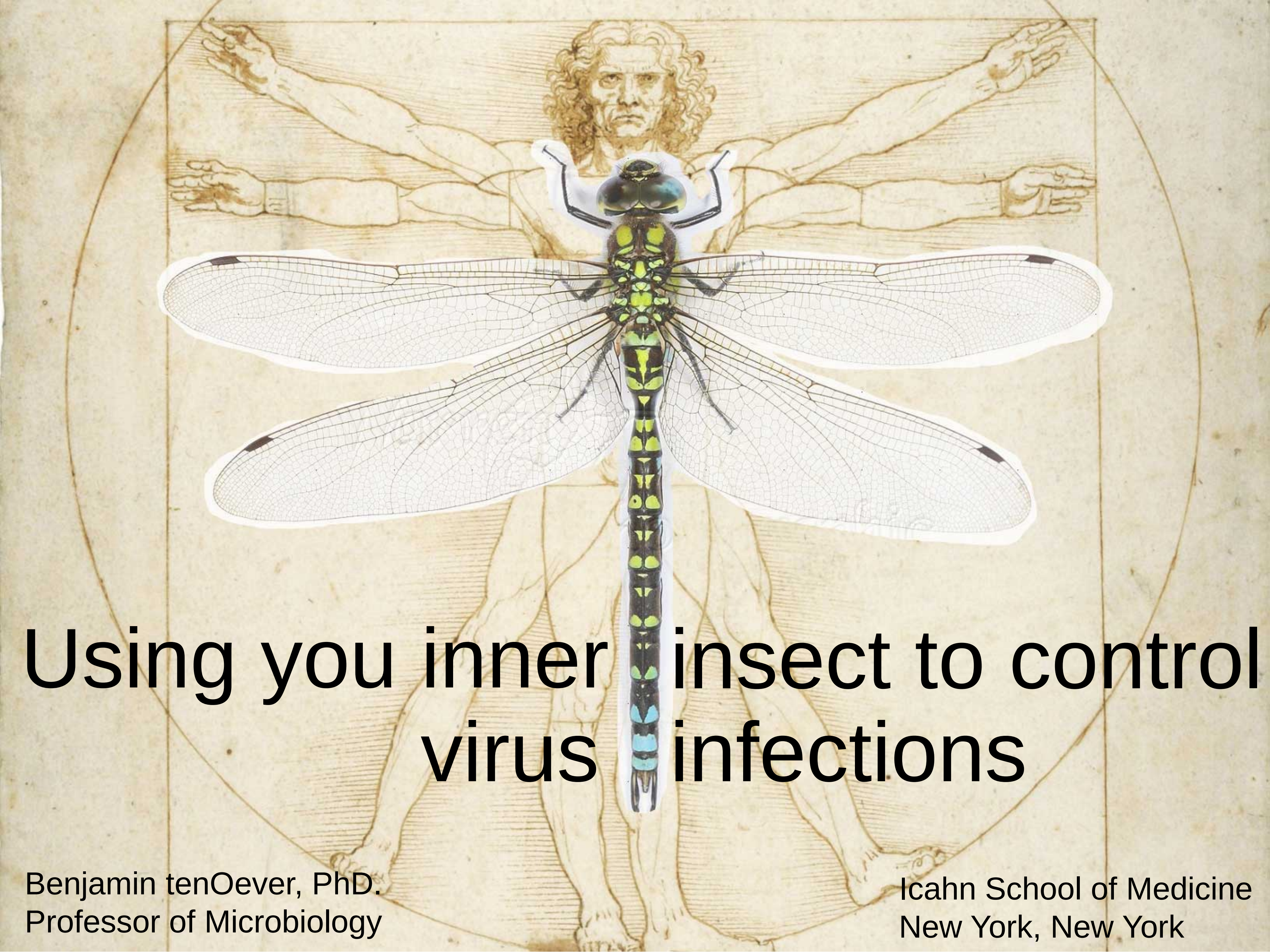
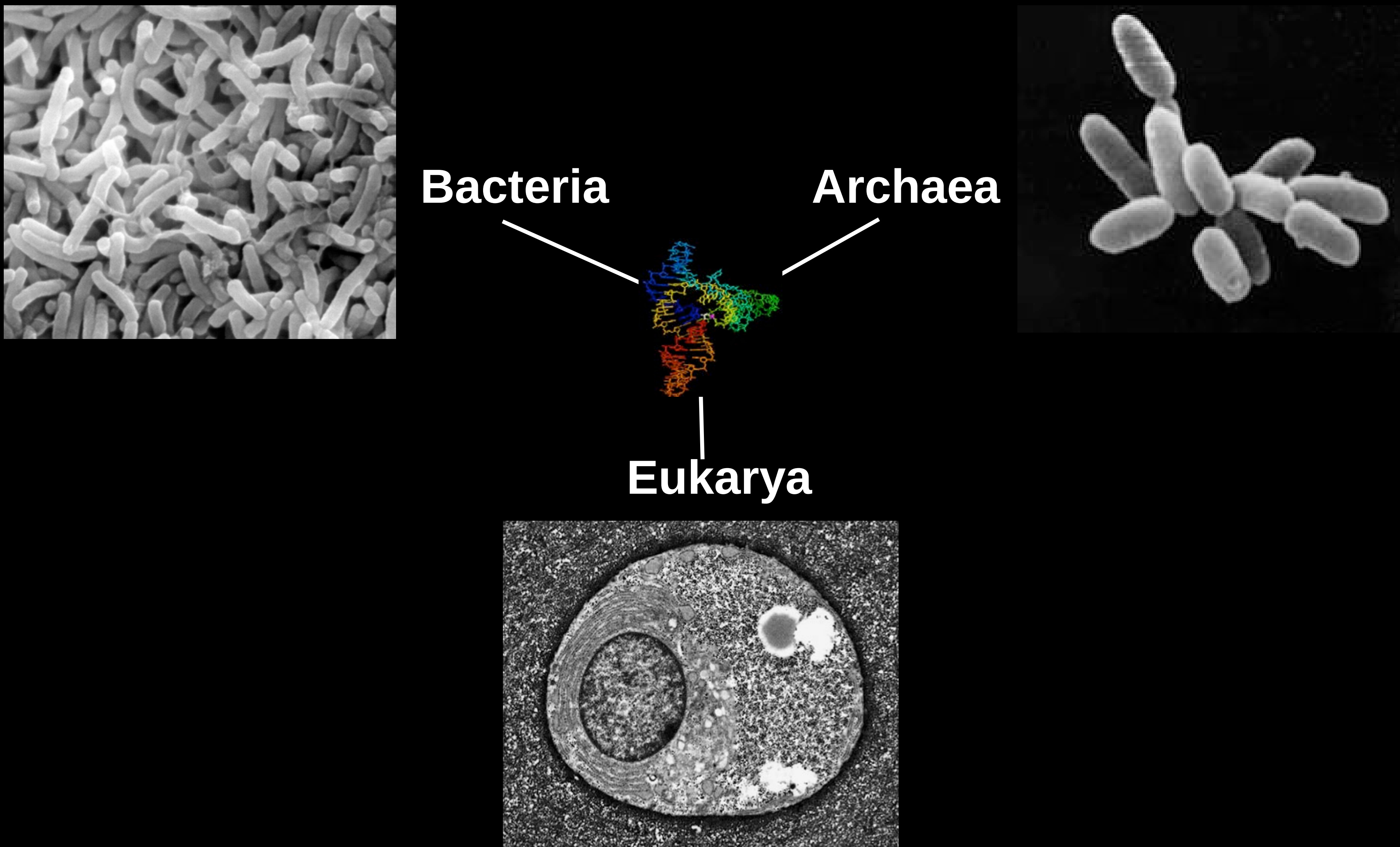


Using you inner insect to control virus infections

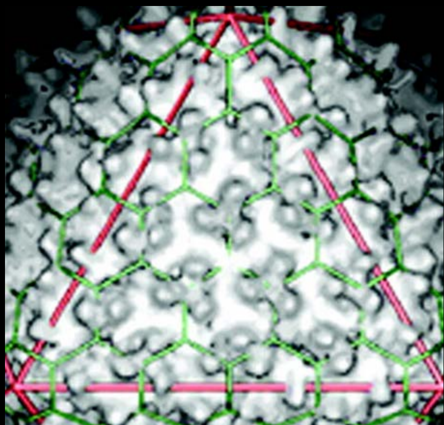
Benjamin tenOever, PhD.
Professor of Microbiology

Icahn School of Medicine
New York, New York

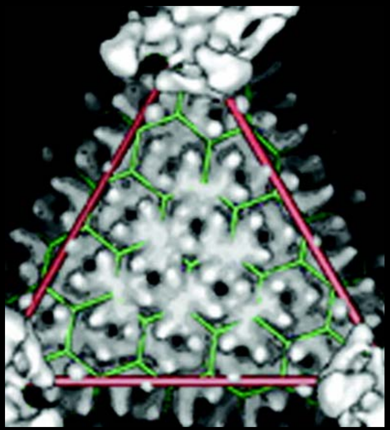
The evolution of life



The evolution of life and viruses



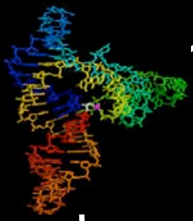
T4 Phage



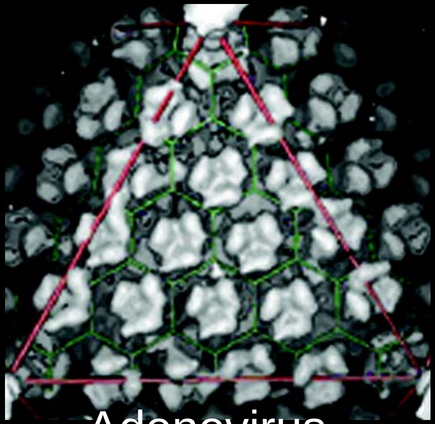
Sulfolobus virus

Bacteria

Archaea

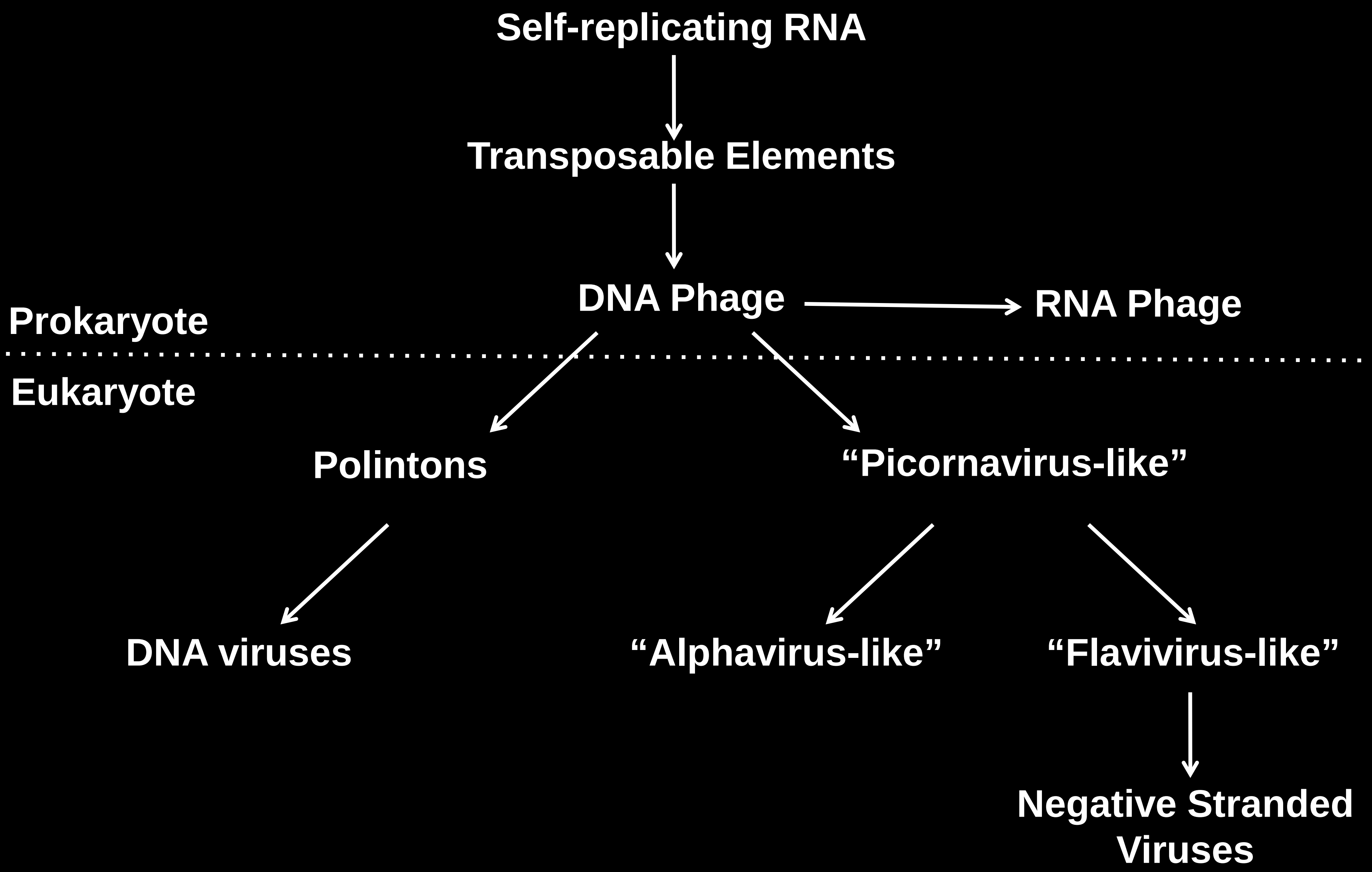


Eukarya

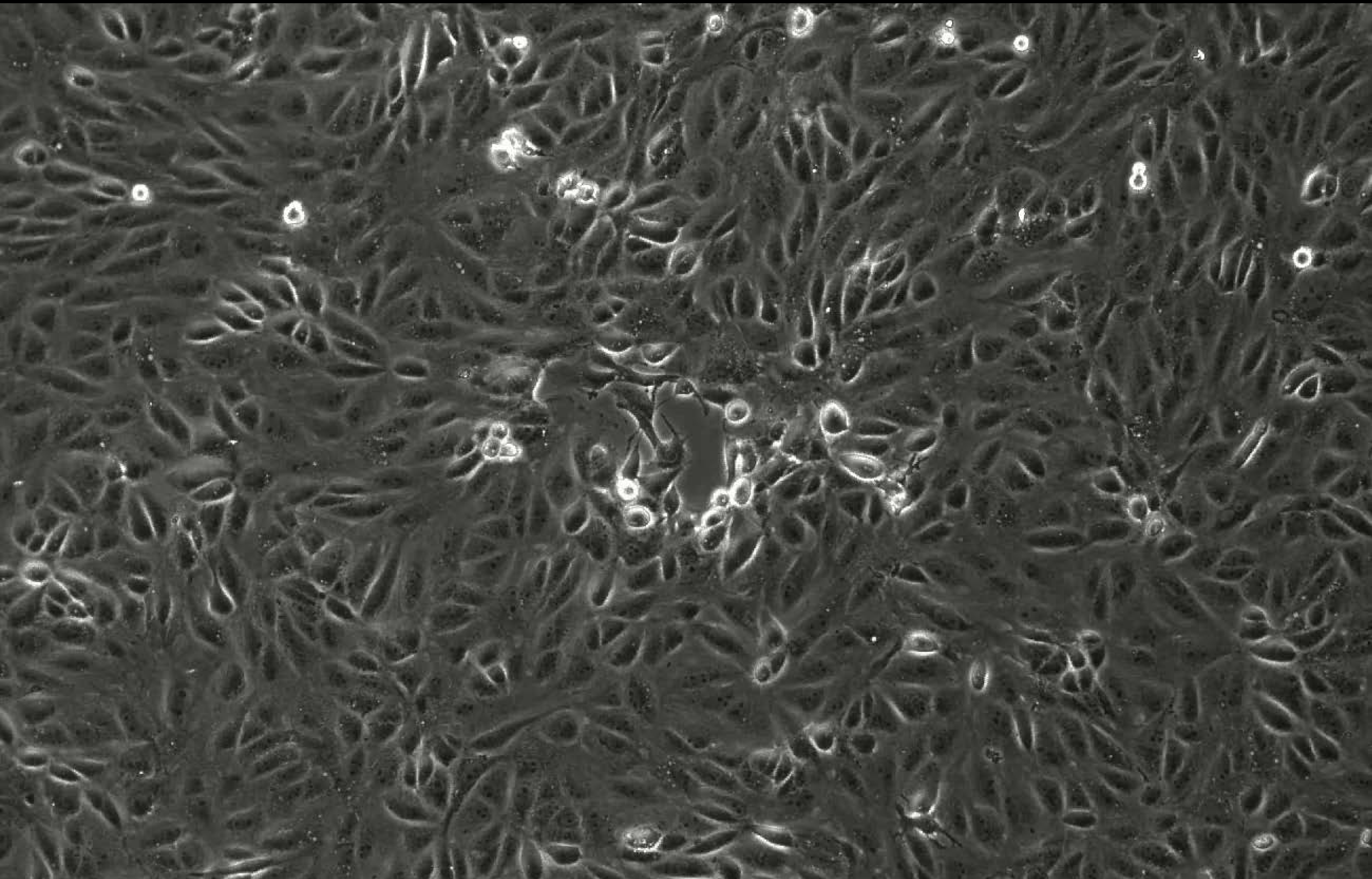


Adenovirus

The evolution of viruses

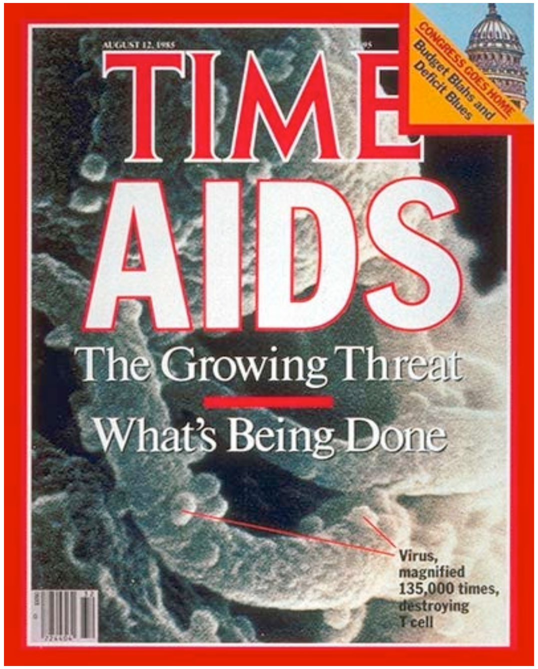


Viruses impose significant selective pressure on their host



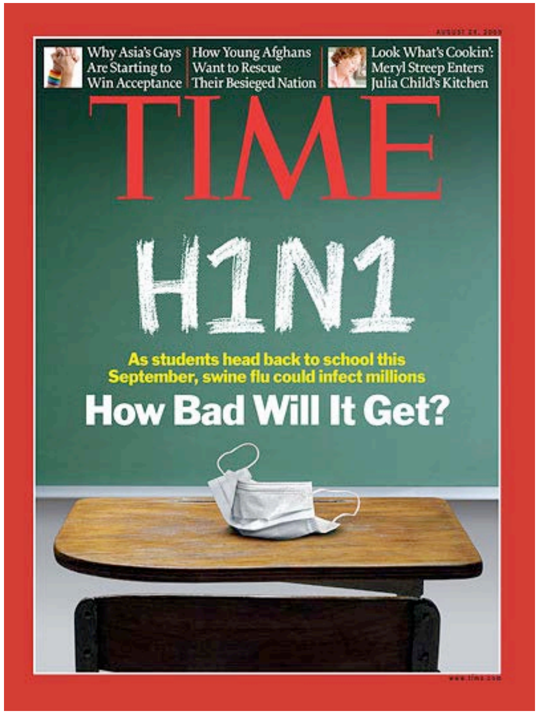






AUGUST 12, 1985
TIME
CONGRESS GOES HOME
Budget Blahs and Deficit Blues
AIDS
The Growing Threat
What's Being Done
Virus, magnified 135,000 times, destroying T cell


MAY 5, 2003
TIME
THE TRUTH ABOUT SARS
WHY the virus spreads
China's COVER-UP
How SCARED should you be?


FEBRUARY 9, 2004
TIME
BIRD FLU
Is Asia hatching the next human pandemic?


Why Asia's Gays Are Starting to Win Acceptance
How Young Afghans Want to Rescue Their Besieged Nation
Look What's Cookin': Meryl Streep Enters Julia Child's Kitchen
TIME
H1N1
As students head back to school this September, swine flu could infect millions
How Bad Will It Get?


TIME
STOPPING EBOLA
THE RISKS AND REALITIES OF THE OUTBREAK


TIME
The Zika Virus
It's a mysterious illness with devastating effects.
Is the next public health crisis in your backyard?

The continual threat of emerging pathogens demands the capacity to study them

There is a growing concern and mistrust of the public concerning this type of research

Chilling new details on cold-storage smallpox

Hoai-Tran Bui and Alison Young, USA TODAY 6:58 p.m. EDT July 17, 2014

HEALTH NEWS | Wed Feb 15, 2012 | 11:53am EST

How secure are labs handling world's deadliest pathogens?

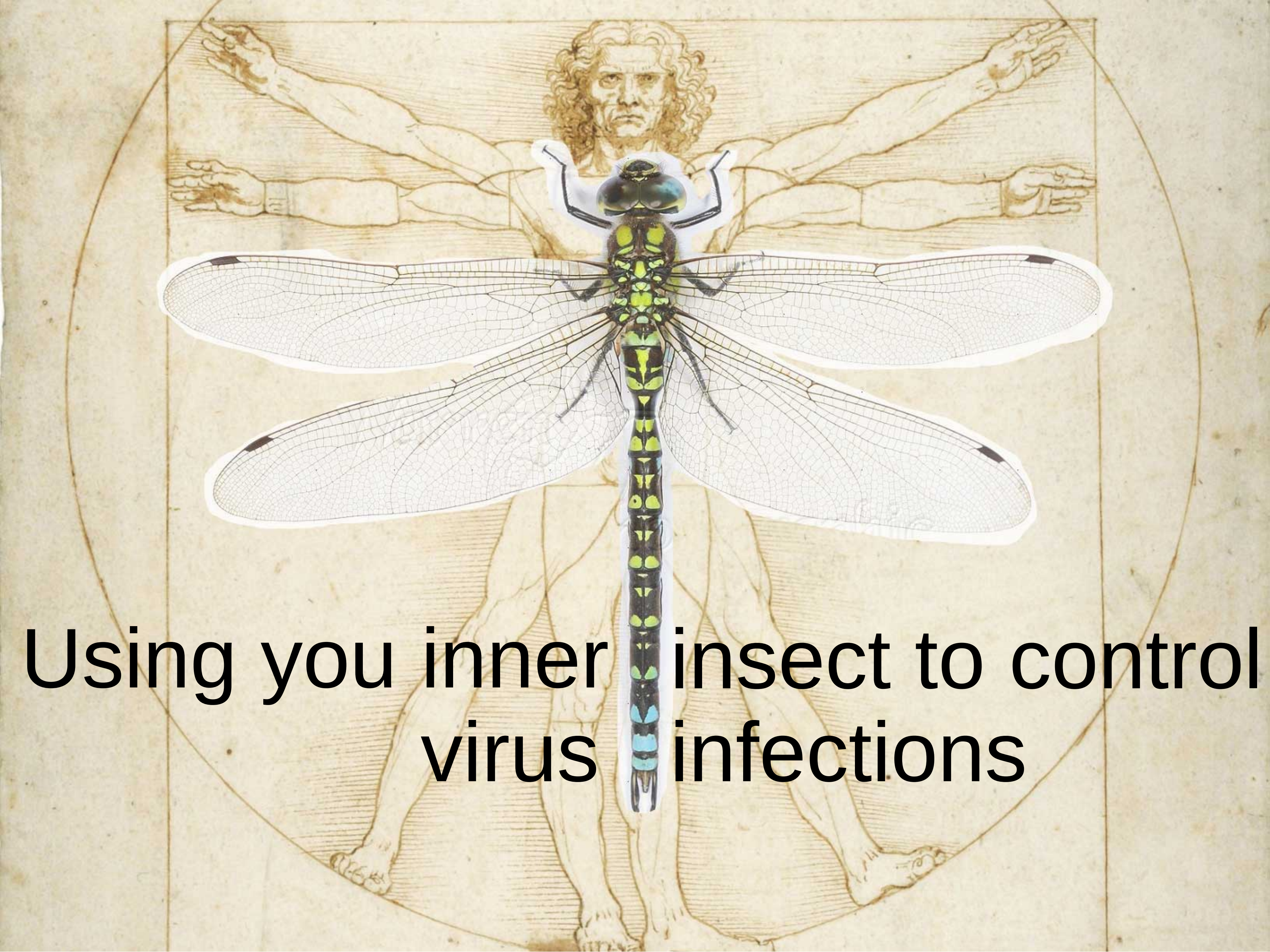
ANNALS OF MEDICINE MARCH 12, 2012 ISSUE

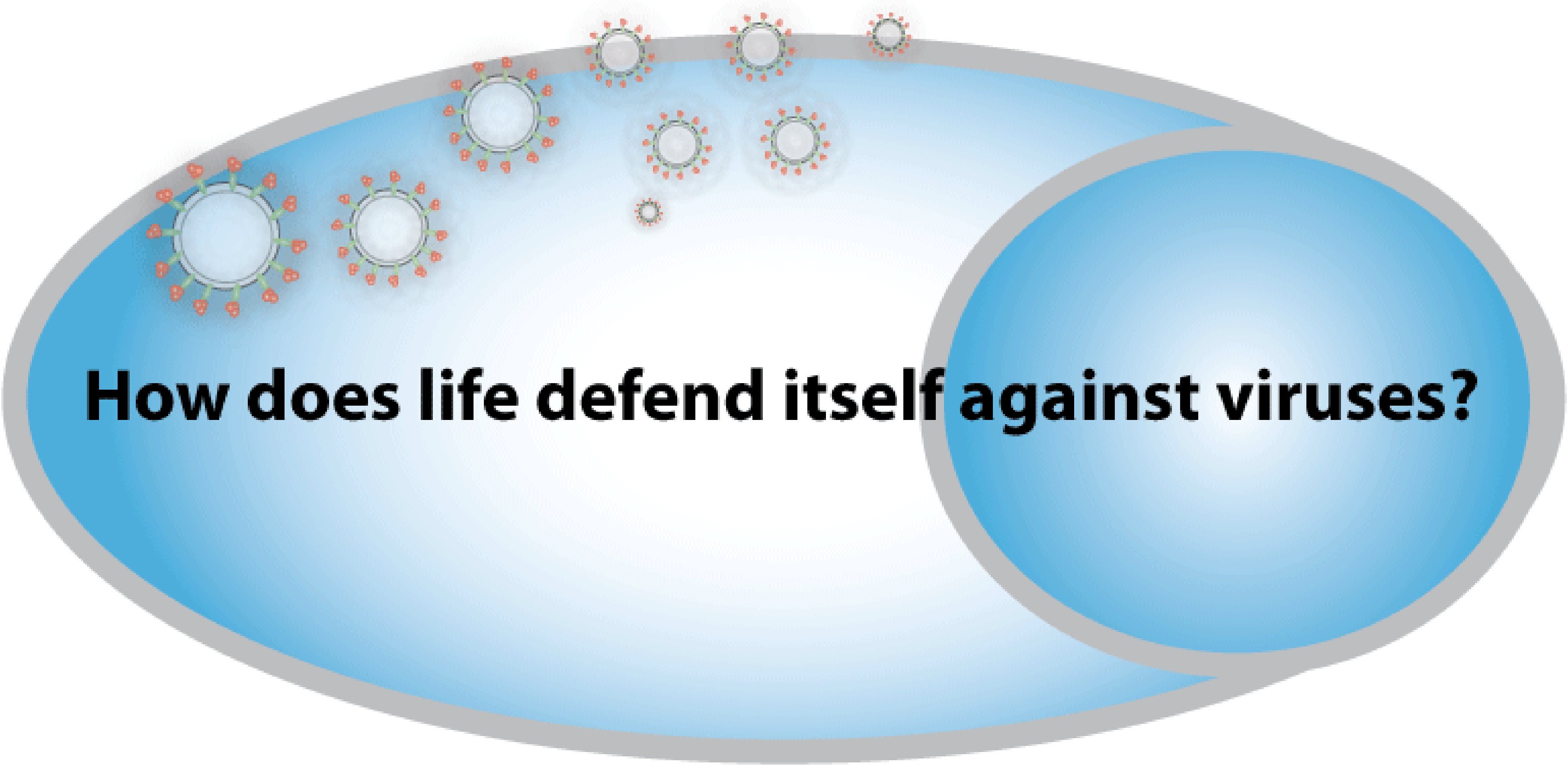
THE DEADLIEST VIRUS

Did a scientist put millions of lives at risk—and was he right to do it?

