A NEW TWO STRANDED HELICAL STRUCTURE: POLYADENYLIC ACID AND POLYURIDYLIC ACID

(J. American Chemical Association, 78, 3458-3459)

SECTION ON PHYSICAL CHEMISTRY
NATIONAL INSTITUTE OF MENTAL HEALTH
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ALEXANDER RICH
DAVID R. DAVIES

RECEIVED JUNE 8, 1956

The Secondary Structure of Complementary RNA
E. Peter Geiduschek, John W. Moohr, and Smauel B. Weiss, Proceedings of The National Academy of Sciences, 48, 1078-1086, 1962.

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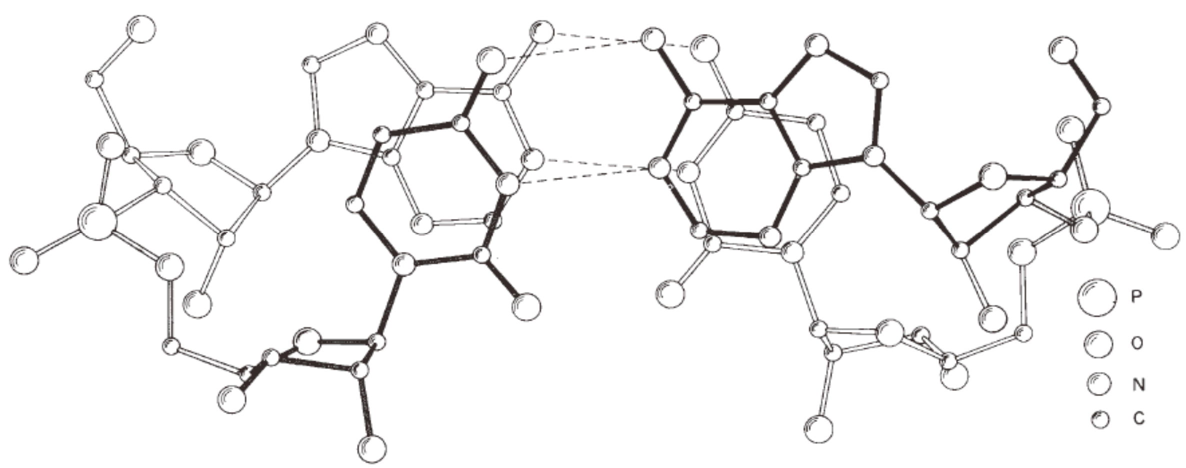
Homology test between the nucleic acid of an RNA virus and the DNA in the host cell.
Science 1962 Dec 14 1270-2.

MONTAGNIER L, SANDERS FK.
REPLICATIVE FORM OF ENCEPHALOMYOCARDITIS VIRUS RIBONUCLEIC ACID.
Nature. 1963 Aug 17;199:664-7.

WARNER RC, SAMUELS HH, ABBOTT MT, KRAKOW JS. (1963) Ribonucleic acid polymerase of Azotobacter vinelandii, II. Formation of DNA-RNA hybrids with single-stranded DNA as primer. Proc Natl Acad Sci U S A. 49:533-8.

NATURE VOL. 243 MAY 18 1973



JOHN M. ROSENBERG, NADRIAN C. SEEMAN, JUNG JA PARK KIM, F. L. SUDDATH, HUGH B. NICHOLAS* & ALEXANDER RICH
Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Double-Stranded Ribonucleic Acid Formation in vitro by MS 2 Phage-Induced RNA Synthetase

CHARLES WEISSMANN
PIET BORST
Department of Biochemistry, New York University School of Medicine, New York
25 September 1963

Virus-Specific Double-Stranded RNA Poliovirus-Infected Cells

(Science 143, 1034-1036, March 6, 1964)

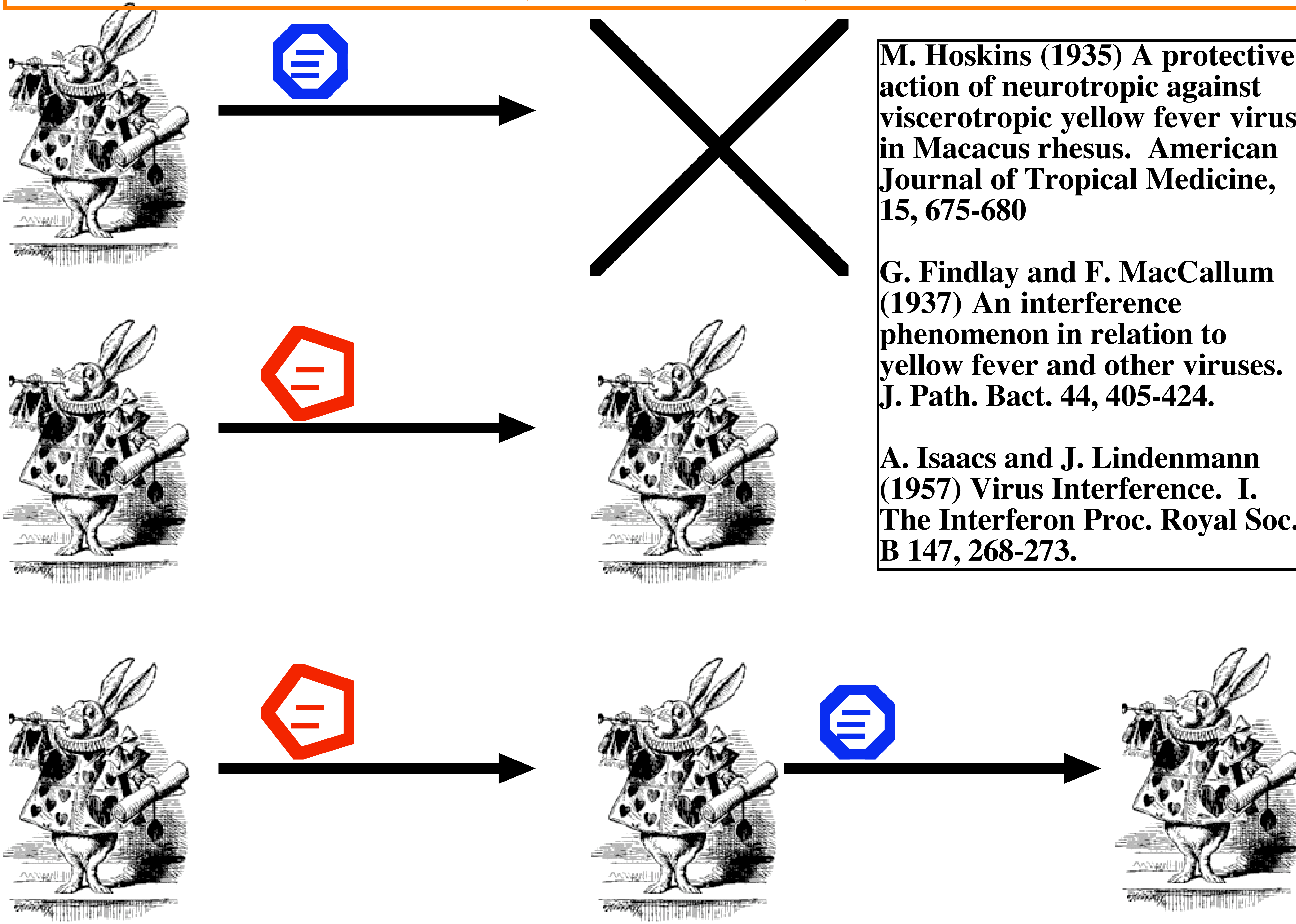
D. BALTIMORE *
Y. BECKER †
J. E. DARNELL
Department of Biology, Massachusetts Institute of Technology, Cambridge

Double Stranded RNA as a Specific Biological Effector

December 8, 2006

Karolinska Institute, Stockholm, Sweden

Viral interference (Interferon) effects in animals



M. Hoskins (1935) A protective action of neurotropic against viscerotropic yellow fever virus in Macacus rhesus. American Journal of Tropical Medicine, 15, 675-680

G. Findlay and F. MacCallum (1937) An interference phenomenon in relation to yellow fever and other viruses. J. Path. Bact. 44, 405-424.

A. Isaacs and J. Lindenmann (1957) Virus Interference. I. The Interferon Proc. Royal Soc. B 147, 268-273.

INDUCERS OF INTERFERON AND HOST RESISTANCE, I. DOUBLE-STRANDED RNA FROM EXTRACTS OF PENICILLIUM FUNICULOSUM

By G. P. Lampson, A. A. Tytell, A. K. Field, M. M. Nemes, and M. R. Hilleman

DIVISION OF VIRUS AND CELL BIOLOGY RESEARCH, MERCK INSTITUTE FOR THERAPEUTIC RESEARCH, WEST POINT, PENNSYLVANIA

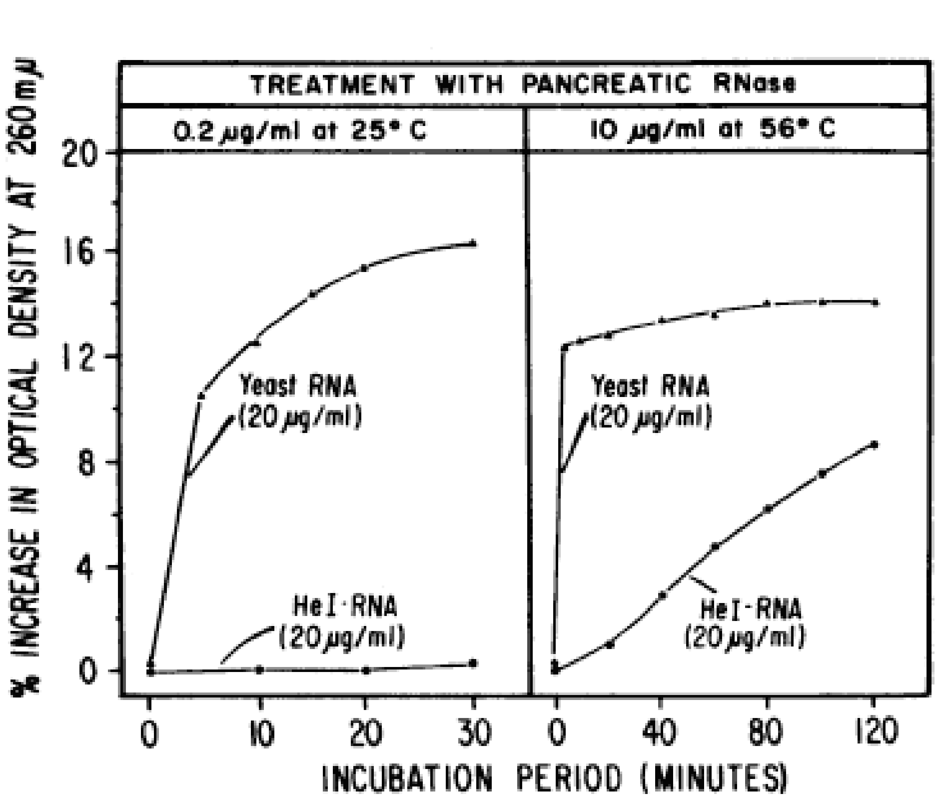


Fig. 2.—Comparative rates of degradation of HeI-RNA and of yeast RNA by RNase.

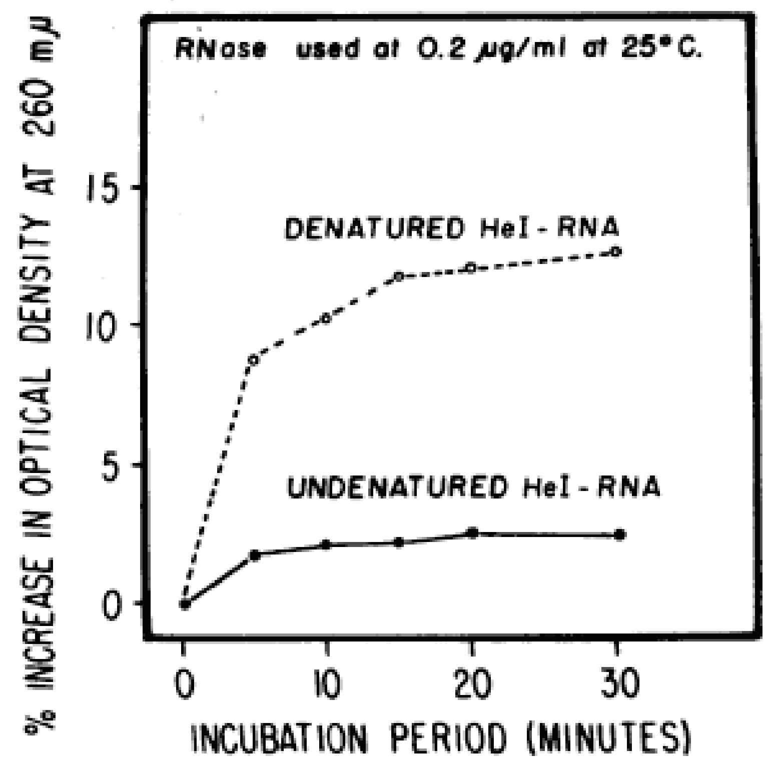


Fig. 3.—Effect of heat denaturation on rate of degradation of HeI-RNA by RNase.

