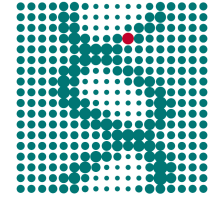




**Universität
Zürich** ^{UZH}



**UniversitätsSpital
Zürich**



MPIMG

Reverse Transcriptase-RNase H

Karin Moelling
Zürich/Berlin



Heinrich-Pette-Institut
Leibniz-Institut für Experimentelle Virologie



Wissenschaftskolleg zu Berlin



www.moelling.ch

KMoelling, Salzburg 2014

www.academia-net.org

Viruses and antiviral defense



RNA tulip virus



silencing

Summary

Reverse Transcriptases

RNases H

Viruses and antiviral defenses

siRNA

CRISPR, siDNA

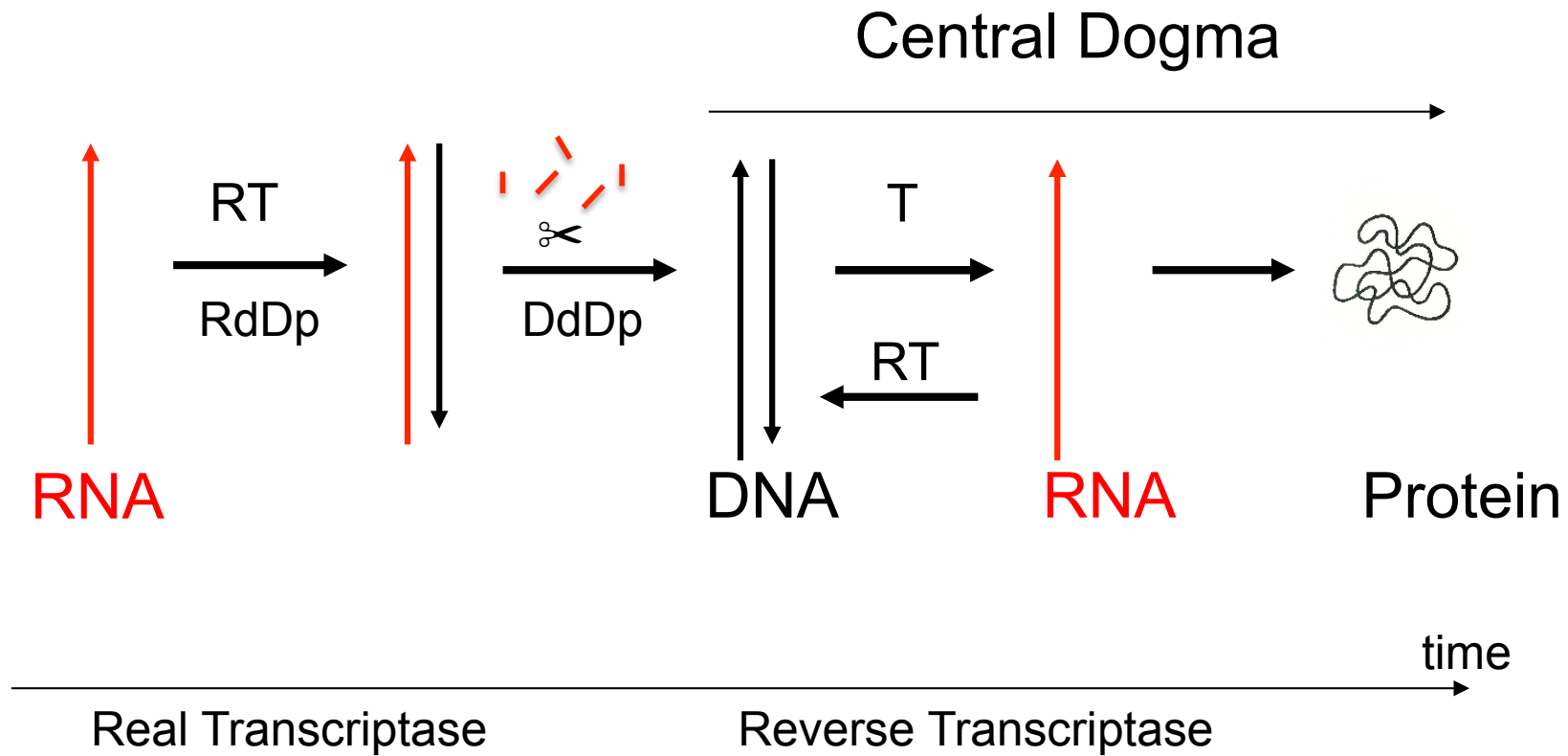
IFN, IgG,

„RNases H“

Surprises! Synopsis

Speculations

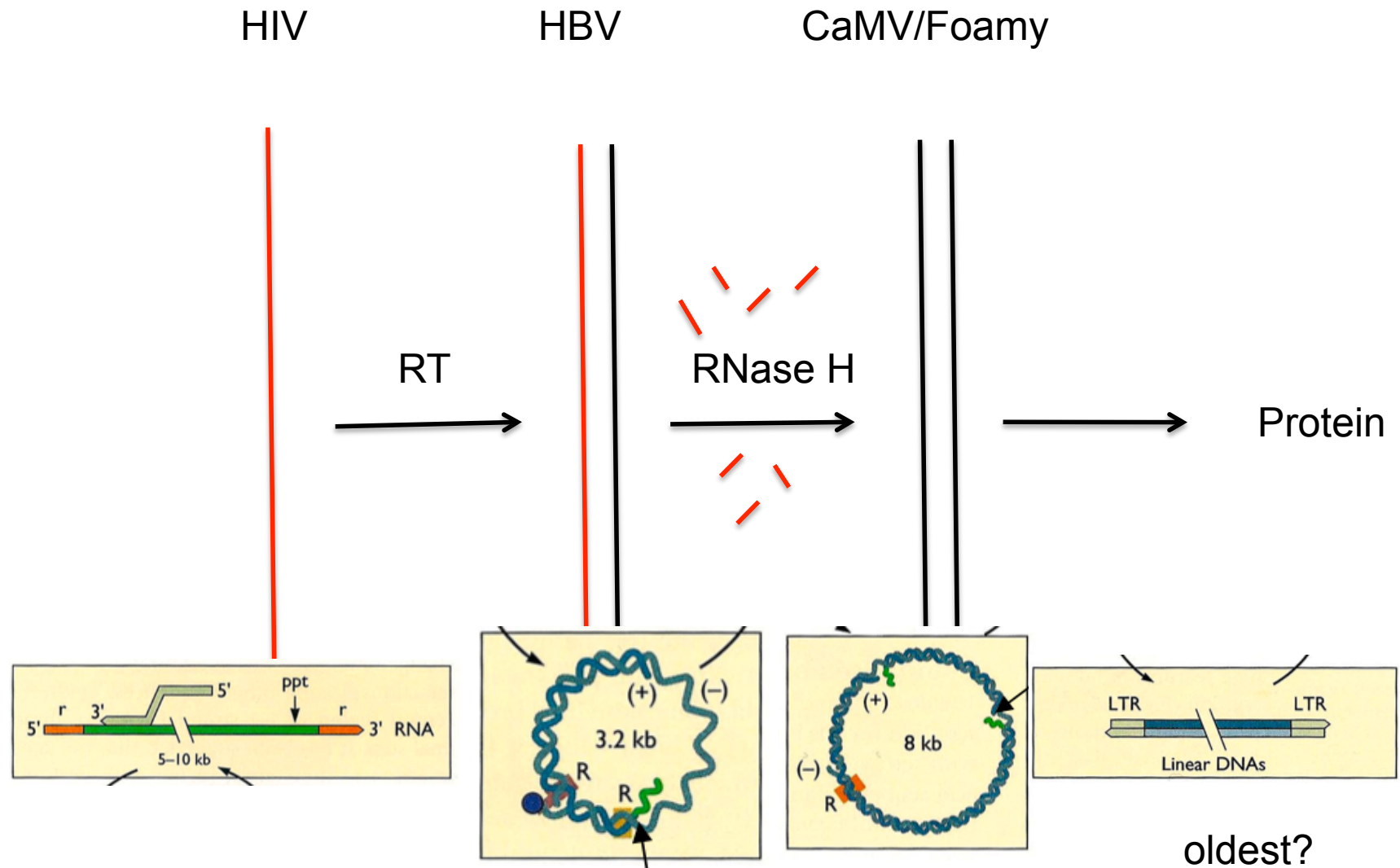
some own data



Retroviruses/Retrotransposons degenerate over time

| | | Deduced amino acid sequence | | | | | | | | | | | |
|----|----------------------------------|-----------------------------|-----|----------------|----------------|----|----|---|----------------|----------------|----|----------------|---------|
| | | LTRs | PBS | CA | NC | PR | RT | T | RH | H/C | IN | Env | Poly(A) |
| I | Retroviruses | + | + | + | + | + | + | + | + | + | + | + | + |
| | Orphan class | + ^a | — | — | — | — | + | — | + | — | — | — | — |
| | Retrotransposons (Metaviridae) | + | + | + ^b | — ^c | + | + | — | + | + | + | — ^d | + |
| | Caulimoviruses | — | + | + ^b | + | + | + | — | + | — | — | — | + |
| | Hepadnaviruses | + ^e | — | + ^b | — | — | + | — | + | — | — | — | + |
