

SilentMutations (SIM): a tool for analyzing long-range RNA-RNA interactions in viral genomes and structured RNAs

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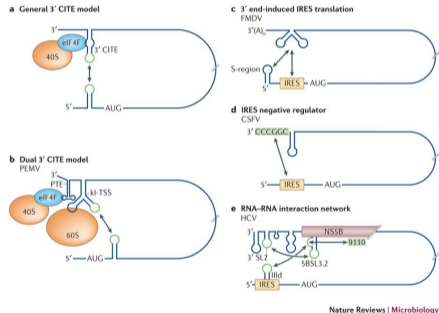
daniel.desiro@uni-jena.de

February 11, 2019

Long-range RNA-RNA Interactions

Translation Initiation

- 3' cap-independent translational enhancers
- internal ribosome entry sites



[Nicholson and White, 2014, Functional long-range RNA-RNA interactions in positive-strand RNA viruses.]

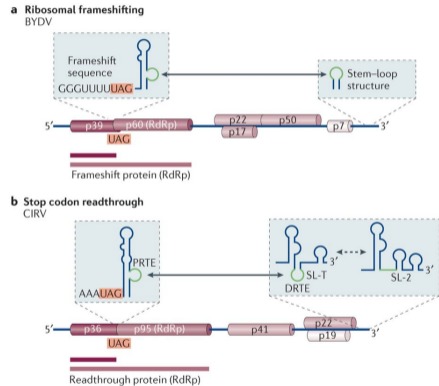
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Translational Recoding

- ribosomal frameshifting
- stop codon readthrough



Nature Reviews | Microbiology

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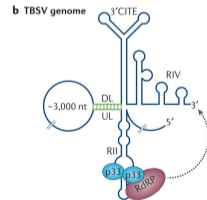
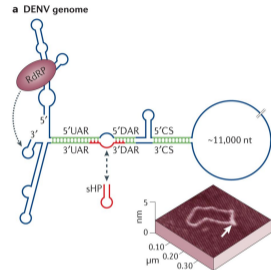
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Genome Replication

- autonomous
- protein factors



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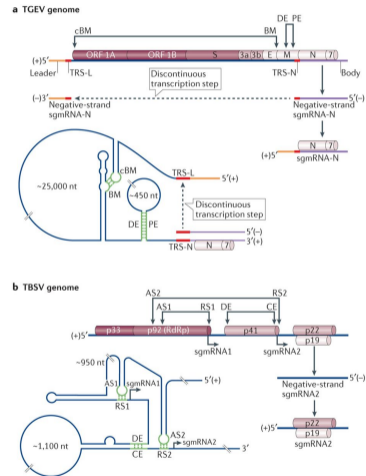
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sgmRNA Transcription

- discontinuous template synthesis
- premature RdRp termination



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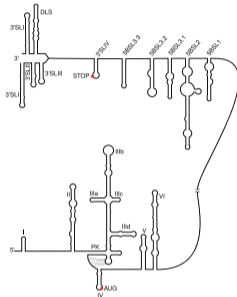
Virus (genus)	RNA-RNA interaction	Viral process regulated	Refs
Many plant viruses in the Tombusviridae family and the Umbravirus and Luteovirus genera	3' CITE-5' UTR or 3' CITE-5' coding region	Translation initiation ⁵⁴	5,6
BYDV (Luteovirus)	3' CITE-5' UTR	Translation initiation	21,25,30
	Frameshift site-3' UTR	Ribosomal frameshifting	47
CIHV (Tombusvirus)	3' CITE-5' UTR	Translation initiation	22,28
	PRTE- DRTE	Stop codon readthrough	50
TBSV (Tombusvirus)	3' CITE-5' UTR	Translation initiation	26,27
	UL-DL	Genome replication	52
	AS1-RS1; AS2-RS2; DE-CE	sgmRNA transcription	81-84
FMDV (Aphthovirus)	IRES-3' UTR	Translation initiation	32,33
	S-region-3' UTR	Possibly genome replication	33
CSFV (Pestivirus)	IRES-3' terminus	Translation	34
HCV (Hepacivirus)	IRES-5BSL3.2	Translation initiation	35-37
	5BSL3.2-3' UTR	Genome replication	38-42
DENV and WNV (Flavivirus)	5'UAR-3'UAR; 5'DAR-3'DAR; 5'CS-3'CS	Genome replication	53-61,65,66
TGEV (Coronavirus)	DE-PE; cBM-BM	sgmRNA-N transcription	77-79