By Michael Specter


Can we do more to mitigate the risk
of studying deadly viruses?


Using you inner insect to control virus infections


How does life defend itself against viruses?

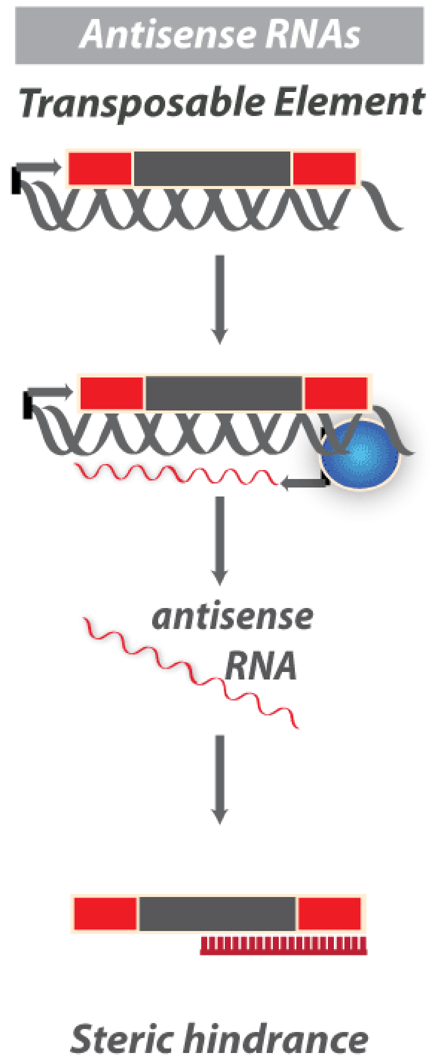
The evolution of viruses

Self-replicating RNA

↓

Transposable Elements

Antiviral defenses of prokaryotes – DNA targeting



The evolution of viruses

Self-replicating RNA

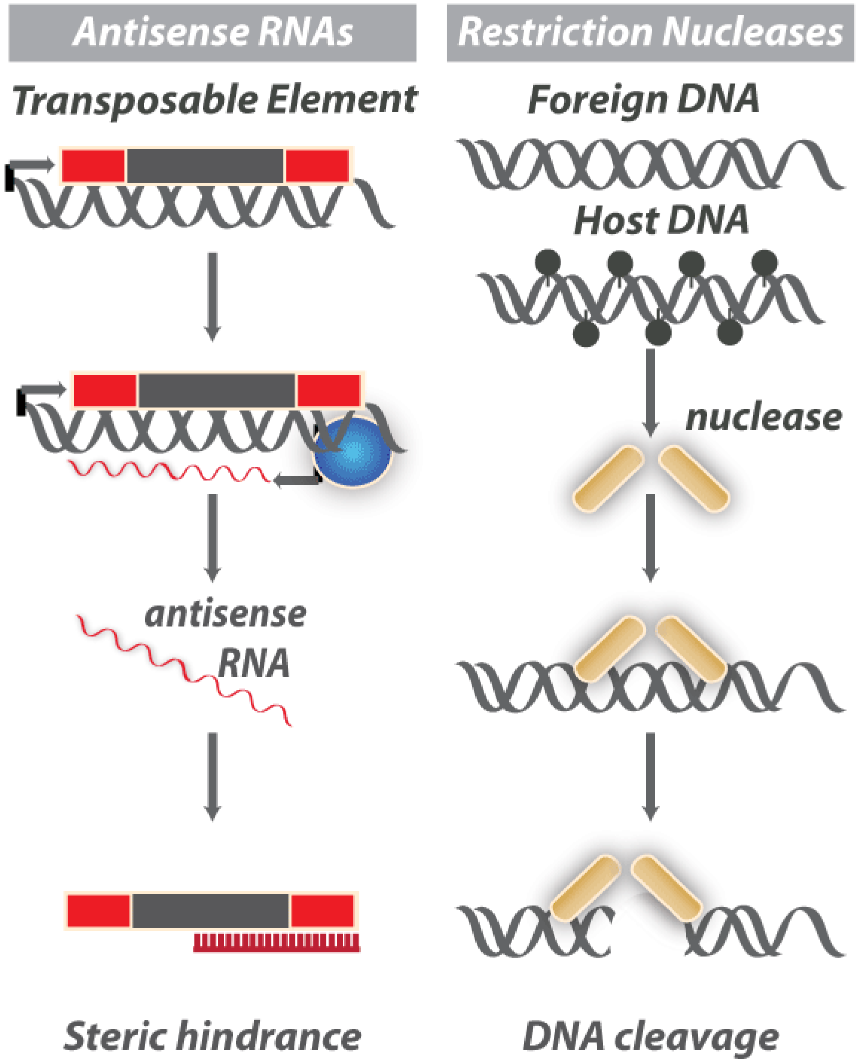
↓

Transposable Elements

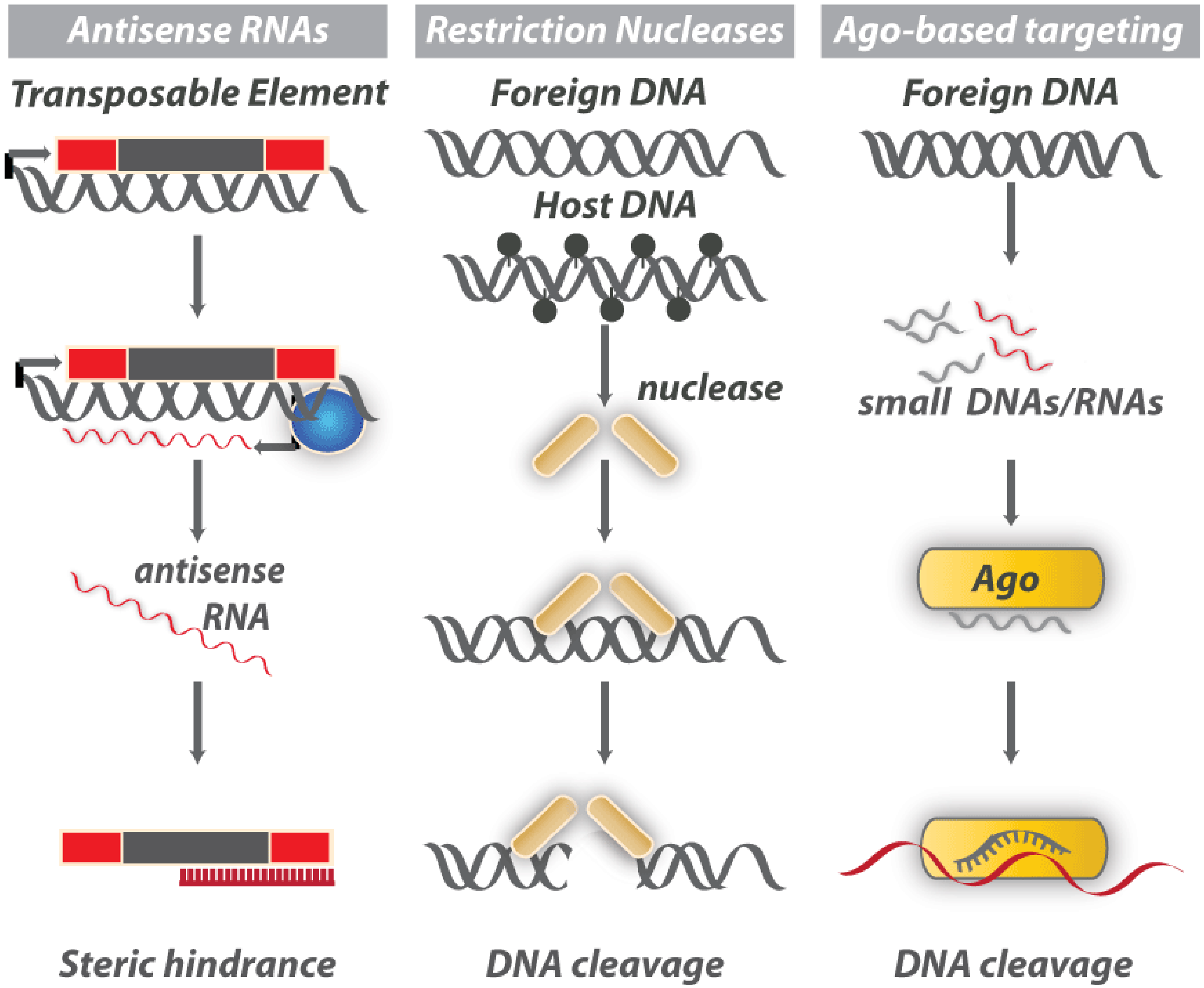
↓

DNA Phage

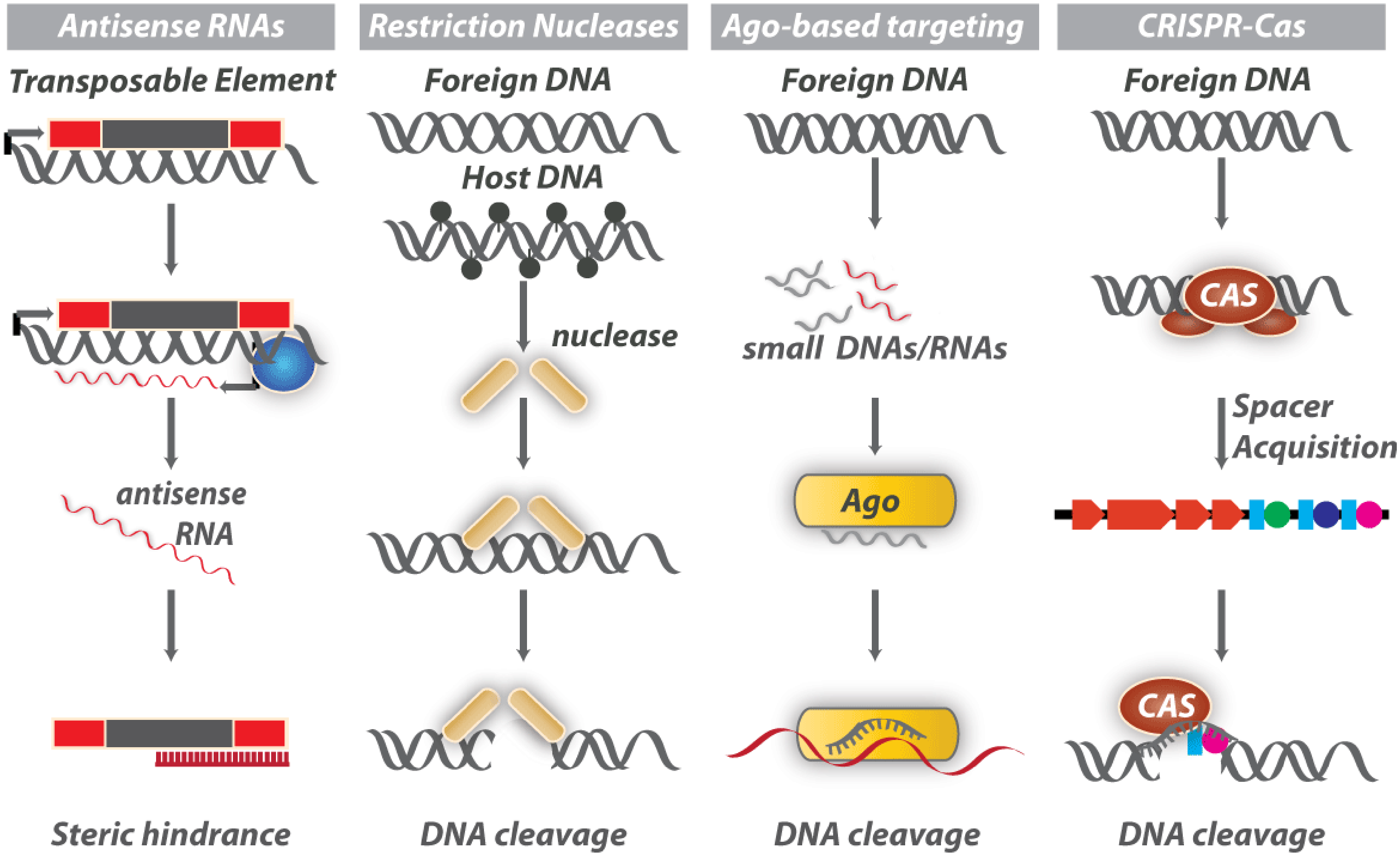
Antiviral defenses of prokaryotes – DNA targeting



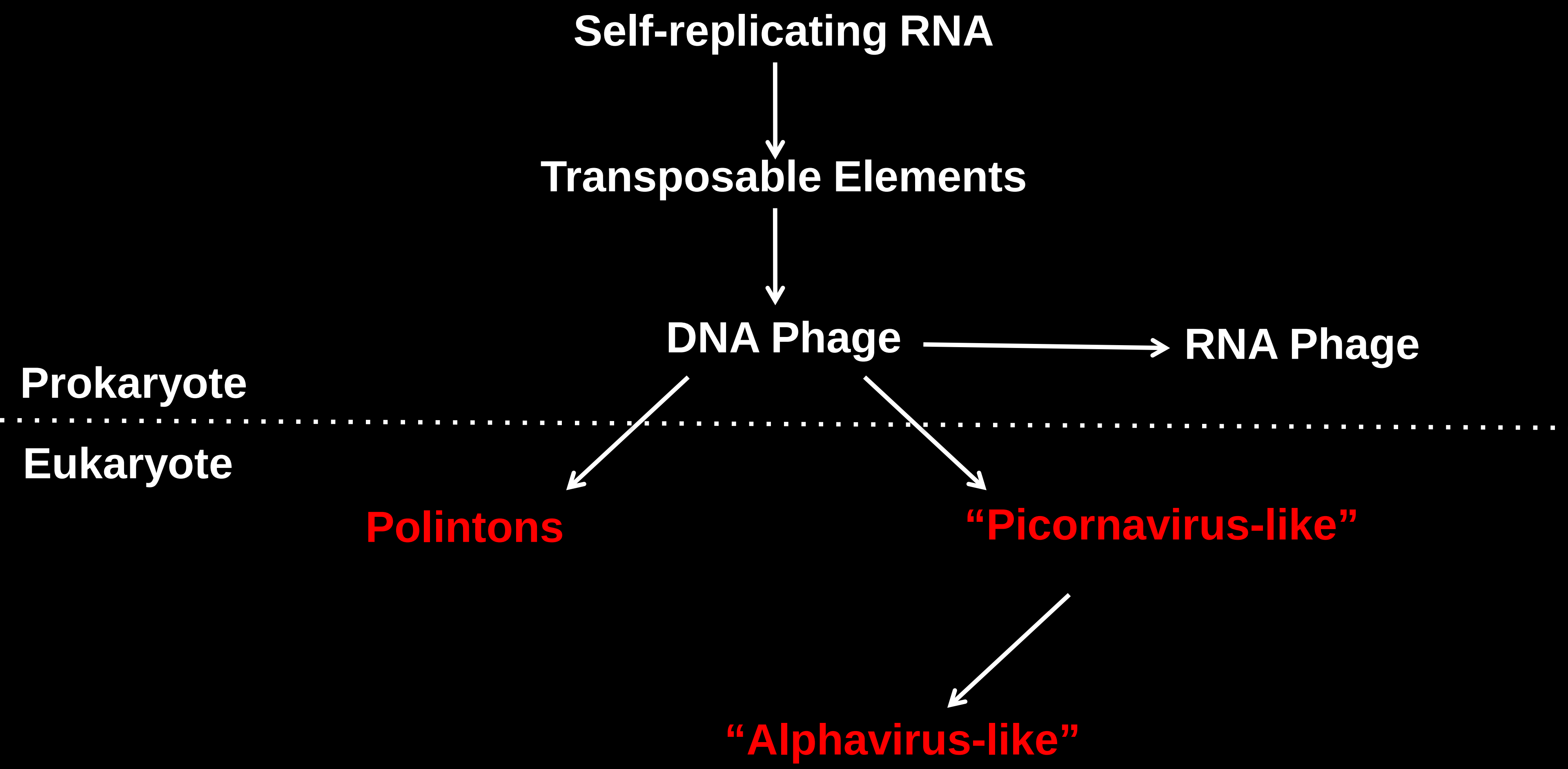
Antiviral defenses of prokaryotes – DNA targeting



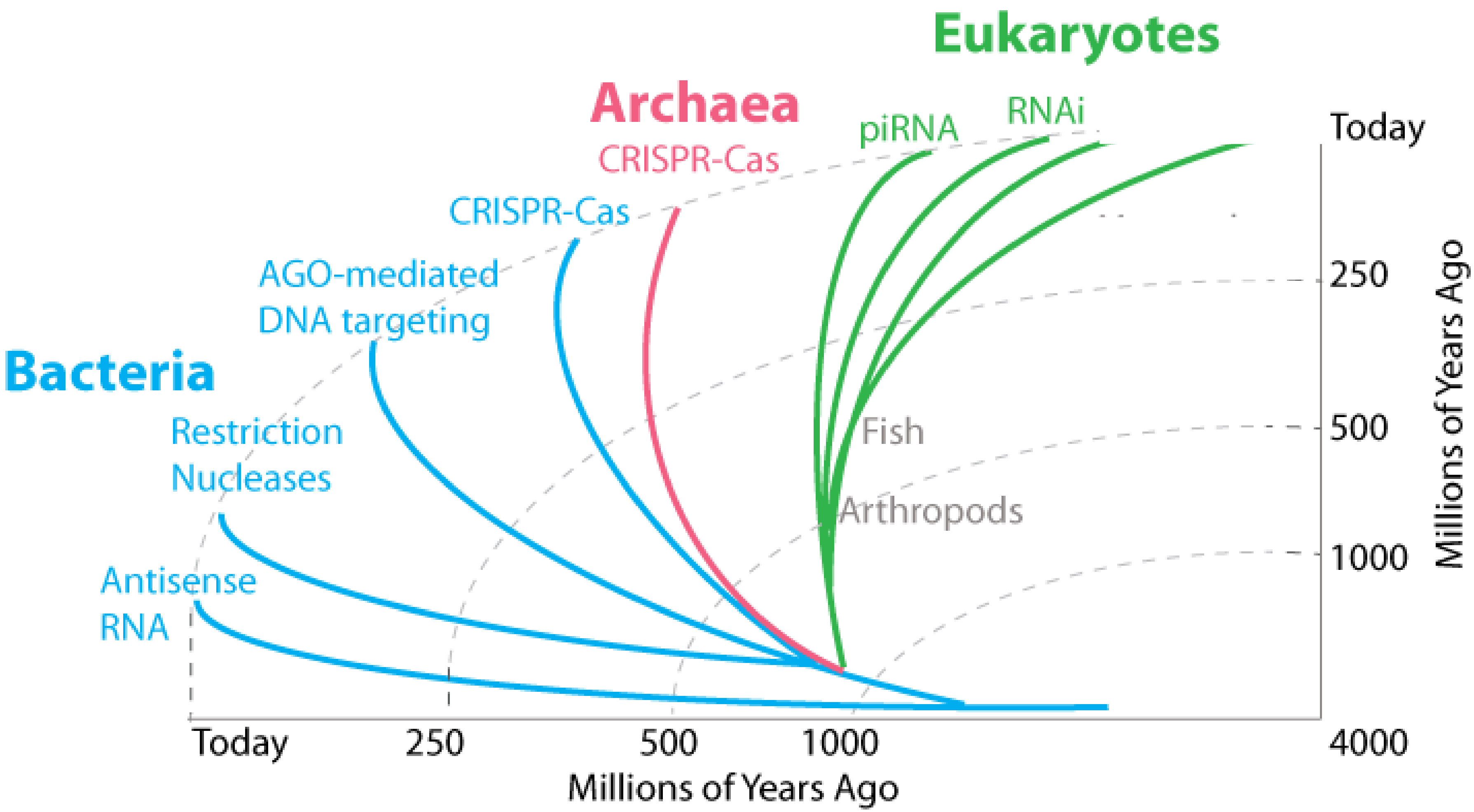
Antiviral defenses of prokaryotes – DNA targeting



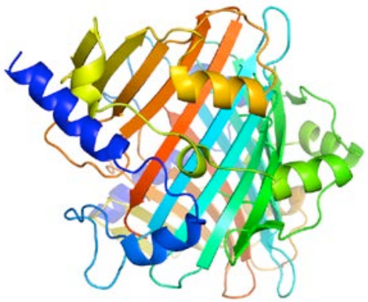
The evolution of viruses

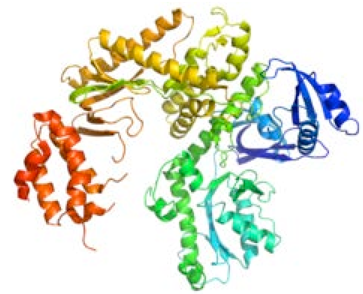


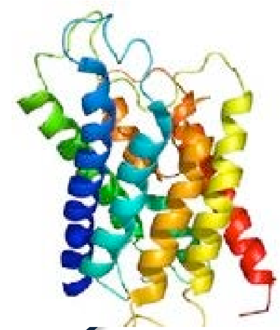
Antiviral defense evolution: new viruses = new defenses