| | Retrotransposons (Pseudoviridae) | + | + | + ^b | + ^c | + | + | — | + | + | + | — | + |
| | LINEs | — | — | + | — | — | + | + | + | — | — | — | + |
| | | — | — | + | + ^f | — | + | + | + | — | — | — | + |
| | | — | — | + | — | — | + | — | — | + ^g | + | — | + |
| | LINEs | — | — | + | + ^f | — | + | + | + | — | — | — | + |
| II | Group II introns | — | — | — | — | + | + | + | — | + | — | — | — |
| | Group II plasmids | — | — | — | — | — | + | — | + | — | — | — | — |
| | Retrons | — | — | — | — | — | + | — | + ^h | — | — | — | + |
| | Telomerase | — | — | — | — | — | + | — | — | — | — | — | + |

Retro-and Para-retroviruses



HIV uses RT after release and for infection, HBV uses RT before release, no integration

Structures of some RTs

Group II intron

D for catalysis and mobility
En endonuclease
IEP intron expressed Proteins

YADD, DDE

Retrotransposon

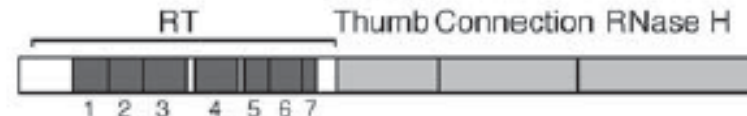
NHN, RuvC, holliday j.

RT in disguise

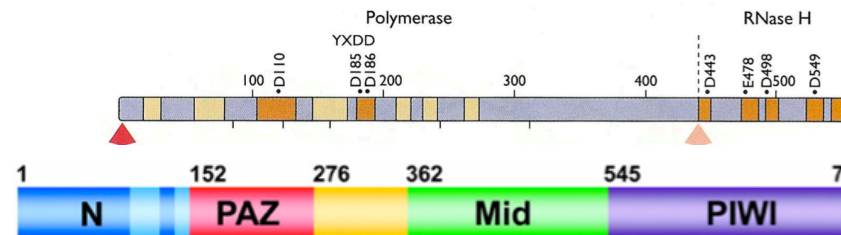
Apobec,
Adeaminase



IEP



RT



HIV-RT

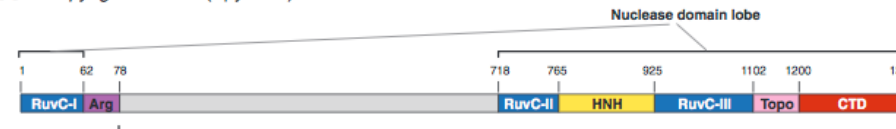
Argonaut

copy-and-paste

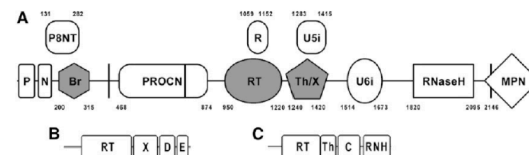
Retrovirus-like retrotransposons [LTR-retrotransposons] (6-11 kb)



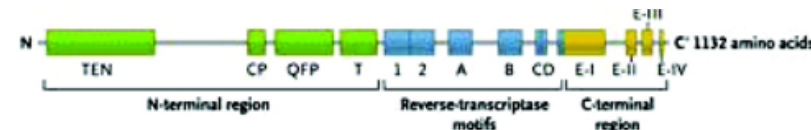
A *S. pyogenes* Cas9 (SpyCas9)



Cas9
(45 family members)

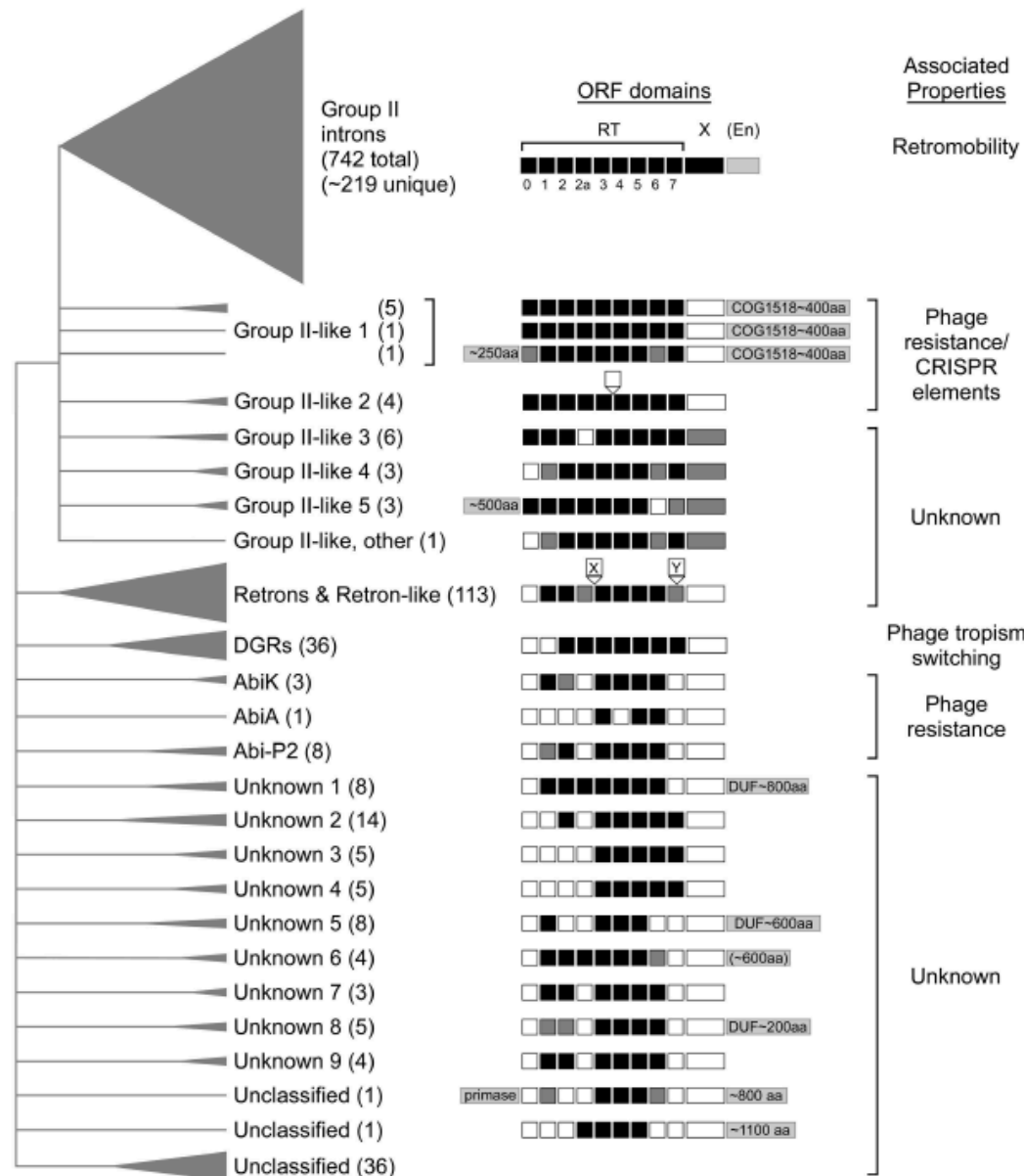


Prp8



Telomerase

1021 RTs identified in eubacterial, archaeal and phage



Surprise: RT in Bacteria?

