CONSERVATION OF SNORNA GUIDING FUNCTION

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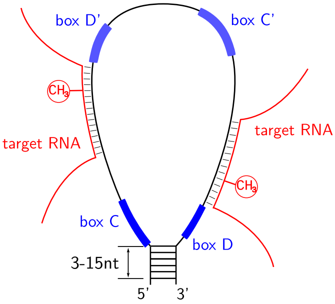
OUTLINE

1. SNORNAS
2. METHOD
3. INTERACTION CONSERVATION INDEX
4. RESULTS

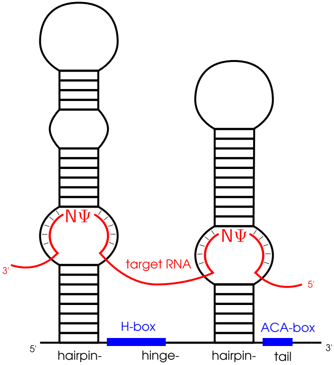
BOX C/D SNORNAS

- ca. 80nts long
- small terminal stem, large loop
- C-box: RTGATGA & D-box: CTGA
- two variant copies: D'- & C'-box
- 2'-0-ribose methylation adjacent to D and/or D'-box
- 7 - 20 nts long antisense element
- small duplex



BOX H/ACA SNORNAS

- ca. 130nts long
- hairpin-hinge-hairpin-tail structure
- H-box: ANANNA & ACA-box
- pseudoknotted interaction
- target RNA bind into pseudouridylation pocket
- bipartite antisense element
- one pseudouridylation per hairpin possible



FUNCTIONS

- main function is modification of:
    - ribosomal RNAs 18S, 28S and 5.8S
    - spliceosomal RNAs U2, U6, U5, U1, U12
    - modifications are clustered around active sites
- cleavage of pre-rRNAs
- alternative splice regulation of mRNA
- miRNA precursor

target RNA	N_m	Ψ
18S	40	36
28S	61	58
5.8S	2	2
snRNA	22	22

FROM KNOWN MODIFICATIONS TO CONSERVATION

- alignments of target RNA sequences with `RNAsalsa` [1]
- target prediction for the all snoRNA sequences
    - for box C/D snoRNAs with PLEXY [2]
    - for box H/ACA snoRNAs with RNAsnoop [3]
- map predicted positions to alignments
- evaluate the conservation of interaction between a modified position and a snoRNA family

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[1] Stocsits, R. R. and Letsch, H. and Hertel, J. and Misof, B. and Stadler, P. F., Accurate and efficient reconstruction of deep phylogenies from structured RNAs. , Nucleic Acids Res, 37(18):6184-93,Oct:2009

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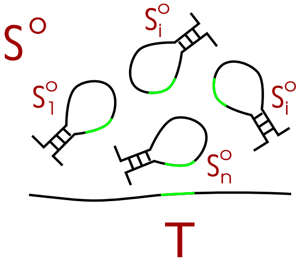
INTERACTION CONSERVATION INDEX



Target T is predicted to be modified by a set of snoRNAs in organism O

$$\bar{I}_T^o = \frac{1}{|\mathcal{S}^o|} \sum_{i \in \mathcal{S}^o} \underbrace{MFE(s_i^o, T)}_{\substack{\text{minimum free energy} \\ \text{of interaction between} \\ \text{snoRNA } s_i^o \text{ and target T}}}$$

- T ... specific target site
- $\mathcal{S} = \{s_1, .., s_n\}$ ... set of snoRNA families
- $\mathcal{O}$ ... set of organisms
- $\mathcal{S}^o$ ... subset of $\mathcal{S}$, contains snoRNAs interacting with T in organism o
- $\bar{I}_T^o$ ... average interaction energy with target site

