What is RNA interference? And what's up with it?

Henry Paulson, M.D., Ph.D.
University of Iowa, Carver College of Medicine
Interdisciplinary Programs in Genetics, Neuroscience and Molecular Biology

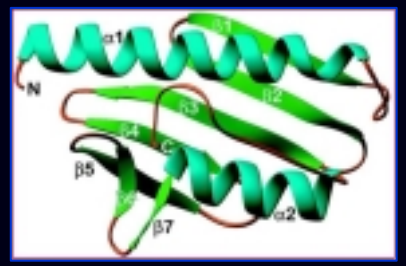
Molecular Biology's Central Dogma

DNA 

RNA

AAAA

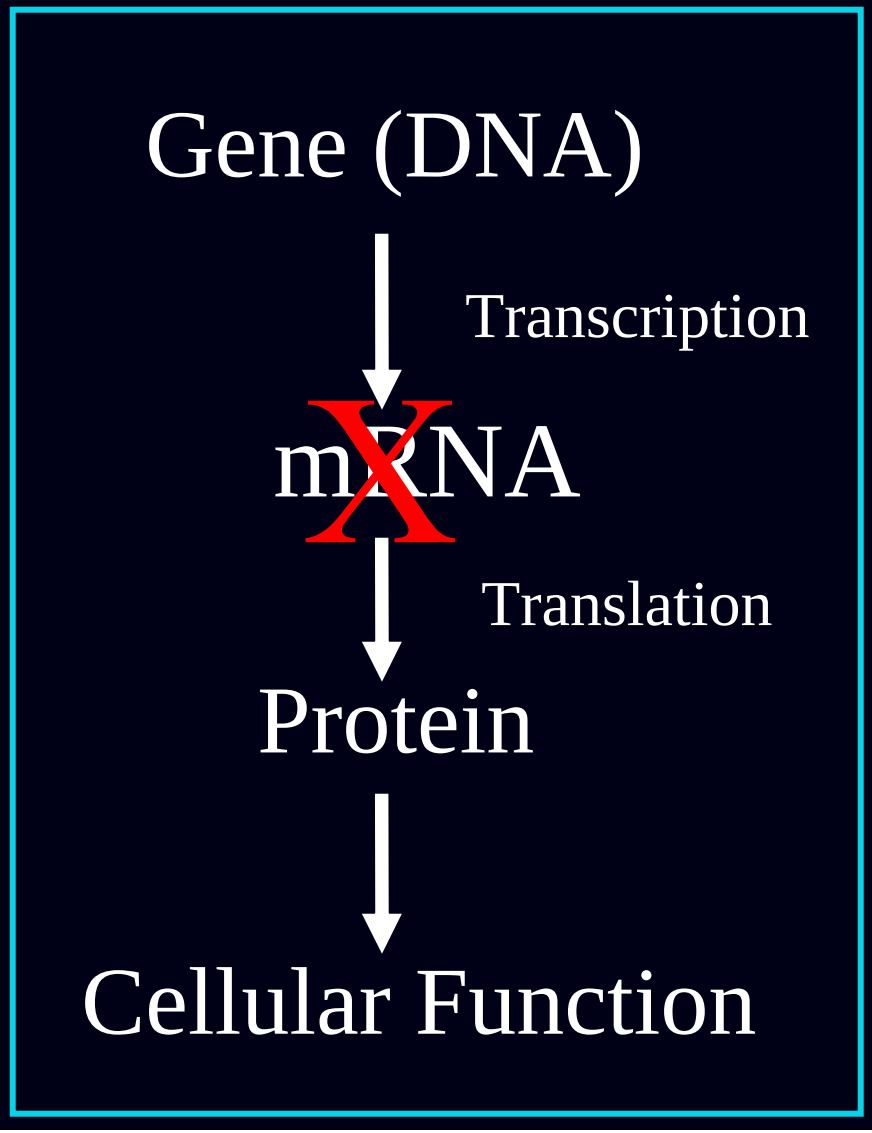
messenger RNA
ribosomal RNA
transfer RNA
other noncoding RNAs



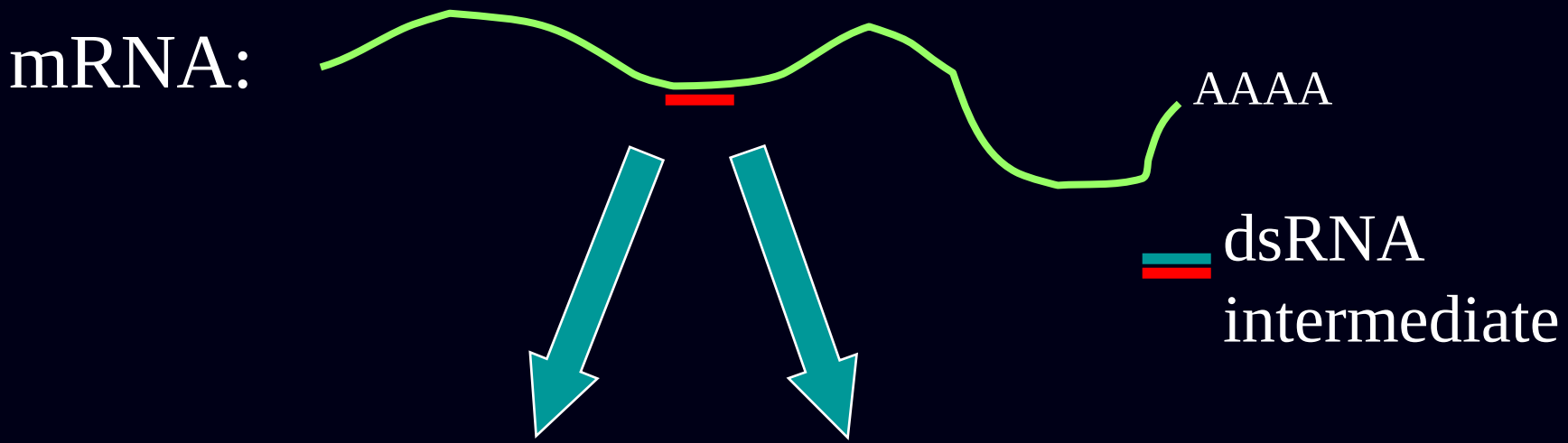
protein

RNAi: Shooting the messenger

RNAi is based on conserved biological machinery found in organisms from yeast to humans.



RNAi is accomplished with small (~20 bases) noncoding RNAs complementary to the targeted gene

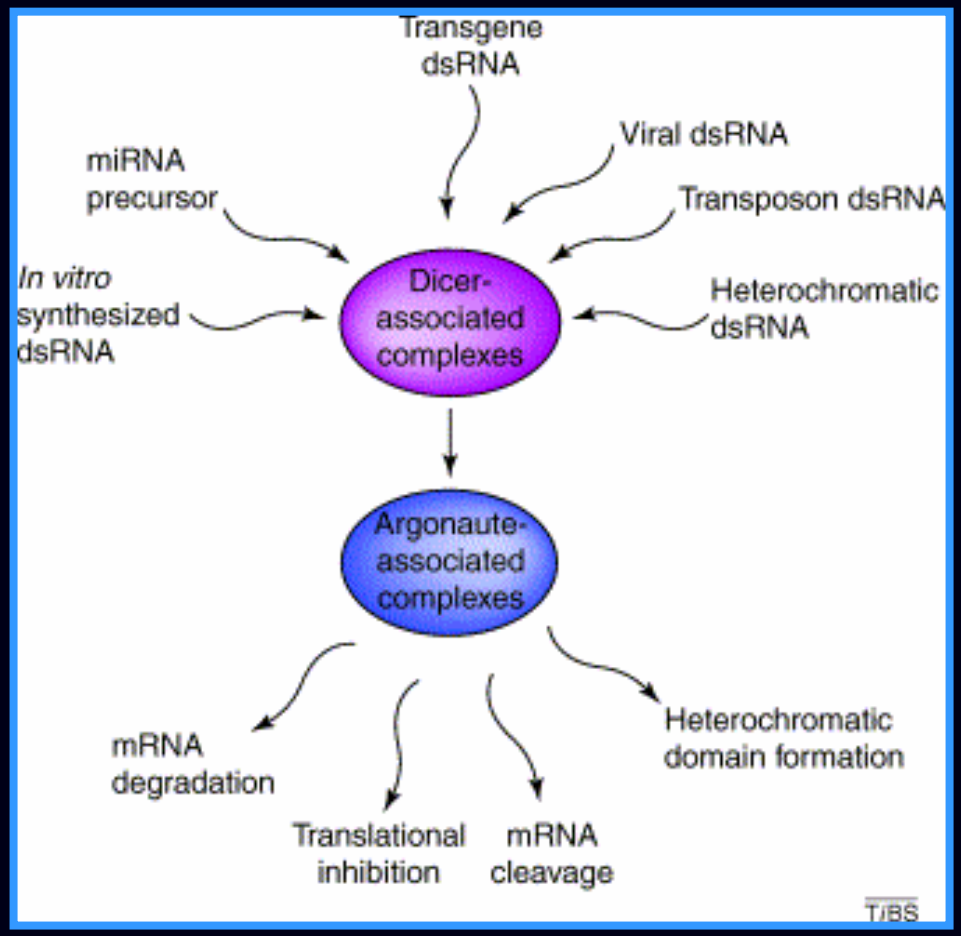


History of RNA-mediated suppression

Curious findings in plants led the way…

Adding a pigment gene to petunias made them less pigmented! Biology works in mysterious ways…

