# Viroids \& the RNA World: from genomic scale (RNA) to atomic scale (ribozyme) 

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# Viroids 

## the RNA World

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# Viroids 

## \&

## the RNA World

from genomic scale (RNA)


## Introduction

- evolution (RNA world) \& genomics
- RNA biology \& RNomics (molecular and cellular functions)
- structural biology \& structural bioinformatics (structural basis for functions)
- enzymology \& computational enzymology (catalysis)


## Prebiotic \& RNA Worlds



# Virus World \& <br> <br> RNA World 

 <br> <br> RNA World}

## viroids <br> 

Archaea with plasmids, group I introns

Bacteria with plasmids, retrons, group I \& II introns

dsDNA and RCR viruses, plasmids
retrons, group II introns
positivestrand, ds RNA viruses
«The ancient Virus World and evolution of cells»

Koonin et al., Biol. Direct, 2006


## Viroids: « survivors from

 the RNA World»

Holmes, J. Virol., 2011

- small size
functions
- error-prone replication
- high GC
- circRNA
- periodicity
- no proteincoding
- ribozyme features
- replication
- replication fidelity
- genome assembly
- ribosomefree



## Viroids: Plant Parasites

## $\%$ ASBVd

2) Replication


Ding \& Itaya, Mol Plant Microbe Interact, 2007.

## Viroids: Replication



Rz: hammerhead ribozyme


10 Ding \& Itaya, Mol Plant Microbe Interact, 2007.

## Viroids: Pospiviroids



Hammann \& Steger, RNA Biol., 2012.

## Viroids: Avsunviroids



## Viroids \& RNA silencing



## ASBVd: 2D Structures


(-) ASBVd


Ding \& Itaya, Mol Plant Microbe Interact, 2007.

## ASBVd(-): 2D Structures




Flores et al., Adv, Virus Res., 2000.

## HHR motifs in life forms



## A prototype for RNA

## Catalysis

## "Minimum" Hammerhead Ribozyme



## Self-cleaving Sell-Splicing



SN2(P) reaction




3'-5'-exonuclease

## Catalytic Metal Ions: One/

## $5^{\prime}$ $\vdots$ $\square$ $\mathrm{N}_{\mathrm{i}}$ YWO X-ray (active)

$\mathrm{O}^{2}$

experimental: Pontius et al., 1997; Lott et al, 1998 theoretical: Boero et al., JCTC, 2005

## Contribution of Metals to

 Catalysis

## HHR: reaction mechanism



## Folding Metal Ions Models



## Metal Binding Sites in the Hammerhead Ribozyme?



Chartrand et al., RNA, 1997


Hansen et al., RNA, 2008

# Minimum/Full-Length HHR 



Wang et al., Biochem., 1999
de la Peña et al., EMBO J., 2003

Khvorova et al., Nat. Struct. Biol., 2003
Canny et al., JACS, 2004

## Variations in HHR motifs



Perreault et al., PLoS Comput. Biol., 2011

Loop-Loop interactions in HHR folding


Penedo et al., RNA, 2004.

# Loop-Loop interactions in HHR catalysis 



## Minimum HHR, Tertiary

## Contact and Catalysis

(a) $\quad 45-\mathrm{NT}$



 Core

$$
\begin{aligned}
& { }^{15,3} \mathrm{C}-\mathrm{G}^{16,3} \\
& { }^{15.4} \mathrm{C}-\mathrm{G}^{16.4} \\
& { }^{15.5} \mathrm{~A}-\mathrm{U}_{5^{16,5}} \\
& { }_{1}^{4}
\end{aligned}
$$

O’Rourke et al., JMB, 2015
(b)

Stem III



Stem I


## HHR morphing

Highly active form


# A Nucleobase 

## Catalyst




## Metal Catalysts in the

 2 'OH activation?

## Metal Catalysts in the

 Hammerhead Ribozymes?



Osborne et al., Biochem., 2009

## Cooperative Models in

## Self-Cleaving?



## Cooperative Models in

## Self-Cleaving?



## Metal Ions back in the Hammerhead Catalysis



Ward \& DeRose, RNA, 2011

## HHR: Active Conformation and Metal Ions

A



B



Mir et al., Biochem., 2015

## HHR: Metal Ions and Catalysis

A





Mir \& Golden, Biochem., 2015

# ASBVd(-)/HHR(-): SANS 

## Study

HHR(-)



$\left(0_{0}\right) \mathrm{du} \boldsymbol{D}_{\perp}$



Leclerc et al., Sci. Rep., 2016.

HHR(-) dimerization/

## catalysis

| $\mathrm{E}_{\mathrm{a}} \sim 8 \mathrm{kcal} / \mathrm{mol}$ | $\mathrm{E}_{\mathrm{a}} \sim 20 \mathrm{kcal} / \mathrm{mol}$ |
| :--- | :--- |
| $\mathrm{E}_{\mathrm{a}} \sim 6 \mathrm{kcal} / \mathrm{mol}$ | $\mathrm{E}_{\mathrm{a}} \sim 30 \mathrm{kcal} / \mathrm{mol}$ |



Leclerc et al., Sci. Rep., 2016.