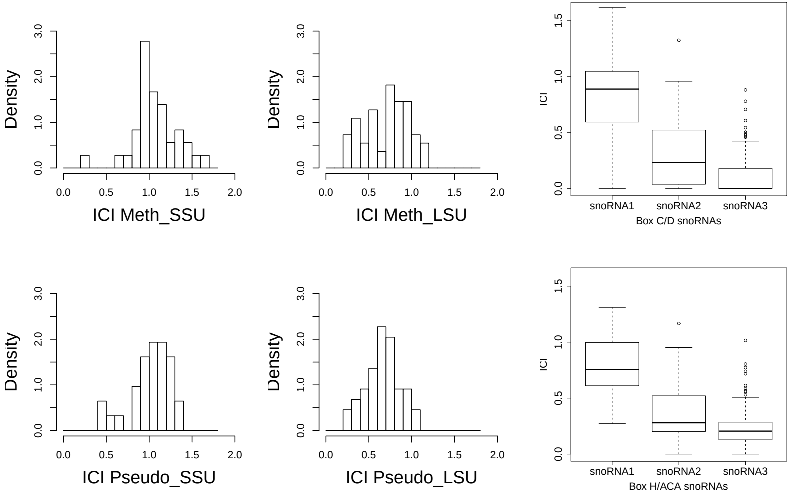
INTERACTION CONSERVATION INDEX

$$ICI_{s_x,T} = \frac{1}{|o|}\sum_{o\in\mathcal{O}} \underbrace{MFE(s_x^o, T)/\bar{I}_T^o}_{\substack{\text{if } >1 \rightarrow s_x^o \text{ better} \\ \text{than average}}},$$

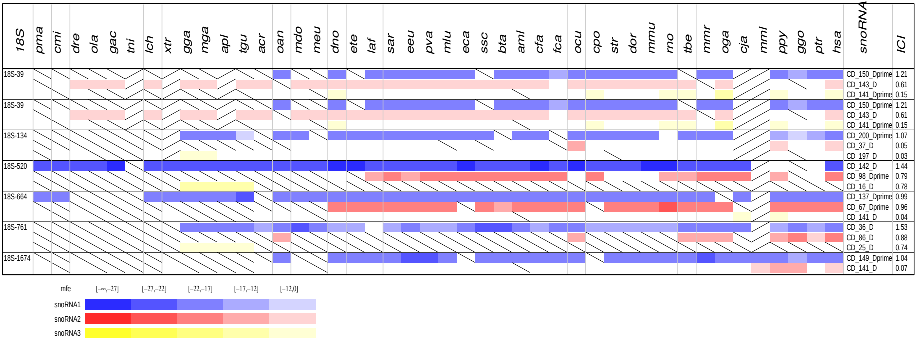
$$MFE(s_x^o, T) = 0, \text{if } s_x \notin \mathcal{S}^o$$

- interaction between a snoRNA family $s_x \in \mathcal{S}$ and a target site T throughout species
- $MFE(s_x^o, T)$ ... mfe of interaction between snoRNA family member and target
- compare interaction of s_x with other putative guides of T

$ICI_{s_x,T}$

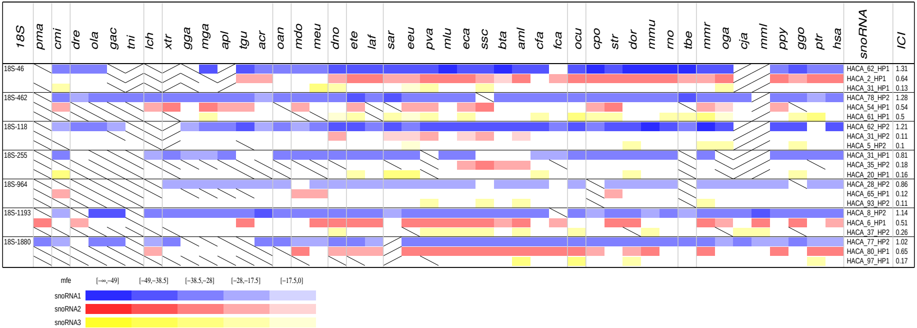

Density
ICI Meth_SSU
Density
ICI Meth_LSU
ICI
snoRNA1
snoRNA2
snoRNA3
Box C/D snoRNAs
Density
ICI Pseudo_SSU
Density
ICI Pseudo_LSU
ICI
snoRNA1
snoRNA2
snoRNA3
Box H/ACA snoRNAs
0.0
0.5
1.0
1.5
2.0
3.0