Proceedings of the National Academy of Sciences, USA, Volume 58, Pages 782-789. 1967

JOURNAL OF AGRICULTURAL RESEARCH

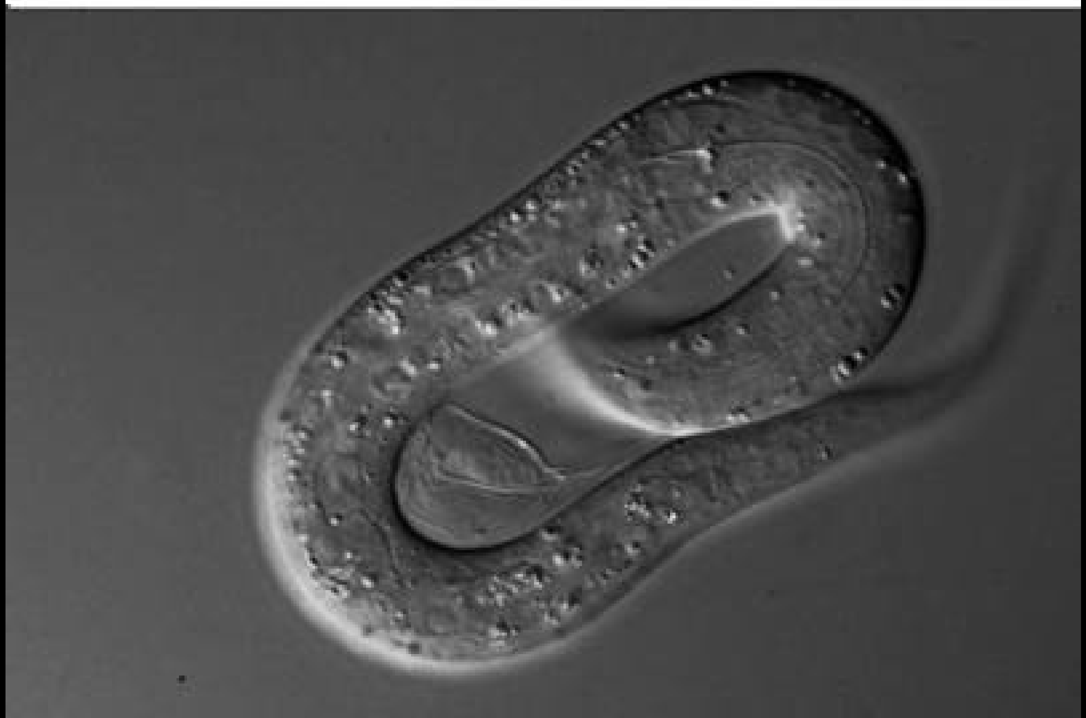
Vol. 37 WASHINGTON, D. C., AUGUST 1, 1928 No. 3

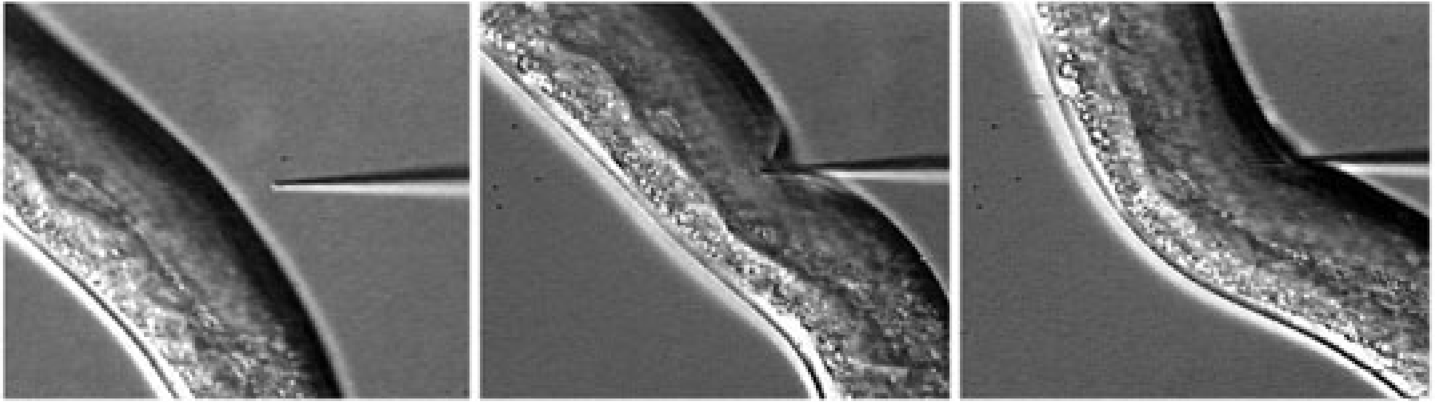
HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS[1]

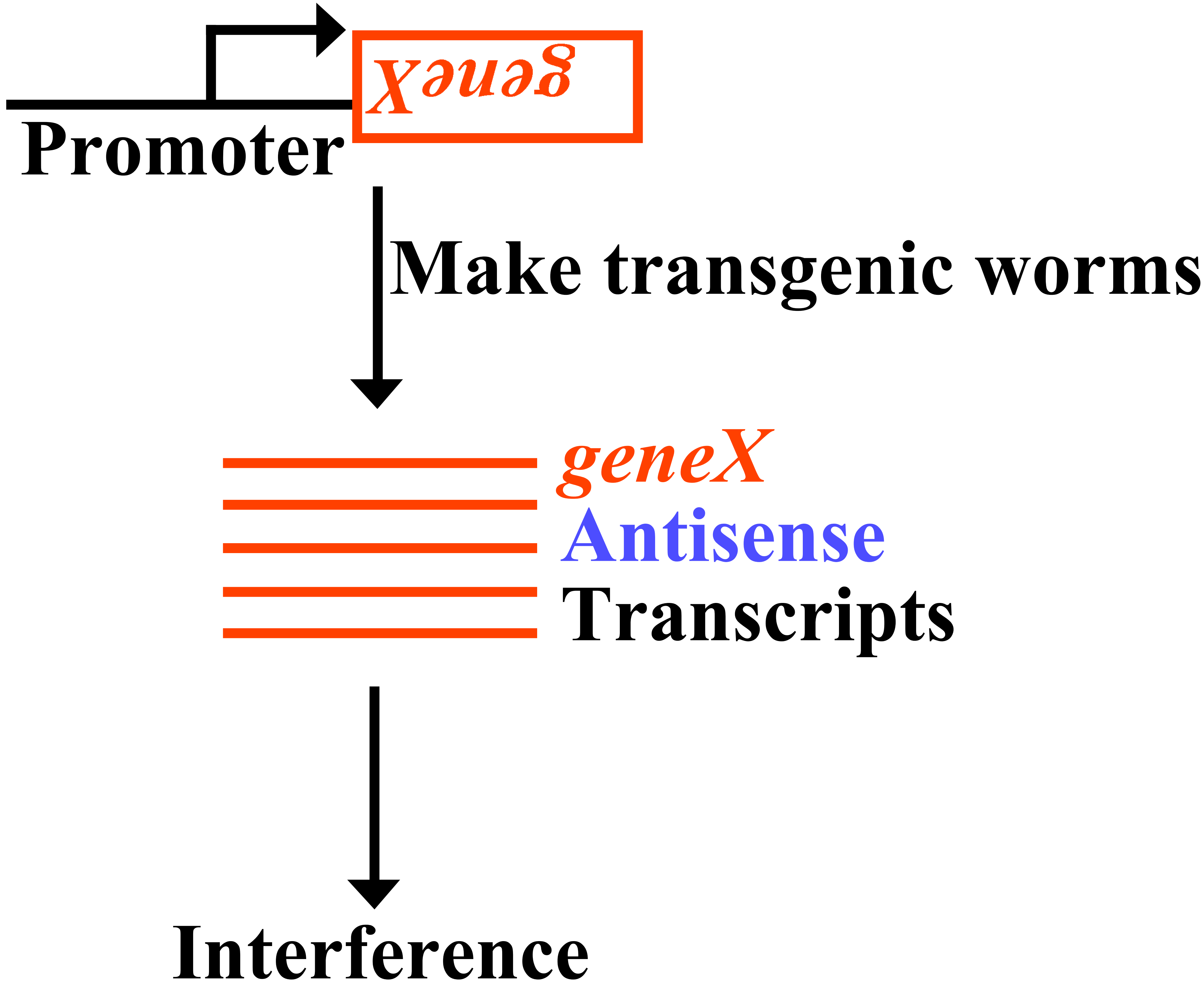
By S. A. WINGARD[2]

Associate Plant Pathologist, Virginia Agricultural Experiment Station

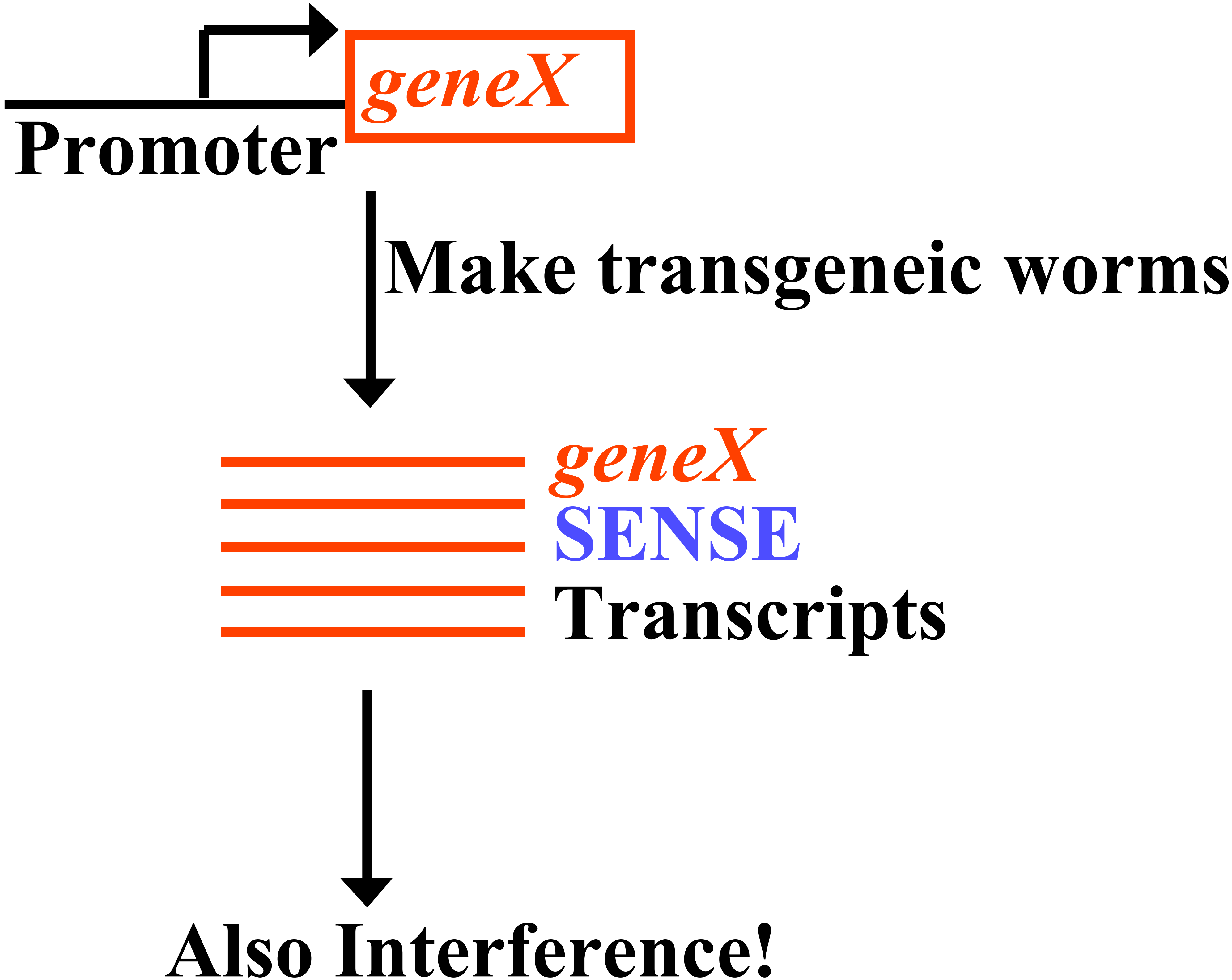
FIG. 7.—Turkish tobacco plant 23 days after inoculation with ring-spot. Note the gradual decline in the development of ring-spot symptoms on the upper leaves until finally the top leaves appear perfectly normal. Much reduced



