Kemian Nobel 2009: Ribosomien atomitason rakenne perustutkimuksen ja lääketieteen palveluksessa

Mikko Frilander Helsingin Yliopisto, Biotekniikan Instituutti



"for studies of the structure and function of the ribosome"



b. 1952 (in Chidambaram, Tamil Nadu, India)



Credits: Michael Marsland/Yale University

Thomas A. Steitz () 1/3 of the prize USA Yale University New Haven, CT, USA; Howard Hughes Medical

b. 1940



Credits: Micheline Pelletier/Corbis

| Ada E. Yonath                                       |
|---|
| () 1/3 of the prize                                 |
| Israel  |
| Weizmann Institute of<br>Science<br>Rehovot, Israel |

b. 1939







### The eukaryotic gene expression pathway

- **1. Transcriptional regulation:**
- + Transcription factors
- + Chromatin structure
- 2. pre-mRNA processing
- + Splicing
- + Polyadenylation
- + Regulated splicing/polyadenylation
- **3. mRNA export to cytoplasm**

- 4. Translation and its regulation + miRNA regulation
- **5. mRNA stability**
- 6. Protein modification, stability, and localization

# **Central Dogma of Molecular biology**



Crick, F. (1970). Central Dogma of Molecular Biology Nature, 227:561-563

# **Central Dogma of Molecular biology**



Crick, F. (1970). Central Dogma of Molecular Biology Nature, 227:561-563

### **Central dogma of Molecular Biology**

DNA RNA Protein

#### **Replication: DNA polymerase**

Nobel 1959 (Mechanism of replication): Severo Ochoa and Arthur Kornberg

**Transcription: (DNA-dependent) RNA polymerase** Nobel 2006 (Structure of RNA pol II): Robert Kornberg

#### **Translation: Ribosome**

Nobel 1968 ( Genetic code): Robert Holley, Har Gobind Khorana Marshall Nirenberg

Crick, F. (1970). Central Dogma of Molecular Biology Nature, 227:561-563

### Keskeinen Dogma



Components of the translation - tRNA (adaptor) - ribosomes

### tRNA



tRNA cloverleaf folds to a L-shaped structure.

The anticodon loop (function in decoding the triplet code) is on the one end and while the 3' end of the molecule with conserved CCA motif form the other end of the molecule. The 3' end is attached to the amino acid.

### Ribosome



# Nobel discovery timelime

# **Ribosome crystallization highlights: finding crystals diffracting to high resolution**

| <b>Yonath, A.,</b> Mussig, J., Tesche, B., Lorenz, S., Erdmann, V.A. and Wittmann, H.G. (1980) Crystallization of the large ribosomal subunits from <i>Bacillus stearothermophilus</i> . Biochem. Int. <b>1</b> , 428–435   | 1980 |
|---|------|
| Shevack, A., Gewitz, H.S., Hennemann, B., <b>Yonath, A.</b> , and Wittmann, H.G. (1985).<br>Characterization and crystallization of ribosomal particles from Halobacterium marismortui.<br>FEBS Lett <i>184</i> , 68-71.  | 1985 |
| Trakhanov, S.D., Yusupov, M.M., Agalarov, S.C., Garber, M.B., Ryazantsev, S.N.,<br>Tischenko, S.V. and Shirokov, V.A. (1987) Crystallization of 70S ribosomes and 30S<br>ribosomal subunits from <i>Thermus thermophilus</i> . FEBS Lett. <b>220</b> , 319–322  | 1987 |
| von Böhlen, K., Makowski, I., Hansen, H.A.S., Bartels, H., Berkovitch-Yellin, Z.,<br>Zaytzev-Bashan, A., Meyer, S., Paulke, C., Franceschi, F. and Yonath, A. (1991)<br>Characterization and preliminary attempts for derivatization of crystals of large ribosomal<br>subunits from <i>Haloarcula marismortui</i> diffracting to 3 Å resolution. J. Mol. Biol. 222,<br>11–15 | 1991 |

#### **Cryo-EM**

Frank, J., Zhu, J., Penczek, P., Li, Y., Srivastava, S., Verschoor, A., Radermacher, M., Grassucci, R., Lata, R.K., and Agrawal, R.K. (1995). A model of protein synthesis based on cryo-electron microscopy of the E. coli ribosome. Nature *376*, 441-444.

