RNAi and Development in *C. elegans*

Craig C. Mello

Stockholm

December 10, 2006

UMass

Caenorhabditis elegans

Bob Goldstein

Sydney Brenner

David Hirsh

Bob Horvitz

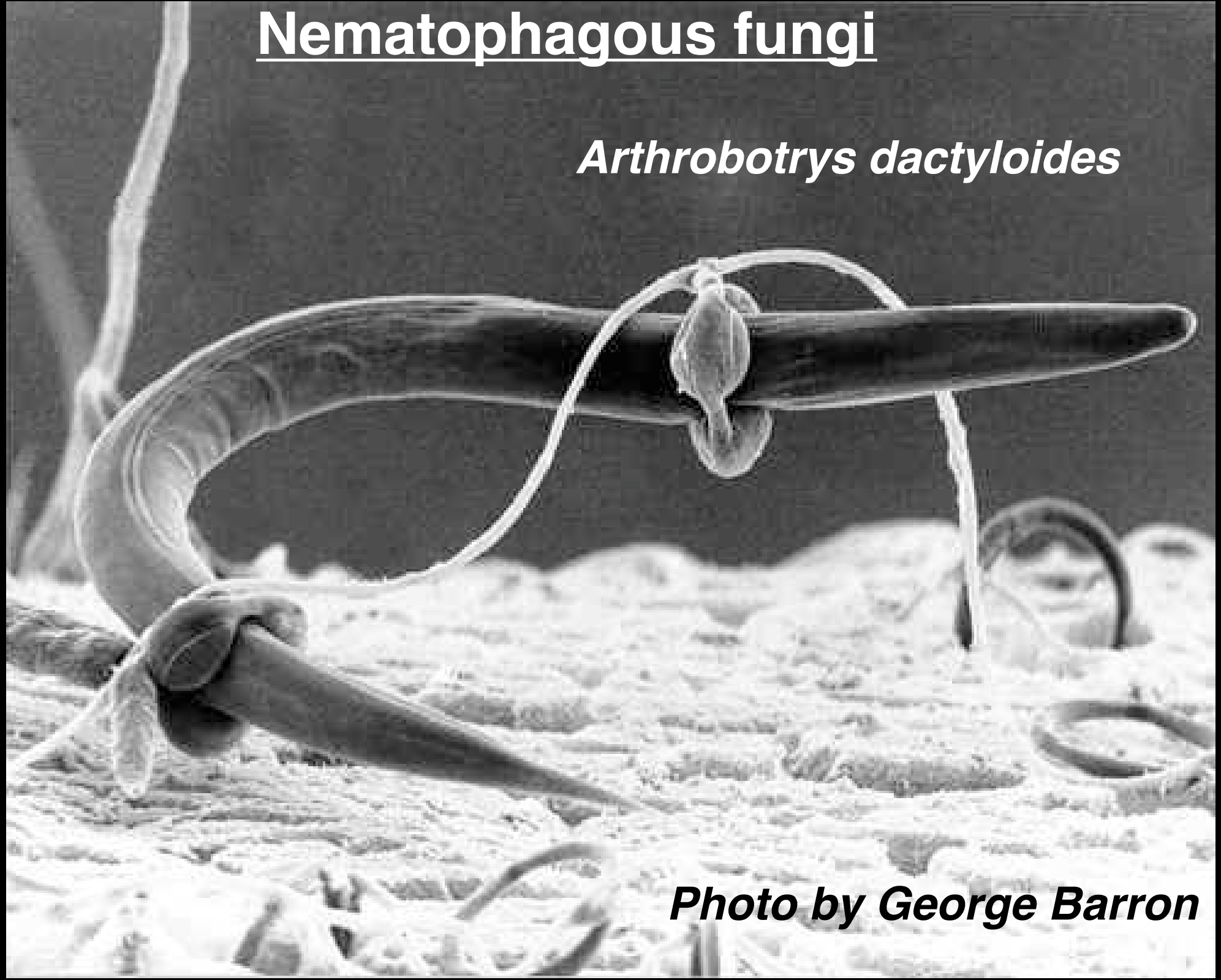
John Sulston

Dan Stinchcomb

Victor Ambros

Jim Priess

Nematophagous fungi



Arthrobotrys dactyloides

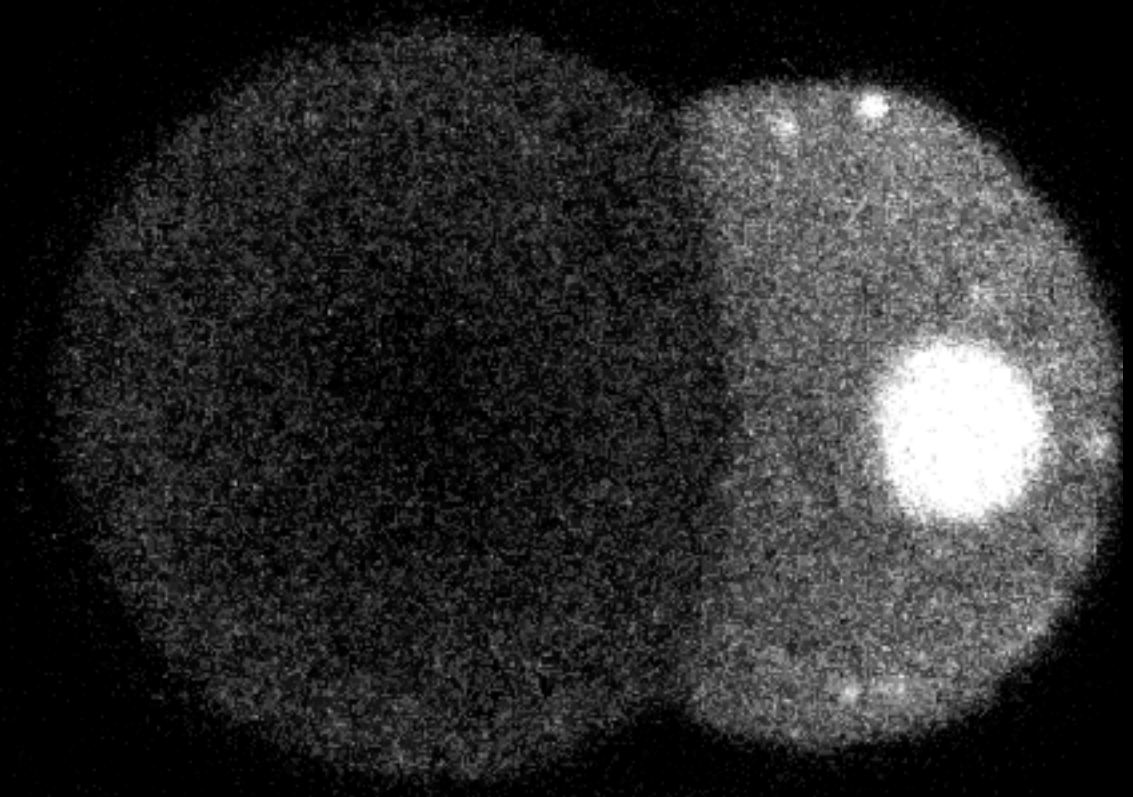
Photo by George Barron

Arthrobotrys dactyloides can catch *C. elegans*

Mark Alkema


Nova Science Now
DNA

Return to the RNA world



small RNAs & development

Evolution?

RNA-directed inheritance?

Weismann's theory (ala Ernst Mayr/& post RNAi).

(1)There is a special particle *(with siRNAs)* for ***some*** traits.

siRNAs

contain ***siRNAs.***

siRNA

siRNAs

NOT WRONG.

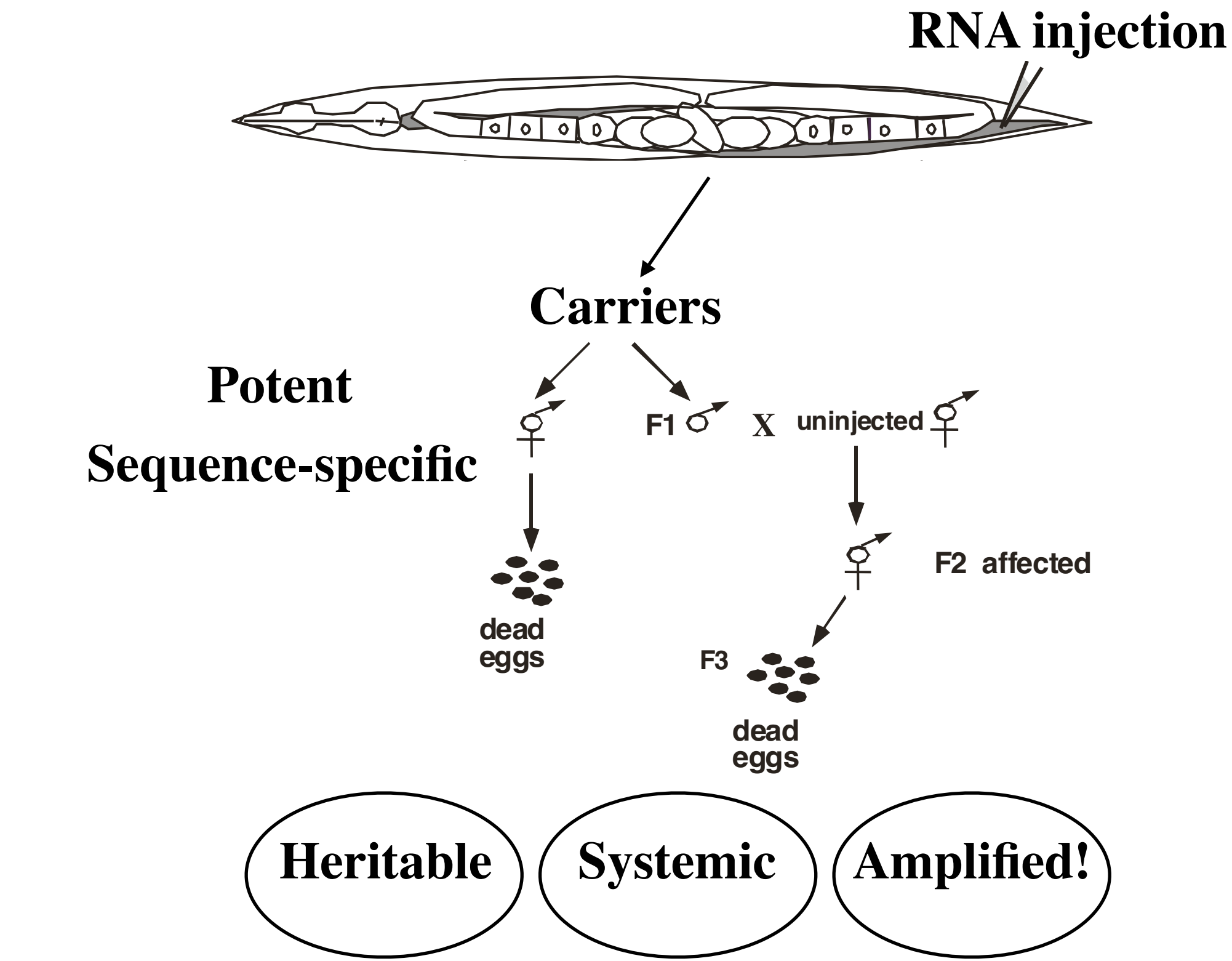
RNA may play a role in inheritance and evolution.