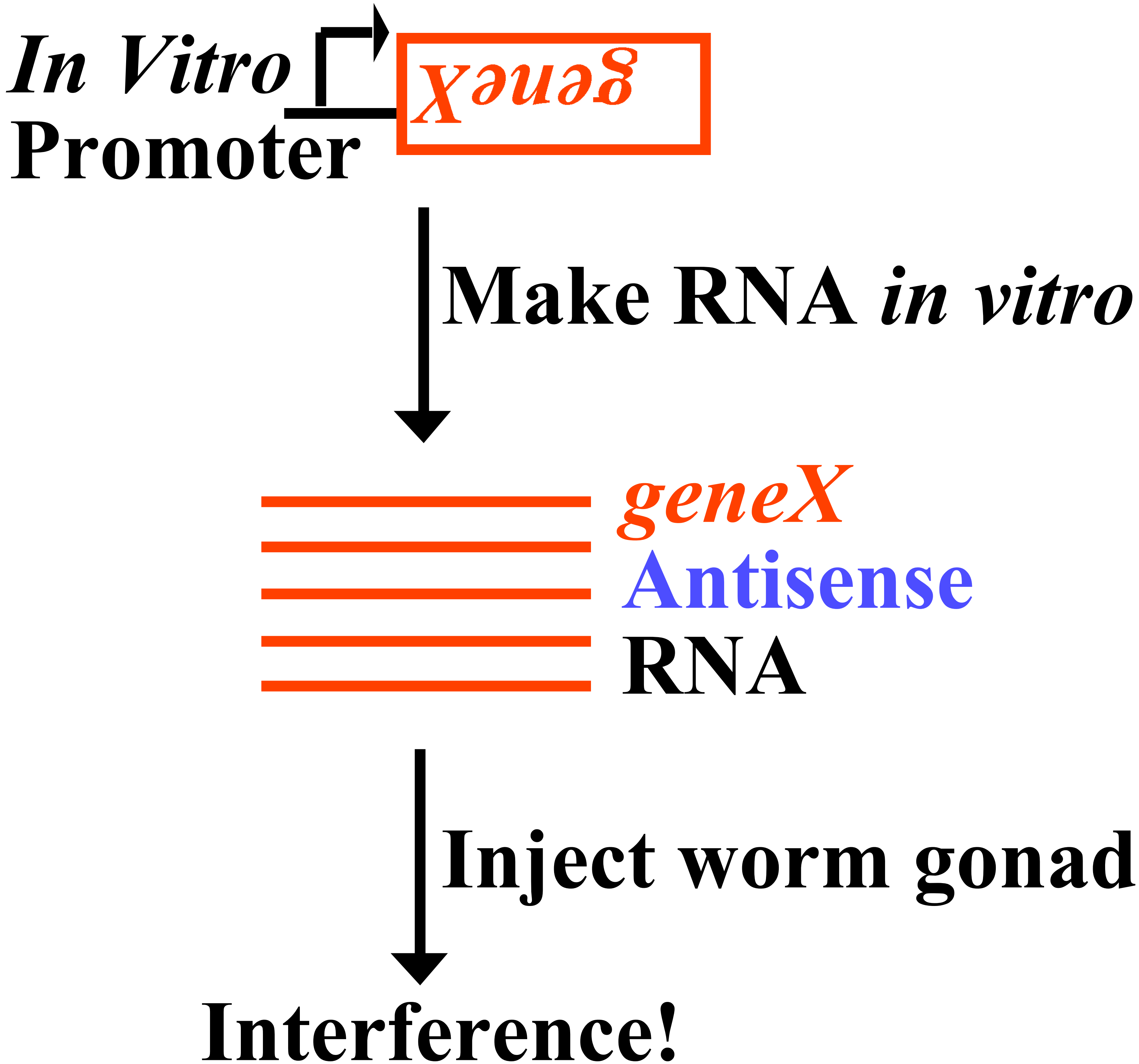




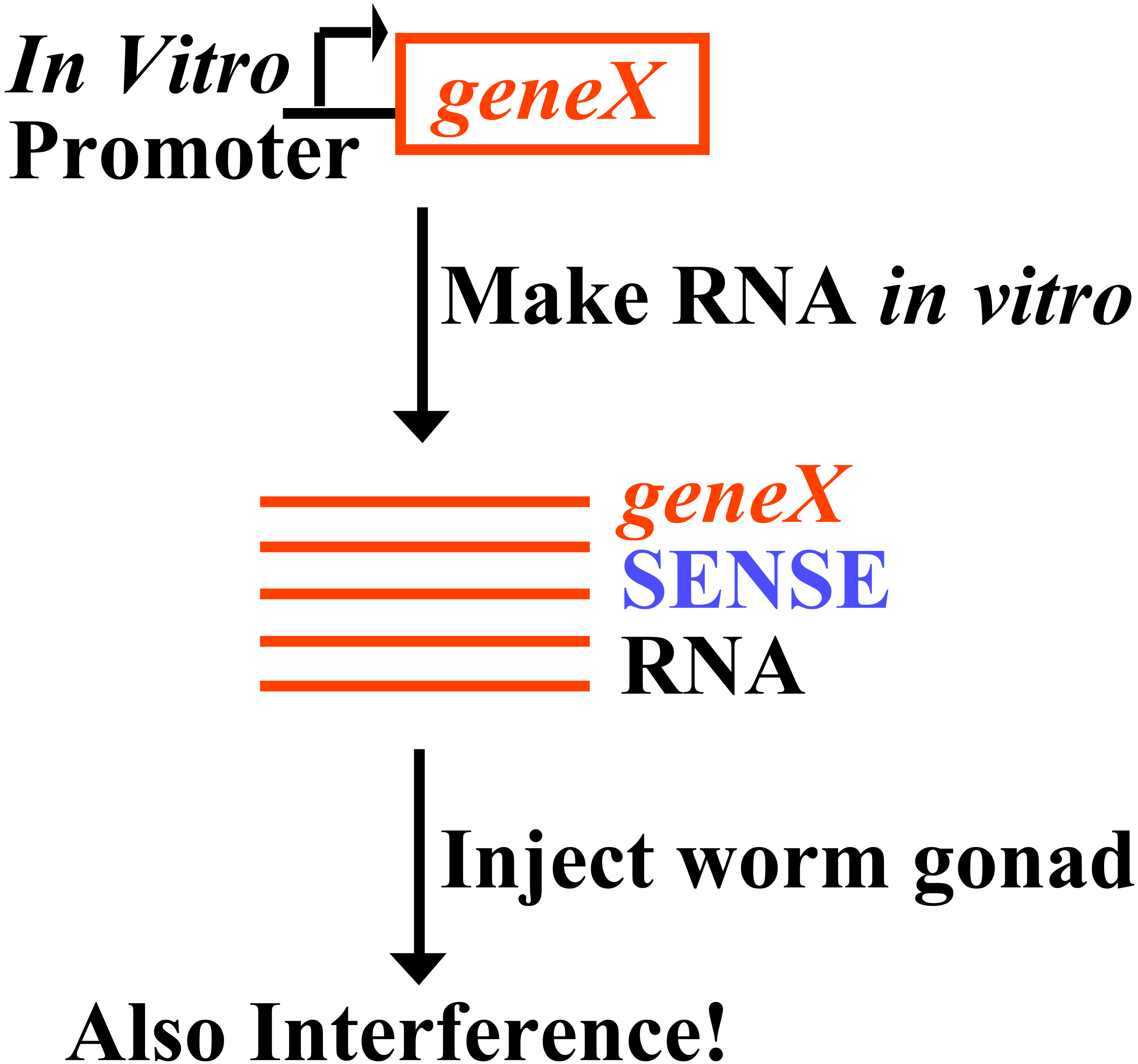
(Development 113:503 [1991])



(Development 113:503 [1991])



(Guo and Kemphues, 1995)



(Guo and Kemphues, 1995)

Craig Mello's RNAi Workshop: 1997 *C. elegans* meeting, Madison USA

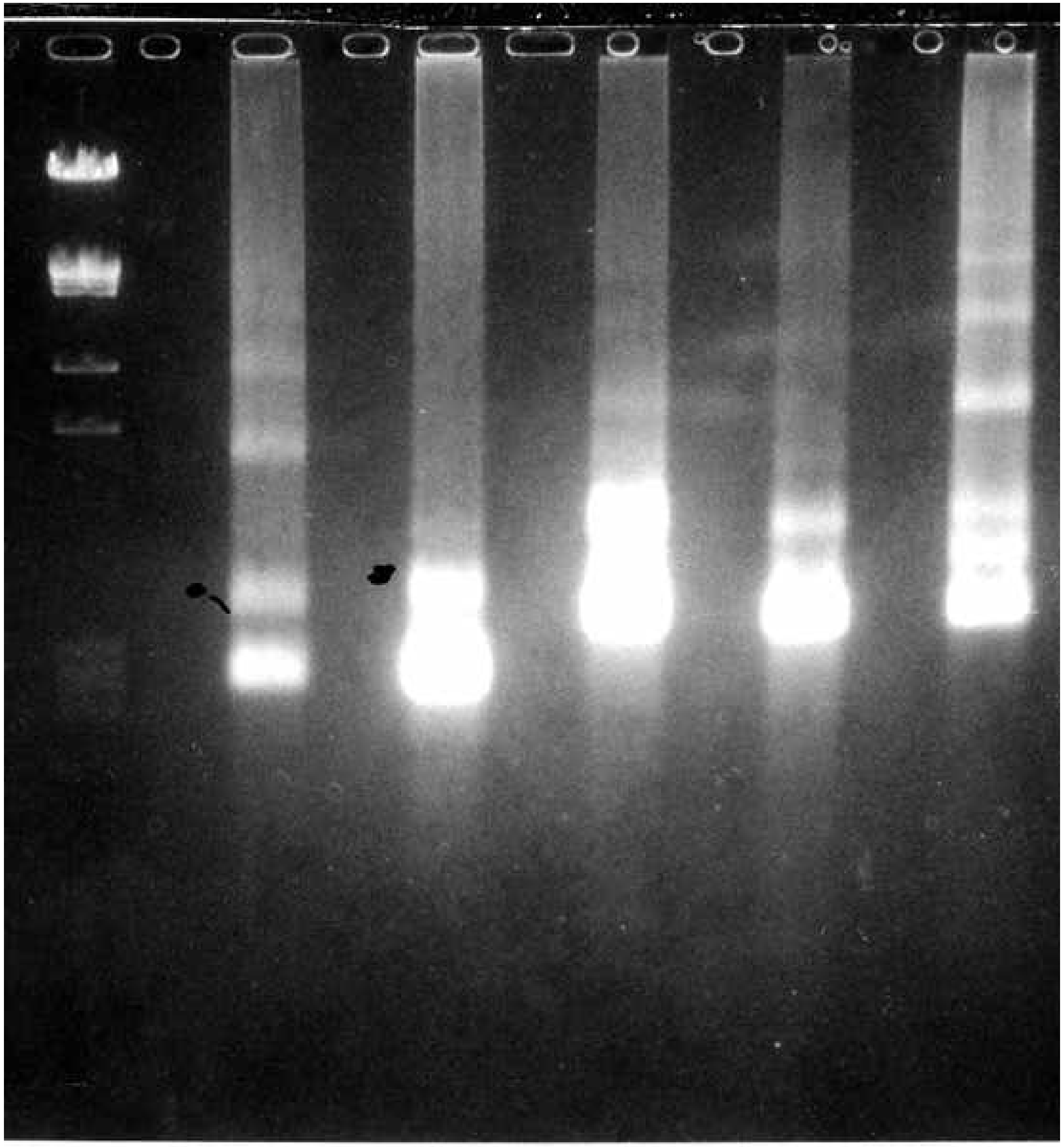
C. elegans RNAi: a mystery and a tool

- **An effective means to block gene function in the early embryo**
- **Used for scores of genes to answer interesting functional questions**
- **Specificity and potency are remarkable and puzzling**
- **Interference can cross cell boundaries**

Two puzzles to investigate for the summer of 1997:

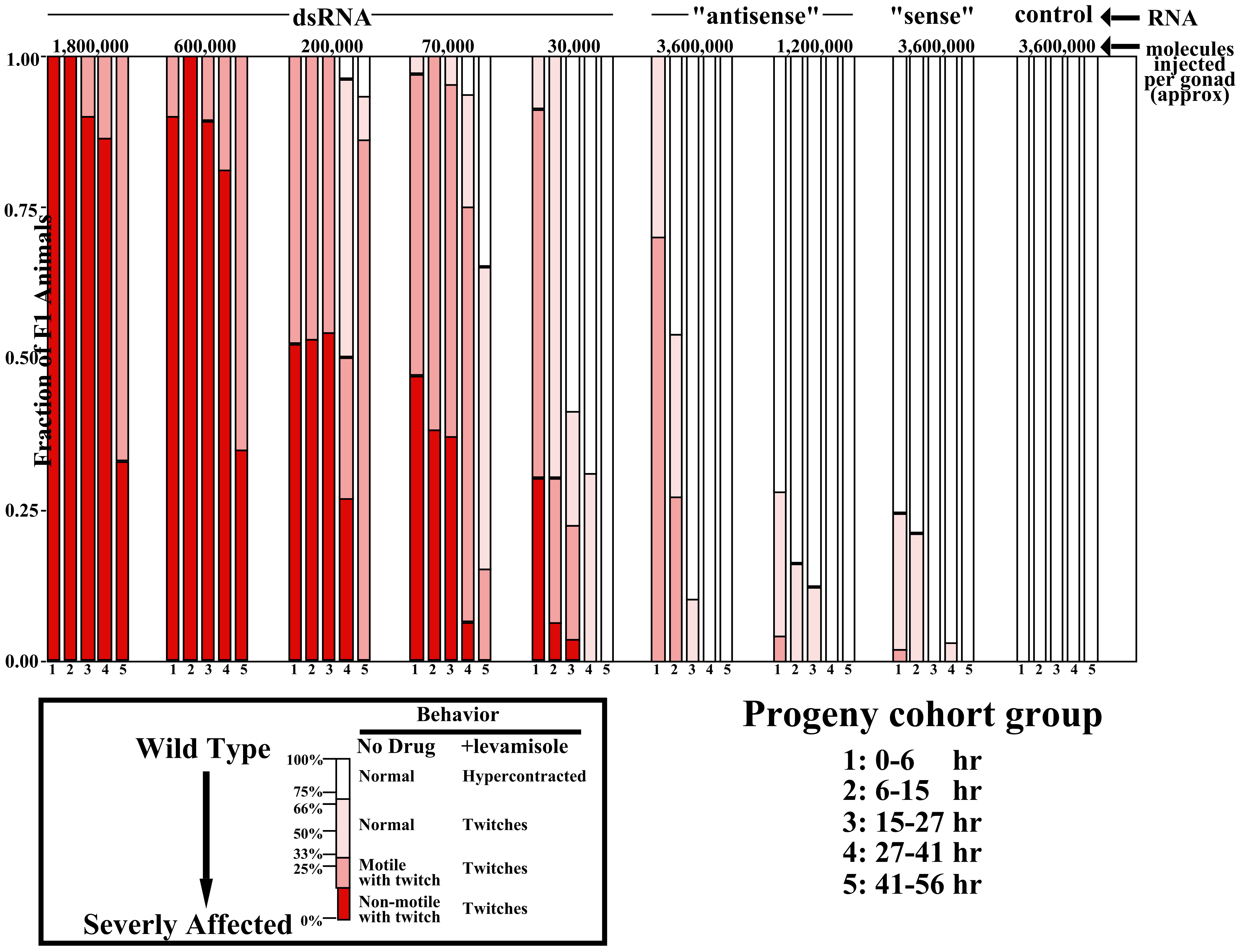
- **How could both "Sense" and "Antisense" RNA produce interference?**
- **Why should injected RNAs outlast normal mRNAs in the same embryo?**

Is the interfering RNA a "contaminant" with stable structure?



