Presenter Disclosure

Ryan L. Boudreau, Ph.D

The following relationships exist related to this presentation:

No relationships to disclose.

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RNAi-based Strategies for Silencing Disease

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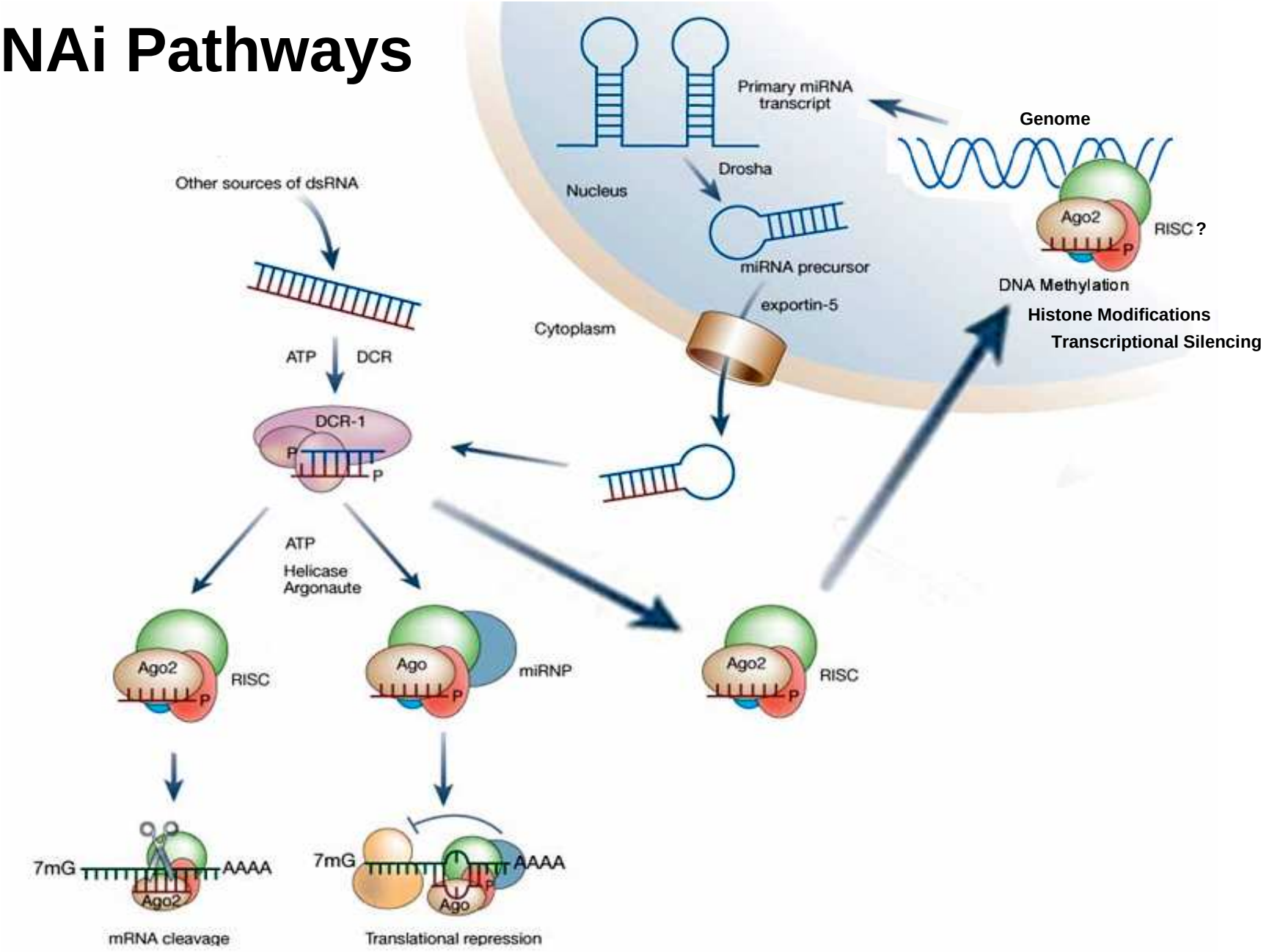
RNA Interference (RNAi)

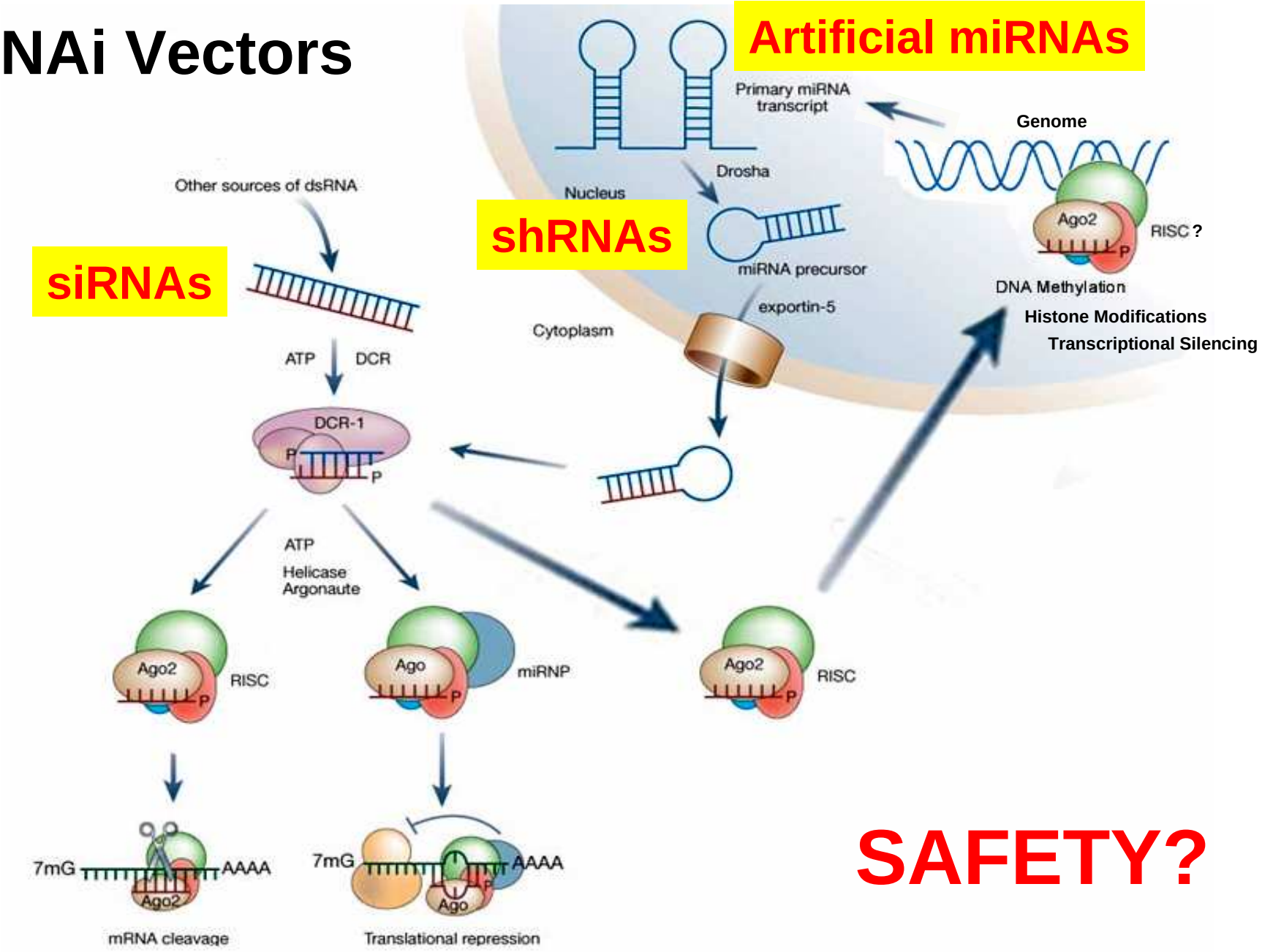
- Process of gene regulation mediated by small dsRNAs
- Utilized as a common biological tool to study gene function
- Under investigation as a therapeutic to treat various diseases for which silencing a target may provide benefit
  - Viral infection (e.g. HIV, RSV, HBV and SARS among others)
  - Cancer (e.g. glioma, melanoma or targets common to most cancers)
  - Genetic diseases (e.g. Huntington's, Alzheimers, ataxias, etc.)
- RNAi Delivery or Expression
  - Exogenous synthetic siRNAs (transient gene silencing)
  - Vectors expressing stem-loop RNAs (persistent gene silencing)
- Absolute specificity is questionable
  - Cellular responses to dsRNA (IFN-β, PKR, OAS1)
  - Off-target effects due to saturation of RNAi machinery or via partial complementarity with unintended mRNAs
  - Supports the need for optimizing RNAi vectors and potentially developing tissue-specific and regulated expression strategies

