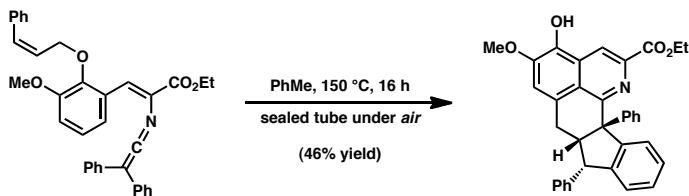


*Mechanism*

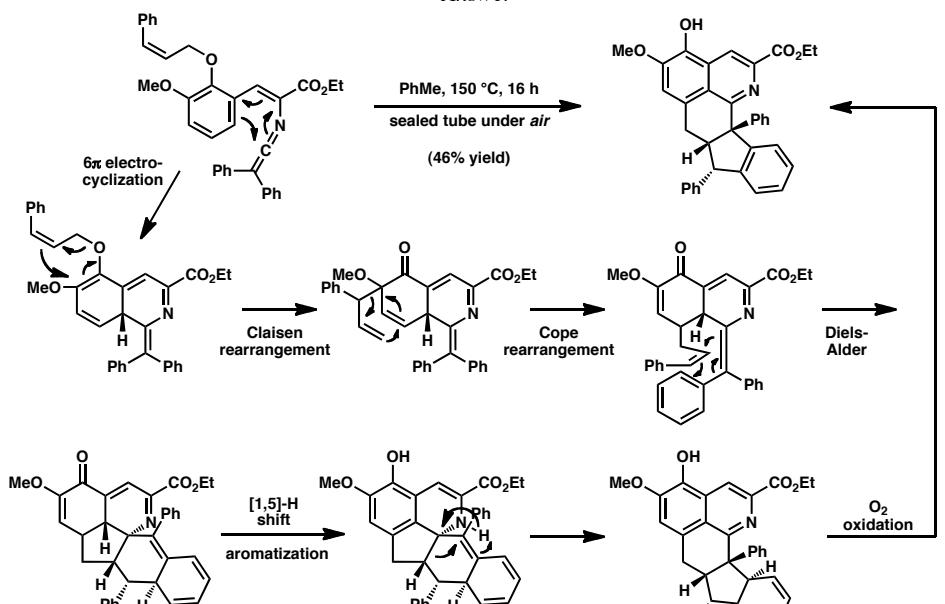
*Answer*



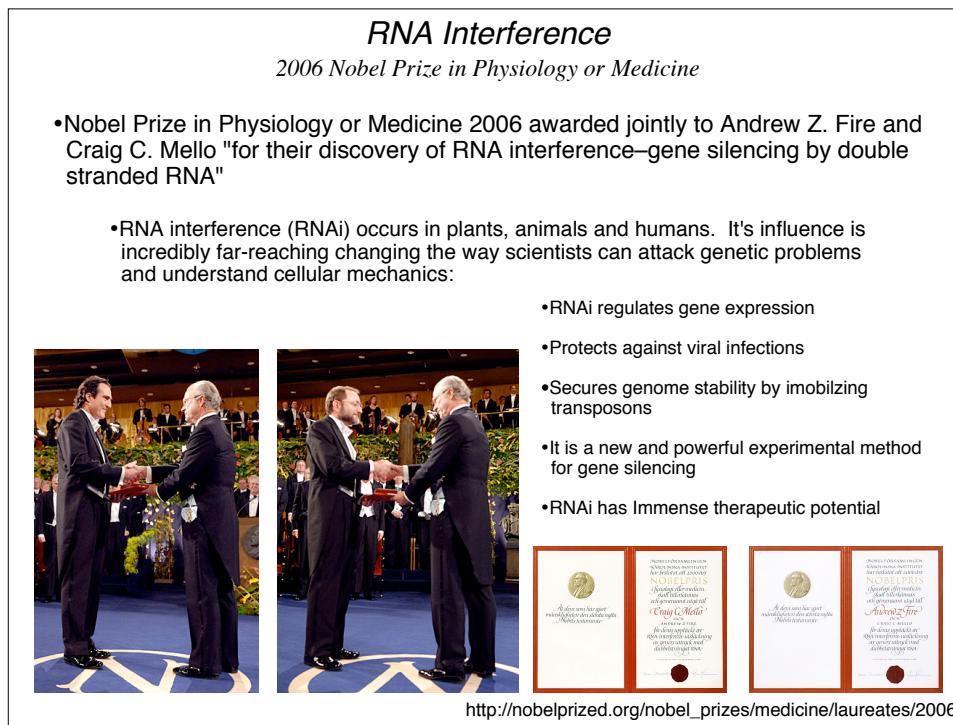
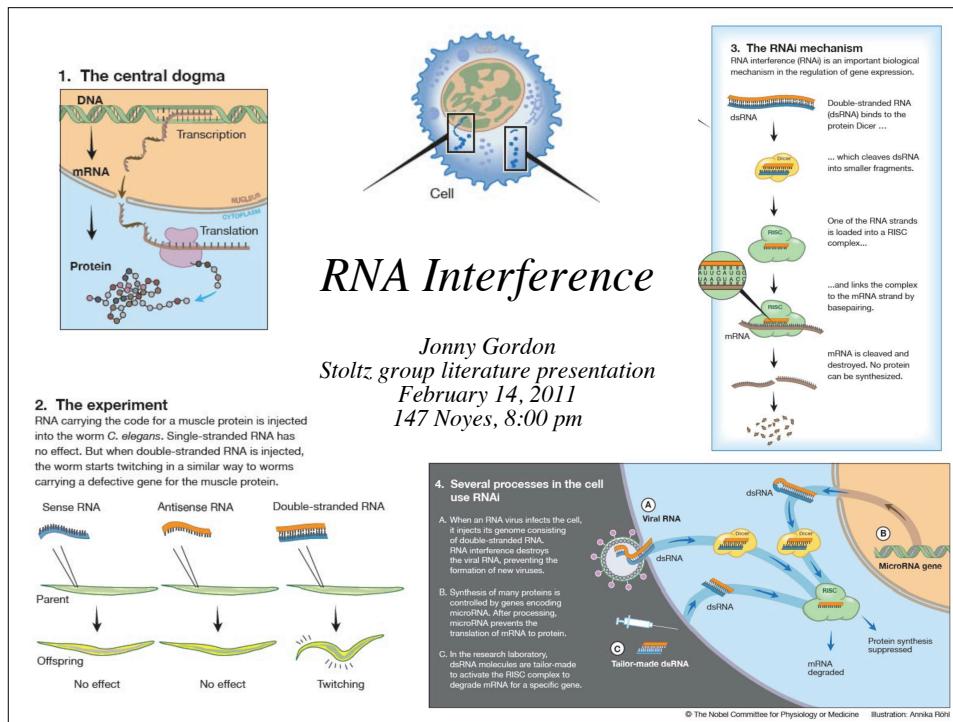
Molina, P.; Alajarín, M.; Vidal, A. *J. Org. Chem.* 1991, 56, 4008.