General Observation



→ conserved snoRNA guiding function in vertebrates

REDUNDANT GUIDING SYSTEM



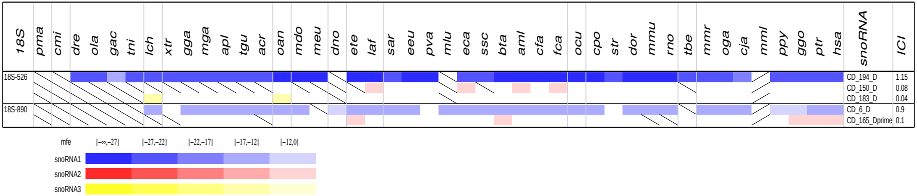
18S	snoRNA	ICI
18S-46	HACA_62_HP1	1.31
	HACA_2_HP1	0.64
	HACA_31_HP1	0.13
18S-462	HACA_78_HP2	1.28
	HACA_54_HP1	0.54
	HACA_61_HP1	0.5
18S-118	HACA_62_HP2	1.21
	HACA_31_HP2	0.11
	HACA_5_HP2	0.1
18S-255	HACA_31_HP1	0.81
	HACA_35_HP2	0.18
	HACA_20_HP1	0.16
18S-964	HACA_28_HP2	0.86
	HACA_65_HP1	0.12
	HACA_93_HP2	0.11
18S-1193	HACA_8_HP2	1.14
	HACA_6_HP1	0.51
	HACA_37_HP2	0.26
18S-1880	HACA_77_HP2	1.02
	HACA_80_HP1	0.65
	HACA_97_HP1	0.17

- one snoRNA family guides specific modification
- a second snoRNA emerges during evolution, guiding the same modification

METHYLATION

- orphan snoRNA SNORD83
- ’orphan’ methylated adenine 18S-526 (human 18S-449)
  - average mfe: $-24.8[kcal/mol]$
  - ICI_{MOD} : 1.15

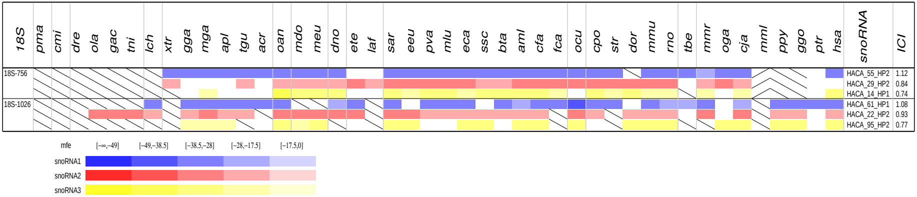
- orphan snoRNA CD_6 (chicken GGgCD20)
- ’orphan’ methylated cytosine 18S-890 (human 18S-778)
  - average mfe: $-14[kcal/mol]$
  - ICI_{MOD} : 0.9



PSEUDOURIDYLATION

- 'orphan' ψ 18S-756 (human 18S-681)
- two snoRNA guides
  - SNORA55
  - ICI_{MOD} : 1.12
  - orphan snoRNA SNORA29
  - ICI_{MOD} : 0.84

- 'orphan' ψ 18S-1026 (human 18S-918)
- two snoRNA guides
  - SNORA61
  - ICI_{MOD} : 1.08
  - snoRNA SNORA22
  - ICI_{MOD} : 0.93



FROM SNORNA TO CONSERVATION

- an analogous score which starts from a snoRNA family and averages the mfes of all putative targets to evalute the individual interactions
- sums over all organsims again
- useful to identify functions apart from rRNA and snRNA modification
- e.g. mRNA targets that influence alternative splicing

THANK YOU

- Sebastian Bartschat
- Hakim Tafer
- Jana Hertel
- Peter Stadler