mRNA Archeology

Laboratory of RNA Archeology. **IPBLN-CSIC**
Granada-SPAIN

ARQUERNA LAB

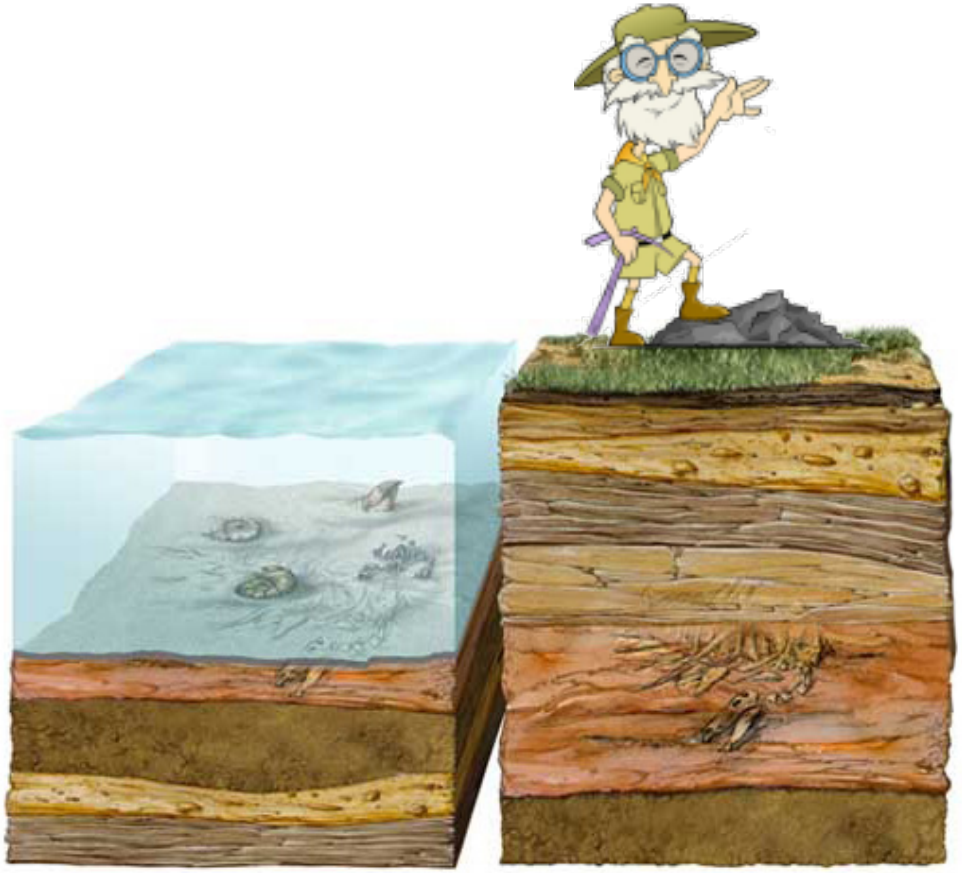
ciberehd

JUNTA DE ANDALUCIA
Consejería de Innovación, Ciencia y Empresa

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Archaeology deals with the strata



Celular World

Eukaryotes
Archaea, Bacteria

RNA-DNA-protein World

Parasitic RNA, retro and
DNA elements

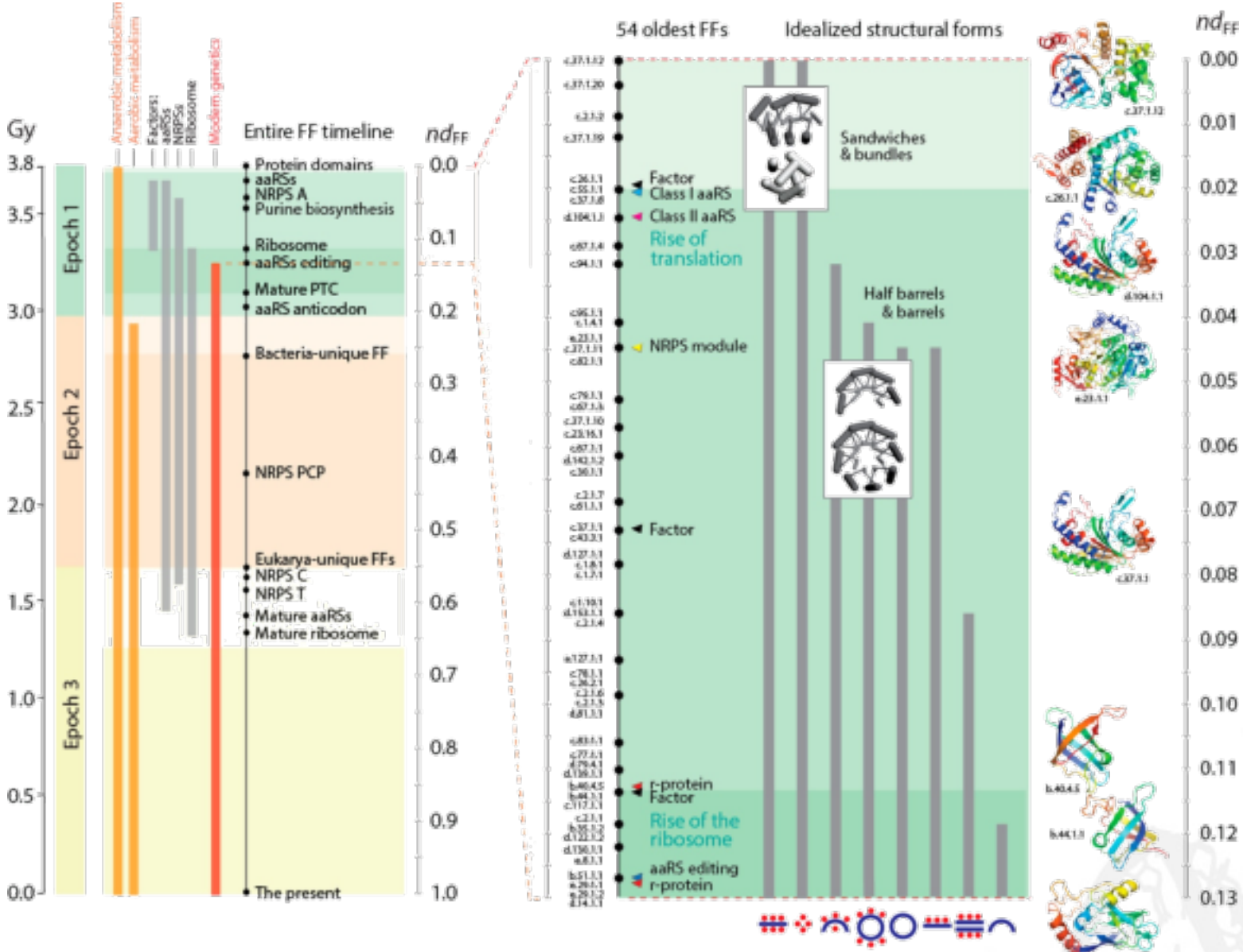
RNA-protein World

RNA virus-like
parasites

RNA World/peptides

Viroid-like
RNA parasites

Structural domains of primordial metabolism and translation



Caetano-Anollés D and Caetano-Anollés G, *Life* (2016)

RNA from early stages

Make Reconstructions

Analysis of RNA lineages (rRNAs, tRNAs…)

Continuity

Theoretical

Detection of Irruptions : ancient-like RNA motifs within present RNAs

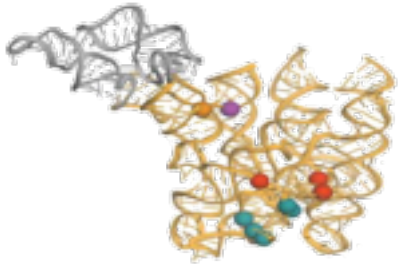
Search RNA structural motifs recognized by activities that deal with ancient RNA elements.

