Non-coding genes: promising targets for molecular biomarkers and disease modifying epilepsy treatments

European Forum on Epilepsy Research
Dublin, May 25-27th 2013
Topic 4: New targets for innovative diagnostics and treatment

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Pathogenesis of epilepsy

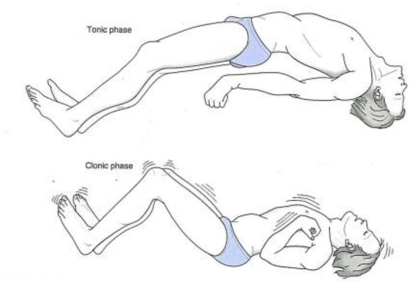


Figure 8–2. Generalized tonic-clonic seizure, illustrating the appearance of the patient in the tonic (stiffenir
clonic (shaking) phases.

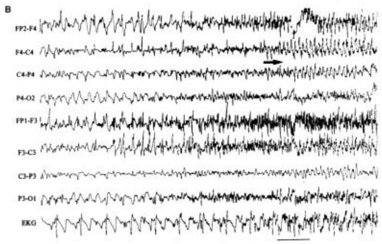


FIGURE 12. Generalized tonic–clonic seizure. A: The seizure begins with a generalized spike-and-wave burst (*arrow*) that is replaced by polyspike and wave. B: The polyspike and wave is succeeded by generalized continuous fast activity, which is the start of the tonic phase of the seizure. The latter half of this page begins to show tonic muscle artifact indicative of a generalized increase in continuous muscle contraction.

Treatment:

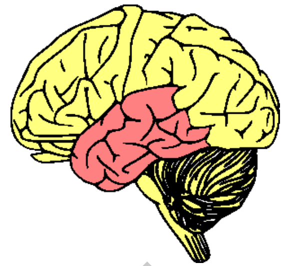
- AEDs (symptomatic only)
- Surgery
- Other (diet, brain stimulation)

Key goals:

Better understanding of <u>epileptogenesis</u>

<u>Disease-modifying</u> treatments

<u>Biomarkers</u> of epileptogenesis



Neuronal death
Gliosis
Inflammation
Plasticity
(axonal, dendritic)
Neurogenesis
Extracellular matrix
Metabolism

Large-scale changes in gene expression

Epileptic seizures

Animal trials

Glutamate antagonists (excitability, neuroprotection)

Neuropeptides (inhibition)

Growth factors (various)

Celecoxib (inflammation)

Rapamycin (structure, metabolism)

[protein-coding] gene targets in epilepsy

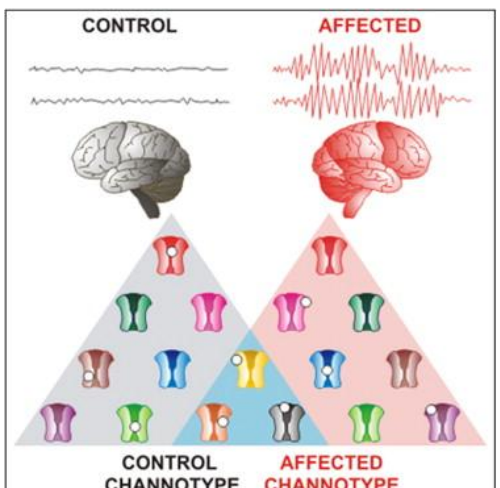
Despite major insights and progress…….
errors in protein-coding genes do not explain the majority of cases of epilepsy

Exome Sequencing Followed by Large-Scale Genotyping Fails to Identify Single Rare Variants of Large Effect in Idiopathic Generalized Epilepsy

Erin L. Heinzen,[1,2,*] Chantal Depondt,[3] Gianpiero L. Cavalleri,[4] Elizabeth K. Ruzzo,[1] Nicole M. Walley,[1]

Exome Sequencing of Ion Channel Genes Reveals Complex Profiles Confounding Personal Risk Assessment in Epilepsy

Tara Klassen,[1] Caleb Davis,[1] Alica Goldman,[1] Dan Burgess,[1] Tim Chen,[1] David Wheeler,[4] John McPherson,[3,4] Traci Bourquin,[4] Lora Lewis,[4] Donna Villasana,[4] Margaret Morgan,[4] Donna Muzny,[4] Richard Gibbs,[3,4] and Jeffrey Noebels[1,2,3,*]



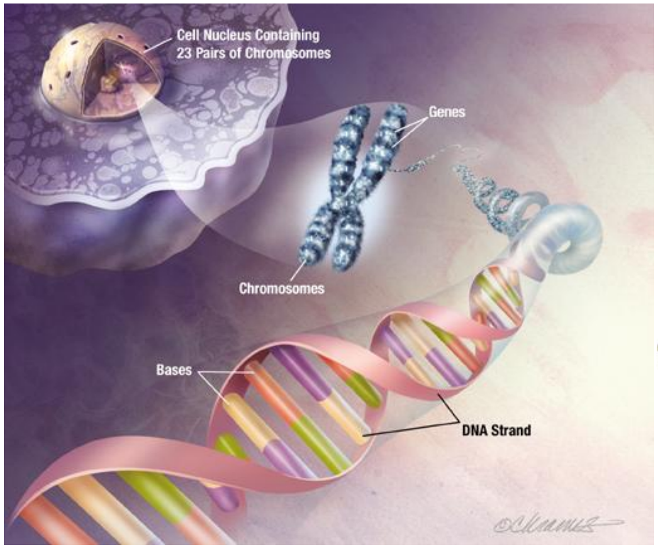
Disappointing anti-epileptogenesis trials in animals

