler, Transcriptional and translational regulation of BACE1 expression--implications for Alzheimer's disease. Prog Neurobiol, 2006. 79(2): p. 95-111.

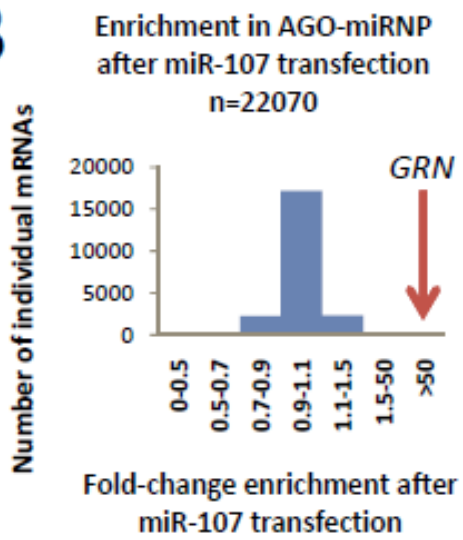
GRN/PGRN was identified as miR-107 target using unbiased high-throughput screening method

GRN was by far the strongest target for miR-107 using this technique

A



B



C

	Fold mRNA enrichment relative to negative control miRNA transfection				
	anti-Ago Co-IP		Lysate		
Gene Symbols	miRNA transfected				Target Prediction?
	miR-107	miR-320	miR-107	miR-320	
GRN	52.4	0.8	0.5	0.8	None
IER3IP1	33.4	0.9	0.7	1.0	None
RPLP0	8.0	2.3	0.7	1.1	None
C22orf13	3.8	1.1	0.4	0.9	None
INSIG1	3.6	0.9	1.0	1.2	None
ZDHHC4	3.3	1.0	0.6	0.9	None
METRNL	2.8	1.1	1.5	1.4	None
RPL27	2.7	1.3	1.0	1.1	None
SAT1	2.6	1.6	2.8	1.2	None
CSNK1G2	2.5	1.4	0.3	1.0	TS, PT
NINJ1	2.5	3.2	0.8	1.0	None

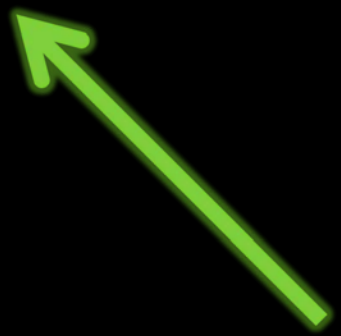
Am J Pathol, 2010

GRN/Granulin

aka

Progranulin (PGRN)
Acrogranin
Glycoprotein 88kDa (Gp88)
Proepithelin
PC cell-derived growth factor (PCDGF)
Epithelial transforming growth factor (TGFe)
Granulin-epithelin precursor (GEP)