Eukaryotes needed a defense for RNA pathogens



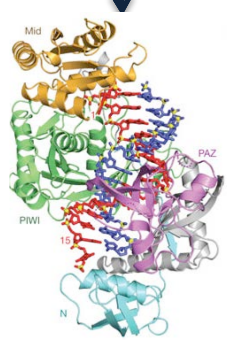




Archaea Hef proteins (DNA Replication) → Dicer

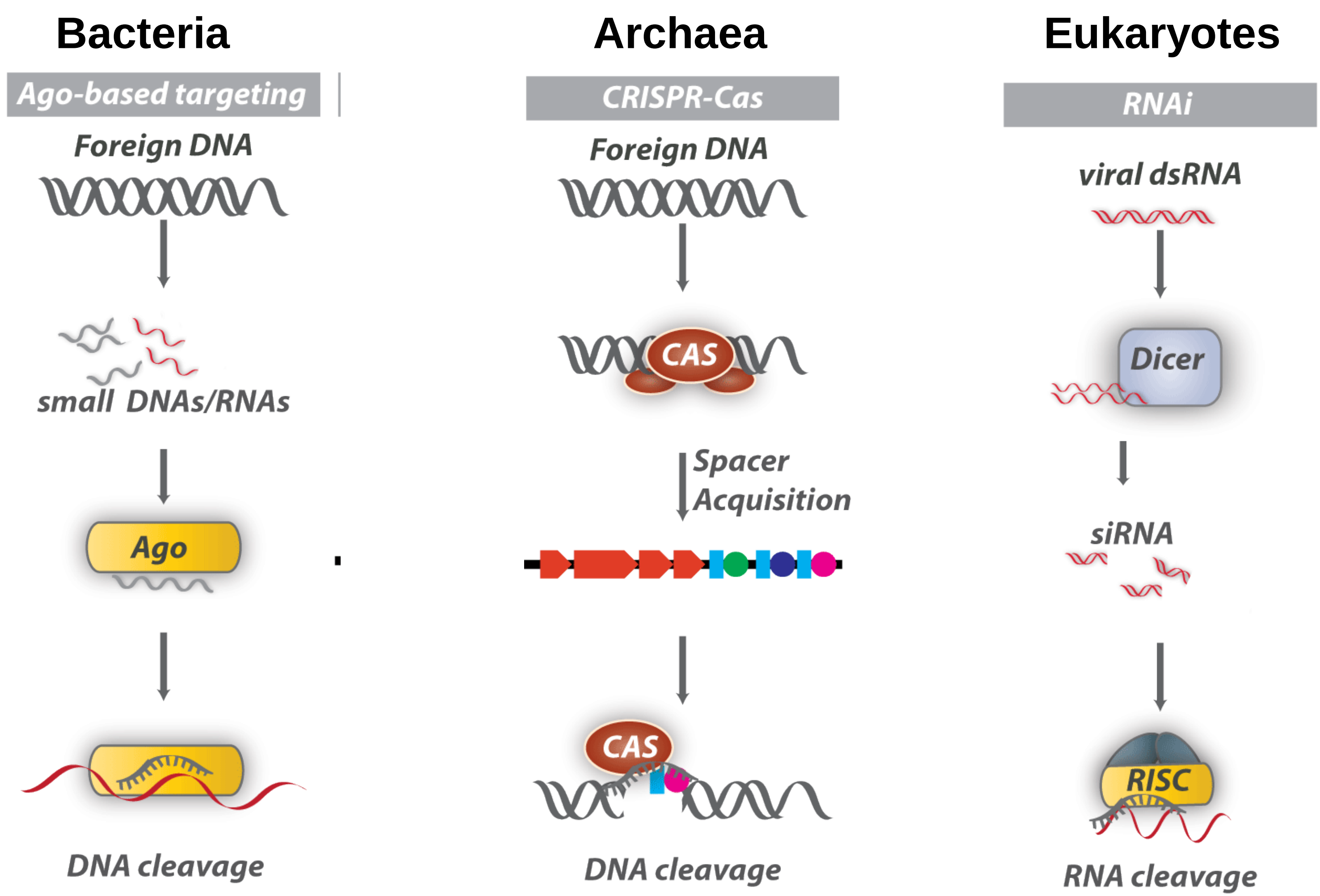
Bacteriophage DdRp → RdRp

Archaea Ago proteins (Chromatin remodelling) → Ago

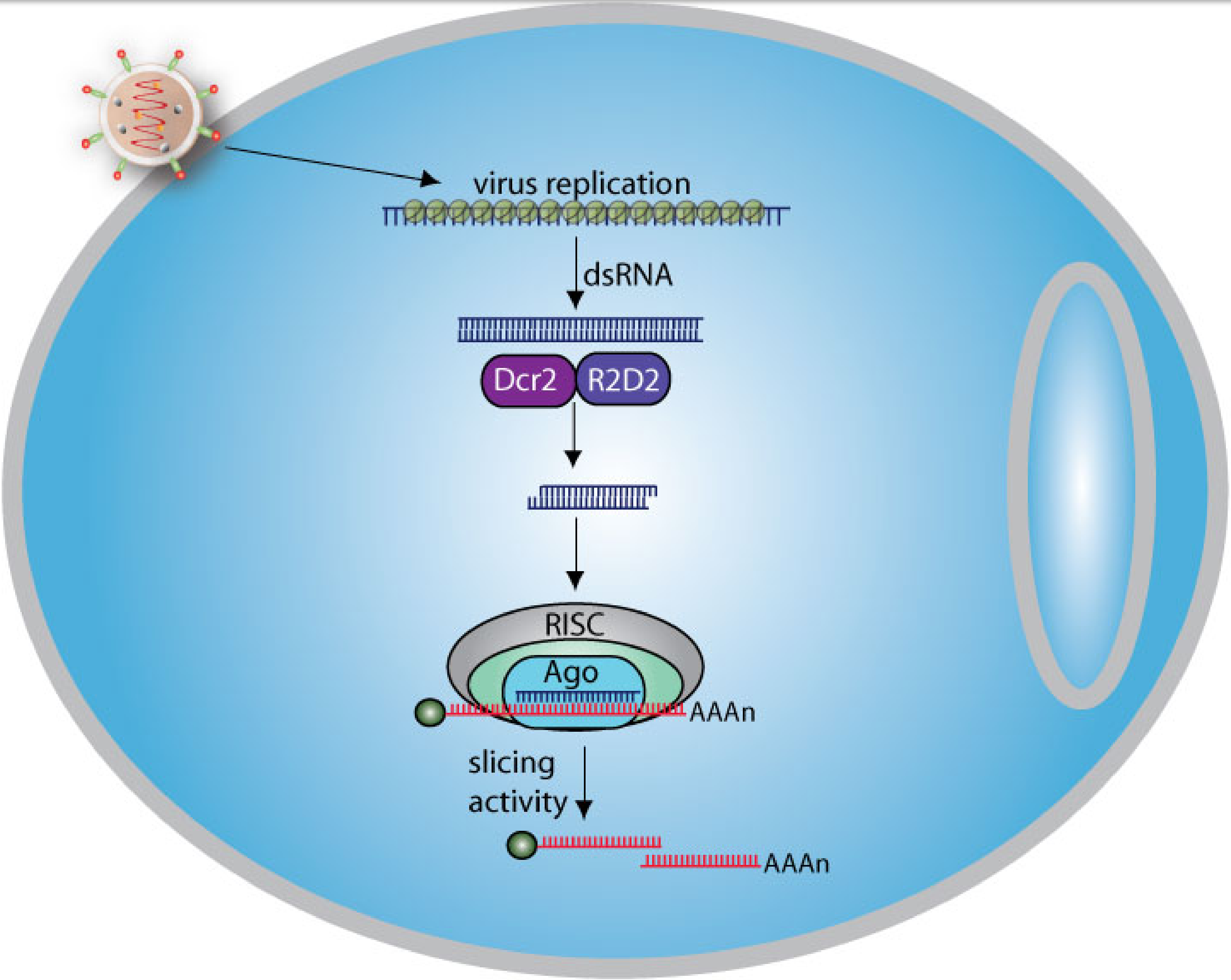


The RNAi Machinery

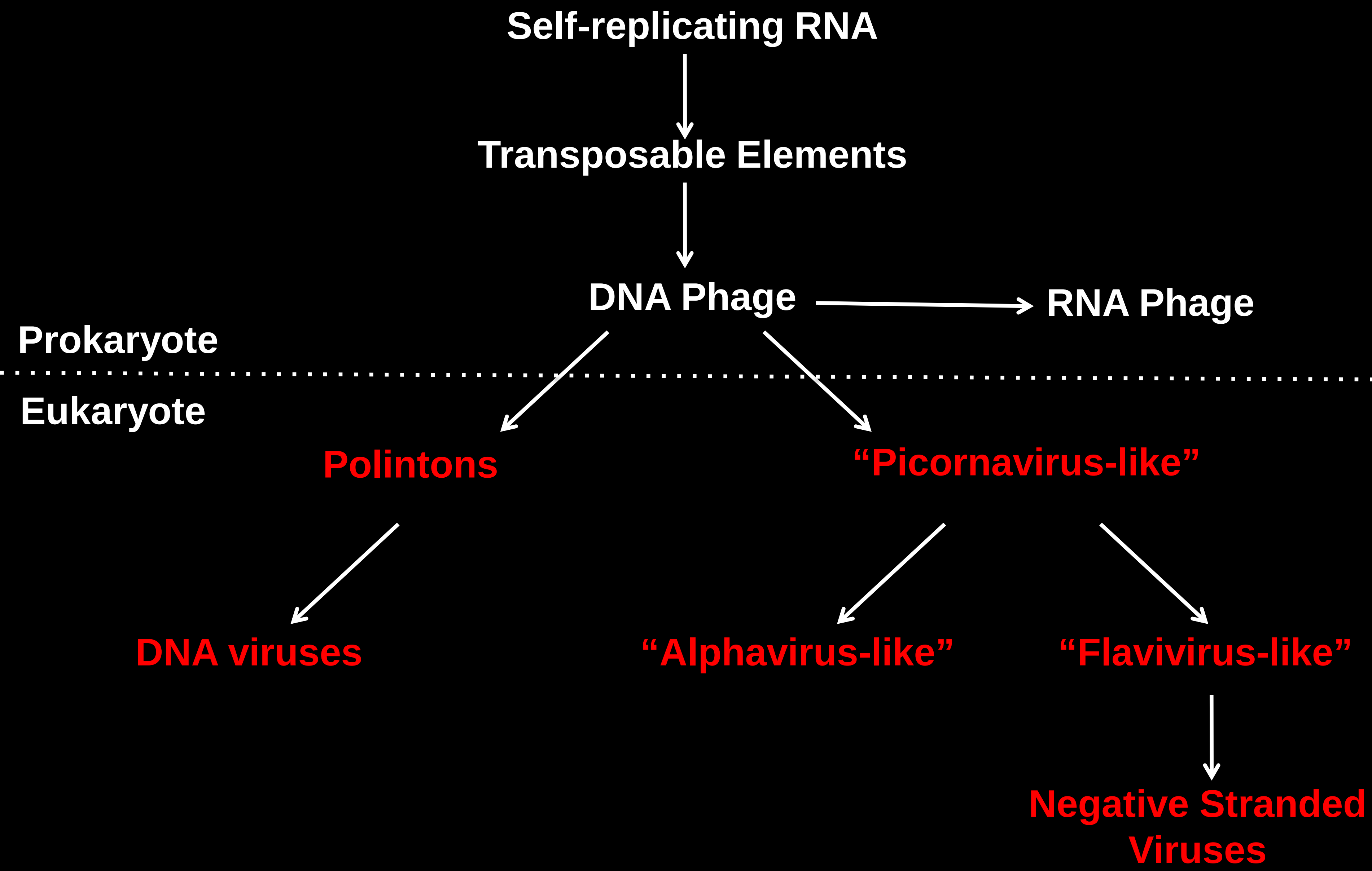
The evolution of small RNA-mediated defenses emerged in all three domains of life



Antiviral defenses of arthropods – RNA targeting



The evolution of viruses

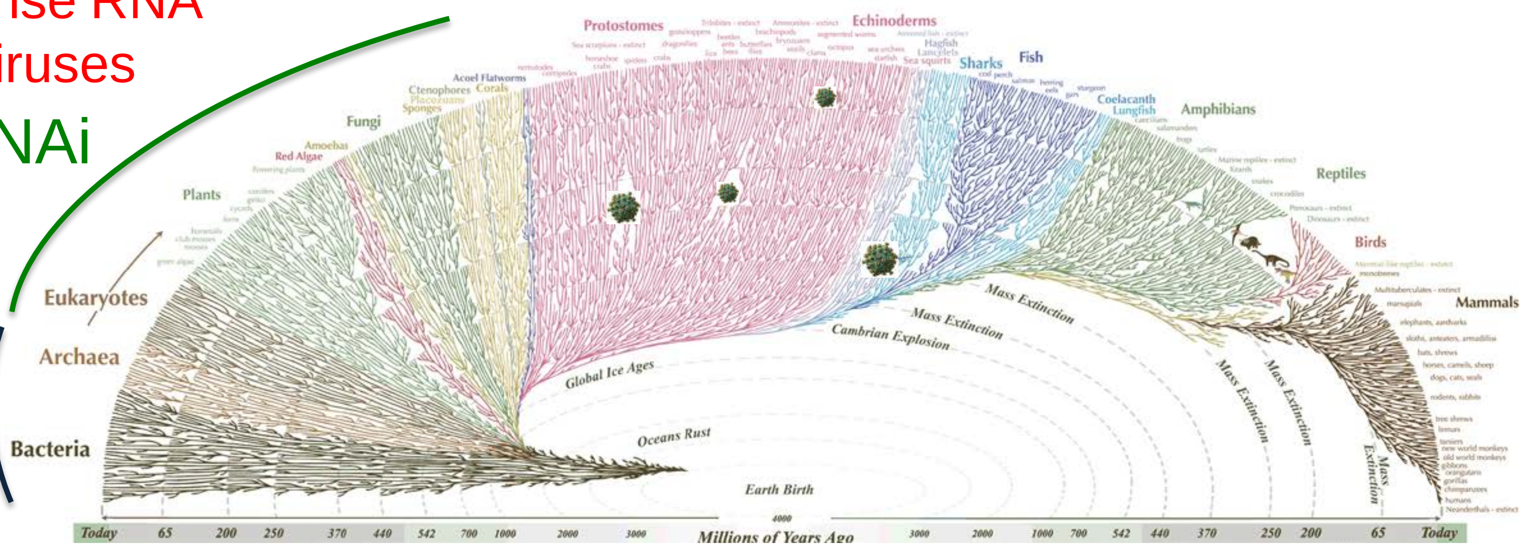


Virus expansion and defense modifications: Beyond plants and insects

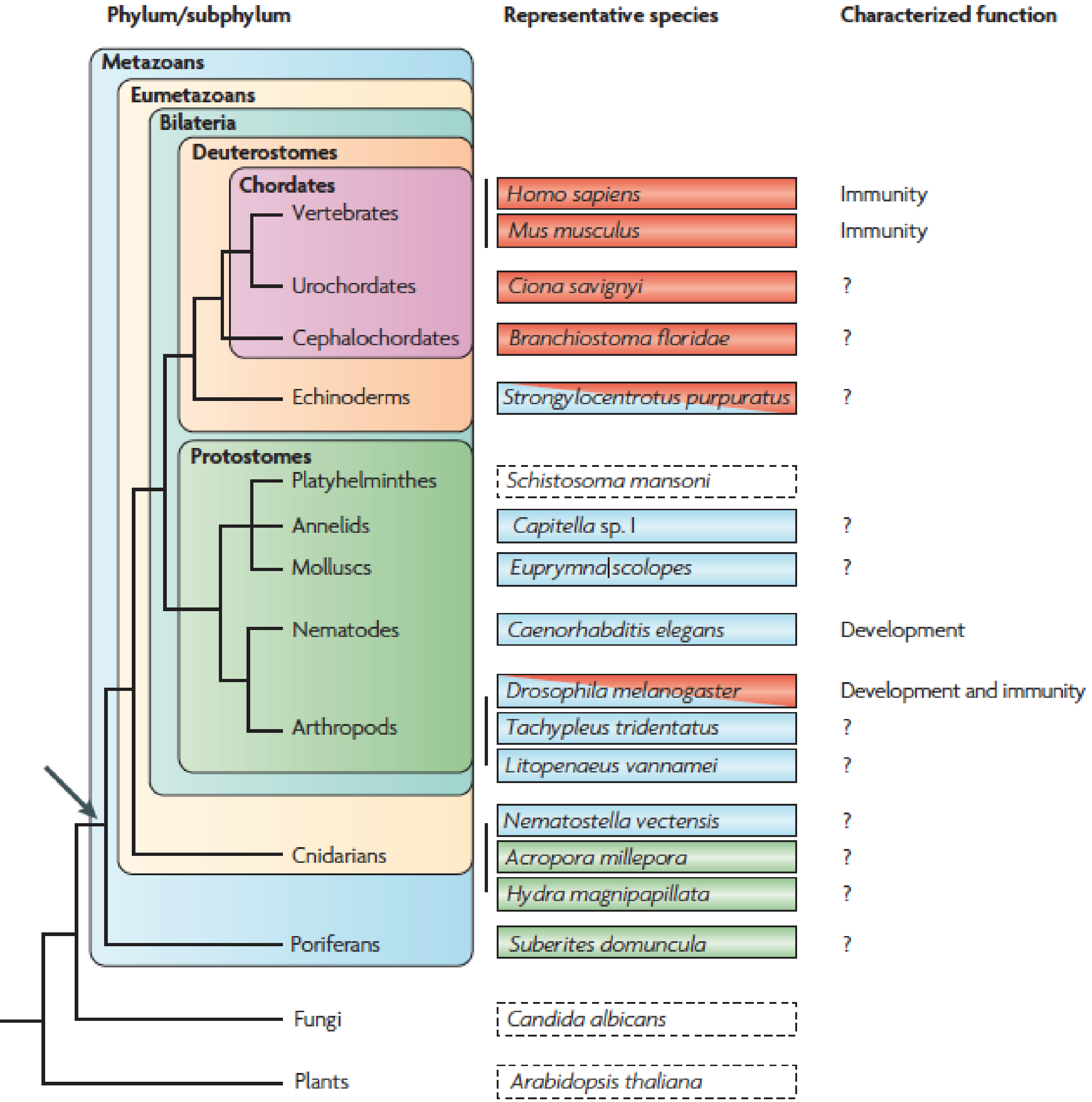
DNA virus
+/- sense RNA viruses

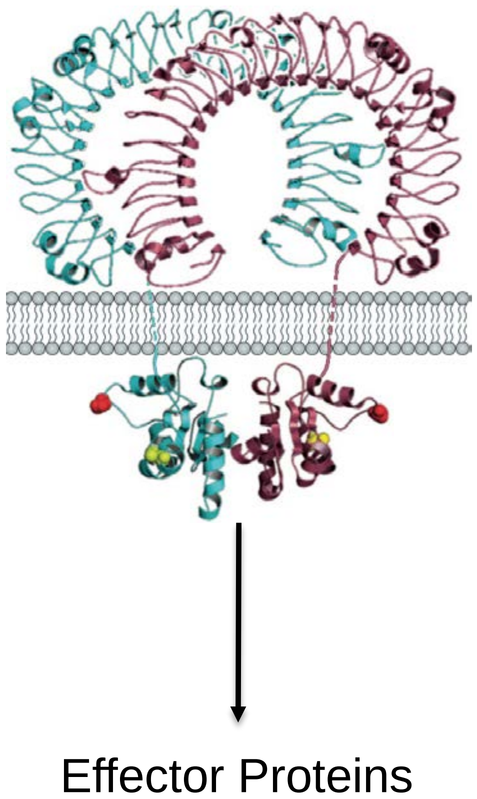
+sense RNA viruses
RNAi

DNA viruses
CRISPR



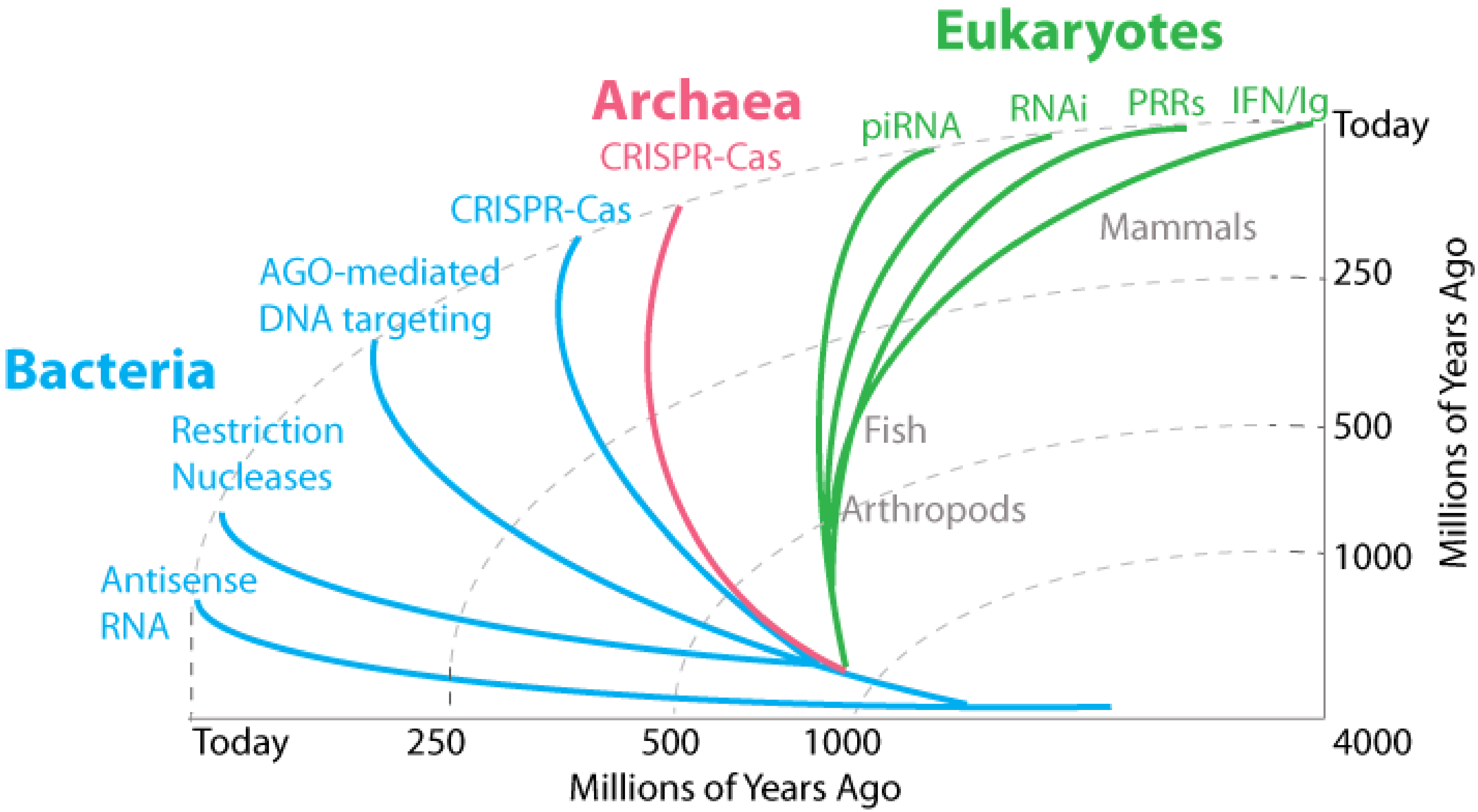
Chordates invent a new defense for both DNA and RNA viruses by repurposing TLRs



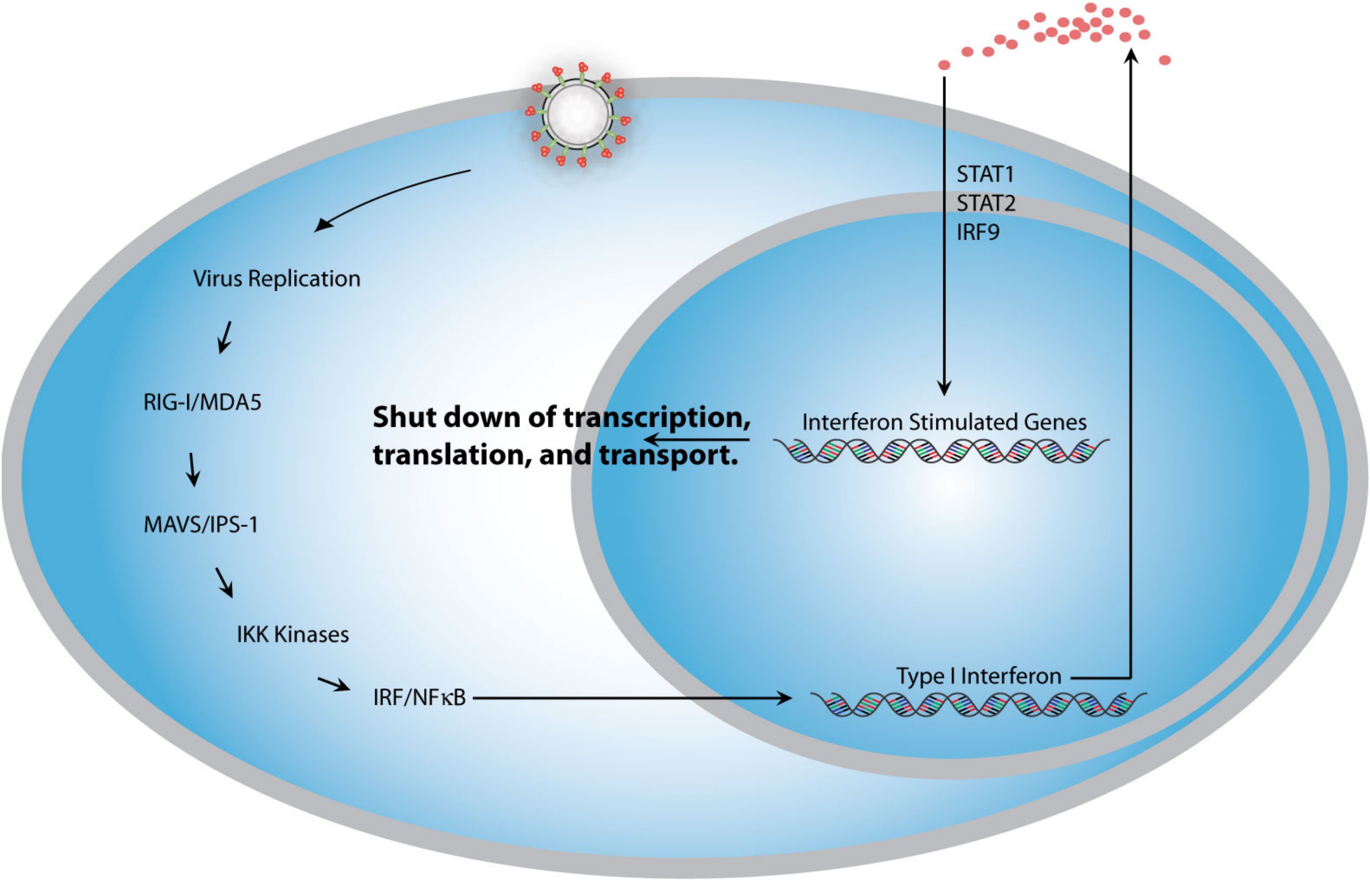


Adapted from Leulier and Lemaitre, Nature Reviews Genetics 2008

TLRs and the emergence of the Interferon system



The mammalian response to virus infection



Loss of RNAi did not mean the loss of small RNA-mediated regulation

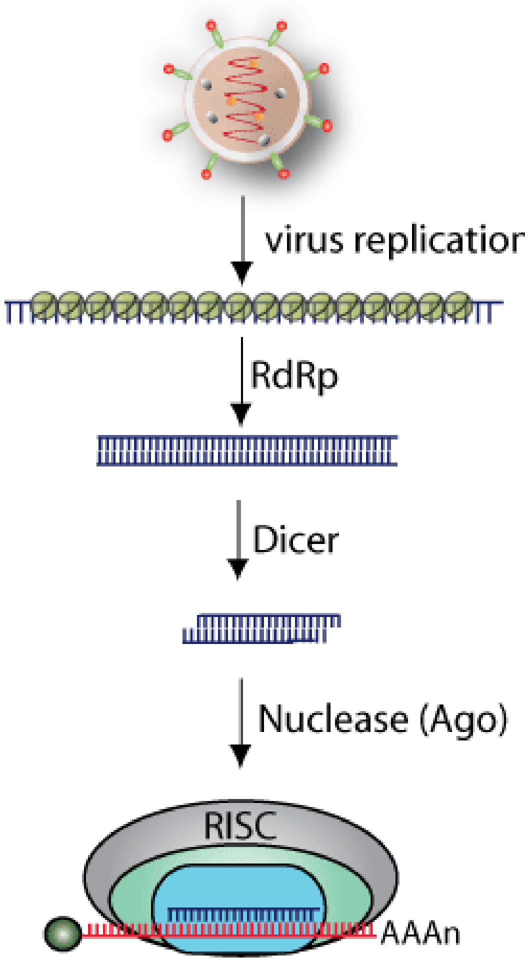
Antiviral RNAi

Small RNA-mediated trancriptional control

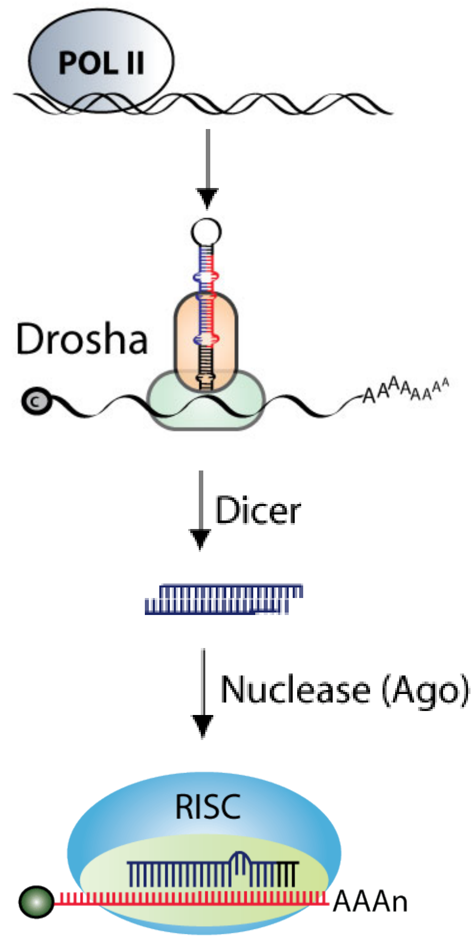
microRNAs

Small RNA usage in eukaryotes

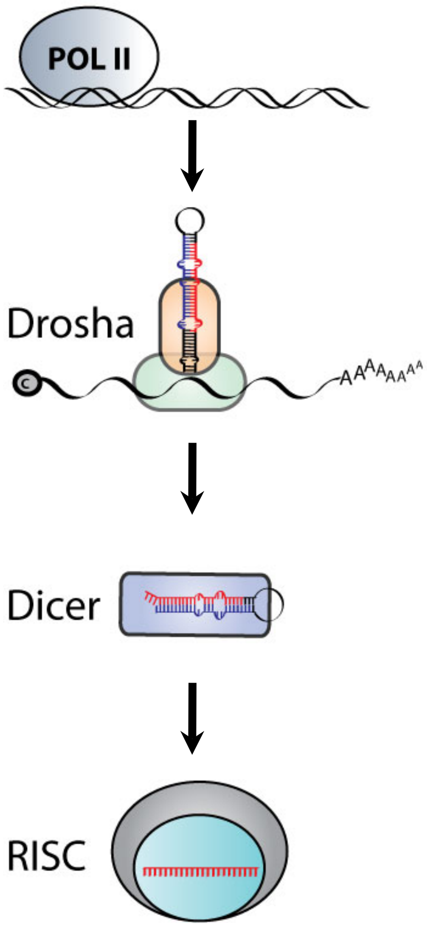
Antiviral RNAi



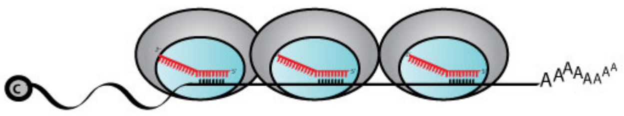
microRNAs



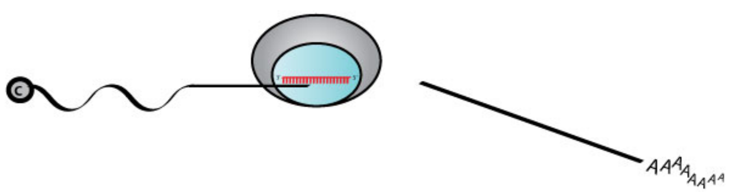
microRNA biology



Imperfect Complementarity
(stochiometric repression)