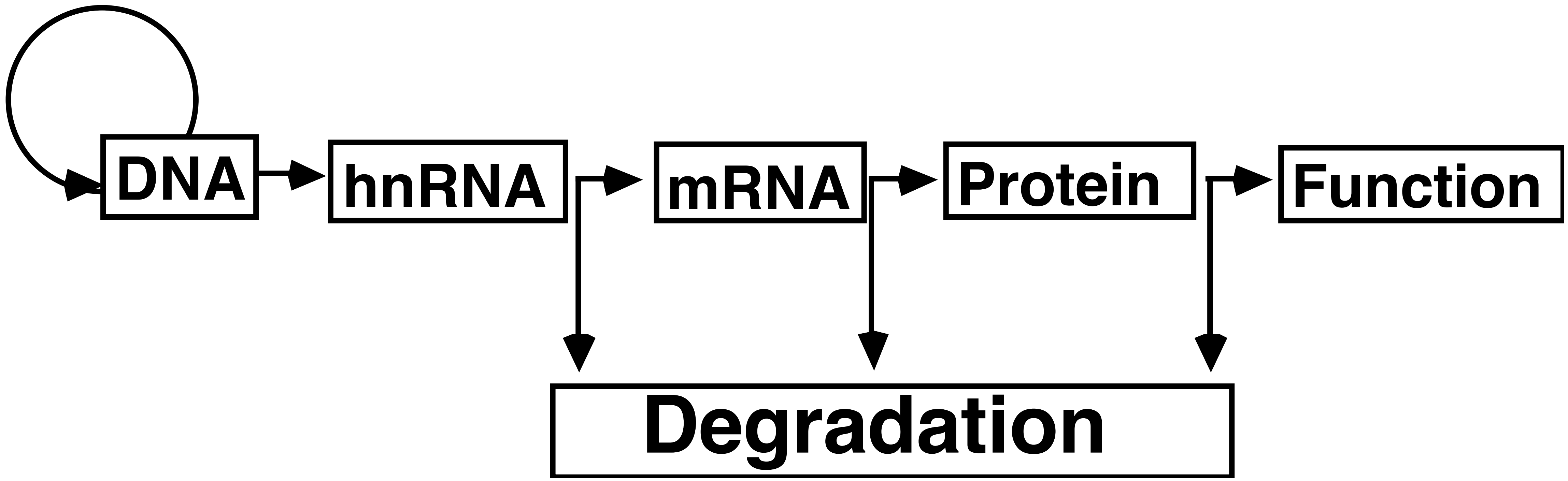
Quantitative assays for silencing: *unc-22*

- **dsRNA is >100-fold more effective than sense or antisense**
- **dsRNA can produce interference at a few molecules per cell**



Progeny cohort group

1: 0-6 hr
2: 6-15 hr
3: 15-27 hr
4: 27-41 hr
5: 41-56 hr

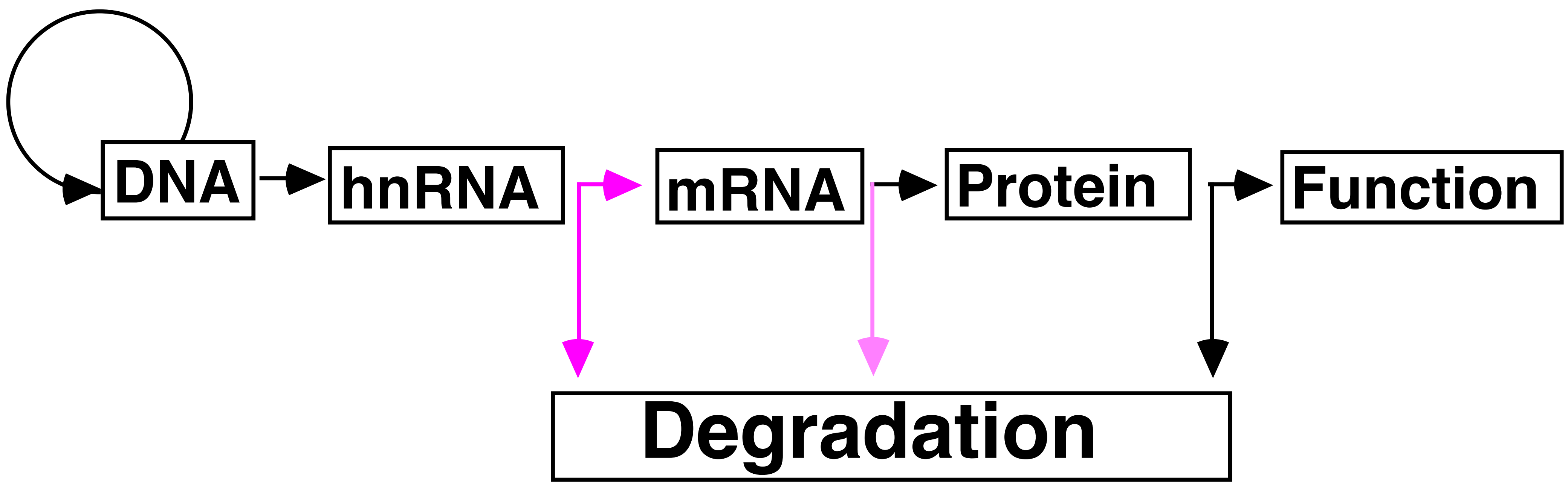

DNA
hnRNA
mRNA
Protein
Function
Degradation

mex-3 mRNA

control **+dsRNA**

RNAi effects on target RNAs

- mRNA is absent
- hnRNA is greatly decreased, but not absent



Levels of (im)precision in RNA delivery

S. Guo (Cornell): RNA into gonad --> gonadal affect

Levels of (im)precision in RNA delivery

S. Guo (Cornell): RNA into gonad --> gonadal affect

S. Driver (UMass): RNA into body cavity --> gonadal affect

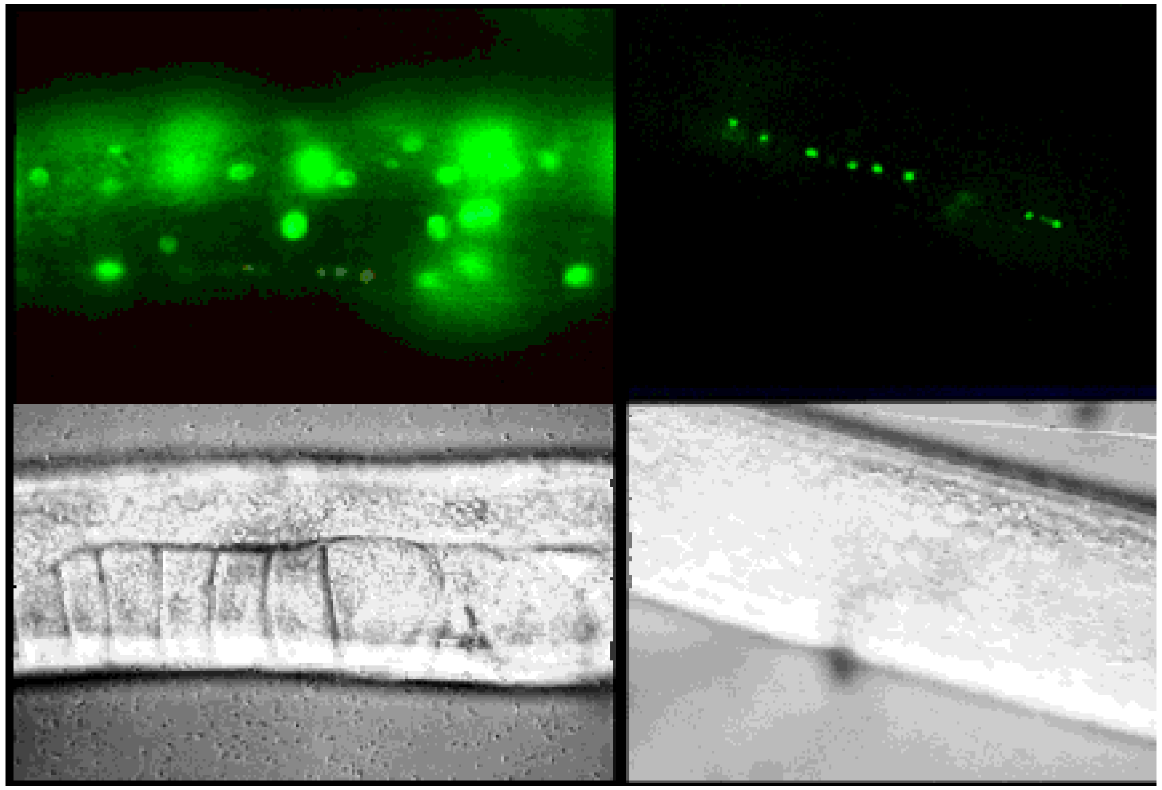
Levels of (im)precision in RNA delivery

S. Guo (Cornell): RNA into gonad --> gonadal affect

S. Driver (UMass): RNA into body cavity --> gonadal affect

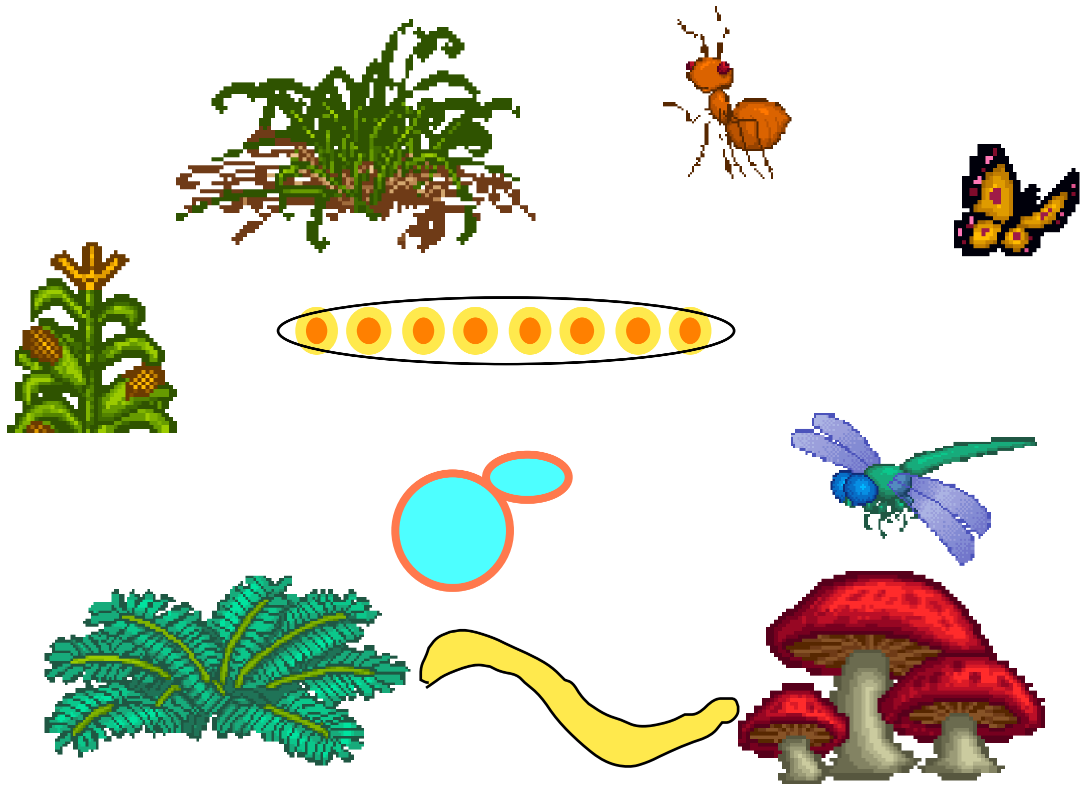
L. Timmons (Carnegie): Feed [dsRNA+ bacteria] to worms