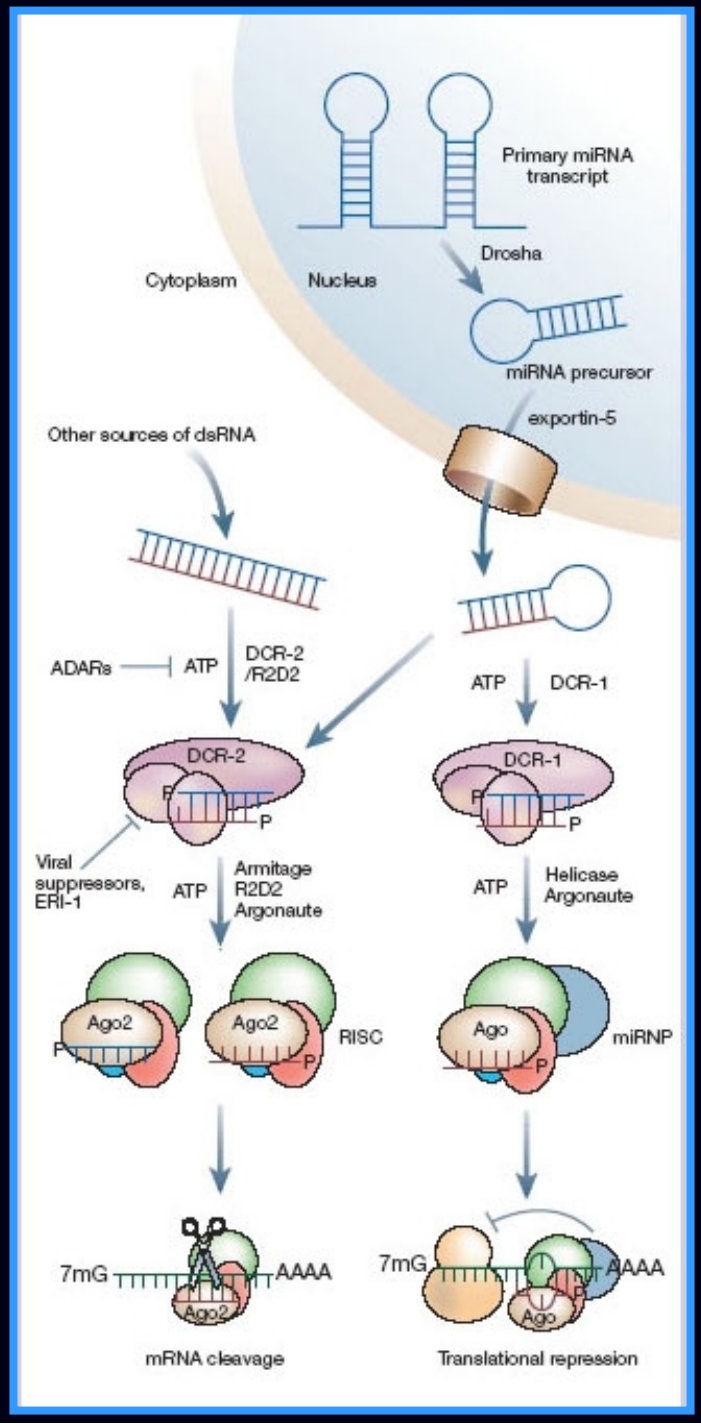
Why do organisms have this machinery?



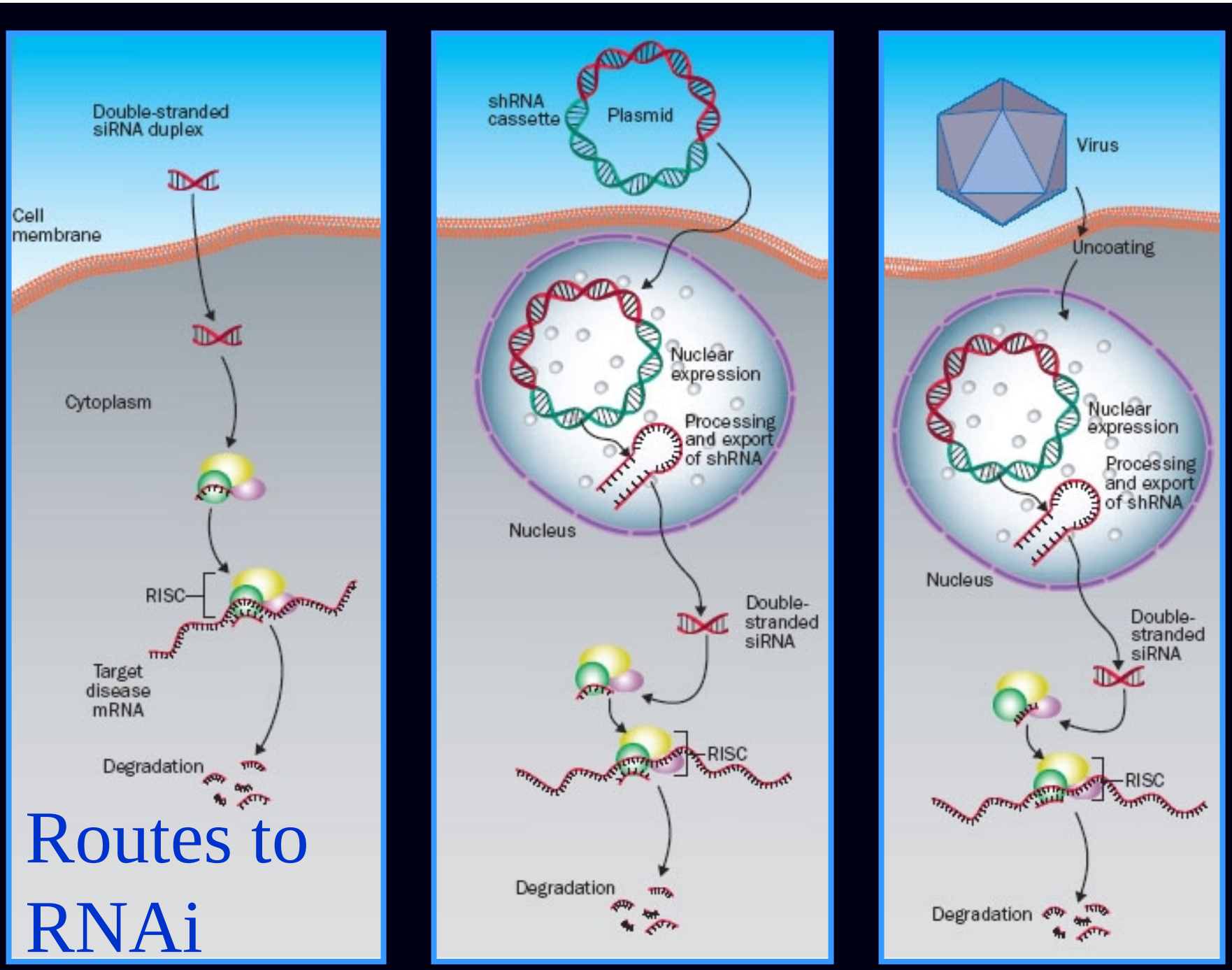
RNAi serves as a cellular defense against foreign DNA/RNA

There are naturally occurring RNAi molecules called “microRNAs”

miRNAs represent a novel class of genes that regulate gene expression during development



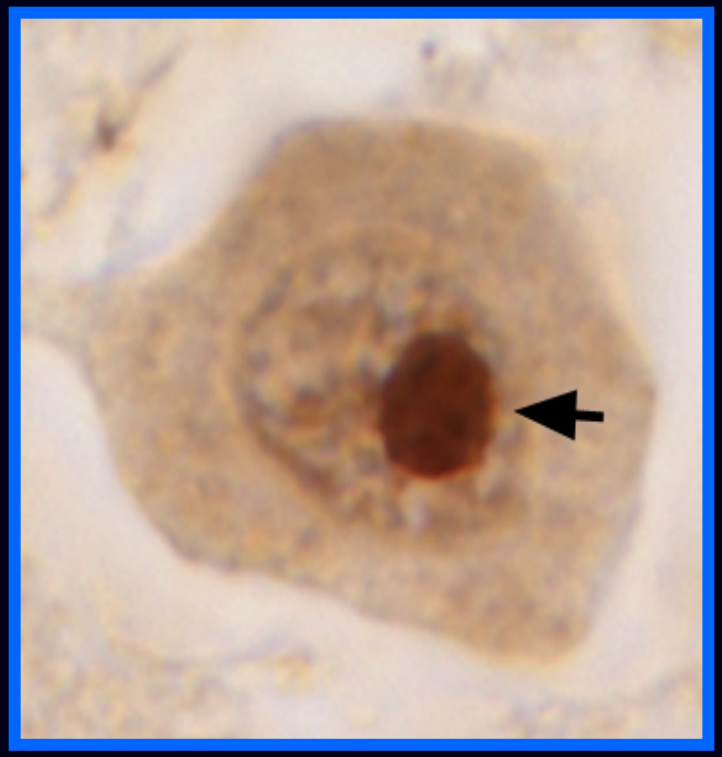
With RNA interference, we are copying the natural biology of microRNAs and applying this to our gene of interest