COG1518 CRISPR-associated protein for RNA interference clusters of orthologous group/CRISPR related)

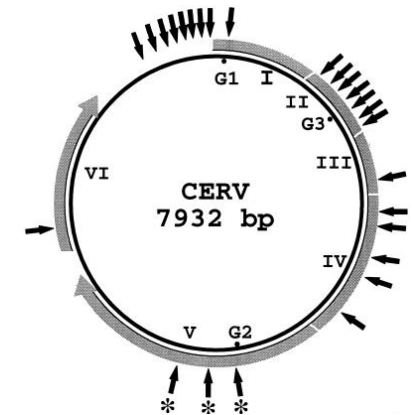
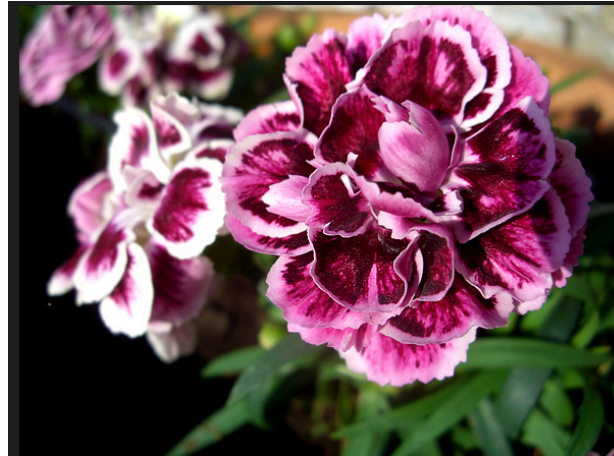
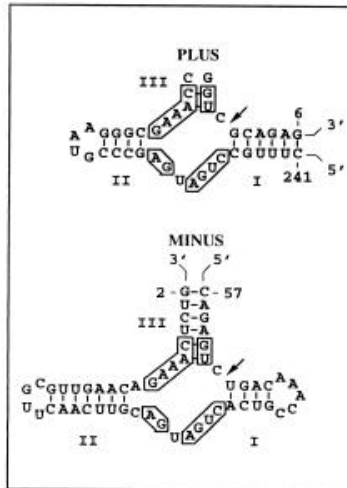
Group II introns: code for endonuclease domains (HNH) for mobility, Ribozyme-based splicing and retromobility
Intron mobility is mediated by RNP (lariat RNA and two molecules of intron-encoded proteins), which site-specifically recognizes and Inserts intron sequence into a DNA target, Target-primed TP- RT

DGRs not mobile, diversifies DNA, Bordetella pertussis, RE (RT, RNA proteins, Variable region VR) for changes in phage tail, contact region for infection of bacterial host, DGR mediates tropism switching, and allows phage to infect cells with altered surface composition)

Retrons: unexplained bacterial RE, RT, multycopy ssDNA/RNA (msDNA/msRNA)
RT reverse transcribes the RNA in a branched RNA/DNA molecule, msDNA accumulates, function?

Lactococcus 20 systems to block phage replication (TRIM5a!!!)

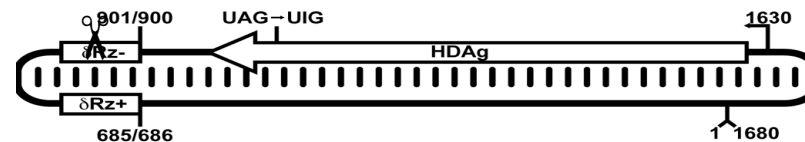
Retroviroids in plants and HDV get help of para-RV



Can there be desoxyribozymes?

Carnation small viroid-like RNA (CarSV RNA) and its homologous DNA are the two forms of a unique plant retroviroid-like system.

HDV



27KD and 29KD
overlooked stop codon?

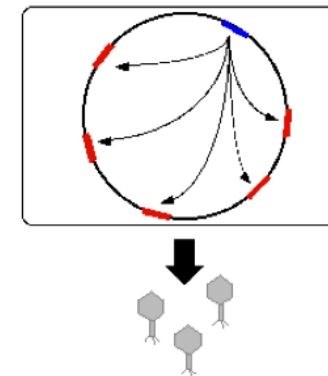
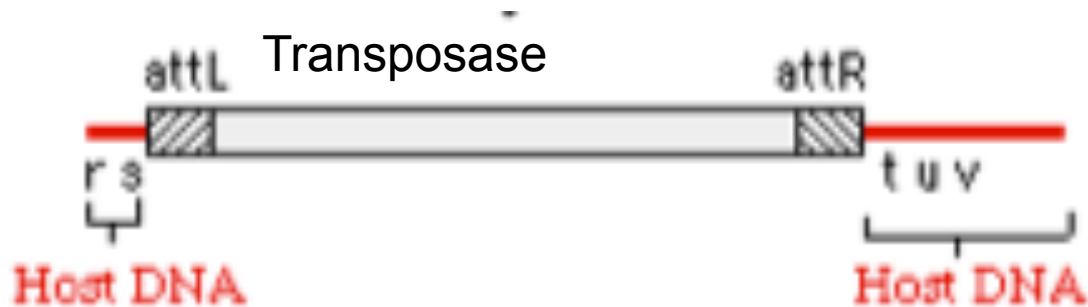
The DNA of a plant retroviroid-like element is fused to different sites in the genome of a plant pararetrovirus and shows multiple forms with sequence deletions. Vera A, Daròs JA, Flores R, Hernández C. J Virol. 2000 Nov;74(22):10390-400.

T.Taylor

Two retrophages:

Mu (RNase H, Integrase), Model for integration, gene therapy,
B McClintock, high frequency, Integrase Inhibitor, Raltegravir

Mu phage



Bordetella phage (RT and Integrase) (MTD, Major Tropism Determinant),
mutagenesis on effect receptor of bacteria, interference, IS