1995

# **Ribosome crystallization highlights:** a race to high resolution structures...

#### 50S subunit

| Ban, N., Freeborn, B., Nissen, P., Penczek, P., Grassucci, R.A., Sweet, R., Frank, J., Moore, P.B. and Steitz, <b>T.A.</b> (1998) A 9 Å resolution x-ray crystallographic map of the large ribosomal subunit. Cell 93, 1105–1115                        | <b>1998</b> |
|---|-------------|
| Ban, N., Nissen, P., Hansen, J., Capel, M., Moore, P.B., and Steltz, T.A. (1999). Placement of protein and RNA structures into a 5 Å-resolution map of the 50S ribosomal subunit. Nature 400, 841-847.  | 1999        |
| Ban, N., Nissen, P., Hansen, J., Moore, P.B., and Steitz, T.A. (2000). The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. Science 289, 905-920.  | 2000        |
| Nissen, P., Hansen, J., Ban, N., Moore, P.B., and Steitz, T.A. (2000). The structural basis of ribosome activity in peptide bond synthesis. Science 289, 920-930.   | 2000        |
| 30S subunit   |             |
| Clemons, W.M., Jr., May, J.L., Wimberly, B.T., McCutcheon, J.P., Capel, M.S., and Ramakrishnan, V. (1999). Structure of a bacterial 30S ribosomal subunit at 5.5 A resolution. Nature <i>400</i> , 833-840.   | 1999        |
| Carter, A.P., Clemons, W.M., Brodersen, D.E., Morgan-Warren, R.J., Wimberly, B.T., and Ramakrishnan, V. (2000). Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. Nature <i>407</i> , 340-348. | 2000        |
| Wimberly, B.T., Brodersen, D.E., Clemons, W.M., Jr., Morgan-Warren, R.J., Carter, A.P., Vonrhein, C., Hartsch, T., and <b>Ramakrishnan</b> , V. (2000). Structure of the 30S ribosomal subunit. Nature <i>407</i> , 327-339.                            | 2000        |
| Schluenzen E. Tocili A. Zariyach R. Harms I. Gluehmann M. Janell D. Bashan A. Bartels H.  | 2000        |

Agmon, I., Franceschi, F., and Yonath, A. (2000). Structure of functionally activated small ribosomal

subunit at 3.3 angstroms resolution. Cell 102, 615-623.

### ...and beyond

#### Whole 70S ribosome

Cate, J.H., Yusupov, M.M., Yusupova, G.Z., Earnest, T.N., and Noller, H.F. (1999). X-ray crystal structures of 70S 1999 ribosome functional complexes. Science 285, 2095-2104.

Yusupov, M.M., Yusupova, G.Z., Baucom, A., Lieberman, K., Earnest, T.N., Cate, J.H., and Noller, H.F. (2001). 2001 Crystal structure of the ribosome at 5.5 A resolution. Science 292, 883-896.

Schuwirth, B.S., Borovinskaya, M.A., Hau, C.W., Zhang, W., Vila-Sanjurjo, A., Holton, J.M., and Cate, J.H. (2005). 2005 Structures of the bacterial ribosome at 3.5 A resolution. Science *310*, 827-834.

Selmer, M., Dunham, C.M., Murphy, F.V.t., Weixlbaumer, A., Petry, S., Kelley, A.C., Weir, J.R., and Ramakrishnan, 2006 V. (2006). Structure of the 70S ribosome complexed with mRNA and tRNA. Science *313*, 1935-1942.

#### **Fidelity**

Ogle, J.M., Brodersen, D.E., Clemons, W.M., Jr., Tarry, M.J., Carter, A.P., and Ramakrishnan, V. (2001). 2001 Recognition of cognate transfer RNA by the 30S ribosomal subunit. Science 292, 897-902.

Ogle, J.M., Murphy, F.V., Tarry, M.J., and Ramakrishnan, V. (2002). Selection of tRNA by the ribosome 2002 requires a transition from an open to a closed form. Cell *111*, 721-732.

#### **Catalysis**

Schmeing, T.M., Huang, K.S., Kitchen, D.E., Strobel, S.A., and Steitz, T.A. (2005a). Structural insights into the roles of water and the 2' hydroxyl of the P site tRNA in the peptidyl transferase reaction. Mol Cell 20, 437-448.