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Conserved LRIs in Hepatitis C Viruses

HCV Genome

- ssRNA+ ~10kb
- single polyprotein
- UTRs highly structured



[Fricke et al., 2015, Conserved RNA secondary structures and long-range interactions in hepatitis C viruses.]

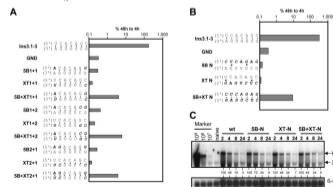
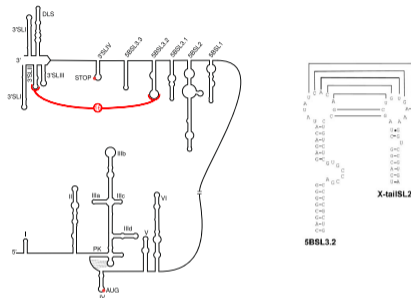
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Replication

- 3' X-tail structure
- LRI between 3'SLII and 5BSL3.2



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[Friebe et al., 2004, Kissing-loop interaction in the 3' end of the hepatitis C virus genome essential for RNA replication]

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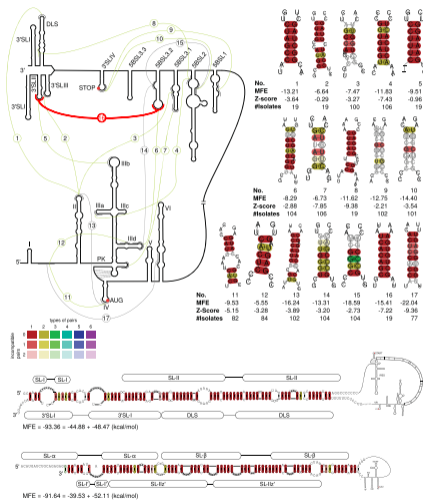
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LRIs can

- detected multiple LRIs
- verify several known
- identify several novel possible

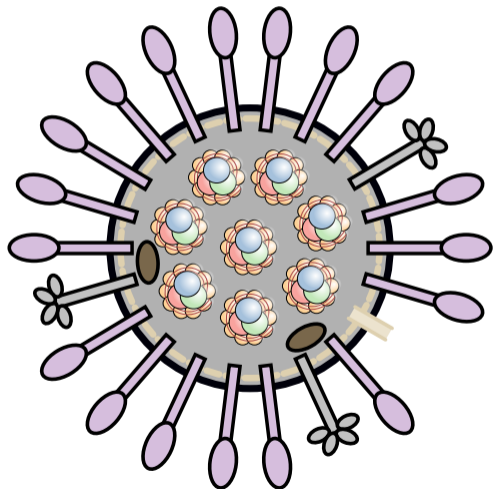
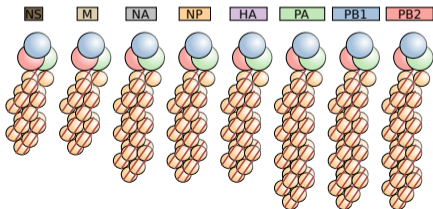


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Influenza A Virus (IAV)

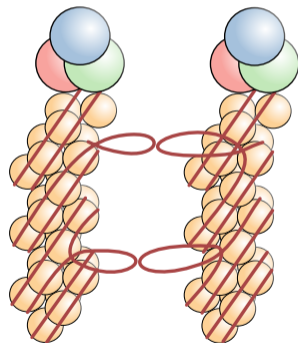
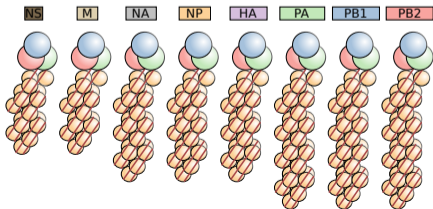
- polymerase basic proteins (PB2, PB1)
- polymerase acidic protein (PA)
- nucleoproteins (NP) + vRNA
- nuclear export proteins (NS)
- matrix protein (M) + lipid bilayer
- neuraminidase (NA) + hemagglutinin (HA)



[Eisfeld et al., 2015, At the centre: influenza A virus ribonucleoproteins.]