Routes to RNAi

Davidson and Paulson, Lancet Neurology 2004

Applying RNAi to dominant ataxia

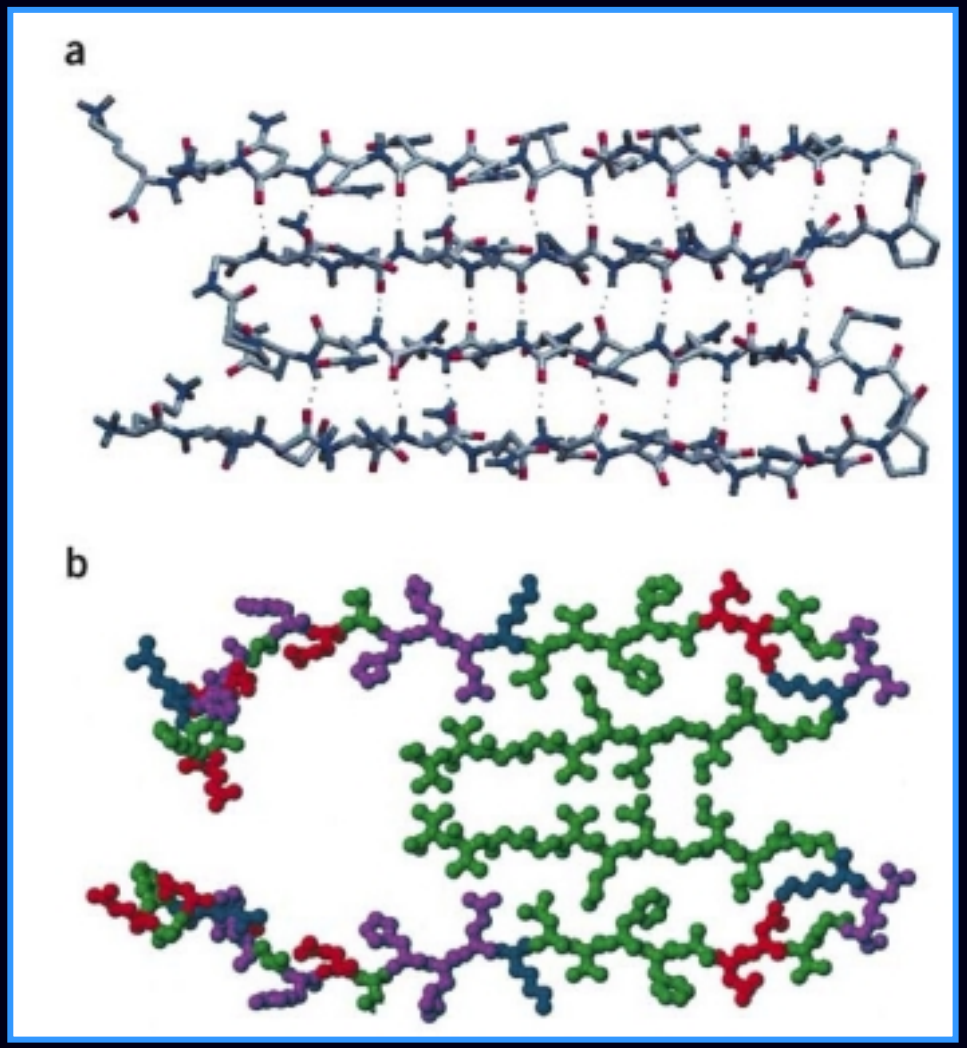


Many dominant ataxias, including those caused by expanded polyglutamine, involve abnormally folded or processed protein (e.g. SCA1,2,3,6,7,17)

“Toxic folds” in neurodegenerative diseases

polyglutamine

A-β amyloid



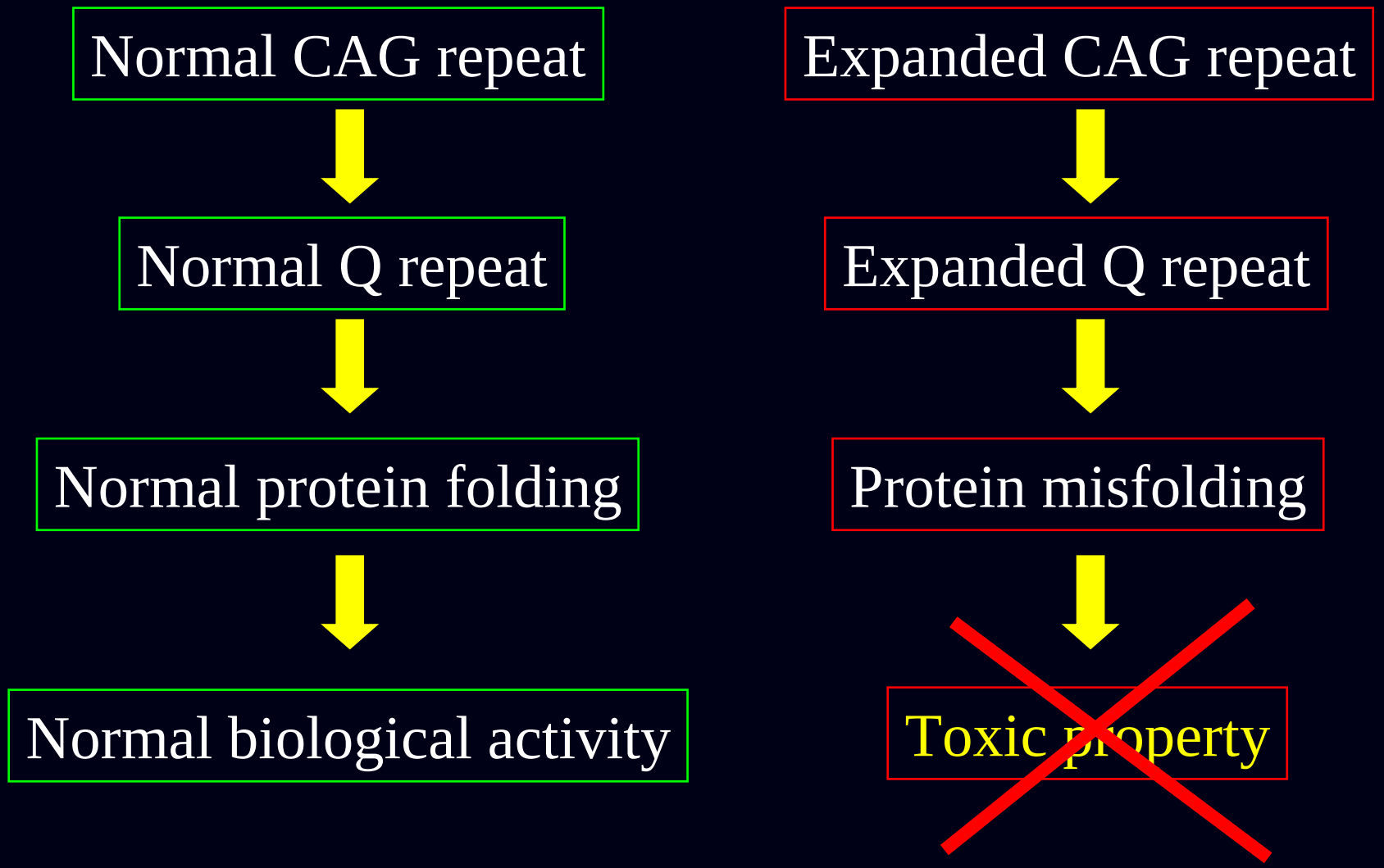
Harnessing RNAi for dominant ataxia

- Can siRNAs effectively silence dominant acting ataxia genes?
- Can RNAi reduce expression of a disease allele while sparing the normal allele?

