Liquid Crystal Self-Assembly of dNTPs and rNTPs as Autocatalytic Pathway for Ribozymes Formation

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Veli Lošinj - Sept 26th, 2017
How to get long RNA prebiotically?

Even if we assume nucleotides were available on early earth, we still have more than one problem:

- Concentration problem
- Solubility problem
- Reactivity problem
- Hydrolysis problem
- Stability problem
How to get long RNA prebiotically?

"How RNA could possibly have emerged from the clutter without a "guiding hand" would baffle any chemist. It seems possible only by selection, a process that presupposes replication.[...] The need seems inescapable for some autocatalytic process such that each lengthening step favors subsequent lengthening. Only in this way could the enormous kinetic obstacle to chain elongation be surmounted. [...] Any invoked catalytic mechanism must accommodate the participation of a template, for there can have been no emergence of true RNA molecules without replication"

*Singularity* - *Landmarks on the pathways of life.*

**C. De Duve** 2005, Cambr. Univ. Press.

We still lack of a process capable to allow the onset of the RNA world starting from simple building blocks conceivably available on early Earth
Soft-matter physics and Origin Of Life: a pathway of self-organization

**SELF-ASSEMBLY** (favors stable aggregates)

**PHASE TRANSITION** (introduces new symmetries)

**PHASE COEXISTENCE** (selects molecules by partitioning)

**CATALYTIC ENVIRONMENTS** (favors chemical reactions)

AND EVERYTHING IS SOFT, MISCIBLE, REVERSIBLE, MARGINALLY STABLE

The guiding hand can be written in RNA chemical - physical properties
DNA/RNA: a physical point of view

Interaction between fragments of DNA and RNA double helices

end-to-end attraction

lateral repulsion
DNA/RNA BECOMES LIQUID CRYSTAL BECAUSE OF ITS ELONGATED SHAPE AND ITS RIGIDITY

Livolant, Nature (1986)
DNA & LIQUID CRYSTALS: A RECURRING CONNECTION

A Structure for Deoxyribose Nucleic Acid
Watson J.D. and Crick
F.H.C.
Nature 171, 737-738 (1953)

The highly concentrated liquid-crystalline phase of DNA is columnar exagonal
Livolant, Levelut, Doucet, Benoit

X-ray diffraction pattern obtained by M H F Wilkins and R Gosling in late 1950 showing a clear crystalline arrangement.
Short DNA/RNA and Liquid Crystals

6 to 20 bases

complementary oligomers

hydrophilic

hydrophobic

nanoDNA...
Short DNA/RNA and Liquid Crystals

6 to 20 bases

5'-CGATCG-3'

end-to-end stacking...

nanoDNA...

5'-CGATCG-3'

hydrophilic

complementary oligomers

stacking between distinct helices

hydrophobic...

pairing and stacking between overhangs
Short DNA/RNA and Liquid Crystals

Nakata et al. Science 2007
Zanchetta et al. JACS 2008

6 to 20 bases

5'-CGATCG-3', 5'-CGATCG-3'

nanoDNA...

end-to-end stacking...

forms liquid crystal phases

uniaxial columnar (C_U)

complementary oligomers

hydrophilic

stacking between distinct helices

pairing and stacking between overhangs

SHORT DNA/RNA BECOMES LIQUID CRYSTAL BECAUSE OF A HIERARCHY OF SELF-ASSEMBLY STEPS
LC ordering is a phase transition

THE LIQUID CRYSTAL ORDERING STABILIZES THE AGGREGATES

C. De Michele, T.B., F. Sciortino, Macromolecules, 45 (2012) 1090
DNA/RNA LCs in Molecular Mixtures

LC formation promotes the entropy driven phase separation of DNA double helices from single strands or flexible polymers (PEG).

Phase separation and liquid crystallization of complementary sequences in mixtures of nanoDNA oligomers.

LC phase coexistence -> SEGREGATION
-> selection of double strands
Random-sequence DNA oligomers

A pool of oligomers with random sequences is the most likely outcome of a hypothetic random chemical ligation of mononucleotides that could have taken place in early Earth.

20mer: 5’-NNNNNNNNNNNNNNNNNNNNNNNNN-3’

10^{12} different strands

Liquid Crystal Self-Assembly of Random-Sequence DNA Oligomers.