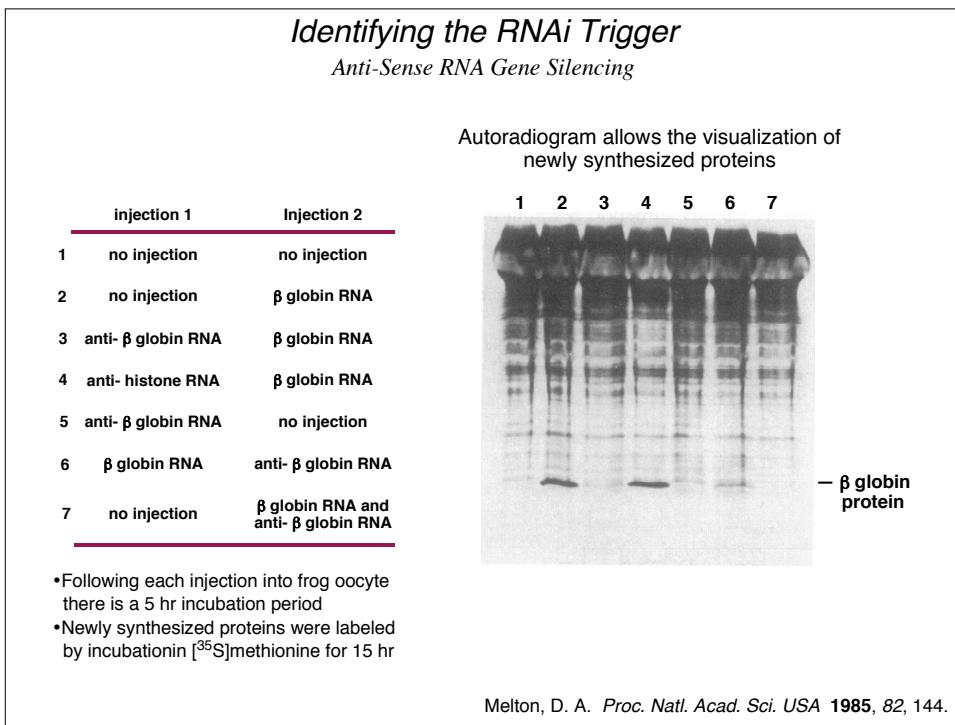
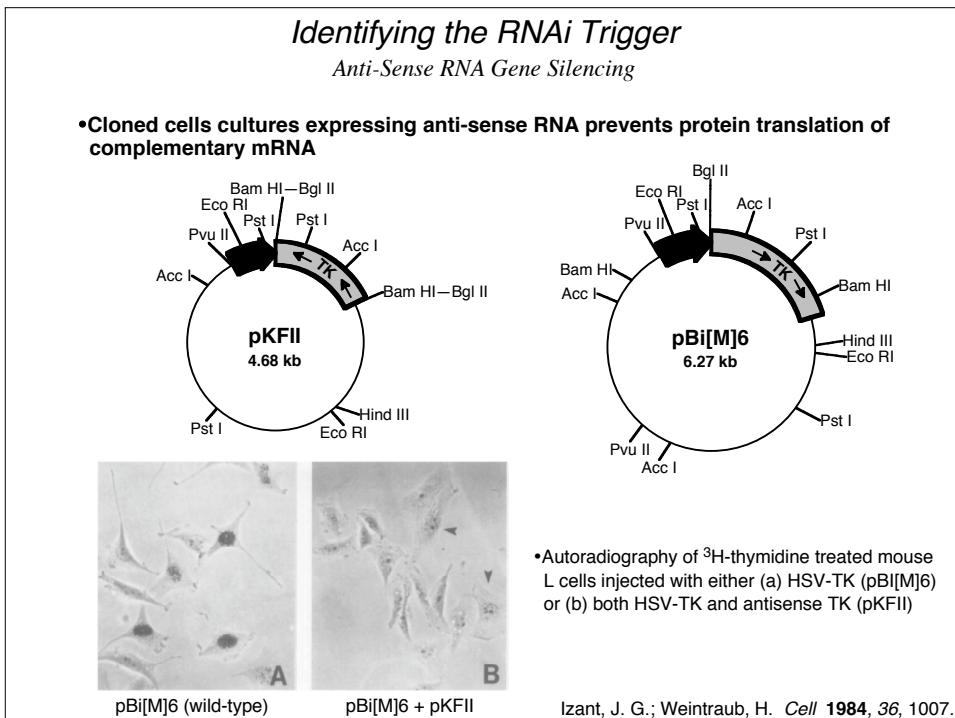
*Mechanism*