Strong growth factor
Inflammation
Wound repair
Neoplasia



GRN/Granulin

GRN/Granulin

haploinsufficiency

Frontotemporal dementia

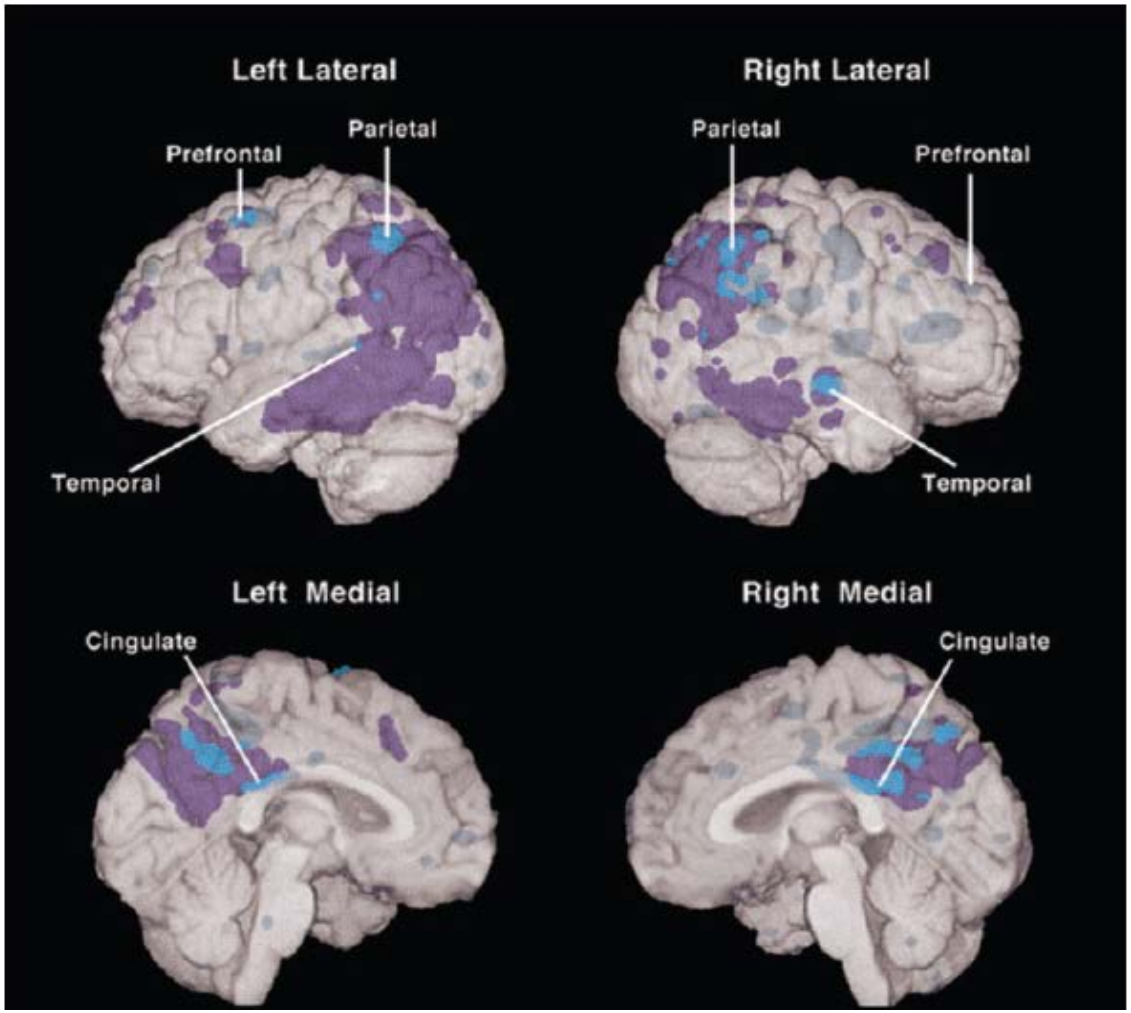
Is there a plausible hypothesis to explain significance of miR-107 decrease in AD brains?

Is there a plausible hypothesis to explain significance of miR-107 decrease in AD brains?