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Verification Experiment

Interaction

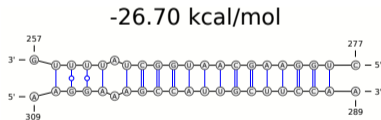
- avian H5N2
- 4 silent, trans-complementary mutations
- PB1 and NS
- reassort wild-type and mutant

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LRIsScan



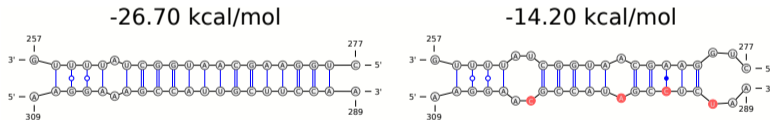
[Gavazzi et al., 2013, A functional sequence-specific interaction between influenza a virus genomic RNA segments.]

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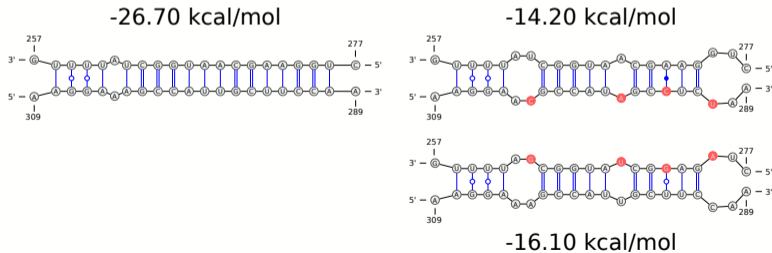
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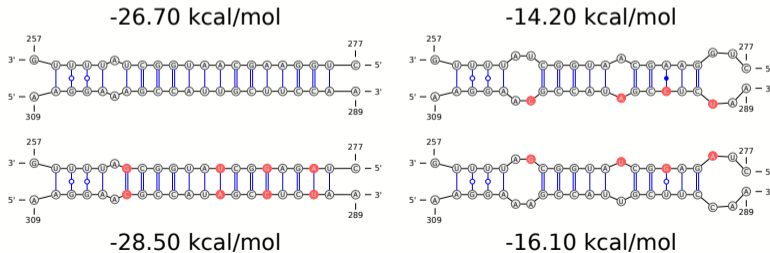
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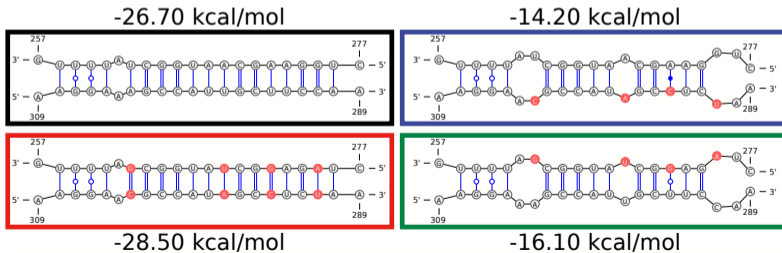
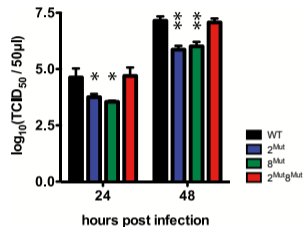
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Gavazzi et al.