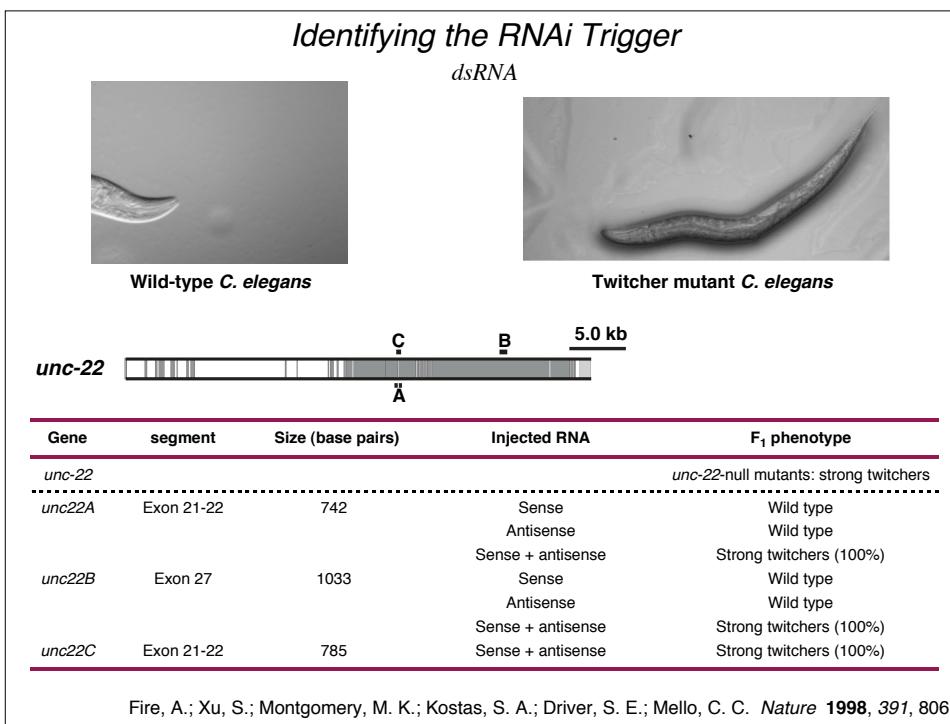
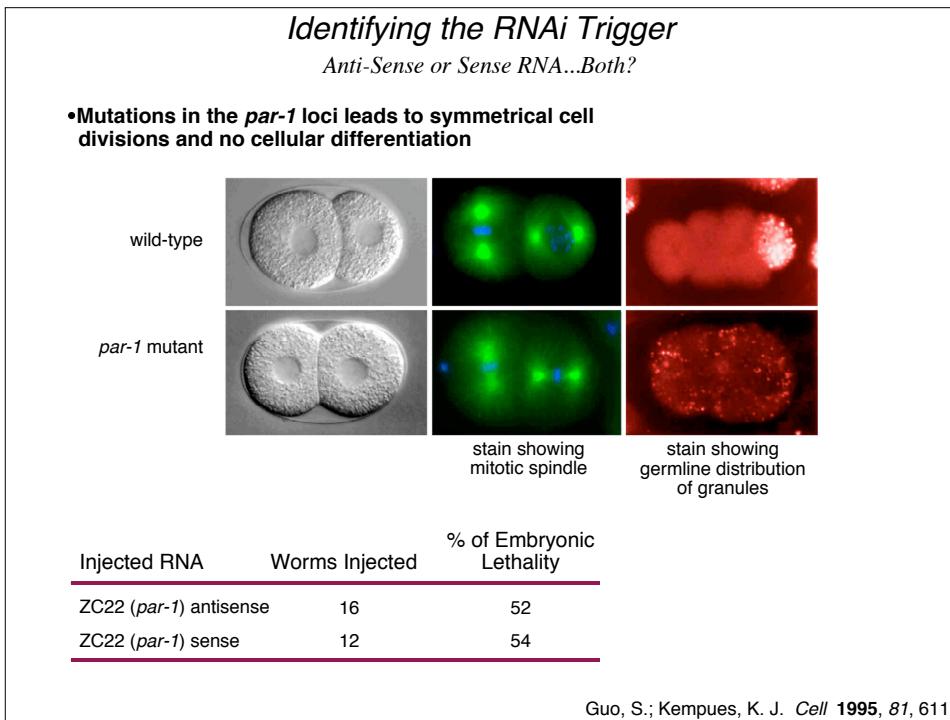
*Answer*



Molina, P.; Alajarín, M.; Vidal, A. *J. Org. Chem.* 1991, 56, 4008.