3 Clues

1. Genomics
2. Glucose (Tang et al, Sabire Ozcan Lab)
3. Brain Trauma (Redell et al, Pramod Dash Lab)

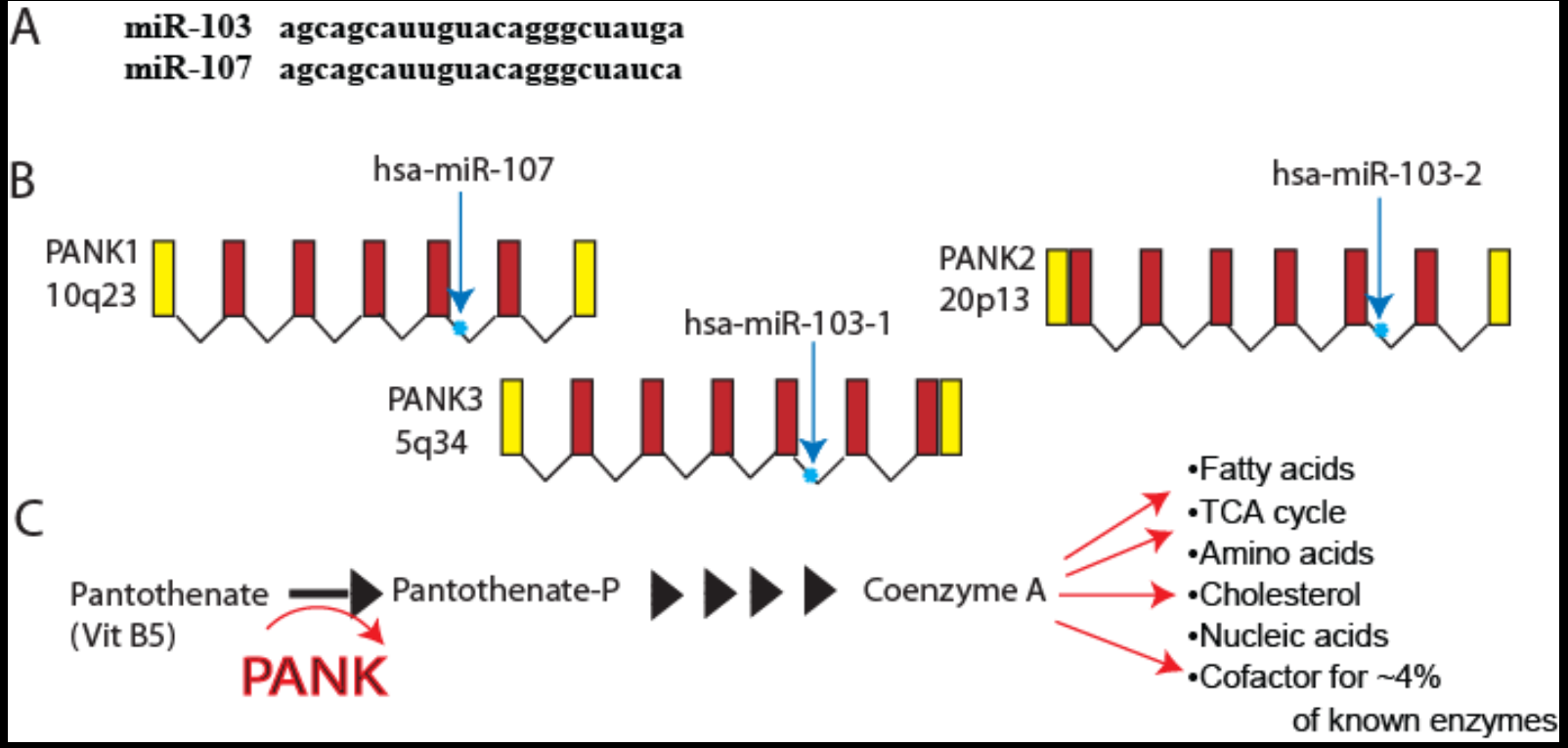
($P < 0.005$, uncorrected for multiple comparisons) (6, 9). The purple areas are regions in which CMRgl was abnormally low only in the patients with AD, the bright blue areas are regions in which CMRgl was abnormally low in both the young adult ε4 carriers and patients with probable AD, and the muted blue areas are regions in which CMRgl was abnormally low only in the ε4 carriers. (Lines point to the locations of the ε4 carriers' most significant CMRgl reductions and



Young (20-39 y.o.) ApoE4 carriers at genetic risk for developing AD show decreased glucose metabolism in a pattern reminiscent of AD brains

Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia

Eric M. Reiman[a,b,c,d,e], Kewei Chen[a,d,f,g], Gene E. Alexander[d,h], Richard J. Caselli[d,i], Daniel Bandy[a,d], David Osborne[d,j], Ann M. Saunders[k,l], and John Hardy[m,n]