RNAi Pathways



Adapted from: Nature. 2004 Sep 16;431(7006):343

RNAi Vectors

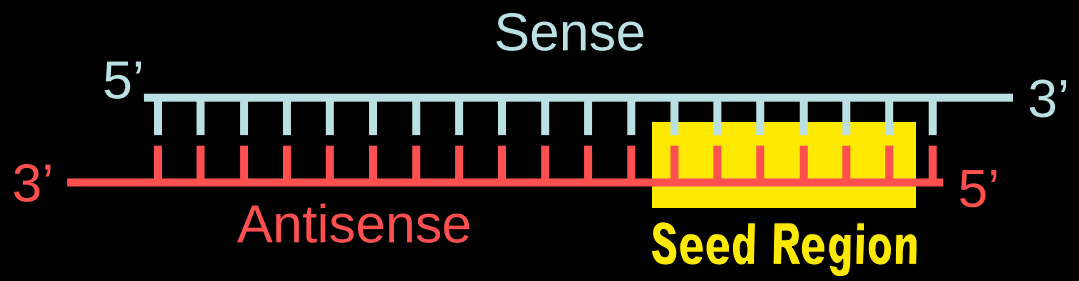


SAFETY?

Adapted from: Nature. 2004 Sep 16;431(7006):343

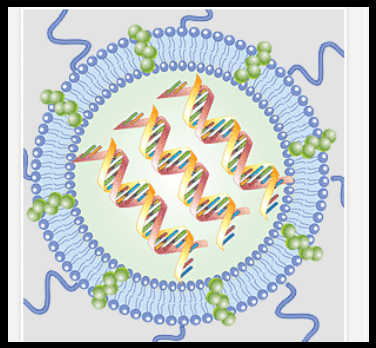
siRNAs

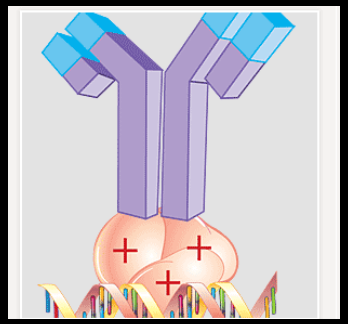
- Synthetic small duplex RNAs (19-27 bp) with di-nucleotide 3’ overhang
- Chemical modifications
  - Improve serum stability
  - Minimize immunostimulation
  - Reduce off-target gene silencing (strand & seed)
  - Moieties for monitoring biodistribution
- Processed by Dicer and RISC (saturation concerns)
- Transient silencing following single-dose (duration)
- Readily dosable and can terminate Rx if adverse events
- Numerous algorithmic design tools on-line

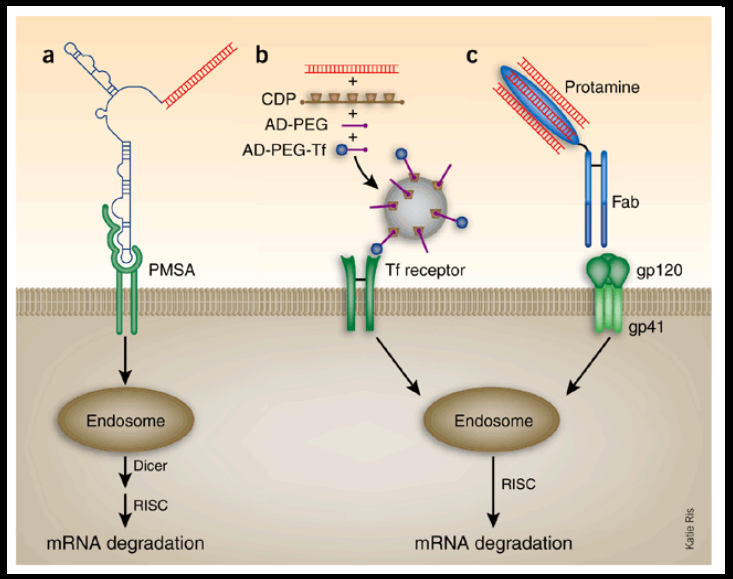


siRNA Delivery Hurdle

- Cationic liposomes or polymers
- Cholesterol-conjugates
- Peptide- and antibody-conjugates
- Aptamer-conjugates
- Various routes (direct injection or infusion, intravenous, inhalation)





