Polar bears, Unpaved roads, Everest Climbing and Ribosomes in Action

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DNA

Replication

Transcription

mRNA

Translation

protein

The Ribosome
Why X-ray crystallography?

SIZE X 1,000,000

ATOM

AMINO ACID, SMALL MOLECULE

PROTEIN

BACTERIA

CELL

INSECT

100KM

1000KM

1000MILES

LIGHT MICROSCOPE

ELECTRON MICROSCOPE

X-RAYS
Lessons from POLAR BEARS

Hibernating polar bears pack their ribosomes orderly just before their winter sleep, indicating that:

Ribosomes can be orderly packed

While densely packed, ribosomes can maintain their integrity and functional activity for months, despite their natural tendency to deteriorate within days.
The Dead Sea
Haloarcula marismortui
Haloarcula marismortui
The first ribosomal (B50S) microcrystals
and their powder diffraction pattern

1980

Exp. 30 B150 T100m pH 6.2

D = 1000

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<th>cÅ</th>
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Aimed
The first ribosomal (B50S) microcrystals and their powder diffraction pattern

1980 - the first microcrystals of the large ribosomal subunit from *Bacillus stearothermophilus*
An electron micrograph of a section of a crystal of the large ribosomal subunit from *Bacillus stearothermophilus*.
"Powder" diffraction to 3.5Å Spacings match solution scattering.
ESRF synchrotron in Grenoble
Crystal decay by irradiation after 0.1 seconds at -10 deg C
PDB Depositions

Introducing biological cryo-crystallography & MAD (for phasing) & Better detectors

Hakon Hope

X10³

'72 '75 '78 '81 '84 '87 '90 '93 '96 '99 '02 '05 '08
The first cryo bio crystallography experiment, SSRL, Stanford 1986
The way to structure determination was long and demanding, we frequently felt as if we are climbing high mountains, just for discovering that a higher Everest is still in front of us.

We hardly felt like Archimedes discovering the “bath principle” and rushing out shouting "Eureka! Eureka! (I've found it! I've found it!)“

Introducing cryo bio crystallography and discovering that crystals can acquire almost eternal life, was one of these rare moments.
# Number of Exposed Crystals

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<th>Until Conditions for High Resolution Were Found</th>
<th>After Conditions for High Resolution Were Found</th>
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<tr>
<td><strong>Haloarcula marismortui</strong></td>
<td>50S</td>
<td>&gt;2500 Pioneering CRYO - biocrystallography</td>
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<td><strong>Thermus thermophilus</strong></td>
<td>30S</td>
<td>&gt;1300 Crystal Stabilization</td>
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<tr>
<td>Deinococcus Radiodurans</td>
<td>50S</td>
<td>75 Freezing conditions</td>
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Eureka!
Ribosome in Action

Based on crystallographic studies, Yonath's group, The Weizmann Institute, Rehovot, Israel, and Max-Planck research Unit, Hamburg, Germany
Using three dimensional image reconstruction of tilt series of two-dimensional sheets we* found that the PTC is situated above the entrance to an internal tunnel.

Based on previous biochemical studies, Malkin & Rich, 1967 Sabatini & Blobel, 1970 we suggested that this is the nascent protein exit tunnel.

* (Milligan and Unwin, 1986; Yonath et al., 1987).
The ribosome returns

Peter B. Moore

In the longer term, however, the need for high resolution information about ribosome structure is clear. The progress made by Yonath and collaborators in their crystallographic investigations in the past year is encouraging in this respect. They recently proposed a startling model for the 50S ribosomal subunit which they postulate a large channel through the centre of the particle. This model was based on a three-dimensional reconstruction from electron micrographs of crystalline sheets of ribosomal subunits from Halobacterium marismortui, a bacterium that lives only in saturated, or near-saturated salt solutions. It would be interesting to know whether this model is consistent with, for example, the solution-scattering data available for large subunits from E. coli. More significant than this model, however, is the fact that Yonath and
The nascent protein exit tunnel
Trigger Factor

- Unique to eubacteria and chloroplasts
- First chaperone to encounter nascent chains
- Prevents aggregation of cytosolic proteins
- Acts co-translationally
- More?
Trigger Factor

binds to ~ 90% of the translating ribosomes

N-terminal (TFa)