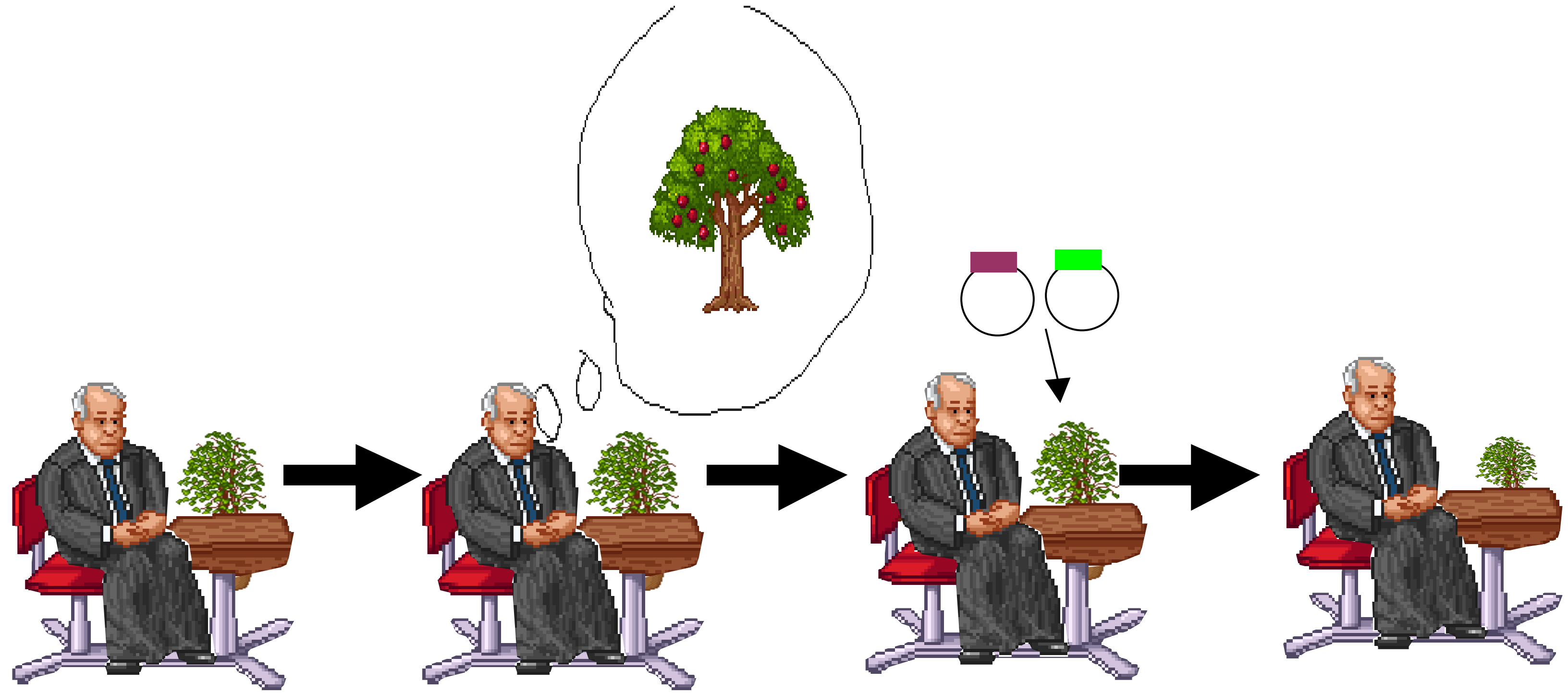
Eating control cells   Eating GFP dsRNA



let-858::gfp

brightfield





Silencing Phenomena in Plants (e.g., Napoli et al., 1990, deCarvalho et al, 1992)

Transgenes are often silent

Big Surprise: homolgous plant gene can also be silent *("Cosuppression")*

Observed with "sense" and "antisense" transgenes

Sequence-specific RNA decay (also...)

Diffusible: Silencing spreads between host and graft

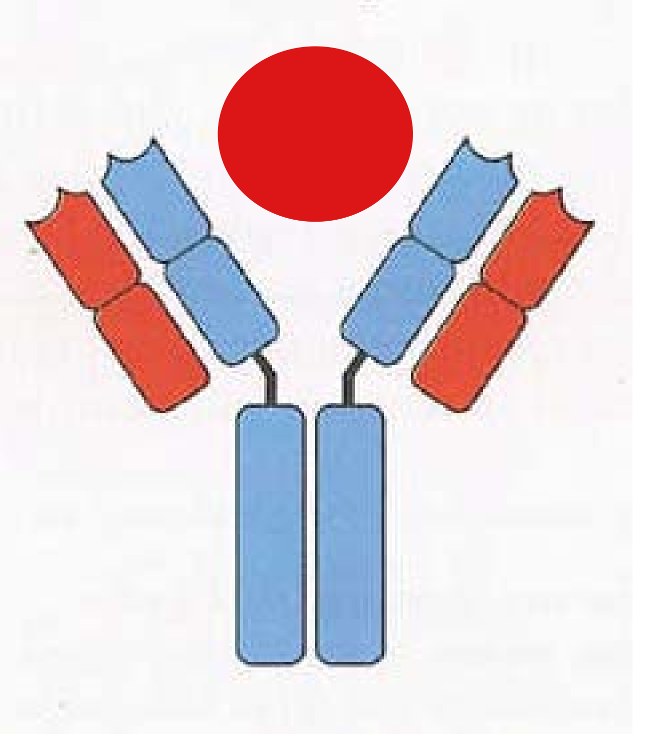
Many lessons from RNAi-like processes in plant systems

I. Plants teach us that RNAi is an anti-viral mechanism

- Viral RNAs can be targets
- Spreading allows systemic antiviral response
- Many viruses produce anti-silencing proteins
- Plants without silencing can be viable
- Silencing- plants can show more severe symptoms of viral infection
- Where are all the nematode RNA viruses to test this in *C. elegans*?

Sources: Baulcombe, Vaucheret, Vance, Carrington Labs (many papers throughout 1990's)

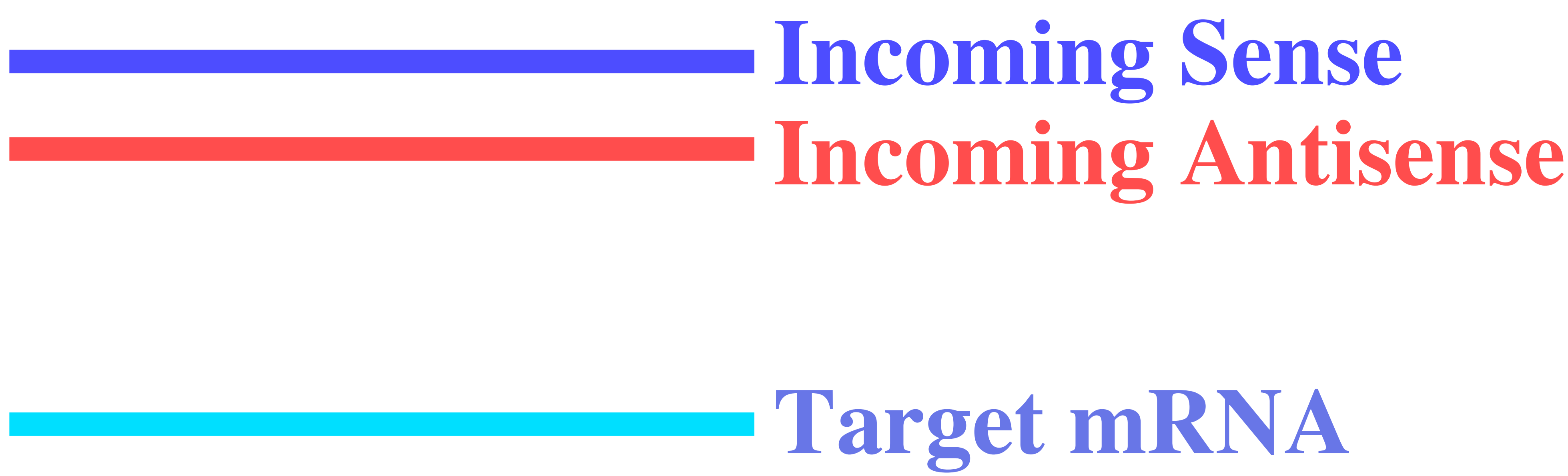
- ***What is the unit of recognition for RNA-based immunity?***

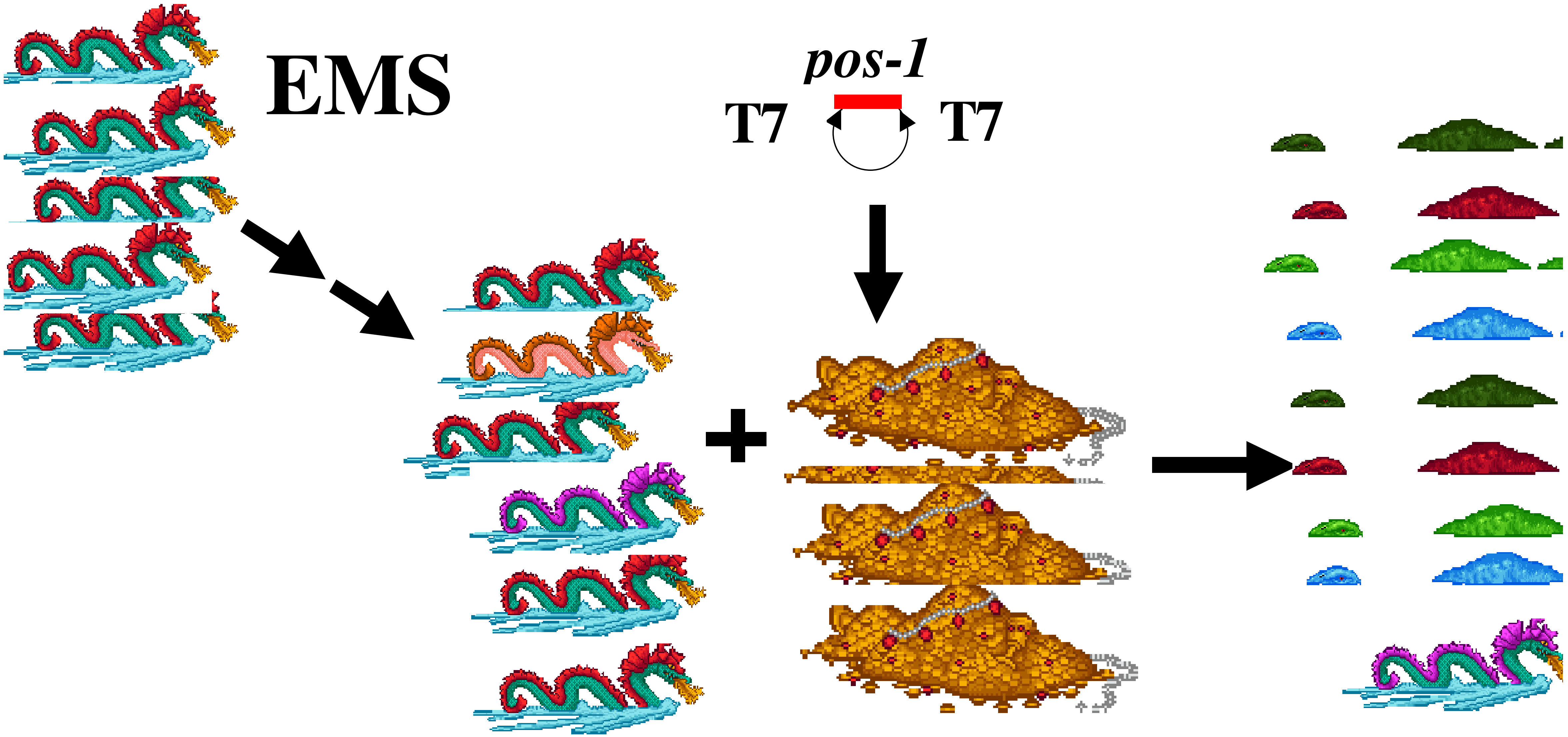


Conclusions from Trigger Analysis

- Highly matched duplex in a region of target homology is required
- dsRNAs as short as ~25nt have can trigger specific RNAi responses
- '+' and '-' trigger strands contribute differentially to RNAi

The three strand problem





A mutational Screen for trans-acting factors involved in RNAi

See: Tabara, H., Sarkissian, M., Kelly, W., Fleenor, J., Grishok, A., Timmons, L., Fire, A., and Mello, C. (1999) "The rde-1 gene, RNA interference, and transposon silencing in *C. elegans*." Cell 99:123-132

Criteria in selecting which mutations to analyze first

- **Null mutations should eliminate RNAi**
- **Effects should occur in all tissues**
- **Minimal set of additional phenotypes**

Biochemistry to the rescue

Short RNAs associated with plant PTGS (Hamilton and Baulcombe, 1999)