Bordetella

Defense Systems

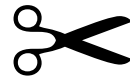
RNA → DNA → Protein

Rz,
siRNA

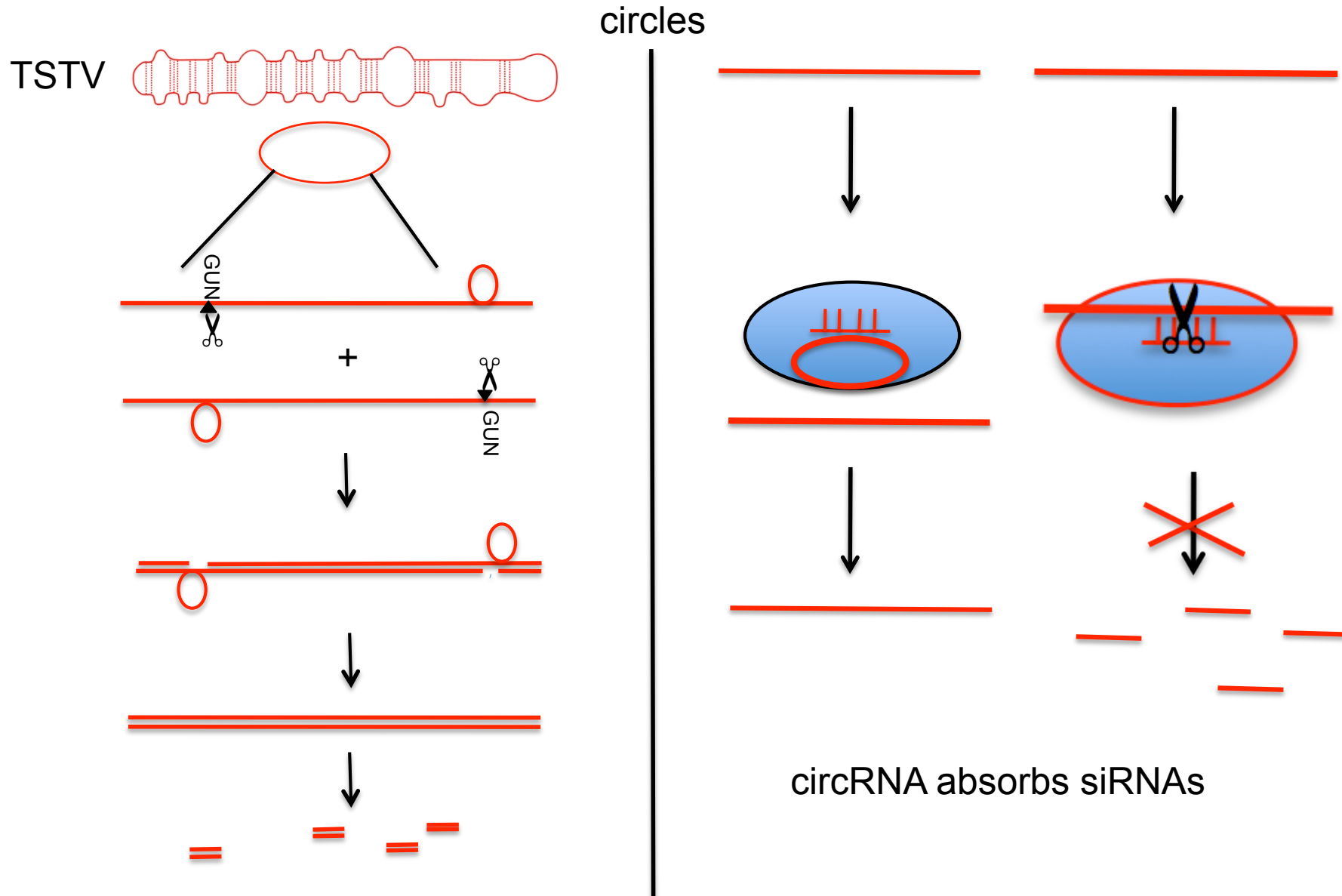
CRISPR,
siDNA

IFN, IgG,
HERV

All based RNases H ?



Ribozymes, catalytic RNAs, viroids, HDV, circRNA – relationships?



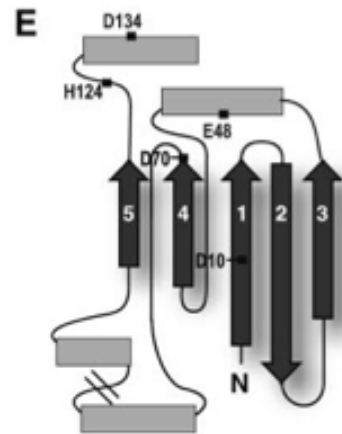
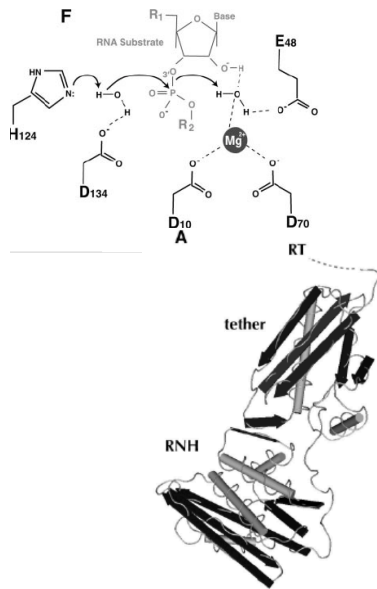
Ancient proteins

Table 2

The most widely shared folds and corresponding occurrences in domain and family spaces constituted by relatively early proteins

| Order ^a | Folds | Occurrence in domain space | Occurrence in family space |
|--------------------|-------------------------------------------------------------|----------------------------|----------------------------|
| 1 (1) | P-loop containing nucleoside triphosphate hydrolases (c.37) | 155 | 64 |
| 2 (3) | TIM beta/alpha-barrel (c.1) | 90 | 65 |
| 3 (4) | NAD(P)-binding Rossmann-fold domains (c.2) | 87 | 42 |
| 4 (5) | Ferredoxin-like (d.58) | 67 | 44 |
| 5 (7) | Ribonuclease H-like motif (c.55) | 49 | 31 |
| 6 (6) | Flavodoxin-like (c.23) | 46 | 22 |

^a The fold emerging order inferred by Caetano-Anolles and co-workers [7] is listed in parenthesis.



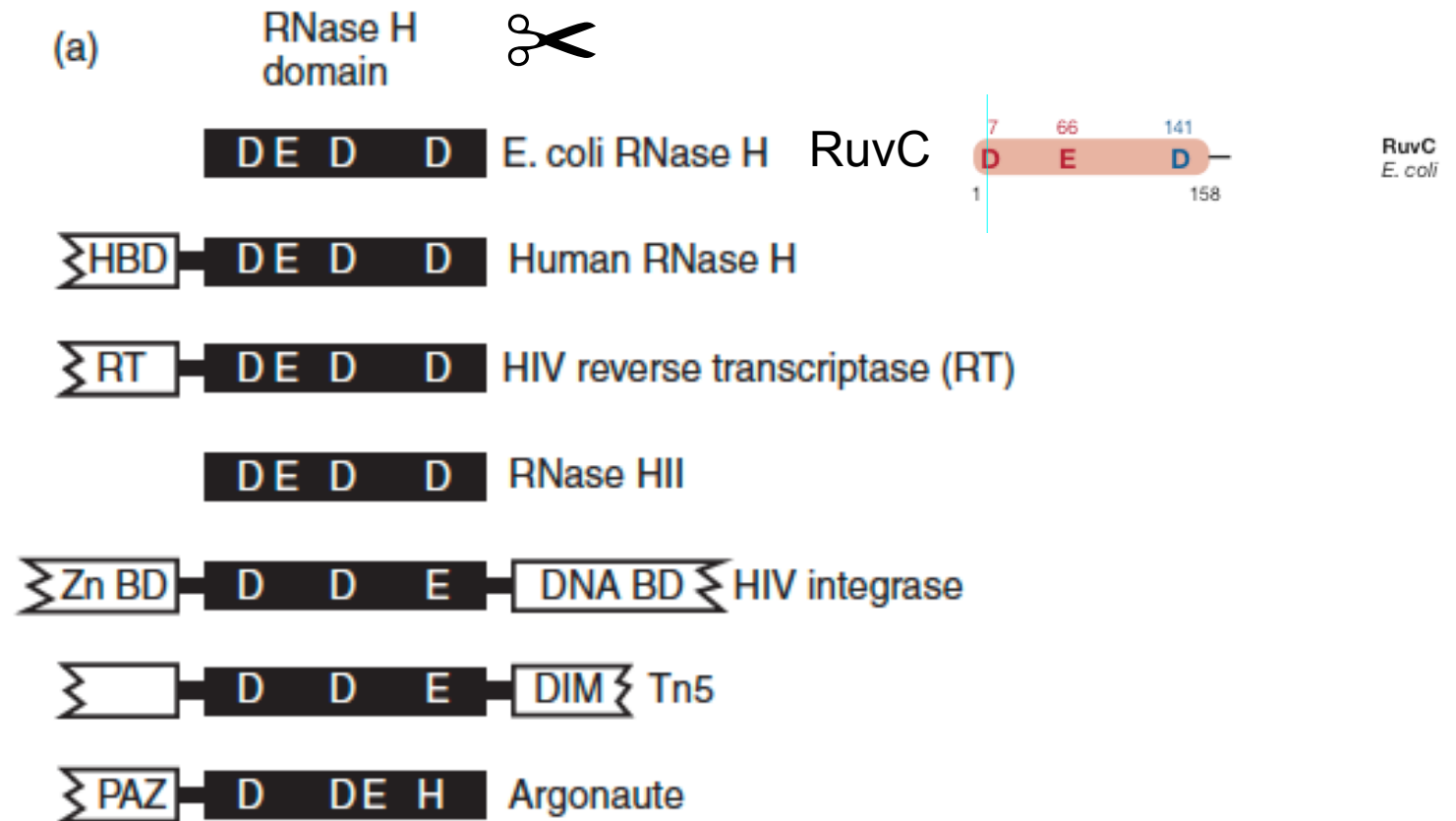
- Ancient
- 5th most abundant protein
- 5 beta-turns, alpha-helices A-E
- divalent ions coordinate structure DDE triad
- endonuclease
- No sequence-specificity topology-recognition
- Cleavage RNA in RNA-DNA hybrids, dsRNA, opposite/at junctions
- Fusion to other domains, imposing functions (binding, processivity)
- 3'-OH, 5'-P
- Primer and primer removal for DNA-synthesis
- single nucleotide removal