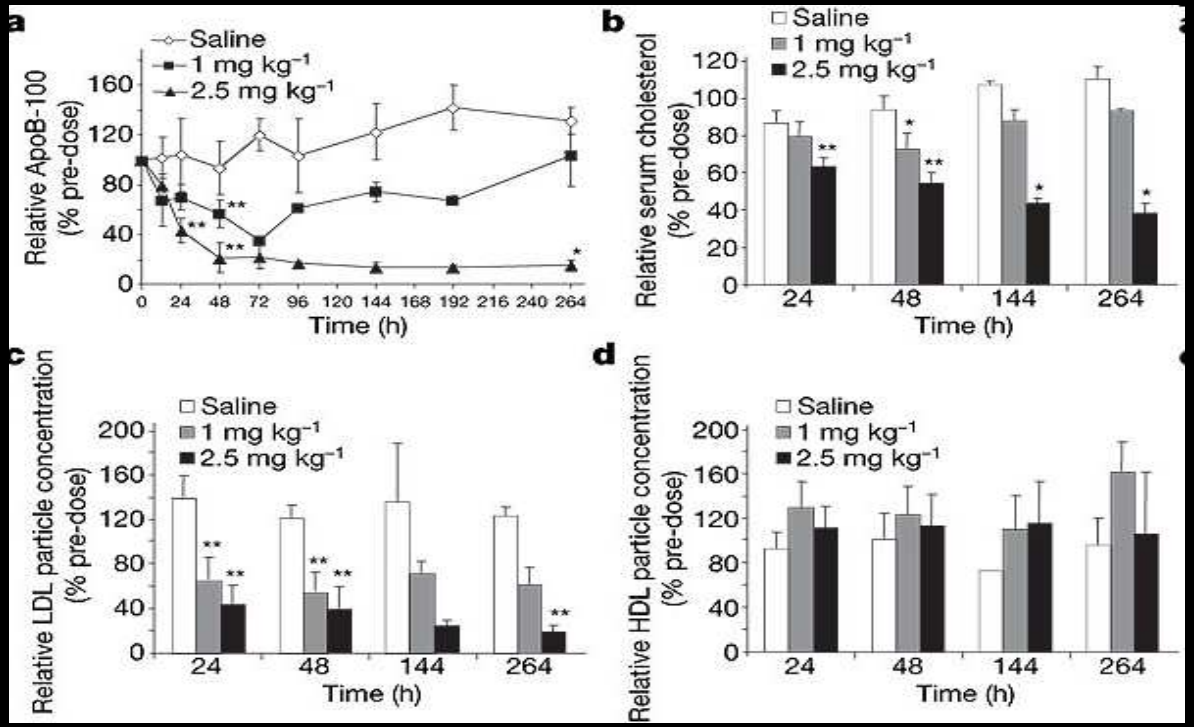


Some Promising siRNA Results to Date

- Silencing by siRNA infusion into mouse brain
  Thakker et al. (2004) *PNAS*
- Nasally administered siRNA inhibit respiratory virus infection in mice
  Bitko et al. (2005) *Nat Med*
- Lipoplexed siRNA prevented lethal infection of HSV-2 in mice
  Palliser et al. (2005) *Nature*
- siRNA treatment improves macular degeneration (caveat)
  Kleinman et al. (2008) *Nature*
- Systemic delivery of lipoplexed siRNA in mouse and non-human primate silences ApoB in liver
  Zimmermann et al. (2006) *Nature*
- Systemic delivery of siRNA appears to work in humans
  Davis et al. (2010) *Nature*

Systemic siRNA in Non-Human Primate

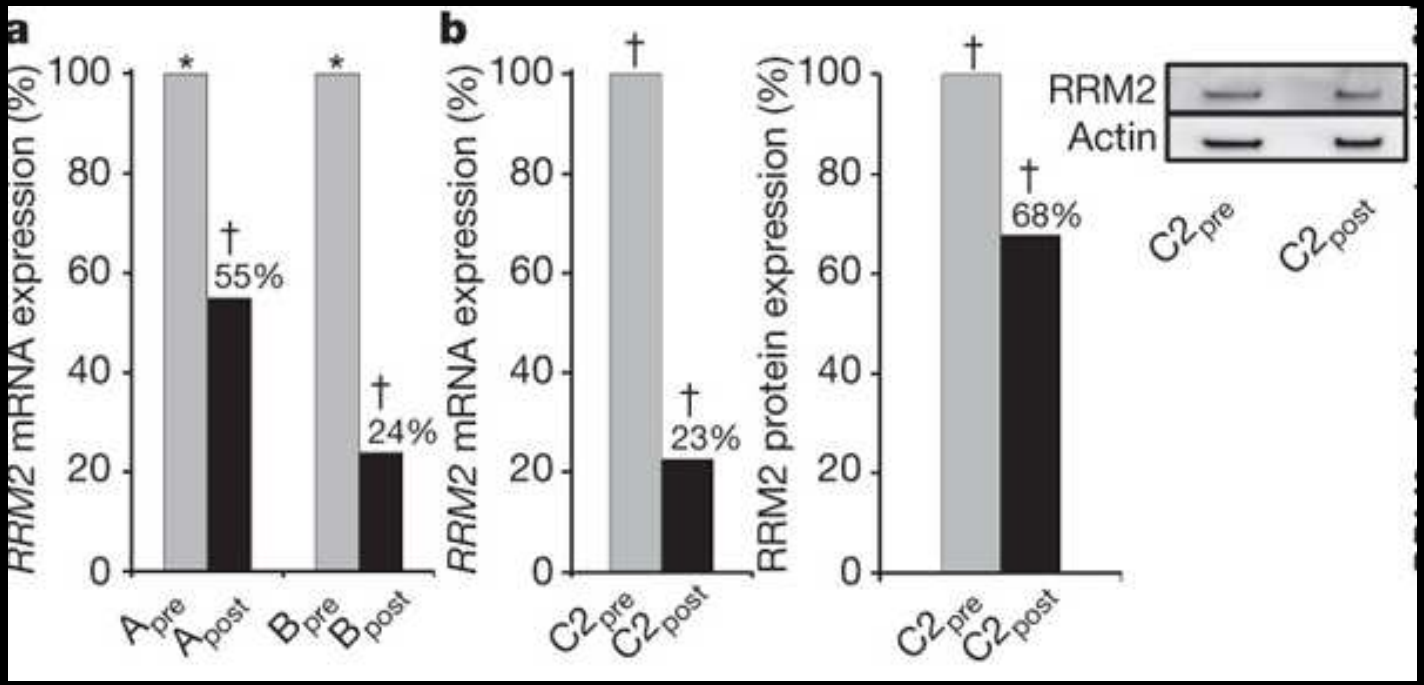
- Cynomolgus monkeys were treated with single bolus i.v. injections of lipoplexed siApoB via the saphenous vein
- ApoB is expressed in the liver and is essential for secretion of VLDL and LDL, which are required for the transport and metabolism of cholesterol