Tools

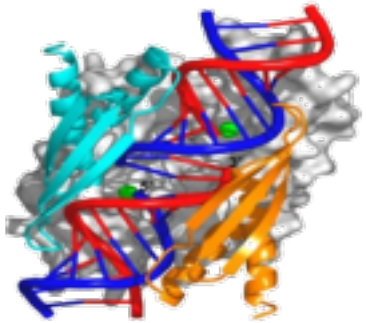
Substrates of study

Tools

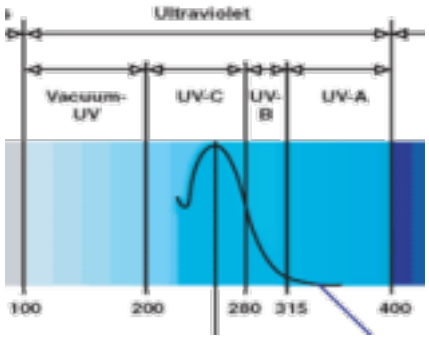
RNase P



RNase III



UV-C light



Properties

Natural

Direct

Covalent modification

Studies

Specificity

Modification site

Ribonuclease P (RNase P)

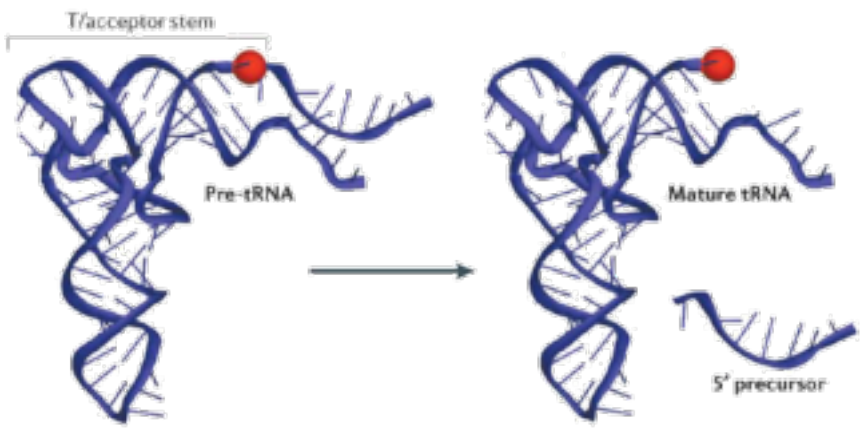
1) Essential. Funciontionally conserved

2) Maturation of 5' end of tRNA precursors.

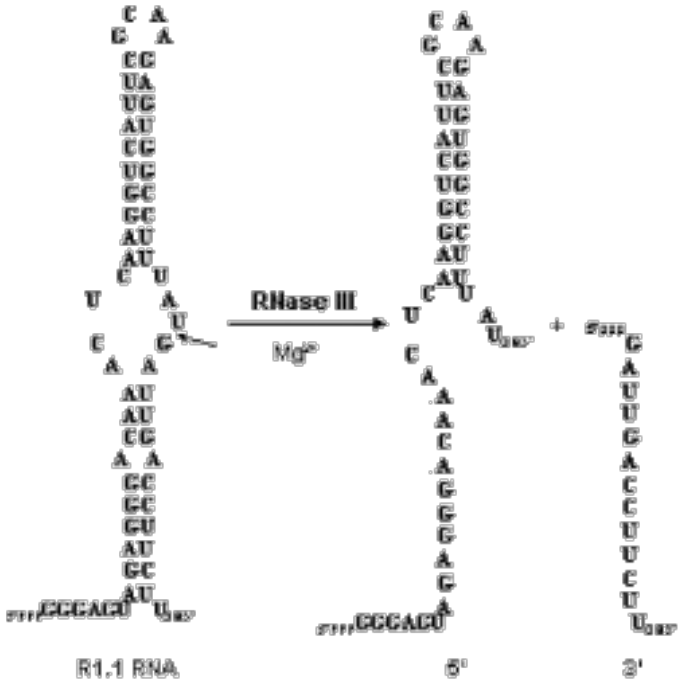
3) Ribonucloprotein.

4) Divalents ion as cofactors (Mg^{2+}).

5) Cleavage chemistry: 5'-P y 3'-OH.



E.coli Ribonuclease III (RNase III)

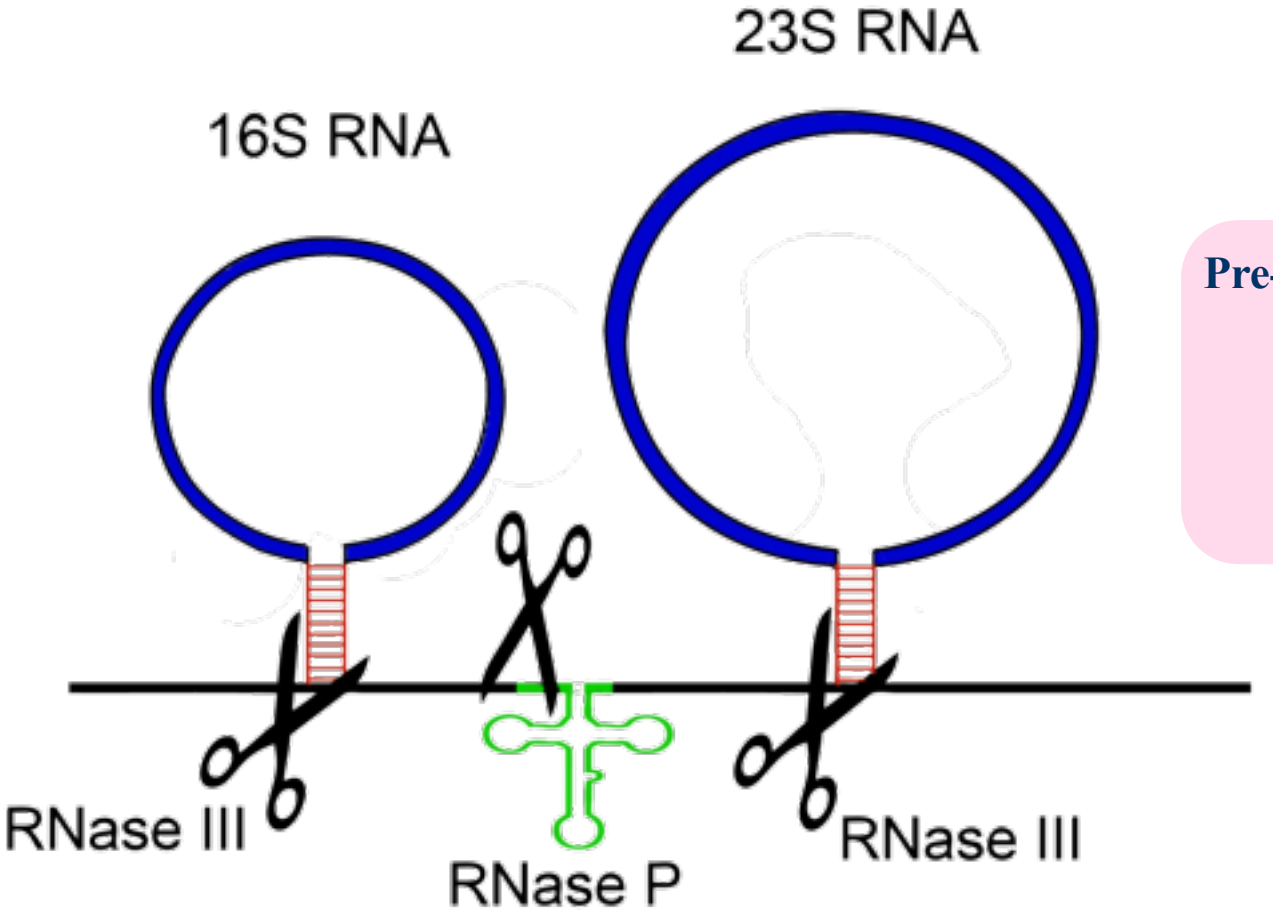


1) Protein enzyme. Recognizes perfect dsRNA > 15 pb or with alterations.

2) Biological function: pre-rRNA processing and de *E.coli* phage mRNA processing.