experience.
cbs news

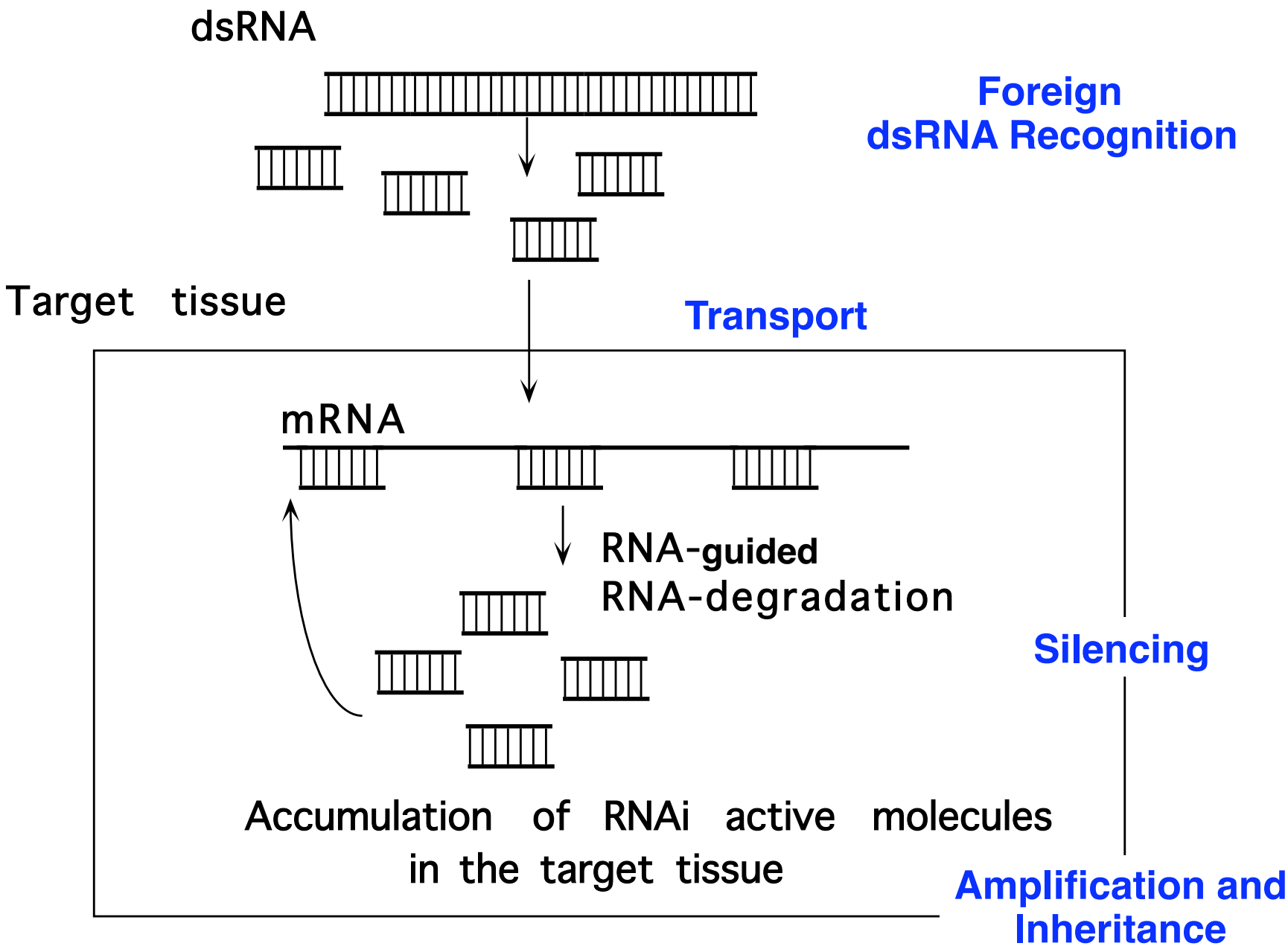
Nova Science Now

Robert Krulwich

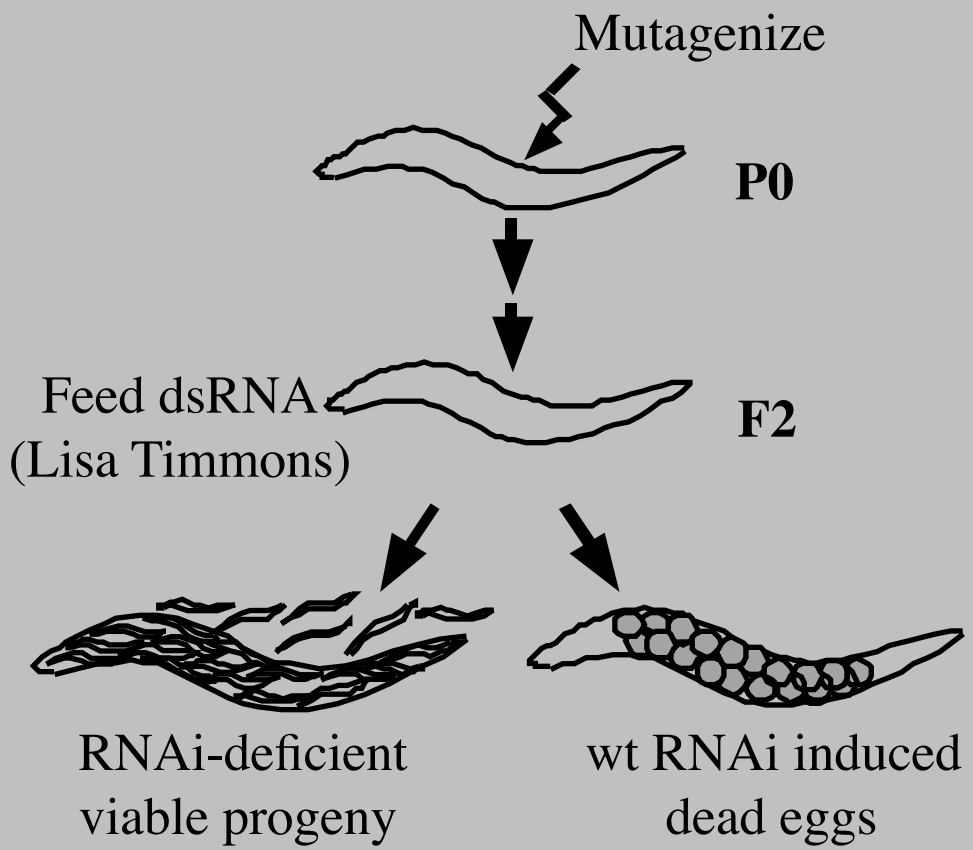
Eric Lander


RNA injection
Carriers
Potent
Sequence-specific
F1
X
uninjected
F2 affected
dead
eggs
F3
dead
eggs
Heritable
Systemic
Amplified!

RNAi


dsRNA
Foreign
dsRNA Recognition
Target tissue
Transport
mRNA
RNA-guided
RNA-degradation
Silencing
Accumulation of RNAi active molecules
in the target tissue
Amplification and
Inheritance

Hiroaki's screen for RNAi deficient mutants (1998-1999)

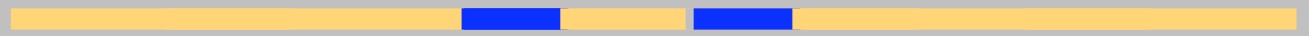


Hiroaki Tabara

Analysis of Hiroaki's RNAi Deficient Mutants

	# alleles	Other Phenotypes
rde-1	6	none
rde-2	3	sterile/him/mutator
rde-3	3	sterile/him/mutator
rde-4	2	none
rde-5	1	sterile/him/mutator
rde-6	2	sterile/him/mutator
mut-7	1	sterile/him/mutator

RNAi



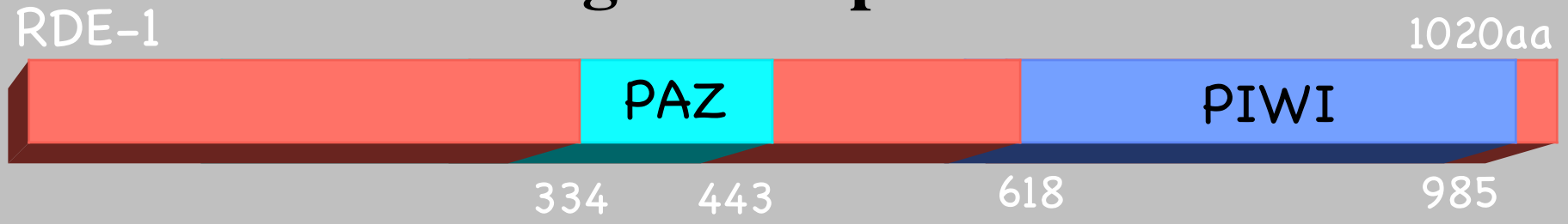
Transposons

Transgenes

What about RDE-1?

Hypothesis: Related silencing pathways with distinct triggering mechanisms

Argonaute protein



C. elegans	*Drosophila*	*Arabidopsis*	*Neurospora*
RDE-1	Aubergine/Sting	AGO1	QDE-2
↓	↓	↓	↓
RNAi	Development and Epigenetic silencing	Development Gene silencing	Quelling (transgene silencing
(Tabara et al., 1999)	(Schmidt et al., 1999 Wilson et al., 1996)	(Fagard et al., 2000 Bohmert et al., 1998)	Cogoni and Macino, 2000

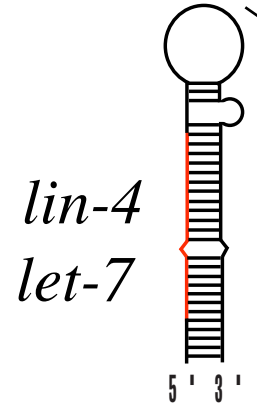
RDE-1 Cloning reveals links to development and gene silencing in insects, plants and fungi!

Alla Grishok

Victor Ambros
Lee et al., 1993

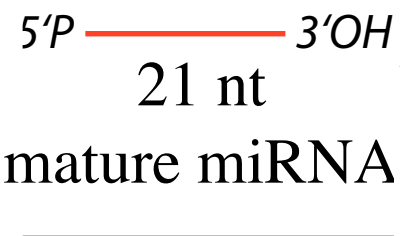
Gary Ruvkun
Reinhart et al., 2000
Pasquinelli et al., 2000