Ferbitz et al. *Nature* 2004
In the physiological complex, the bound TFa undergoes conformational rearrangements.
A hydrophobic Pocket Opens upon Binding to the Ribosome
Combination of real-time experiments (Kaiser et al., 2006) & crystallographic analysis (Baram et al., 2005)
Binding of TFα to D50S in a physiological complex

Modeled Nascent Peptide
Early deaths caused by infectious diseases and no antibiotics

Keats (1795-1821)  Kafka (1883-1924)  Orwell (1903-1950)
Mozart (1756-1791)  Schubert (1797-1828)  Chopin (1810-1849)

Adapted from V. Ramakrishnan
Because of the fundamental role played by the ribosomes, many antibiotics target them.
Erythromycin in D50S tunnel
Over 40% of the antibiotics inhibit protein biosynthesis.
Most of them bind to the ribosome.

The main problems in the clinical use of the antibiotics are selectivity and resistance.
All antibiotics induce resistance.
Most antibiotics are not fully selective.
Synergism of ribosomal antibiotics:
The combination of two antibiotics drugs that can interact with each other and enhance activity.
Synergcid

Streptogramin $A$

dalfopristin

Streptogramin $B$

quinpristin

**synergid** Function: *noun*, Etymology: New Latin *synergida*, from Greek *synergos* working together
Synergism opens the gates for:

(a) Introduction of further species-specific anchors, thus increasing selectivity

(b) Providing alternative interactions, thus reducing the rate of the appearance of resistance
All antibiotic binding sites on the ribosome are of functional relevance.
David and Goliath: How do the tiny antibiotics paralyze the giant ribosome?
Antibiotics Targeting Ribosomes

Based on crystallographic studies, Yonath's group, The Weizmann Institute, Rehovot, Israel, and Max-Planck research Unit, Hamburg, Germany
The ribosome translates the genetic code into proteins

- Universal cellular assembly of rRNA and r-proteins
- Total mol weight 2.5 - 4 Mega Dalton (for prokaryotes & eukaryotes, respectively)
- Two subunits: in bacteria called 30S and 50S

Total mol weight 2.5 Mega dalton in prokaryotes: One RNA chain of ~1600 nucleotides (16S RNA) and ~ 21 different proteins (called S1….S21)

Total mol weight 1,5 Mega dalton in prokaryotes: Two RNA chain of total ~3000 nucleotides (5S RNA & 23S RNA) and ~ 34 different proteins (called L1….L34)
We identified the ancient (prebiotic) translation apparatus within the contemporary ribosome.
2D representation of the 23S RNA from *D. radiodurans*
The sizable symmetry related region (180 nucleotides) within the large ribosomal subunit
A similar symmetry related region was detected in all known structures.
The tRNA molecule

Anti-codon loop

Double Helical Regions

The bond connecting the helical region and the single strand almost overlaps with the two fold axis

CCA-aa

The Universal Single Strand CCA (3'end) carries the amino-acid (A-site) or the nascent peptide (P-site)
tRNA motions

The independent-correlated motions:

1. A shift of the helical part
2. Rotation of the 3'-end

around the bond connecting it with the tRNA acceptor stem, which coincides with the PTC 2-fold axis
THE RIBOSOME IS A POLYMERASE,
HENCE IT HAS TWO CATALYTIC TASKS:

1. The creation of the peptide bond

2. The elongation of the nascent protein
The ribosome architecture provides a symmetrical the frame for the tRNA 3’end end (CCA-aminoacid), which accords with the early finding of symmetrical relationship of the ribosome substrates*

* (Nissen et al., 2000)
The ribosome architecture provides a symmetrical frame for the tRNA 3’end (CCA-aminoacid), which accords with the early finding of symmetrical relationship of the ribosome substrates*

The tRNA 3’end rotatory motion is part of the mRA/tRNA translocation and can occur regardless of the nature of this motion (simple sideways shift, hybrid motion, etc) It also does not depend on global ratcheting.