From Wilfred B et al, Mol Genet Metab. 2007

MiR-107 gene(s) are located within introns of PANK gene in all vertebrates

PANK is the universal, rate-determining enzyme in CoA formation

PANK is regulated transcriptionally in response to metabolic requirements

MiR-107 is predicted to participate in metabolic pathways in synchrony with PANK function

May link metabolic and pathologic pathways in Alzheimer's disease

RNA

A PUBLICATION OF THE RNA SOCIETY

Identification of glucose-regulated miRNAs from pancreatic β cells reveals a role for miR-30d in insulin transcription

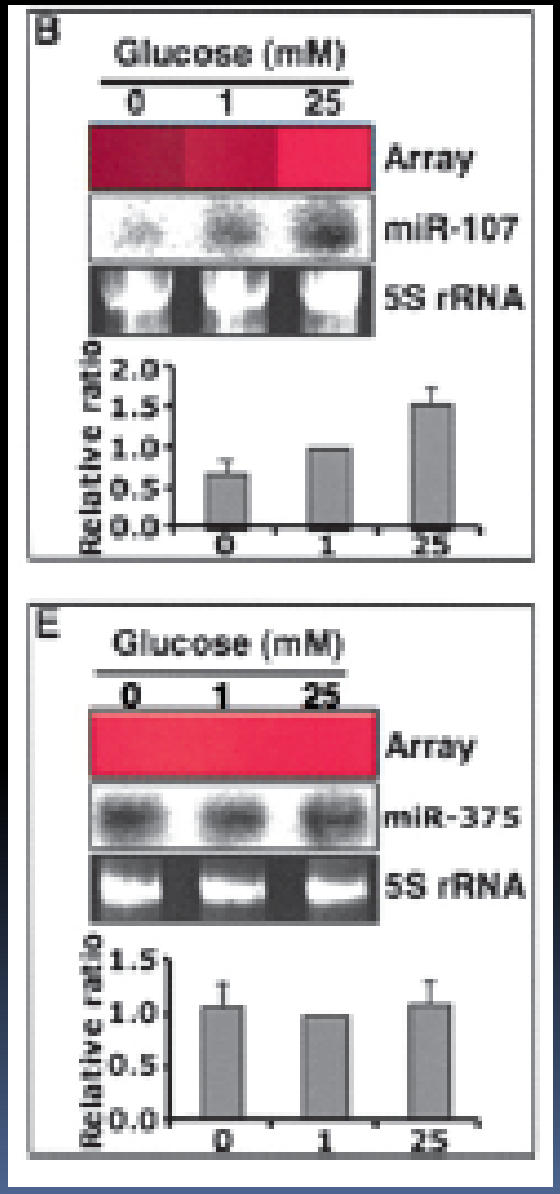
Xiaoqing Tang, Latha Muniappan, Guiliang Tang, et al.