Zimmermann et al. (2006) *Nature*

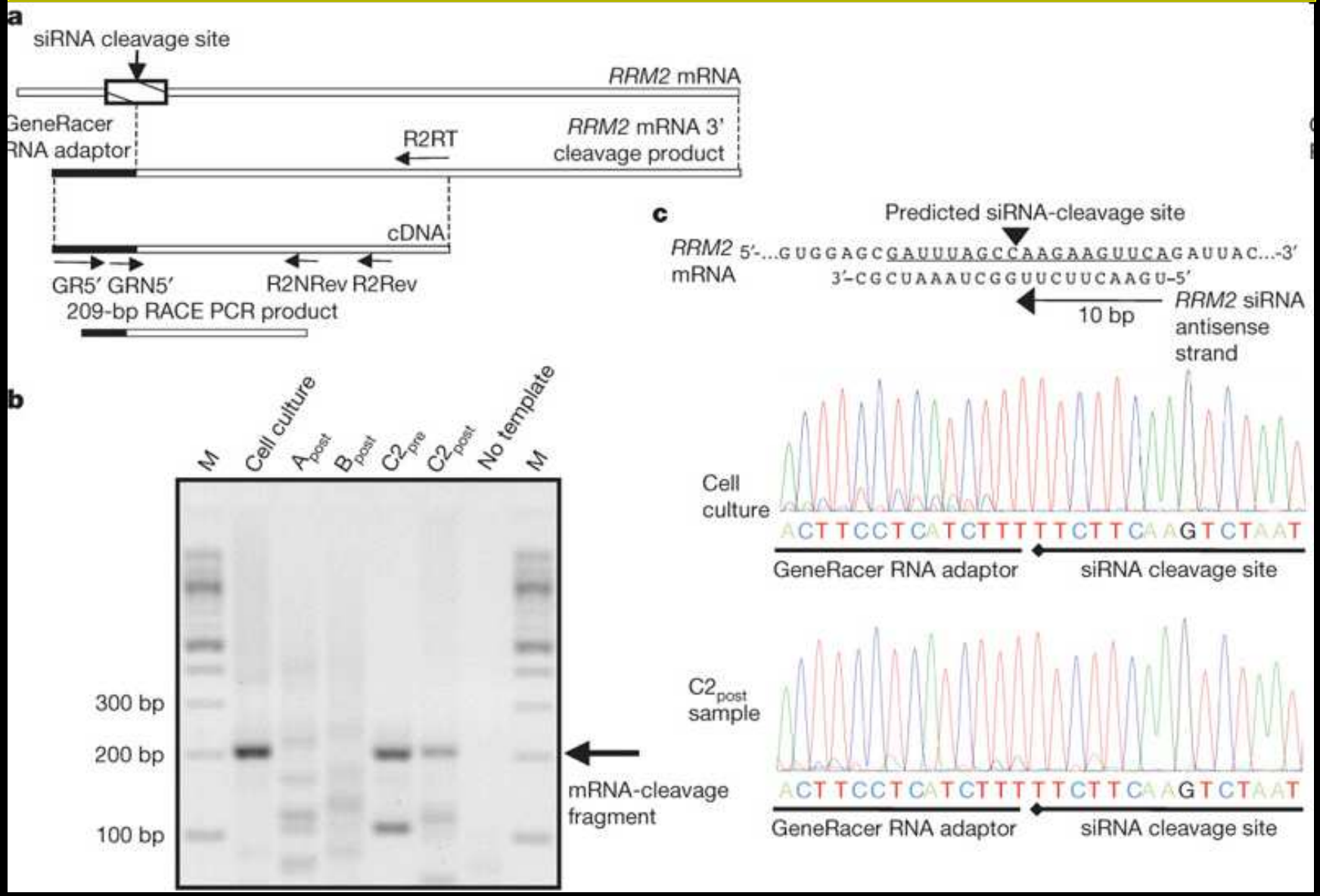
Systemic siRNA in Human

- siRNA clinical trials for macular degeneration, viral infection, cancer and skin disease are underway
- Systemic RNAi delivery remains a major hurdle
- Nanoparticles have recently shown promise in delivery of siRNAs to tumors via a systemic delivery route



