**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 viscerotrophic yellow fever virus in Macacus rhesus. American Journal of Tropical Medicine, 15, 675-680


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,
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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
HOSTS AND SYMPTOMS OF RING SPOT, A VIRUS DISEASE OF PLANTS

By S. A. Wingard
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Fig. 7.—Turkish tobacco plant 22 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.
Promoter $\subseteq$ geneX

Make transgenic worms

geneX

Antisense Transcripts

Interference

(Development 113:503 [1991])
Make transgeneic worms

Promoter

\textit{geneX}

\textbf{SENSE} Transcripts

Also Interference!

\textit{(Development 113:503 [1991])}
In Vitro
Promoter

Make RNA in vitro

geneX
Antisense RNA

Inject worm gonad

Interference!

(Guo and Kemphues, 1995)
In Vitro Promoter

Make RNA in vitro

geneX

SENSE RNA

Inject worm gonad

Also Interference!

(Guo and Kemphues, 1995)
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

![Graph showing quantitative assays for silencing: *unc-22*.](image)
DNA \rightarrow hnRNA \rightarrow mRNA \rightarrow Protein \rightarrow Function

Degradation
mex-3 mRNA

control  +dsRNA

dsRNA in situ probe

mex-3 mRNA
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
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S. Driver (UMass): RNA into body cavity --> gonadal affect
Levels of (im)precision in RNA delivery

S. Guo (Cornell): RNA into gonad \(\rightarrow\) gonadal affect
S. Driver (UMass): RNA into body cavity \(\rightarrow\) gonadal affect
L. Timmons (Carnegie): Feed [dsRNA+ bacteria] to worms

\[\text{Eating control cells} \quad \text{Eating GFP dsRNA} \]

\textbf{let-858::gfp}

\textbf{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

Incoming Sense

Incoming Antisense

Target mRNA
A mutational Screen for trans-acting factors involved in RNAi

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
Target RNA

Additional cellular degradation mechanisms

Cleaved target RNA

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?
RNAi versus Our "Traditional" Immunity

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How to remember where you've been?
DNA VIRUSES

Poxviridae
Asfarviridae
Herpesviridae
Adenoviridae

REVERSE-TRANScribing VIRUSES

Papovaviridae
Paroviridae
Circoviridae

Hepadnaviridae
Retroviridae

RNA VIRUSES

Reoviridae
Birnaviridae
Paramyxoviridae
Rhabdoviridae
Bornaviridae

Filoviridae

Orthomyxoviridae
Bunyaviridae
Arenaviridae
(Coronavirus)
Coronaviridae
(Torovirus)

Arteriviridae
Picornaviridae
Caliciviridae
Astroviridae
Togaviridae
Flaviviridae
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How to remember where you've been?
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 antiviral 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
Target RNA

Additional cellular degradation mechanisms

Cleaved target RNA

Degraded target RNA
siRNA distributions:
- antisense strand bias
- bidirectional transitivity

siRNA terminus structure:
- 5’ triphosphate
- 3’ OH

Pak and Fire, Science, in press
Load siRNAs into RDE1 ‘RISC’ complex

Scan mRNA pool for potential targets

Direct Recruitment of RdRP

End-directed recruitment of RdRP

Make secondary siRNA

Load Secondary RISC Complexes
From: “Transgenes and Gene Suppression: Telling us something new?”
Cleave RdRP Recruit RdRP Recruit Histone Modifiers Block Protein Accumulation Xisc Xisc Xisc Xisc Xisc Ribosomes degrade? block? modify? Ribosomes Xisc HMTase? RNA PolII NS NS NS NS Chromatin
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How to remember where you've been?
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?
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