Alla Grishok
and Amy Pasquinelli
Grishok et al, 2001

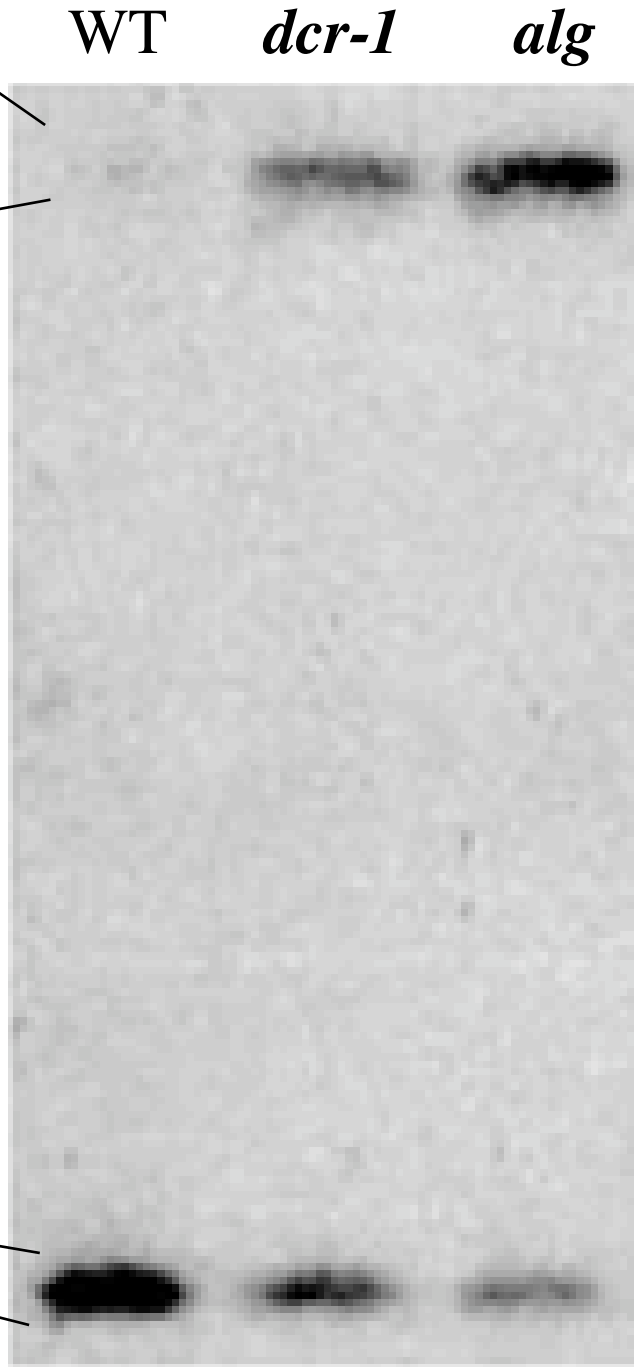


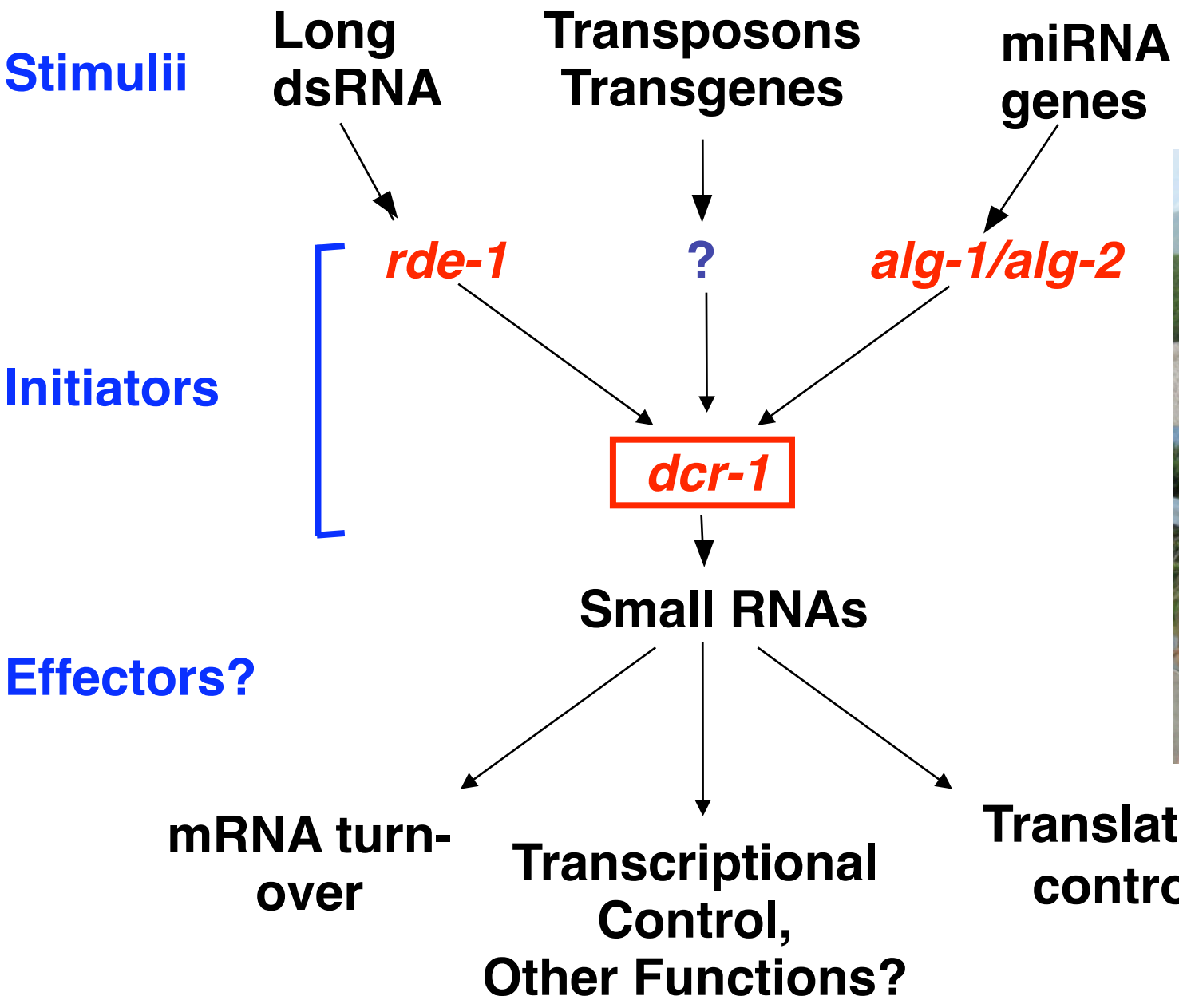
pre-miRNA

ALG-1/ALG-2



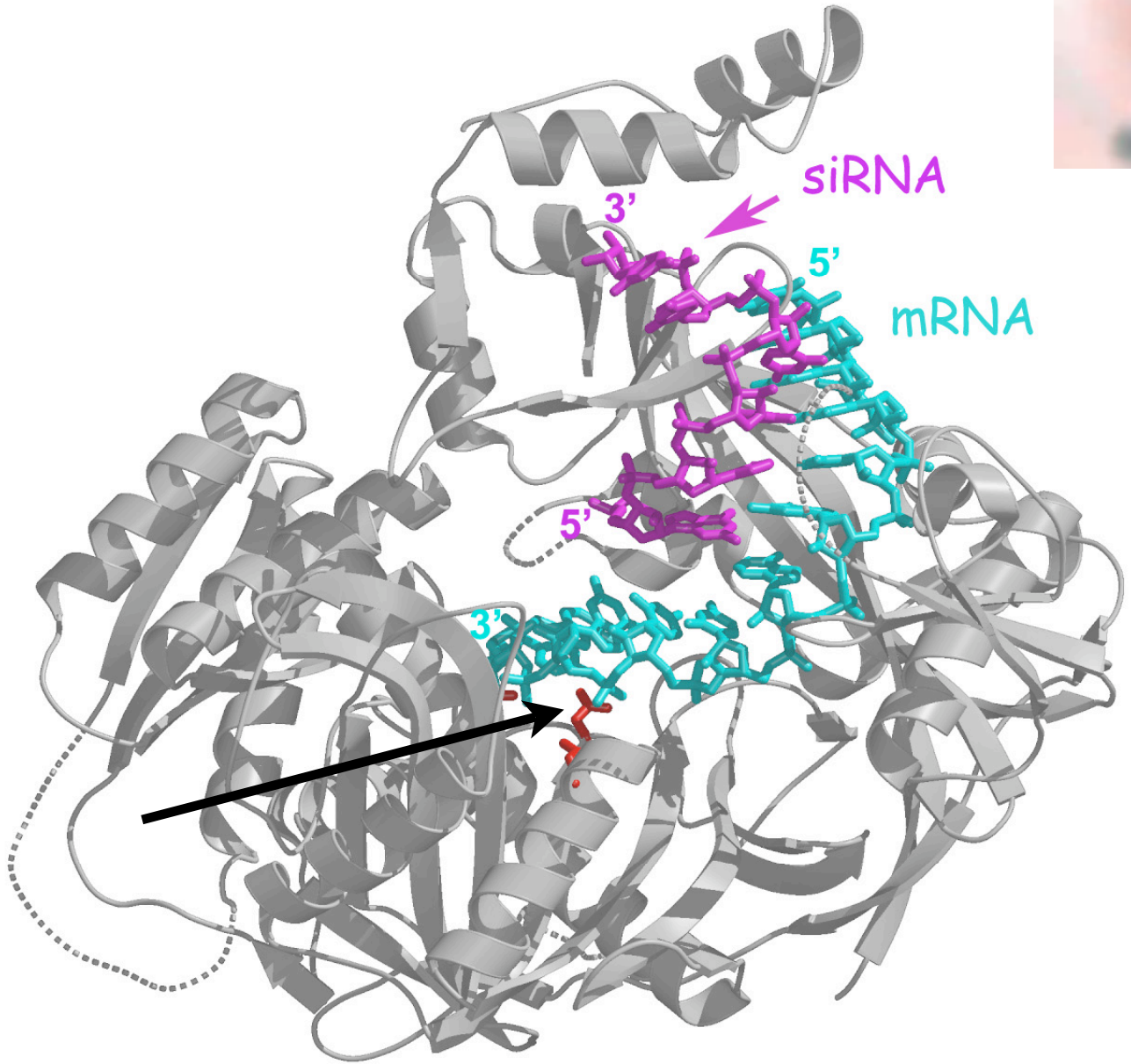
21 nt
mature miRNA





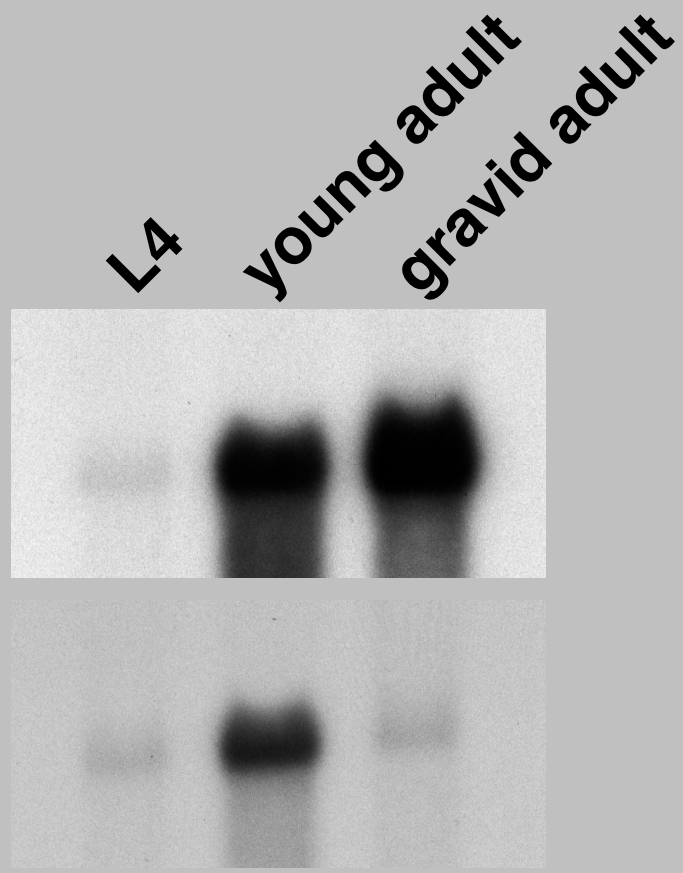
Greg Hannon
with Claire
(Bernstein et al., 2001)