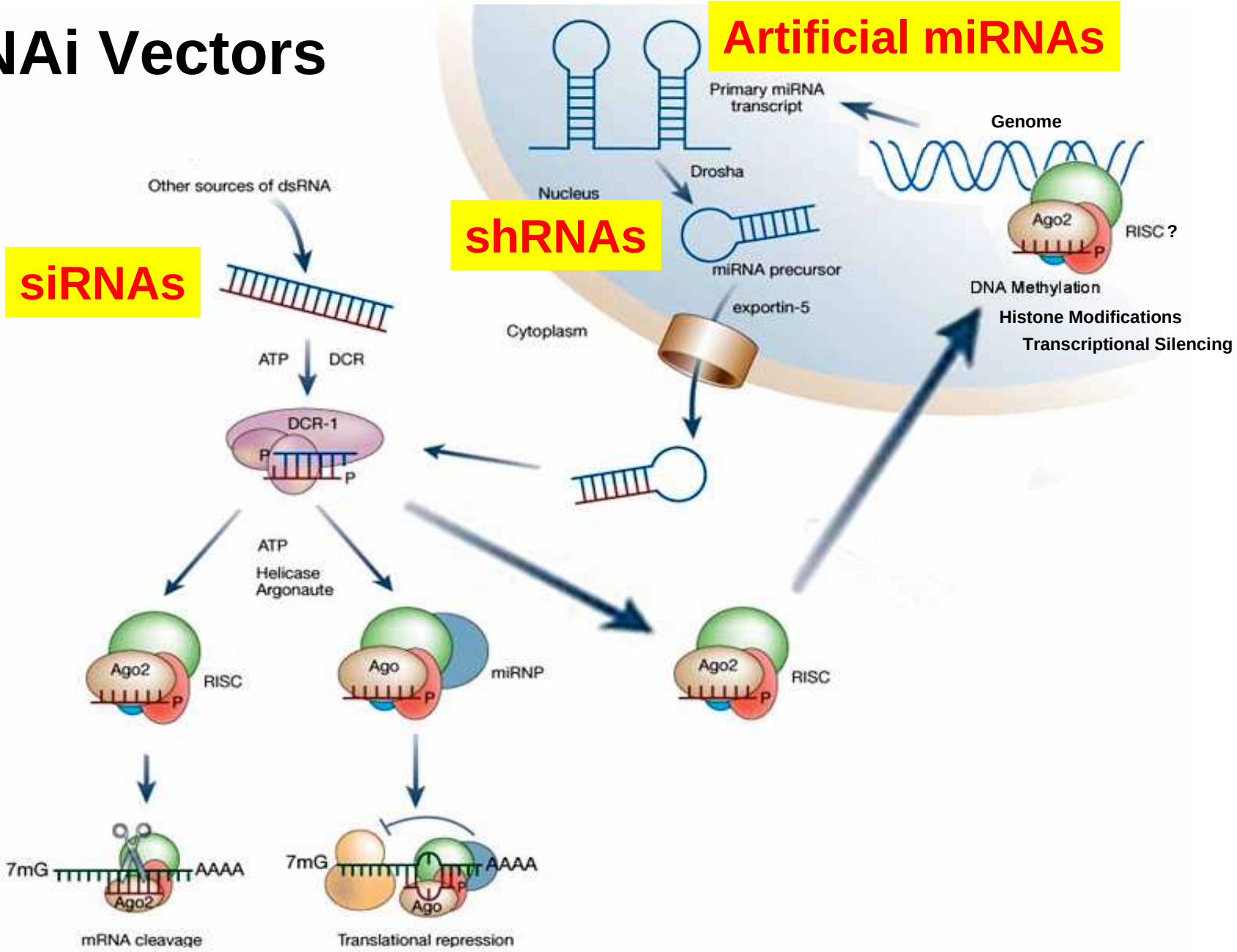
Davis et al. (2010) *Nature*

Systemic siRNA in Human



Davis et al. (2010) *Nature*

RNAi Vectors



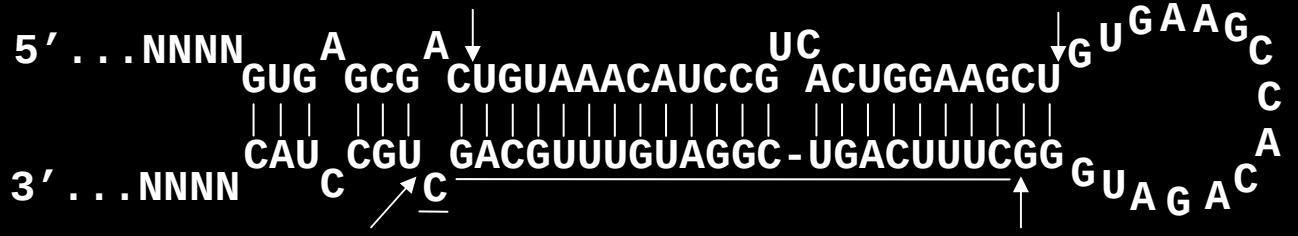
Adapted from: Nature. 2004 Sep 16;431(7006):343

RNAi Expression Vectors

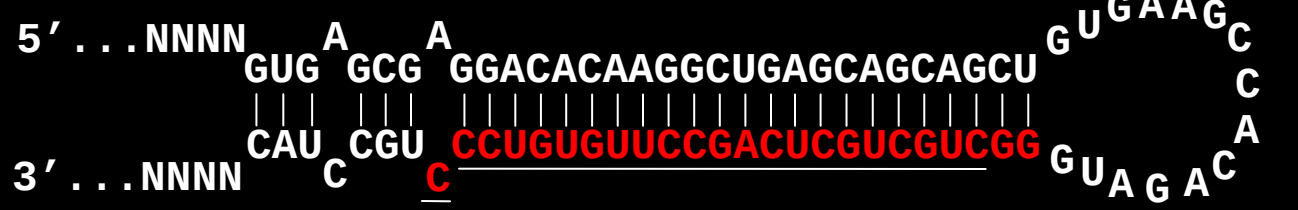
- Vector-based systems (plasmid, viral, etc.) are employed to express sense and antisense sequences to form duplex RNAs – most often in stem-loop formats
- Short-hairpin RNAs (shRNA)
  - Mimic pre-miRNAs (stem-loop with 3' overhang)
  - Substrate for Exportin-5, Dicer and RISC
  - Classically expressed from Pol-III promoters (e.g. U6 or H1) since start and stop transcription sequences should be controlled
- Artificial miRNAs or sh-miRs
  - Mimic pri-miRNA (embed siRNA in naturally occurring pri-miRNAs)
  - Substrate for Drosha, Exportin-5, Dicer and RISC
  - Can be expressed from any Pol-II promoter since transcription start & stop sites can be relatively arbitrary
- Allow persistent expression and long-term gene silencing following a single treatment

RNAi Stem-loop Designs

mir-30



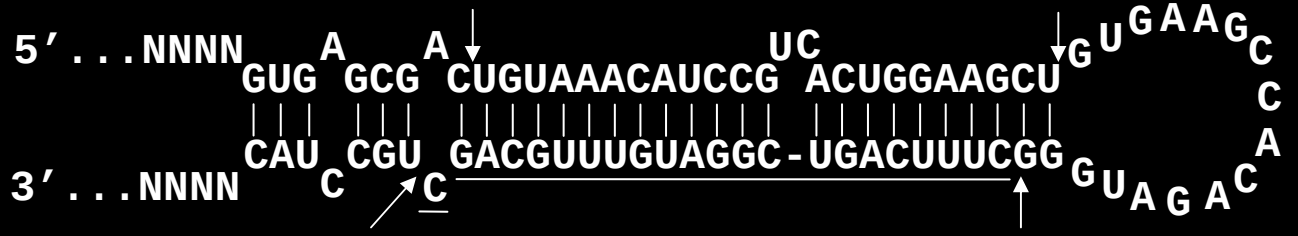
Artificial miRNA



shRNA

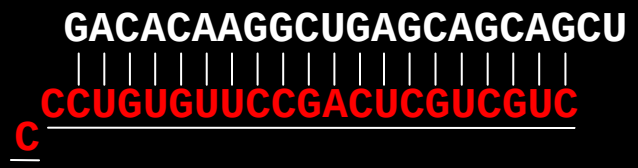
RNAi Stem-loop Designs

mir-30



Artificial miRNA

Drosha



Dicer

shRNA

Dicer

Therapeutic Studies in Mice

Disease / Virus	***Target***	***Delivery Paradigm***	***Results***
Hepatitis B Virus	HBVsAg	Plasmid-shRNA to liver	Inhibited viral replication
HIV	Tat or CCR5	Lentivirus-shRNA to hematopoietic progenitors	Resistance to viral challenge
Cancer	BRAF, EGFR, VEGF, etc.	shRNA expressing viruses	Reduction in tumor volume, improved survival, etc..
Spinocerebellar ataxia type 1	ataxin-1	AAV-shRNA to Cerebellum	Improved histopathology & motor function
Huntington's disease	huntingtin	AAV-shRNA to Striatum	Reduced htt inclusions, improved motor phenotypes
Amyotrophic Lateral Sclerosis	SOD1	Lentiviral-shRNA to lumbar spinal cord	Improved motor neuron survival and function, extended survival
Alzheimer's	BACE1 or APP	Lentiviral-shRNA or AAV-shRNA to hippocampus	Reduced AB-deposition and improved neuropathology and learning & memory

Many more published results and many more studies underway.

RNAi Toxicity in Mice (Liver)

Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways

Dirk Grimm[1], Konrad L. Streetz[1]†, Catherine L. Jopling[2], Theresa A. Storm[1], Kusum Pandey[1], Corrine R. Davis[3], Patricia Marion[4], Felix Salazar[4] & Mark A. Kay[1]