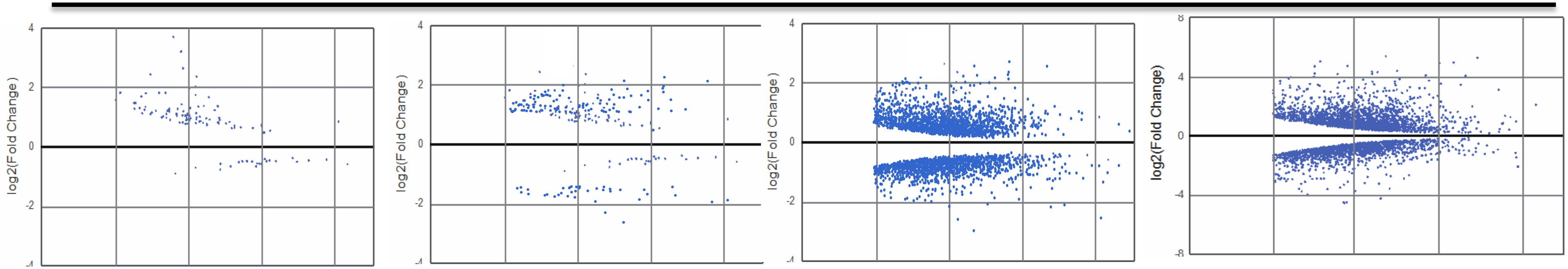
Perfect Complementarity
(enzymatic cleavage)



Consequences of complementarity:
Potent silencing of a single target vs. fine-tuning of 1000s

Days post miRNA depletion

Day One	Day Two	Day Four	Day Nine

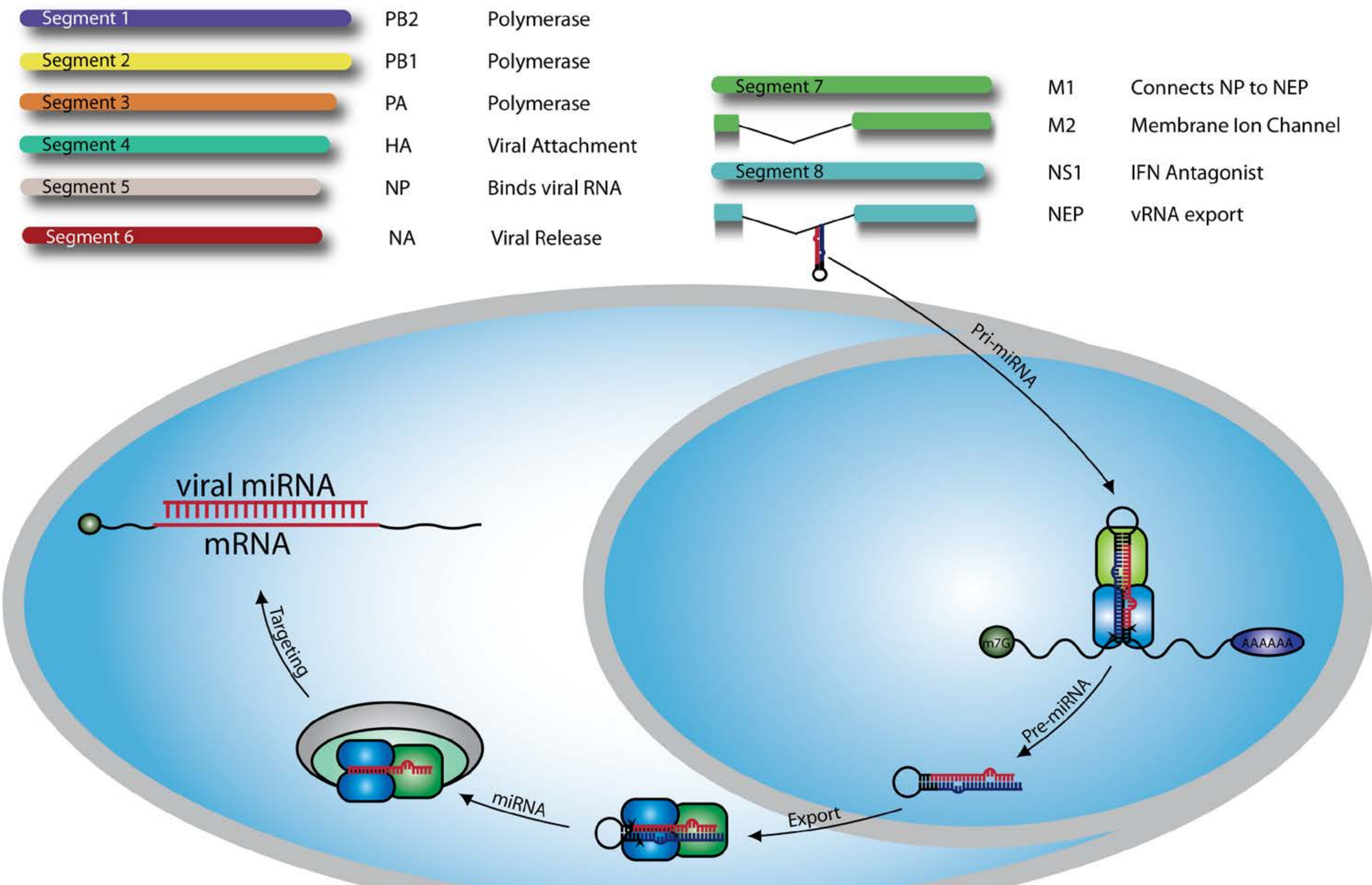


~50 DE genes	~80 DE genes	~500 DE genes	~1000 DE genes

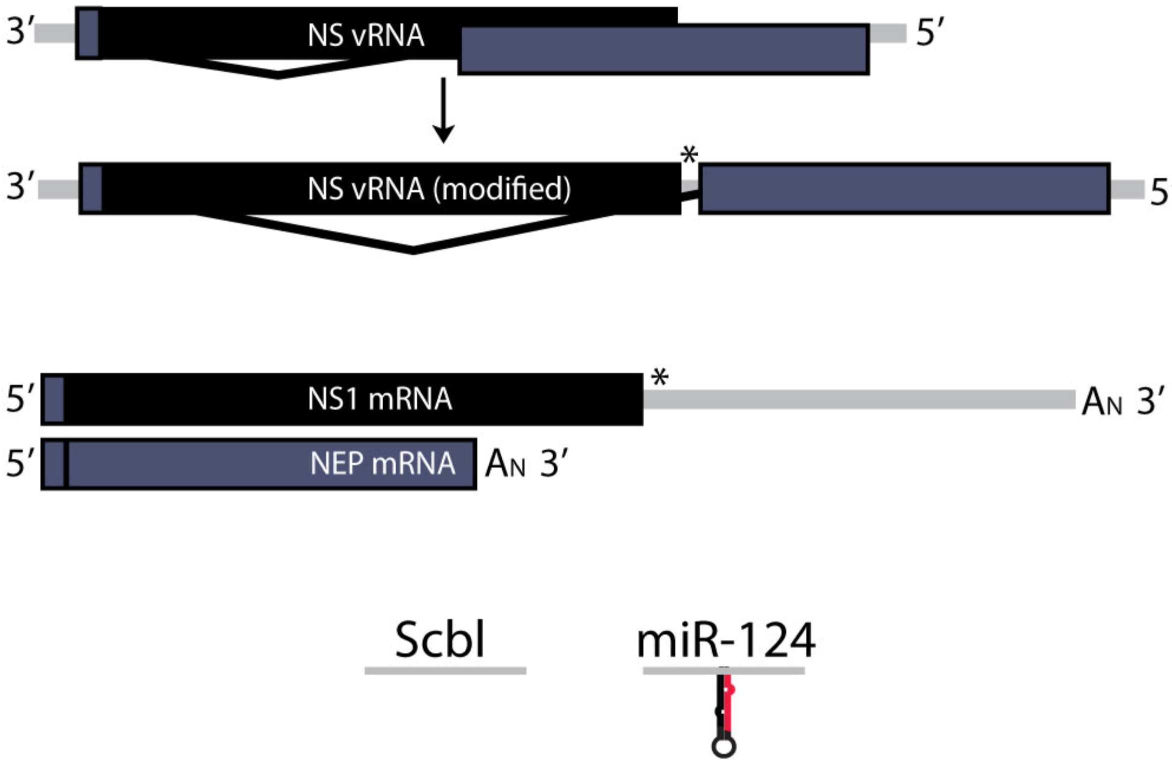
Part I.

Given the successful utilization of RNAi in insects and our expression of similar small RNA machinery, can we artificially engineer viruses to intersect with this biology?

Engineering flu to engage with the miRNA machinery

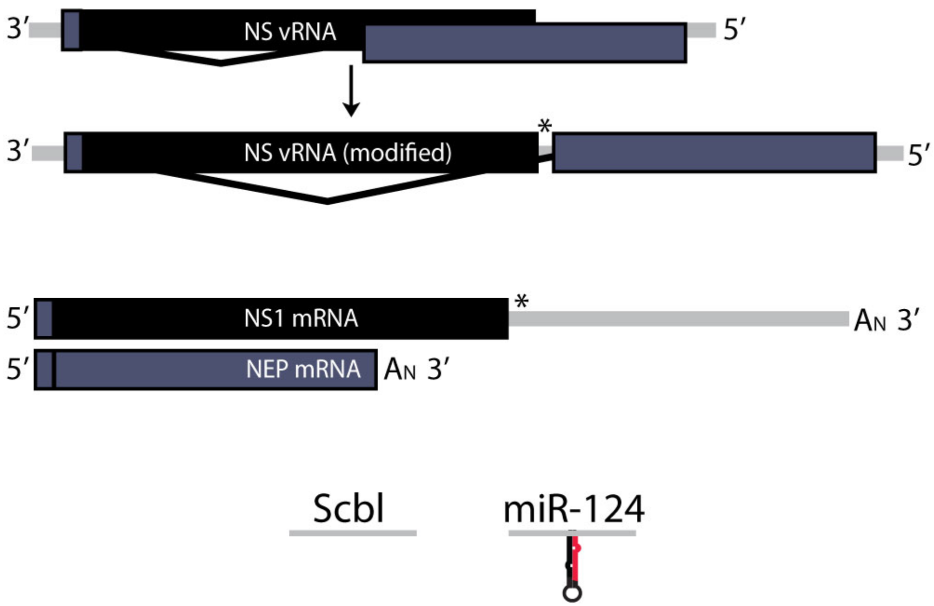


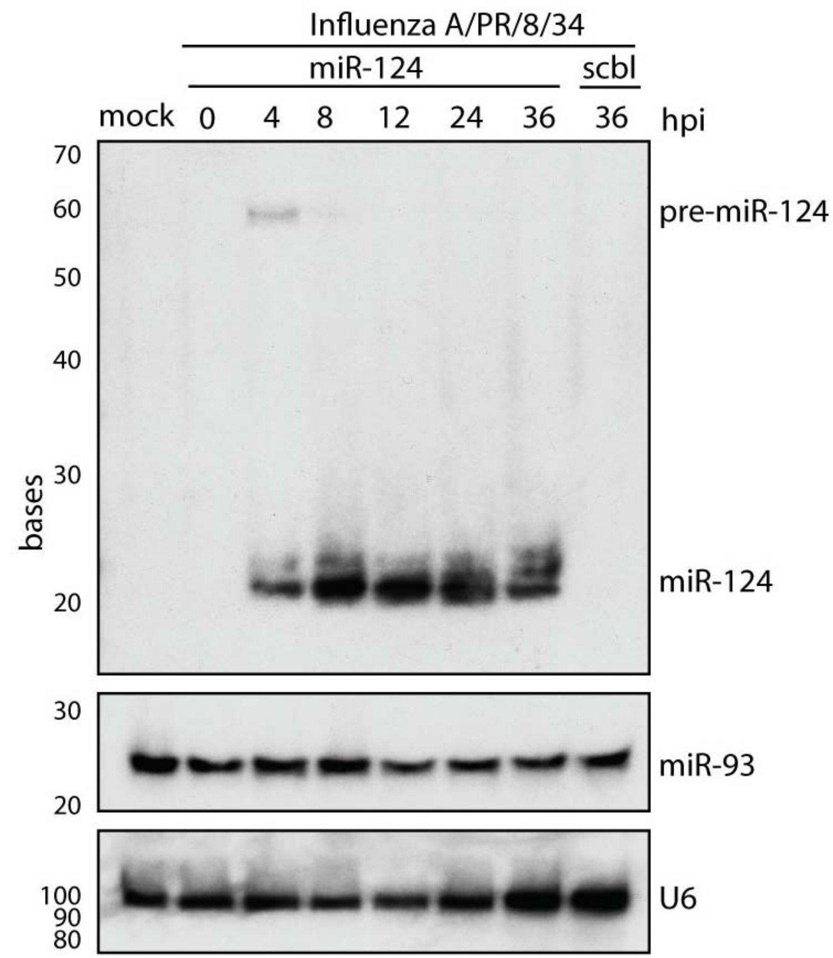
Engineering flu to produce miRNAs



Varble and Chua et al. PNAS (2010) 197, 11519-11524.

Engineering flu to produce miRNAs

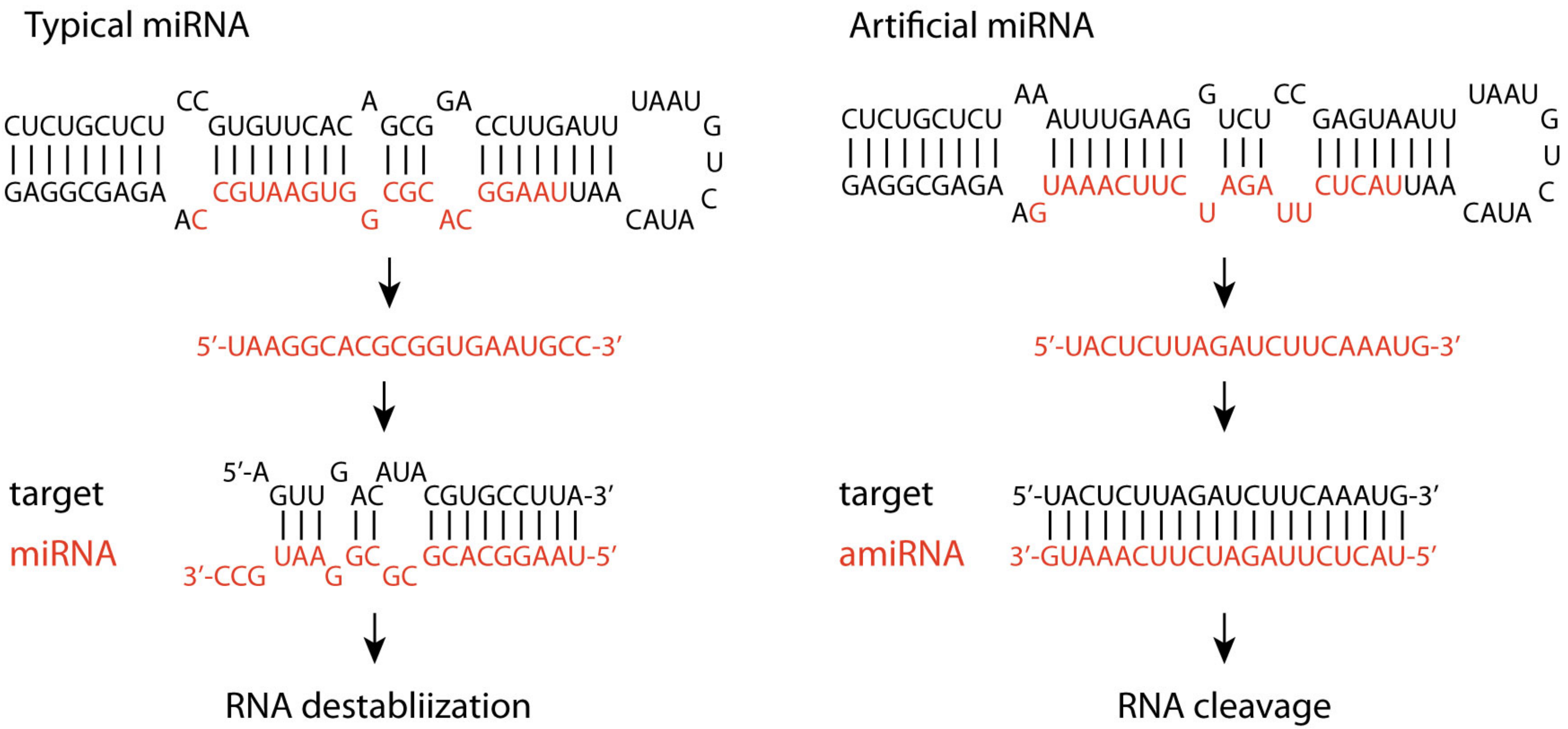




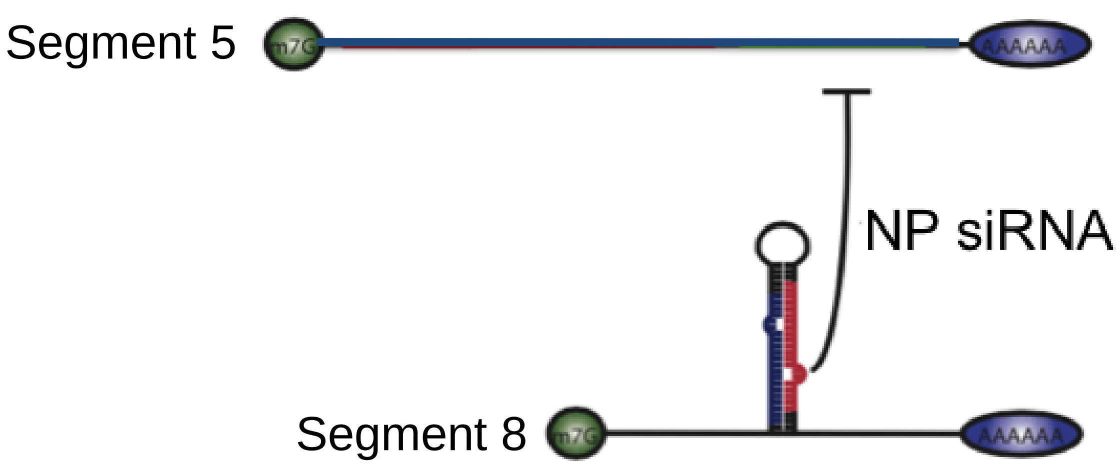
Can we design a self-inactivating virus?

Varble and Chua et al. PNAS (2010) 197, 11519-11524.

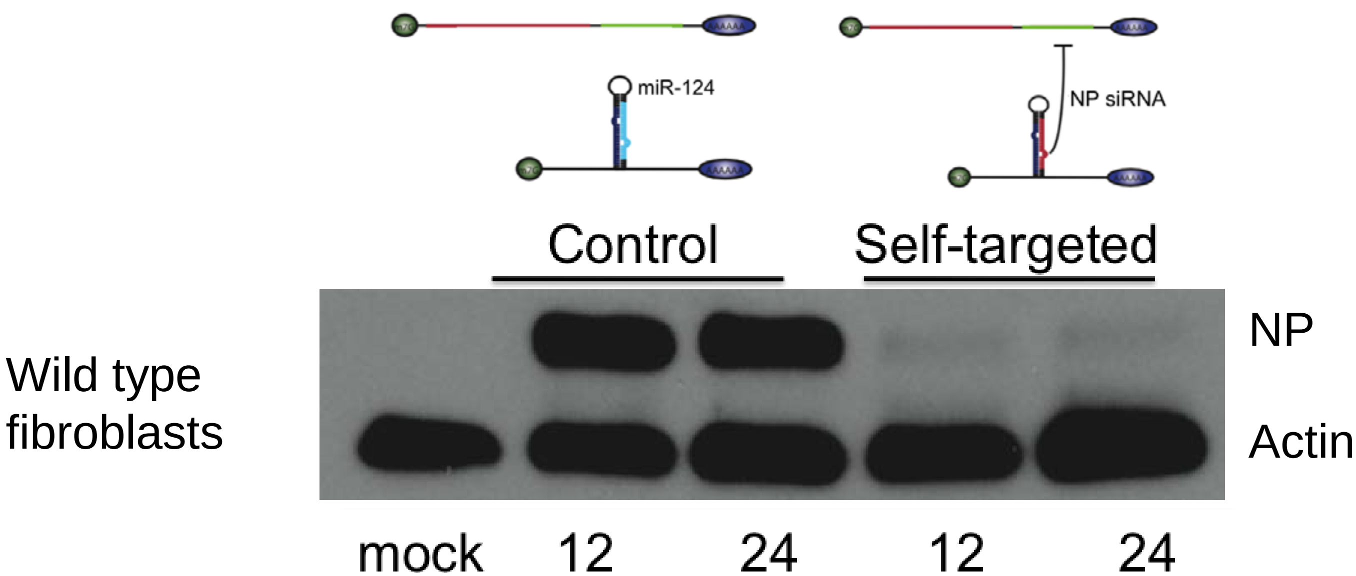
Re-wiring microRNAs



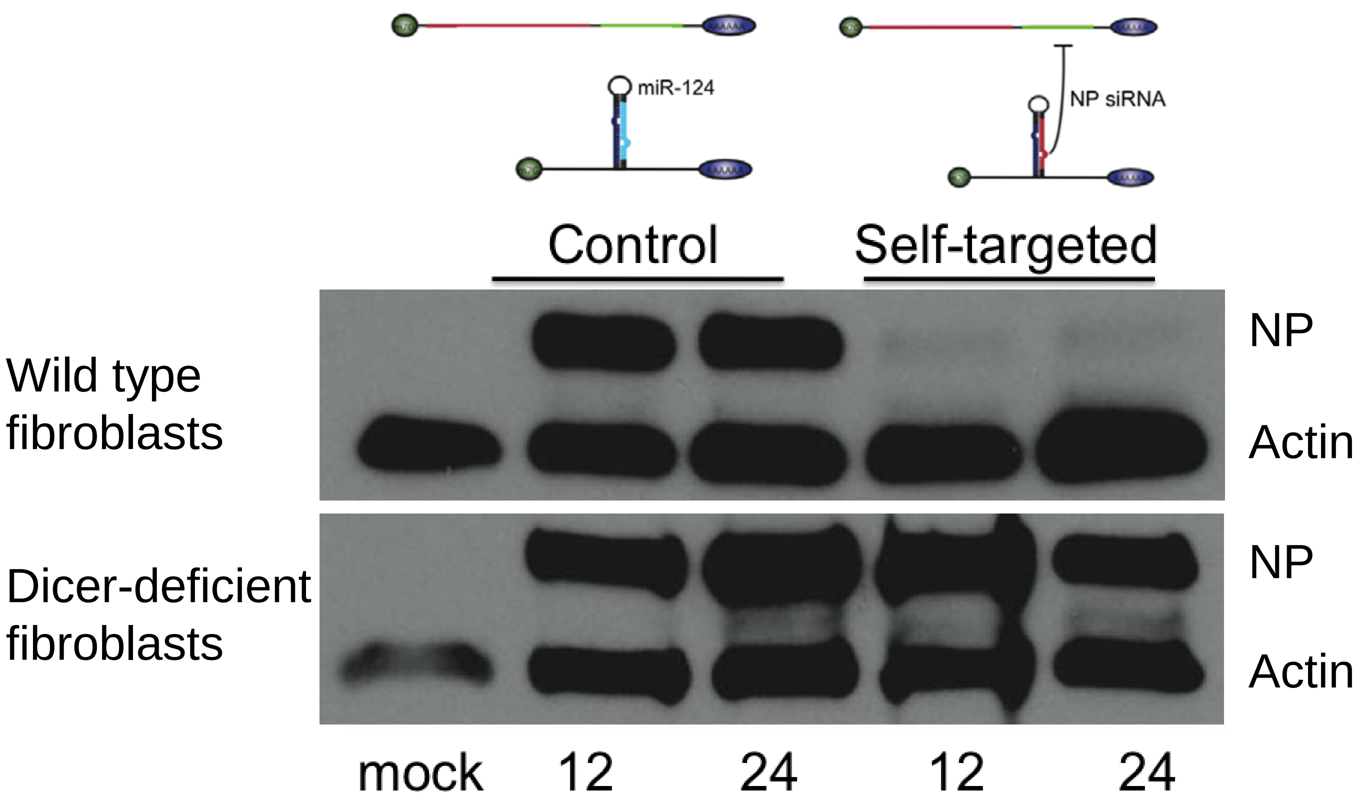
miRNA-mediated self-targeting of influenza