* (Nissen et al., 2000)
The symmetrical region is highly conserved

FREQUENT nucleotides: same in > 95% of ALL sequences

In the symmetrical region: **98%**

In the ribosomal 23S RNA excluding symmetrical region: **36%**

(based on 930 species from all phylogenetic domains)
The high conservation of the symmetrical region indicates that its existence is beyond environmental conditions.
Suggesting that

The proto-ribosome, which was a simple dimeric RNA enzyme, is still embedded in the core the contemporary ribosome
Synthesis of activated pyrimidine ribonucleotides in prebiotically plausible conditions

Powner, Be´atrice Gerland & Sutherland
nature 459, 14 May 2009

Systems chemistry on early Earth
Jack W. Szostak
A new way of looking at the synthesis of RNA

Self-Sustained Replication of an RNA Enzyme
Lincoln and Joyce
SCIENCE, 323, 2009
The SES motif (Stem-Elbow-Stem)

The symmetrical region of the ribosome core contains the SES fold. This motif, which has a tendency to dimerize, seems to be an ancient form of RNA.
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The proto-ribosome was a dimer of RNA chains resembling the contemporary PTC.
We suggest a chemical prebiotic process, originating from an oligonucleotide and proceeding via a self folded unit into a self-assembled dimer, thus producing proto-ribosome pockets.

As RNA can be its own template for replication, the proto ribosome could have evolved by gene fusion or gene duplication.
General RNA Oligonucleotide Construct Design

RNA Oligonucleotide Precursor (SES Motif)
Proto-ribosome constructs:
An example for a “primitive” version
Proto-ribosome constructs: An example for a “still existing” version

the two dimensional diagram of D50S
Non uniform tendency to dimerize.
The preference of selected sequences over very similar, albeit not identical ones, indicate that:
survival of the fittest and natural selection seem to play a major role in the prebiotic world, although these properties are commonly related to the evolution of species
A hierarchical model for evolution of 23S ribosomal RNA
Konstantin Bokov & Sergey V. Steinberg

The symmetrical region = proto-ribosome

Based on the pattern of A-minor interactions within the 23S RNA
Peeling the union: Ribosomes are ancient molecular fossils

Based on the pattern of the RNA tetra-loops within the 23S RNA

Haiso et al., 2009
Conclusions

Analysis of the early steps in protein biosynthesis enabled visualization of a continuous path from the primordial world to contemporary genetic translation.

It also indicates that the ribosome is a naturally occurring ribozyme that outlived the transition from the presumed pre-biotic ‘RNA World’ to contemporary life.
Still open questions (a selected list)

Was there an RNA world?

Why should RNA produce machine for making proteins? Or: Was the proto-ribosome an RNA machine, performing RNA needs, prior to the appearance of amino acids?

What was first: the genetic code or its products?
Nonenzymatic RNA Ligation in Water
Pino, Ciciriello, Costanzo and Di Mauro
J. Biol. Chem. 2008

Efficiency of a self-aminocatlyed ribozyme: Effect of the length and base-composition of its 3' extension
Lehmann, Reichel, Buguin and Libchaber

Charging of tRNA with non-natural amino acids at high pressure
Giel-Pietraszuk Jan Barciszewski

Aminoacyl-RNA Synthesis Catalyzed by an RNA
Ilangasekare, Sanchez, Nickles and Yarus
Science 267 (1995) 643-647
The existence of well performing polypeptides catalyzing fundamental reactions and/or stabilizing the machines producing them may have led to the emergence of the genetic code.
A hypothetical non-coded “enzyme” that can be useful for RNA metabolism

Active site formed by enhanced local concentration of imidazole rings
First amino acid?

Glycine?

Lysine and arginine?

Histidine?
First amino acid?

**Glycine?** Because it is the simplest

**Lysine and arginine?** Because they are basic, thus interacting with RNA

**Histidine?** Because of its imidazole that is synthesized in a pathway similar to RNA bases; and because imidazole can act as a catalyst
Those who believed in us

H.G. Wittmann, & later, Franceschi, MPI, Berlin

Christian Anfinsen, NIH

Alexander Rich, MIT

Michel Sela, Weizmann inst.

Sir John Kendrew, EMBL
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the members of my group

for their devotion and enthusiasm

in good and bad times
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Thanks to the Max-Planck Ribosome Research Unit at the DESY, Hamburg
X-ray data were collected initially at BW6/EMBL/DESY, KEK CHESS/Cornell and SSRL/Stanford

High resolution data were collected at ID14/4 and ID29 ESRF/EMBL, Grenoble, France and 19ID/APS/ Argonne Nat lab, USA

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and, more than to anybody else,

to MY FAMILY

who supported me throughout, with no questions or complains despite my frequent "disappearances" and although, at times, my mind was not solely with them....