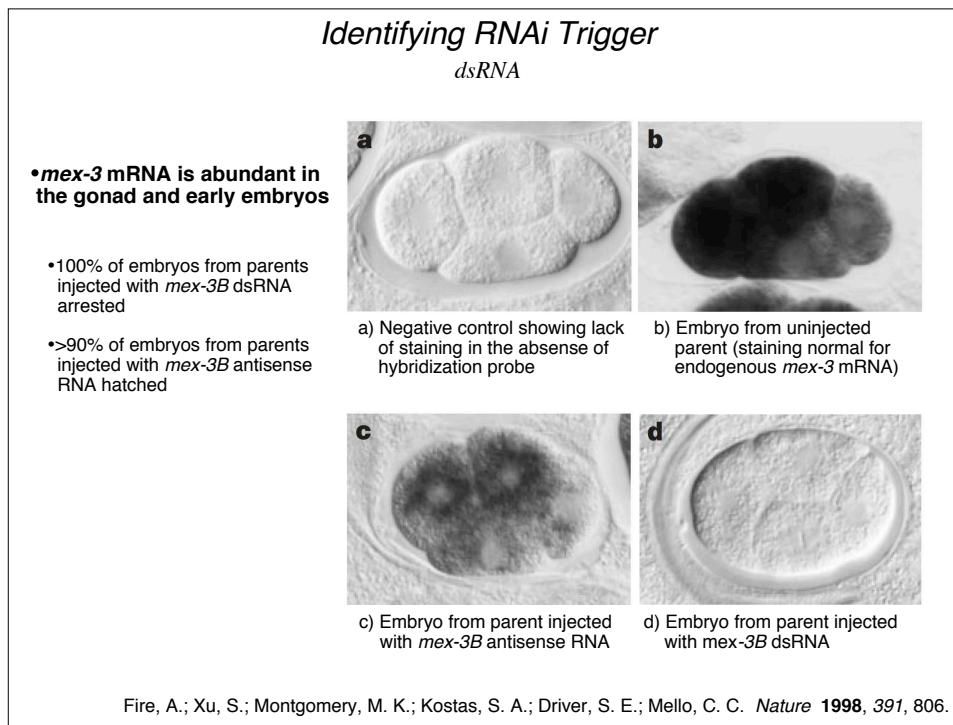


*unc-54*

*hlh-1*

Gene	segment	Size (base pairs)	Injected RNA	F <sub>1</sub> phenotype
<i>unc-54</i> null mutants: paralysed				
<i>unc54A</i>	Exon 6	576	Sense	Wild type (100%)
			Antisense	Wild type (100%)
			Sense + antisense	Paralysed (100%)
<i>unc54B</i>	Exon 6	651	Sense	Wild type (100%)
			Antisense	Wild type (100%)
			Sense + antisense	Paralysed (100%)
<i>unc54C</i>	Exons 1-5	1015	Sense + antisense	Arrested embryos and larvae (100%)
<i>unc54D</i>	Intron 1	567	Sense + antisense	Wild type (100%)
<i>unc54E</i>	Intron 3	369	Sense + antisense	Wild type (100%)
<i>unc54F</i>	Promoter	1015	Sense + antisense	Wild type (100%)
<i>hlh-1</i> null mutants: lumpy-dumpy larvae				
<i>hlh1A</i>	Exons 1-6	1033	Sense	Wild type (<2% lpy-dpy)
			Antisense	Wild type (<2% lpy-dpy)
			Sense + antisense	Lpy-dpy larvae (>90%)
<i>hlh1B</i>	Exons 1-2	438	Sense + antisense	Lpy-dpy larvae (>80%)
<i>hlh1C</i>	Exons 4-6	299	Sense + antisense	Lpy-dpy larvae (>80%)
<i>hlh1D</i>	Intron 1	697	Sense + antisense	Wild type (<2% lpy-dpy)

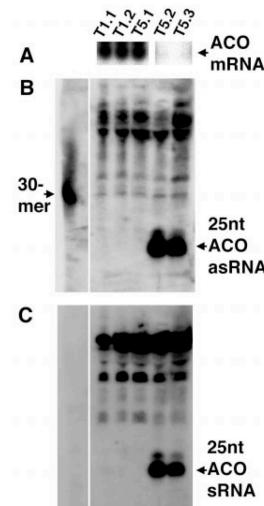
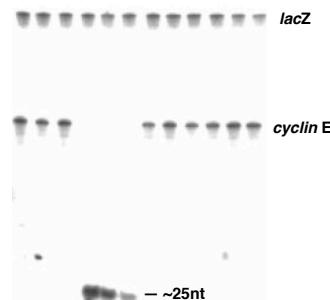
Fire, A.; Xu, S.; Montgomery, M. K.; Kostas, S. A.; Driver, S. E.; Mello, C. C. *Nature* 1998, 391, 806.



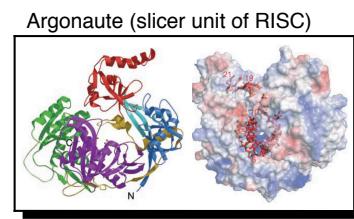
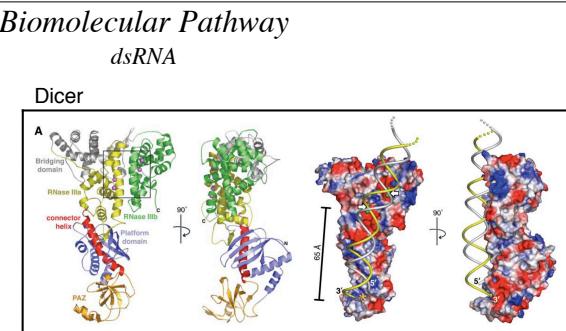
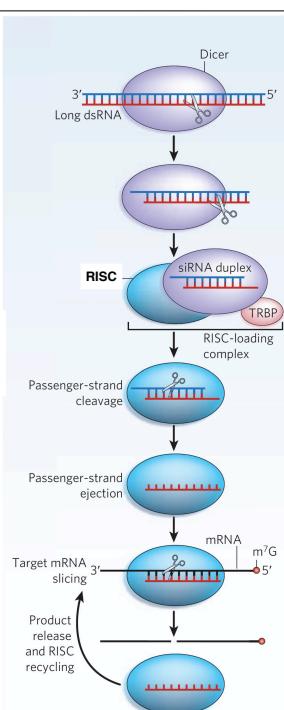
## *Identify the RNAi Trigger*