Self targeting can be achieved using the host small RNA machinery

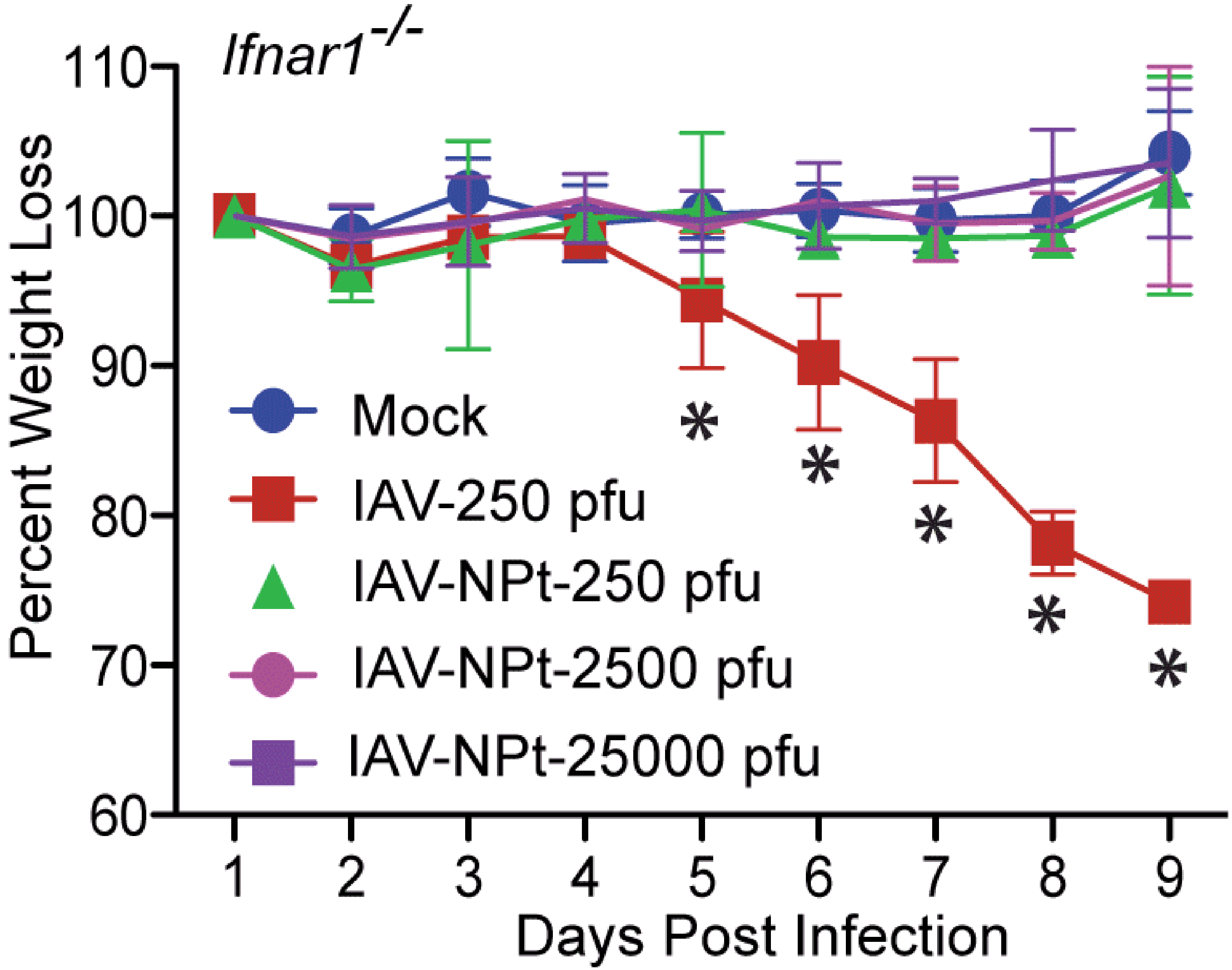


Self targeting can be achieved using the host small RNA machinery



Benitez and Spanko et al.
Cell Reports (2015)

RNAi can replace the mammalian IFN-I response



Benitez and Spanko et al.
Cell Reports (2015)

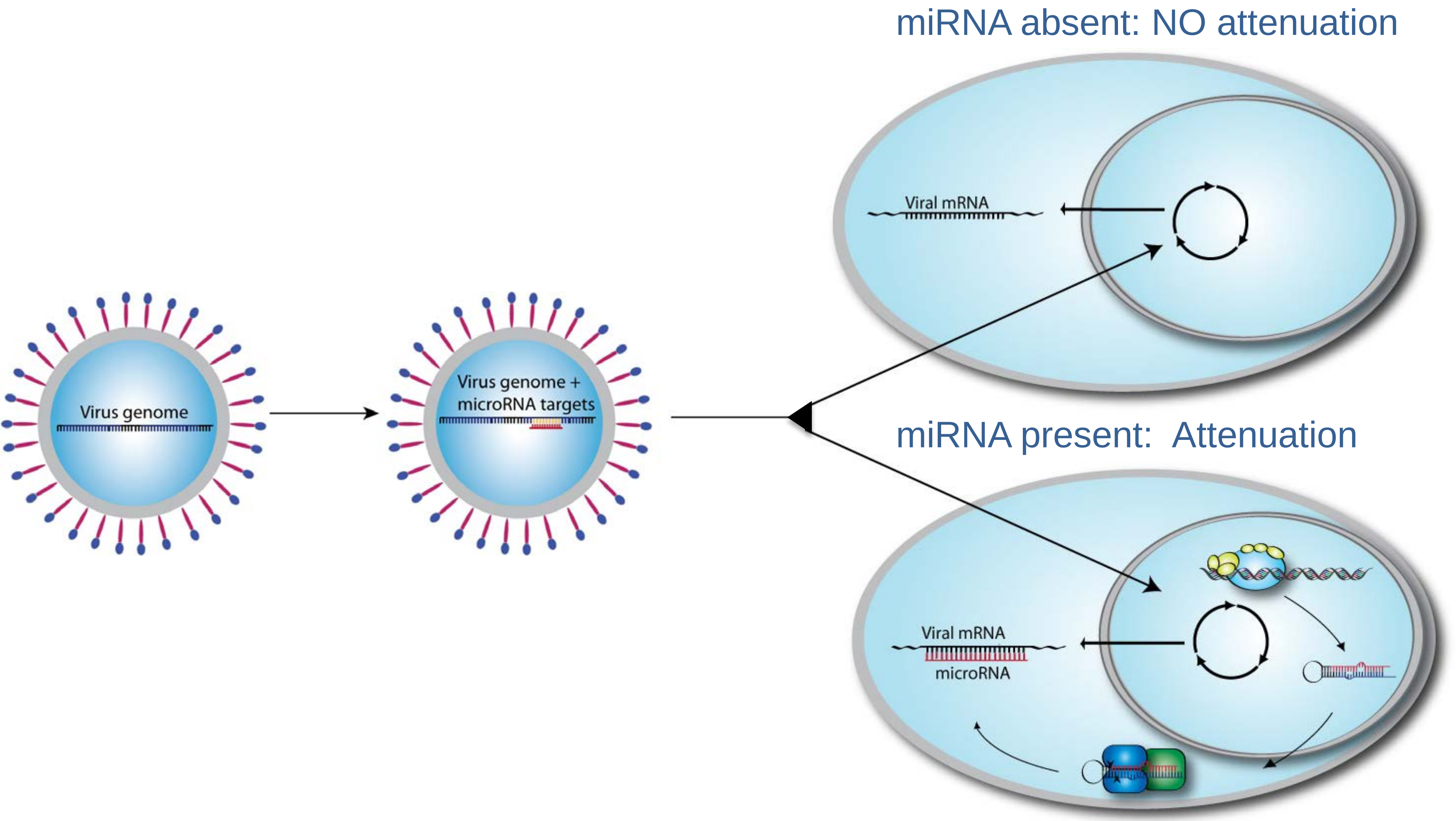
Part I. Conclusion

We can successfully engineer viruses to engage with our small RNA machinery.

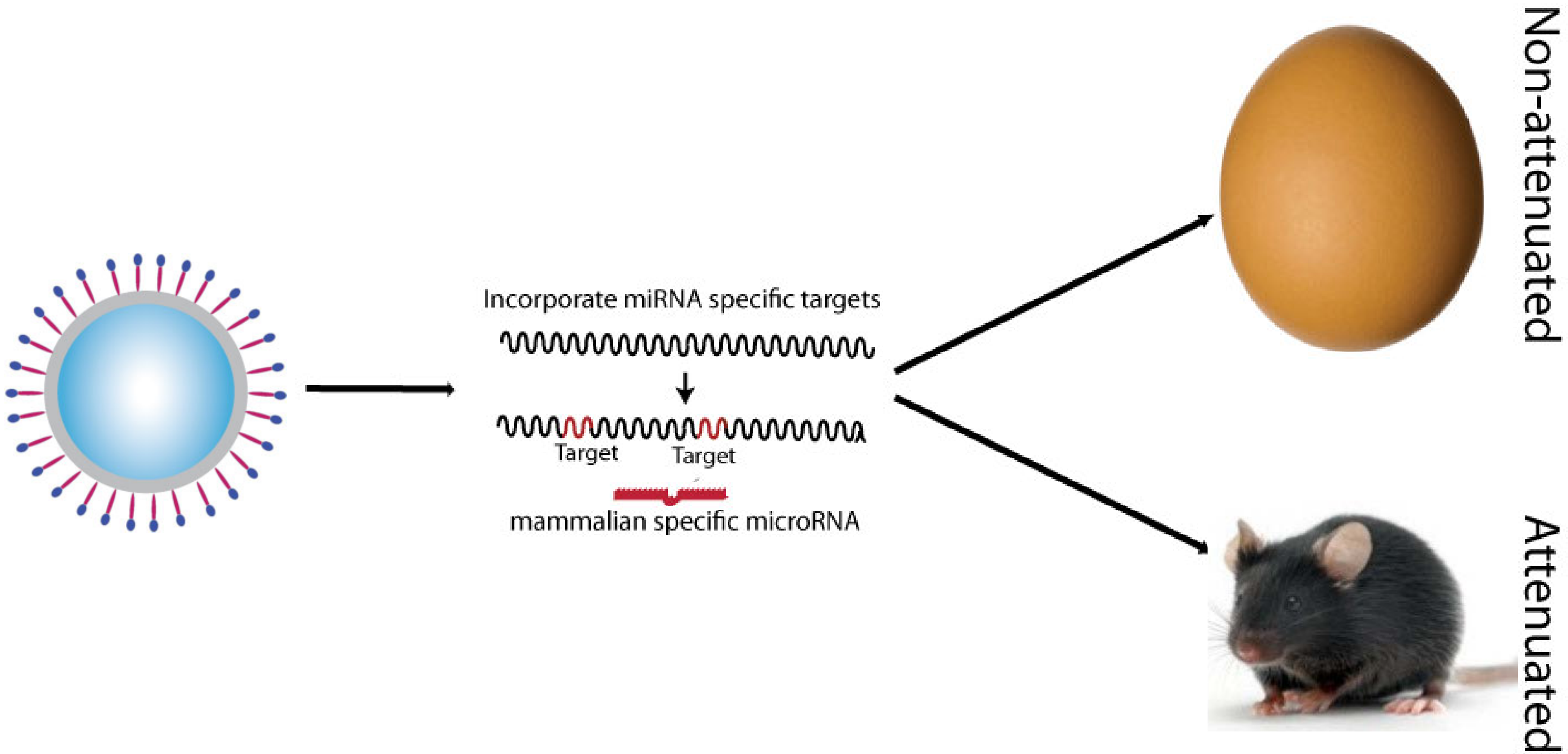
Part II.

Can the host small RNA machinery be co-opted? Can we re-create the antiviral defenses of plants and arthropods in mammals?

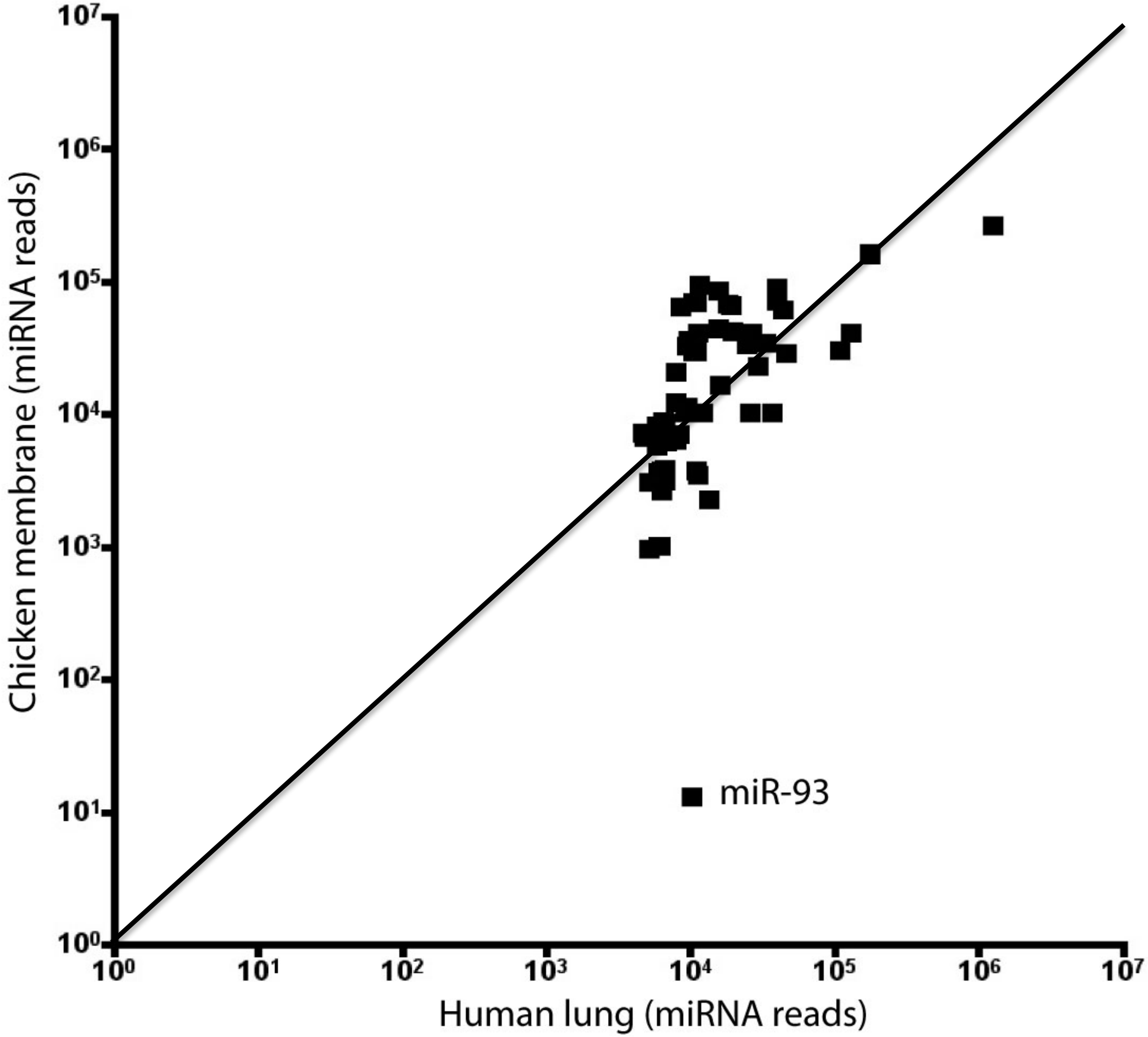
miRNA exploitation to control virus biology



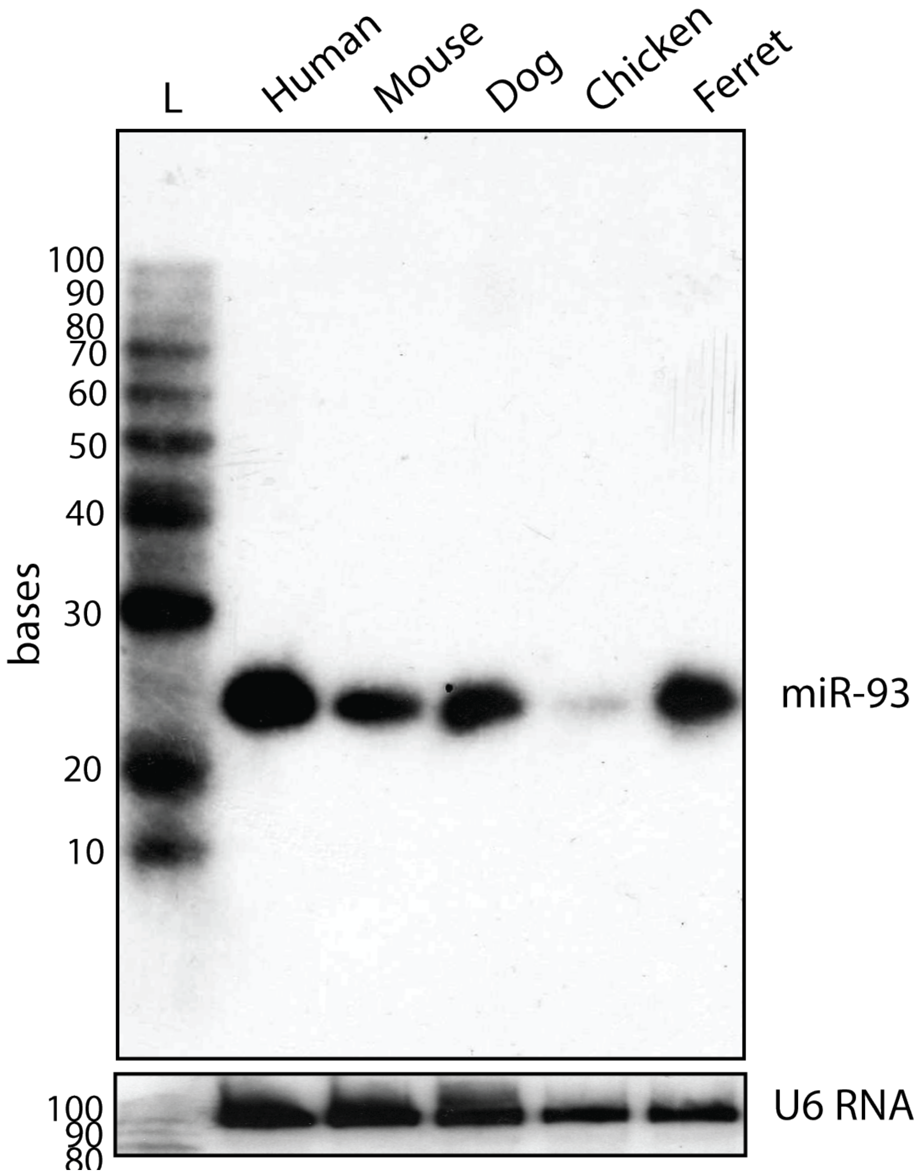
Can we generate a species-specific IAV?



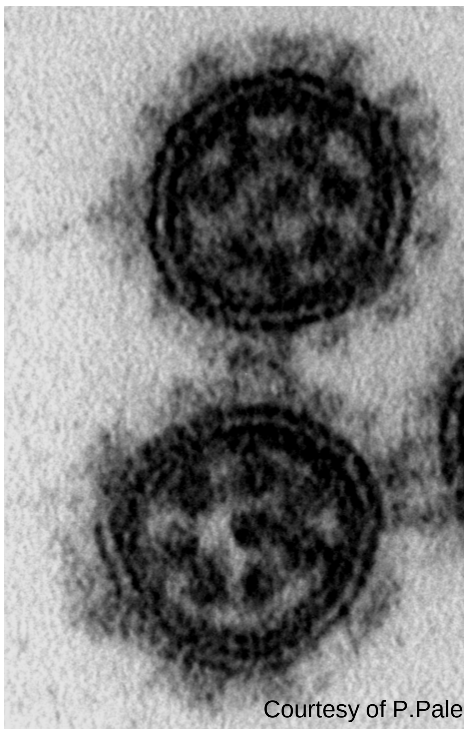
Identifying mammalian-specific miRNAs



miR-93: not expressed in chickens, abundant in mammals

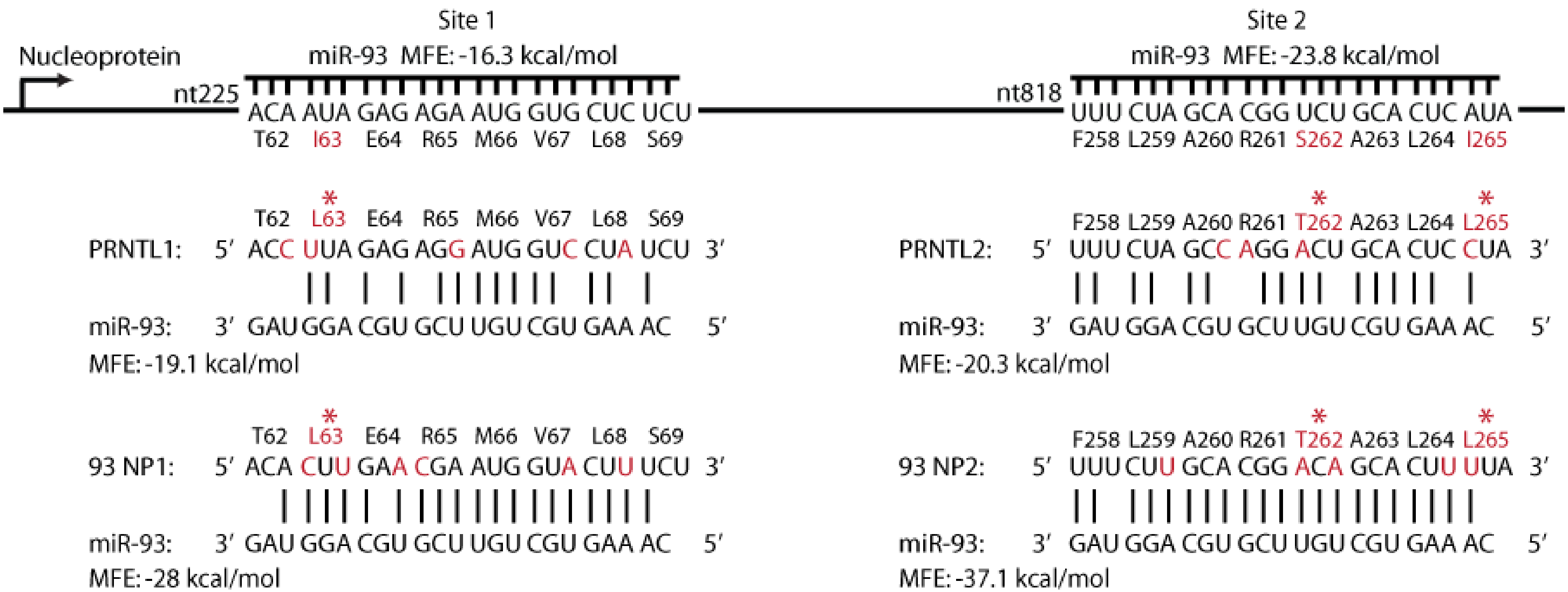


Confining IAV to chickens

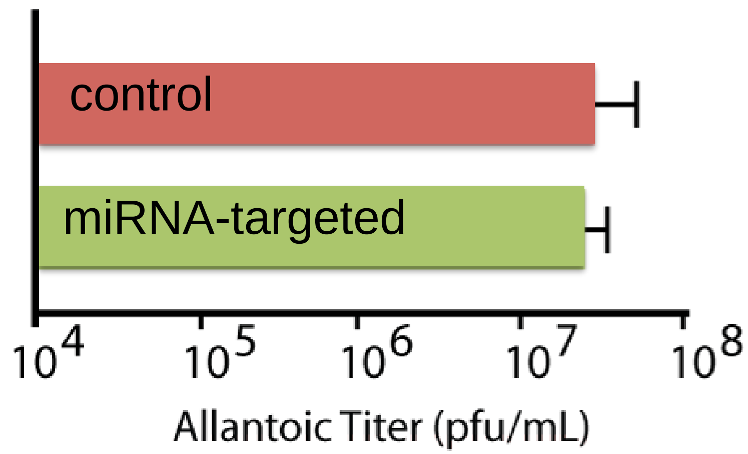

Courtesy of P.Palese

Influenza A Virus Segment	Protein	Function
Segment 1	PB2	Polymerase
Segment 2	PB1	Polymerase
Segment 3	PA	Polymerase
Segment 4	HA	Viral Attachment
Segment 5	NP	Binds viral RNA
Segment 6	NA	Viral Release
Segment 7	M1/M2	Membrane stability
Segment 8	NS1/NEP	Antagonist/RNA export