Schmeing, T.M., Huang, K.S., Strobel, S.A., and Steitz, T.A. (2005b). An induced-fit mechanism to promote 2005 peptide bond formation and exclude hydrolysis of peptidyl-tRNA. Nature *438*, 520-524.

Voorhees, R.M., Weixlbaumer, A., Loakes, D., Kelley, A.C., and Ramakrishnan, V. (2009). Insights into substrate stabilization from snapshots of the peptidyl transferase center of the intact 70S ribosome. Nat Struct Mol Biol *16*, 528-533.

### The path to 50S high-resolution structures

9Å 5Å 2.4Å

Ban, N., Freeborn, B., Nissen, P., Penczek, P., Grassucci, R.A., Sweet, R., Frank, J., Moore, P.B., and Steitz, T.A. (1998). A 9 Å resolution X-ray crystallographic map of the large ribosomal subunit. Cell 93, 1105-1115. Ban, N., Nissen, P., Hansen, J., Capel, M., Moore, P.B., and Steitz, T.A. (1999). Placement of protein and RNA structures into a 5 Å-resolution map of the 50S ribosomal subunit. Nature 400, 841-847. Ban, N., Nissen, P., Hansen, J., Moore, P.B., and Steitz, T.A. (2000). The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. Science 289, 905-920.

### **Atomic resolution structures of small and large subunits**





Schluenzen, F., Tocilj, A., Zarivach, R., Harms, J., Gluehmann, M., Janell, D., Bashan, A., Bartels, H., Agmon, I., Franceschi, F., and Yonath, A. (2000). Structure of functionally activated small ribosomal subunit at 3.3 angstroms resolution. Cell *102*, 615-623. Harms, J., Schluenzen, F., Zarivach, R., Bashan, A., Gat, S., Agmon, I., Bartels, H., Franceschi, F., and Yonath, A. (2001). High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. Cell 107, 679-688.

### **Key discoveries**

1. Mechanism of peptide bond formation
→ Peptide bond formation is catalysed by
- 23S rRNA
- 2'OH of the aa-tRNA at P-site

and assisted by proteins L27 and L16

2. Fidelity of translation

→ 16S rRNA has proofreading mechanism to confirm the geometry of codon-anticodon helix

**3. Structural basis for the antibiotic activity** 

→ rational structure-based desing of new compounds binding to ribosomes and inhibiting pathogen growth

# Mechanism of peptidy transferase activity



# **Ribosome Translation**

#### T. Martin Schmeing Laboratory of Thomas A. Steitz Yale University / HHMI



### Translation mechanism

- 23S rRNA: H-bond network and induced fit  $\rightarrow$  geometry of active site

- P-site-tRNA: 2'OH needed to shuttle protons → substrate catalysis

Also proteins involved:
→ L25 and L16 proteins stabilize CCA-ends of both tRNAs

### Location of tRNAs and mRNA on small subunit



www.mrc-lmb.cam.ac.uk/ribo/homepage/mov\_and\_overview.html

### **Putting it all together:** Translation The Movie



Animation based of true cryo-EM structures of ribosomes in different stages of translation, combined with X-ray structural data plus fair amount of imagination

http://pubs.acs.org/cen/multimedia/85/ribosome/translation\_bacterial.html

# Fidelity

### **Fidelity of translation: how to recognize corret tRNAs**



Ogle, J. M., Carter, A. P., and Ramakrishnan, V. (2003). Insights into the decoding mechanism from recent ribosome structures. Trends Biochem Sci 28, 259-266.

### **Selection of corret tRNAs**



**Two kinetic proofreading step:** 

During initial recognition
During proofreading stage

### **Proofreading in A-minor**



A-minor interactions with codon-anticodon pair are important for the recognition of correct codon-anticodon pair. A-residues from 16S rRNA interact with the minor groove of the tRNA:mRNA complex

### **Proofreading the tRNA:mRNA complex**



Ogle, J. M., Carter, A. P., and Ramakrishnan, V. (2003). Insights into the decoding mechanism from recent ribosome structures. Trends Biochem Sci 28, 259-266.