LC ordering -> SELECTION OF STRUCTURES
-> segregation of linear from non linear polymers
Can it have a role in the prebiotic synthesis of long RNA?
Very short DNA oligomers: 4 base long

5' - GCCGp - 3'
5' - ATTAp - 3'
5' - GCTAp - 3'

5' - GCCp - 3'
5' - AATTp - 3'
5' - GCTAp - 3'

Isotropic (ISO)
Nematic (N*)
Columnar (COL)

Fraccia et al. ACS Nano, 2016

DUPLEX FORMATION, LINEAR AGGREGATION & LC FORMATION ALL AT THE SAME TIME
Even the shortest: LC ordering of dATP - dTTP

Starting solution:

- 50 mM dATP-dTTP
- pH 7.5
- No added salt
- T = 5°C

Under submission
Even the shortest: LC ordering of dATP - dTTP

Starting solution:
50 mM dATP-dTTP
pH 7.5
No added salt
T = 5°C
Even the shortest: LC ordering of dATP - dTTP

Starting solution: 50 mM dATP-dTTP, pH 7.5, No added salt, T = 5°C
Liquid Crystals ordering of dNTPs

LCs seem to know about Watson – Crick pairing...
Liquid Crystals ordering of dNTPs

**GROWING STABILITY**

**GUANOSINE QUADRUPLEXES**

Liquid Crystals ordering of dNTPs

- **dCTP + dGTP**
- **dTTP + dATP**
- **dNTPs mix**

under submission
Which is the arrangement of dNTPs?

LC of single bases
- dATP + dTTP 1:1
- T = 5 °C
- Base stacking distance = 0.334 nm

LC of DNA 12mers
- T = 20 °C

Intercolumnar distance = 2.3 nm

LC of single bases
- LC of DNA 12mers

smaller q
- COLUMNAR PEAK
larger q
- STACKING PEAK

q_h = 0.217 Å⁻¹
- 374 mg/ml

q_h = 0.275 Å⁻¹
- COL

Intercolumnar distance = 2.3 nm
Still duplexed DNA even without backbone

inter-columnar distance

$\text{NA anion internal density: } c_U = M_U / V_U$

slope:
$SL = (8\pi^2/\sqrt{3})L_U / M_U$

duplex DNA polymers

NTPs
duplexes

triplexes
quadraplexes

column of dimers

lines computed from concentration for columns formed by stacks of 2, 3, and 4 bases

$\chi^2 (\text{Å}^2)$

$c$ (mg/ml)

$dGTP$ short range COL

dCMP/dGTP short range COL

dGTP COL
Liquid Crystals ordering of NTPs (almost)

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[dTTP](#)

[UTP](#)

*to be published*
Message:

- DNA and RNA order in LC phases in a very broad range of lengths

- As length increases LCs become more stable
LC order as a template for ligation

Liquid crystal order keeps the terminals of the oligomers in continuous physical contact

Can this proximity favor the formation of chemical bonds?


Non-enzymatic ligation reaction

1st Step: Terminal Phosphate activation

2nd Step: Nucleophilic substitution

3’P DNA ligation in LC phase

- **D1p** (12mer)

- **PEG 8 kDa**

C₀ DNA = 5mM = 19 mg/ml

Cₚ DNA = 80 – 110 mM

Cₚ DNA = 300 - 400 mg/ml

DNA – PEG mixed

DNA – PEG domains

Increasing PEG (osmotic pressure)

PEG 8 kDa
LC forming sequences

D1p/PEG mixture    D1p LC domains

<table>
<thead>
<tr>
<th>c&lt;sub&gt;PEG&lt;/sub&gt; (100 g/l)</th>
<th>DNA/PEG mixture</th>
<th>&lt;N&gt; &lt; 2</th>
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DNA LC domains    <N> ≈ 9
ISO vs LC domains

How to compare ISO and LC phases segregated PEG? D1p/PEG phase diagram is **temperature** dependent...

Increasing Temperature

D1p LC

25°C

D1p ISO

65°C

...and **PEG osmotic pressure** controls LC stability: high PEG -> higher melting T, lower PEG -> lower melting T
ISO vs LC domains

\[ \frac{[\text{PEG}]}{[\text{DNA}]} = 50 \]

Reaction Time = 24h

Temp = 65°C

\[ <N>_{\text{ISO}} \approx 3 \]

\[ <N>_{\text{LC}} \approx 9 \]
Not just concentration dependence
5’P RNA ligation catalyzed by LCs

Reaction conditions:
10 mM RNA 5’P
10 mM HEPES pH 7.5
60 mM MgCl₂
300 mM EDC
0 – 30 %W PEG 8 kDa
24 - 48 h
T = 25 – 40°C

Circular/linear products decreases in LC phase

Fluorescence Intensity

Polymer Size

Fluorescence Intensity

Polymer Size

yield 31%
yield 72%

to be published
Features of Liquid Crystals Autocatalysis

✓ Self-assembly and ordering from monomers to long DNA/RNA

✓ Self-templating Non–Enzymatic ligation, protection from hydrolysis

✓ Complementarity and structural selection through phase separation

✓ Everything is soft, miscible, reversible and marginally stable
LC autocatalysis conjecture
Growing length by non-equilibrium cycling

Structural selection

Folding polymers
LC autocatalysis conjecture

- Folding RNA strands, ribozymes
- LC-assisted chain lengthening feeds the RNA world
- Irreversible collapse of immiscible molecules
- LIQUID CRYSTAL AUTOCATALYSIS
- NA fragments, nucleotides, amphiphilic molecules
- Boundary of marginal solubility
- Solubility in water

- Breaking of chains recycles them through the LCA
- Isotropic dispersion of water-soluble molecules
and thanks to you for listening!