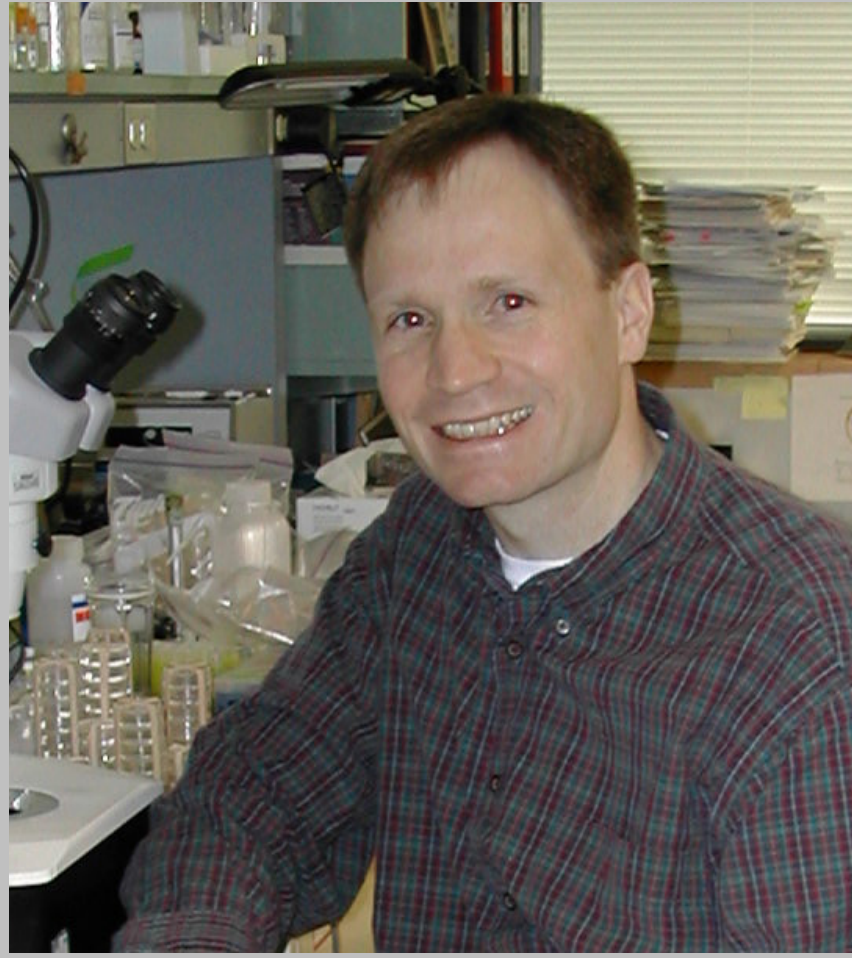
A model for mRNA slicing



Who is the RNAi cop in *C. elegans?*

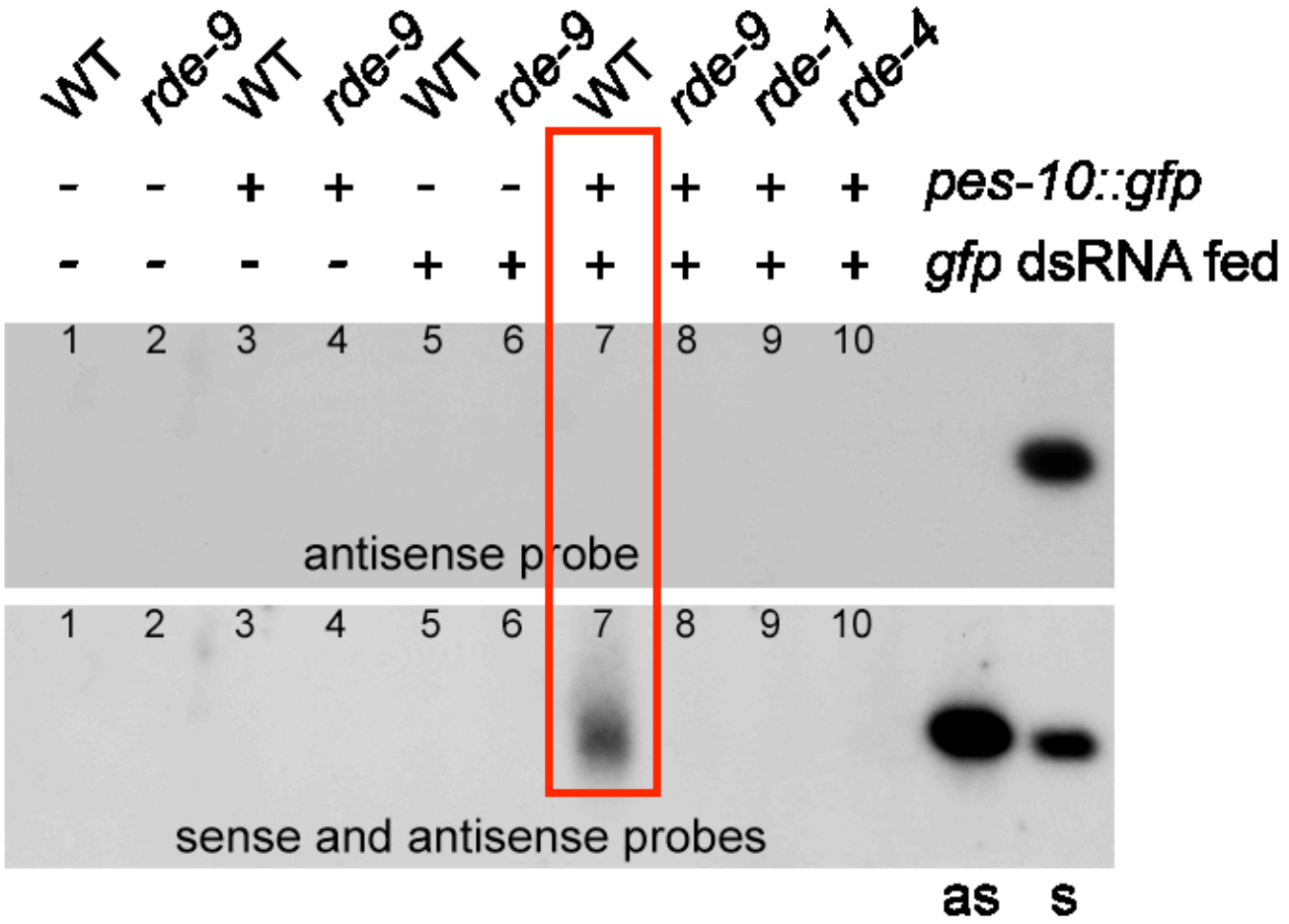
Ji-Joon Song et al., 2004



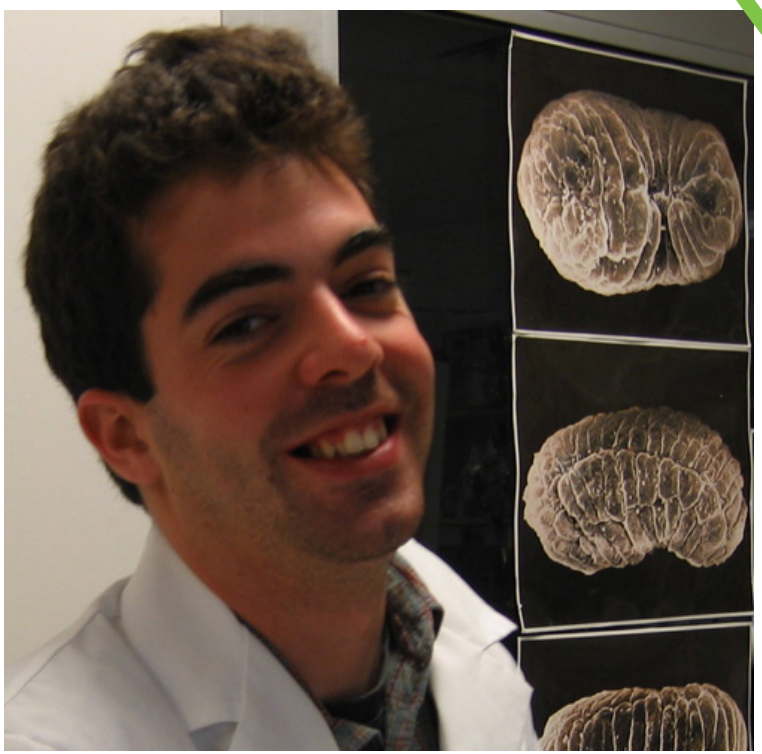


Darryl Conte and
Alla Grishok

Burst of target mRNA expression before silencing



Only antisense siRNAs accumulate. Requires a target mRNA, RDE-1, RDE-4, DCR-1 and RDE-9

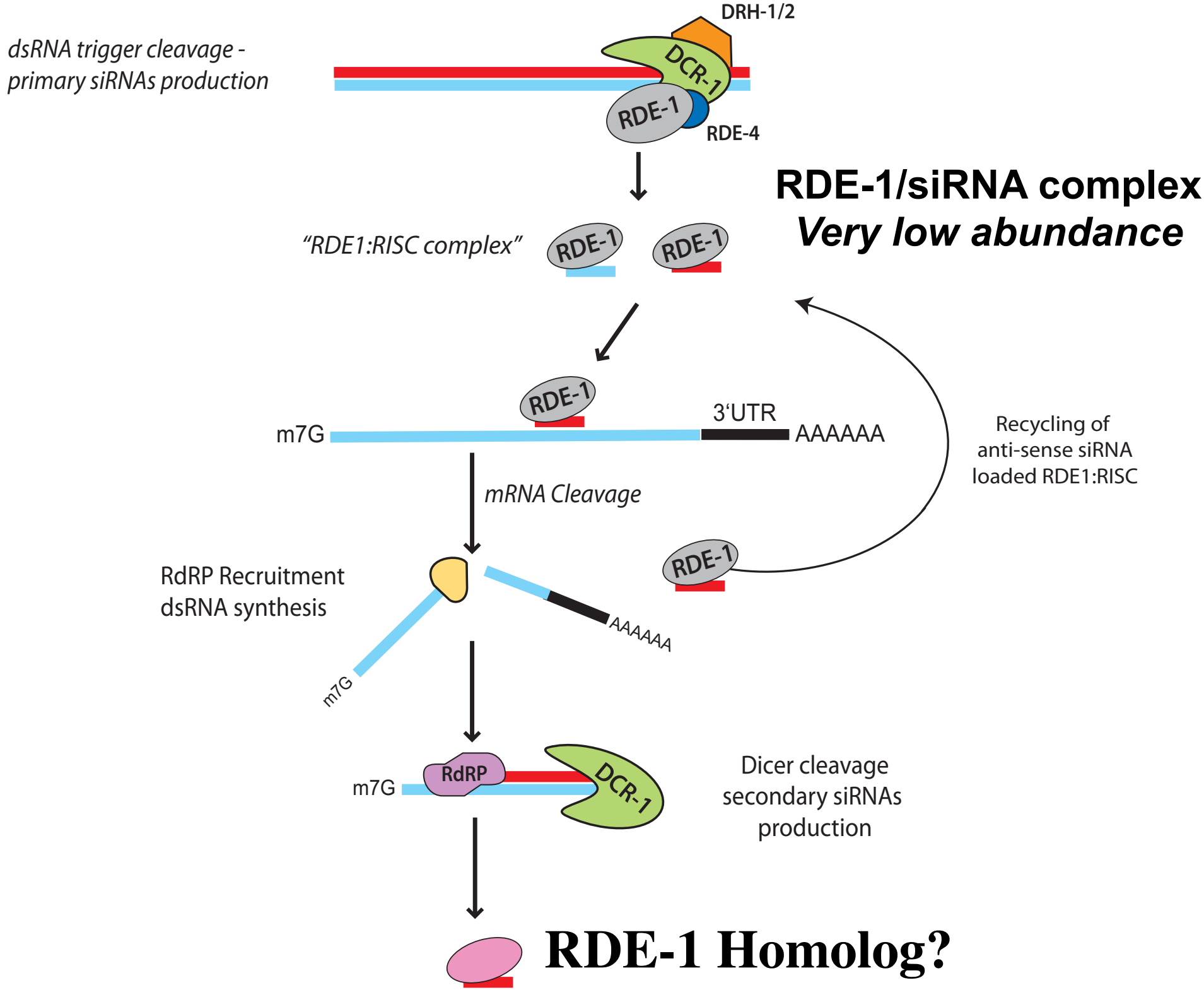


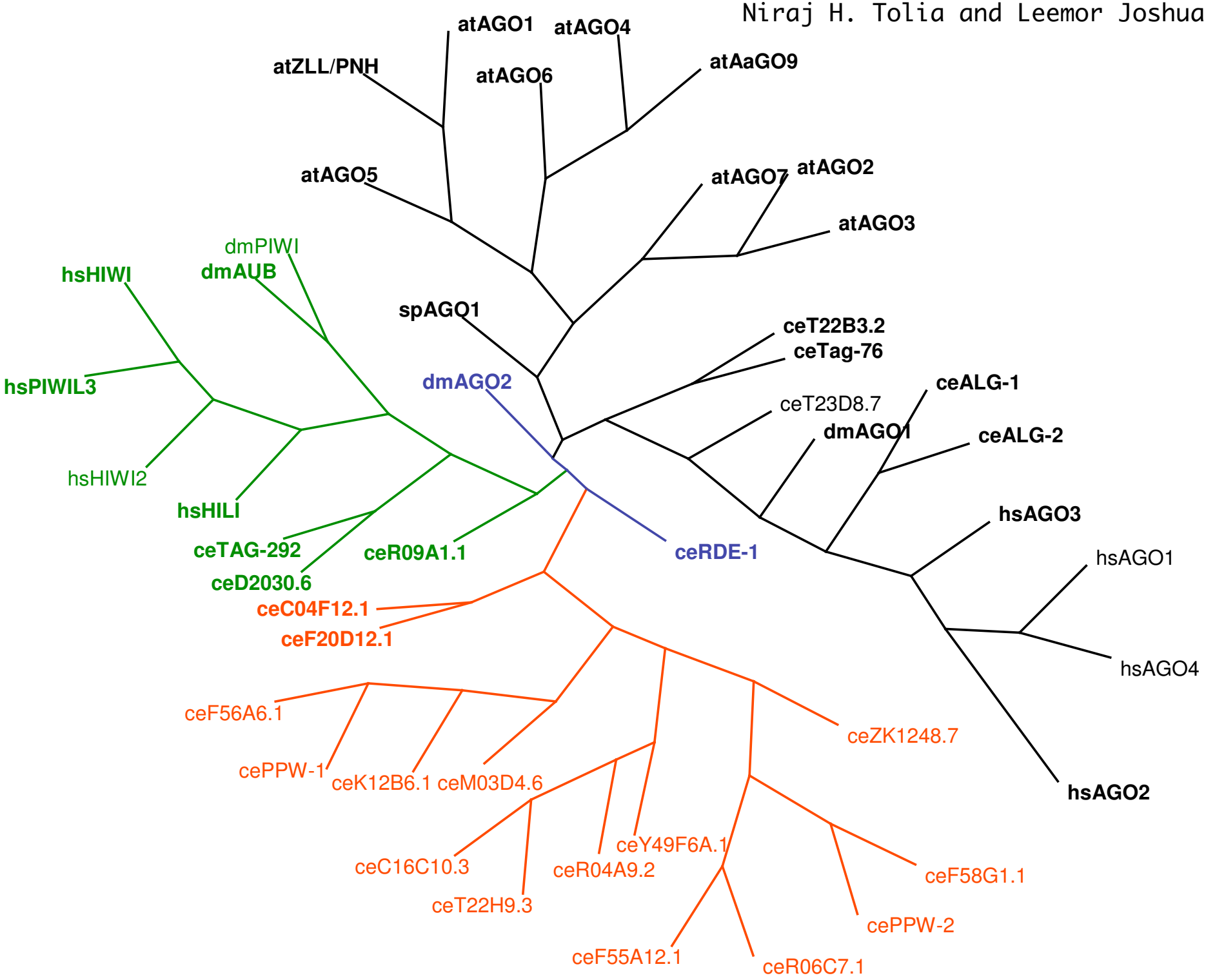
Pedro Batista

homogenize

Affinity Matrix

Western Blot


dsRNA trigger cleavage -
primary siRNAs production
DRH-1/2
DCR-1
RDE-1
RDE-4
RDE-1/siRNA complex
Very low abundance
"RDE1:RISC complex"
RDE-1
RDE-1
RDE-1
m7G
3'UTR
AAAAAA
mRNA Cleavage
Recycling of
anti-sense siRNA
loaded RDE1:RISC
RdRP Recruitment
dsRNA synthesis
RDE-1
AAAAAA
m7G
m7G
RdRP
DCR-1
Dicer cleavage
secondary siRNAs
production
RDE-1 Homolog?