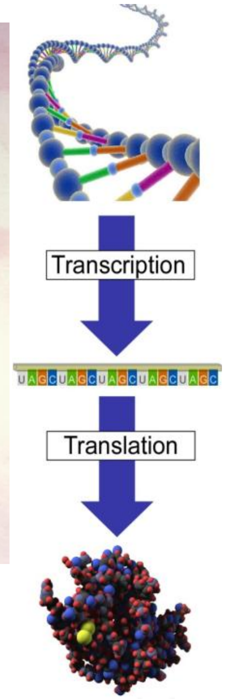
Seizure modification			
Decrease in frequency	Decrease in duration	Milder seizure type	Prevention of progressive increase in seizure frequency
Yes	Yes	Yes	Yes
No	Yes	Yes	Yes
No	No	Yes	..
No	No	..	..
Yes	No	..	..

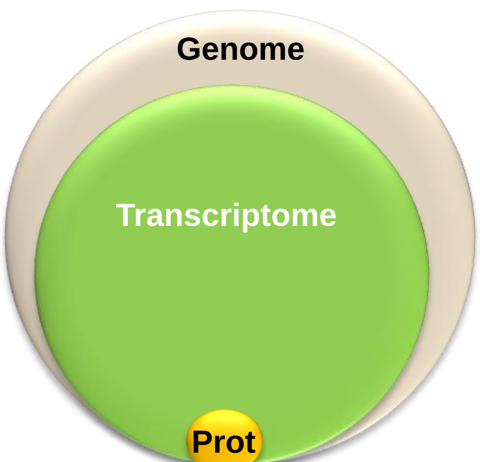
Cure
Increase in proportion of animals that become seizure free
..
..
..
..
No
..
..

Pitkanen & Lukasiuk *Lancet Neurol* (2011)

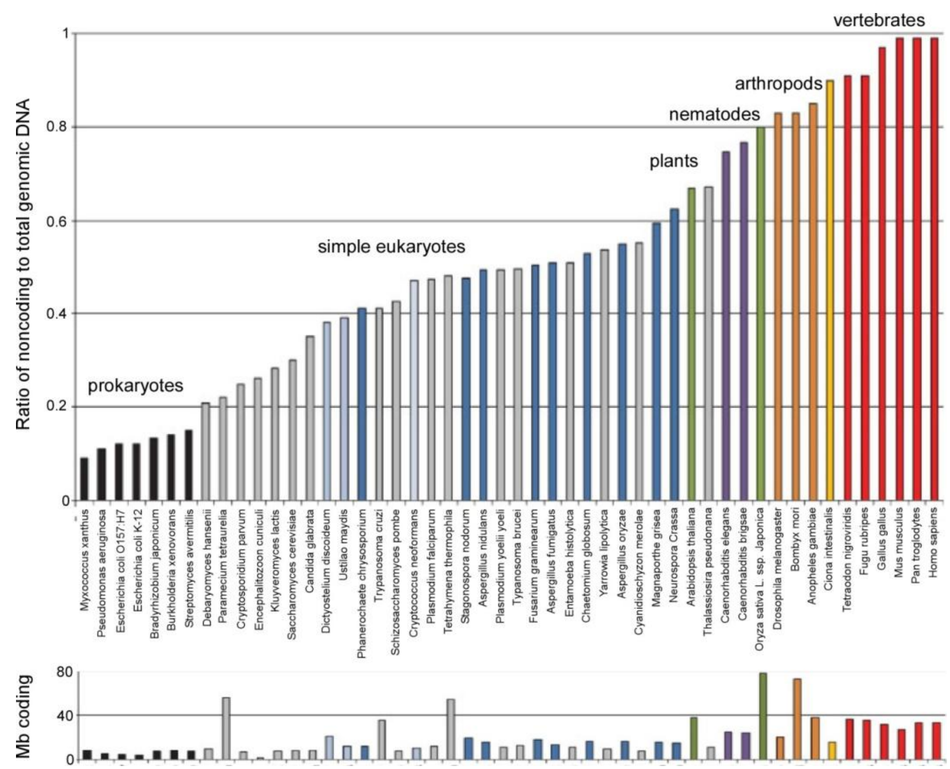
Non-coding RNA – a new gene focus in epilepsy