### Induced fit in 30S subunit: an RNA-based proofreading mechanism



### **Ribosomes and RNA World**

### **Key discoveries**

1. Mechanism of peptide bond formation
→ Peptide bond formation is catalysed by
- 23S rRNA
- 2'OH of the aa-tRNA at P-site

and assisted by proteins L27 and L16

2. Fidelity of translation

→ 16S rRNA has proofreading mechanism to confirm the geometry of codon-anticodon helix

Both the catalysis and proofreading are carried out by RNA, not the proteins  $\rightarrow$  strong support for the RNA world hypothesis

### The RNA World hypothesis

Discovery of RNA-based catalysis by Cech and Altman 1992-1993 (Nobel 1989) challenged the Central dogma and showed that RNA can function both as information molecule and catalytically active entity.

This work suggested that life on earth started from self-replicating RNA molecules → RNA world hypothesis by Walter Gilbert: "The RNA world," p 618 v 319, Nature, 1986.

One of the suggestions in this hypothesis is that few key functions/features present at the RNA world have survived and are actually present in the genomes of current organisms. Those are:

- RNA catalysed translation by the ribosome
- RNA catalysed splicing of introns
- split genes (intron-exon structure)

#### **Nobel Prize work:**

Kruger, K., Grabowski, P. J., Zaug, A. J., Sands, J., Gottschling, D. E., and Cech, T. R. (1982). Self-splicing RNA: autoexcision and autocyclization of the ribosomal RNA intervening sequence of Tetrahymena. Cell 31, 147-157.

Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N., and Altman, S. (1983). The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell 35, 849-857.

Discussion about RNA world: http://en.wikipedia.org/wiki/RNA\_world\_hypothesis http://wiki.cotch.net/index.php/RNA\_world

# **Central Dogma of Molecular biology**



# The RNA World





Information + Function

# The RNA World



EDITED BY Raymond F. Gesteland Thomas R. Cech John F. Atkins

### **Short history of life on earth**

Life first started as self-replicating RNA molecules

- → amino acids and small peptides were used to stabilize ribozymes
  → more efficient catalysis
- $\rightarrow$  life evolved to RNP world

→ appearance of DNA and the rise of modern life forms

# Antibiotics

| Table 1a – Available structures of antibiotics targeting the small ribosomal subunit (30S) |                     |   |  |  |  |  |
|--|---------------------|---|--|--|--|--|
| Proposed mechanism of action   | Antibiotic<br>class | Antibiotic  | Refs.  | PDB<br>ID  | System used for structural determination   |  |
| Bind to A- or P-sites and affect decoding.   | Aminoglycosides     | Apramycin<br>Geneticin<br>Hygromycin B<br>Paromomycin<br>Paromomycin<br>Paromomycin<br>Tobramycin<br>Streptomycin | [66]<br>[67]<br>[26]<br>[48]<br>[25]<br>[50]<br>[26] | 1YRJ<br>1MWL<br>1HNZ<br>1FJG<br>1IBK<br>1J7T<br>1LC4<br>1FJG | RNA fragment<br>RNA fragment<br>T. thermophilus<br>T. thermophilus<br>T. thermophilus<br>RNA fragment<br>RNA fragment<br>T. thermophilus |  |
| Block binding of A-site tRNA   | Tetracyclines       | Tetracycline<br>Tetracycline  | [68]<br>[69]   | 1HNW<br>1197   | T. thermophilus<br>T. thermophilus   |  |
| Inhibit translocation  | Various             | Edeine<br>Pactamycin<br>Spectinomycin   | [69]<br>[68]<br>[26]                                 | 1I95<br>1HNX<br>1FJG   | T. thermophilus<br>T. thermophilus<br>T. thermophilus  |  |