Characters of very ancient proteins.

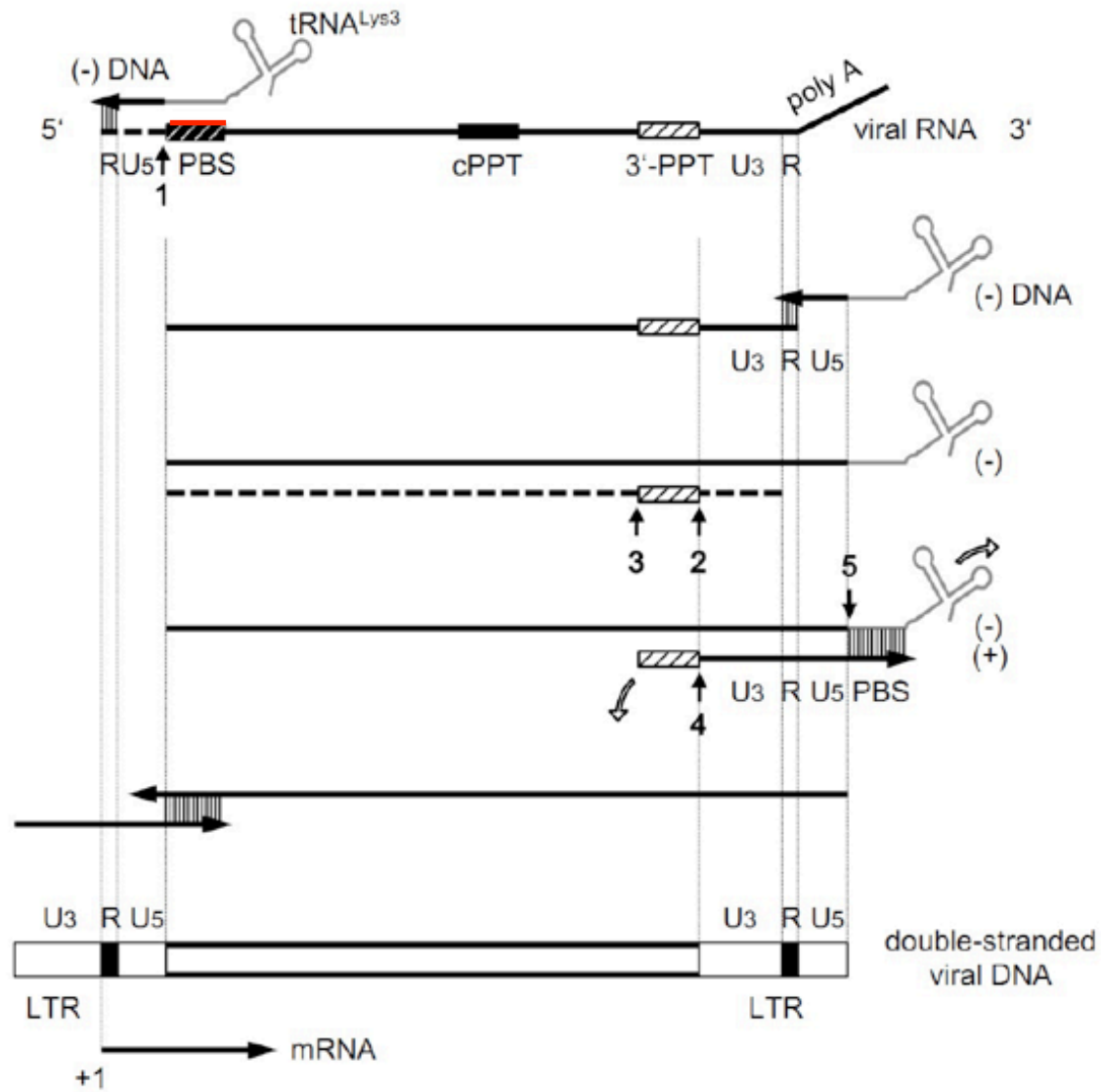
Ma BG, Chen L, Ji HF, Chen ZH, Yang FR, Wang L, Qu G, Jiang YY, Ji C, Zhang HY.
 Biochem Biophys Res Commun. 2008 Feb 15;366(3):607-11.

Moelling et al, Nature NB 1974

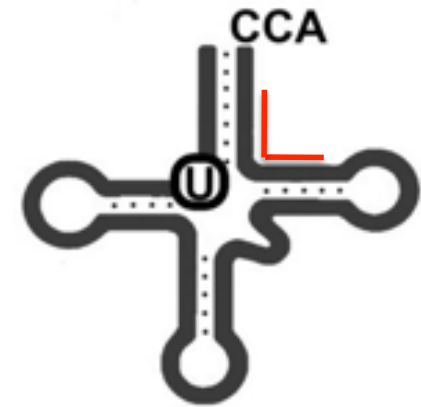
Fusion proteins for new functions



Retroviral replication



Removal of RNA primers



5 RNase H
cleavage events

DDDDRDDD
DDDDDDDD

Structural relationship evolutionary ?

AGO2

N-term

PAZ

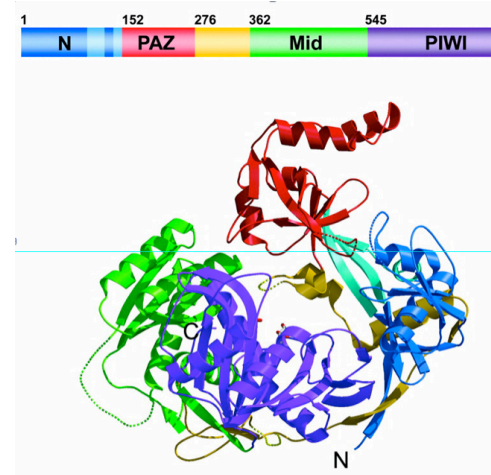
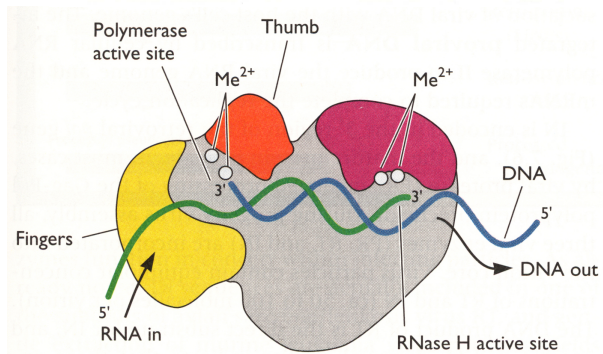
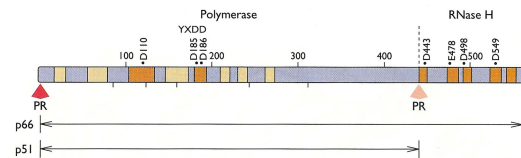
PIWI

HIV

NC?