The amount of non-coding RNA increases as a function of organism complexity



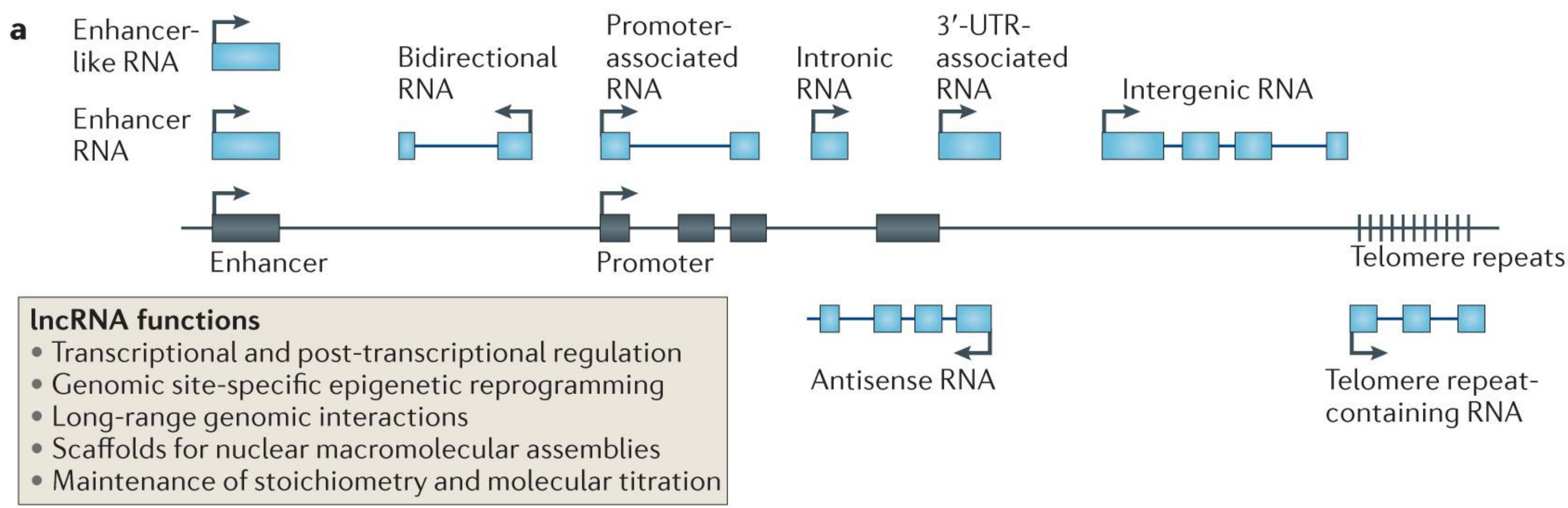
Taft et al. *Bioessays* 2007

Non-coding RNA – forms and functions

Long non-coding RNA (>200 nucleotides)

Largest class of ncRNA in the human genome (**100,000+**), interact with DNA, RNA & protein

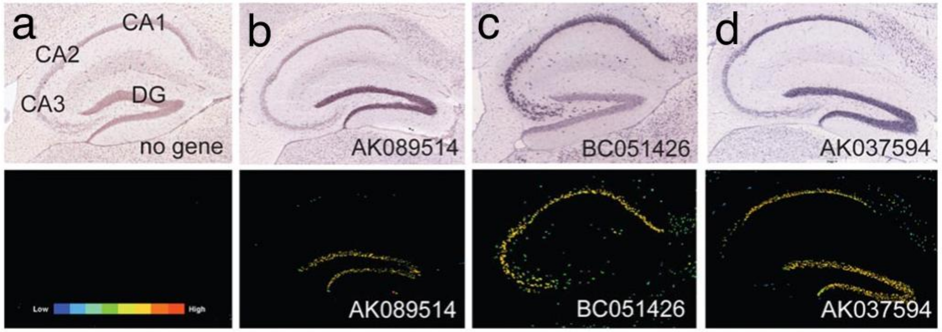
Functions: Epigenetic (guiding methylation); transcriptional/translational interference



Qureshi & Mehler, *Nat Rev Neurosci* 2012

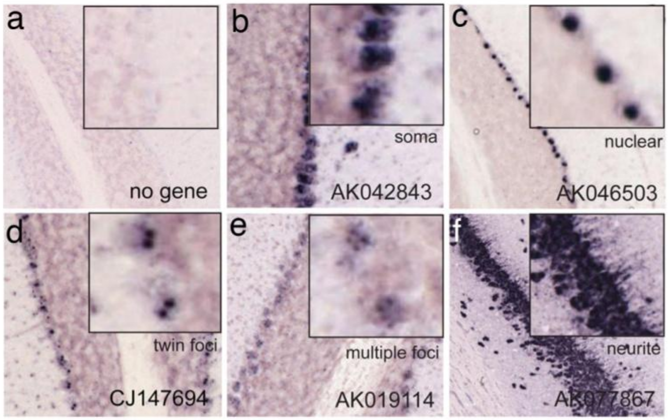
LncRNA; brain enrichment, emerging functions