atAGO1
atAGO4
atZLL/PNH
atAGO6
atAaGO9
atAGO5
atAGO7
atAGO2
atAGO3
dmPIWI
hsHIWI
dmAUB
spAGO1
ceT22B3.2
ceTag-76
hsPIWIL3
dmAGO2
ceALG-1
ceT23D8.7
dmAGO1
ceALG-2
hsHIWI2
hsAGO3
hsHILI
hsAGO1
ceTAG-292
ceR09A1.1
ceRDE-1
ceD2030.6
ceC04F12.1
hsAGO4
ceF20D12.1
ceF56A6.1
ceZK1248.7
cePPW-1
ceK12B6.1
ceM03D4.6
hsAGO2
ceY49F6A.1
ceC16C10.3
ceR04A9.2
ceF58G1.1
ceT22H9.3
cePPW-2
ceF55A12.1
ceR06C7.1

dpy-10 C06 (II) x F58 (II)

hT2(gfp)/rde-9 (I); him-8(IV) x C06 F58 (II)

hT2(gfp)/+ (I); C06 F58 (II); him-8/+ (IV) x C18 F56 (I)

F20/nT1(gfp) (IV) +/nT1(gfp) (V) x C18 F56 (I) C06 F58 (II)

C18 F56/+ (I) C06 F58/+ (II) +/nT1(gfp) (IV) +/nT1(gfp) (V)

x

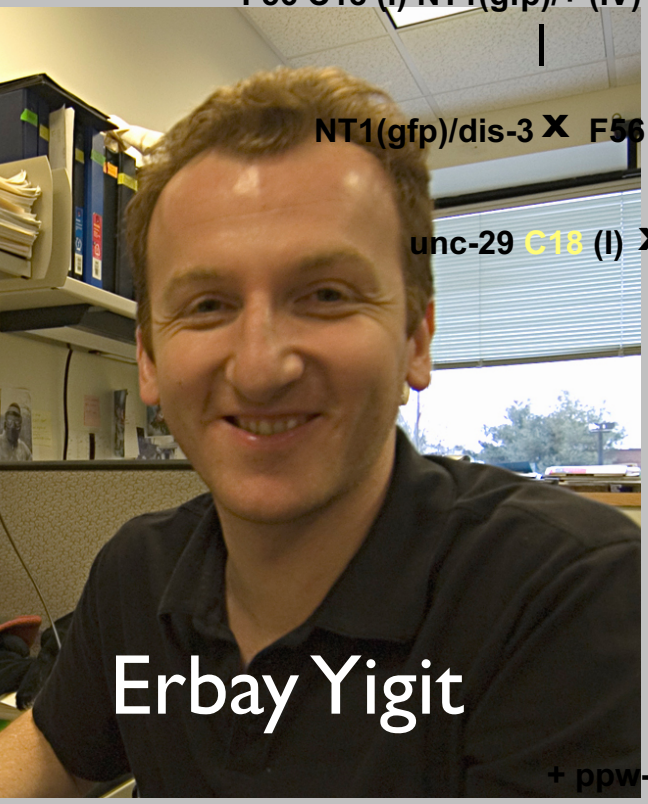
F56 C18 (I) M03 (IV) K12 (V)

F56 C18 (I) NT1(gfp)/+ (IV) NT1(gfp) (V) x M03 (IV) K12 (V)

K12 (V) x M03 (IV)

NT1(gfp)/dis-3 x F56 C18

unc-29 C18 (I) x F56 (I)



Sextuple Argonaute Mutant

C18 F56(I); C06 F58(II); M03(IV) K12(V)

Octuple

x –F56, ppw-2, C18, C04 (I); F58 (II);C06, M03 (IV), K12 V

F56 ppw-2 C18 C04 (I) F58 (II)

F58 x F56 ppw-2 C18 C04 (I); unc-32 (III)

F56 + + + / + ppw-2 C18 C04 (I); unc-32 (III) x hT2(gfp): him-8 (IV)

F56 x ppw-2 C18 C04 (I); unc-32 (III)

ppw-2 C18 (I) + / + + C04; unc-32 x hT2(gfp) (I) hT2(gfp) (III)

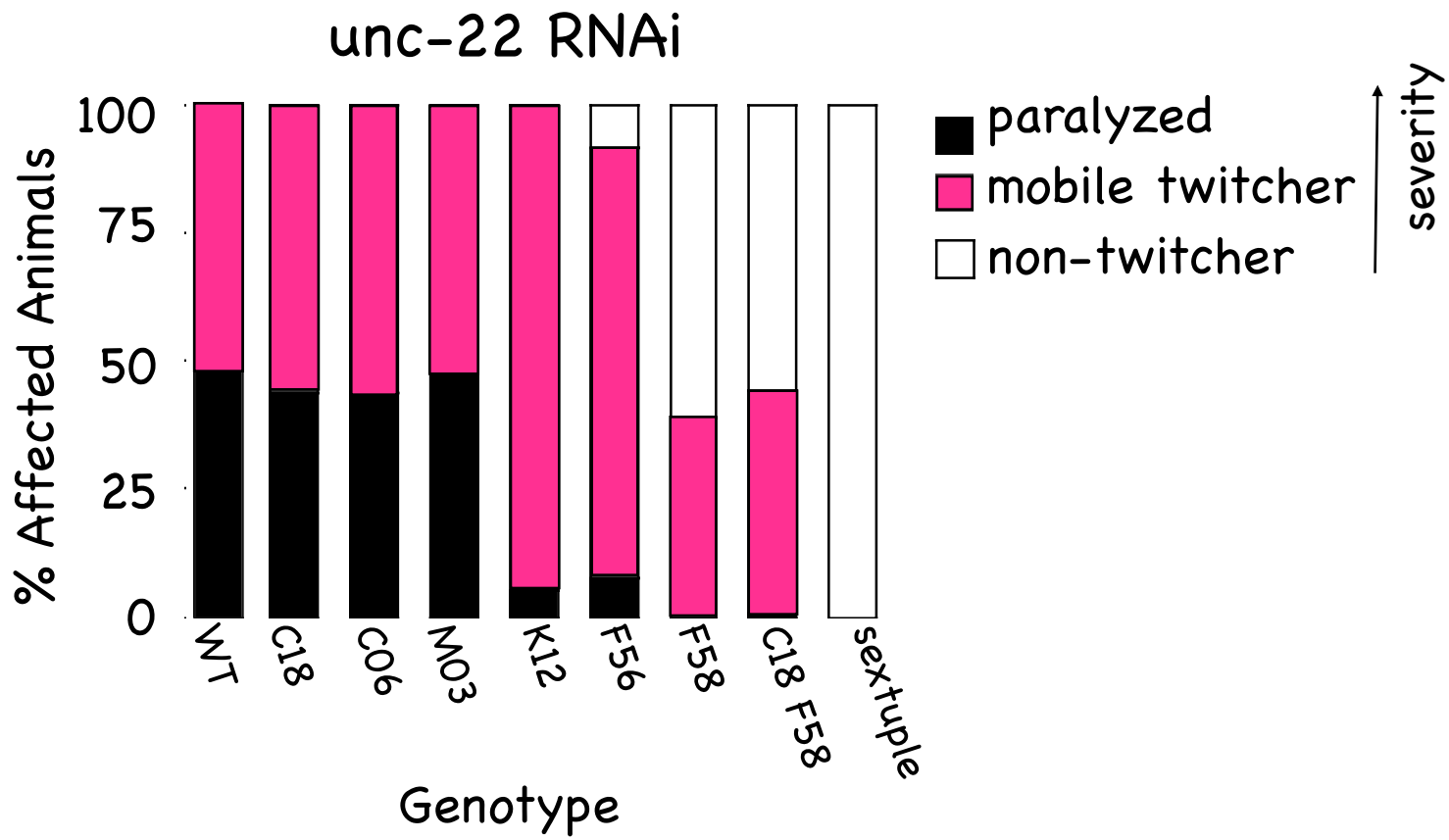
ppw-2 C18 (I) unc-32 (III) x C04 (I)

ppw-2 C18 (I) x dpy-5 (I); rol-6 (II); unc-32 (III)

+ ppw-2 +/ +C18 unc-29 (I) x hT2(gfp)/ego-1 rde-9 (I); hT2/+; him-8 (IV)

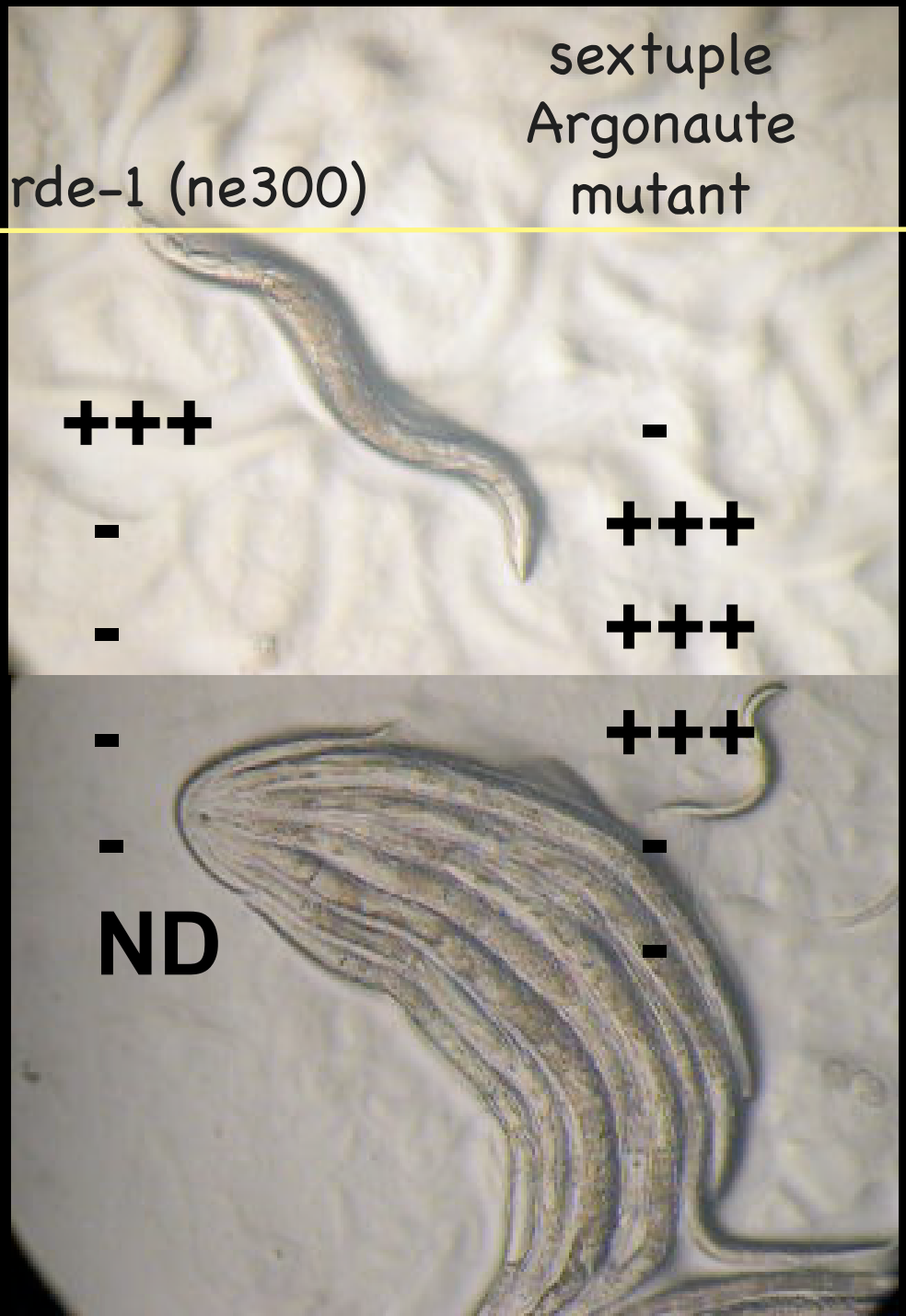
C18 unc-29 (I) x ppw-2 (I)