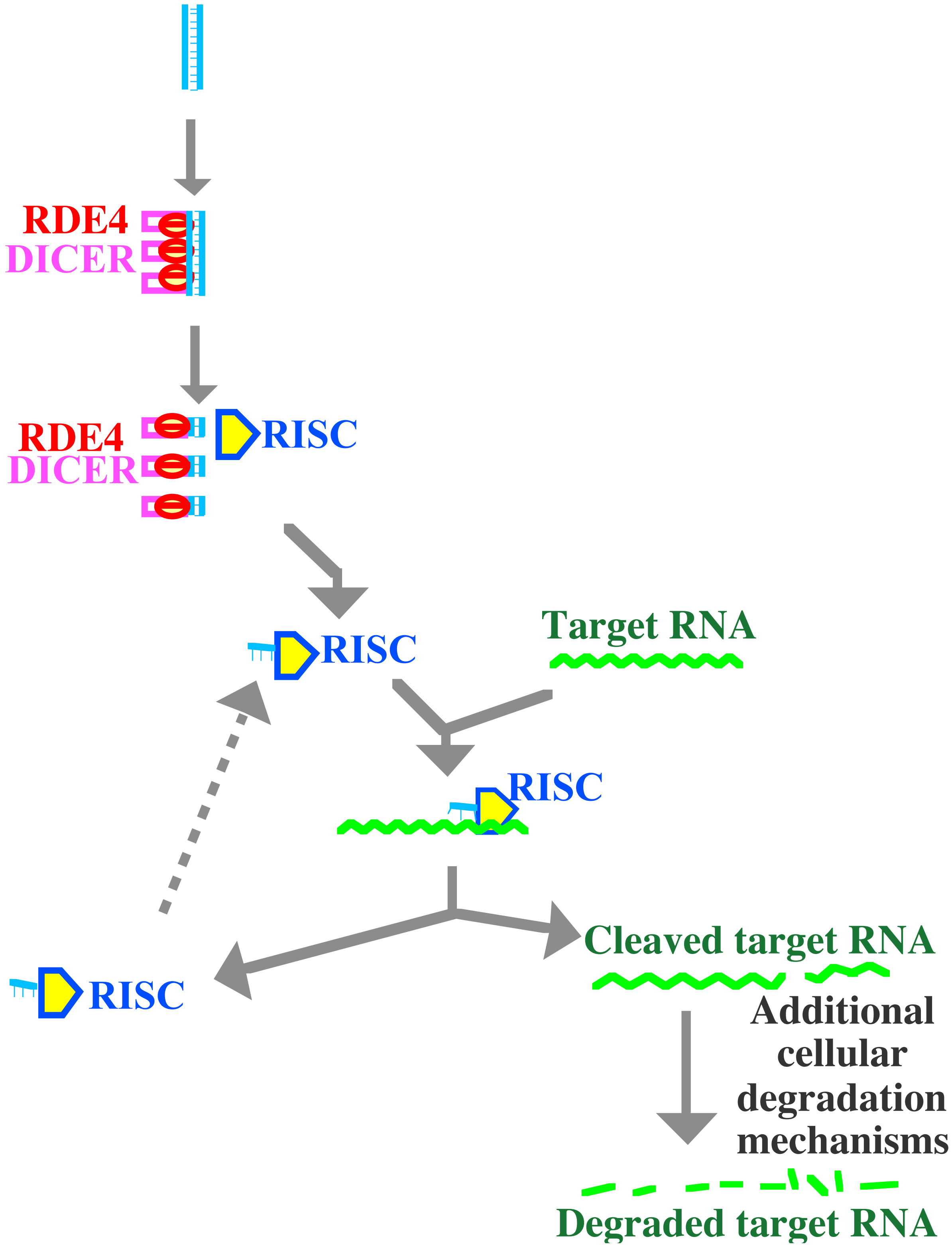
- A population of ~25nt RNAs associated with PTGS
- Related to PTGS?
- Unrelated to PTGS?
- Degraded Target?
- Degraded Trigger?
- Products of RNA-dependent RNA polymerase?

RNaseIII type activity "Dicer" (Zamore et al., 2000, Bernstein; Elbashir et al. 2001)

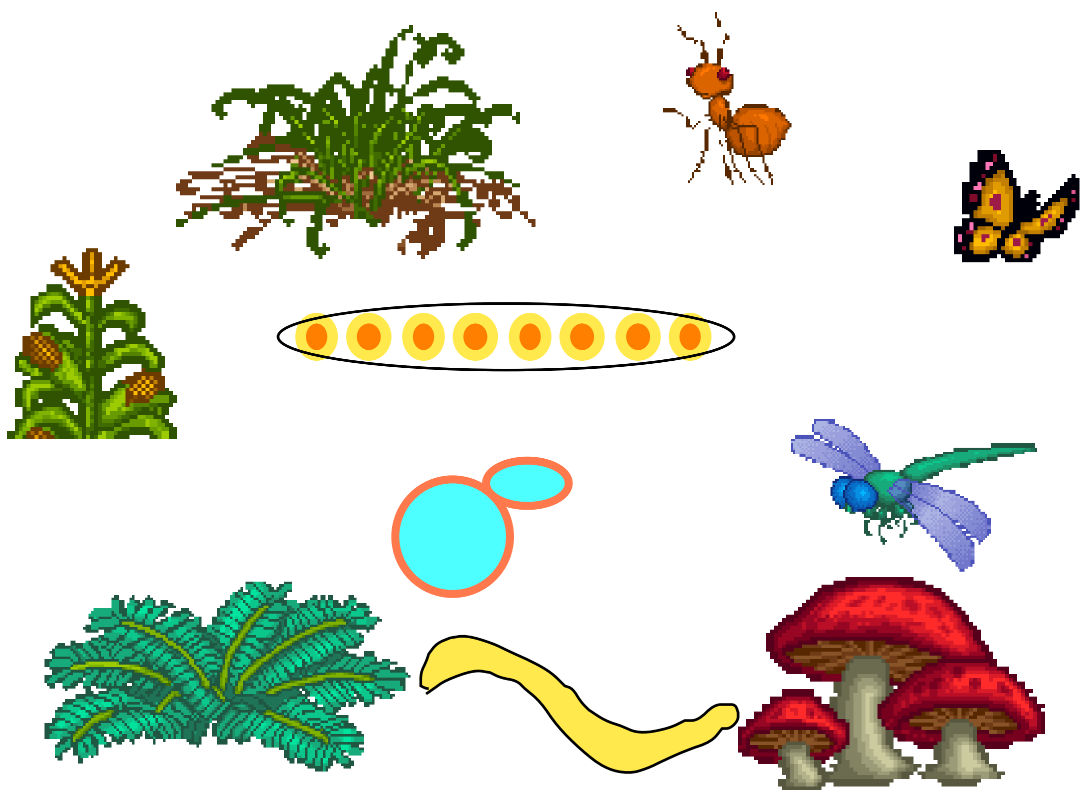
- Trigger dsRNA cleaved every 21-23bp to make ds short RNAs
- Specific structure "siRNA": 5'P + 3'OH, 3' 2 base overhang

siRNA/Protein complex "RISC" (Hammond et al., 2001; Nykanen et al., 2001)

- ATP-dependent RNAse activity copurifies with short RNAs
- Fly RISC complex incorporates RDE1 family member AGO2



RDE4
DICER
RDE4
DICER
RISC
RISC
Target RNA
RISC
RISC
Cleaved target RNA
Additional cellular degradation mechanisms
Degraded target RNA



RNAi versus Our "Traditional" Immunity

Specificity: How to find a "needle in a haystack"?

How to react to diverse pathogens without self-attack?

- Pre-existing "innate" repertoire
- Infection-specific "acquired" repertoire

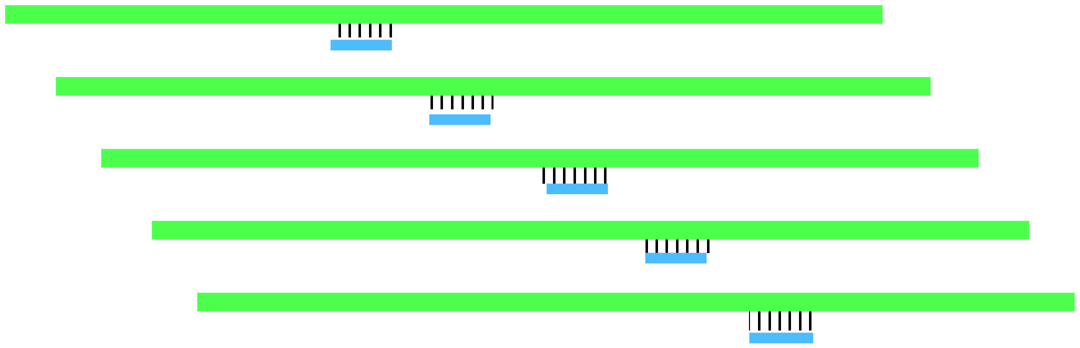
How to focus on small pieces of each pathogen?

How to mount a systemwide response?

How to conserve resources for useful responses?

- by Stabilizing "useful responses"
- by Amplifying "useful responses"
- by Recycling "useful responses"
- by co-dependence of different immune responses

How to remember where you've been?



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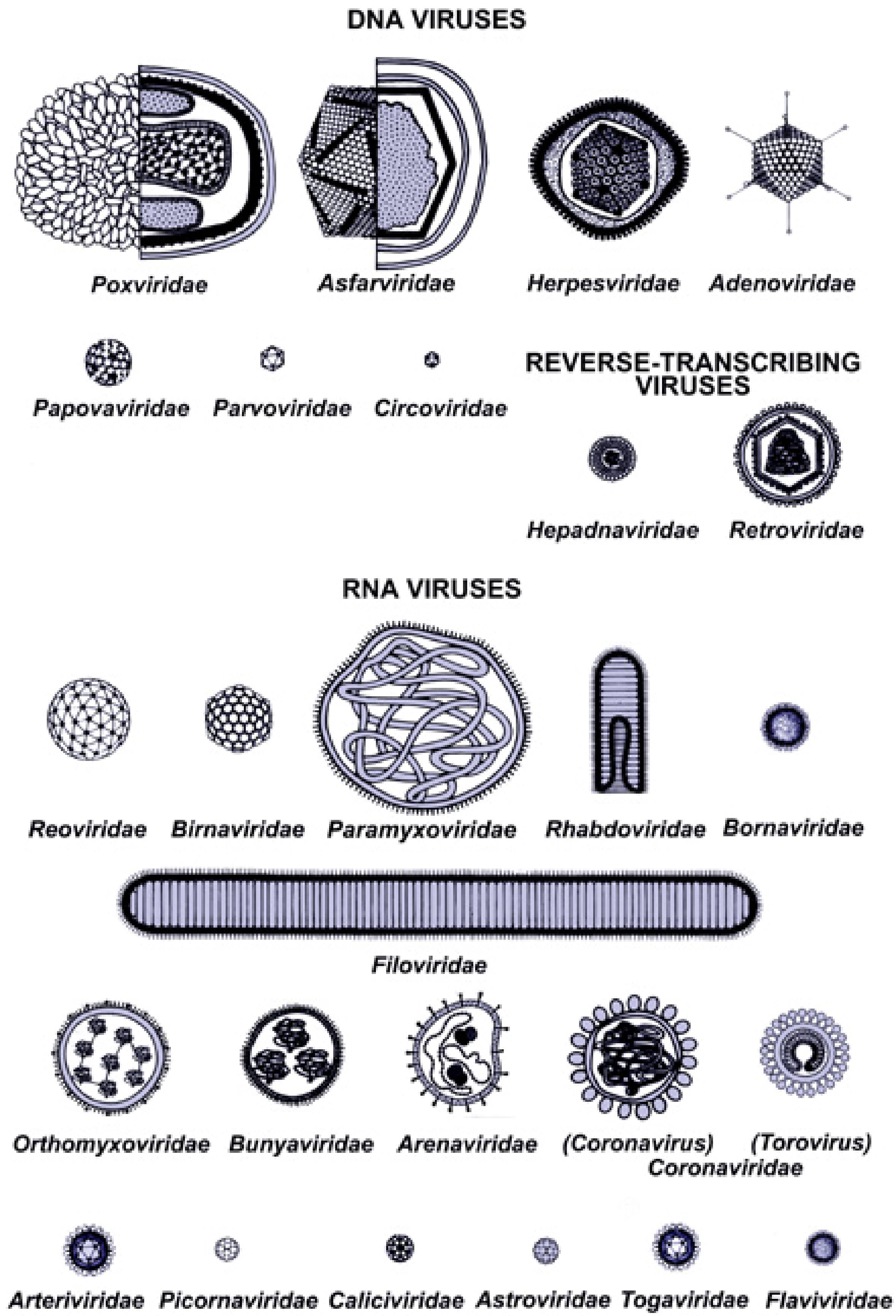
DNA VIRUSES
Poxviridae
Asfarviridae
Herpesviridae
Adenoviridae
Papovaviridae
Parvoviridae
Circoviridae
REVERSE-TRANSCRIBING VIRUSES
Hepadnaviridae
Retroviridae
RNA VIRUSES
Reoviridae
Birnaviridae
Paramyxoviridae
Rhabdoviridae
Bornaviridae
Filoviridae
Orthomyxoviridae
Bunyaviridae
Arenaviridae
(Coronavirus)
(Torovirus)
Coronaviridae
Arteriviridae
Picornaviridae
Caliciviridae
Astroviridae
Togaviridae
Flaviviridae

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Why degrade the RNA trigger to short dsRNAs?