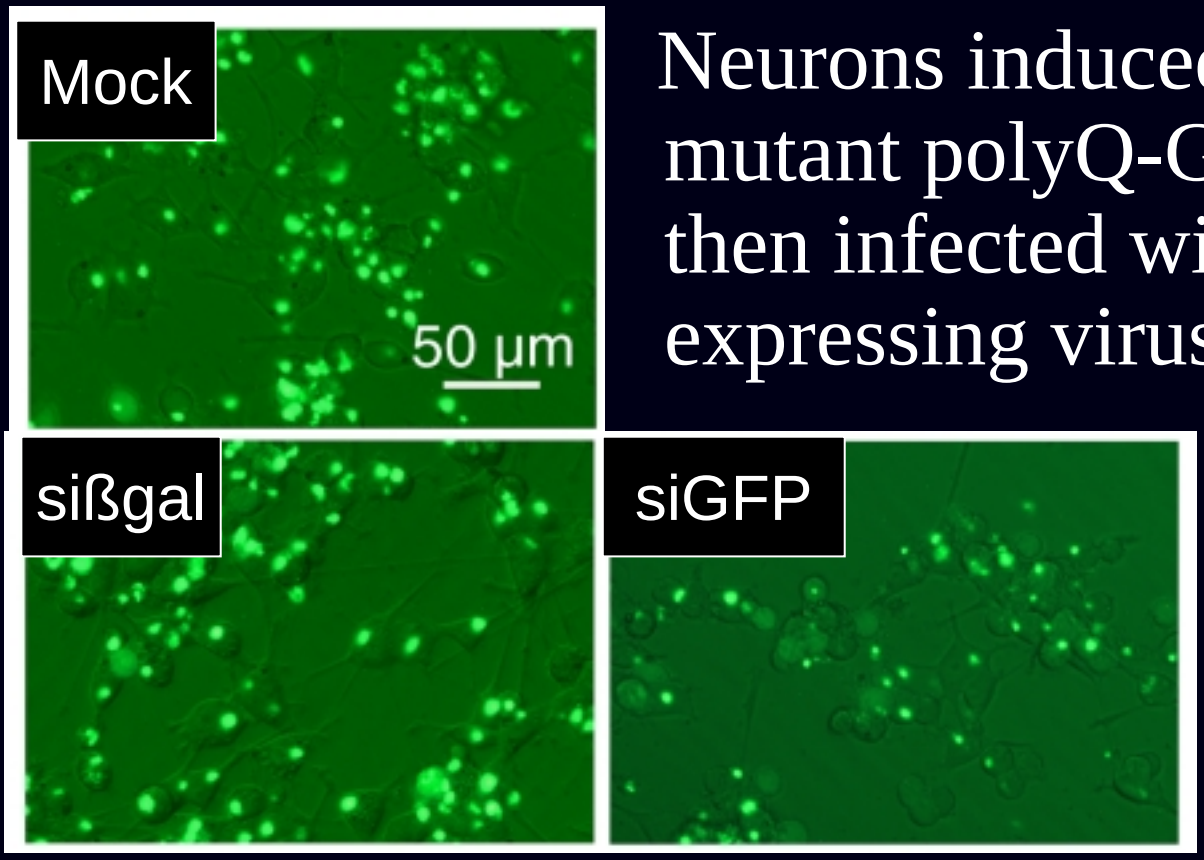
- SCA1
- SCA3/MJD

Both are expanded polyglutamine diseases

Simple view of polyglutamine disease…



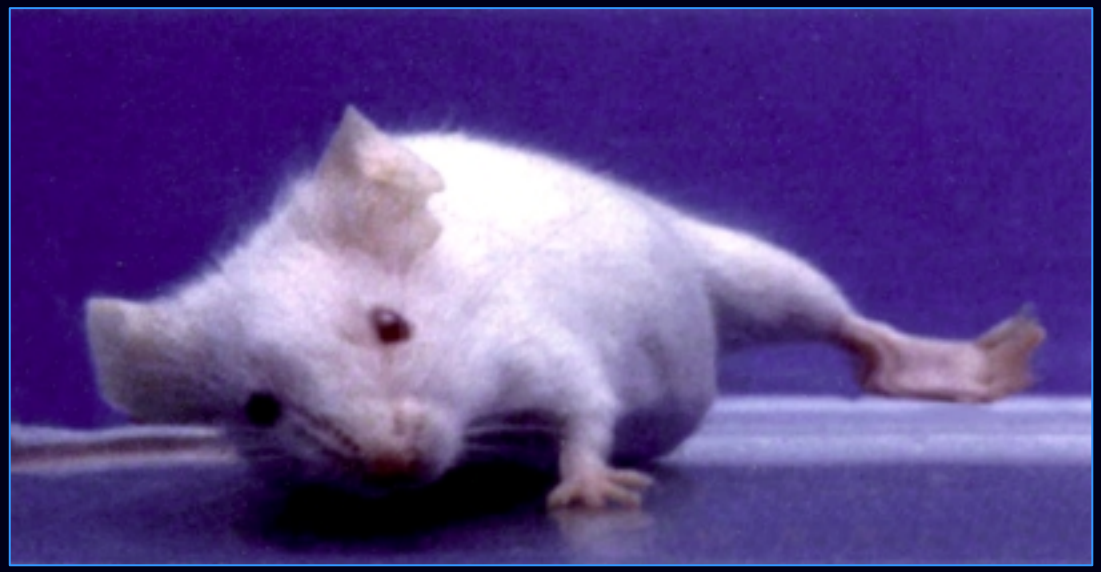
RNAi against preformed polyglutamine aggregates in cells



Neurons induced to express mutant polyQ-GFP were then infected with shRNA-expressing virus

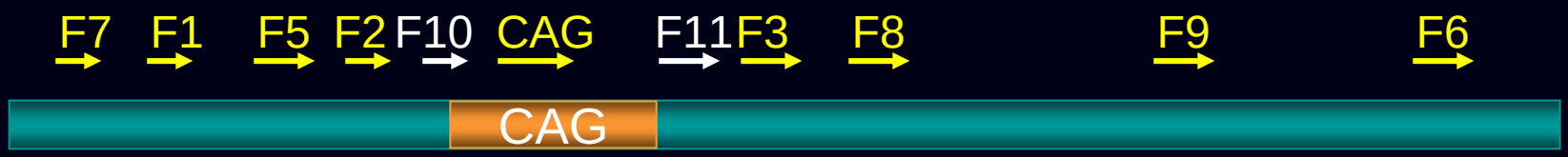
Xia H. et al, Nat Biotech (2002)

Applying RNAi to Spinocerebellar ataxia type 1 (SCA1)

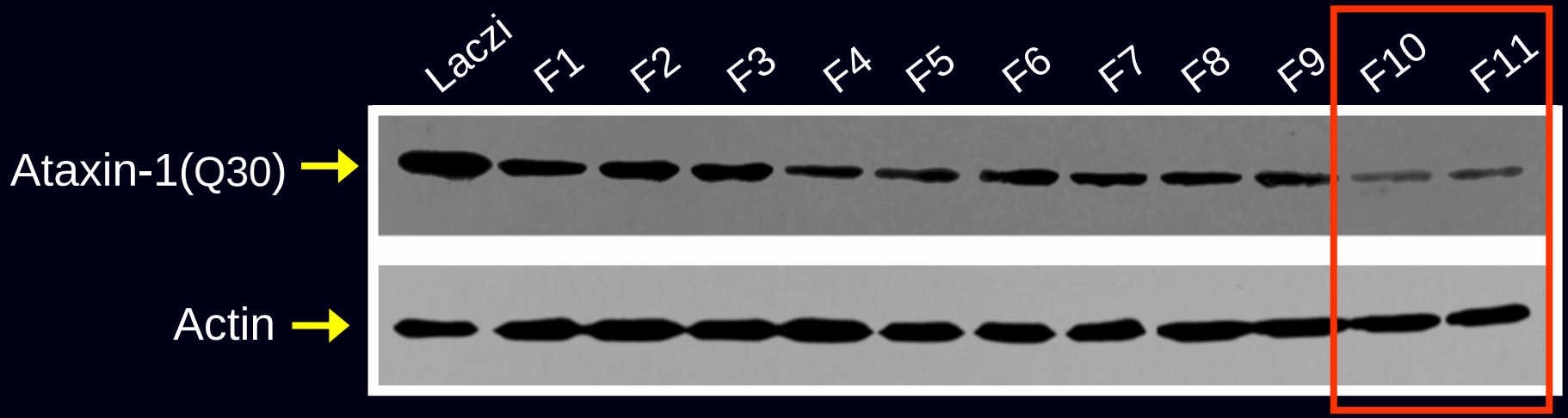


SCA1 transgenic mouse created by Drs. Orr, Zoghbi and colleagues (Burright et al. Cell 1995)

Developing RNAi against SCA1 protein, ataxin-1



Human SCA1 “open reading frame” encoding ataxin-1



Xia et al, Nature Medicine, 2004

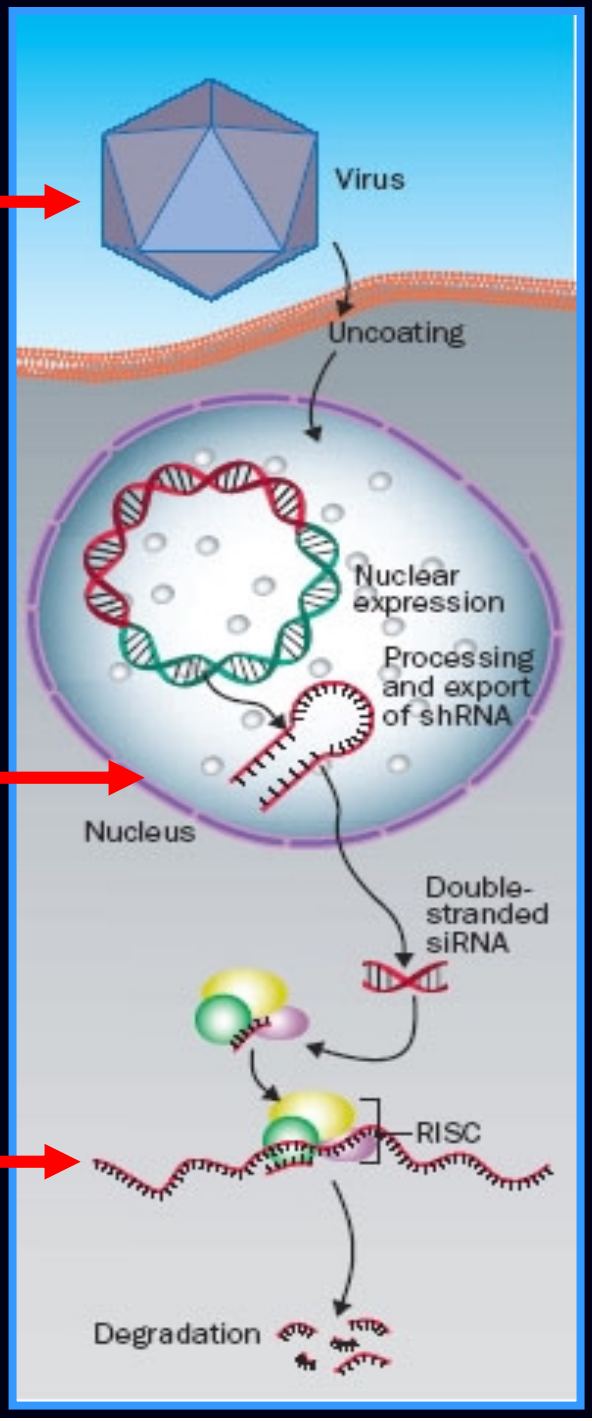
Testing shRNA that targets mutant ataxin-1 in SCA1 mice