Loss of Multiple Argonautes Leads to RNAi deficiency

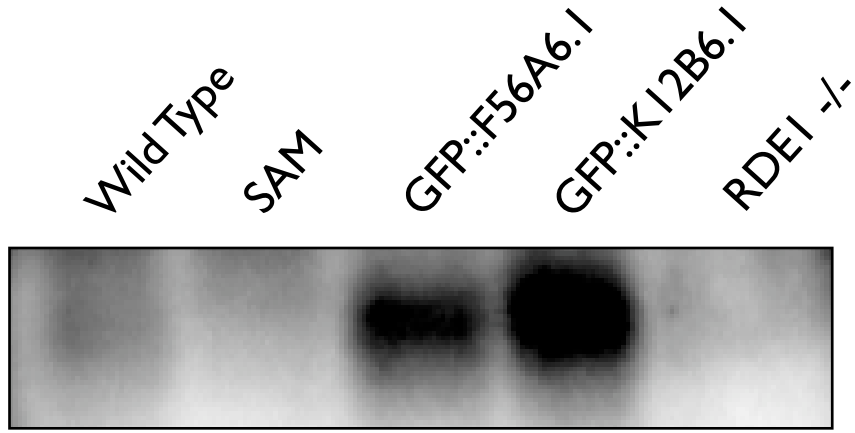


Over-expression of individual Argonautes can Rescue the Sextuple Argonaute Mutant

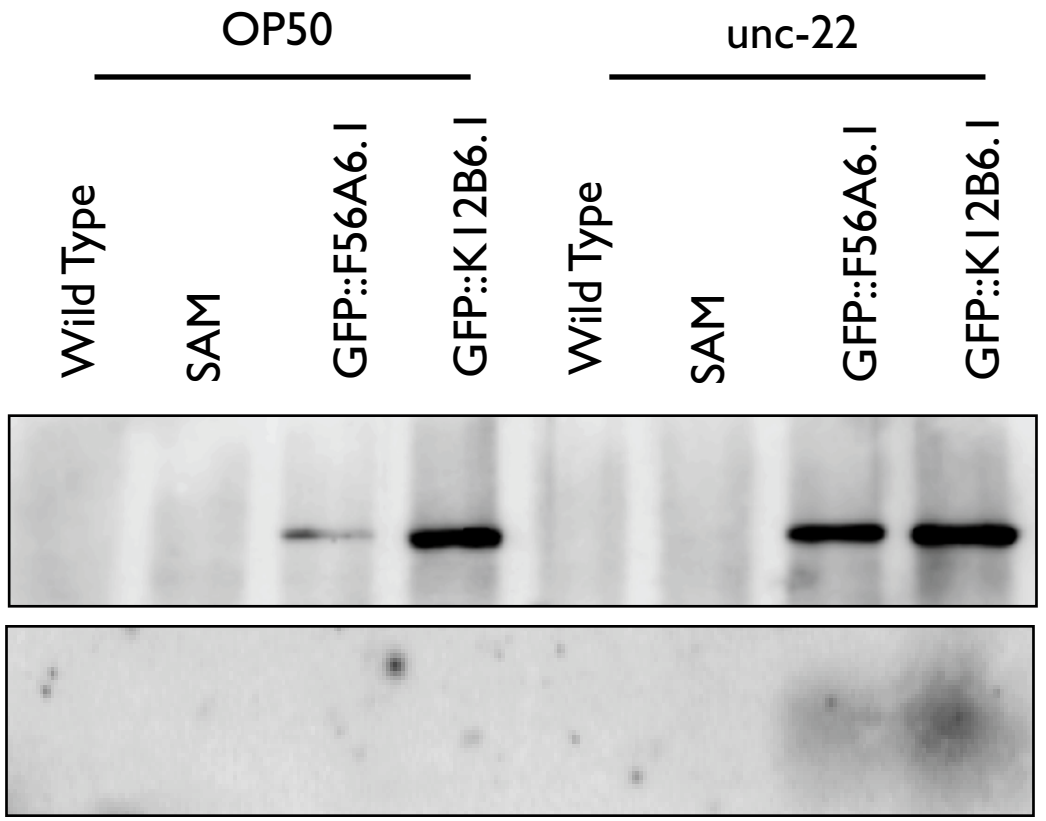
Expression Vector	rde-1 (ne300)	sextuple Argonaute mutant
myo-3::rde-1::HA	+++	-
myo-3::gfp::f56	-	+++
myo-3::ppw-1	-	+++
myo-3::gfp::k12	-	+++
myo-3::prg-1	-	-
myo-3::alg-1	ND	-