Codon changing to create miRNA targets



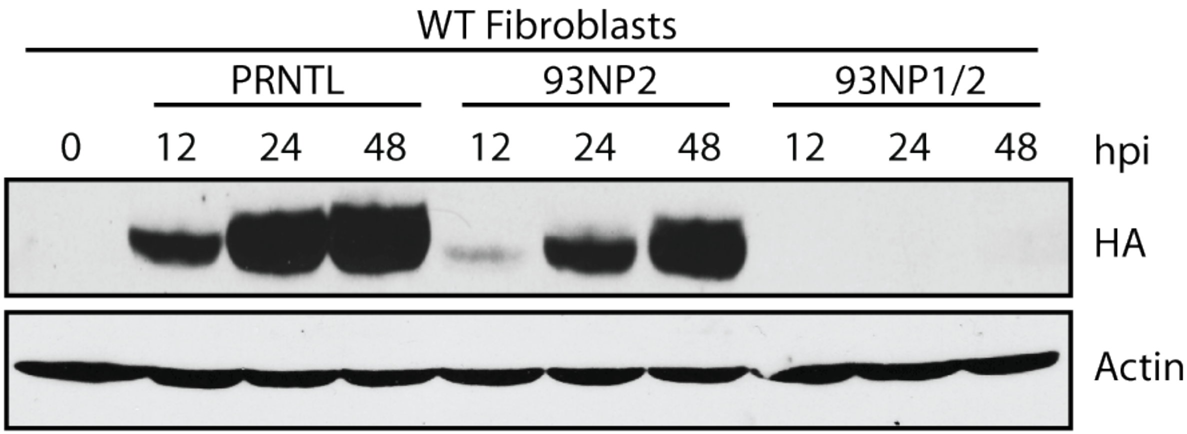
miR-93 targeting has no impact on virus replication *in ovo*



in ovo

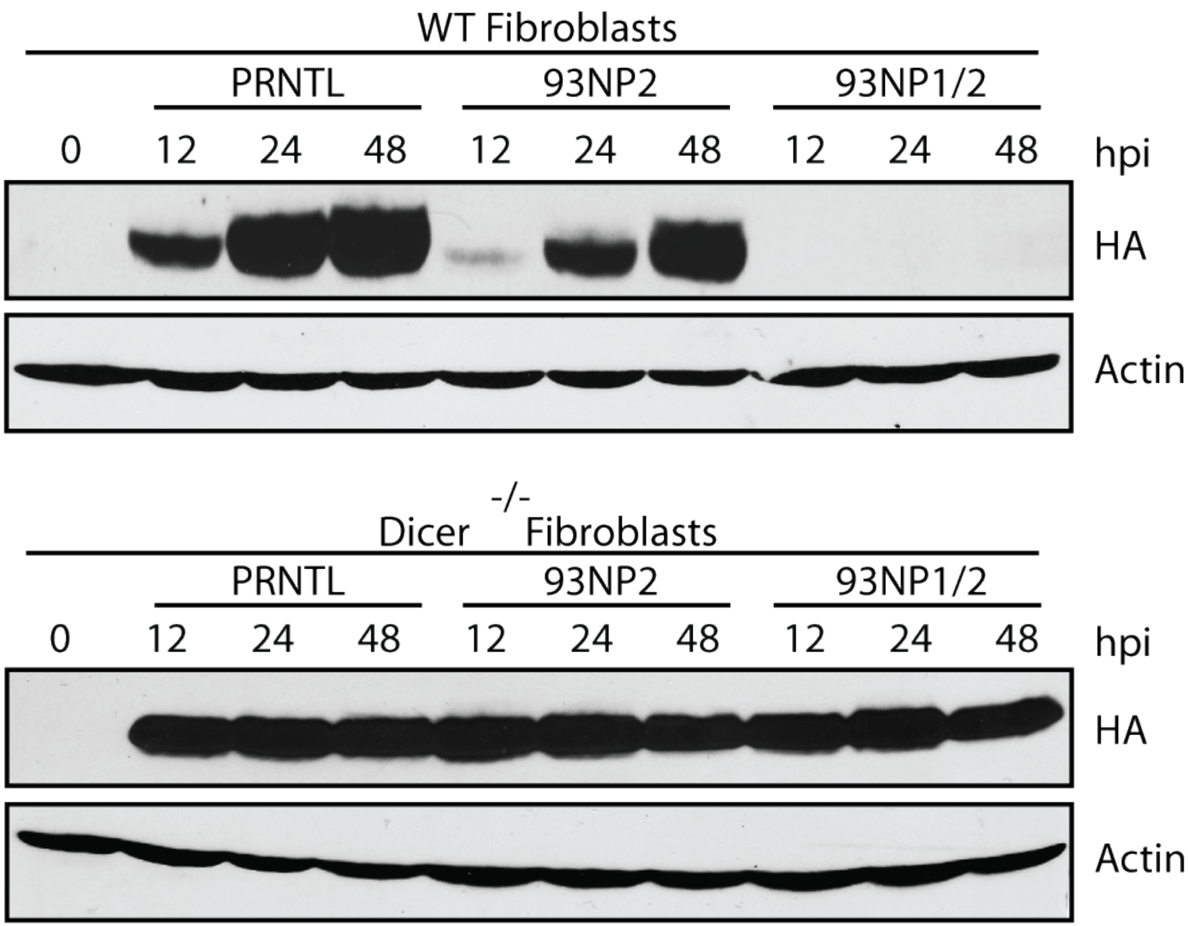
Perez et al. Nat Biotechnol (2009).

miR-93 targeting suppress virus replication in mammals *in vitro*



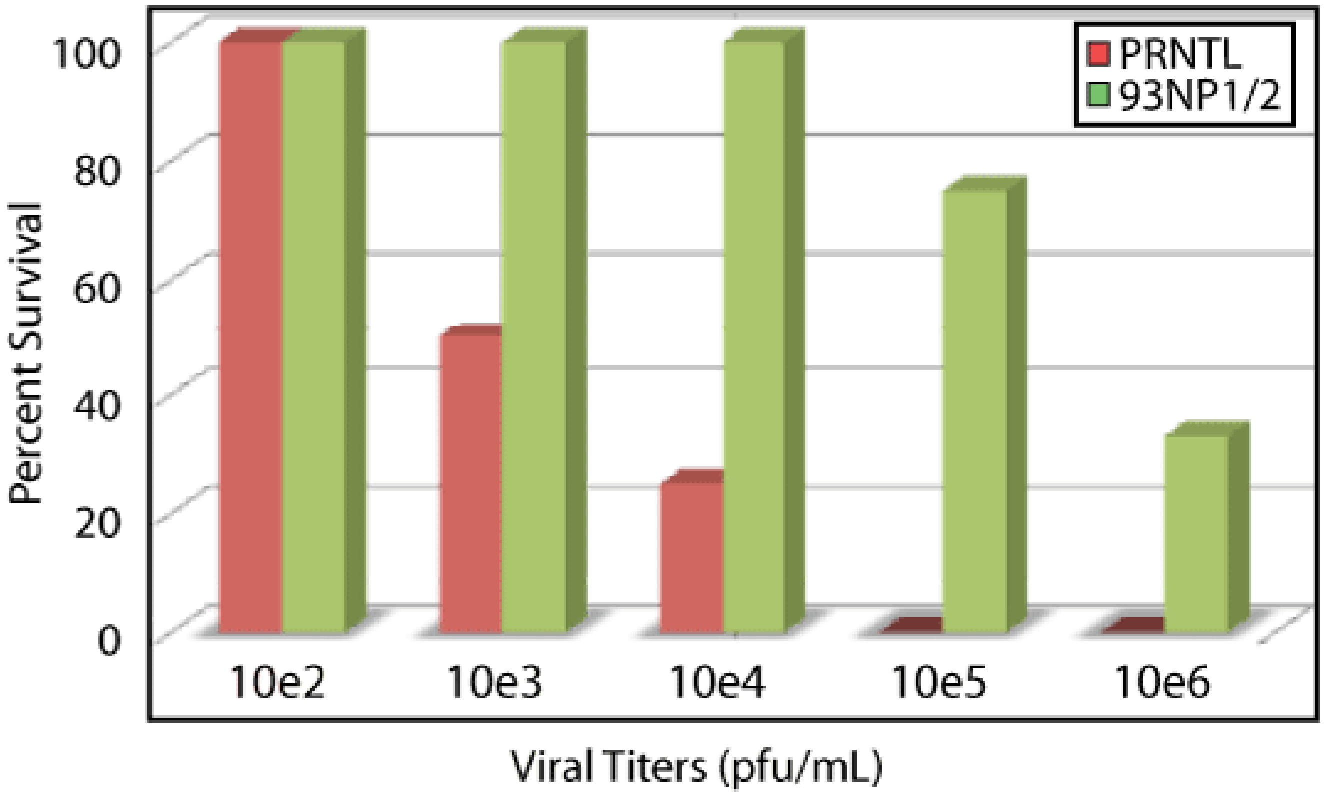
Perez et al. Nat Biotechnol (2009) 27, 572-57

miR-93 targeting suppress virus replication in mammals *in vitro*

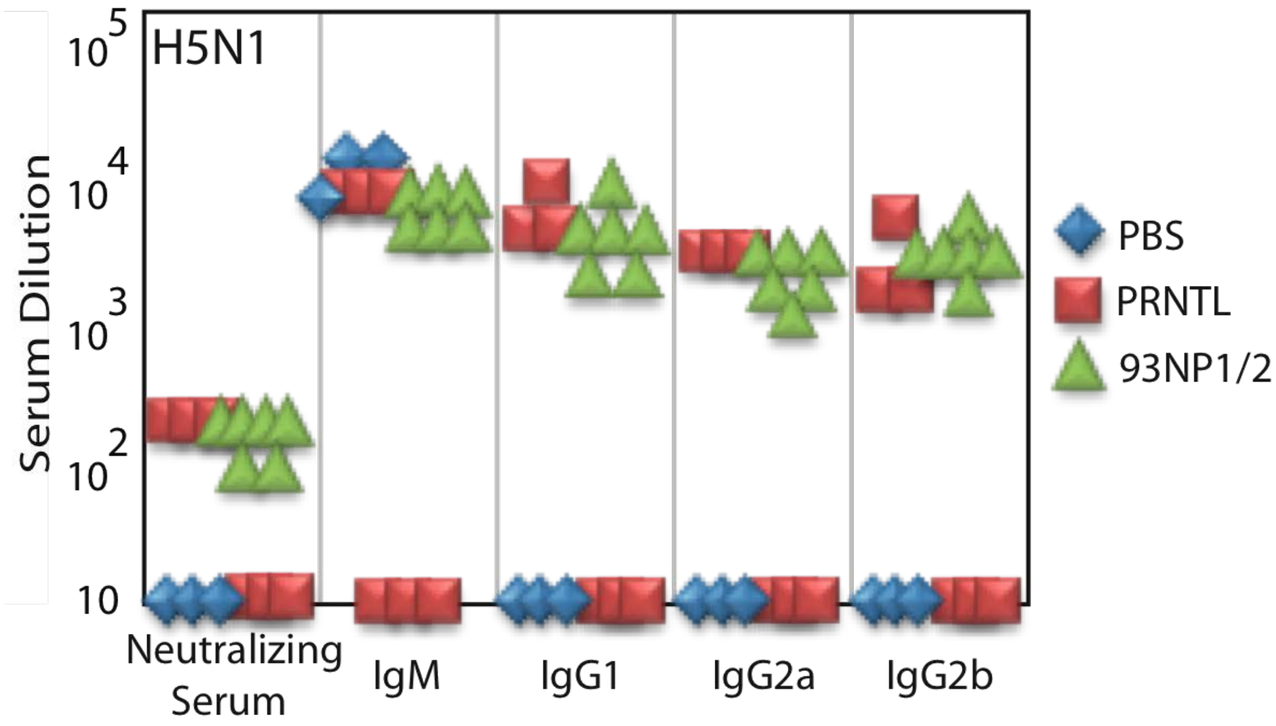


Perez et al. Nat Biotechnol (2009) 27, 572-57

miRNA-mediated, species-specific attenuation



The humoral response to miR-93 targeted virus is indistinguishable from wt infection



Perez et al. Nat Biotechnol (2009) 27, 572-57

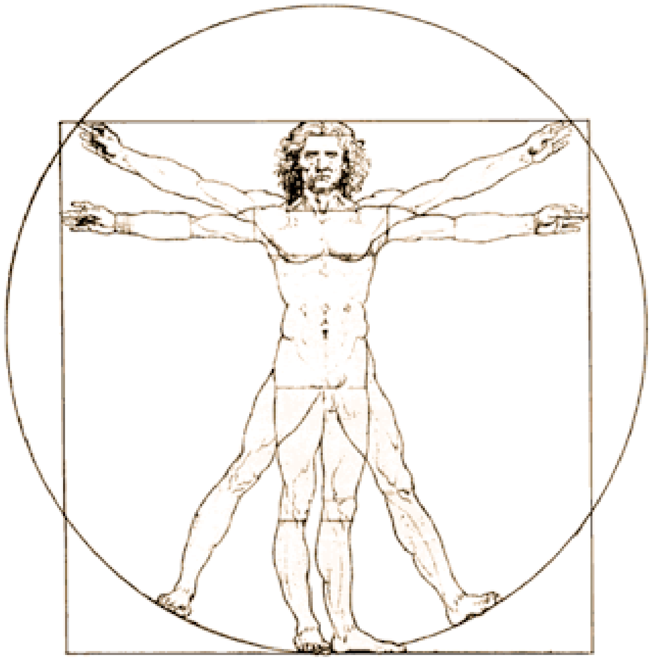
Part II. Conclusion

We can successfully co-opt the expression of host miRNAs to generate species-specific restrictions.

Part III.

How versatile is this idea? What about other viruses? Especially those that have evolved alongside RNAi-based defenses.

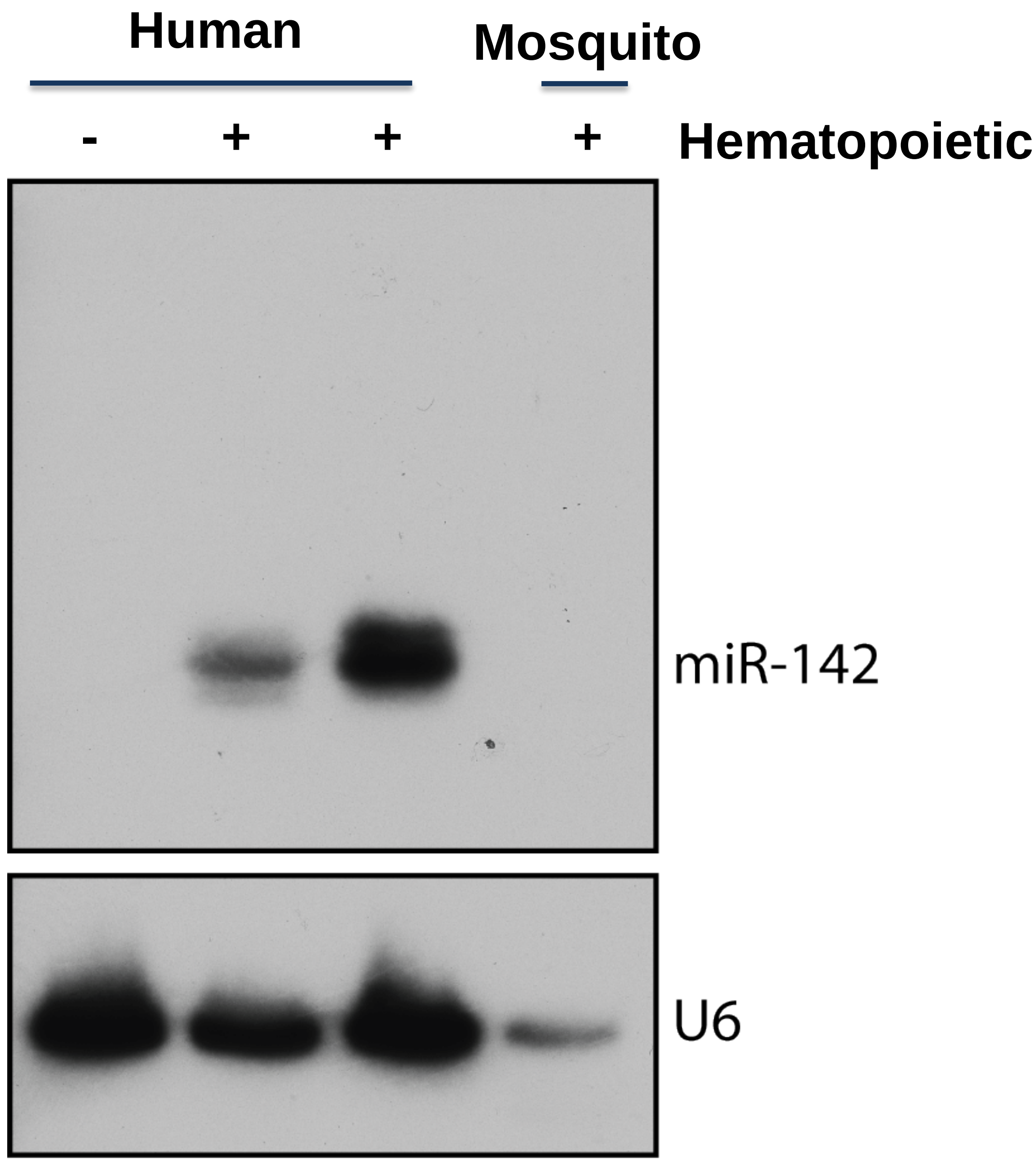
Viruses that transmit between RNAi and IFN systems: Dengue Virus



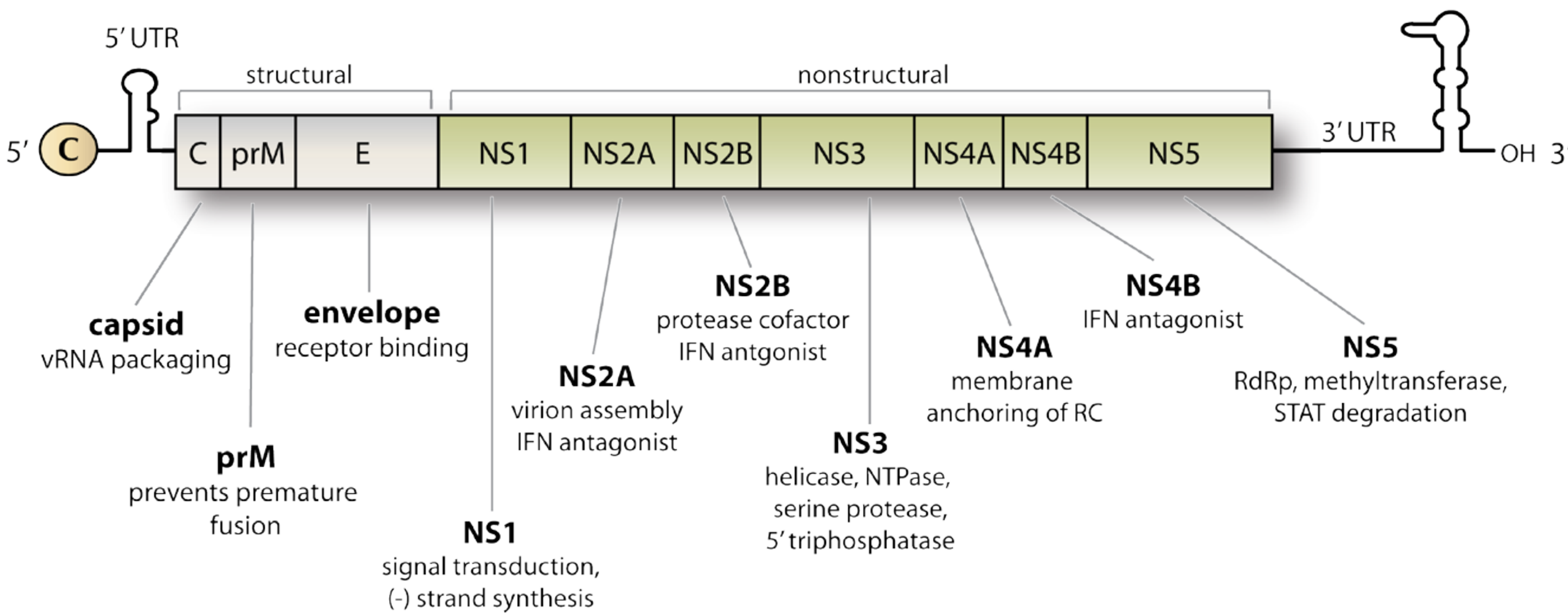
RNAi-based defenses

Interferon-based defenses

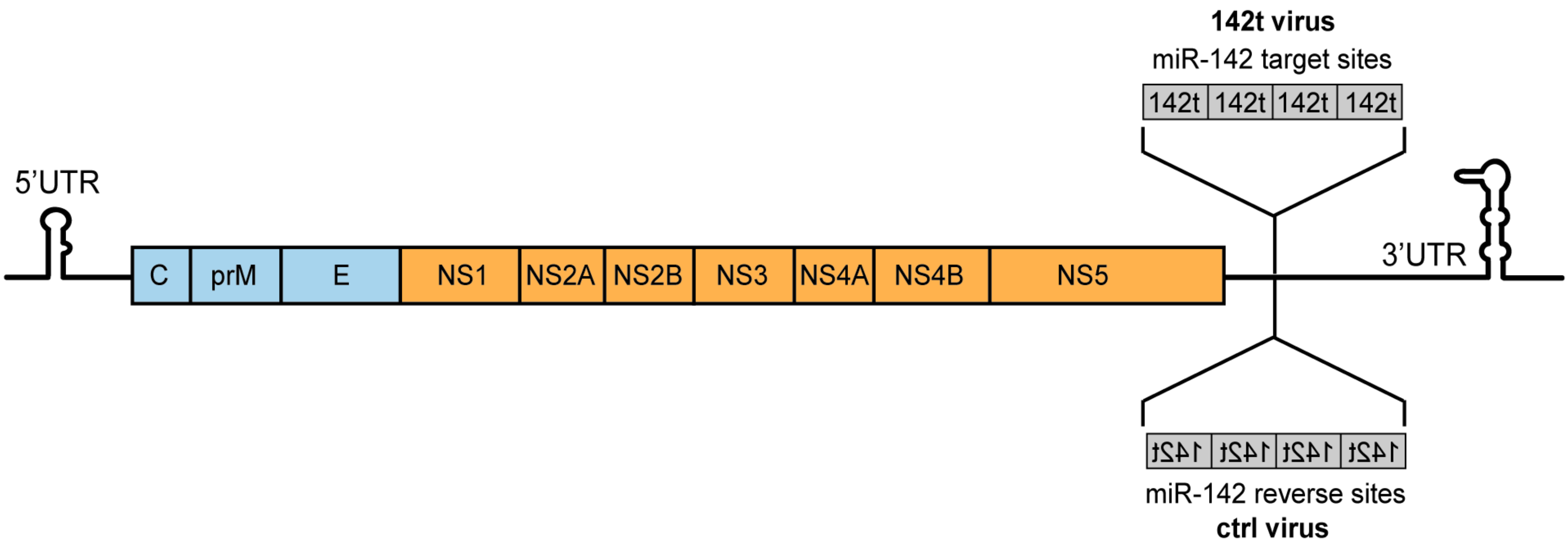
Discerning mosquito and mammalian systems



Dengue virus genomic organization

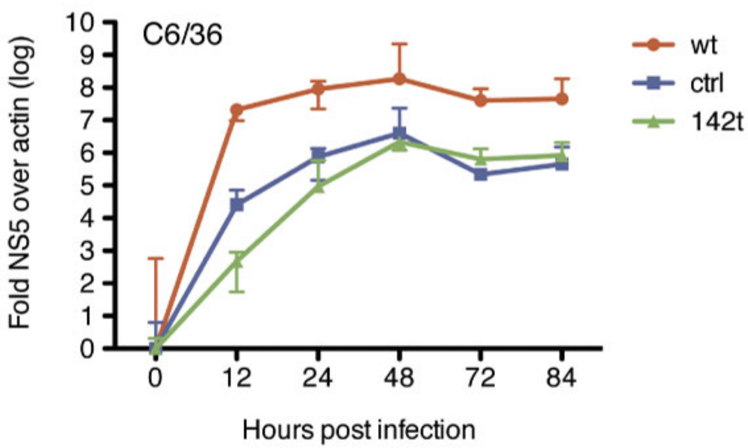


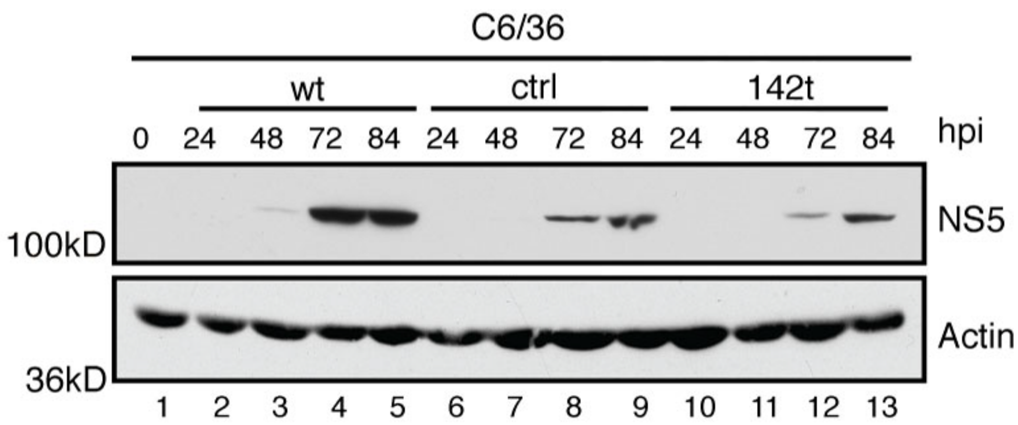
Controlling tropism with tissue-specific miRNAs



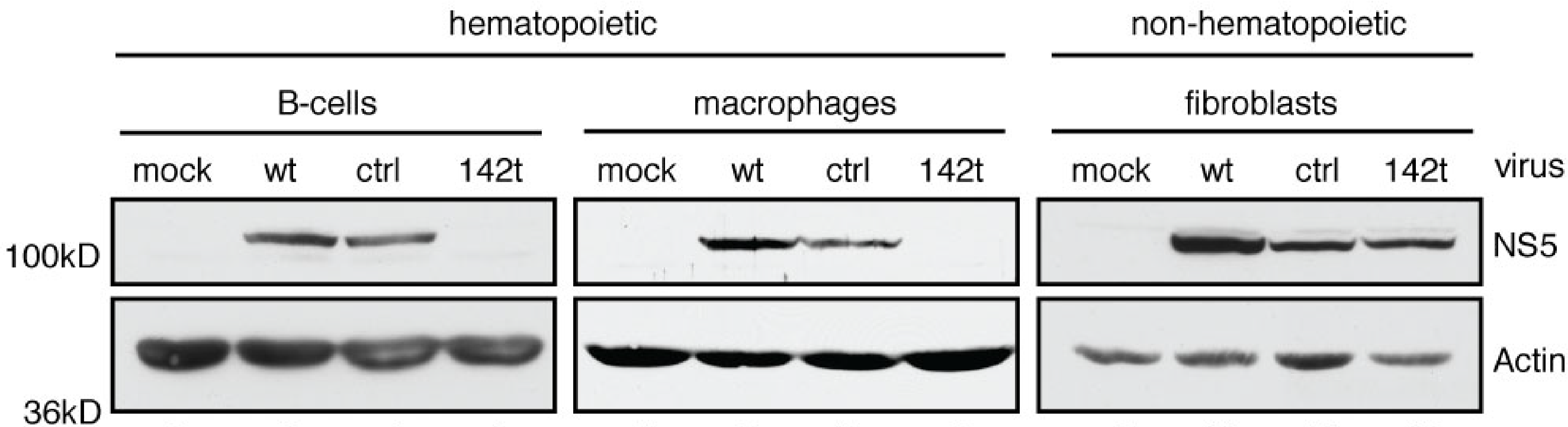
Pham et al. PLoS Path. (2012) e1002465

miR-142 targeting of Dengue had no impact on virus replication in mosquito cultures





miR-142 targeting of Dengue confines replication to non-hematopoietic cells



Part III. Conclusion

miRNA targeting is a versatile tool that can be used to attenuate diverse viruses. **Has now also been demonstrated on ZIKV.