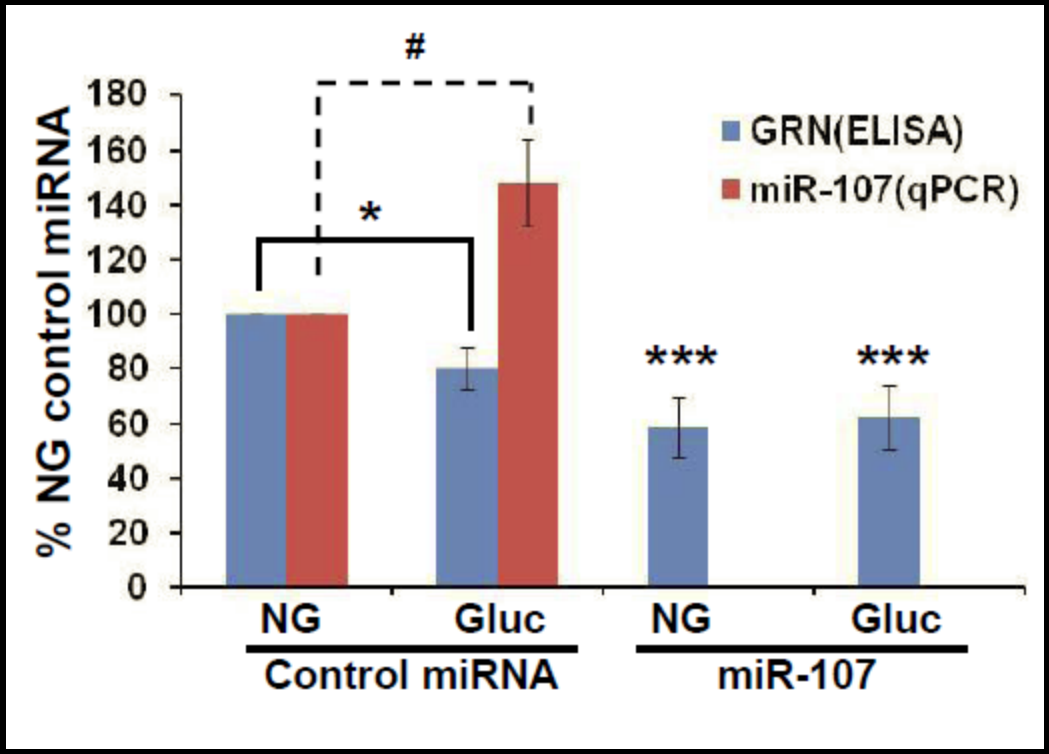
RNA 2009 15: 287-293 originally published online December 18, 2008
Access the most recent version at doi:10.1261/rna.1211209