3) Cleavage chemistry: 5'-P y 3'-OH.

Biological substrates for RNase P and RNase III



Pre-rRNA Bacteria and Archaea

Pre-rRNA 5.8S yeast

tmRNA *E. coli*

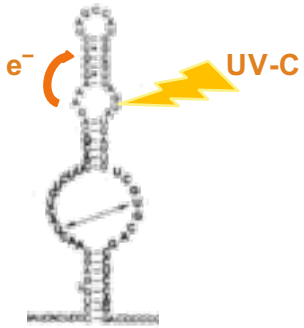
T4 species RNA

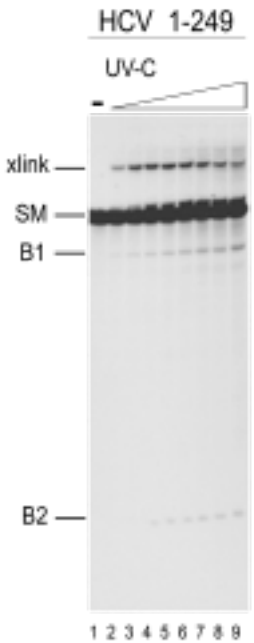
Specific RNA modifications induced by UV-C

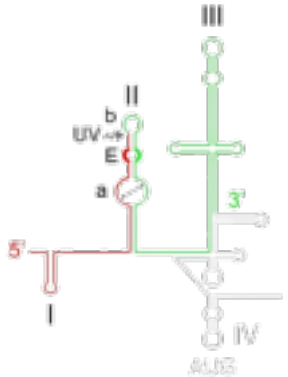
Cross-link

Lyons A.. et al NAR 2001