| Proposed mechanism of action  | Antibiotic<br>class | Antibiotic      | Refs.            | PDB ID        | System used for<br>structural determination |
|-------------------------------|---------------------|-----------------|------------------|---------------|---|
|                               | Macrolides          | Azithromycin    | [70]             | 1M1K          | H. marismortui                              |
|                               |                     | Azithromycin    | [71]             | 1NWY          | D. radiodurans                              |
|                               |                     | Azithromycin    | [19]             | 1YHQ          | H. marismortui (G2058A)                     |
|                               |                     | Erythromycin    | [72]             | 1JZY          | D. radiodurans                              |
|                               |                     | Carbomycin      | [70 <sup>]</sup> | 1K8A          | H. marismortui                              |
|                               |                     | Erythromycin    | [19.79]          | 1YI2          | H. marismortui (G2058A)                     |
|                               |                     | Clarithromycin  | [72]             | 1J5A          | D. radiodurans                              |
|                               |                     | Roxithromycin   | [72]             | 1JZZ          | D. radiodurans                              |
|                               |                     | Spiramycin      | [70]             | 1KD1          | H. marismortui                              |
|                               |                     | Troleandomycin  | [73]             | 10ND          | D. radiodurans                              |
|                               |                     | Tylosin         | [70]             | 1K9M          | H. marismortui                              |
|                               | Ketolides           | ABT-773         | [71]             | 1NWX          | D. radiodurans                              |
| Block peptide bond formation  |                     | Telithromycin   | [74,79]          | 1P9X          | D. radiodurans                              |
| by interfering with A-site or |                     | Telithromycin   | [19]             | 1YIJ          | H. marismortui (G2058A)                     |
| P-site tRNA and/or prevent    | Streptogramins      | Dalfopristin    | [75]             | 1SM1          | D. radiodurans                              |
| the elongation of the         |                     | Quinupristin    | [75]             | 1SM1          | D. radiodurans                              |
| nascent peptide               |                     | Quinupristin    | [19]             | 1YJW          | H. marismortui (G2058A)                     |
|                               |                     | Virginiamycin S | [19]             | 1YIT          | H. marismortui (G2058A)                     |
|                               |                     | Virginiamycin M | [76]             | 1N8R          | H. marismortui                              |
|                               |                     | Virginiamycin M | [19]             | 1YIT          | H. marismortui (G2058A)                     |
|                               | Lincosamides        | Clindamycin     | [72,79]          | 1JZX          | D. radiodurans                              |
|                               |                     | Clindamycin     | [19]             | 1YJN          | H. marismortui (G2058A)                     |
|                               | Pleuromutilins      | Tiamulin        | [77]             | 1XBP          | D. radiodurans                              |
|                               | Phenyl propanoids   | Chloramphenicol | [72]             | 1K01          | D. radiodurans                              |
|                               |                     | Chloramphenicol | [76]             | 1NJ1          | H. marismortui                              |
|                               | Oxazolidinones      | Linezolid       | [61]             | Not available | H. marismortui                              |
|                               | Various             | Puromycin       | [78]             | 1FFZ          | H. marismortui                              |
|                               |                     | Sparsomycin     | [76]             | 1M90          | H. marismortui                              |
|                               |                     | Anisomycin      | [76]             | 1K73          | H. marismortui                              |
|                               |                     | Blasticidin S   | [76]             | 1KC8          | H. marismortui                              |

The PDB ID refers to the Protein Data Bank (PDB) identification code of each structure. The atomic coordinates for each structure can be downloaded from http://www.pdb.org using their respective PDB IDs.

### **Decoding as the target of antibiotics**



Sutcliffe, J. A. (2005). Improving on nature: antibiotics that target the ribosome. Current Opinion in Microbiology *8*, 534-542.

Several antibiotics affecting bacterial growth interact with the decoding site:

Hygromycin BParomomycinNeomycin B

Affect the ribosomal proofreading mechanism and increase the error frequency of bacterial ribosomes.



The Ribosome Company



#### Scientific advisorsory board

**Thomas A. Steitz, Co-Founder and Chair of Scientific Advisory Board** 

John N. Abelson, Co-Founder

William L. Jorgensen, Co-Founder

**Peter Moore, Co-Founder** 

Harry F. Noller, Co-Founder

Venkatraman Ramakrishnan, Medical Research Council



### Conclusions

"Ramakrishnan, Steitz and Yonath have made ground breaking contributions to the crystallography of ribosomes and used highresolution functional ribosome complexes to clarify long-standing and fundamental questions in protein synthesis. Their work has farreaching implications for basic science and medicine."

Scientific Background on the Nobel Prize in Chemistry 2009 STRUCTURE AND FUNCTION OF THE RIBOSOME Uppsala 30 September 2009 Måns Ehrenberg