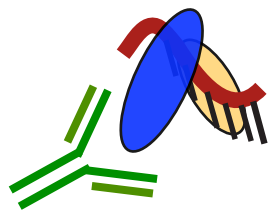


Secondary AGOs Are Limiting for RNAi



Blot for small RNAs
unc-22 siRNA

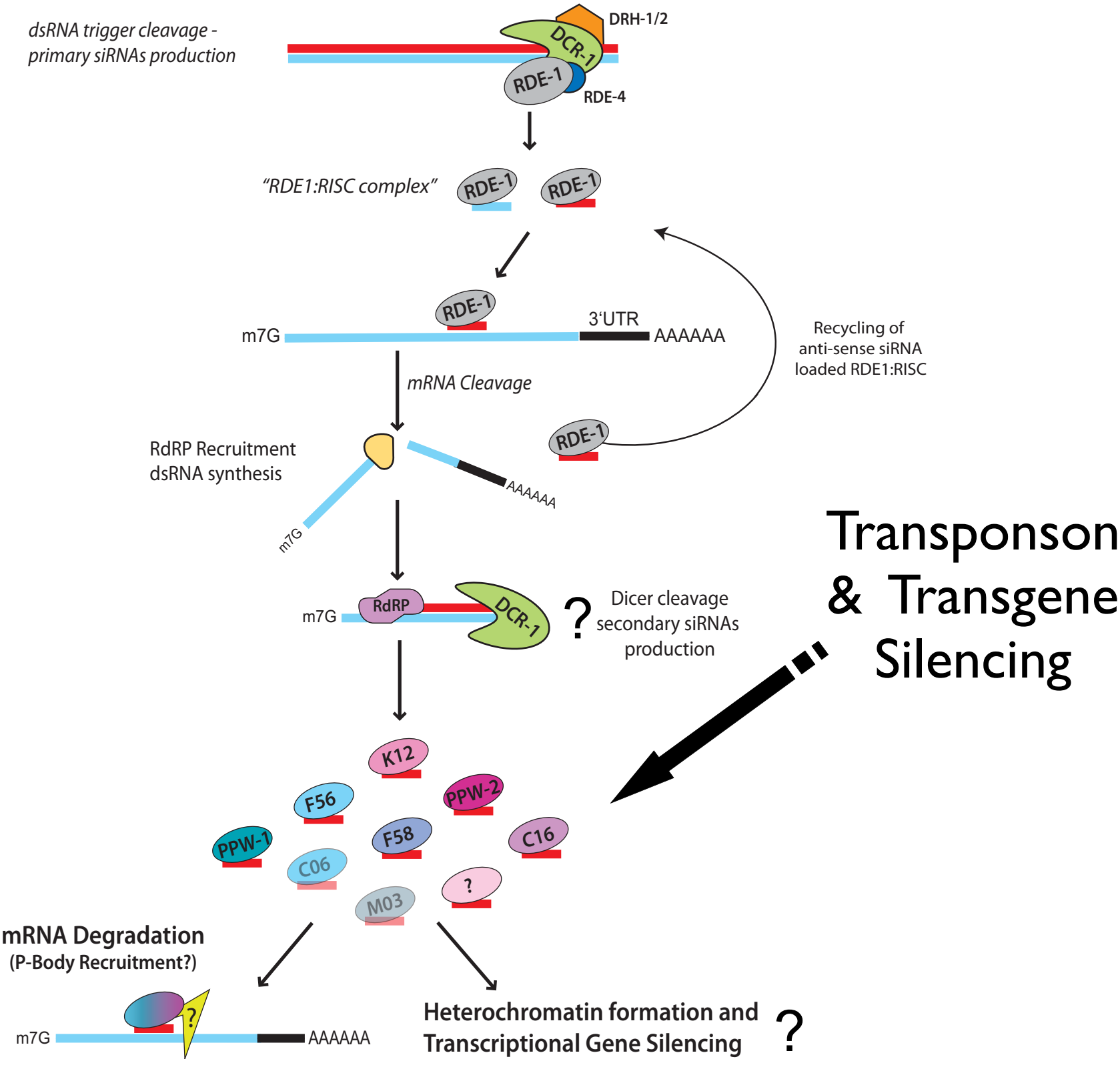


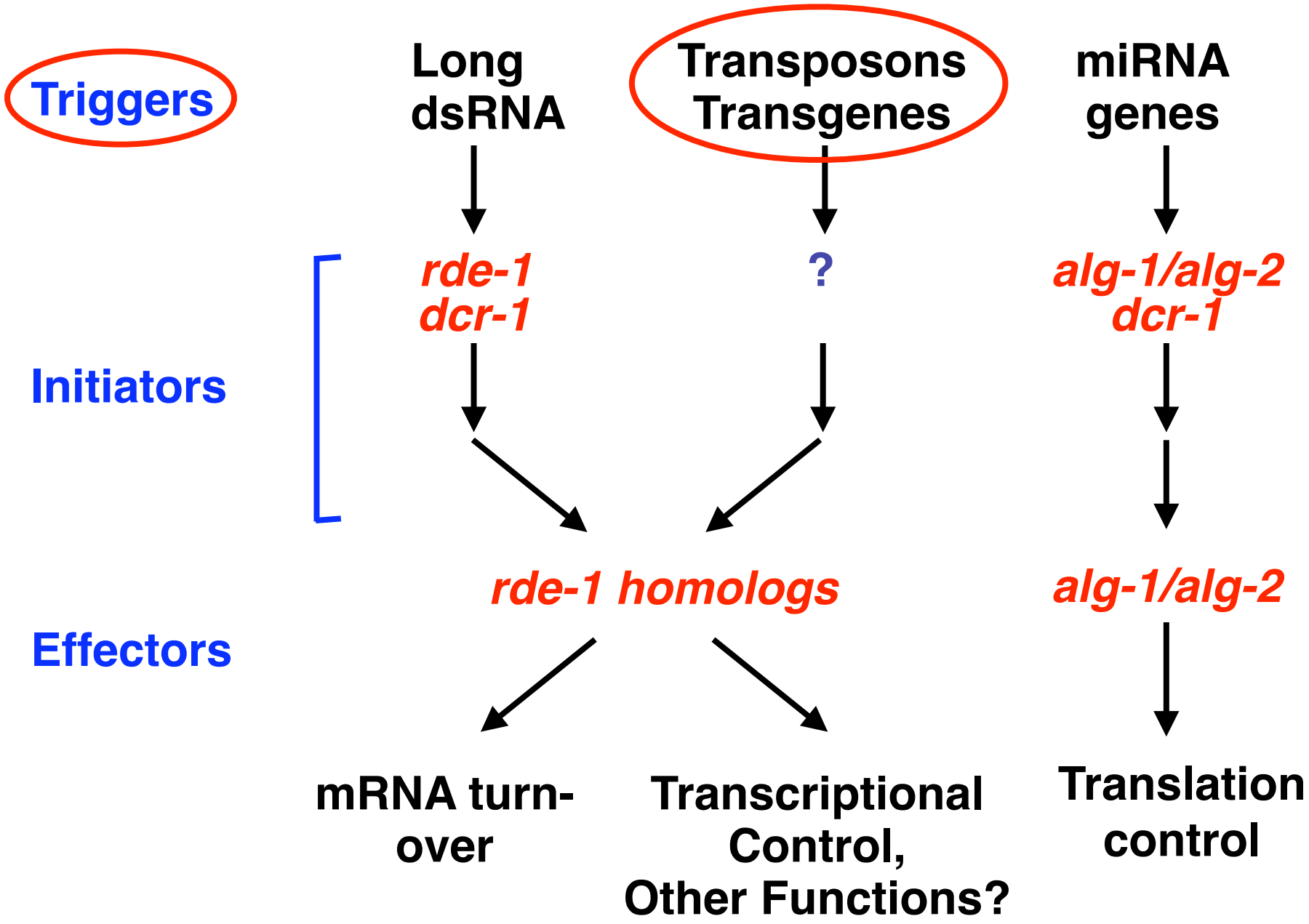


GFP Western

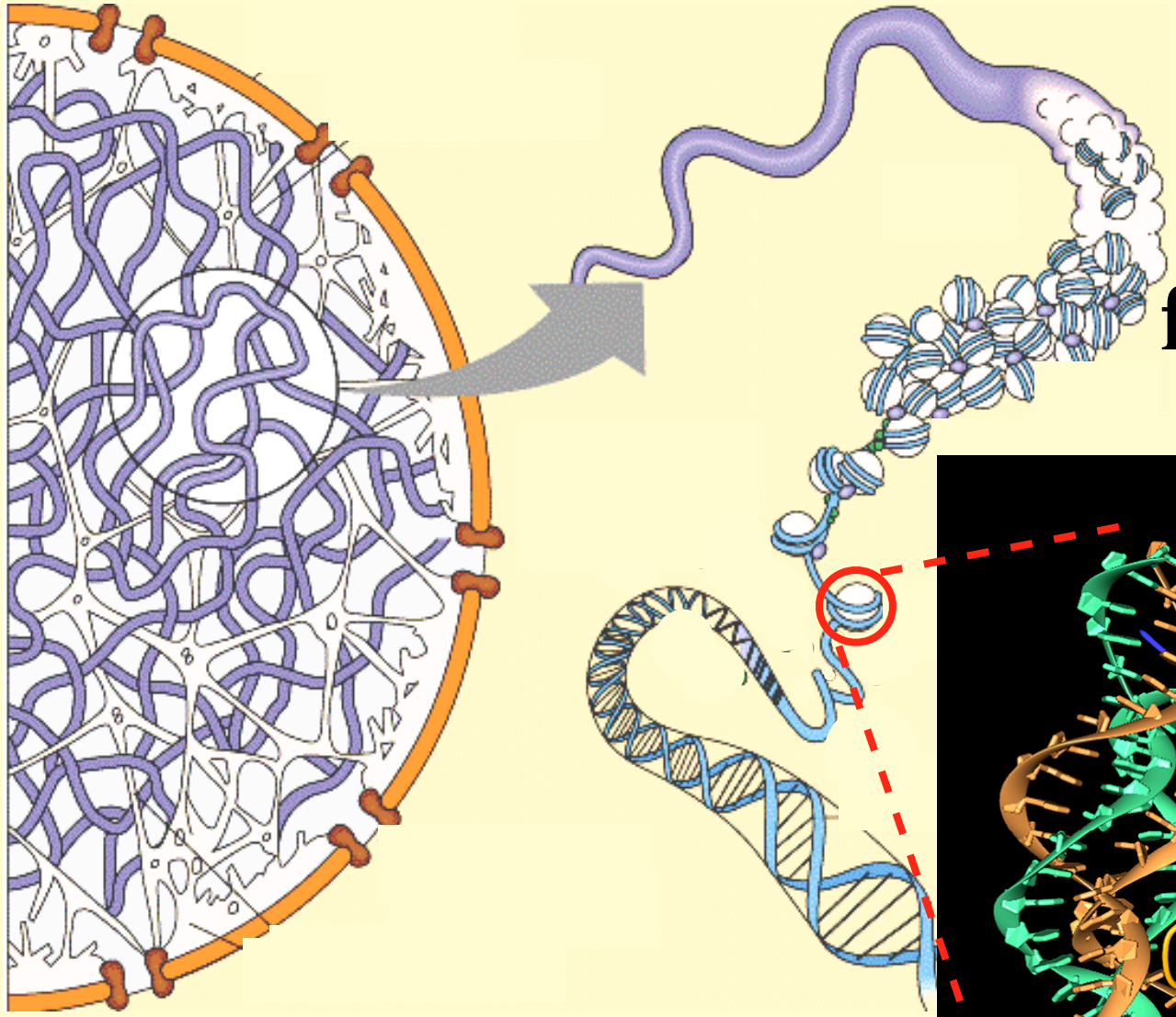
unc-22 siRNA

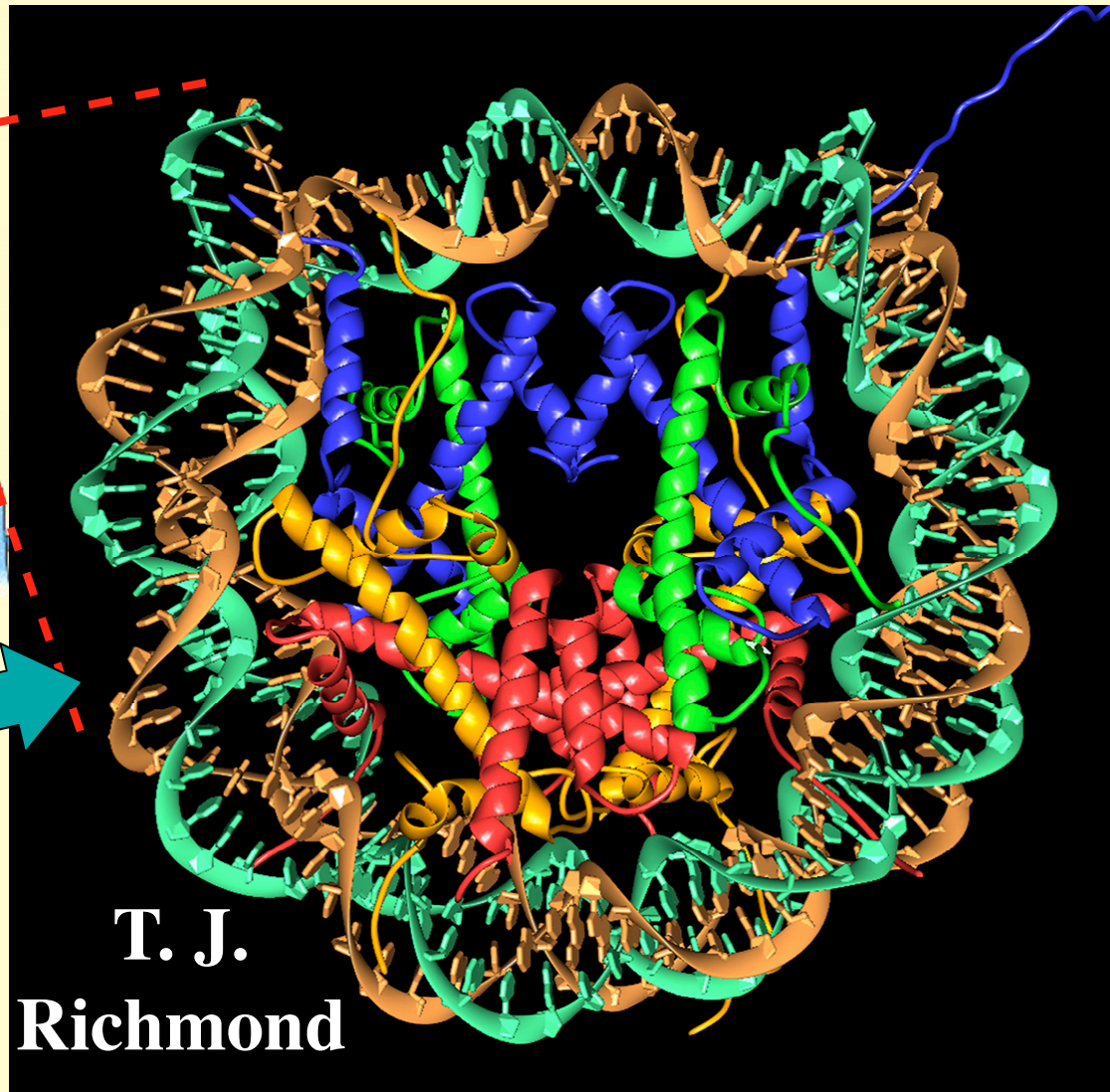
Erbay Yigit and Pedro Batista


dsRNA trigger cleavage -
primary siRNAs production
DRH-1/2
DCR-1
RDE-1
RDE-4
"RDE1:RISC complex"
RDE-1
RDE-1
RDE-1
m7G
3'UTR
AAAAAA
mRNA Cleavage
Recycling of
anti-sense siRNA
loaded RDE1:RISC
RDE-1
RdRP Recruitment
dsRNA synthesis
AAAAAA
m7G
m7G
RdRP
DCR-1
?
Dicer cleavage
secondary siRNAs
production
Transponson
& Transgene
Silencing
K12
F56
PPW-2
PPW-1
F58
C16
C06
?
M03
mRNA Degradation
(P-Body Recruitment?)
m7G
?
AAAAAA
Heterochromatin formation and
Transcriptional Gene Silencing
?


Triggers
Long dsRNA
Transposons Transgenes
miRNA genes
Initiators
rde-1 dcr-1
?
alg-1/alg-2 dcr-1
rde-1 homologs
alg-1/alg-2
Effectors
mRNA turn-over
Transcriptional Control, Other Functions?
Translation control

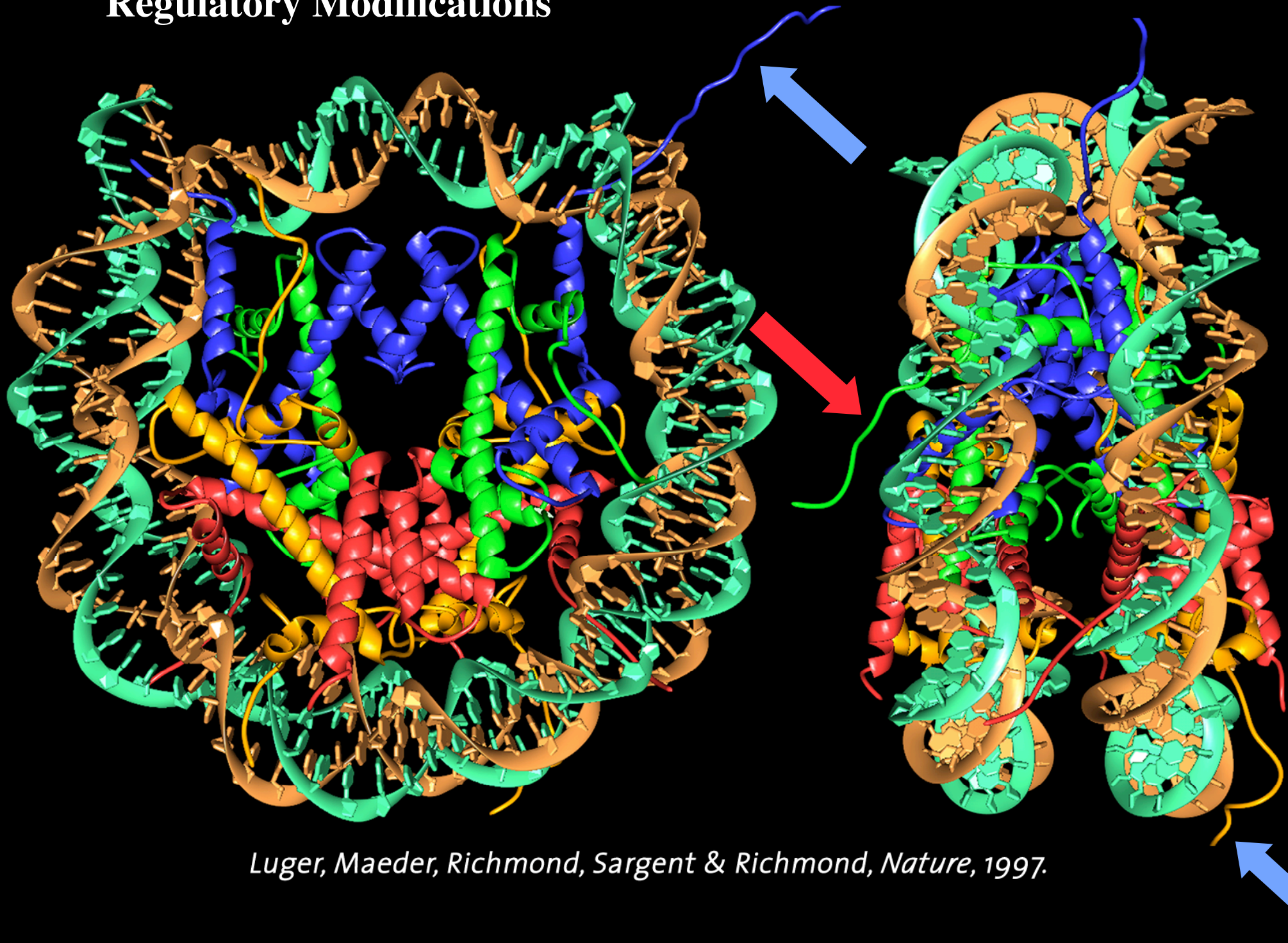
Chromatin: the physiological form of the genome