lncRNAs display specific subfield expression



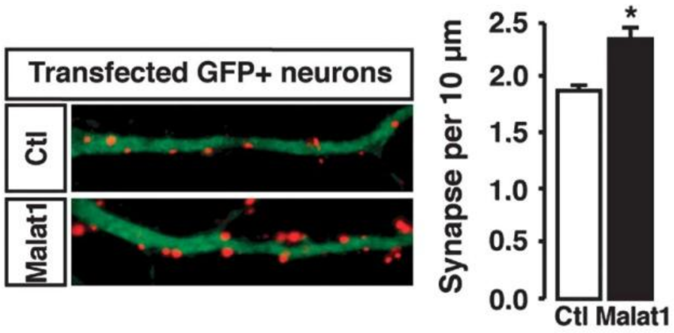
Mercer et al. *PNAS* 2008

…and specific <u>subcellular</u> localization



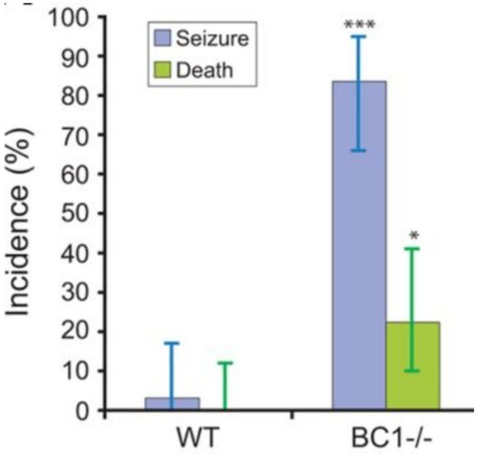
Mercer et al. *PNAS* 2008

lncRNAs regulate <u>synapse formation</u>



Bernard et al. *EMBO J* 2010

….and <u>seizure thresholds</u>



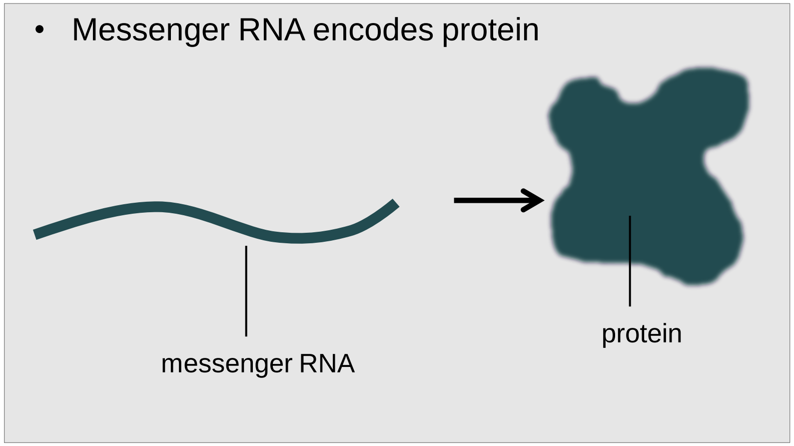
Zhong et al. *J Neurosci* 2009

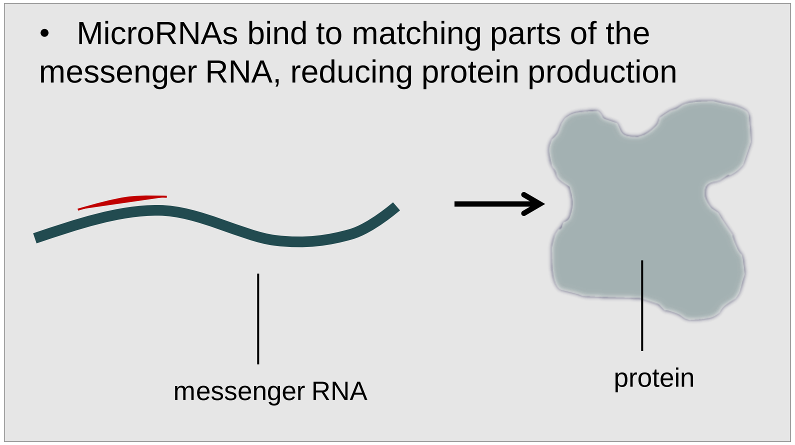
Short non-coding RNA

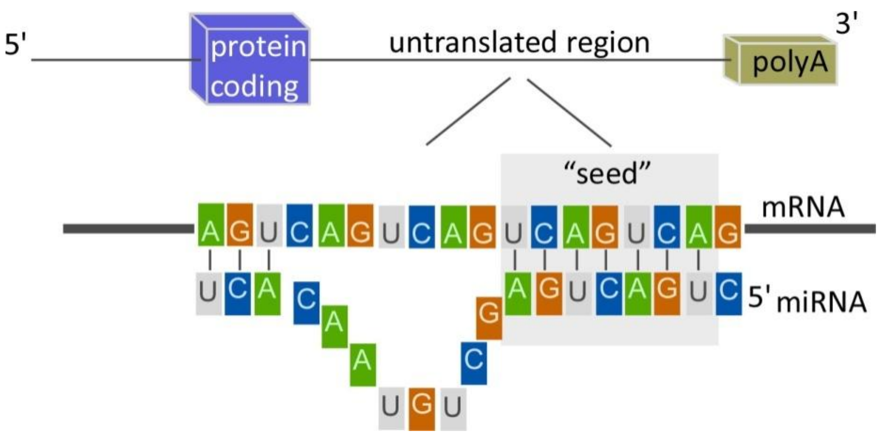
Includes **microRNA** (miRNA) - small non-coding RNAs (~22 nt); **~1500** in humans