Part IV.

How versatile is targeting specificity? Can you design viruses that discern between mammals? If so, could this be used as a ‘molecular biocontainment’ system for gain-of-function studies?

Transmissible H5N1 sparks concerns and a research moratorium

LETTER

doi:10.1038/nature10831

Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets

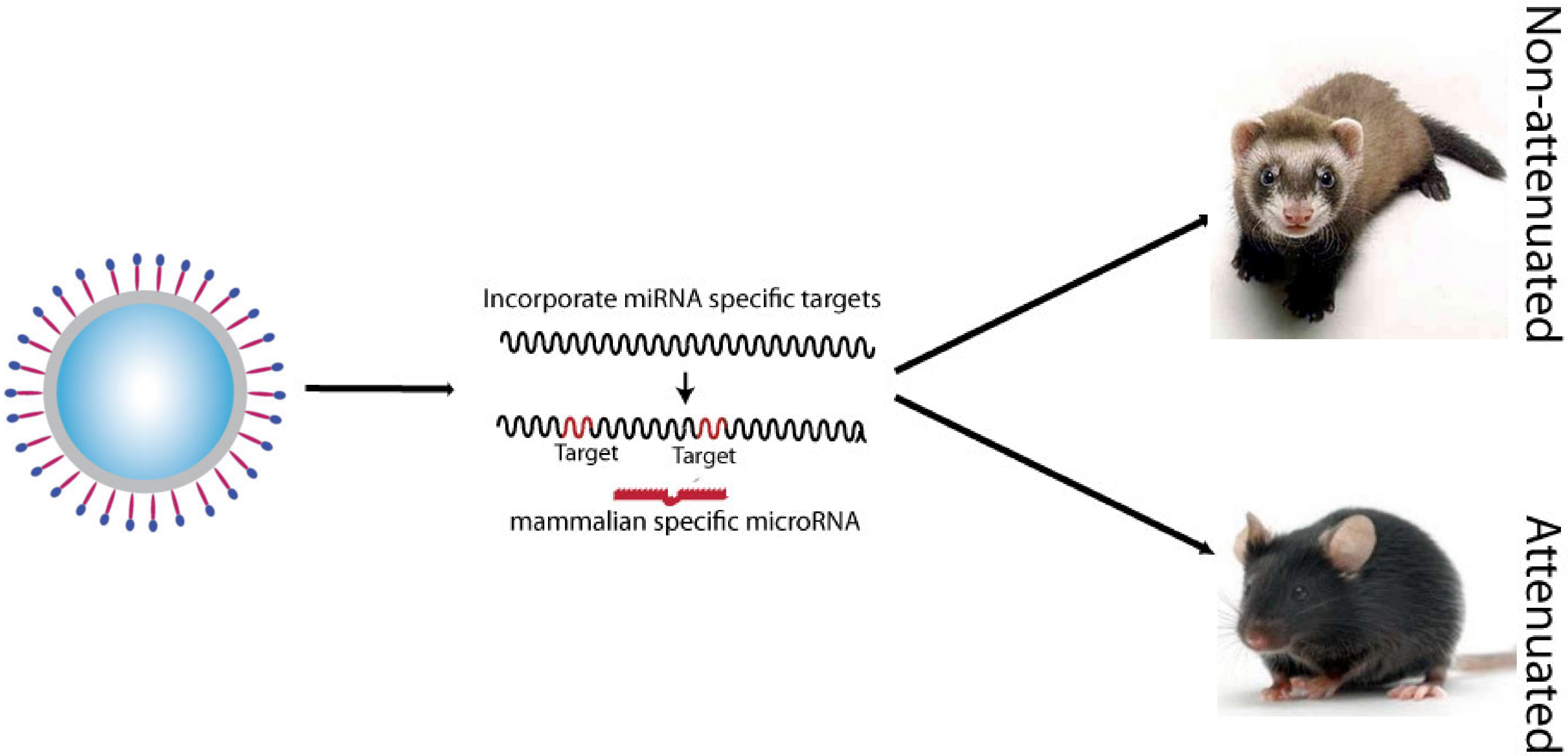
Masaki Imai[1], Tokiko Watanabe[1,2], Masato Hatta[1], Subash C. Das[1], Makoto Ozawa[1,3], Kyoko Shinya[4], Gongxun Zhong[1], Anthony Hanson[1], Hiroaki Katsura[5], Shinji Watanabe[1,2], Chengjun Li[1], Eiryo Kawakami[2], Shinya Yamada[5], Maki Kiso[5], Yasuo Suzuki[6], Eileen A. Maher[1], Gabriele Neumann[1] & Yoshihiro Kawaoka[1,2,3,5]

REPORT

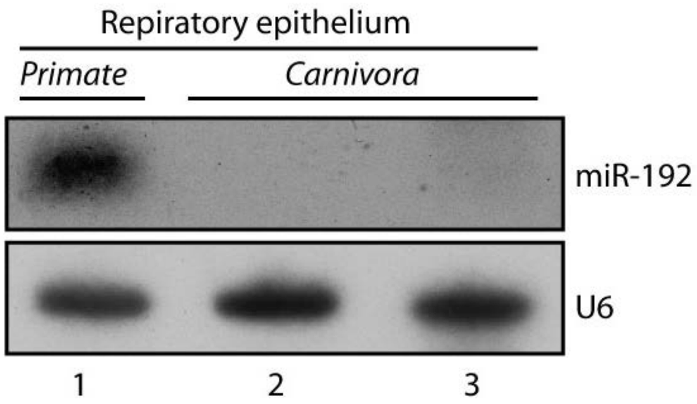
Airborne Transmission of Influenza A/H5N1 Virus Between Ferrets

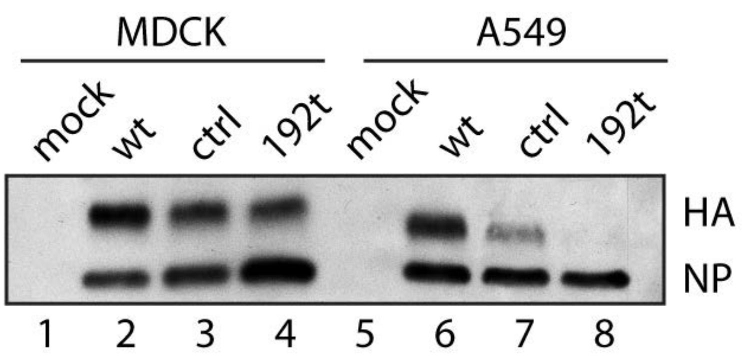
Sander Herfst,[1] Eefje J. A. Schrauwen,[1] Martin Linster,[1] Salin Chutinimitkul,[1] Emmie de Wit,[1]* Vincent J. Munster,[1]* Erin M. Sorrell,[1] Theo M. Bestebroer,[1] David F. Burke,[2] Derek J. Smith,[1,2,3] Guus F. Rimmelzwaan,[1] Albert D. M. E. Osterhaus,[1] Ron A. M. Fouchier[1]†

Restricting IAV replication to ferrets

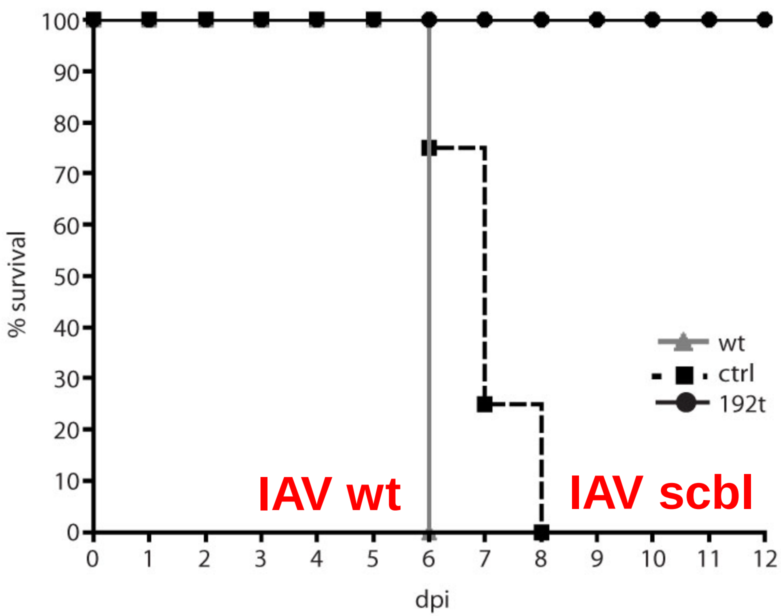


miR-192 targeting of IAV HA



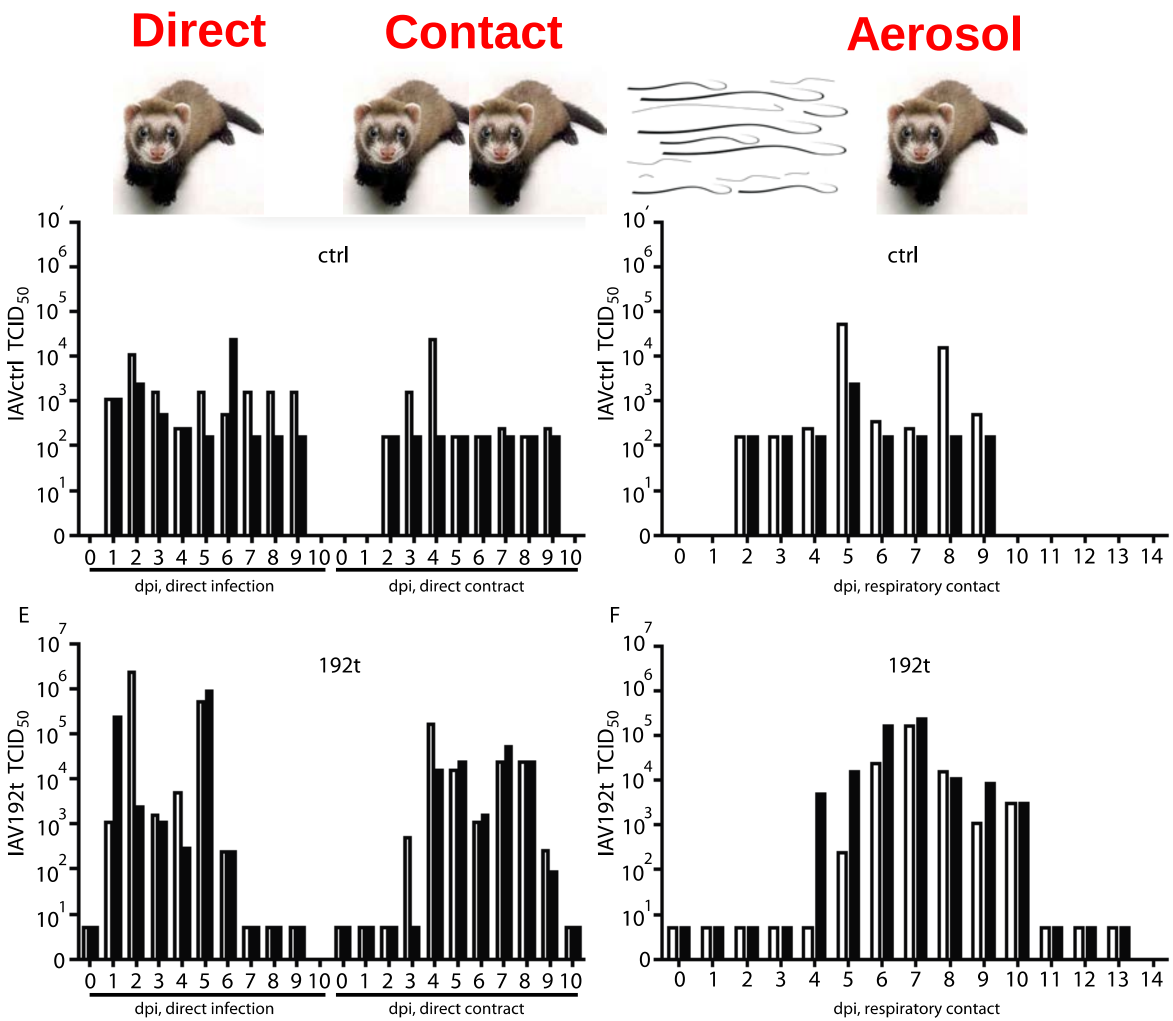


miR-192 targets attenuate flu replication by four logs in mice



Langloiss et al. Nat. Bio (2012)

miR-192 targets do not impact flu replication and transmission in ferrets



Langloiss et al. Nat. Bio (2012)

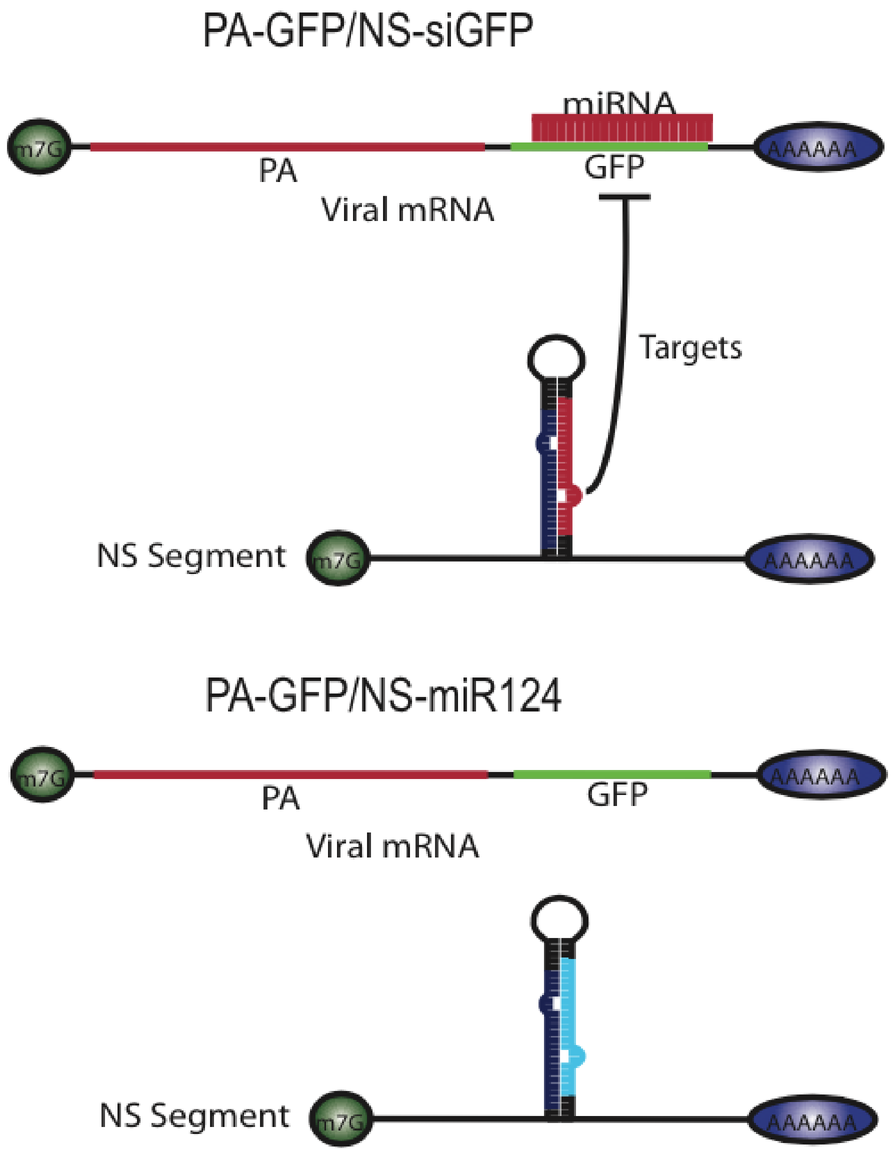
Part IV. Conclusion

miRNA targeting appears to have no limits and can be used as a molecular biocontainment system.

Part V.

What about viral escape?

Can we generate escape mutants?



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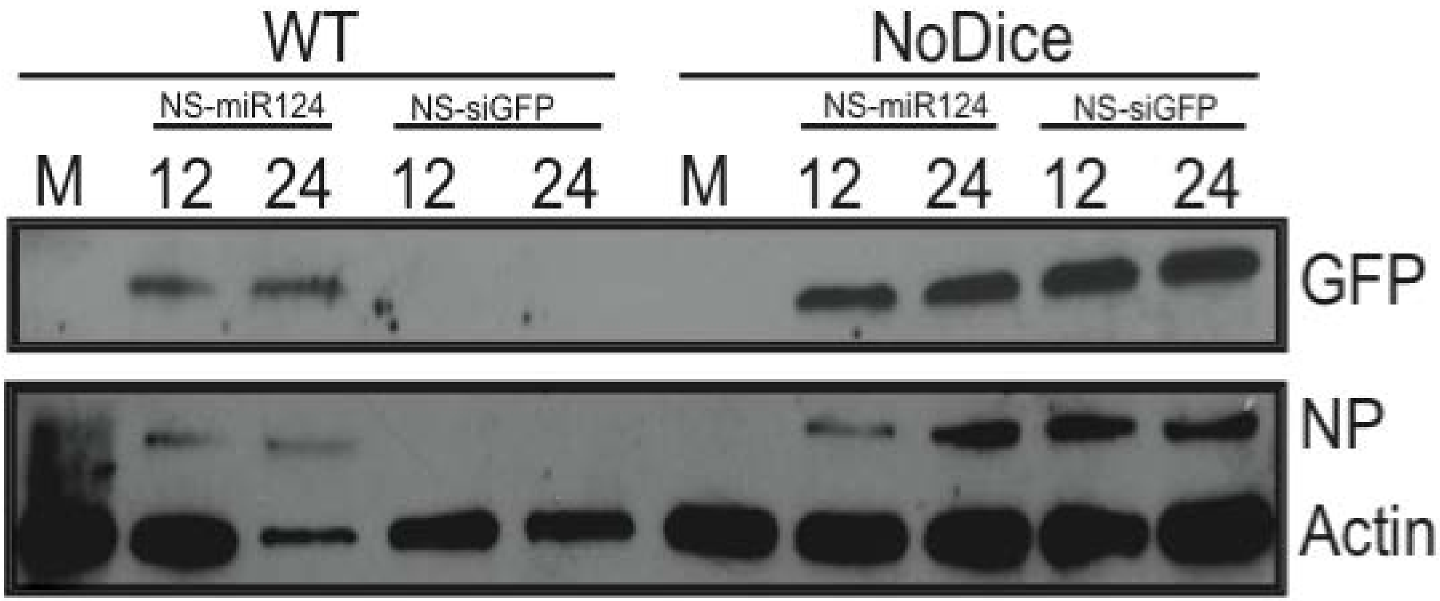
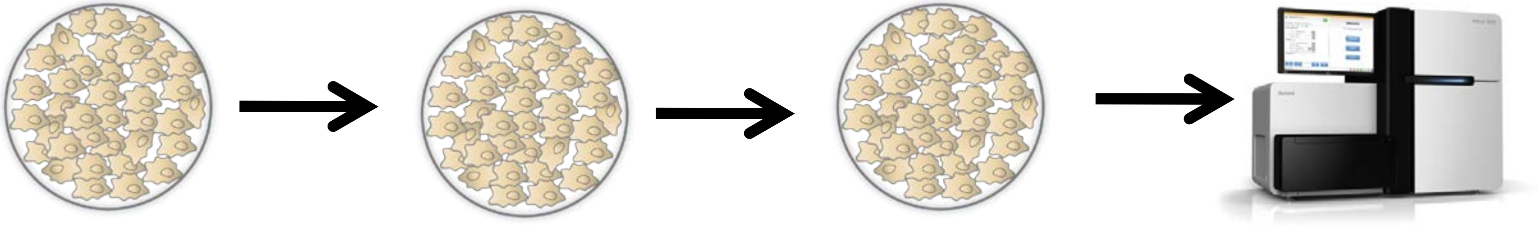


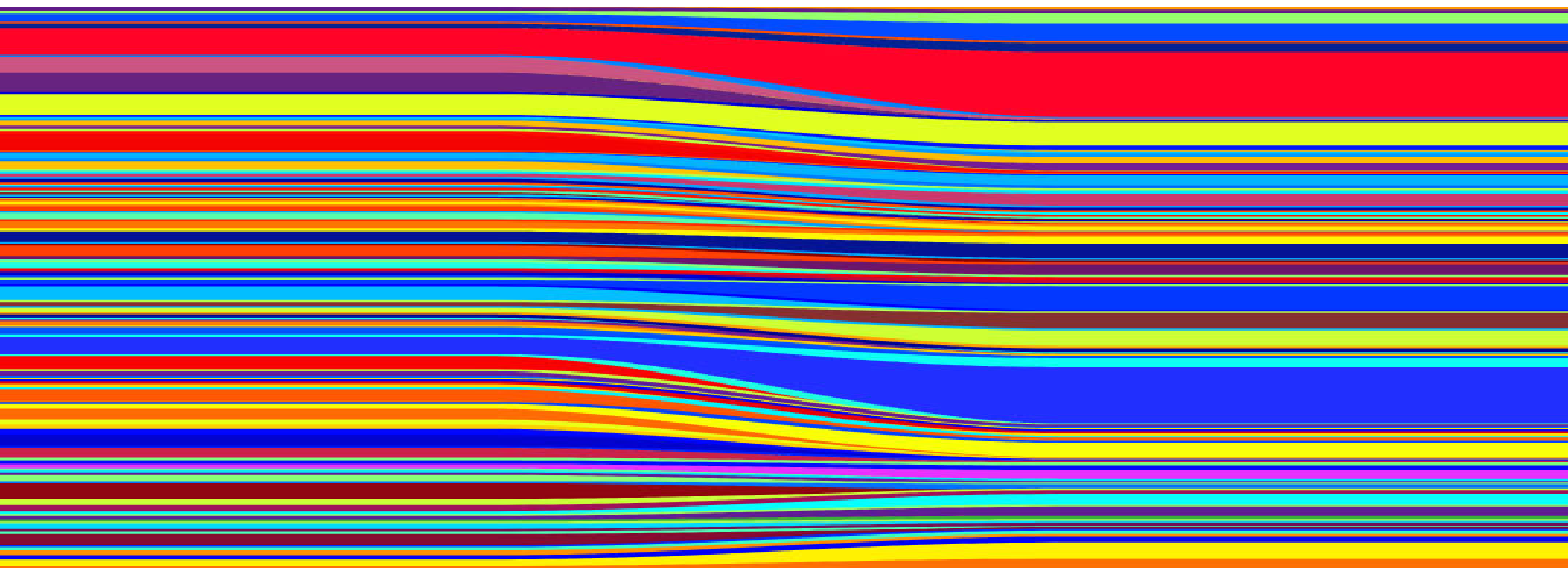
Escape in the absence of selective pressure?

NoDice Cells



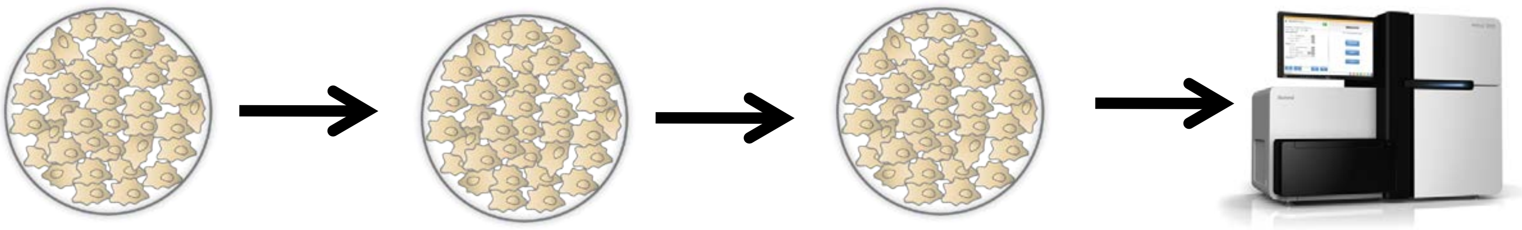
Escape in the absence of selective pressure?

NoDice Cells



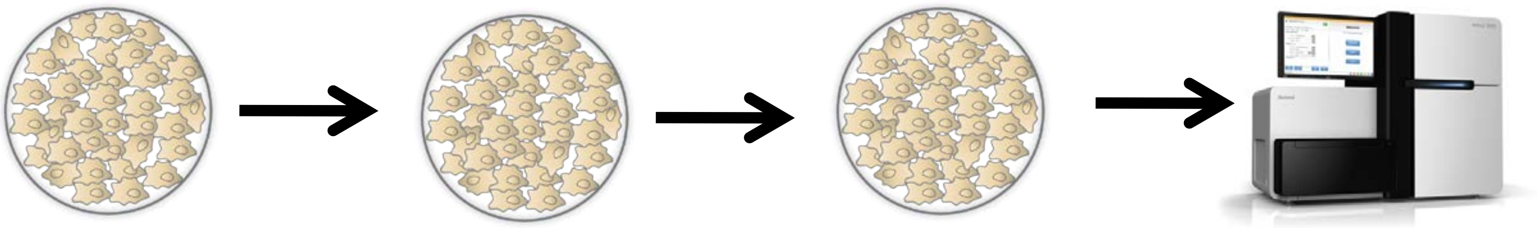
Escape in the presence of selective pressure?