RT

RNaseH



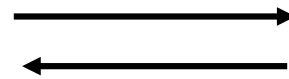
PAZ Piwi/Argonaute/Zwille (protein domain)
PIWI protein domain homologous to piwi proteins (encoded by the
'P-element induced wimpy testis' class of genes in *Drosophila*)

Song et al., Science 305, 1434 (2004)

K. Moelling, Salzburg 2014

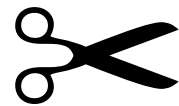
Co-Evolution of RNase H

Virus

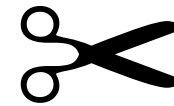


Cell

siRNA

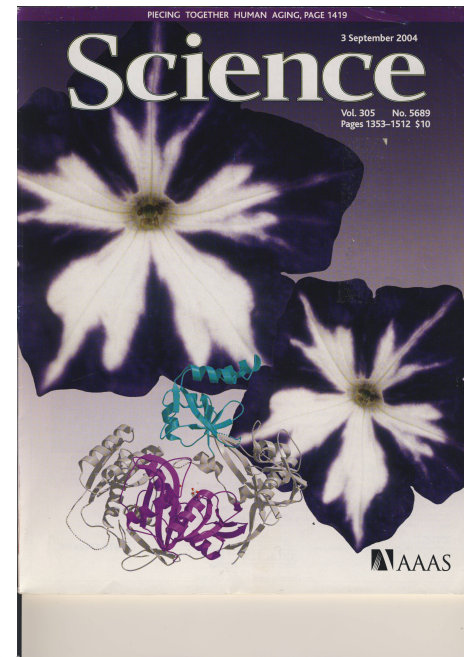


Virus
attack



Anti-viral
defence

RISC



RISC and HIV

21-23mer
sequence-specific
slicer (PIWI)
dsRNA
guidance protein
Ago2 (PAZ and PIWI)
3'-OH and 5'-P
FMRP
staggered
Dicer
(dinucleotide overhangs/endo)
caspase?

TRBP

TRBP: Tar-RNA bp, like TAT

25mer
sequence-specific
RNase H (endo/exo)
hybrid
(?)
RT/RNaseH
id.
nucleocapsid
staggered
integrase
(cleaving and joining)
protease

TRBP/Dicer

Relationship between Retroviral Replication and RNA Interference Machineries

K. MOELLING, A. MATSKEVICH and J.-S. JUNG

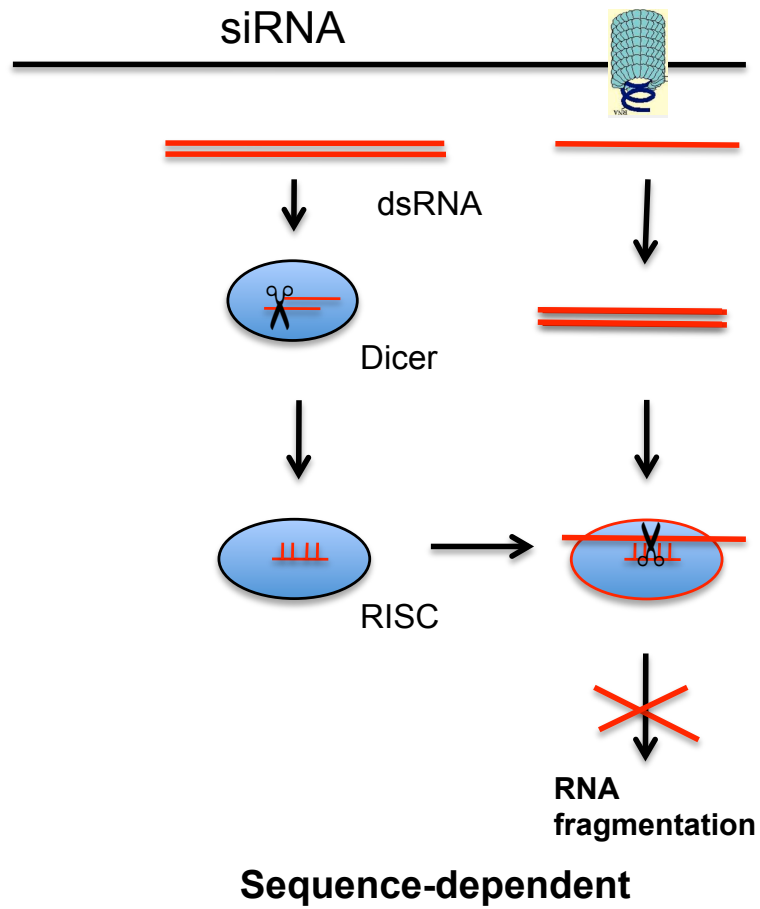
Cold Spring Harb Symp Quant Biol 2006 71: 365-368
Access the most recent version at doi:[10.1101/sqb.2006.71.010](https://doi.org/10.1101/sqb.2006.71.010)



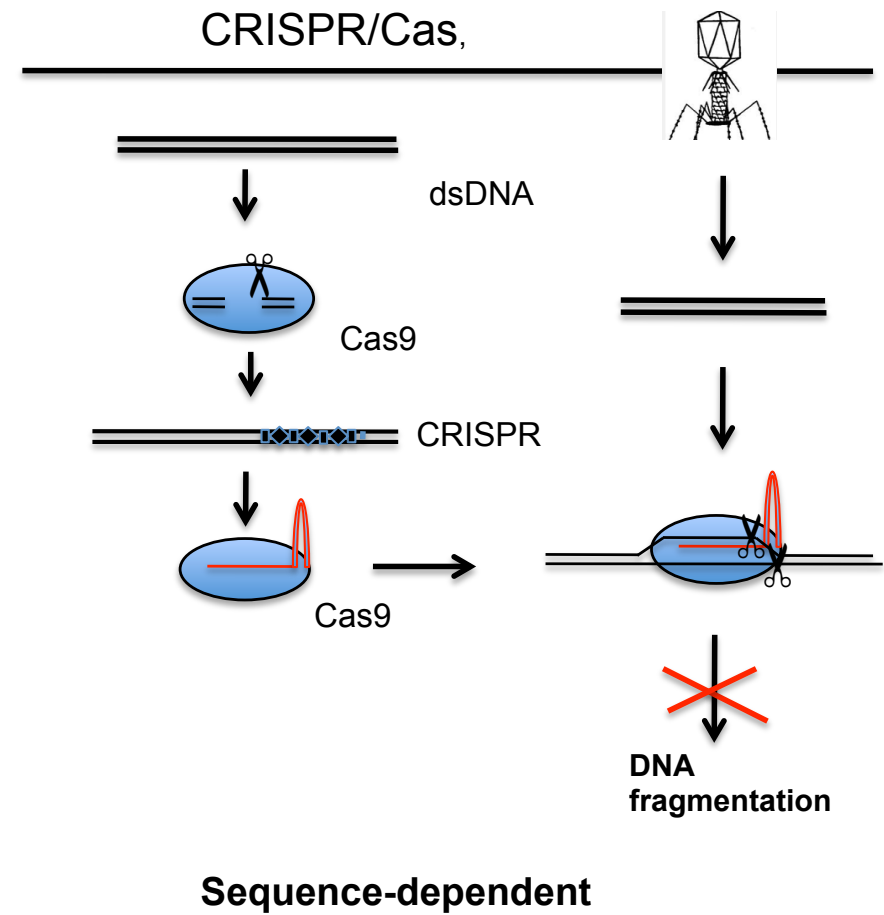
Cold Spring Harbor Symposium
on Quantitative Biology

Cold Spring Harbor Symposium RNAi (2006)