### *dsRNA is processed to siRNA*

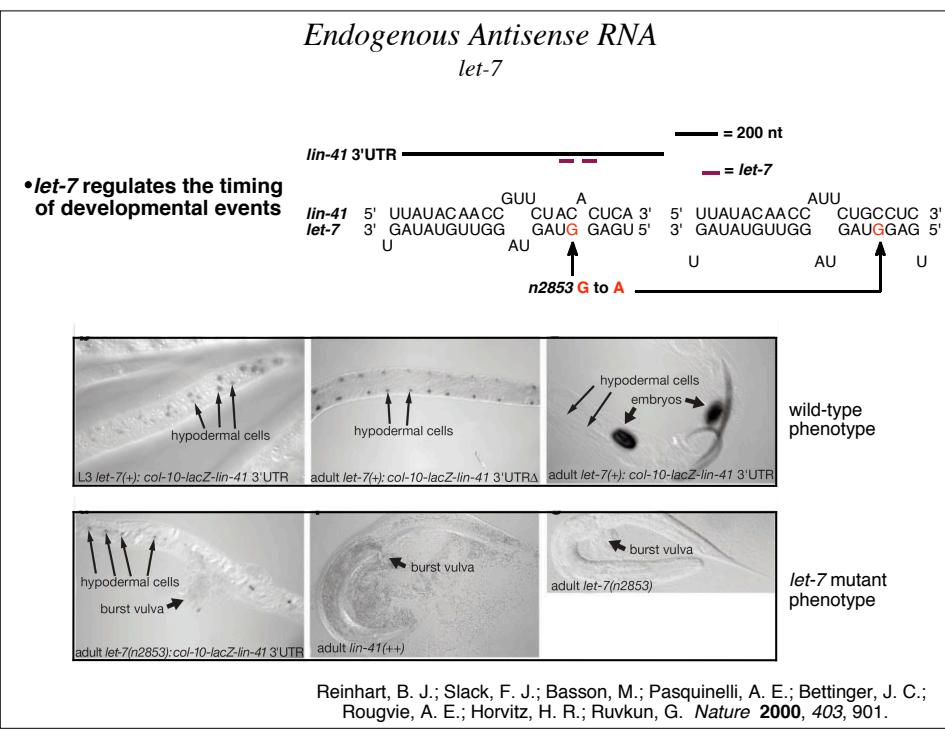
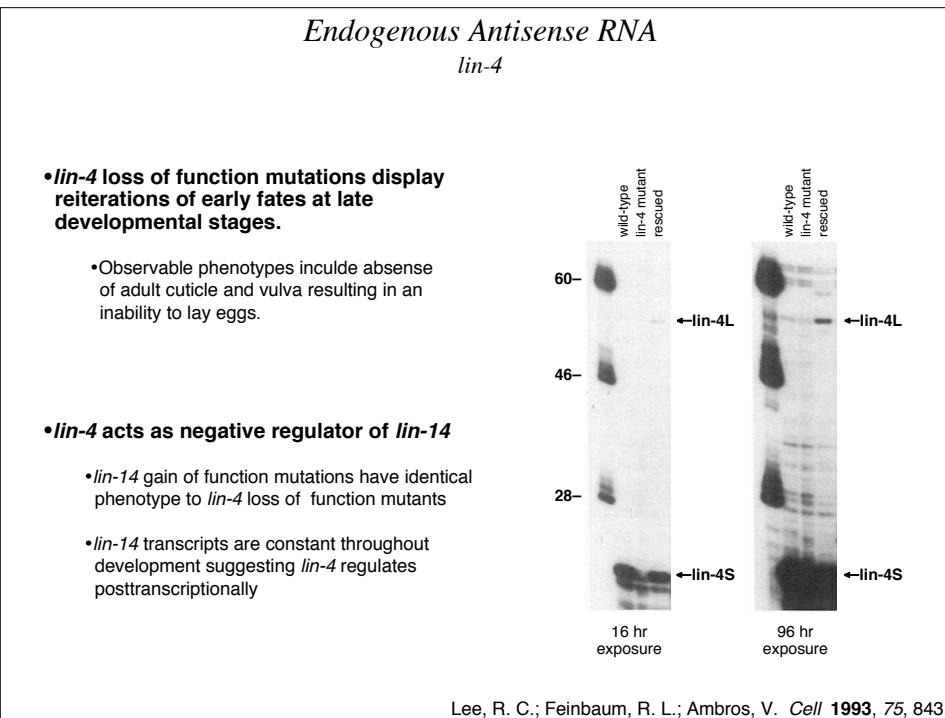
- Anti-sense RNA of a similar size to typical mRNA is not detected in plants or animals
  - Rather antisense RNA strands of roughly 25 nucleotides are found in cells showing gene silencing

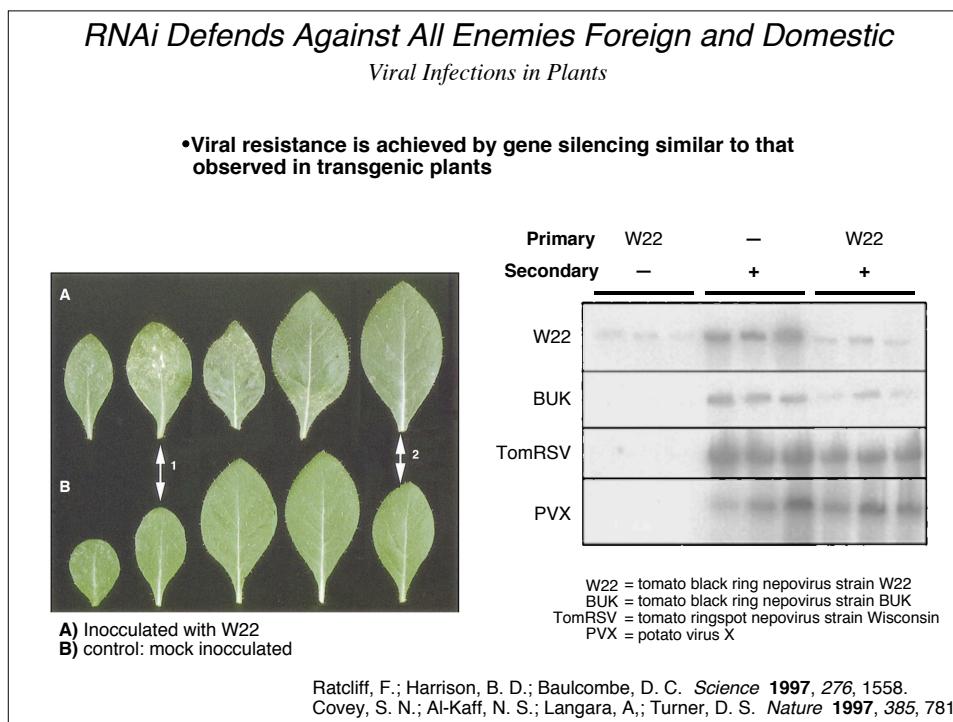
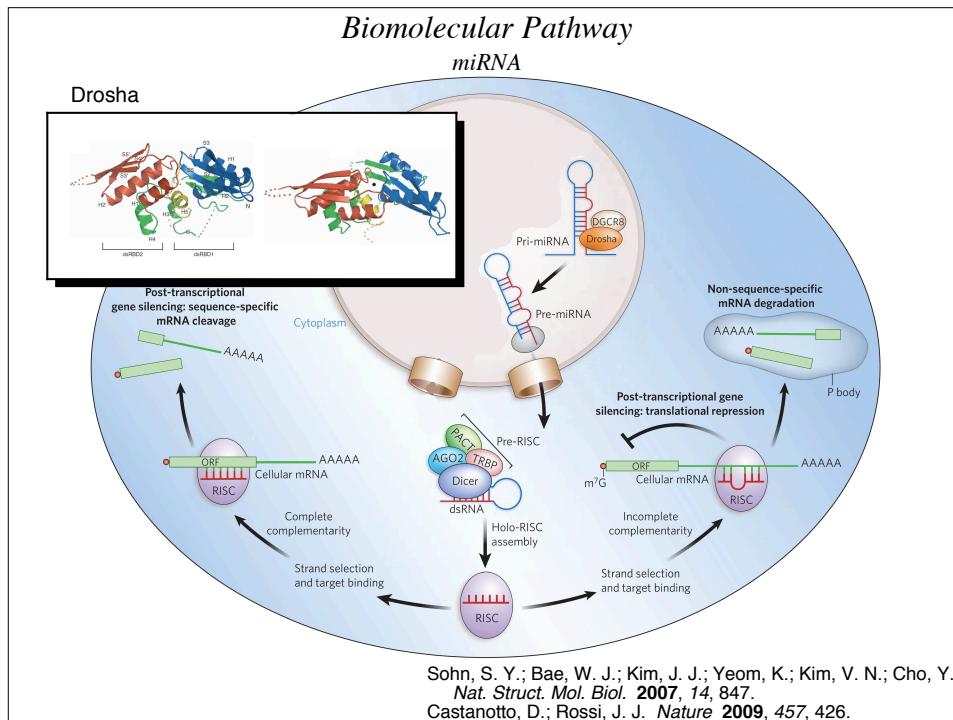