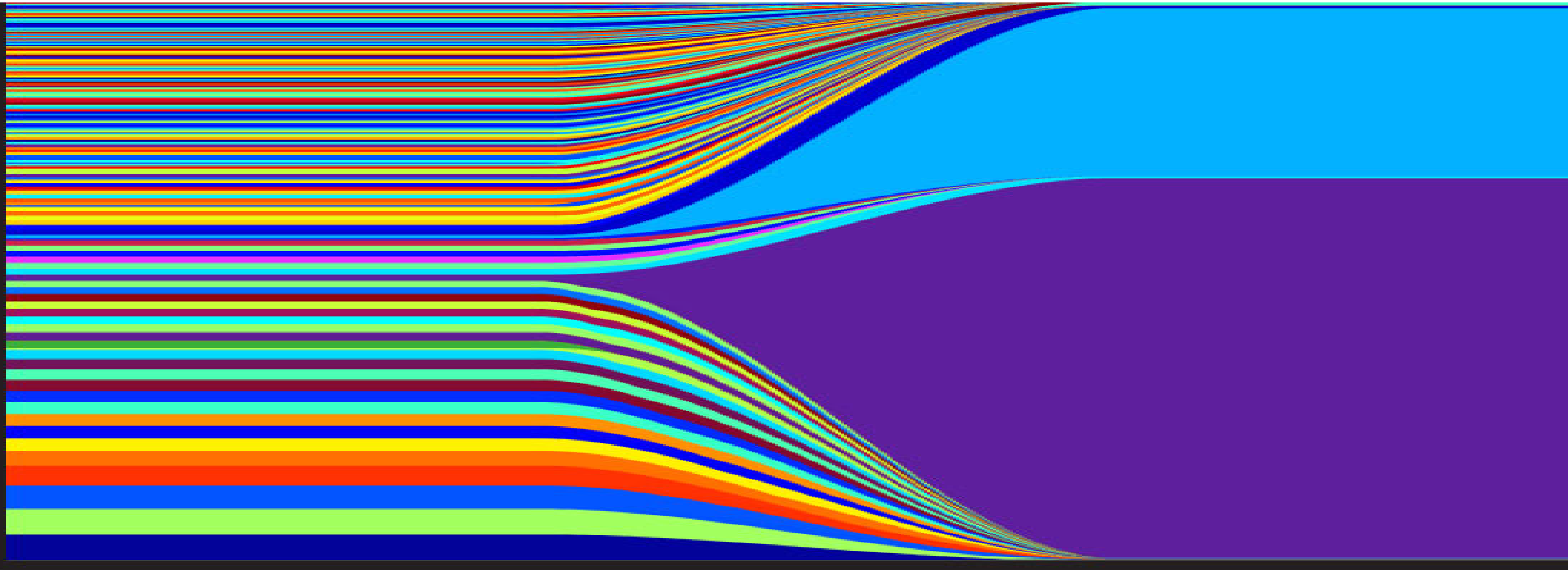
WT Cells



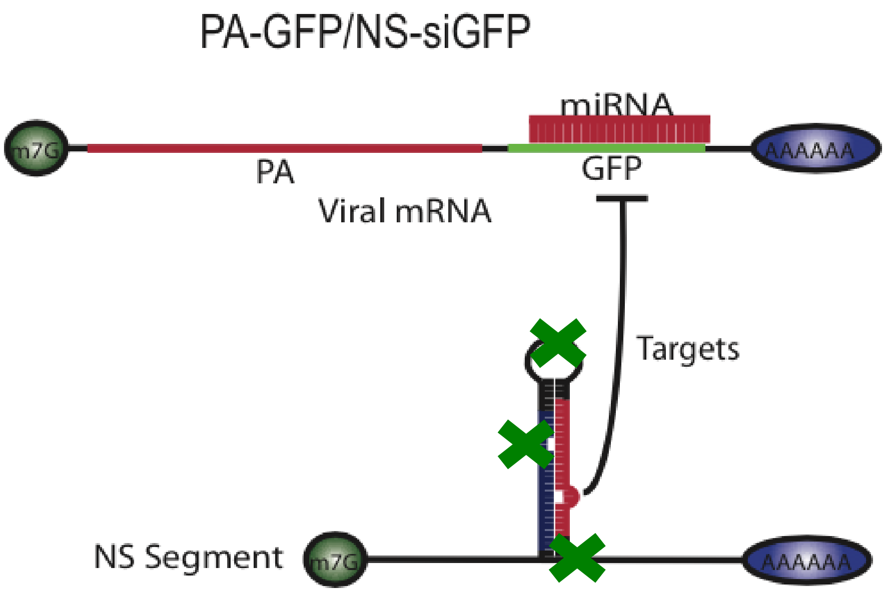
Escape in the presence of selective pressure?

WT Cells



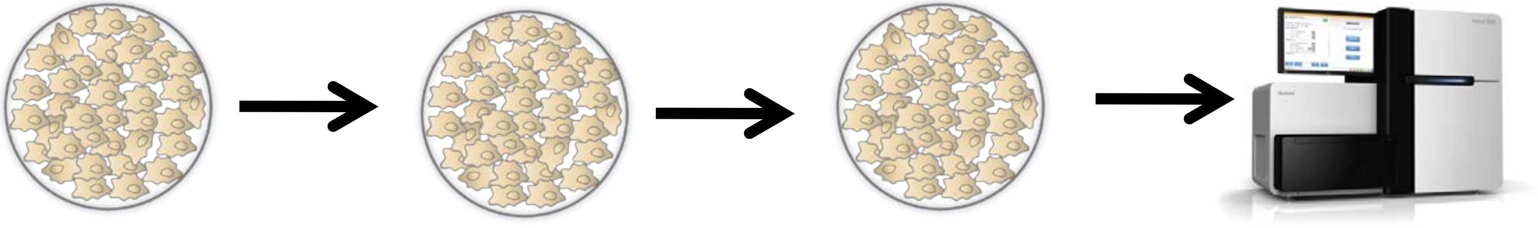


Escape mutants ALL confined to hairpin mutations



What about other viruses? Dengue?

WT Cells



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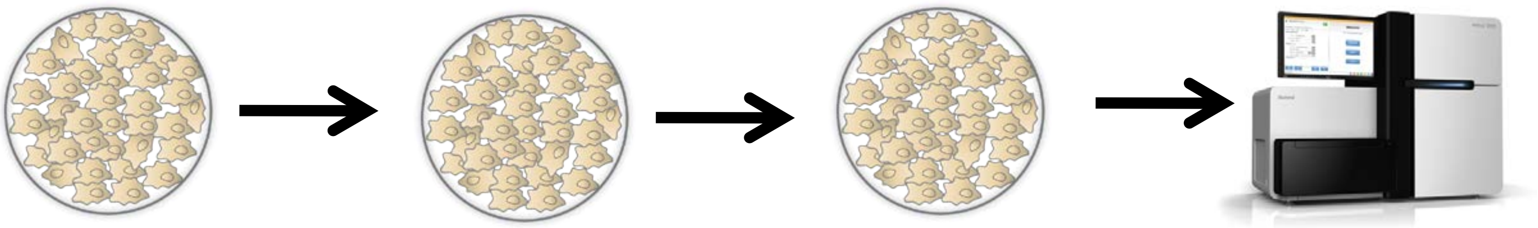
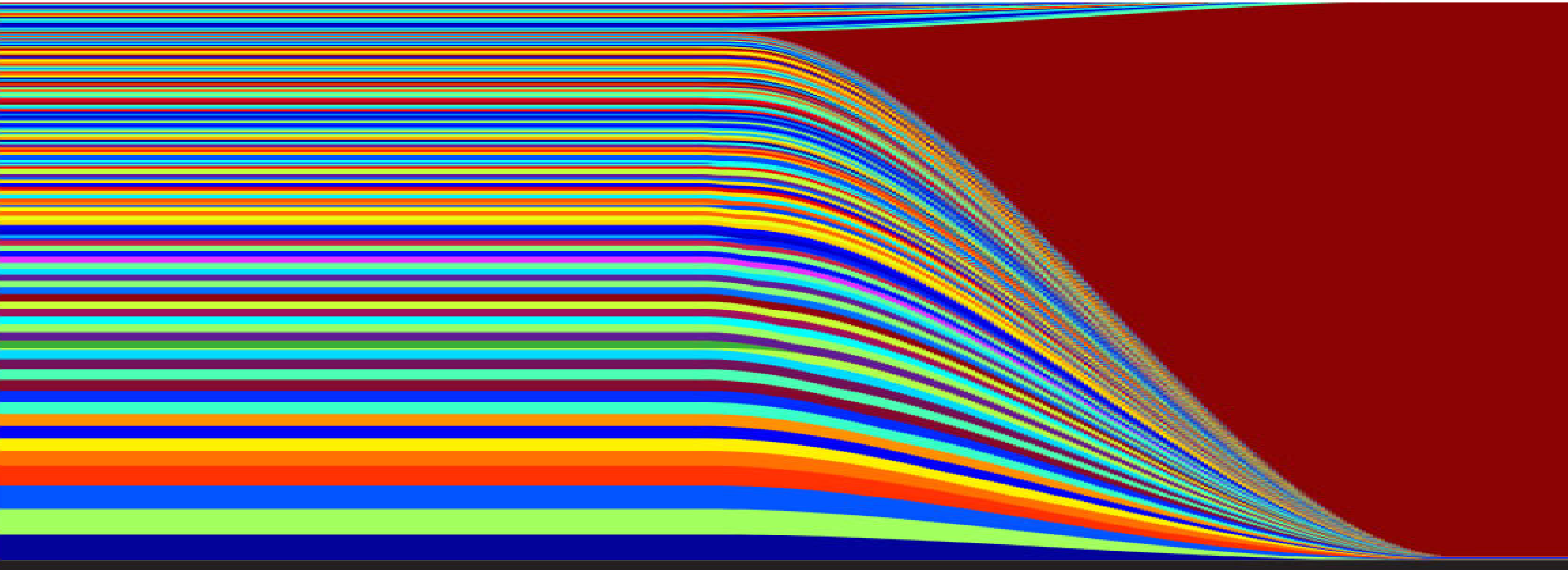
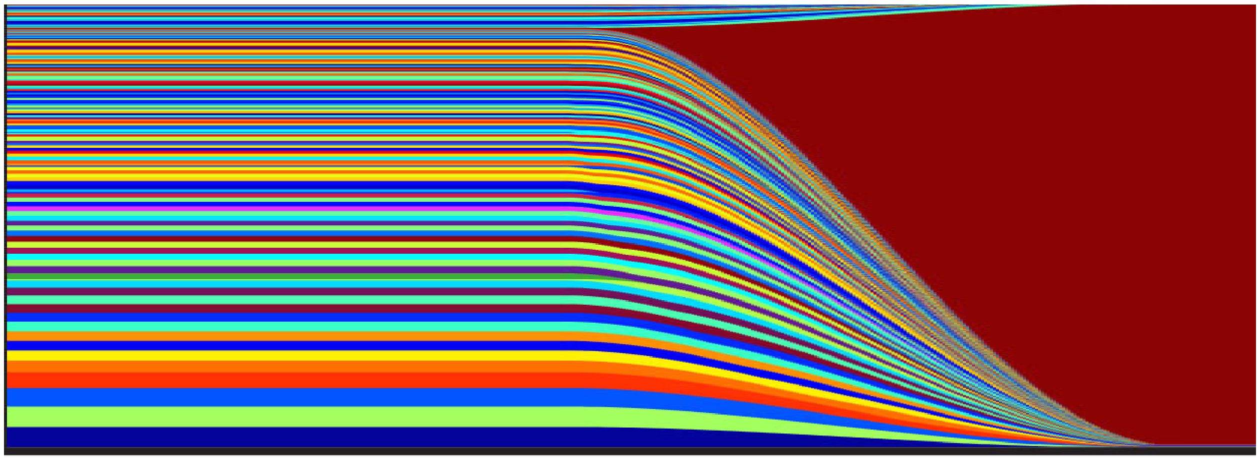




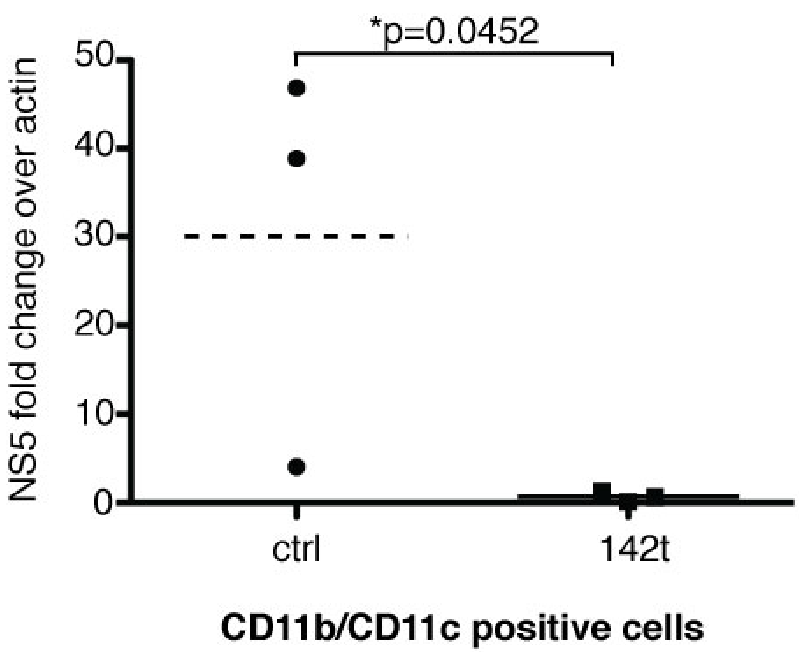
Escape demands target site excision

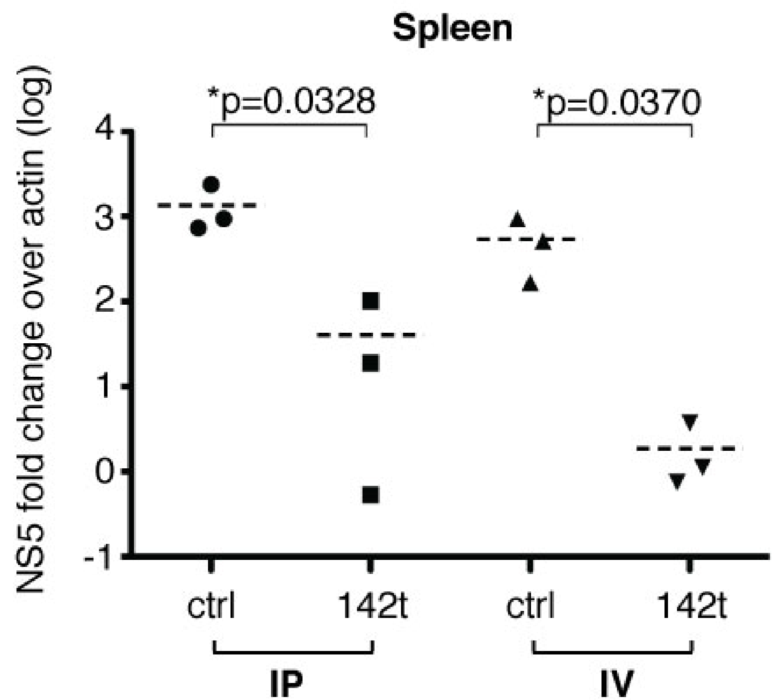


Mutations observed in miR-142 target sites of recombinant DENV viruses

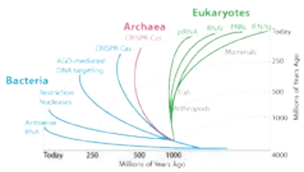
ctrl	142T
UGUAGUGUUUCCUACUUUAUGGA	UCCAUAAAGUAGGAAACACUACA
UGUAGUG**C**UUCCUACUUUAUGGA	**GAGUCUCCUCUAGUUAAGUA** *(insertion of WD67 repeat domain)*
UGUAGUGUUUCCUACUU- AUGGA	
UGUAGUGUUUCCUA**U**UUUAUGGA	*complete excision of miR-142 target sites*
AGUAGUGUUUCCUACUUUAUGGA	
UGUAGUGUUUCCUACUUUA**G**GGA	

Despite the emergence of escape mutants, miRNA-targeted DENV still induces no disease

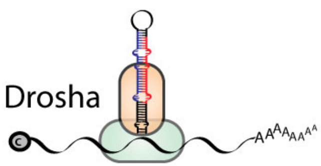




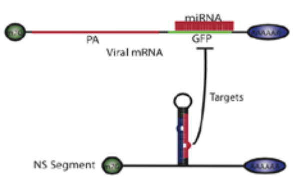
Conclusions



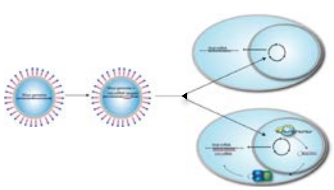
Small RNAs evolved to block both DNA and RNA viruses for much of life's history. Chordates largely replaced this system for interferon.



Mammals lost RNAi but retained microRNAs



The capacity to "self-target" suggests no selective pressure imposed by small RNA machinery



An RNAi-like response can be established in mammals artificially and can be used as a molecular biocontainment system.

Acknowledgments

Icahn School of Medicine at Mount Sinai

The tenOever Lab (present):

- Daniel Melo, Ph.D.
- David Sachs
- Lauren Aguado
- Julie Eggenberger
- John Heard
- Rasmus Moeller
- Maryline Panis
- Ismarc Reyes

The tenOever Lab (past):

- Laura Spanko
- Sonja Schmid, Ph.D.
- Asiel Benitez, Ph.D.
- Jasmie Perez,Ph.D.
- Alissa Pham, Ph.D.
- Andrew Varble, Ph.D.
- Ryan Langlois, Ph.D.
- Simone Backes, Ph.D.

Adolfo Garcia-Sastre, Ph.D.
Randy Albrecht, Ph.D.
Daniel Perez, Ph.D. (U. Maryland)
Bryan Cullen,PhD (Duke)

BURROUGHS WELLCOME FUND

NATIONAL INSTITUTES OF HEALTH

DEPARTMENT OF DEFENSE UNITED STATES OF AMERICA

Why did mammals abandon RNAi?

Science AAAS

Antiviral RNA Interference in Mammalian Cells
P. V. Maillard *et al.*
Science **342**, 235 (2013);
DOI: 10.1126/science.1241930

Science AAAS

RNA Interference Functions as an Antiviral Immunity Mechanism in Mammals
Yang Li *et al.*
Science **342**, 231 (2013);
DOI: 10.1126/science.1241911

Journal of Virology

Replication of Many Human Viruses Is Refractory to Inhibition by Endogenous Cellular MicroRNAs

Hal P. Bogerd[a], Rebecca L. Skalsky[a*], Edward M. Kennedy[a], Yuki Furuse[a], Adam W. Whisnant[a], Omar Flores[a], Kimberly L. W. Schultz[b], Nicole Putnam[b], Nicholas J. Barrows[a], Barbara Sherry[c], Frank Scholle[d], Mariano A. Garcia-Blanco[a], Diane E. Griffin[b] and Bryan R. Cullen[a]

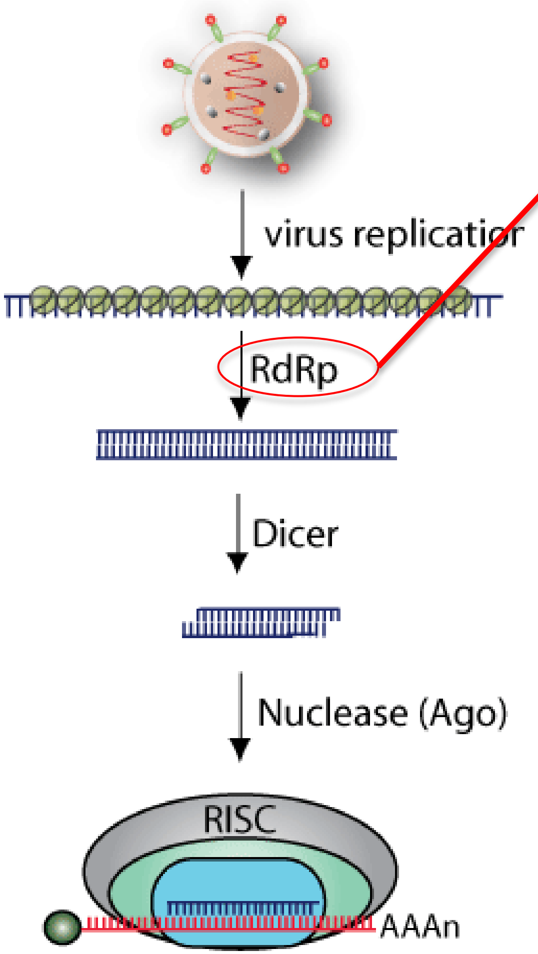
Cell Reports
Article

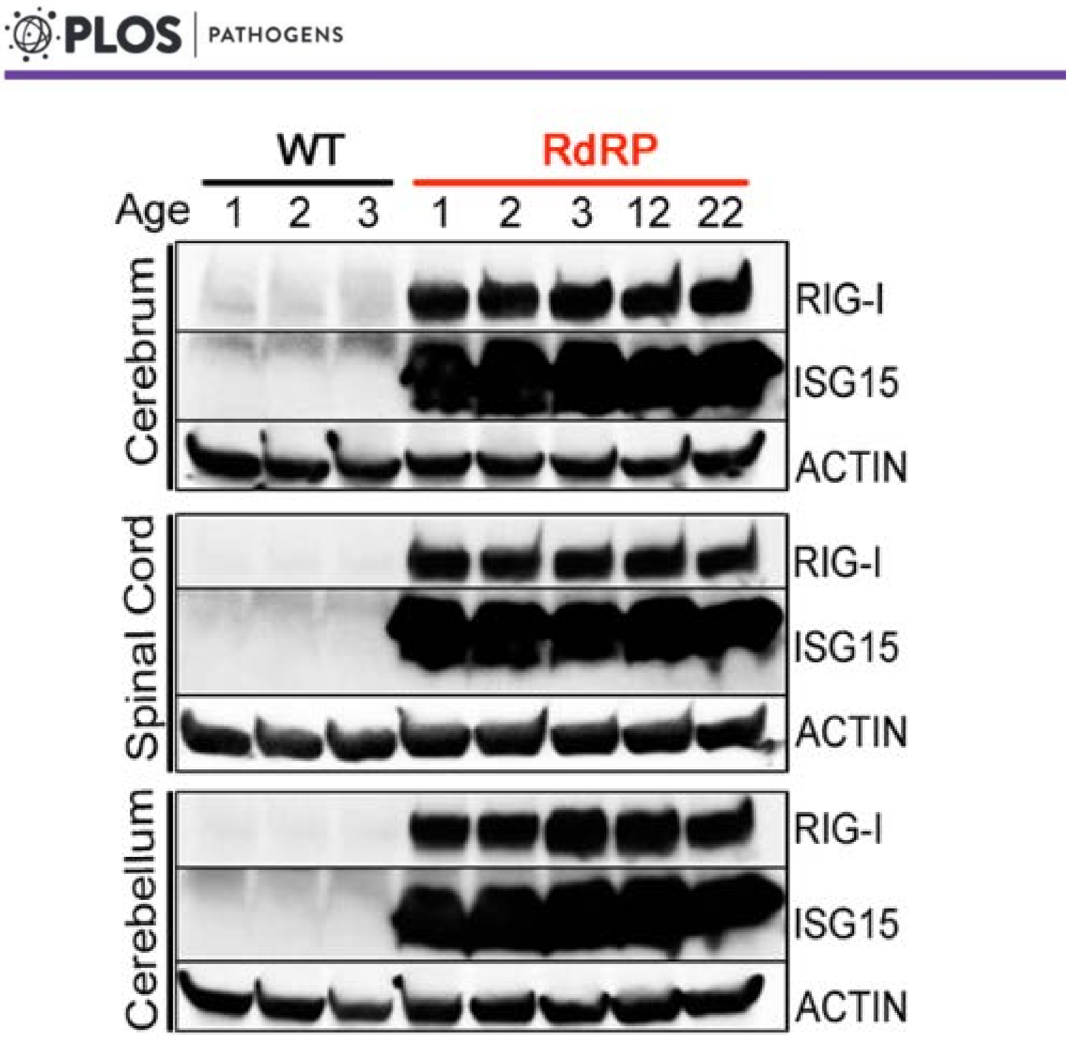
The Mammalian Response to Virus Infection Is Independent of Small RNA Silencing

Simone Backes,[1] Ryan A. Langlois,[1] Sonja Schmid,[1] Andrew Varble,[1] Jaehee V. Shim,[1] David Sachs,[2] and Benjamin R. tenOever[1,3,*]

RdRp Incompatibility

Antiviral RNAi

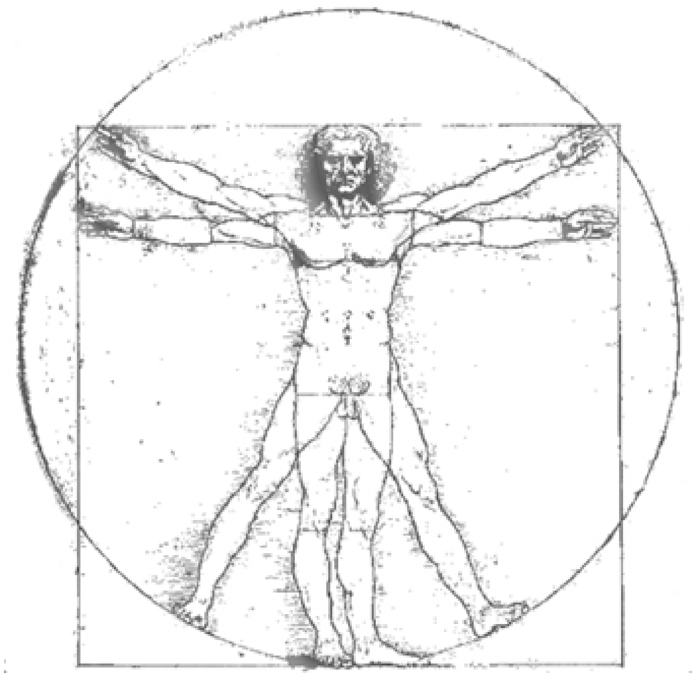




Size and the need for amplification

RNAi
-many RdRps

RNAi
-no RdRp
-transport



~~RNAi~~
-no RdRp
-transport not possible

Cellular Tools for Virus Wareface