Self-cleavage







Ariza-Mateos A., et al NAR 2012

Substrates of study

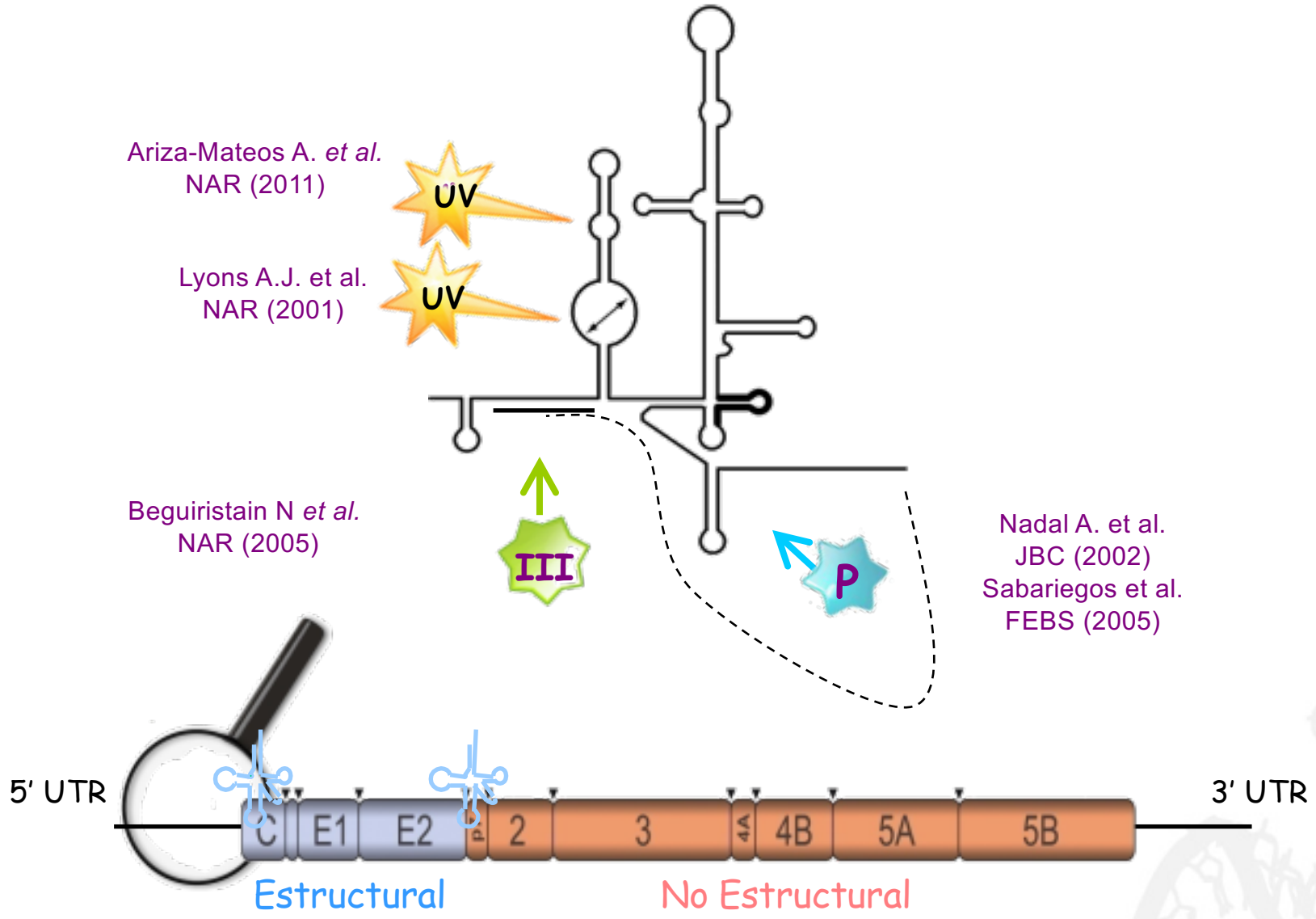
Hepatitis C virus RNA

Flaviviridae RNAs

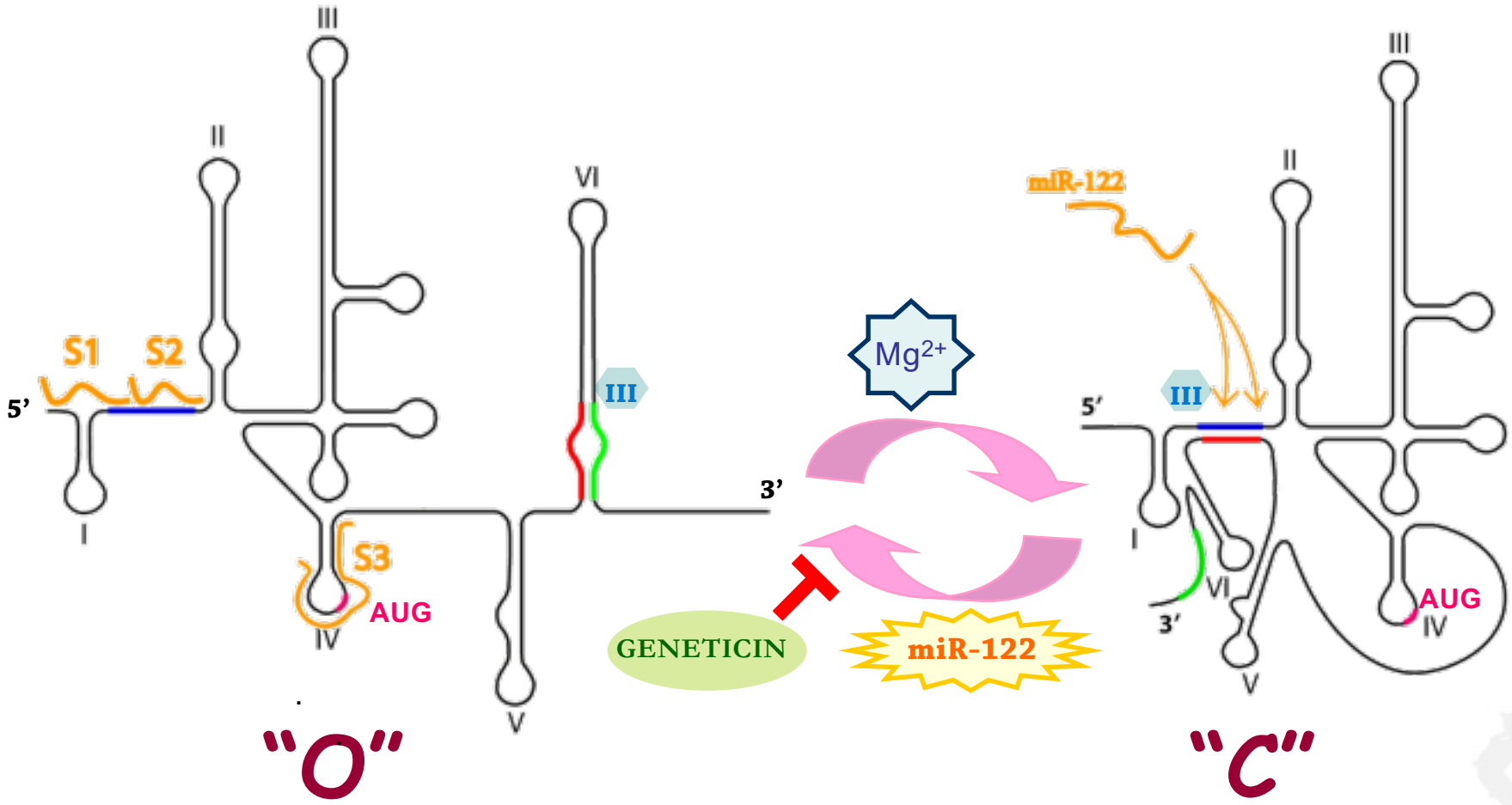
Flavivirus Hepacivirus Pestivirus

Human cellular mRNAs

Hepatitis C virus RNA



RNase III sites are involved in RNA switch



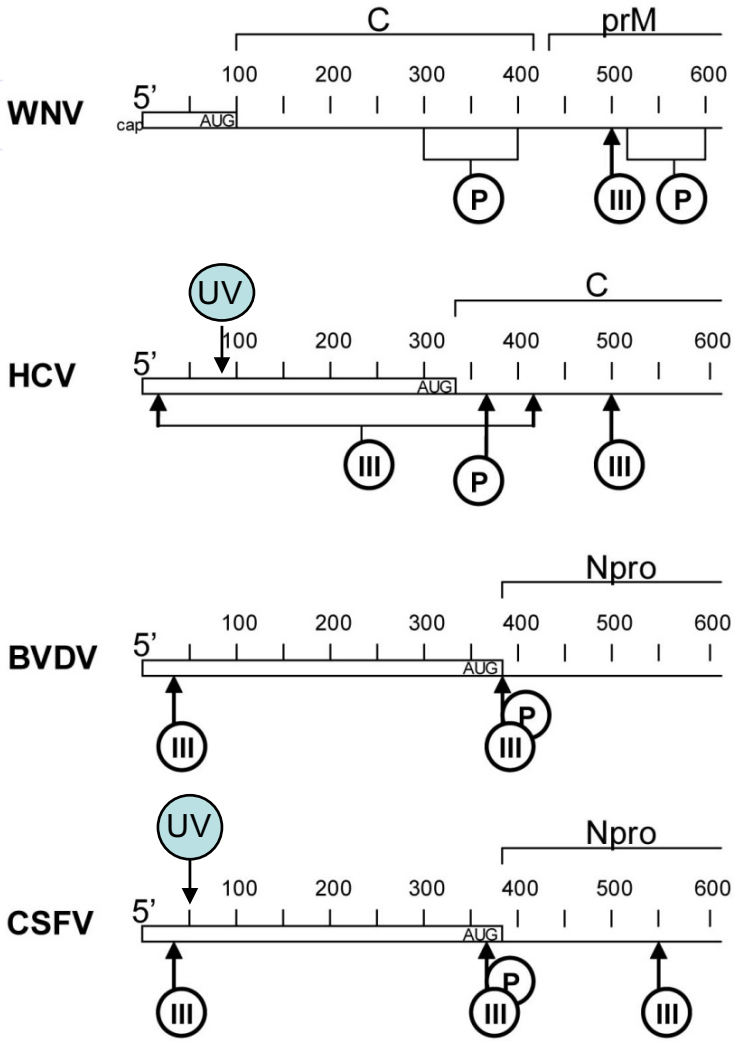