## HHR(-): 2D structures



## 3D Modeling of HHR(-)


loops HI-HII


## HHR folding



## HHR morphing

Highly active form

O'Rourke et al., JMB, 2015
$\mathbf{R}_{\mathrm{g}}(\mathrm{I}) /(\mathrm{F}) \sim 1.1$
ASBVd: $\mathbf{R}_{\mathrm{g}}(\mathrm{I}) /(\mathrm{F}) \sim 1.2$

## Modeling/SANS




monomer 1 HI
HII
HIII
monomer 2

# HHR (-): dimerization 


monomer 1 HI HII monomer 2 $\quad$ 30b


# HHR(-) 

 dimer

## HHR(-): monomer 1




## HHR(-): monomer seeds



## HHR(-) monomers



## HHR: Monomer \& Dimer



C


## Rg \& self-association



## MD setup for HHR dimer

- 300000 atoms
- $\mathrm{K}^{+} \& \mathrm{Cl}^{-}(0.15 \mathrm{M})$
- T = (283K, 313K)
- NAMD



# Dynamics of HHR(-) dimer 



# HHR dimer: MD trajectory 



## ASBVd(-): self-association



monomer 1

# ASBVd(-): self-association 



# ASBVd(-): self-association 

## What did we learn?

- for RNA and ribozymes: «too short» may be bad
- don't forget about dynamics
- SANS \& modeling approaches to infer selfassociation modes
- theoretical approaches to explore reaction mechanisms and pathways


## Viroids: Plant Parasites

Genus Pospiviroids: PSTVd (potato spindle tuber)

Genus Hostuviroids: HSVd (hop stunt)

Genus Cocadviroids
CCCVd (coconut cadang-cadang)

Genus Apscaviroids:
ASSVd (apple scar skin)


Genus Coleviroids:
CbVd 1 (coleus blumei 1)

Genus Avsunviroids: ASBVd (avocado sunblotch)


Genus Pelamoviroids:
PLMVd (peach latent mosaic)


# Viroid \& HDV: Hepatitis D 



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相沮
TW


NITA SAHAI and HUSSEIN KADDOUR, Guest Editors


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## Assembling the RNA World



Prebiotic chemical synthesis

- How can nucleotides be synthesized in appreciable quantities?
- How can nucleotides be polymerized into strands long enough to be functional?


## b

## Finding functional ribozymes

- How small is the fraction of random sequences that can act as functional ribozymes, and how long do functional sequences have to be?
- Are there ribozymes that can make accurate copies of themselves by any mechanism?
c


## Evolutionary theory and modelling

- Is it easier to begin life with an autocatalytic set or a single polymerase?
- What is the error threshold for cooperative trans-acting replicators in a spatially structured model?


## Viroids: Phylogeny

## REPLICATION <br> ASYMMETRIC <br> PATHWAY

SYMMETRIC
PATHWAY

## FAMILY


Avsunviroidae

SUBFAMILY

|  |
| :---: |
| Pospiviroinae |
|  |
|  |



## Coleviroinae

GENUS


```
Hostuviroids
```

```
Hostuviroids
```



Coleviroids

## Avsunviroids

Pelamoviroids

## SPECIES

Potato spindle tuber (PSTVd)
Mexican papita (MPVd)
Tomato planta macho (TPMVd)
Chrysanthemum stunt (CSVd)
Citrus exocortis (CEVd)
Tomato apical stunt (TASVd)
Iresine 1 (IrVd 1)
Columnealatent (CLVd)

Hop stunt (HSVd)

Coconut cadang-cadang (CCCVd)
Coconuttinangaja (CTiVd)
Hop latent ( HLV )
CitrusIV (CVd-IV)

```
Apple scar skin (ASSVd)
Citrus III (CVd-III)
Apple dimple fruid (ADFVd)
Grapevine yellow speckle 1 (GYSVd 1)
Grapevine yellow speckle 2 (GYSVd 2)
Citrus bent leaf (CBLVd)
Pearblister canker (PBCVd)
Australian grapevine (AGVd)
```

Coleus blumei 1 (CbVd 1)
Coleus blumei 2 (CbVd2)
Coleus blumei 3 ( CbVd 3 )

## Avocado sunblotch (ASBVd)

Peach latent mosaic (PLMVd)
Chrysanthemum chlorotic mottle (CChMVd)

## Modeling RNA-RNA Interactions



- $10 \quad 30 \quad 60 \quad 120$
B.






## HHR motifs




Type II prokarotes


Type ||| $\begin{aligned} & \text { Prokaryotes } \\ & \text { Plants }\end{aligned}$

# HHR (-): symmetric dimerization 



# ASBVd(-): symmetric self-association 



# ASBVd(-): symmetric self-association 



# ASBVd(-): symmetric self-association 



## HHR design \& engineering



## Glms ribozyme



## Viroid Pathogenicity \& Localization



## Viroid\& RNA silencing



## Viroid disease symptoms



Potato spindle tuber viroid


Apple viroid disease


chrysanthemum stunt viroid


Avocado Sunblotch Viroid