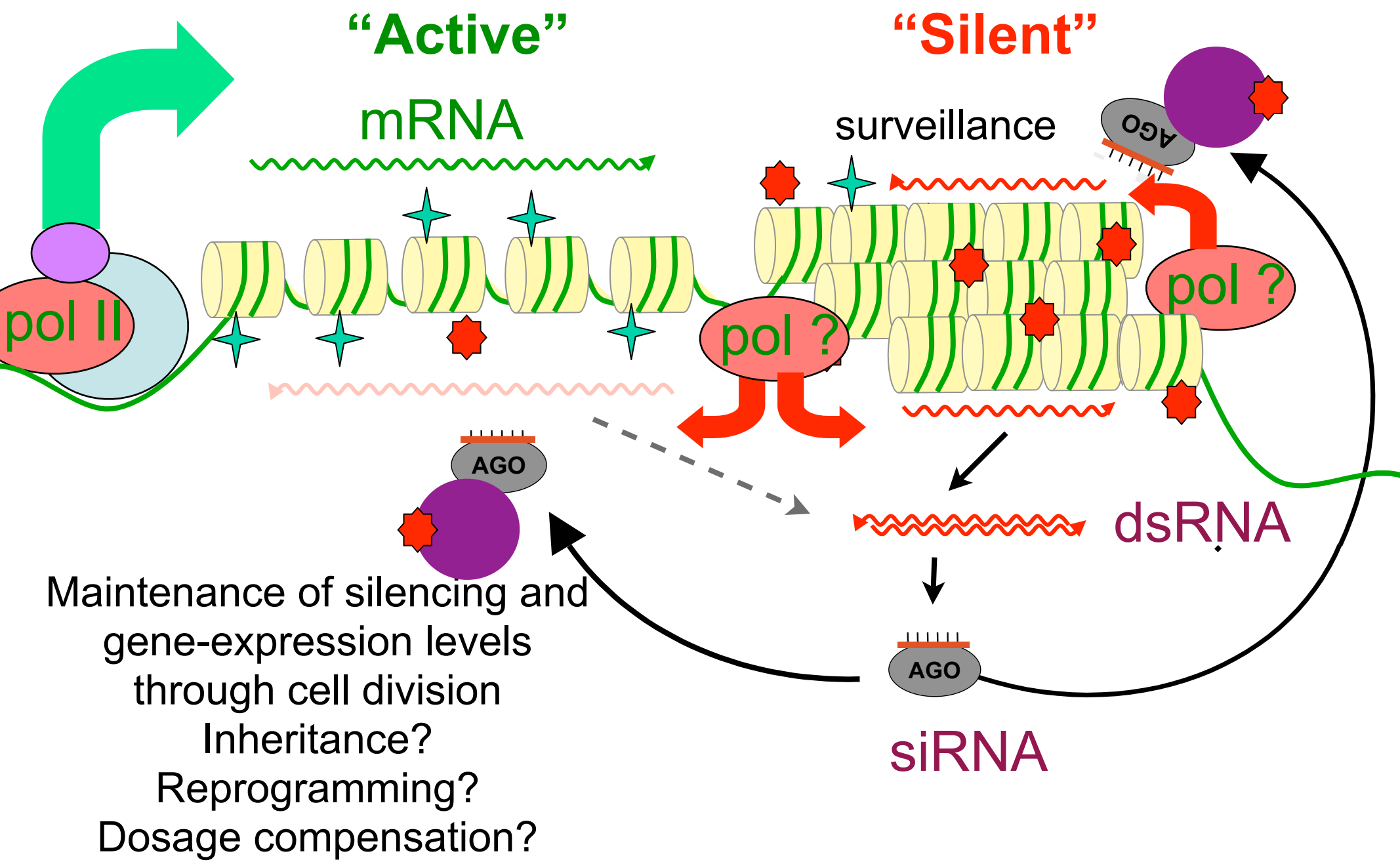
Nucleosomes: the building blocks of Chromatin

Regulatory Modifications

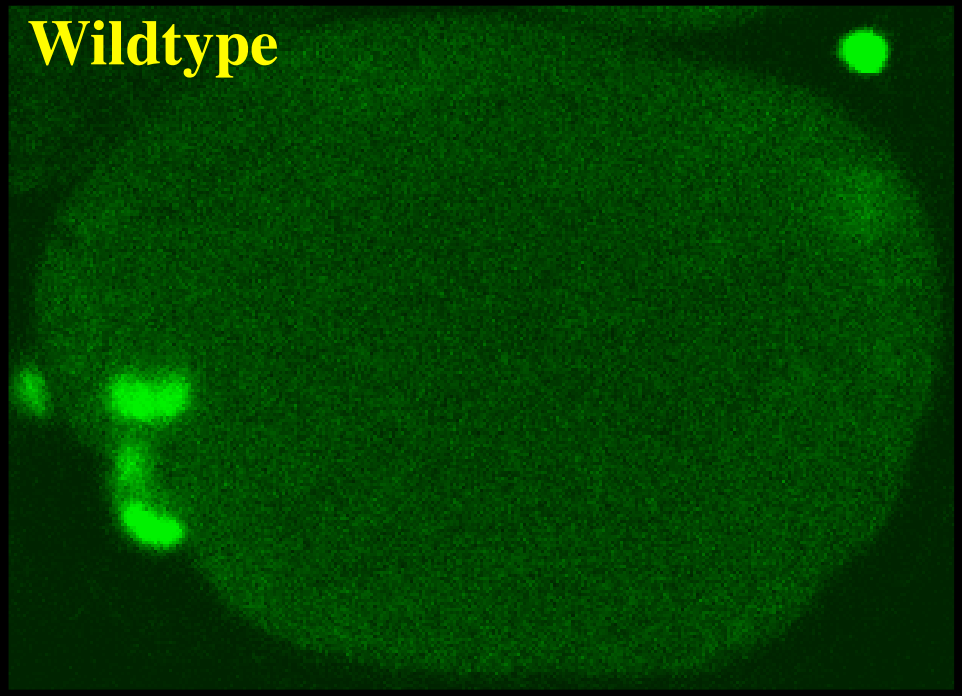


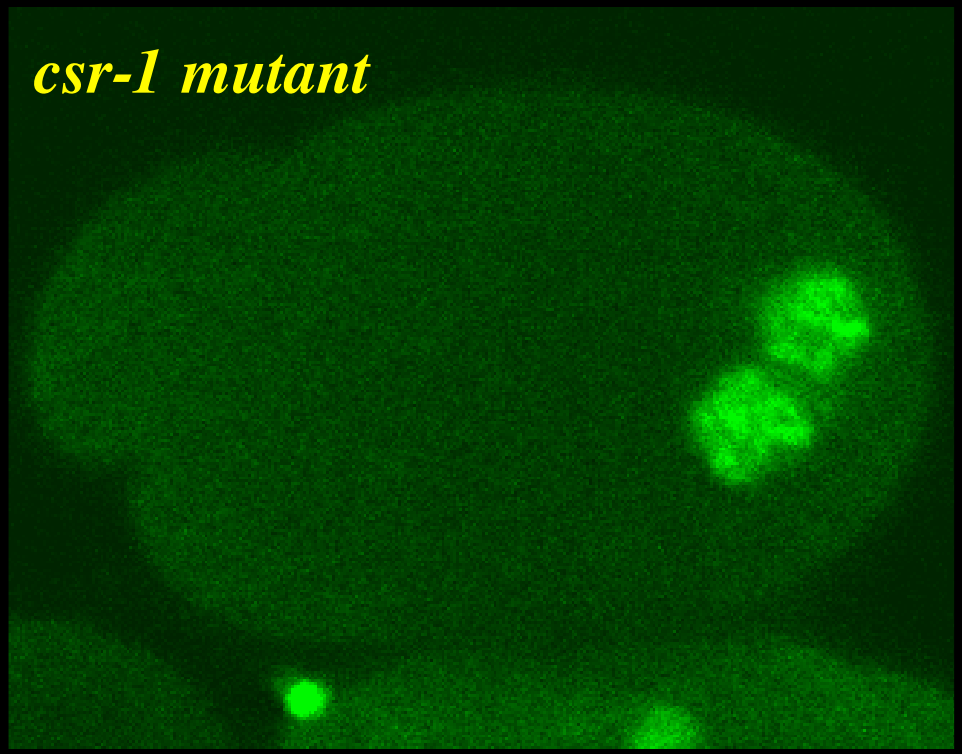
Luger, Maeder, Richmond, Sargent & Richmond, Nature, 1997.

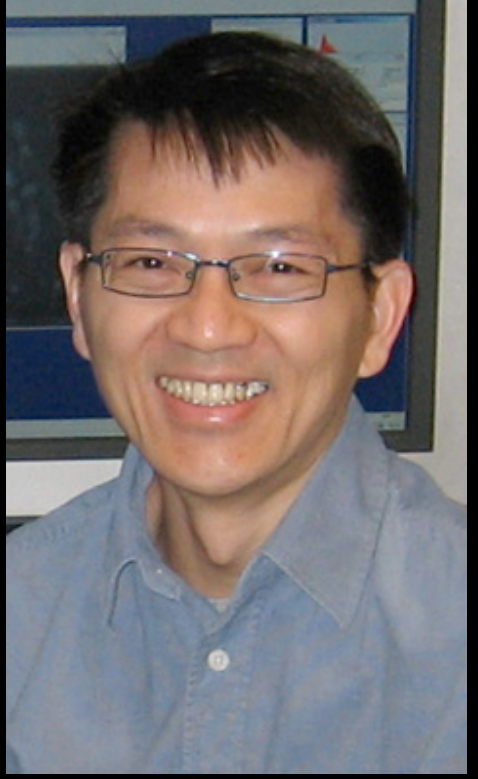
A Chromatin-RNA Feedback Loop



Julie Claycomb

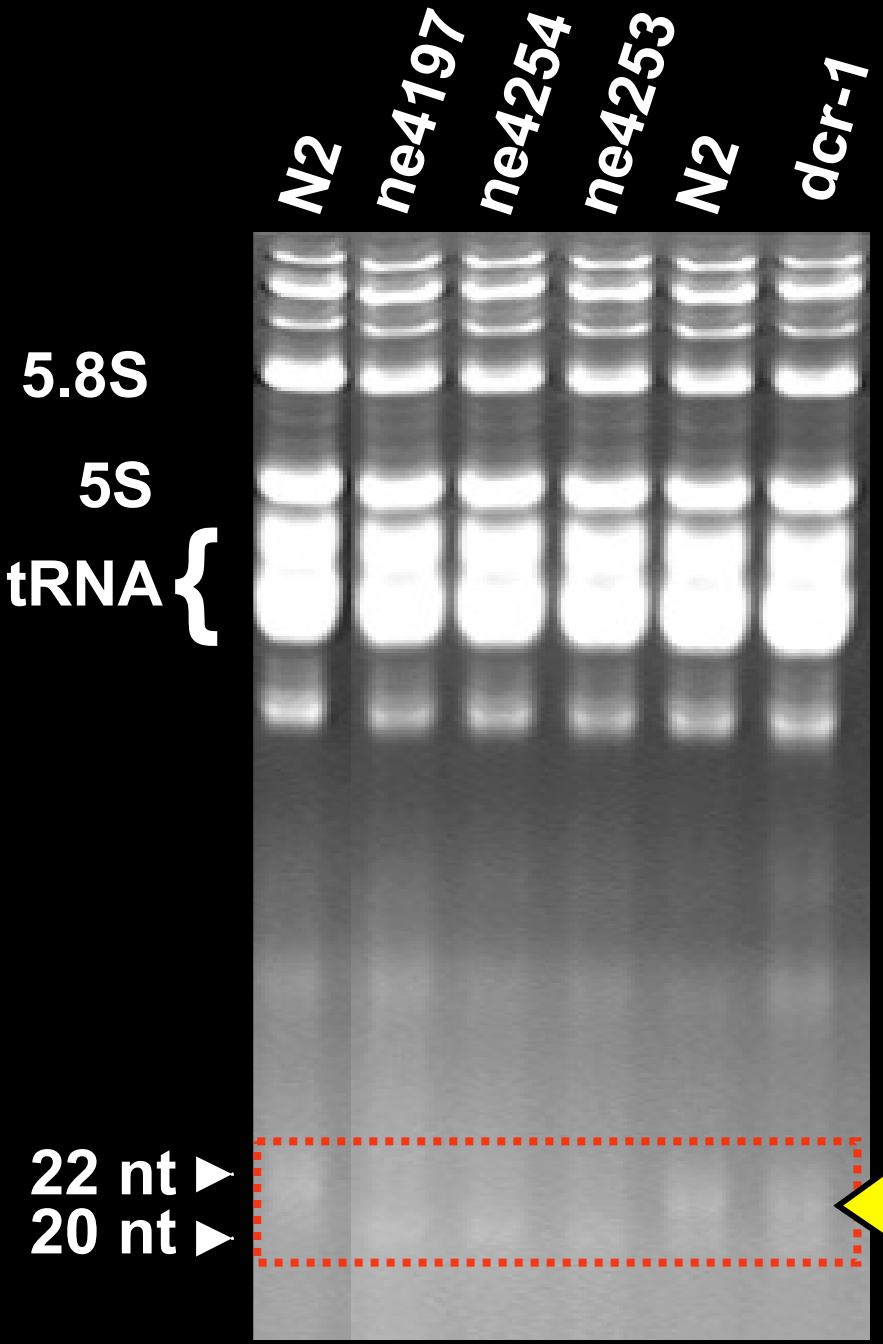






Ka Ming Pang

An abundant DCR-independent species of endo-siRNA




Nova Science Now
Eric Lander

THANK YOU!

Andy!

University of Massachusetts

Howard Hughes Medical Institute

National Institutes of Health, USA

Pew Scholars Program

March of Dimes

American Cancer Association

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