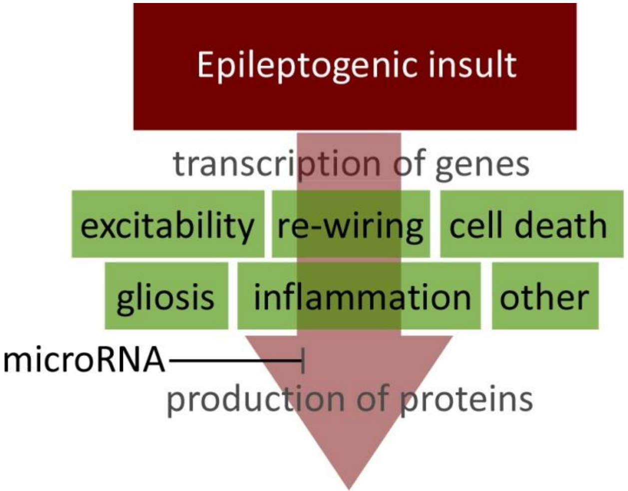
Function to fine-tune protein output

“meta” controllers of gene expression – single miRNA may target ~200 mRNAs

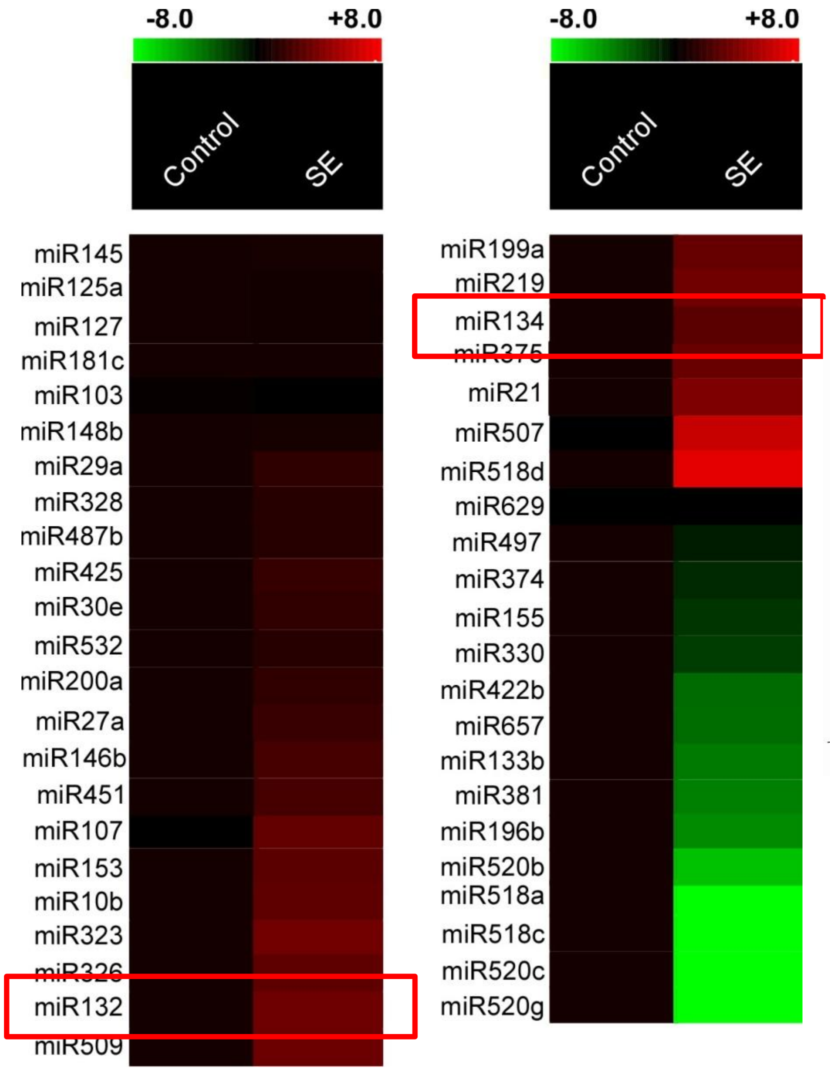




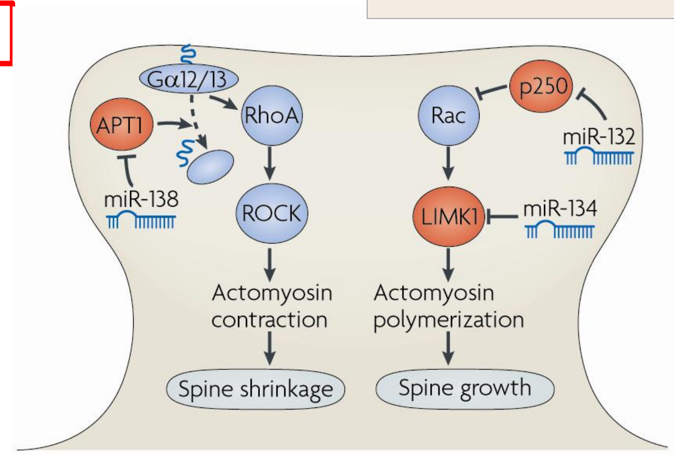




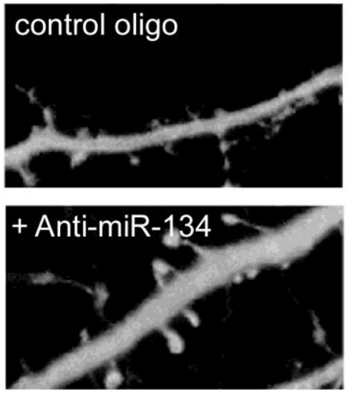
Altered miRNA levels after *status epilepticus* and in epilepsy