Díaz-Toledano R. NAR 2009

García-Sacristán A. NAR 2014

Ariza-Mateos A. AAC 2015

Flaviviridae RNAs

No IRES



Flavivirus

Hepacivirus

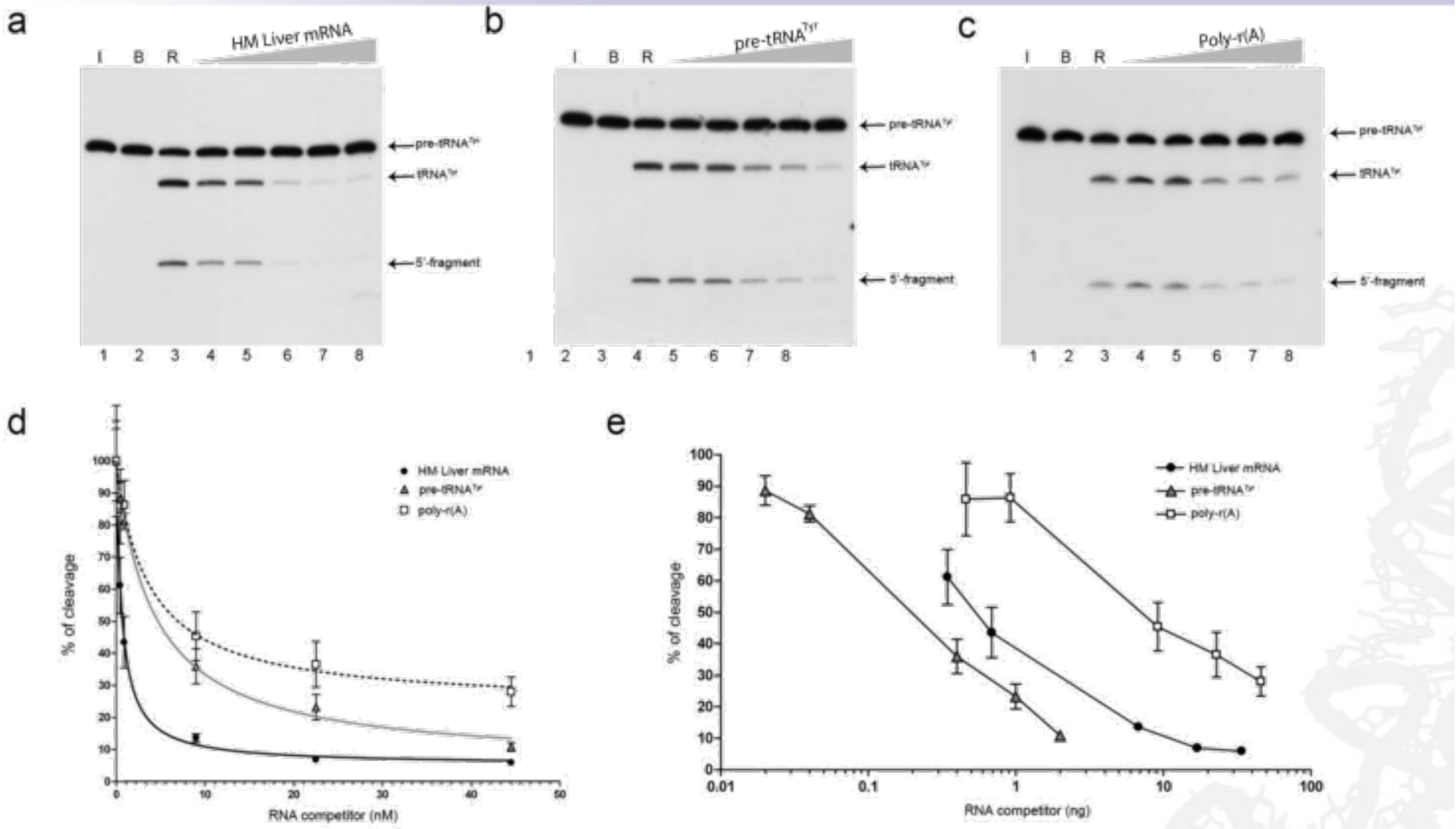
No annealing

Pestivirus

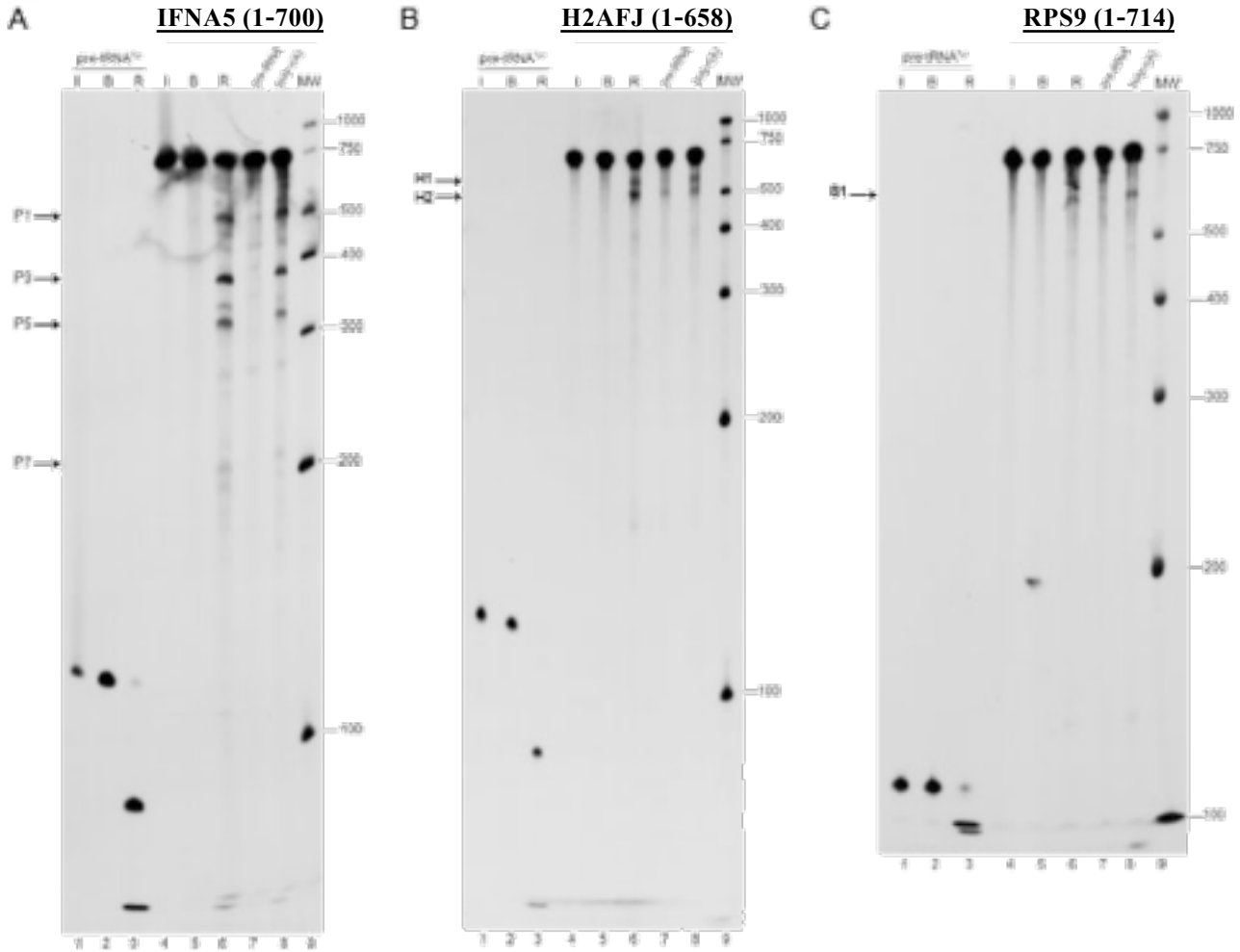
Human cellular mRNAs

There might be commonly organized information within the viral and cellular mRNA structures