Potency: More trigger molecules to do RNAi

Dissemination: Smaller molecule to distribute

Immune Effect: Reduce risk of helping a virus

Other fragmentation mechanisms in immunity

Protein fragmentation in vertebrate immune system antigen presentation

Program fragmentation in antivral software

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Cellular RNA-directed RNA polymerases (cRdRPs)

Activity in many plants (First detected: Cabbage, 1971: Astier-Mann et al.)

Initial debate: cellular enzyme or viral "contaminant"?

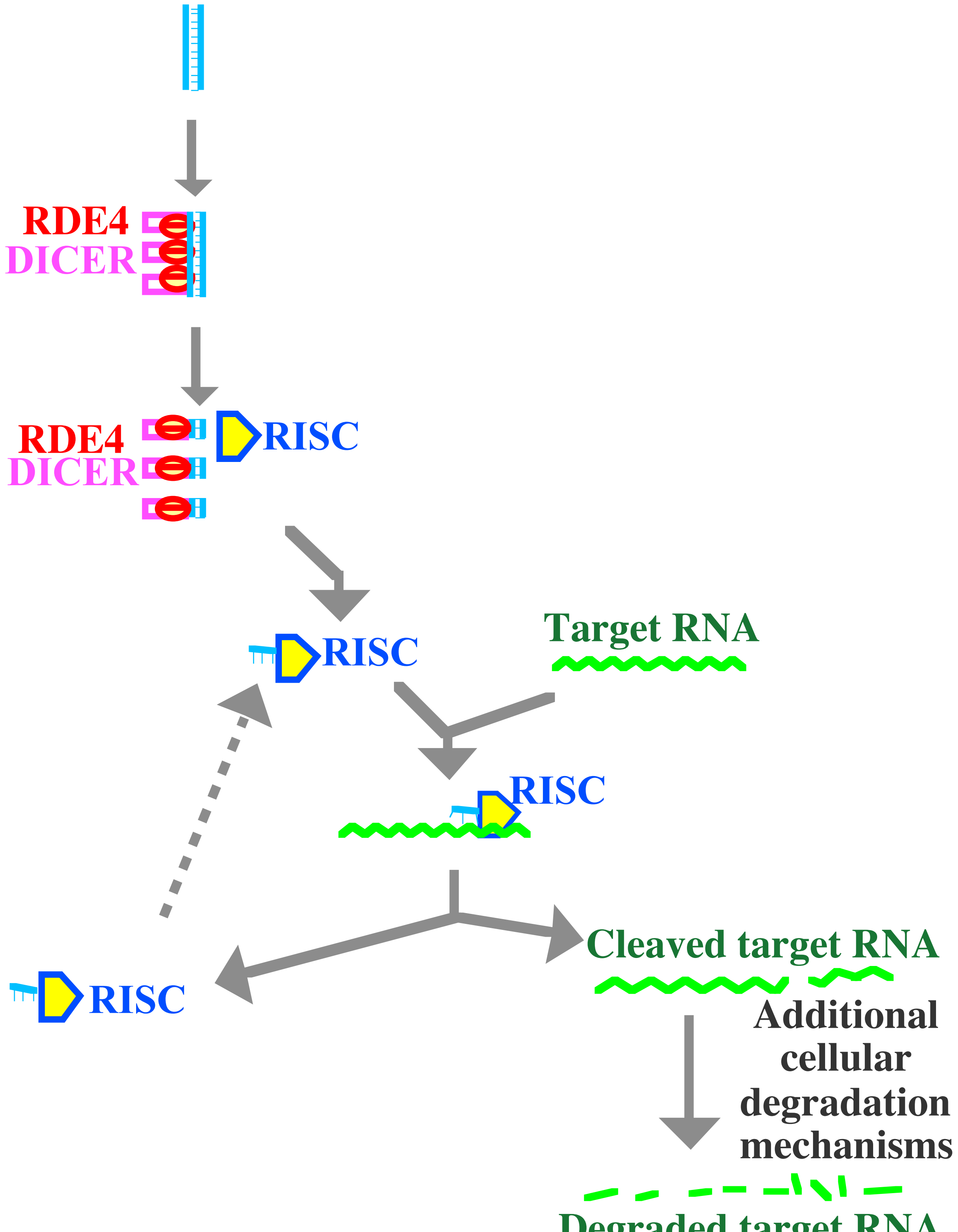
One tomato enzyme purified, cloned (Schiebel et al. 1993, 1998)

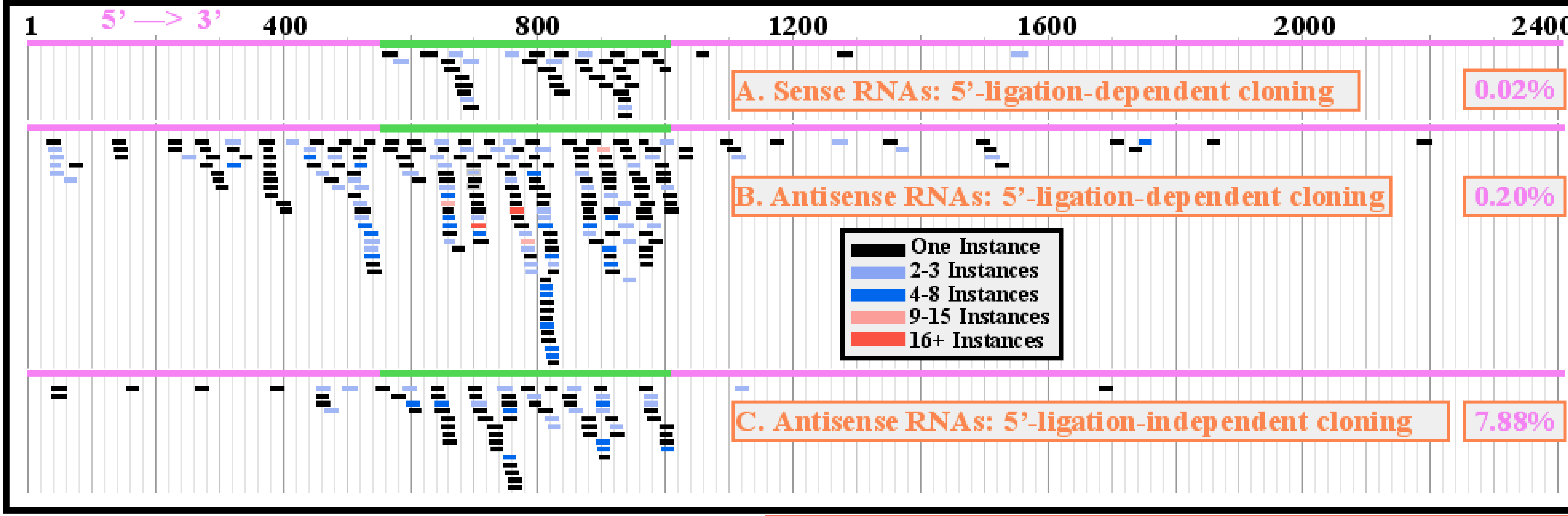
Homologs required for RNA-triggered silencing

- **Neurospora qde-1** (Cogoni, Macino, 1999)
- **C. elegans ego-1/rrf-1** (Smardon et. al. 2000; Conte & Mello; Simmer, Plasterk)
- **Arabidopsis sgs2/sde1** (Mourrain et al; Dalmay et al. 2001)

Other Homologs: S. Pombe, Many plants

No homologs found in S. Cerevisiae, Drosophila, Vertebrates


RDE4
DICER
RDE4
DICER
RISC
RISC
Target RNA
RISC
RISC
Cleaved target RNA
Additional cellular degradation mechanisms
Degraded target RNA

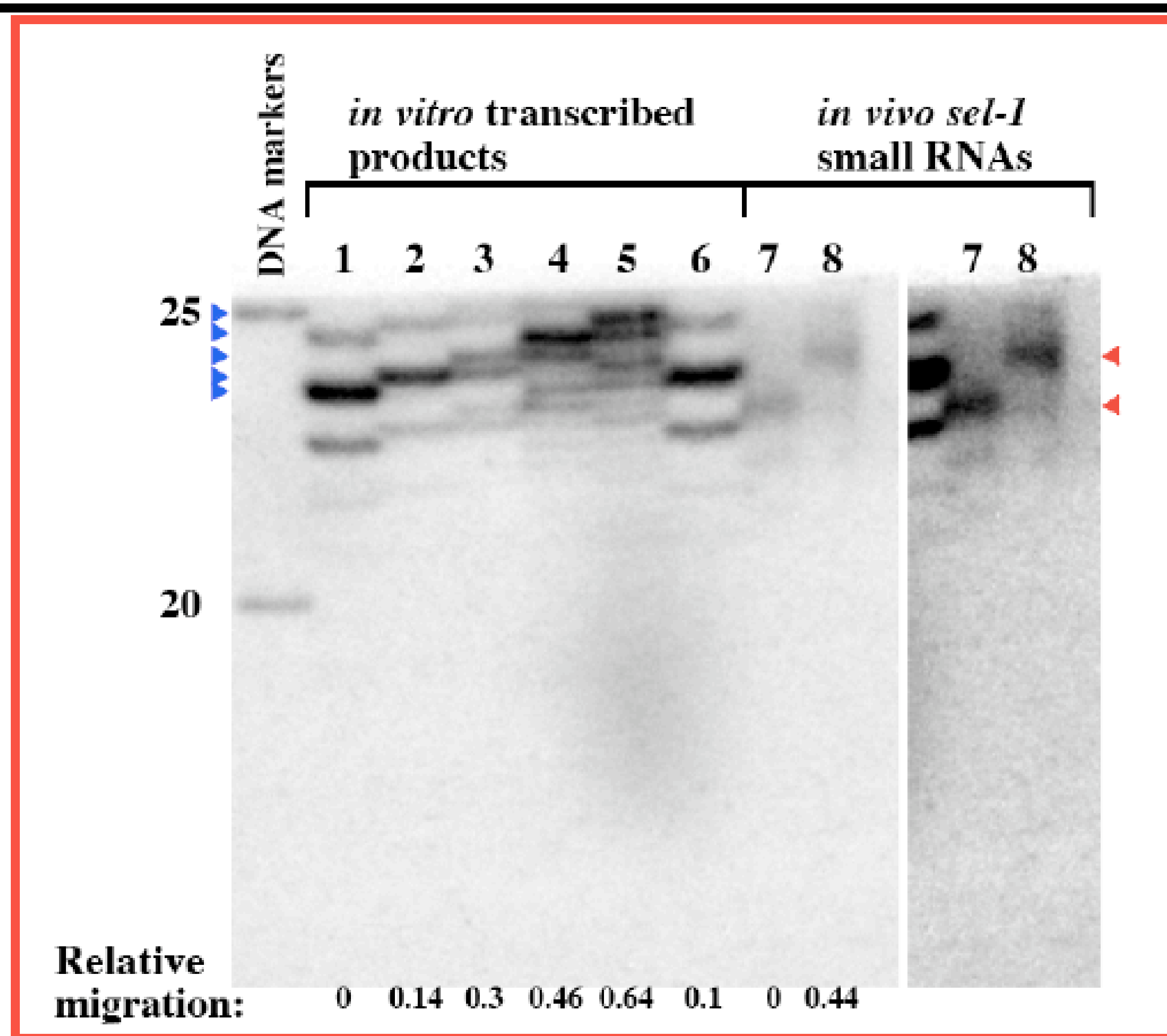


siRNA distributions:

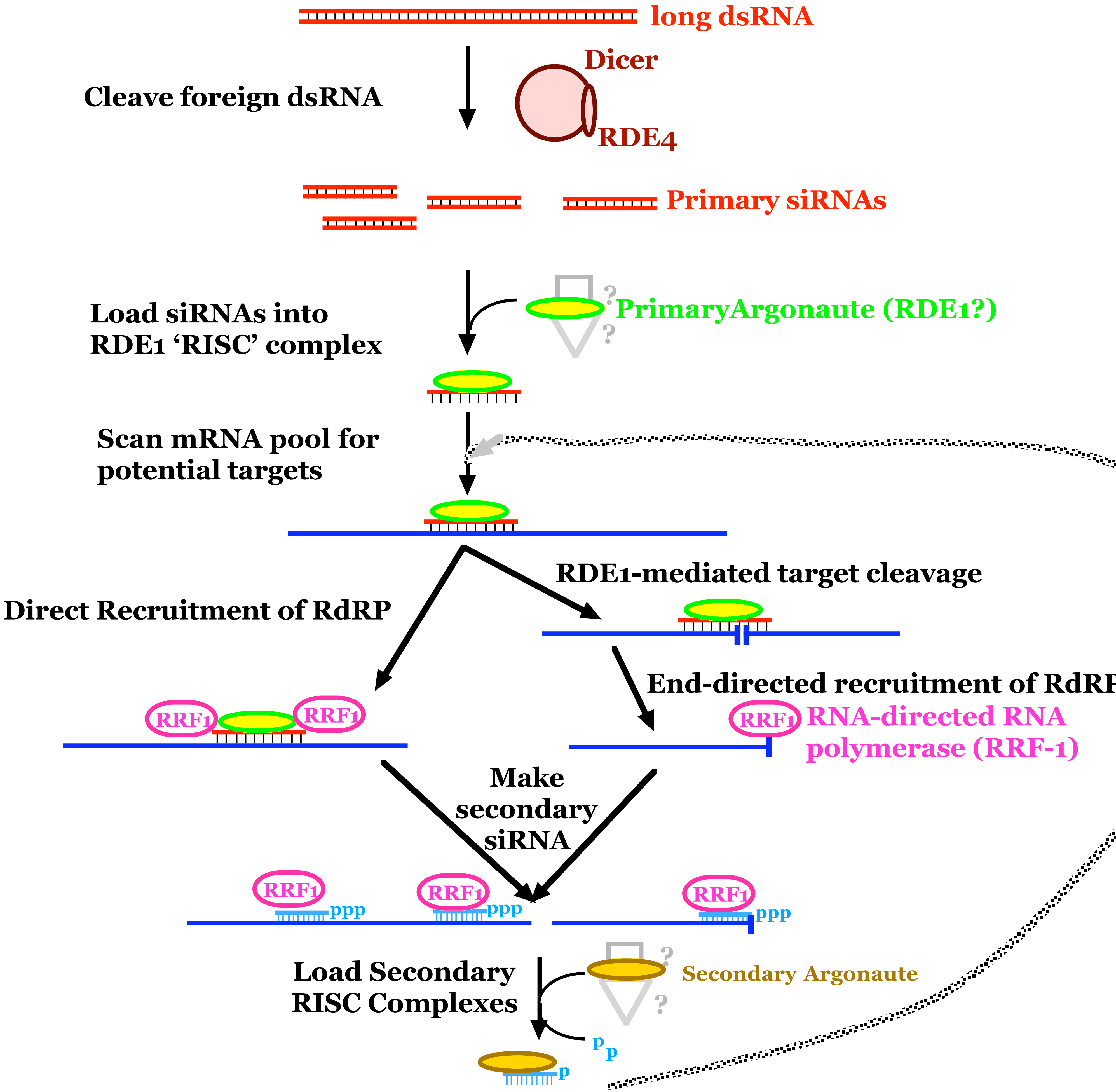
- antisense strand bias
- bidirectional transitivity

siRNA terminus structure:

- 5' triphosphate
- 3' OH



Pak and Fire, Science, in press


long dsRNA
Cleave foreign dsRNA
Dicer
RDE4
Primary siRNAs
Load siRNAs into
RDE1 'RISC' complex
PrimaryArgonaute (RDE1?)
Scan mRNA pool for
potential targets
RDE1-mediated target cleavage
Direct Recruitment of RdRP
End-directed recruitment of RdRP
RRF1
RNA-directed RNA
polymerase (RRF-1)
Make
secondary
siRNA
ppp
Load Secondary
RISC Complexes
Secondary Argonaute
p

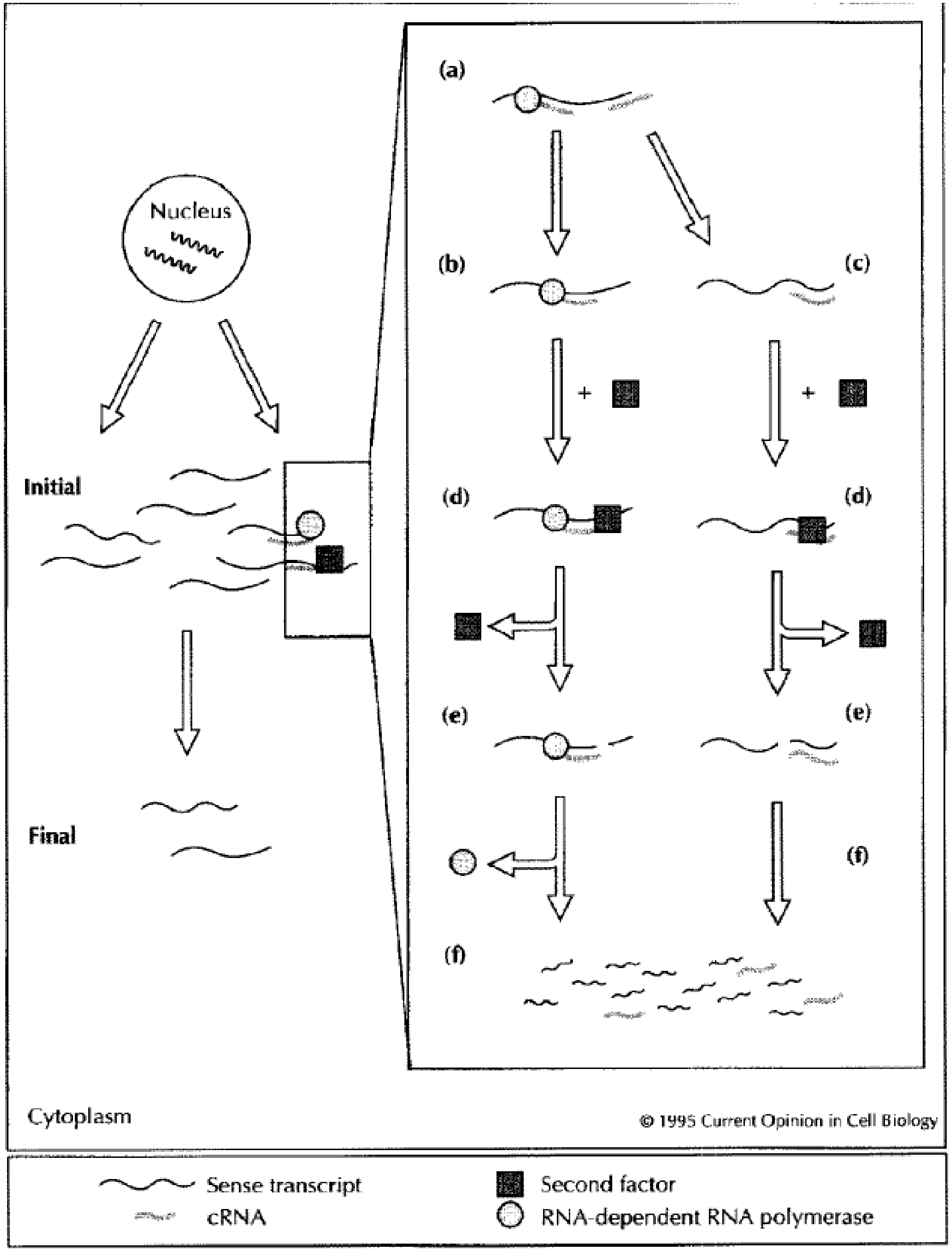
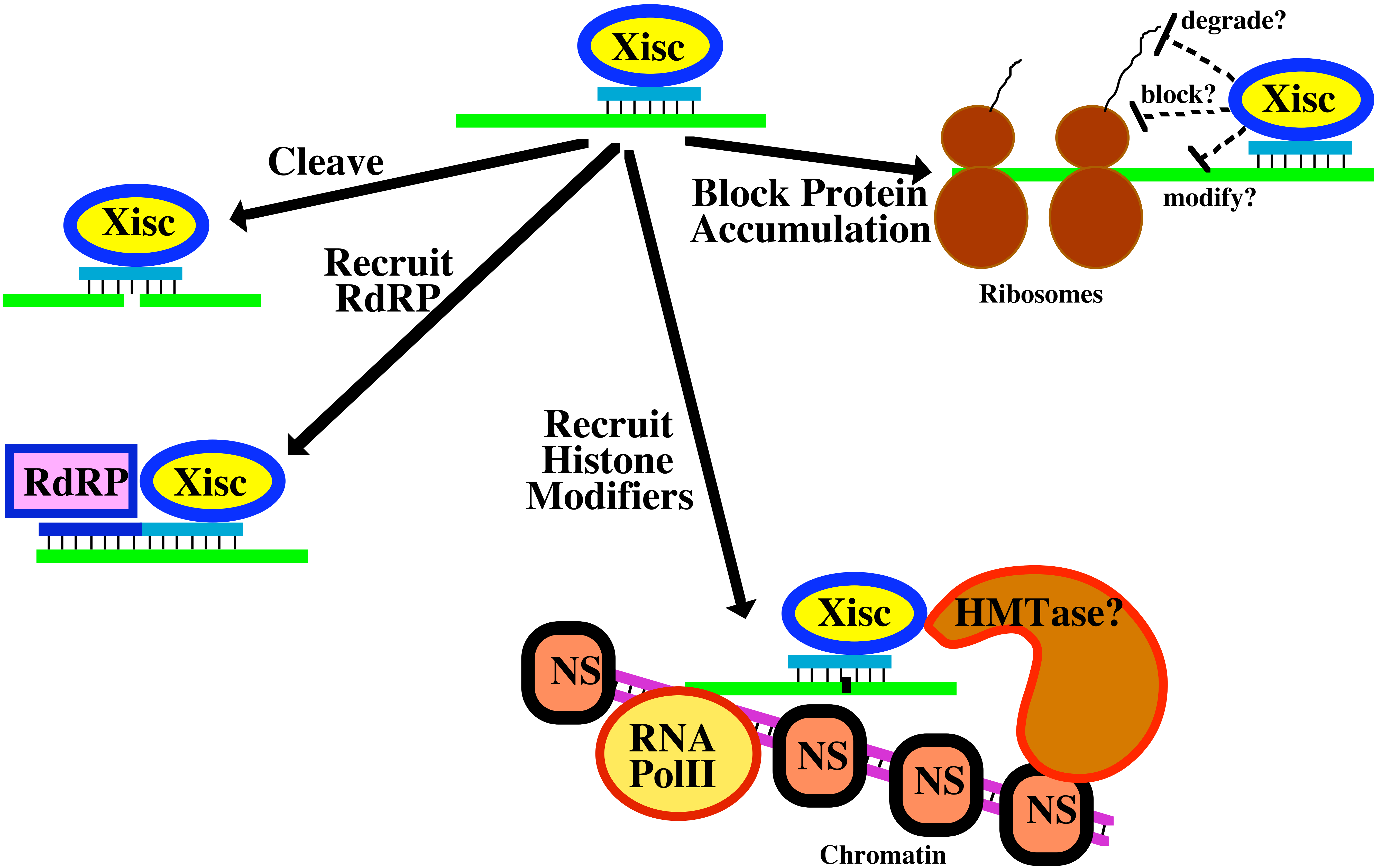


Fig. 1. A schematic portrayal of an RNA-driven mechanism underlying sense suppression. The model suggests a cytoplasmic system is present which surveys RNA species. Aberrant or overexpressed RNAs are the normal targets for such a system. With selected transgenic organisms, however, sense transcripts (or antisense transcripts — see Fig. 2) are present at an elevated level (initial) and the cell attempts to downregulate transcripts to the steady-state level (final). The entire process would be sequence-driven by small RNA molecules and the system is unable to differentiate between an identical nucleotide sequence contained in an RNA transcript derived from a exogenous or endogenous nuclear gene or contained in a viral genome. The size of these RNAs must be sufficiently small to allow binding and dissociation (<20 nt?) but must be long enough (>10 nt?) to account for the observed specificity. In sense suppression, the transgene-derived sense RNAs are available for an RNA-dependent RNA polymerase activity to bind and copy **(a)** segments of the transgene transcript to make small complementary RNAs (cRNAs). The cRNAs can function in *cis* or *trans* fashion. In *cis* **(b)**, the cRNAs are made and remain bound to the target RNA; their length is not important. Alternatively, some of the smaller cRNAs may dissociate from the template (i.e. target) after synthesis and rebind in a *trans* fashion **(c)** to another target mRNA molecule. The cRNA–target mRNA complexes are recognized by a second factor **(d)** and a nucleolytic cleavage event takes place **(e)**. Once an internal cleavage occurs, other exonucleases would continue the degradation process **(f)**.

From: " Transgenes and Gene Suppression: Telling us something new? "
W. Dougherty and D. Parks. Current Opinion in Cell Biology, 1995 (7) 399-405


Xisc
Cleave
Xisc
Recruit
RdRP
RdRP
Xisc
Block Protein
Accumulation
degrade?
block?
Xisc
modify?
Ribosomes
Recruit
Histone
Modifiers
Xisc
HMTase?
NS
RNA
PolII
NS
NS
NS
Chromatin

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Some open questions on RNAi and Immunity

Does RNAi in animals function as an anti-pathogen response?

What physiological factors modulate RNAi to allow maximal response to pathogen RNAs?

Do small endogenous RNAs act as a layer of innate immunity?

Can RNAi be manipulated to provide protective immunization?

Are RNAi-related mechanisms responsible for a subset of the gene silencing events that occur during tumorogenesis?

The Carnegie Institution RNAi Crew and Collaborators
Jamie Fleenor (Now @ DHS)
Steve Kostas (Now @ ICTY)
Mary Montgomery (Now @ Macalester College)
Susan Parrish (Now @ NIH)
Lisa Timmons (Now @ Kansas University)
Susan White Harrison (Now @ University of Kentucky)
SiQun Xu (Now @ Washington University)
Natasha Caplen, Rick Morgan (NIH), Farhad Imani (JHU)
Craig Mello, Sam Driver, Hiroaki Tabara (UMass)
Titia Sijen, Femke Simmer, Karen Thijsen, Ronald Plasterk (Utrecht)

The 2006 Fire Lab- Stanford University School of Medicine
Rosa Alcazar*
Daniel Blanchard*
Scott Boyd
Jonathan Gent
Steve Johnson
Chaya Krishna
Weng-Onn Lui
Jay Maniar
Cecilia Mello
Julia Pak
Poornima Parmeswaran
Kyle Sha*
Fred Tan*

Supporting Institutions:
National Institutes of Health- NIGMS/NICHD
Carnegie Institution of Washington
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