Critical for excitatory signals

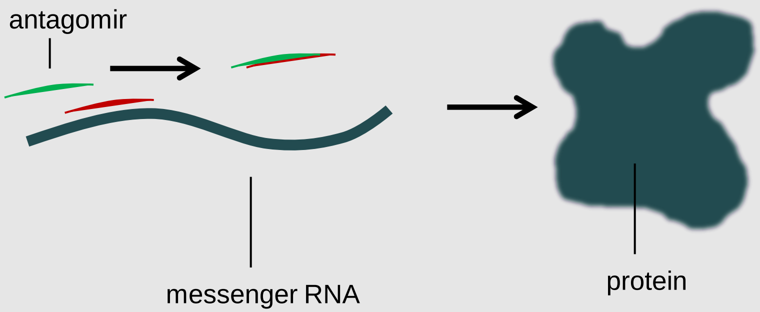


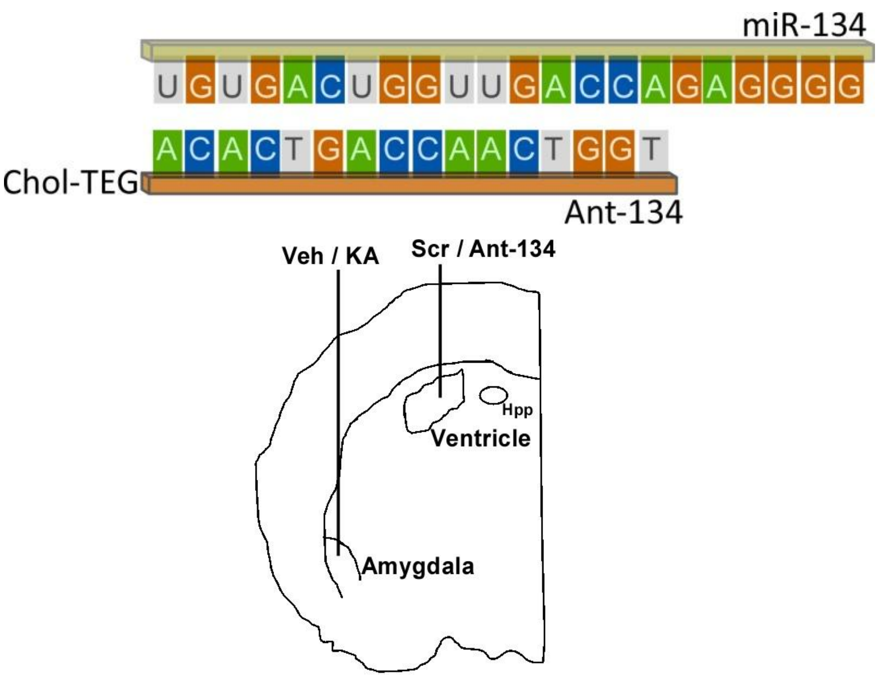
Schratt et al. *Nat Rev Neurosci* 2009

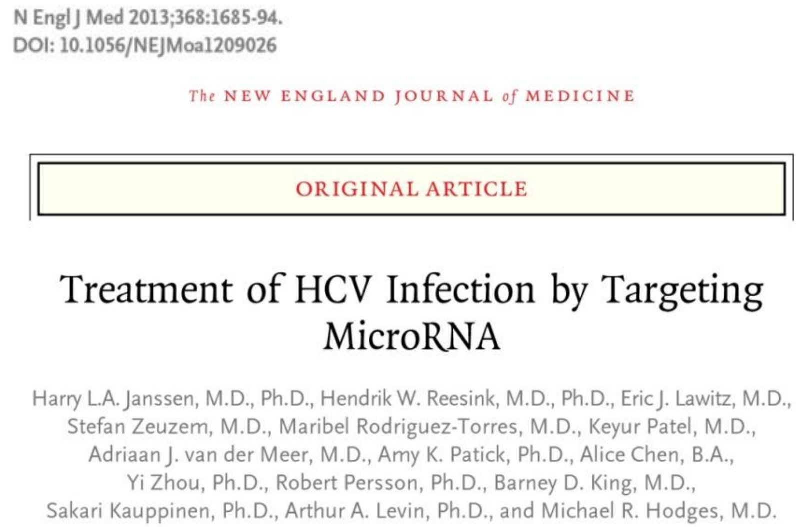


Targeting miRNAs Locked nucleic acid-modified DNA antisense oligonucleotides

- Antagomirs bind to miRNAs, allowing protein production to resume





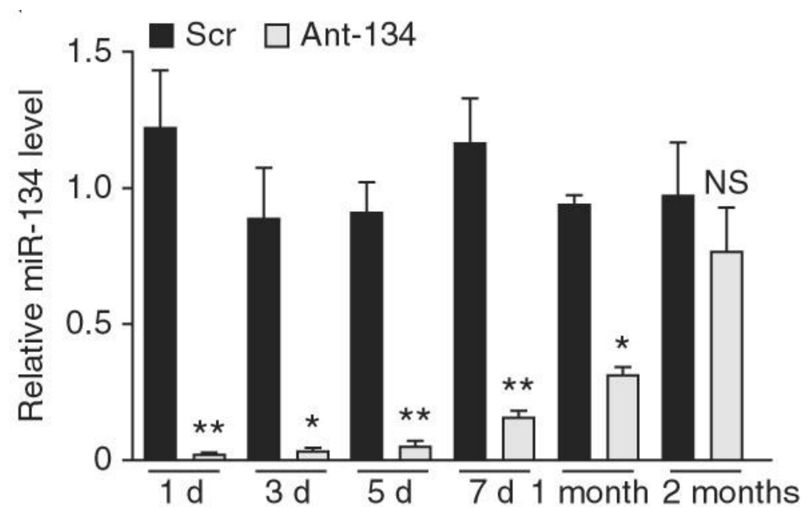

N Engl J Med 2013;368:1685-94.
DOI: 10.1056/NEJMoa1209026

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