tRNA-mimic elements in celullar mRNAs

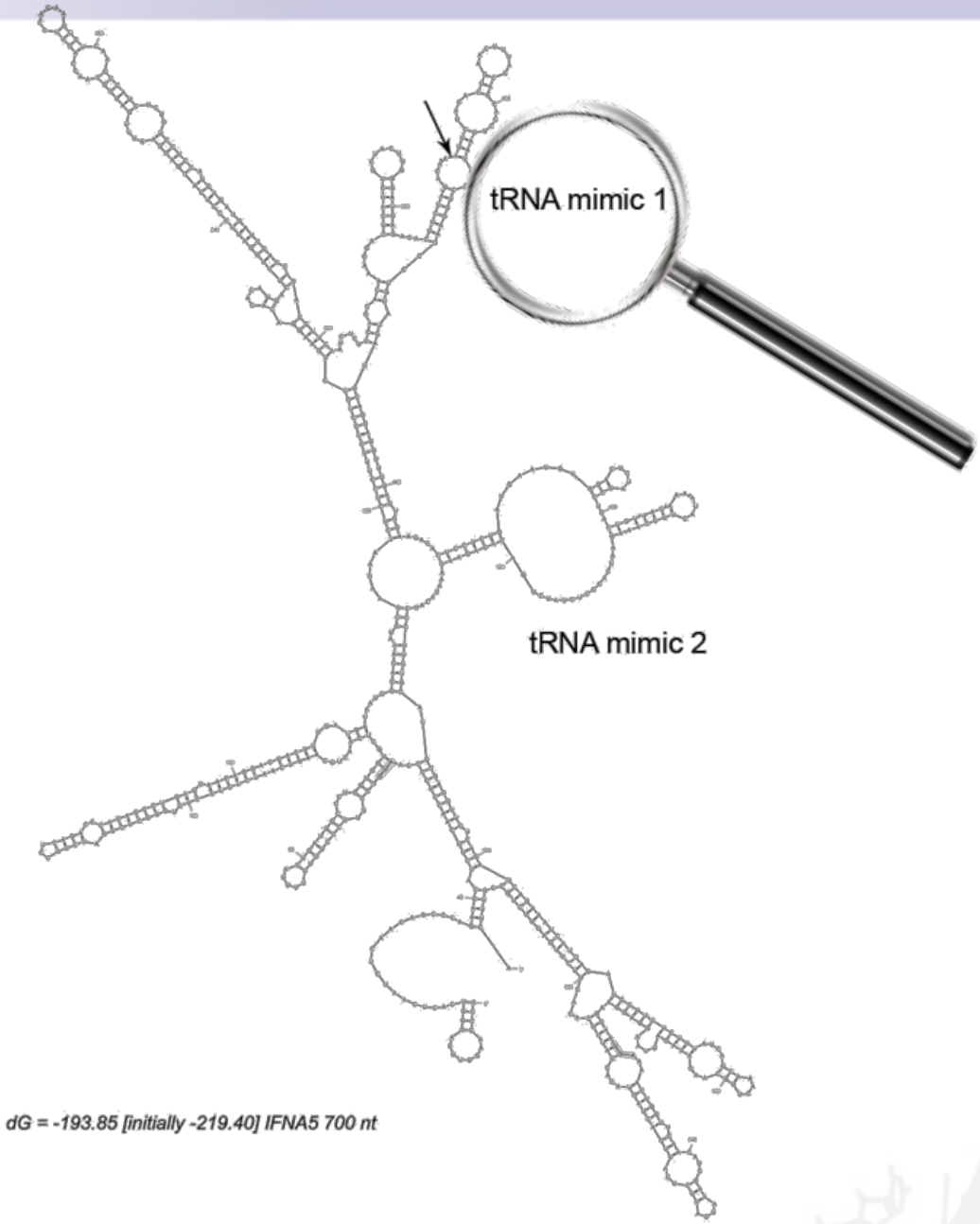


Cleavage, competition and minimal length



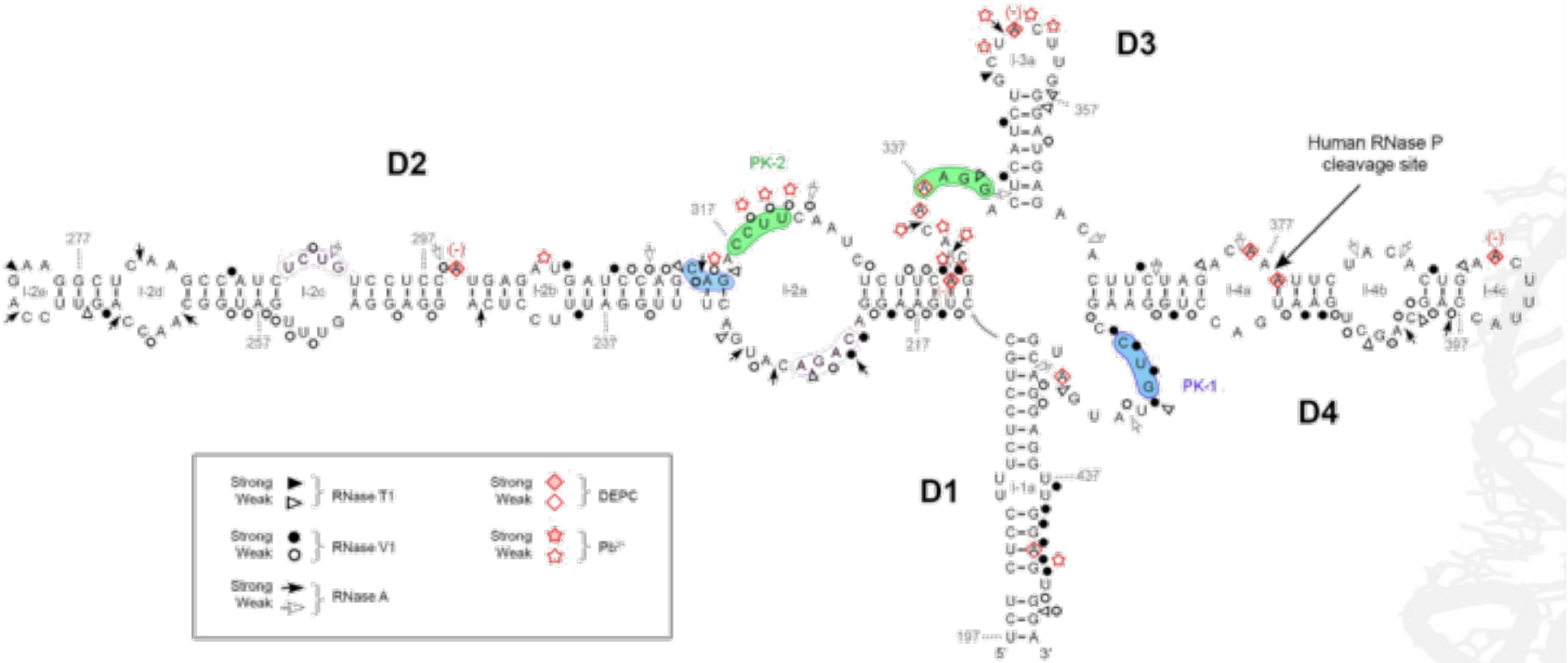
Precise cleavage position	+	+	?
Minimal substrate length (~200 nt)	+	+	+
Reverse competition	+	+	+
End group chemistry	+	?	?

IFNA5 secondary structure

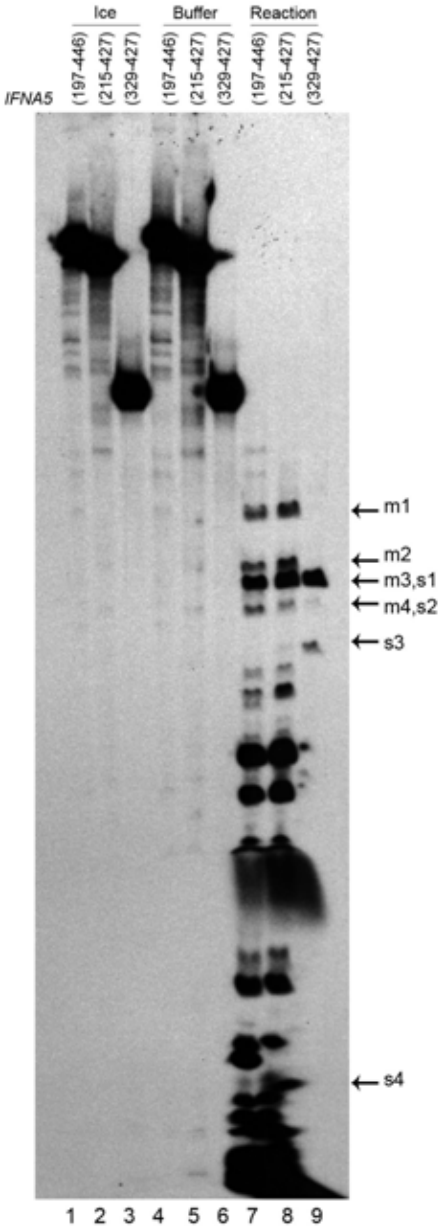


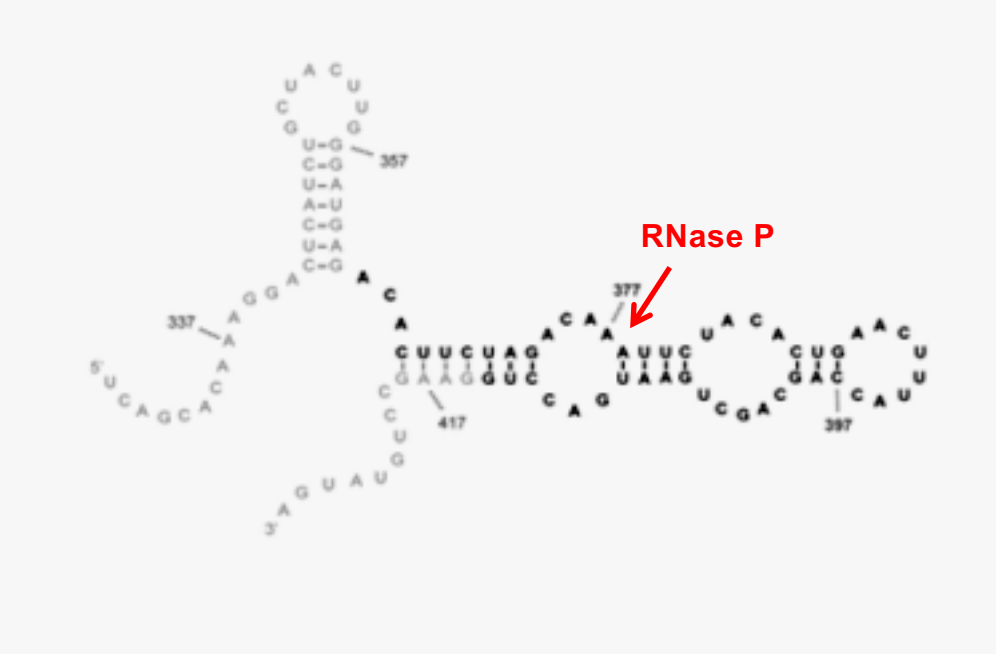
Summary of enzymatic and chemical probing

IFNA5-RNA (197-446)