Hamilton, A. J.; Baulcombe, D. C. *Science* **1999**, *286*, 950.  
Hammond, S. M.; Bernstein, E.; Beach, D.; Hannon, G. J. *Nature* **2000**, *404*, 293.



Song, J.; Smith, S. K.; Hannon, G. J.; Joshua-Tor, L. *Science*, **2004**, *305*, 1434.  
MacRae, I. J.; Zhou, K.; Li, F.; Repic, A.; Brooks, A. N.; Cande, W. Z.; Adams, P. D.; Doudna, J. A. *Science* **2006**, *311*, 195.  
Wang, Y.; Sheng, G.; Juraneck, S.; Tuschl, T.; Patel, D. J.; *Nature* **2008**, *456*, 209.  
Jinek, M.; Doudna, A. *Nature* **2009**, *457*, 405.





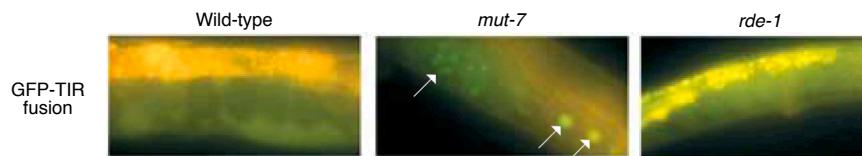
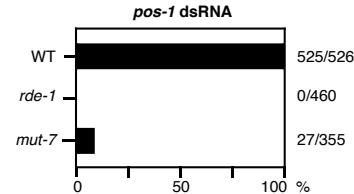
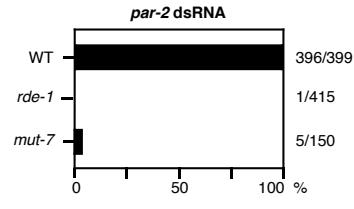
## RNAi Defends Against All Enemies Foreign and Domestic

### Transposons

- Several RNAi deficient strains display transposon activity

- Transposon activity is accompanied by an increase in endogenous transposon siRNAs

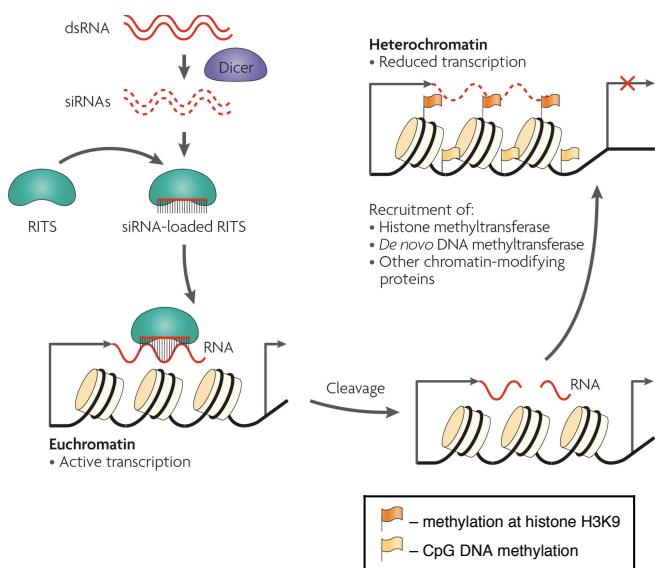
Genotype	% Transposon Activation
+/+	4.3 (2/46)
<i>mut-7</i> / <i>mut-7</i>	73.3 (33/45)
<i>rde-1</i> / <i>rde-1</i>	0 (0/34)