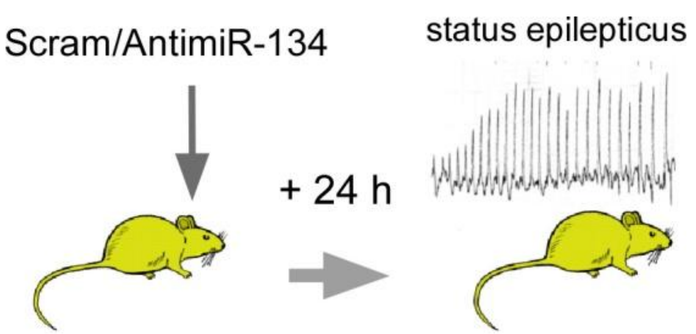
Treatment of HCV Infection by Targeting MicroRNA

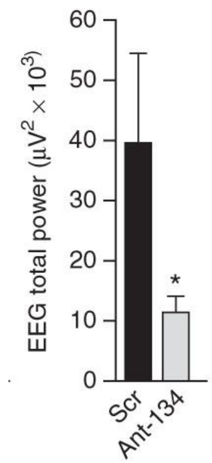
Harry L.A. Janssen, M.D., Ph.D., Hendrik W. Reesink, M.D., Ph.D., Eric J. Lawitz, M.D., Stefan Zeuzem, M.D., Maribel Rodriguez-Torres, M.D., Keyur Patel, M.D., Adriaan J. van der Meer, M.D., Amy K. Patick, Ph.D., Alice Chen, B.A., Yi Zhou, Ph.D., Robert Persson, Ph.D., Barney D. King, M.D., Sakari Kauppinen, Ph.D., Arthur A. Levin, Ph.D., and Michael R. Hodges, M.D.