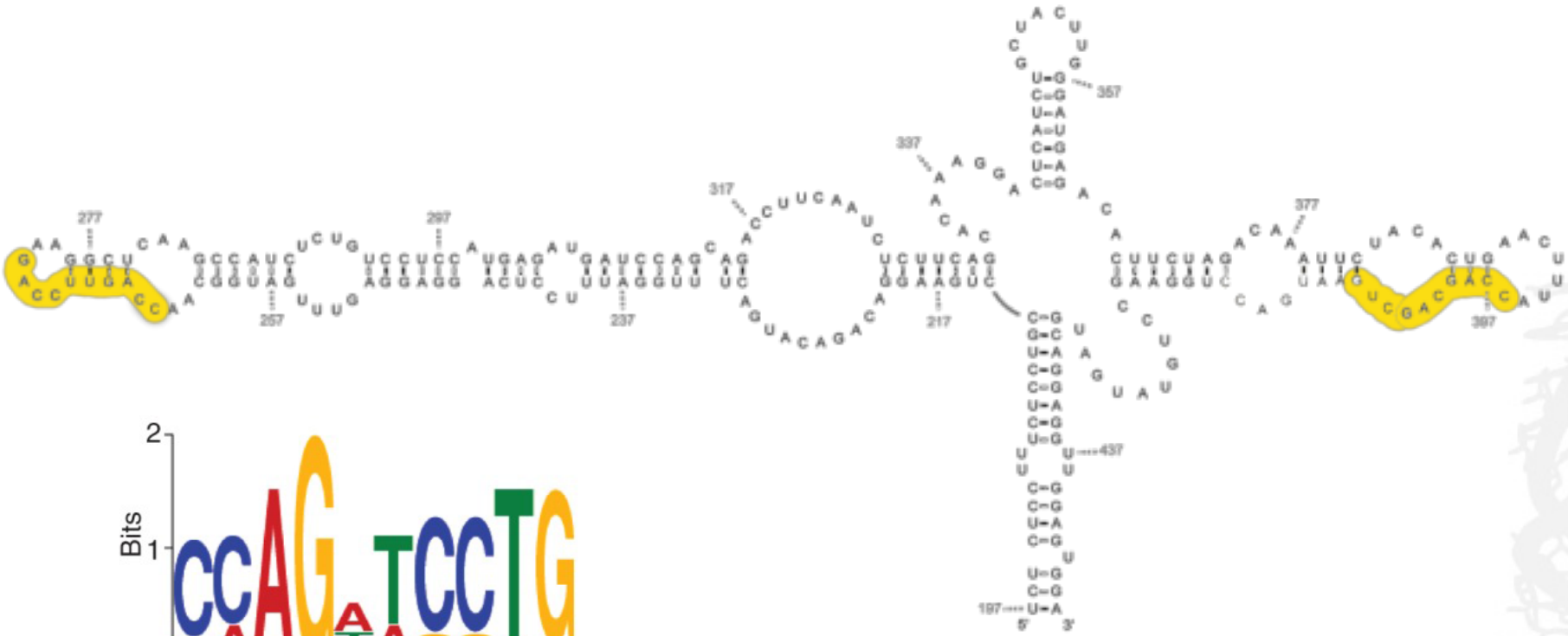


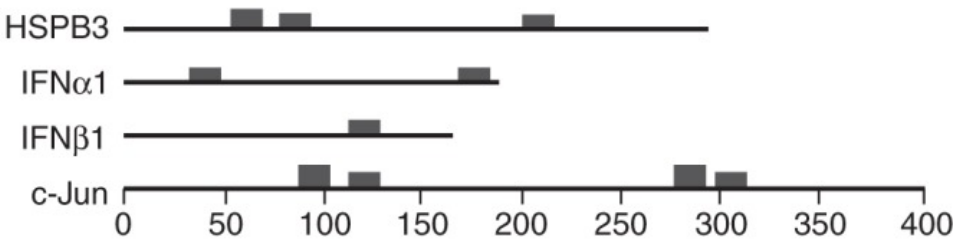
RNase T1 protection



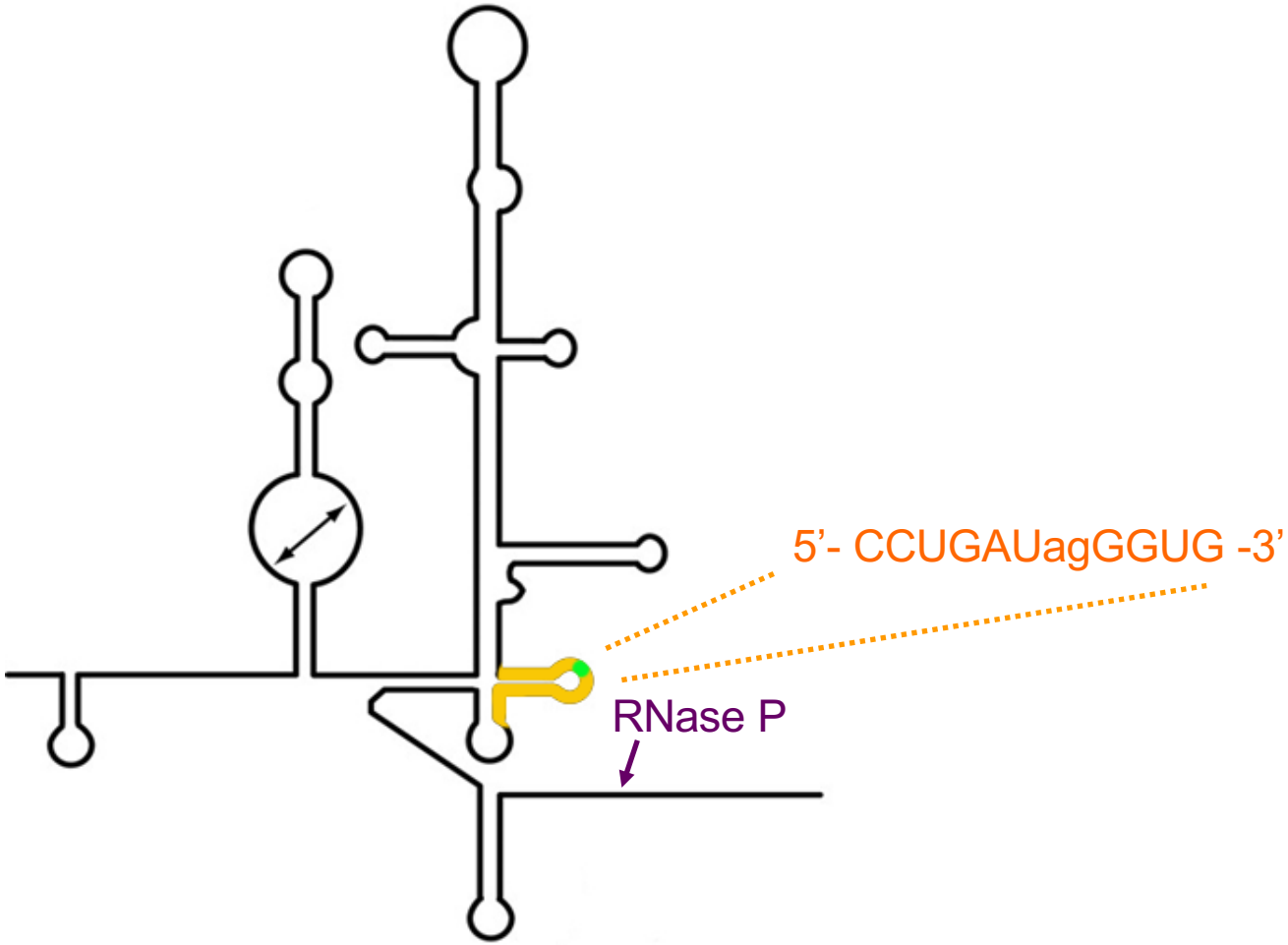


IFNA CAR signal is a tRNA-like element

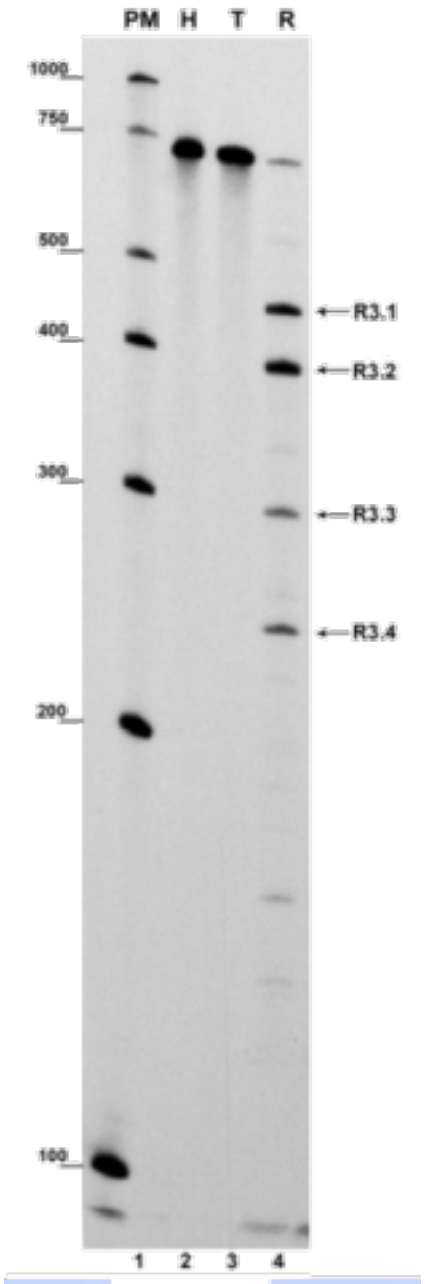


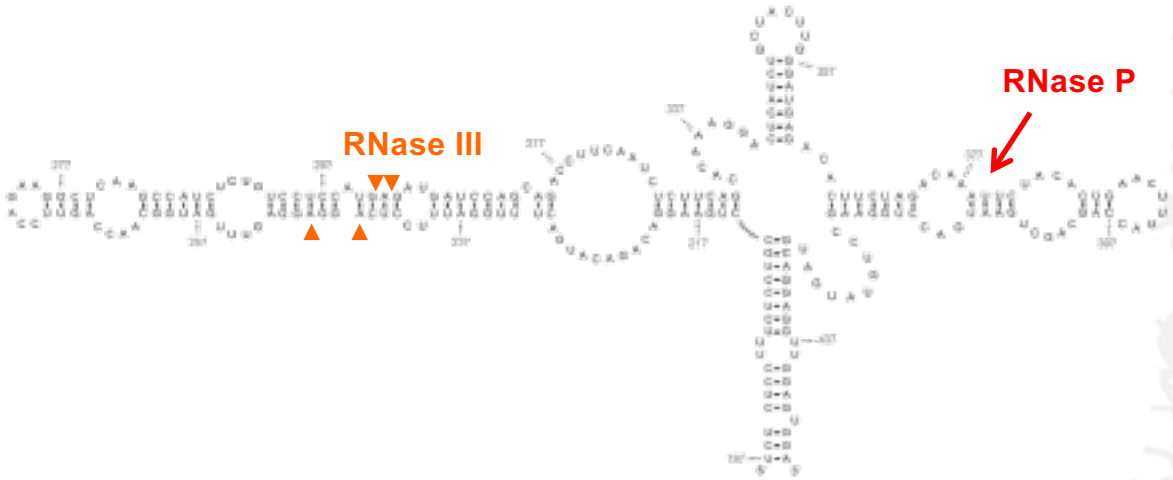


HCV tRNA-like might be a CAR signal

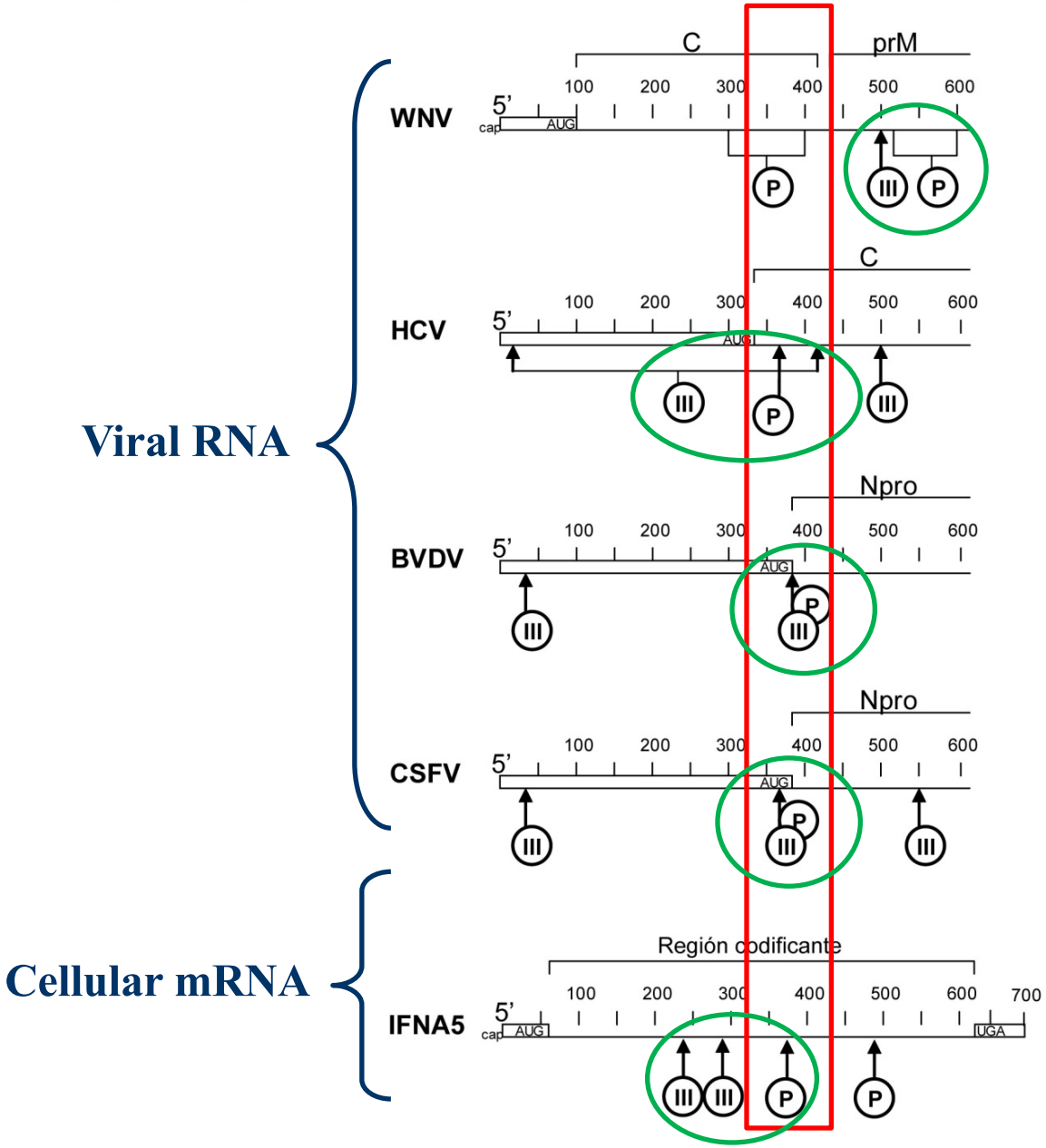


RNase III cleavage of IFNA5 mRNA





RNase III-P motifs



New archaeological method

RECONSTRUCTION		IRRUPTION
Structure	Evolution	Identification of "remote past" RNA elements
Representation		Material imprint
Similarity		Mimicry
	Common ancestor Convergence Capture	No distinction
High-resolution		Superficial knowledge

Detection of irruptions

Each individual mRNA is the vehicle for ancient RNA motifs

The meaning of every RNA motif is dependent of

- mRNA flanking context
- mRNA expression
- Protein interaction
- microRNA interaction
- Stress, cell cycle, cell death....
- Virus infection

- Sponge effect
  - tRNA-like
  - dsRNA-like
  - tRNA-dsRNA-like
- Derepression → social roles

Conclusions

- **Information layer at the RNA structure level within cellular and viral mRNAs**
- **(dsRNA-tRNA)-like motifs within cellular mRNAs**
- **Virus may reveal ontological repression**

Thank you!

Dra. Rosa Díaz Toledano

Dra. Mª Ascensión Ariza-Mateos