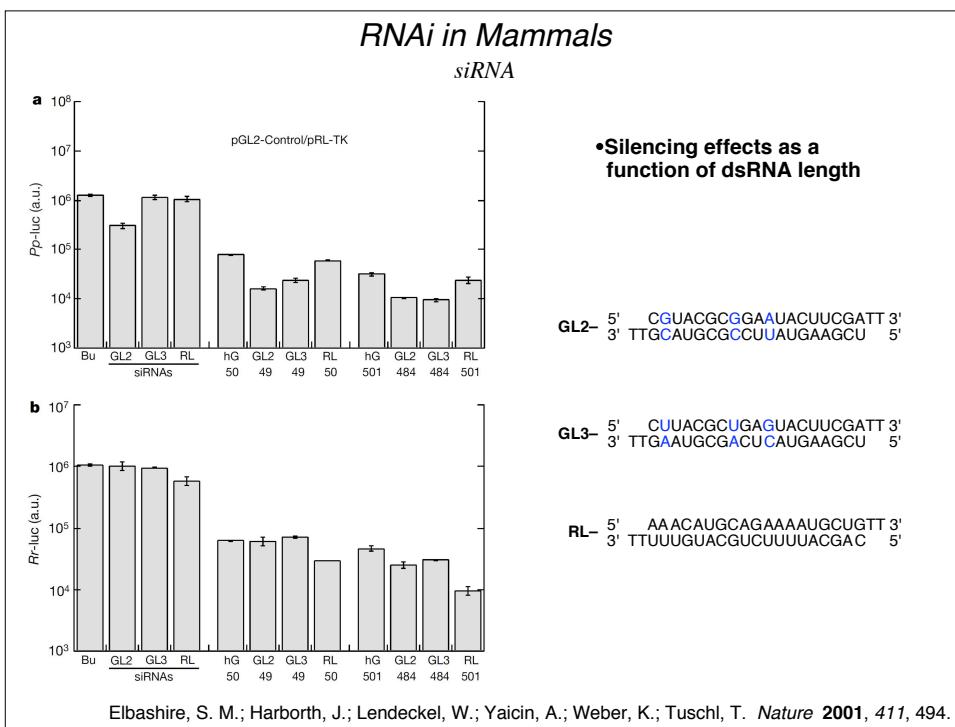
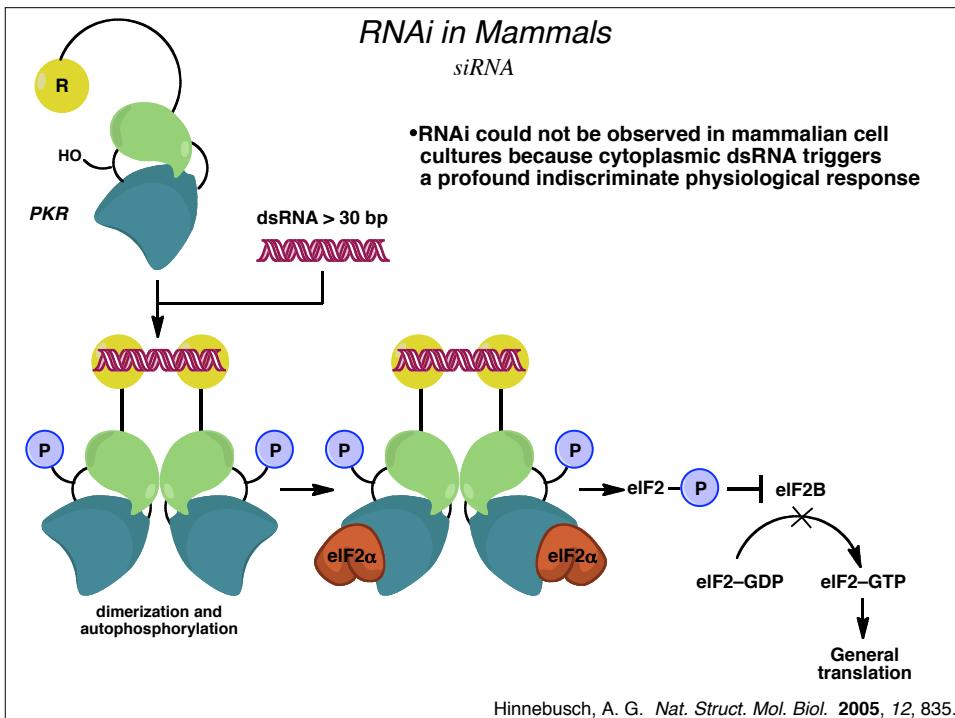
Tabara, H.; Sarkissian, M.; Kelly, W. G.; Fleenor, J.; Grishok, A.; Timmons, L.; Fire, A.; Mello, C. C. *Cell* 1999, 99, 123.  
Sijen, T.; Plasterk, R. H. A. *Nature* 2003, 426, 310.

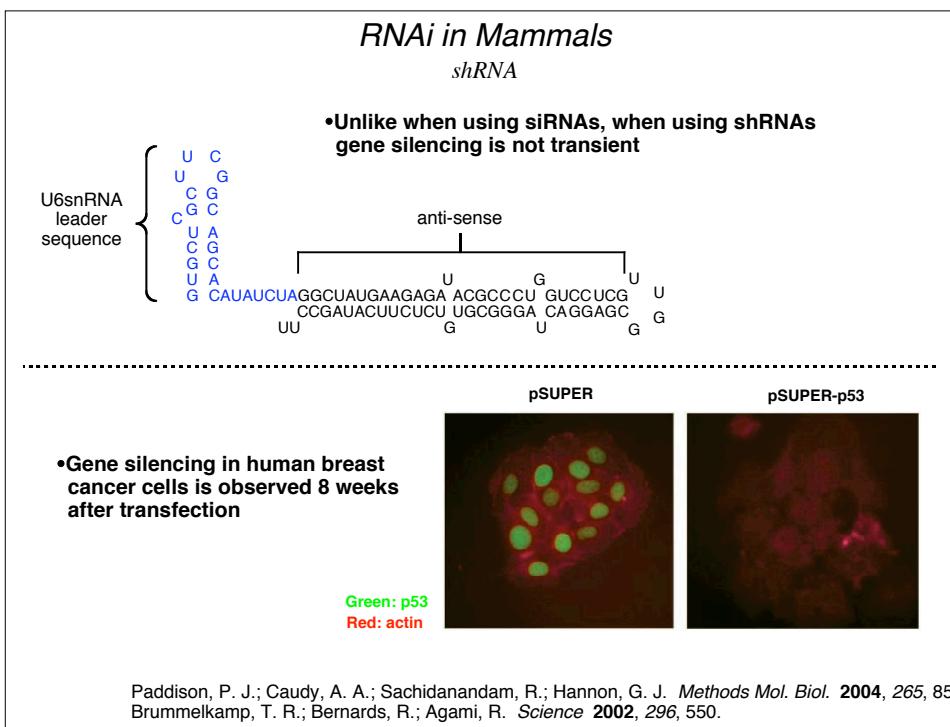
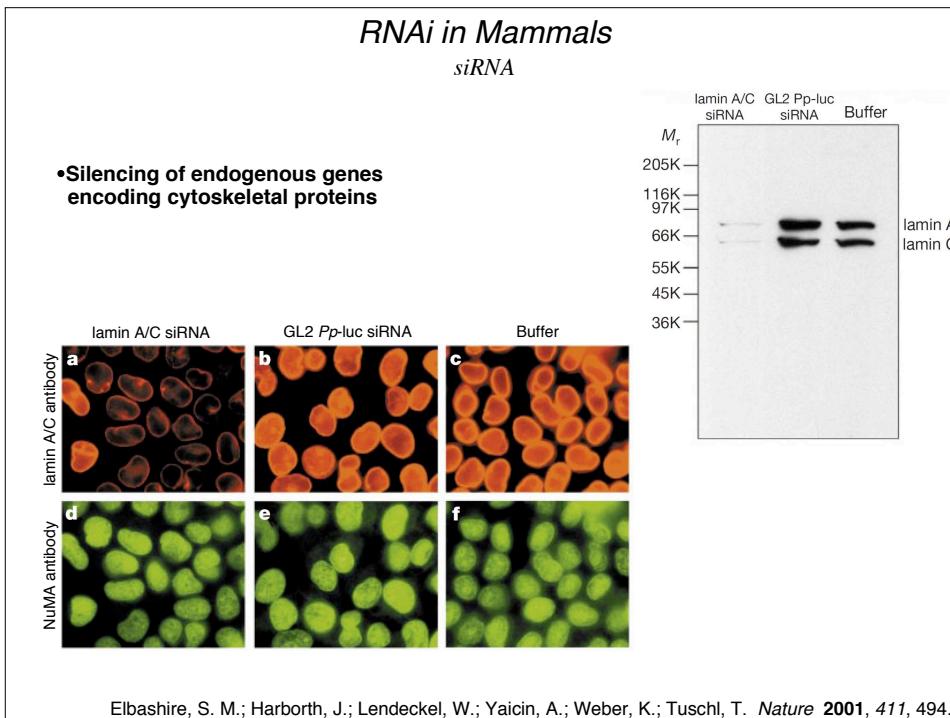
## RNAi Defends Against All Enemies Foreign and Domestic