RNA viral defense



Phage DNA-defense

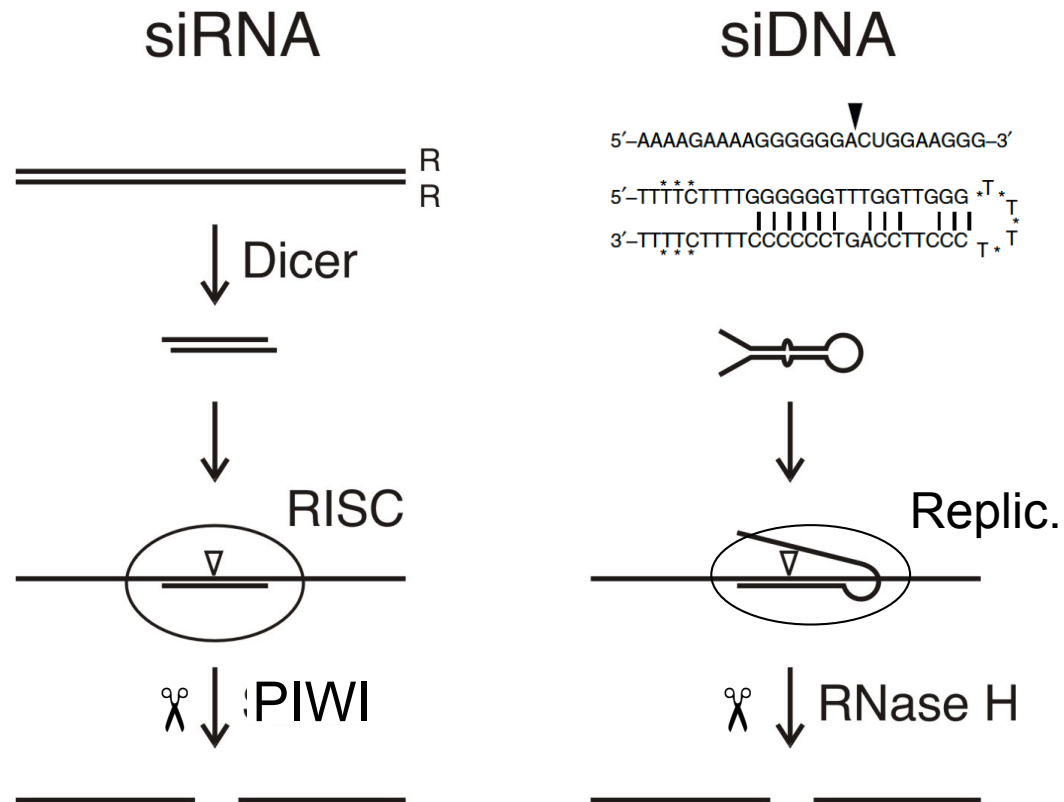


RISC:RNA-Induced Silencing Complex

KMoelling, Salzburg 2014

CRISPR: Clustered Regularly Interspaced
Short Palindrome Repeats, Cas: CRISPR-associated
s

siRNA versus siDNA



RNA silencing

Relationship between Retroviral Replication and RNA Interference Machineries

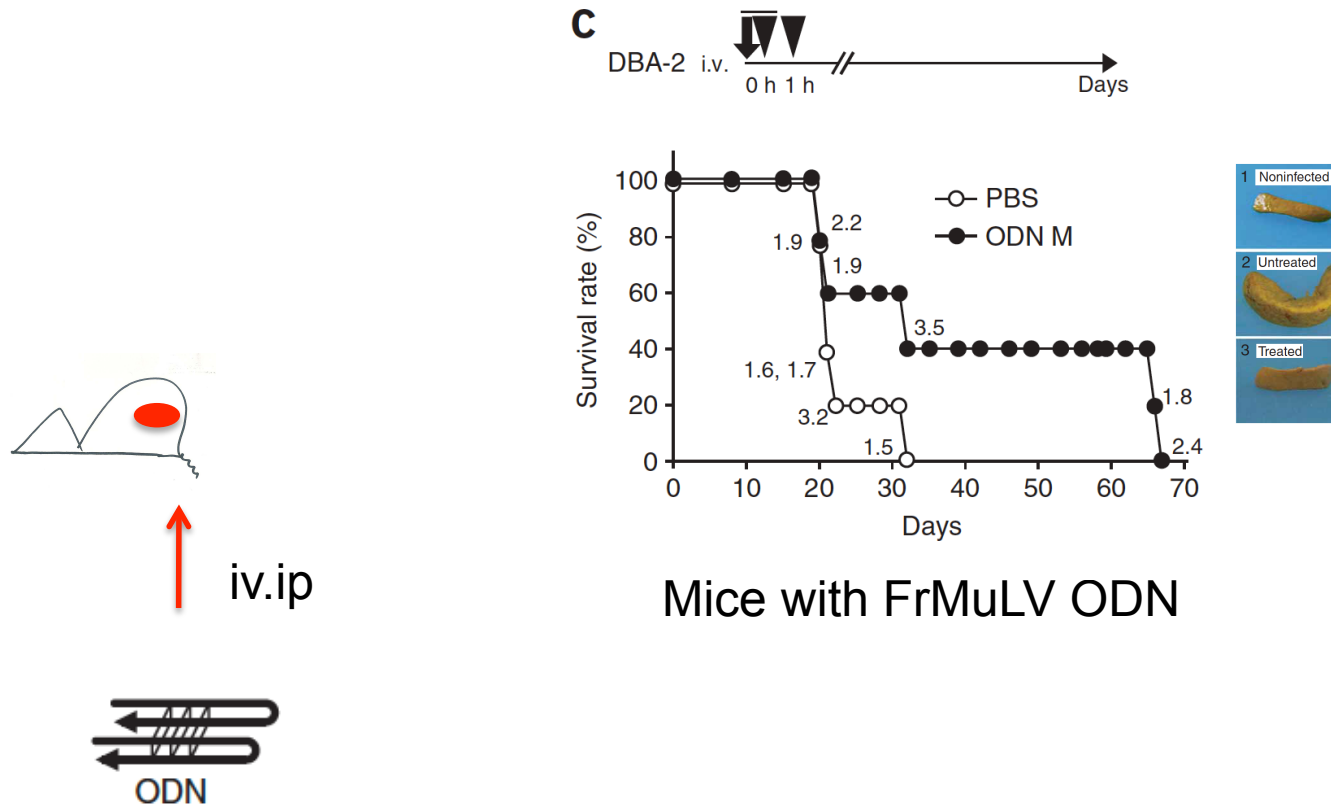
K. MOELLING, A. MATSKEVICH and J.-S. JUNG

Cold Spring Harb Symp Quant Biol 2006 71: 365-368
Access the most recent version at doi:[10.1101/sqb.2006.71.010](https://doi.org/10.1101/sqb.2006.71.010)



Cold Spring Harbor Symposium
on Quantitative Biology

„Driving HIV into suicide“ by „siDNA“



Matzen et al Moelling RNase H-mediated retrovirus destruction in vivo triggered by oligodeoxynucleotides.
[Nat Biotechnol.](#) 2007 :669-74.

Moelling et al Silencing of HIV by hairpin-loop-structured DNA oligonucleotide.
FEBS Lett. 2006,;580:3545-50.