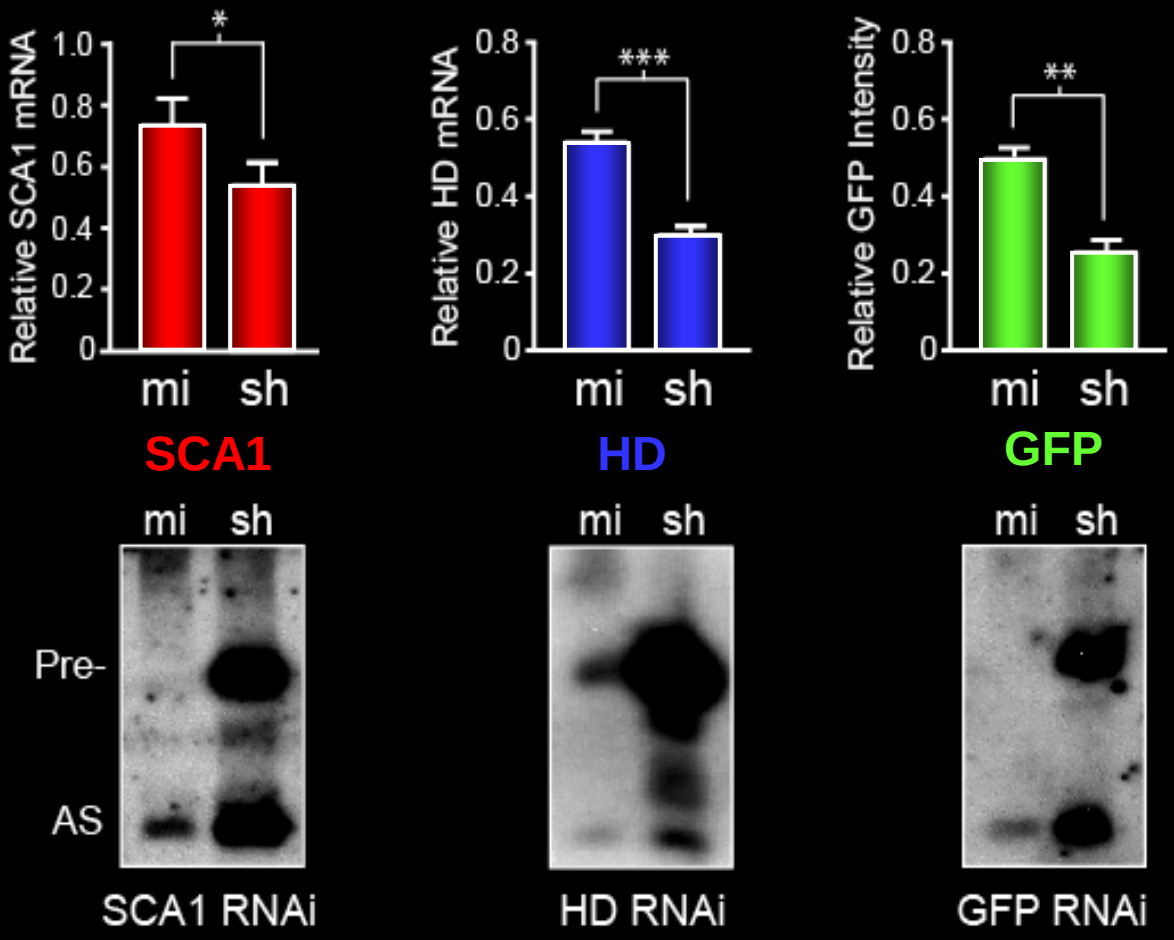
- Excessive doses of AAV8-shRNA vectors were delivered to the liver
- Mice receiving toxic shRNAs died within 30 days
- Toxicity correlated with shRNA expression levels and was, in part, due to saturation of RNAi processing machinery thus disrupting endogenous miRNA biogenesis and function. Off-targeting?