- Expression in Purkinje cells
- Recapitulates features of disease:
  - Dendritic atrophy, Purkinje cell loss, ataxia

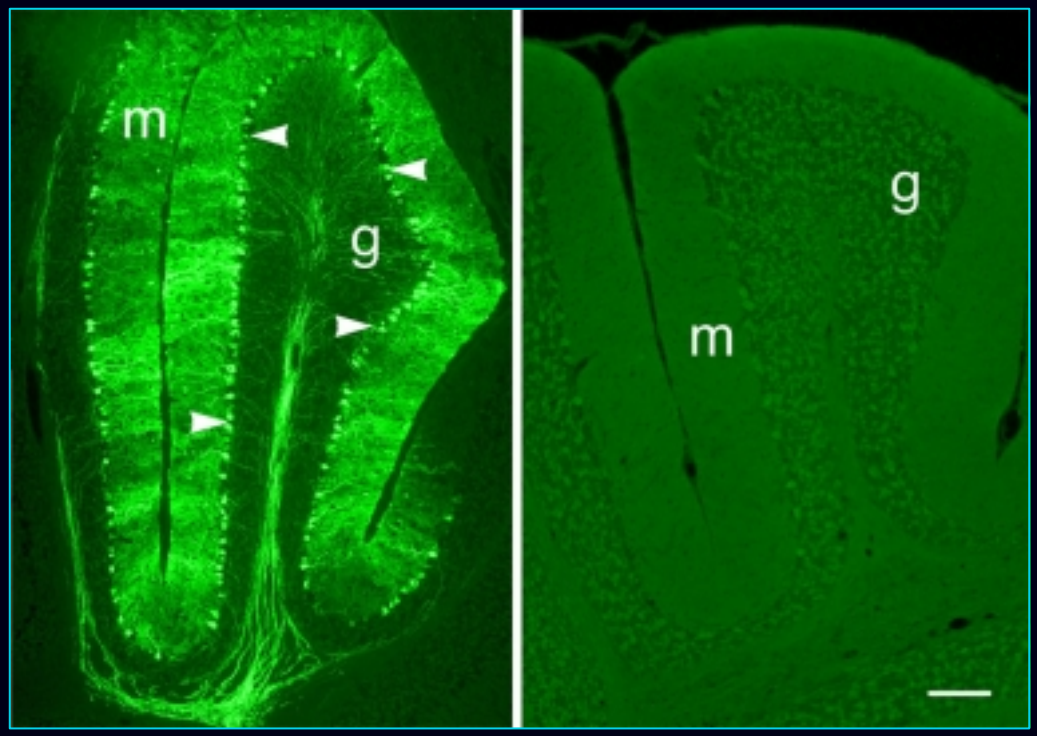
recombinant short hairpin RNA-expressing virus

shRNA targeting ataxin-1 mRNA

ataxin-1 mRNA

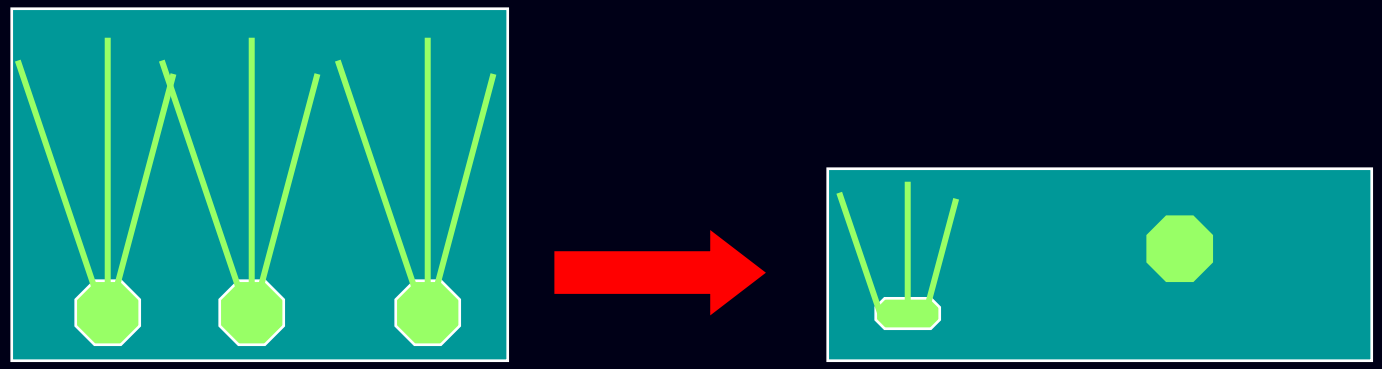


Can we deliver virus to the target cells?



Yes!! Delivery to Purkinje cells of recombinant adeno-associated virus (AAV) expressing green reporter protein