RNase H family members

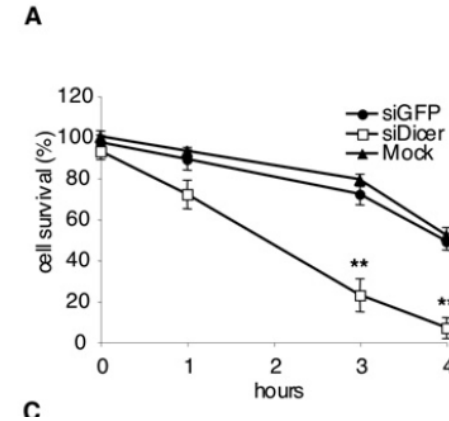
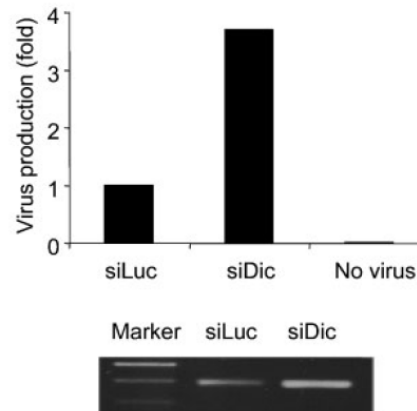
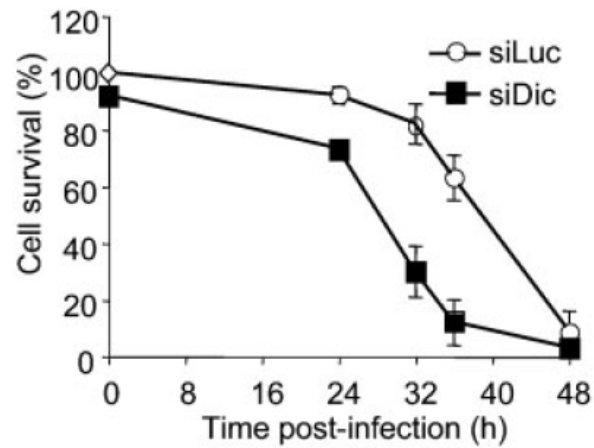


- Viruses: RT-RNase H, integrase
- RNase H1 (mitochondria)
- RNase H2A, B, C Diseases: AGS
- Group II introns (all species)
- RNase H1, II, III
- siRNA, Argonaute, Dicer (plants and more!)
- CRISPR/Cas9
- RuvC (Holliday junctions)
- Transposons: Mu, Tn3, 5, SB, Ty3 (Gypsy), LOTUS
- Retrotransposons: LINE, L1
- Interferon, RNase L?
- IgG : Rag1, 2, V(D)J
- HERVs (Poster)

Are these „RNasesH“-related: En, dsRNA, DNA in Hybrids,

Both, IFN and siRNA ex in Humans

A. A. Matskevich and K. Moelling



Without siRNA Influenza increases fourfold

Dicer is involved in protection against influenza A virus infection

Alexey A. Matskevich and Karin Moelling

Biochem. J. (2008) **412**, 527–534 (Printed in Great Britain) doi:10.1042/BJ20071461

Stimuli-dependent cleavage of Dicer during apoptosis

Alexey A. MATSKEVICH¹ and Karin MOELLING

Institute of Medical Virology, University of Zurich, Gloriastrasse 30/32, CH-8006 Zurich, Switzerland



Inheritable Immunesystem also in Humans? Are the HERVs?!



TRIM5a

FV-1



FV-1 in mice und Trim5 alpha in humans against HIV Ebola and Borna viruses, Filo, Circo, Parvo



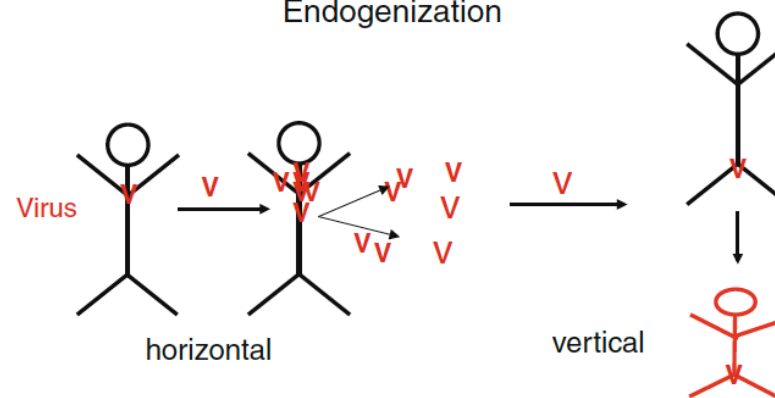
Koalas endogenization and resistance against GALV

Bordetella tropism change by phage???

35 Mio years ago
Borna depression



Endogenization



GALV



Rag 1 and VDJ related to Transposons

VDJ evolved from transposons joined to
form the variable Antigen-binding site

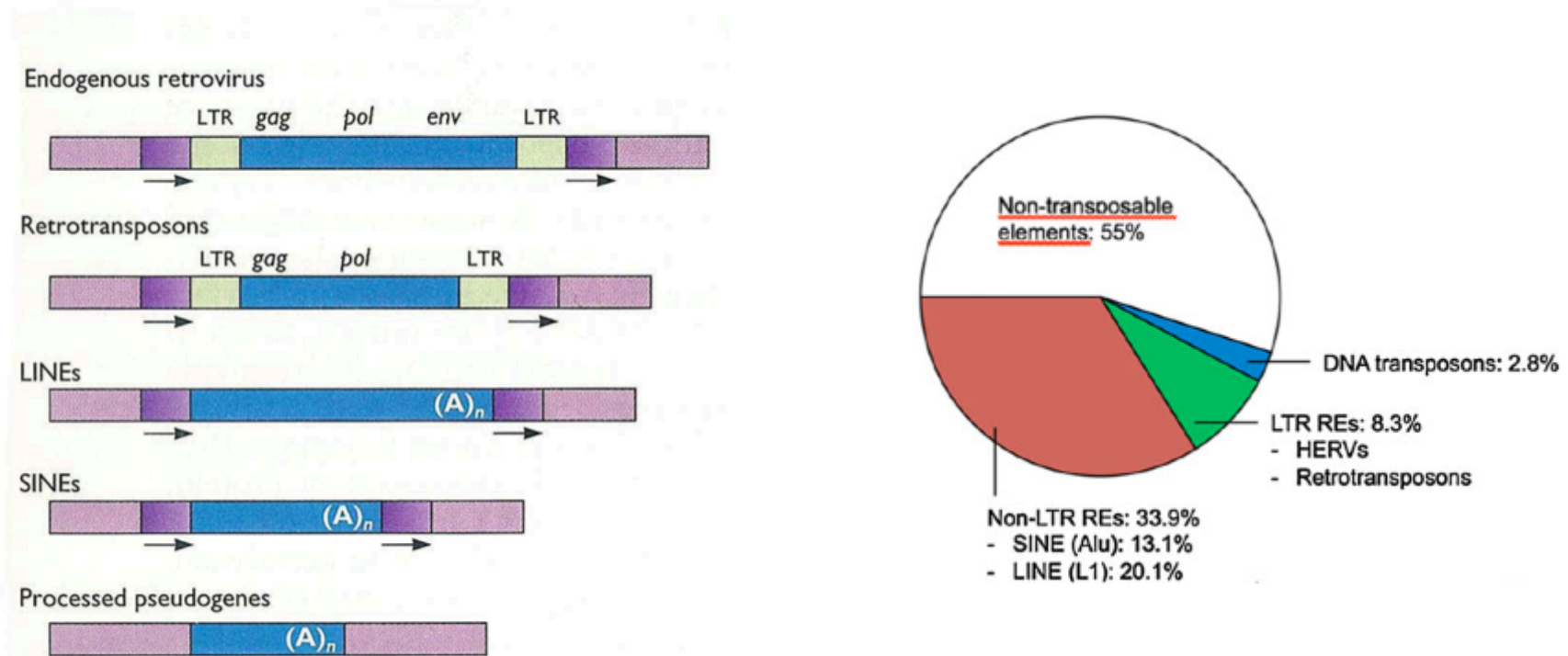
TIR and RSS are related

Hydra; RAG1 and Transposase related

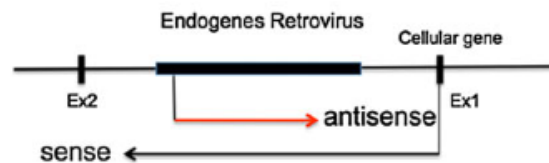
RAG1 Core and V(D)J Recombination
Signal Sequences Were Derived from
Transib Transposons, Plos Biology3,2005
Vladimir V. Kapitonov, Jerzy Jurka

Retroviral Elements

(50% : could it have been 100%? of human Genome)



Broecker et al Nat Comm under modify



Finis
Thank your for your attention