### Transposon Silencing can Occur by Chromatin Modification



Slotkin, R. K.; Martienssen, R. *Nat. Rev. Genet.* 2007, 8, 272.





## Tapping RNAi for Therapeutics

Direct Delivery of Naked siRNA

- Local injections of siRNA in saline were the first RNAi treatments to enter clinical trials



- The Eyes

- Choroidal neovascularization (CNV) from wet age-related macular degeneration (AMD) (phase III clinical trial)



- The Lungs

- Respiratory syncytial virus (RSV) (phase II clinical trial)



- The Nervous System

- Chronic neuropathic pain (Mouse Model)

Dorn, G.; et al. *Nucleic Acid Res.* 2004, 32, e49.  
Castanotto, D.; Rossi, J. J. *Nature* 2009, 457, 426.  
De Fougerolles, A. R. *Hum. Gene Ther.* 2008, 19, 125.

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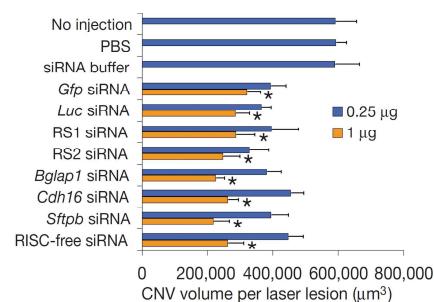
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- The Nervous System

- Chronic neuropathic pain (Mouse Model)

### CNV inhibition is not caused by RNAi!



Kleinman, M. E.; et al. *Nature* 2008, 452, 591.

Dorn, G.; et al. *Nucleic Acid Res.* 2004, 32, e49.  
Castanotto, D.; Rossi, J. J. *Nature* 2009, 457, 426.  
De Fougerolles, A. R. *Hum. Gene Ther.* 2008, 19, 125.

## Tapping RNAi for Therapeutics

### Systemic Delivery

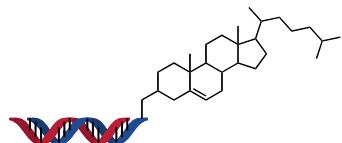
- Naked siRNA has a  $t_{1/2}$  of 6 min and a corresponding plasma clearance ( $C_L$ ) of 17.6 mL/min

- Hydrodynamic tail vein injection methods are not clinically applicable

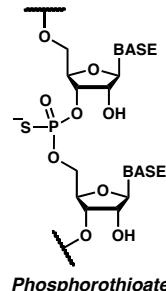
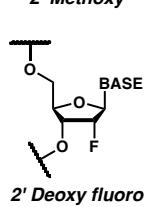
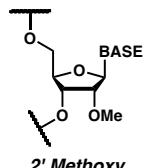
- Chemical modifications to the sugar or phosphate backbone



- siRNA with appended cholesterol has increased pharmacokinetics:



$t_{1/2}$  of 95 min and  $C_L$  of 0.5 mL/min

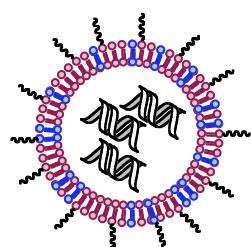


Song, E.; et al. *Nat. Med.* 2003, 9, 347.  
Soutscheck, J.; et al. *Nature* 2004, 432, 173.  
Bumcrot, D.; Manoharan, M.; Kotielansky, V.;  
Sah, E. W. Y. *Nat. Chem. Biol.* 2006, 2, 711.

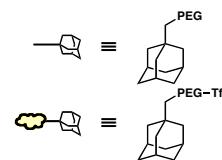
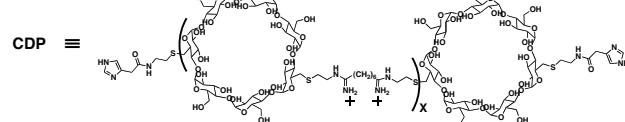
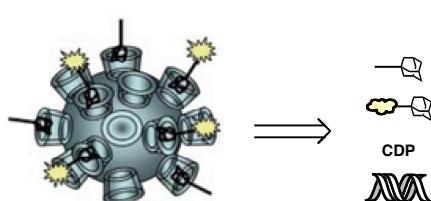
## Tapping RNAi for Therapeutics

### Systemic Delivery

- Lipid Based Nanoparticle



- Polymer Based Nanoparticle



Davis, M. E. *Mol. Pharm.* 2009, 6, 659.  
Davis, M. E.; et al. *Nature* 2010, 464, 1067.

*RNA Interference*  
*Conclusions*

- RNAi is triggered by dsRNA
- RNAi is utilized by the cell to regulate gene expression
- RNAi in plants can lead to viral resistance
- RNAi is used to silence transposons
- RNAi has great potential as a therapeutic