Cerebellar atrophy occurs during course of disease



healthy cerebellum

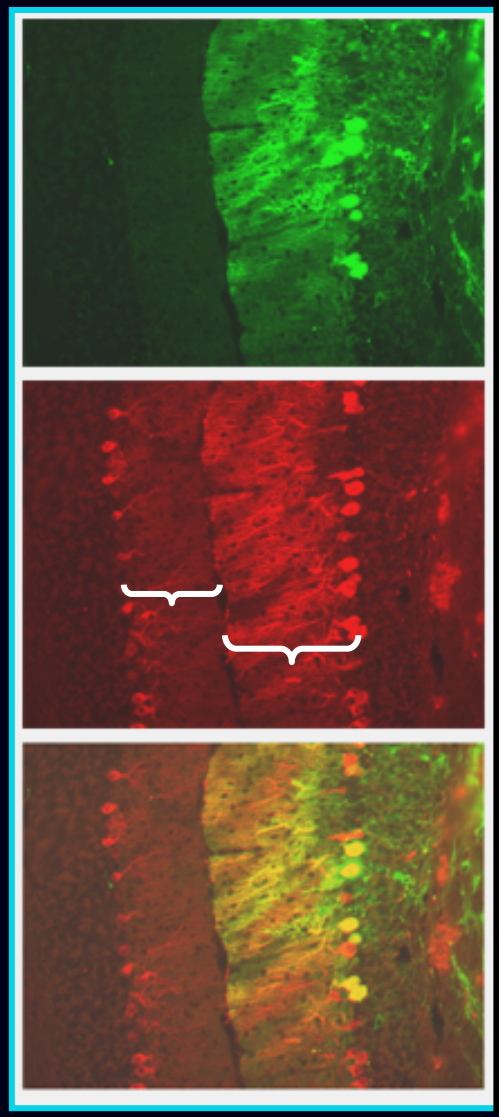
diseased cerebellum

SCA1/F10

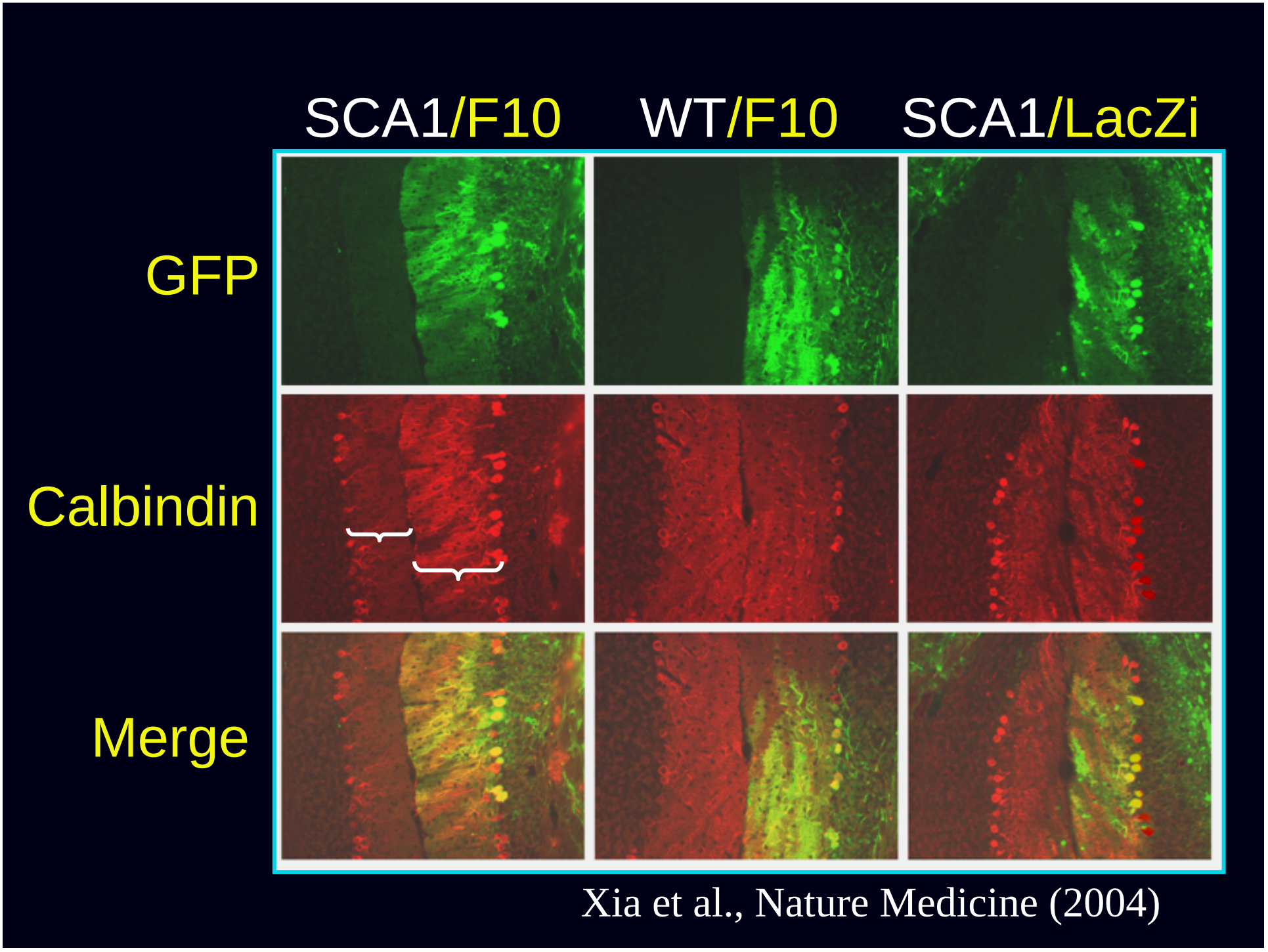
GFP

Calbindin

Merge

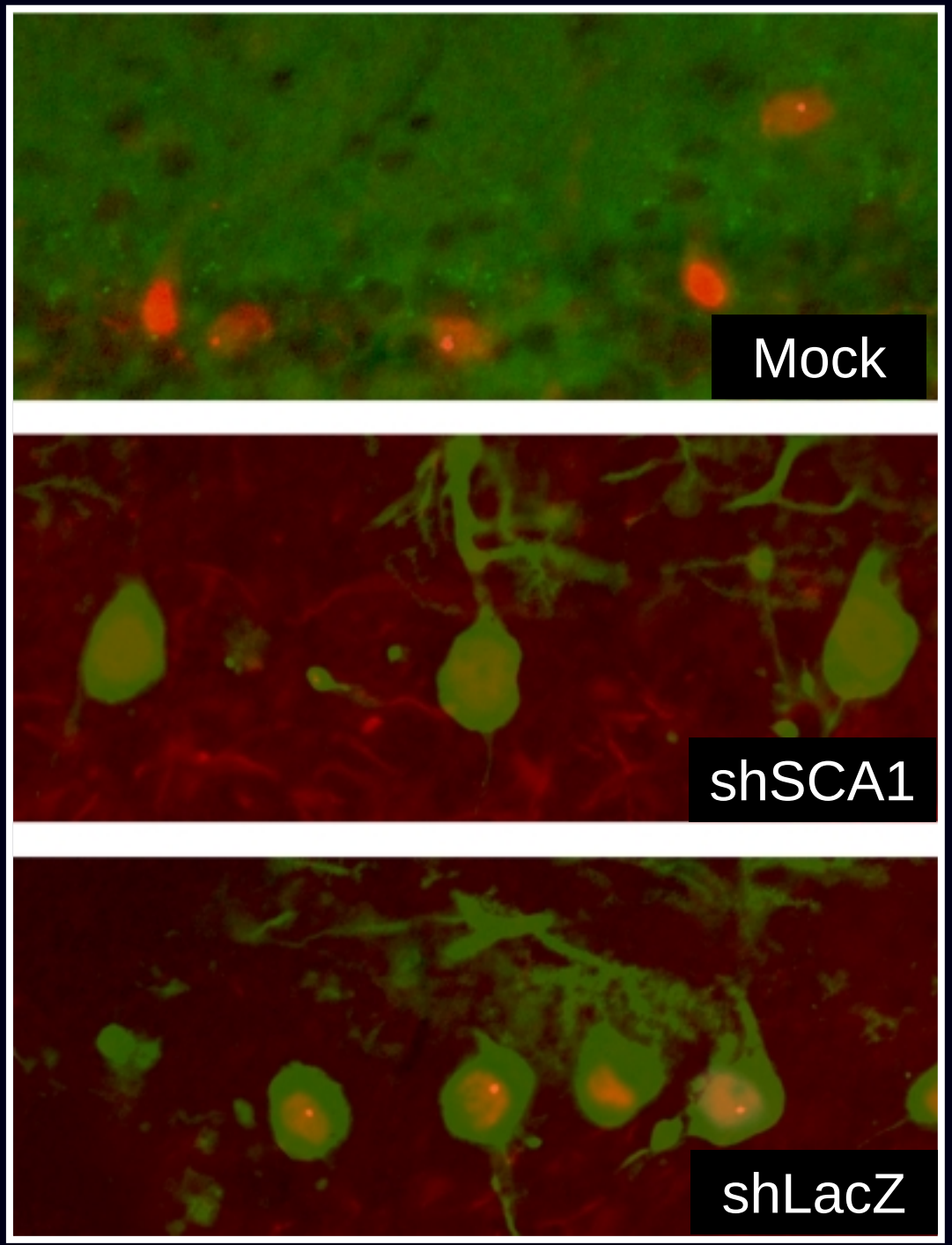


Xia et al., Nature Medicine (2004)

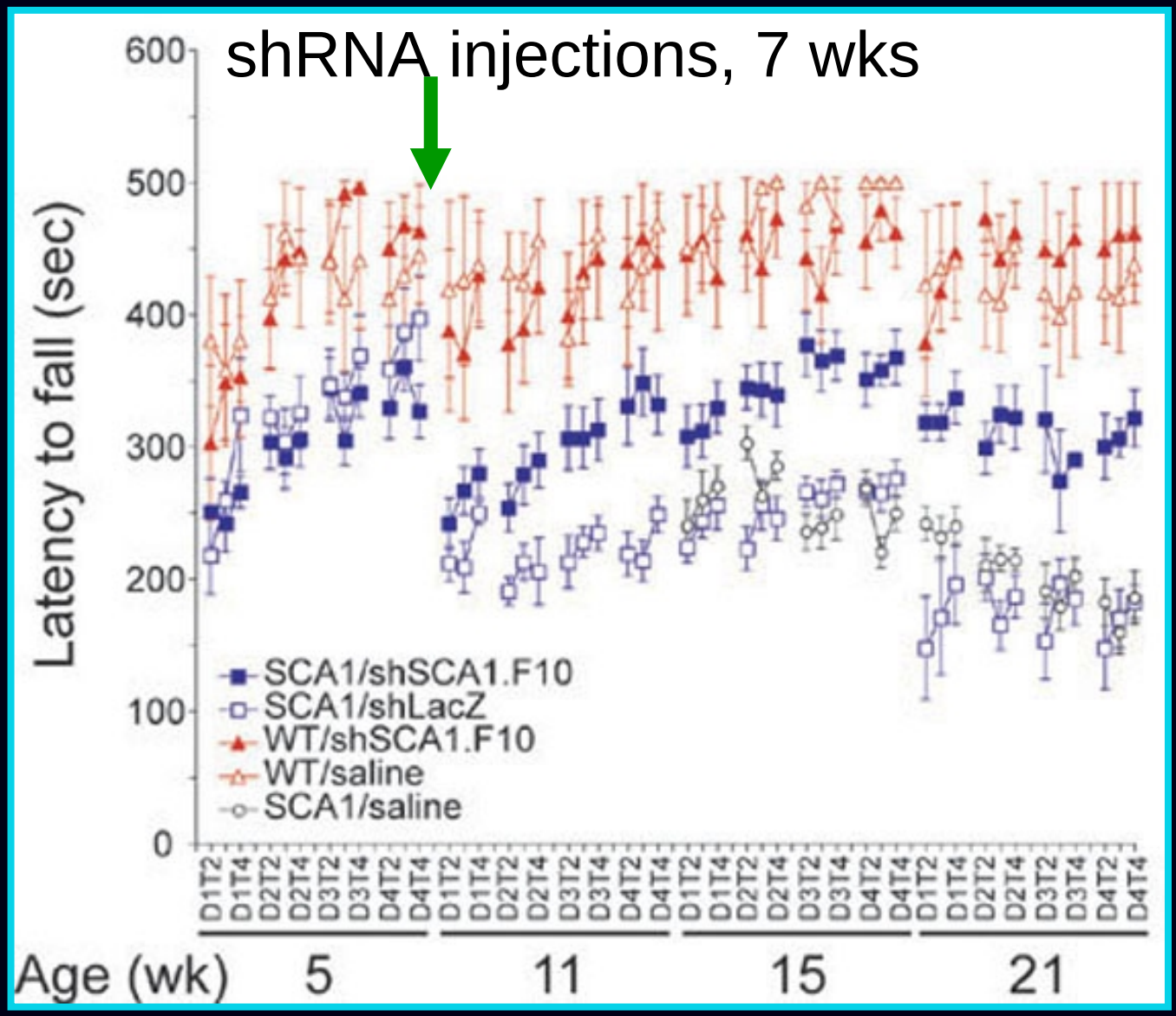


Xia et al., Nature Medicine (2004)

RNAi virus reduces disease protein expression and inclusions



Treated mice are not as clumsy!