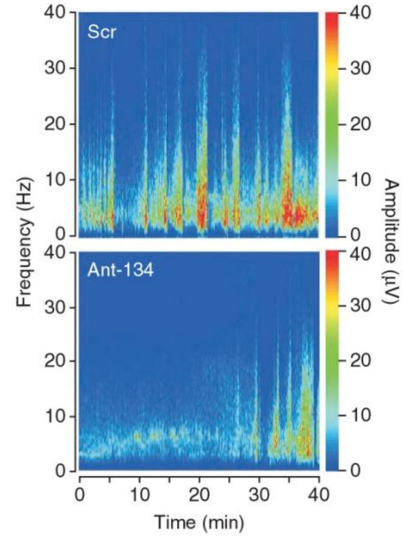


Jimenez-Mateos et al. *Nat Med* 2012

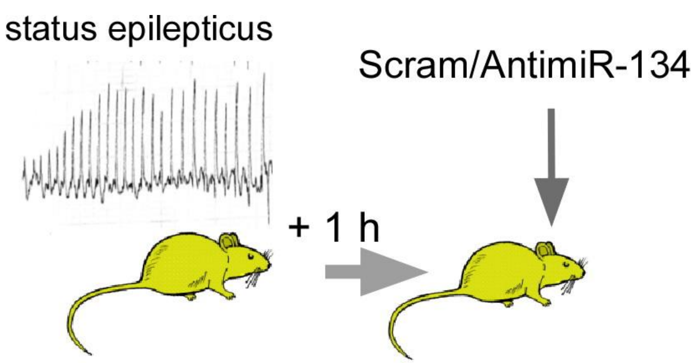
Silencing miR-134 reduces seizures

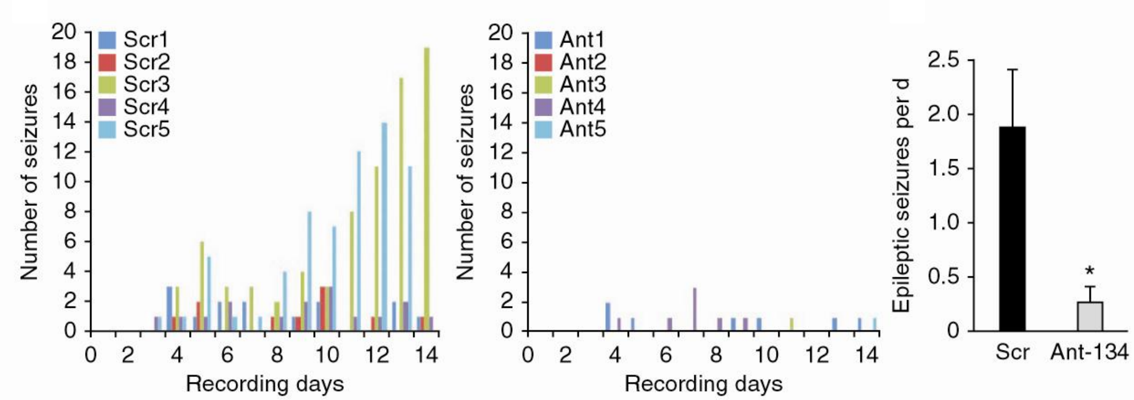






92% reduction in epileptic seizures





Jimenez-Mateos et al. *Nat Med* 2012

miRNAs and seizures

Increasing numbers of miRNAs linked to seizures and/or epilepsy pathology

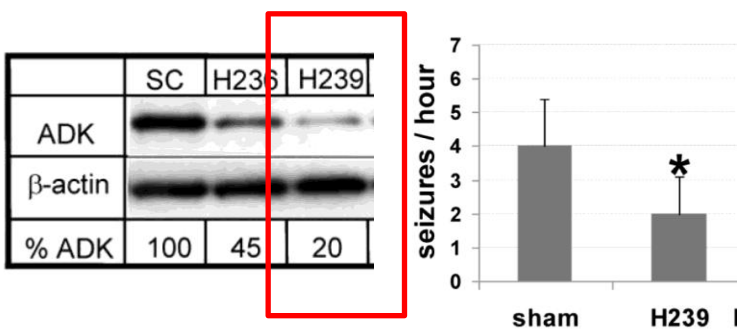
miRs 21, 34a, 132, 138, 134, 146a, 155, 184…..

Silenced miR	Brain role	EEG	Damage
miR-34a	apoptosis	—	✓
miR-132	spines	—	✓
miR-134	spines	✓	✓
miR-184	?	—	×

New ways to target miRNAs

Viral approaches?

Mesenchymal stem cell ADK-targeting miRNAs reduce seizures



Ren et al. *Exp Neurol* 2007; Li et al. *Epilepsy Res* 2009

Which are the best targets?

How can we "medicinize"?

- Are they safe?
- Administration routes
- Effects on established epilepsy?

Other future focuses on non-coding RNA in epilepsy

miRNA as diagnostic biomarkers?

- Stable in biofluids, pH and freeze-thaw resistant
- Detectable in multiple biofluids (blood, CSF)
- Signal-carrying paracrine functions
- Identify at risk patients, track epileptogenesis, support prognosis

Journal of Cerebral Blood Flow & Metabolism (2010) 30, 92–101

www.jcbfm.com

Brain and blood microRNA expression profiling of ischemic stroke, intracerebral hemorrhage, and kainate seizures

JOURNAL OF NEUROTRAUMA 29:1379–1387 (May 1, 2012)

MicroRNA Let-7i Is a Promising Serum Biomarker for Blast-Induced Traumatic Brain Injury

Non-coding RNA variation in human epilepsy?

- Sequence variation in ncRNA including miRNA may function as a risk factor

Future

1. Non-coding RNA is a largely <u>unknown</u> contributor in epileptogenesis

Which short and long ncRNAs are pathogenic vs adaptive/useful/not important?
What is their mechanism? What controls their expression?

2. Genetics of non-coding RNA

Do ncRNA variants have a role in explaining disease risk?

3. Sources of molecular biomarkers

Are miRNAs disease biomarkers? Do different precipitating injuries elicit unique signatures?
What is the best biofluid source and ncRNA "pool"?

4. Next generation of disease-modifying treatments?

Are drugs acting on ncRNA safe for use in the CNS? How can they be delivered?
Do AEDs or other drugs have any ncRNA-modifying effects?