RNA Tertiary Structure as a Proto-Language for Nano-Construction

“RNA is more than mathematics”

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Control of RNA information

- Control of synthesis
- Control of assembly
- Control of binding events
- Control of catalysis
- Control of movement

Regulation of timing
Multilayers of expression of information
In an evolutionary context

Selection pressure

Bottom-up causation

Top-down causation

Auletta, Ellis, Jaeger (2007) submitted
Structural network A
(eg. RNase P RNA Type A)

Structural network B
(eg. RNase P RNA Type B)

<table>
<thead>
<tr>
<th>Equivalence classes</th>
<th>Operations</th>
<th>Functions</th>
</tr>
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<tbody>
<tr>
<td>(=) (=) (=)</td>
<td>Assembly recognition</td>
<td>RNA assembly</td>
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<tr>
<td>(=) (=)</td>
<td>Assembly recognition Substrate recognition</td>
<td>pre-tRNA binding</td>
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<td>(=) (=)</td>
<td>Assembly recognition Catalysis</td>
<td>pre-tRNA cleaving</td>
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<tr>
<td>(=) (=)</td>
<td>Assembly recognition Substrate recognition Catalysis</td>
<td>pre-tRNA maturation</td>
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</tbody>
</table>
Conformational search is highly sequence dependent. The key elements of folding that control the precise orientation and stacking of helices are recurrent structural features or motifs.
Exploring the relationship between sequence and tertiary structure

RNA structures at atomic resolution (X-ray and NMR)

5' AUGCUACGGAUCAUCAUGAUUC
GUCAUGCAUCUUAUACGGGCC
CAUACCUGGAACUAACUACUAA
GAUCAGCGGUCAGCAGCAGCAG
UAUCAUUUGACAGCGGUAUUA
UACGCUAACUGACUAUCGGCUA
UCUAUCUACUGCUACUCCUACC
CUACUCGCCCUCUACUGCUCCUAC
UGCCUCAGCUCCUACCGGCU
CUCCUAACACACCGUCUC-3'

RNA sequences

Structural and sequence comparative analysis

Identification of set of conserved and semi-conserved nucleotides defining sequence signatures of specific RNA conformers

Recurrent conformer sequence signature

Erik Verzemnieks & Luc Jaeger, in preparation
Different structural motifs, different modes of operation, but same assembly function

Class of structural equivalence

Bacterial RNase P RNAs

Type A

Type B

Establishing the rules for prediction and rational design of RNA 3° structure

Specified by:

Watson-Crick pairing

Sequence signatures of 3° structure motifs

Watson-Crick pairing and sequence signatures of 3° structure motifs

**RNA nano-construction**

Tektonikos (Greek): "from the carpenter"

Architectonics (English): scientific study of architecture

RNA architectonics ⇔ RNA LEGO® game

(Leqt Godt = Play well, in latin: study, put together)

- Knowledge of the building blocks and of the rules governing RNA self-assembly into complex objects
- Rational design of novel RNA architectures

Westhof, Masquida and Jaeger (1996) Folding and Design (structure), 1, R78-R88
Design strategy (reverse folding 3D->2D->1D)

GAAA loop/receptor assembling module

Known X-ray structure (P4P6 RNA domain)

3D Molecular Design

tectoRNA 1

RNA Helical Packing in Solution: NMR Structure of a 30 kDa GAAA Tetraloop–Receptor Complex

2D Noesy NMR data for tectoRNA dimer

1 mM RNA, 40 mM Na\(^+\), pH 6.5, 10°C

In vitro selection

3D models building of tectoRNA from known RNA molecules

- P4P6 domain
- Group I ribozyme
- Hairpin ribozyme
- Loop-receptor interfaces
- 4 way-junction

TEM images of self-assembling RNA filaments and particles

Controlling the geometry of RNA helices with 3° structure motifs

This approach allows to determine the autonomous folding properties of RNA motifs
The RNA Architectonics Methodology
(3°->2°->1°)

X-ray or NMR
3° structures of RNAs

1. 3° structural motifs

2. TectoRNA 3° model

3. (three-dimensional modeling)

4. 4° building block model

5. (sequence design)

6. Experimental characterization of tectoRNA self-assembly properties
   (Native PAGE, TGGE, chemical probing, AFM, TEM, cryoEM)

7. Blue print

Chworos and Jaeger (2007) Book chapter 10 in Foldamers
Optimization of the RNA sequences

Criteria:

(1) Optimization of the stability predicted by mfold or RNAfold for the secondary structure (clear uncoupling of folding of 2D versus 3D structures)

(2) Minimization of occurrence of stable alternative folds
DDG(pred.fold1 - pred. fold2) should be large (> 3-4 kcal/mol)
Hierarchical stepwise assembly

16 RNAs

0.2 mM Mg$^{2+}$

4 tectosquares

Adressable 2D RNA particles

First step
0.2 mM Mg$^{2+}$

Square side of ~15 nm
Central hole 7-9 nm

Hierarchical stepwise assembly

16 tectoRNAs

0.2 mM Mg\(^{2+}\)

4 tectosquares

15 mM Mg\(^{2+}\)

Second step
15 mM Mg\(^{2+}\)

9 distinct patterns created with 22 TS (49 tectoRNAs)

Applications: towards informational materials

Fully addressable nucleic acid based nano-patterns and scaffoldings as templates for 1D, 2D and 3D connected networks

-> very precise immobilization of various nano-particles or other functionalities (nano-patterns and nano-circuits)

Chworos & Jaeger (2007) in "Foldamers"
Controllable Spacing of Cationic Gold Nanoparticles by Nano-Crown RNA

Alexey Y. Koyfman, Gary Braun, Sergei Magonov, Arkadiusz Chworos, Norbert O. Reich, & Luc Jaeger

*(JACS 2005, 127, 11886-11887)*

*Mixing inorganic with bio-organic components*
Construction and characterization of a gold nanoparticle wire assembled using Mg$^{2+}$-dependent RNA-RNA interactions

Andrew D. Bates, Benjamin P. Callen, Jonathan M. Cooper, Rick Cosstick, Cody Geary, Andrew Glidle, Luc Jaeger, John L. Pearson, Maria Proupin-Perez, Cigang Xu and David R. S. Cumming

Fundamental questions

RNA architectonics

Applications in biomaterial and nanosciences
Acknowledgments

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