Sabire Ozcan's lab

Pancreatic beta-cell line

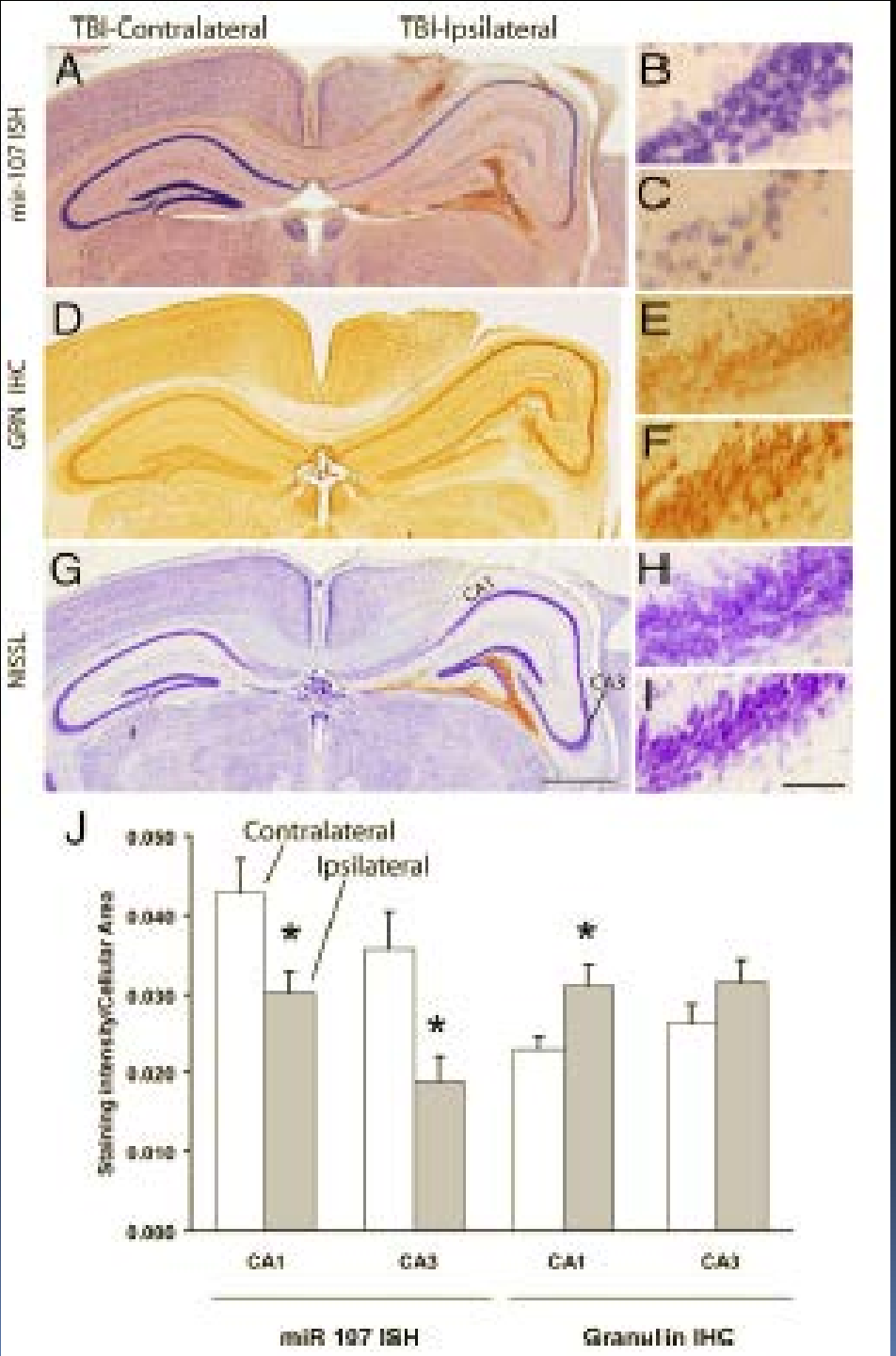
Glucose → miR-107 expression





High glucose → Increased miR-107
Decreased GRN

Am J Pathol, 2010



Traumatic brain injury

Ipsilateral side:

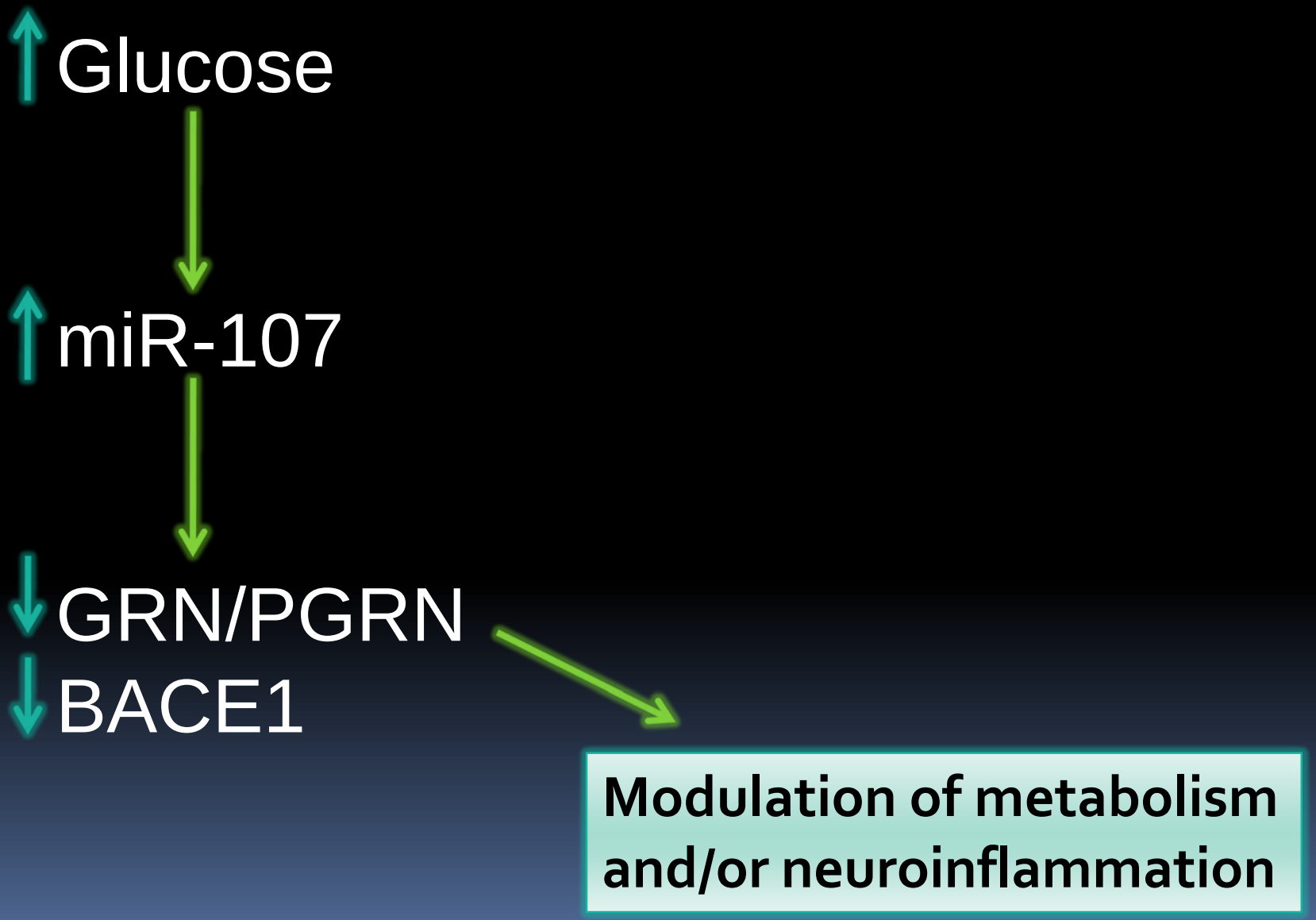
-Decreased miR-107

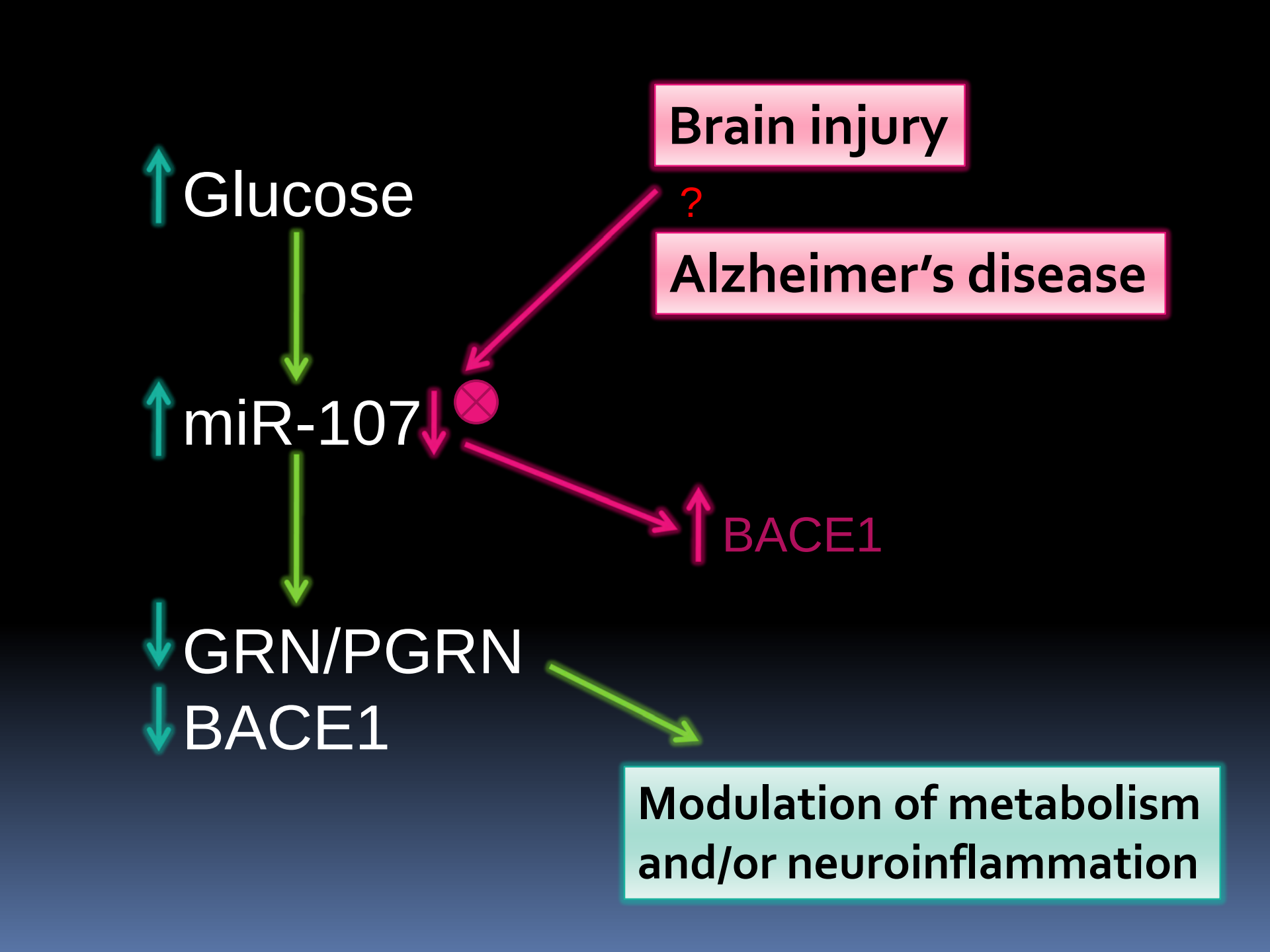
-Increased GRN/PGRN

Compatible with hypothesis
that miR-107 regulates GRN/PGRN
and this is relevant to neural injury

Thanks to Dr. Kathy Saatman and Dr. Sindhu Kizhakke

Am J Pathol, 2010


Glucose
miR-107
GRN/PGRN
BACE1
Modulation of metabolism and/or neuroinflammation


Brain injury
?
Alzheimer's disease
Glucose
miR-107
BACE1
GRN/PGRN
BACE1
Modulation of metabolism and/or neuroinflammation

Summary

1. mRNA translation is key node of gene expression regulation
2. miRNAs are a key regulator of translation
3. In miRNA profiling, technical details are important
4. miRNA expression is systematically altered in AD
5. miRNAs dysregulated in AD impact genes implicated in AD and FTLD

WE HAVE ONLY BEGUN TO SCRATCH THE SURFACE OF microRNA BIOLOGY IN HUMAN BRAIN

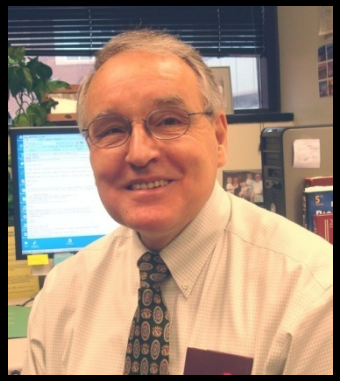
Lab

Neuropathology Core

UK ADC

Erin Abner, MPH

Dick Kryscio, PhD



Fred Schmitt, PhD

Greg Jicha, MD PhD

Linda Van Eldik, PhD

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Dr. William Markesbery

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NIH/NIA	P30 Grant

http://www.mc.uky.edu/coa/faculty/nelson.html