shRNAs vs. Artificial miRNAs

Comparison of silencing efficacies and RNAi processing

shRNA
TOXIC

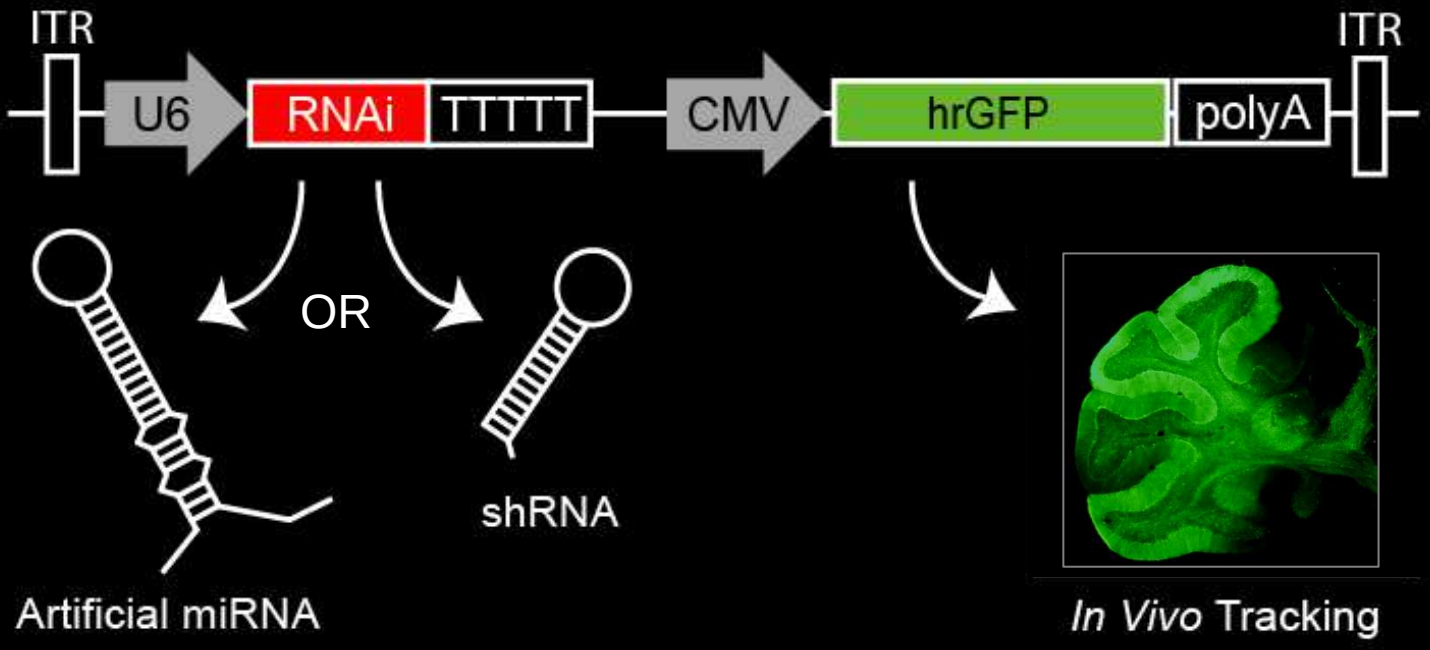
Artificial miRNA



RNA. 2008 Sep;14(9):1834-44

Evaluating RNAi Vector Safety and Efficacy in Mouse Brain

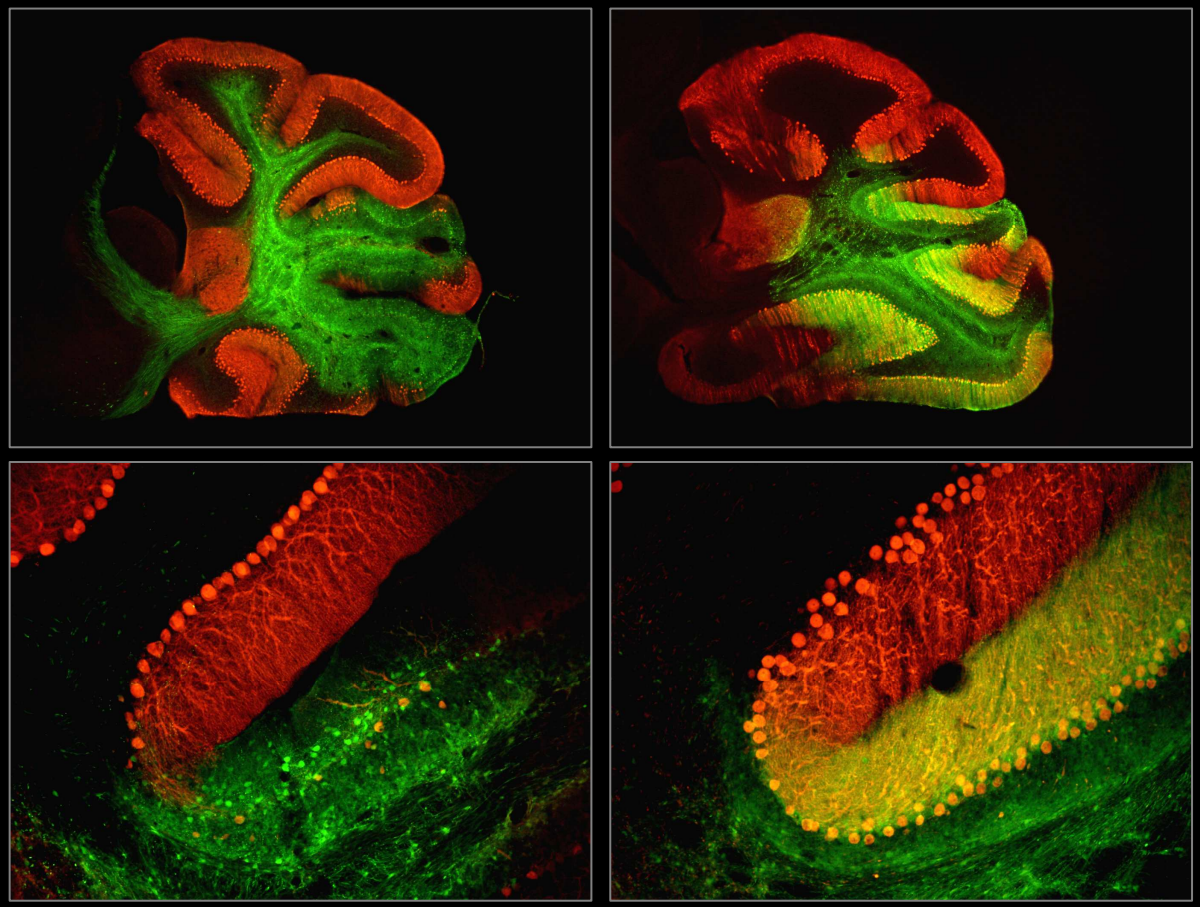
Engineered AAV Genome



Clinically relevant vector system.