Normal mice

SCA1 mice, treated

SCA1 mice

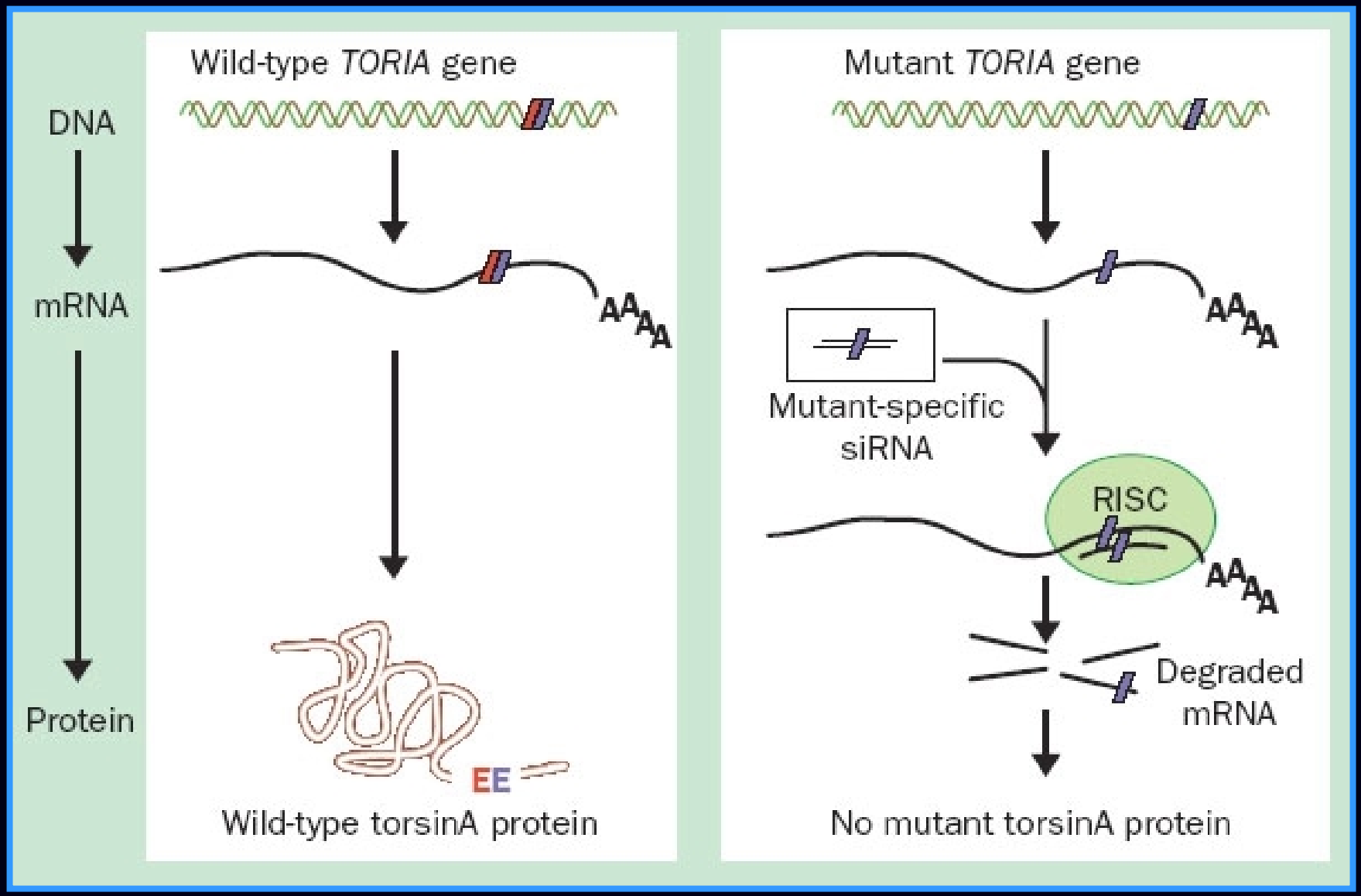
Summary in SCA1 mice

- Long-term suppression of ataxin-1 expression
- Successfully reduces protein inclusions, dendritic atrophy and ataxia
- RNAi delivery to wild type mice seems to be well tolerated

But what if we must target the disease allele selectively?

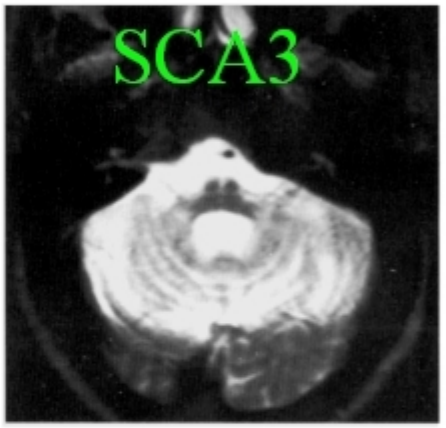
- What if the protein coded by disease gene is important to brain function…
- … then knocking down both the normal and disease copy could be bad
- So … can RNAi distinguish the normal and disease copies of the gene?
- Let's test this with disease-linked polymorphism in SCA3/MJD

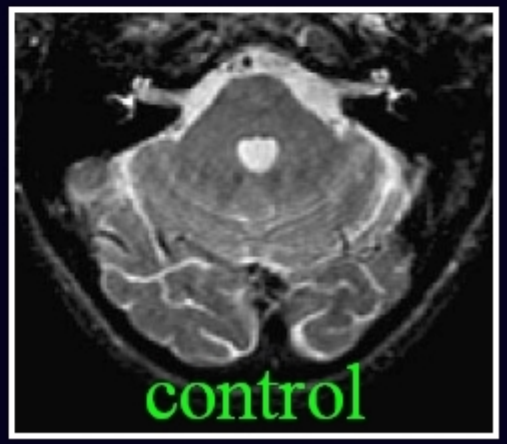
Example of allele-specific silencing



Spinocerebellar ataxia type 3/ Machado-Joseph disease

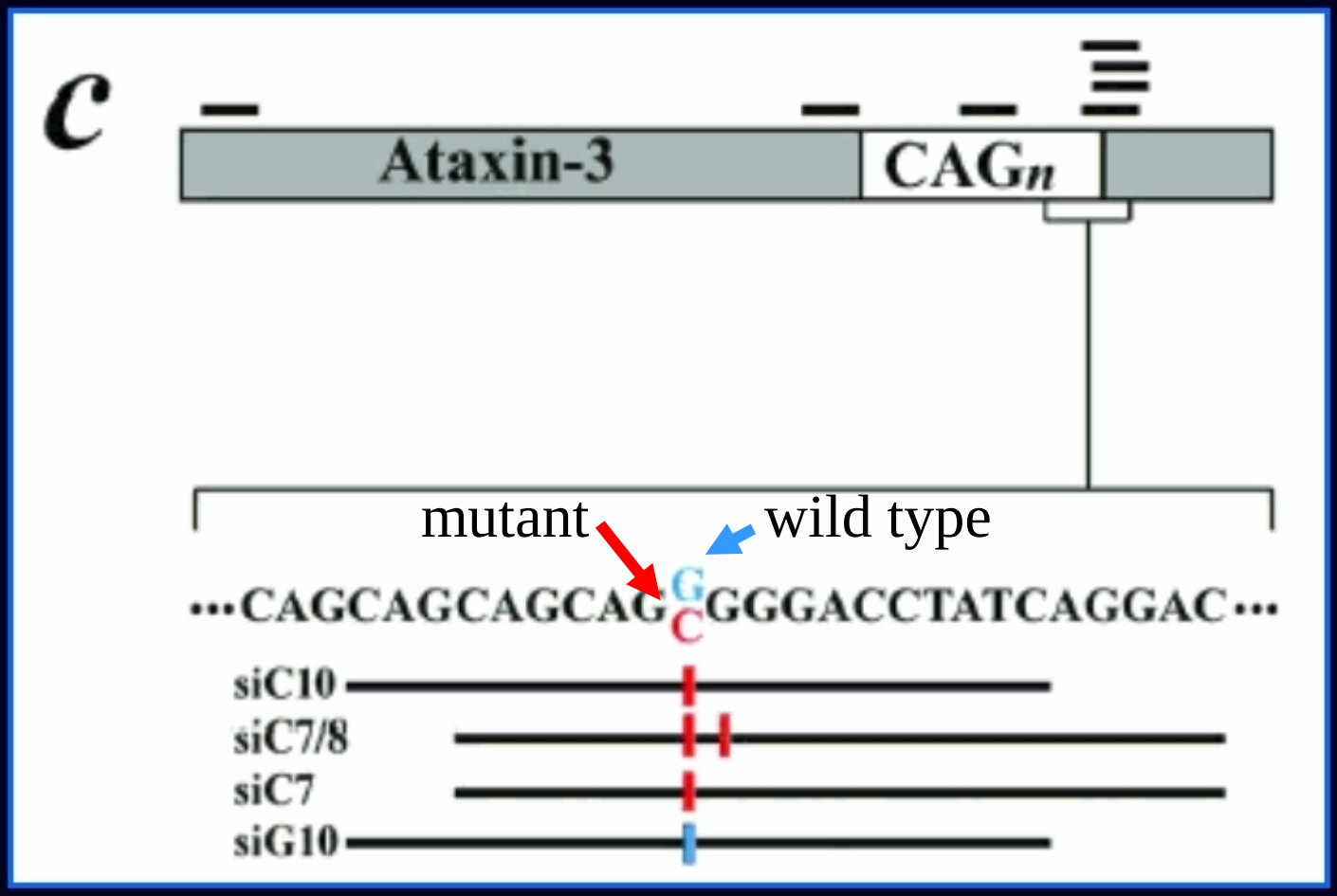
- Most common dominant ataxia
- "Cerebellar-Plus" syndrome
- Highly variable:
  - -early onset dystonia
  - -adult onset ataxia
  - -late onset neuropathy
  - -parkinsonism