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(a) preprocessing

seq1:2:29-45

5' ●●●●●●●●●● CUA ~~AUA~~ CCG ~~AGG~~ AGC ~~AGG~~ ACU ~~GGA~~ AGC ~~AAU~~ GGC ~~UAU~~ UUU ~~GAG~~ GUU ~~UAG~~ ●●●●●●●●●● 3'

5' ●●●●●●●●●● CUA ~~GGA~~ UUC ~~UUC~~ AAG ~~GAA~~ AGC ~~CAU~~ UGC ~~UUC~~ CAA ~~CAC~~ ACA ~~AUC~~ UGU ~~UAC~~ ●●●●●●●●●● 3'

seq2:1:21-42



pre-snip1

5' ACU ~~GGA~~ AGC ~~AAU~~ GGC ~~UAU~~ UUU 3'

3' ~~CAC~~ AAC ~~CUU~~ CGU ~~UAC~~ CGA ~~AAG~~ GAA 5'

pre-snip2



snip1

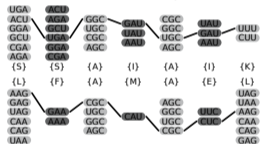
5' ACU ~~GGA~~ AGC ~~AAU~~ GGC ~~UAU~~ UUU 3'

5' AAG ~~GAA~~ AGC ~~CAU~~ UGC ~~UUC~~ CAA 3'

snip2

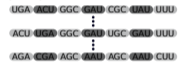
(b) permutation

codons1 (reverse complement)



codons2 (reverse complement)

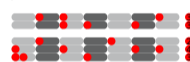
pre-perms1 (P*)



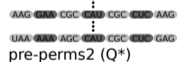
pre-perms1 (P*): 10368



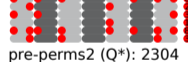
perms1 (P): 2684



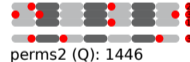
pre-perms2 (Q*)



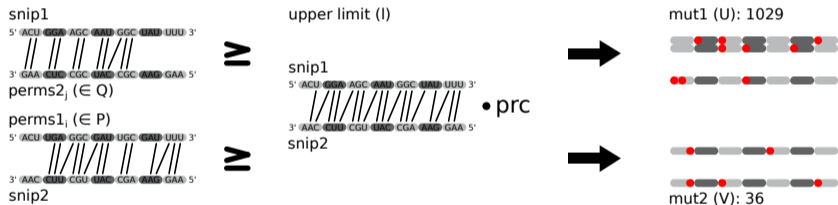
pre-perms2 (Q*): 2304



perms2 (Q): 1446

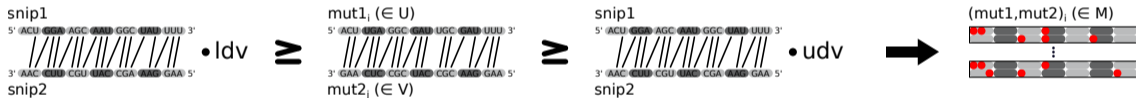


(c) attenuation



[Lorenz et al., 2011, ViennaRNA package 2.0.]

(d) recovery



(e) sampling

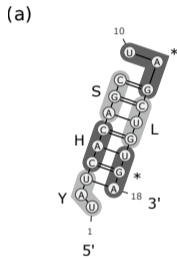
$$\min \left(\text{l-mrg} \geq \begin{array}{c} \text{snip1} \\ 5' \text{ ACU GGA AGC AAU GGC UAU UUU } 3' \\ // // // // // \\ 3' \text{ GAA CUU CGC UAC CGC AAC GAA } 5' \\ \text{mut2}_i \in M \end{array} \geq \text{u-mrg} \quad + \quad \text{l-mrg} \geq \begin{array}{c} \text{mut1}_i \in M \\ 5' \text{ ACU UGA GGC GAU UGC GAU UUU } 3' \\ // // // // // // // \\ 3' \text{ AAC CUU CGU UAC CGA AAC GAA } 5' \\ \text{snip2} \end{array} \geq \text{u-mrg} \right)$$

$$\text{l-mrg} = (mfe(\text{snip1}, \text{mut2}_i) + mfe(\text{mut1}_i, \text{snip2})) \cdot 0.5 \cdot (1 + \text{-mrg})$$

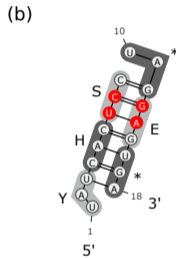
$$\text{u-mrg} = (mfe(\text{snip1}, \text{mut2}_i) + mfe(\text{mut1}_i, \text{snip2})) \cdot 0.5 \cdot (1 - \text{-mrg})$$

[Lorenz et al., 2011, ViennaRNA package 2.0.]