RNAi Safety in Mouse Cerebellum

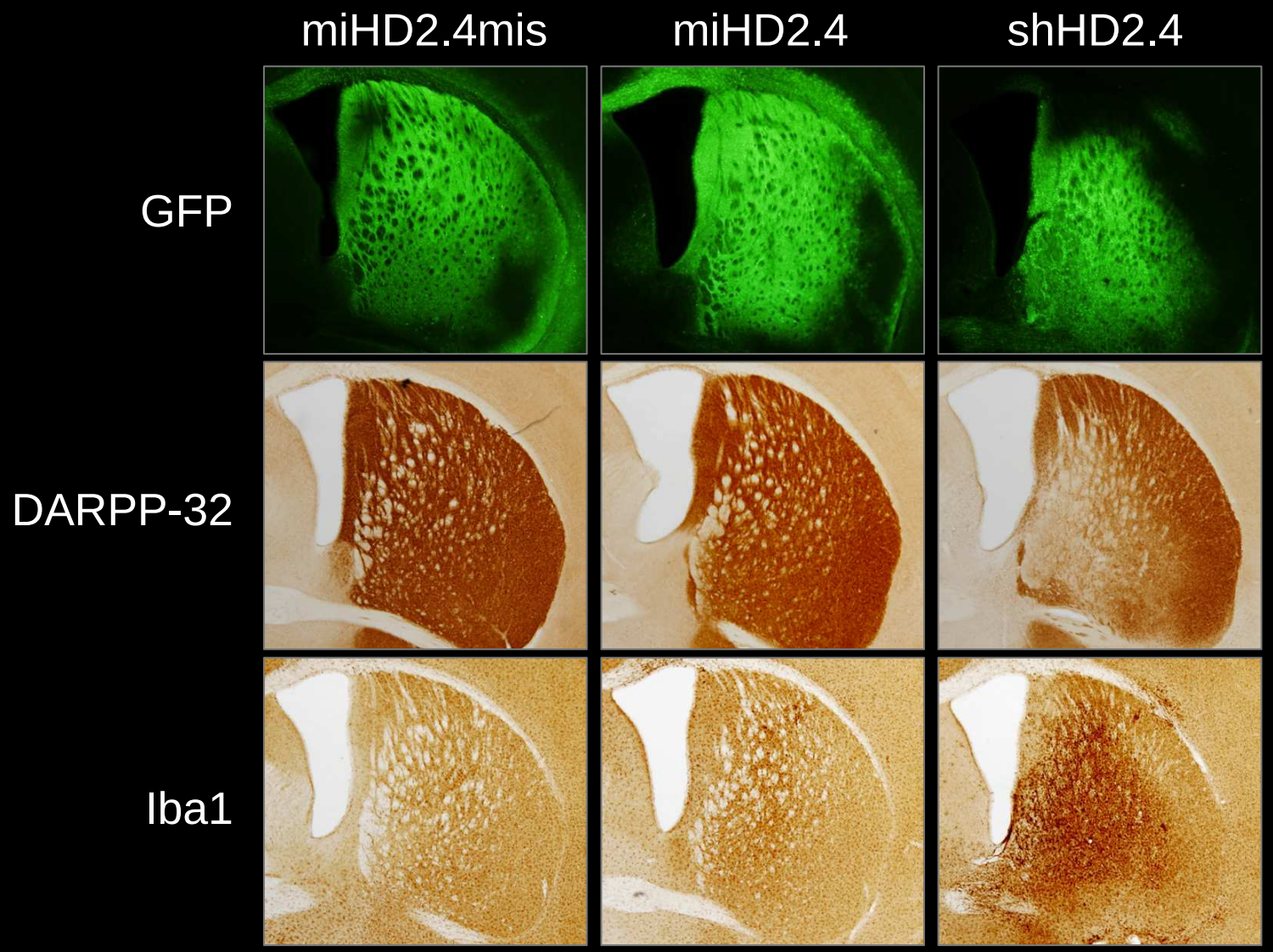
shSCA1 miSCA1



hrGFP-Calbindin

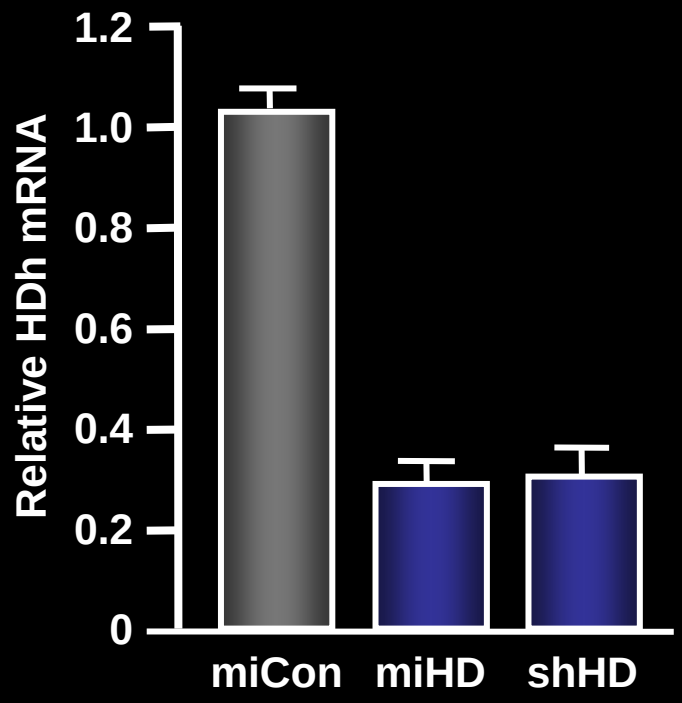
Mol Ther. 2009 Jan;17(1):169-75

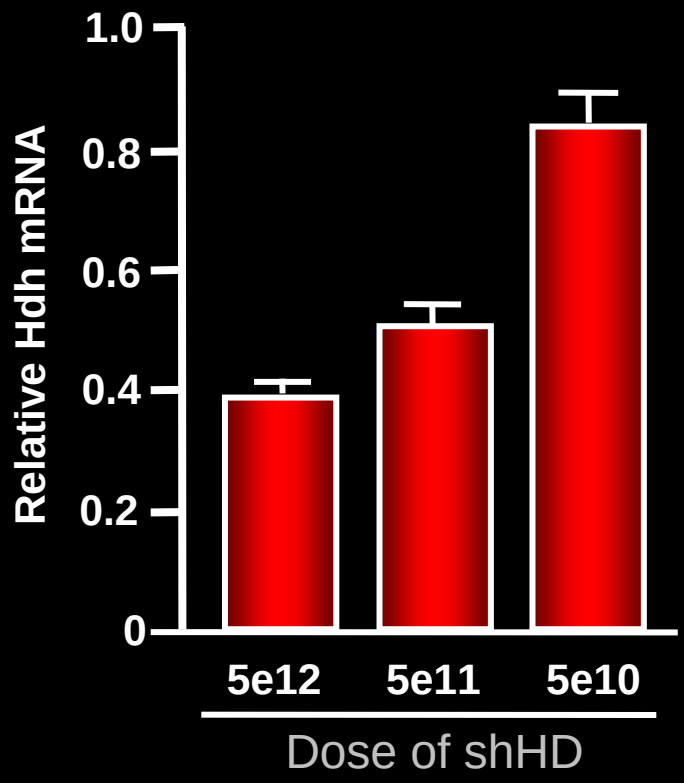
Toxicity in Mouse Striata



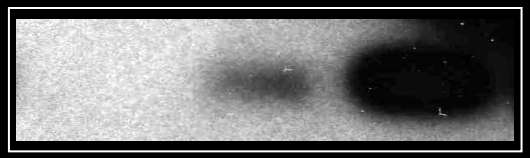
McBride, Boudreau and Harper et al. (2008) *PNAS*

Silencing and Toxicity in Mouse Striata





HD2.4 AS



Lowering shRNA dose improved toxicity, but also led to ineffective target gene silencing.

Why not deliver less shRNA virus?

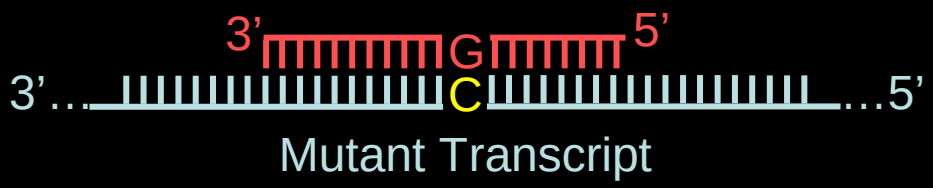
McBride, Boudreau et al. (2008) *PNAS*

Importance of Dose Optimization

- Dose delivered (vector copies per cell)
- Promoter selection
- shRNA vs. artificial miRNA
- Optimization is dependent upon vector platform delivery modality, target tissue/cell population and therapeutic target

Mutant Allele-Specific Silencing

- Relevant to dominant diseases
- If wild-type protein serves vital cellular function
- Target polymorphisms that discriminate wild-type and mutant transcripts
- Specificity can discriminate SNPs
- Has been investigated for a variety of diseases (AD, DYT1, HD, SCA3, SCA7)



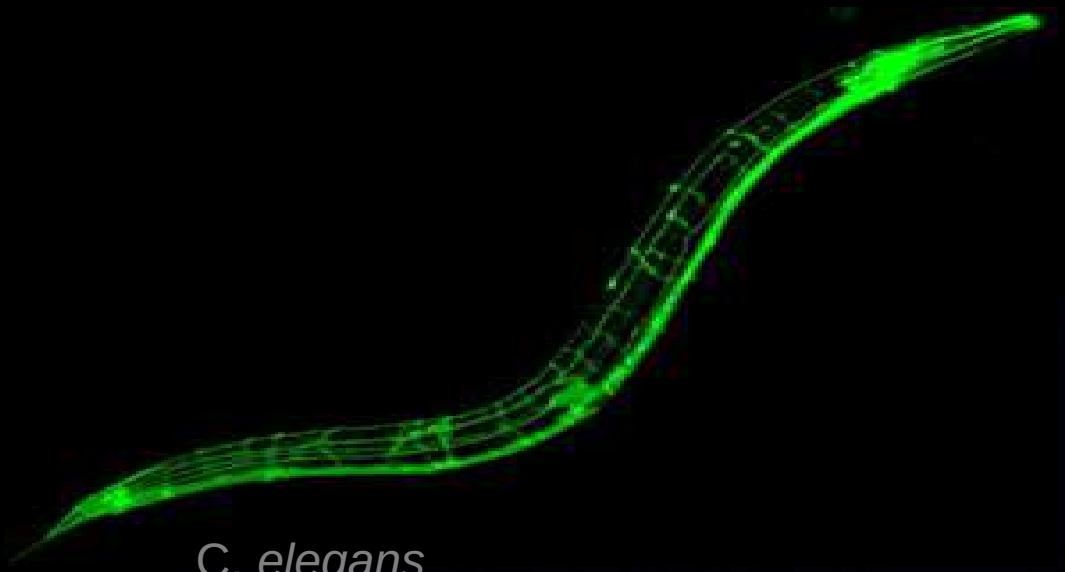
Mutant Transcript

Wild-type Transcript

Future Directions & Considerations

- Delivery, delivery, delivery
- Employing safe and potent sequences will reduce concerns regarding saturation and off-targeting
- Regulated silencing for expression-based RNAi
- Clinical trials for every sequence?

Questions?



C. *elegans*

Petunia