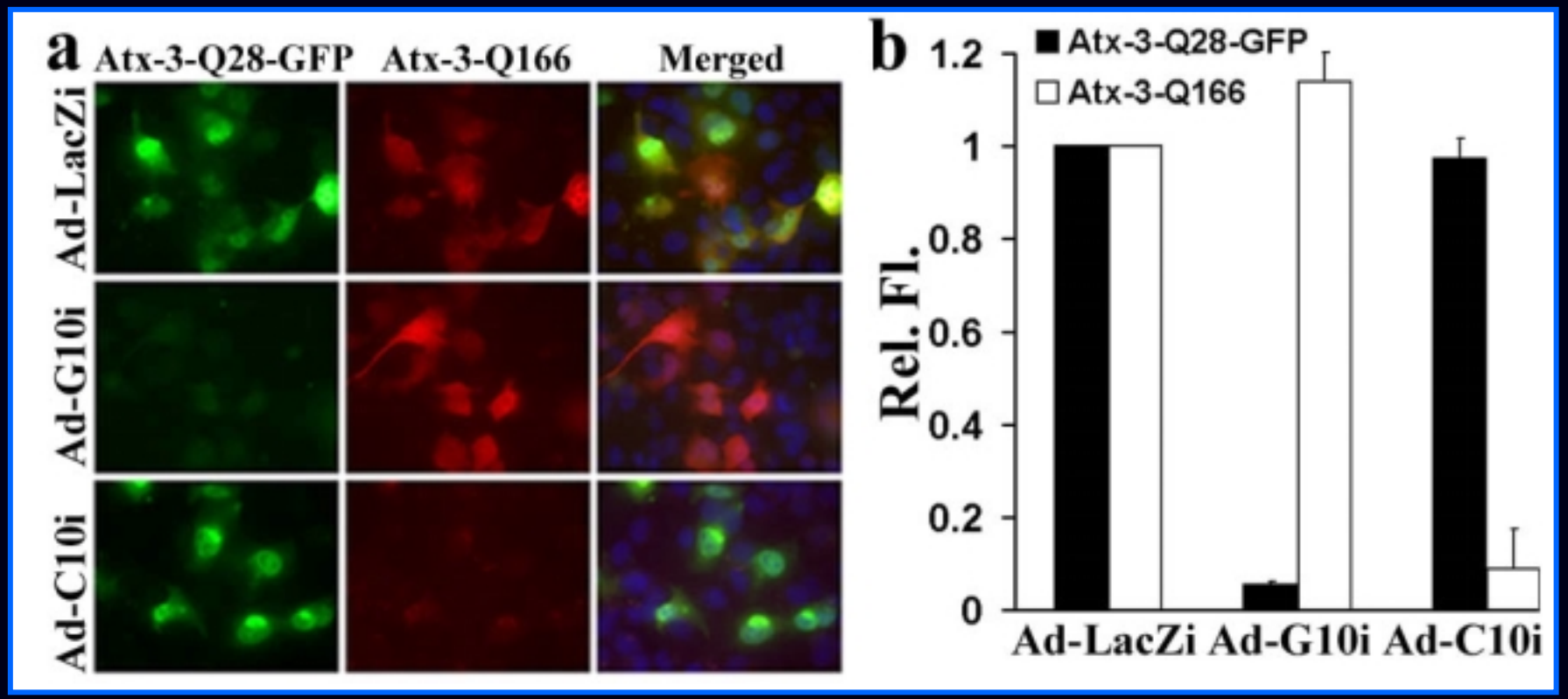
CAG repeat length

Using a linked SNP to suppress expression of SCA3/MJD protein



~70% expanded alleles contain "C"
but most normal alleles contain "G"

Viral-mediated, allele-specific shRNA suppression of ataxin-3



Fluorescence images (L) and quantification (R) of siRNA-viral suppression of normal or mutant ataxin-3

RNAi in neurons from transgenic SCA3/MJD mice

- Using Veronica Colomer's mouse model (published this month)
- Mouse widely expresses mutant ataxin-3
- RNAi virus reduces expression of mutant ataxin-3 while sparing normal mouse ataxin-3
- Beginning in vivo experiments, as described in SCA1 mice

Summary

- RNAi has revolutionized biological research
- siRNAs can silence disease genes differing by as little as a single nucleotide
- First studies suggest viral mediated RNAi is tolerated by the brain
- Further studies in animal models are needed to prove that RNAi has actual therapeutic value

Challenges and Concerns

1. Delivery (how?)
2. Delivery (where?)
3. Delivery (what reagent?)

See Soutschek et al., Nature 432: 173-178 (2004); a brand new paper showing that lipid-modified RNAs can work in mice

Challenges and Concerns

- Can we achieve efficient, sustained expression in brain?
- Will “co-opting” the RNAi biological machinery have untoward effects?
- What about “off-target” effects?

RNAi sounds good, but keep a broad view to other routes to benefit!

- Symptomatic therapy
- Existing drugs and compounds that might have protective effect
- What is happening in related disorders? (e.g. Huntington disease)

Acknowledgements

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- Cynthia Gouvion
- Other members of Paulson lab
- Beverly Davidson lab
- Gloria Lee
- Veronica Colomer

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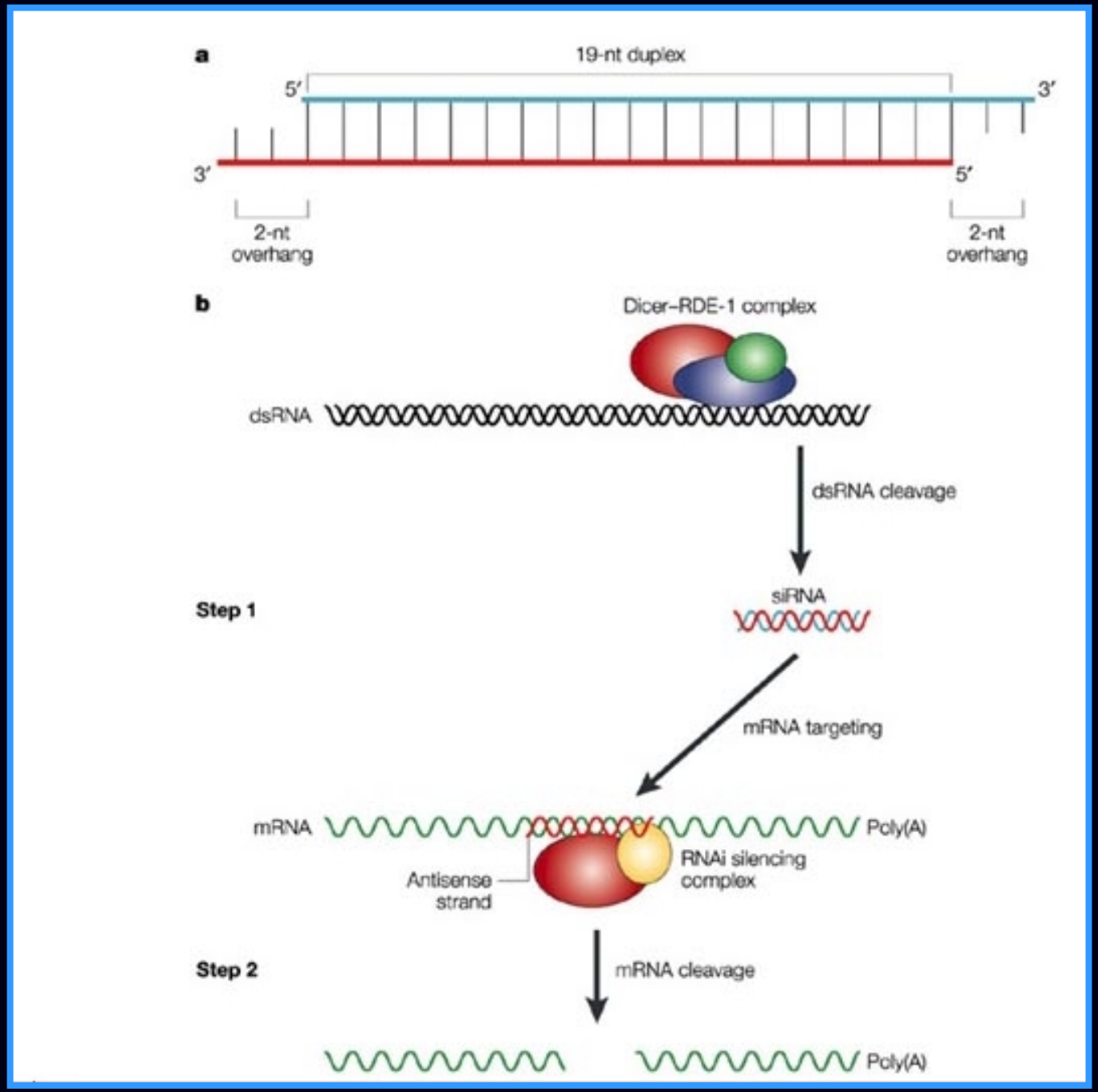

Nat Rev Genet 3:737 (2002)