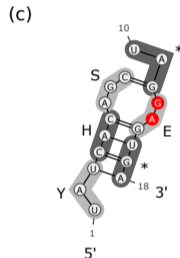
HCV Result



mfe(5BSL3.2_{WT}, SLII_{WT})
= -9.70 kcal/mol



mfe(5BSL3.2_{mut}, SLII_{mut})
= -9.90 kcal/mol



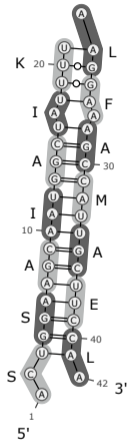
mfe(5BSL3.2_{WT}, SLII_{mut})
= -2.40 kcal/mol



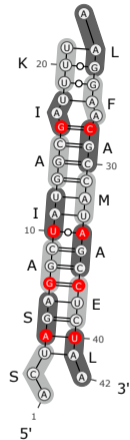
mfe(5BSL3.2_{mut}, SLII_{WT})
= -2.10 kcal/mol

[Darty et al., 2009, VARNA: Interactive drawing and editing of the RNA secondary structure..]

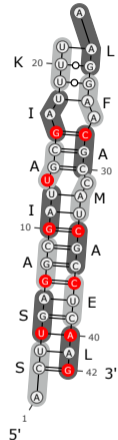
IAV Result



mfe(NS_{WT}, PB1_{WT})
= -24.60 kcal/mol



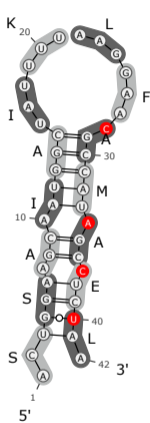
mfe(NS_{mut}, PB1_{mut})
= -26.40 kcal/mol



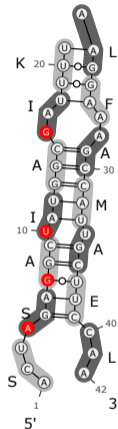
mfe(NS_{mut}, PB1_{mut})
= -23.40 kcal/mol

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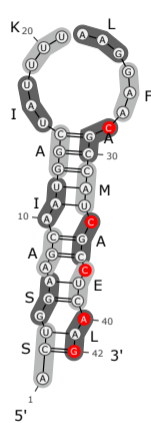
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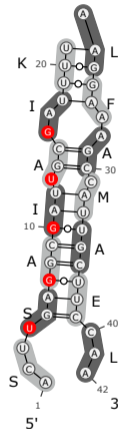
mfe(NS_{mut}, PB1_{WT})
= -11.80 kcal/mol



mfe(NS_{WT}, PB1_{mut})
= -14.00 kcal/mol



mfe(NS_{WT}, PB1_{mut})
= -10.10 kcal/mol



mfe(NS_{mut}, PB1_{WT})
= -11.30 kcal/mol

[Darty et al., 2009, VARNA: Interactive drawing and editing of the RNA secondary structure..]

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- Python (v3.6.5)
- ViennaRNA Package (v2.4)
- VARNA (v3.93) (optional)

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- worst case: $O(c^{\frac{k}{3} + \frac{l}{3}})$
- highly dependent on filter steps
- Example: 10368 · 2304 to 1029 · 36

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Summary

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- ssRNA+ and ssRNA-
- coding and non-coding

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Application

- predict LRIs (LRIScan)
- simulate mutations (SIM)
- create mutants (Lab)

Acknowledgements

- Martin Hölzer
- Bashar Ibrahim
- Manja Marz

