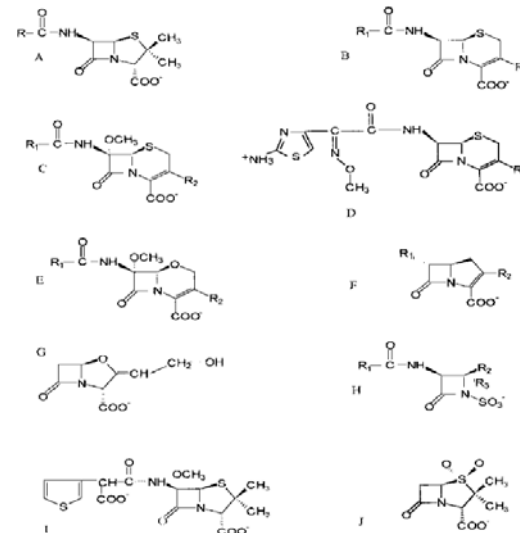
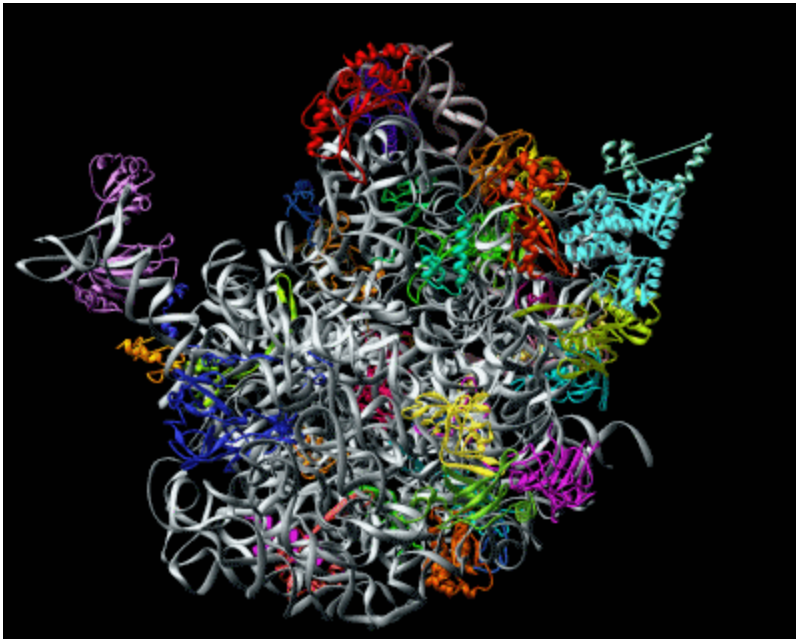
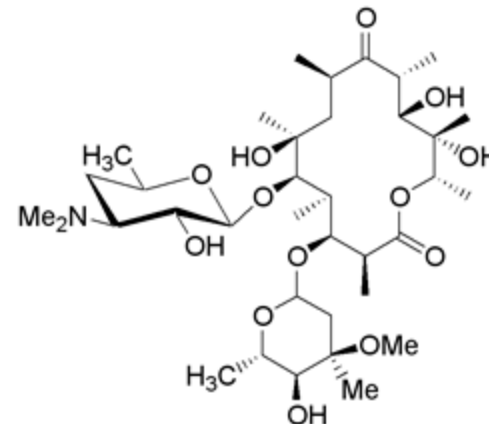


# Ribosomes and Antibiotics



[www.biochemj.org/bj/330/0581/bj3300581.htm](http://www.biochemj.org/bj/330/0581/bj3300581.htm)

Professor Vassie Ware  
Bioscience in the 21<sup>st</sup> Century  
October 31, 2011



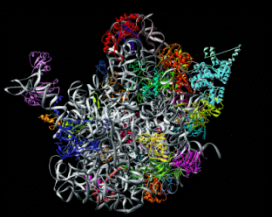
# PERSPECTIVE

- **Widespread use of antibiotics after WWII to improve global health**
- **Increasing antibiotic resistance in bacterial pathogens coupled with a lag in the development of additional antibiotics by pharmaceutical companies poses an escalating problem in the 21<sup>st</sup> century**

**20 years ago: ~13,000 deaths/year from bacterial infections.**

**Today: ~90,000 deaths/year from bacterial infections!!!**

- **Challenge to design effective new generation antibiotics**
- **Use of structure-based drug design to develop novel drugs based on high resolution structures of drug targets and their resistance mutants**
- **The ribosome is the target of over 50% of existing antibacterial drugs. High resolution structures of bacterial ribosomal subunits offers new prospects for developing new drugs with the advent of increasing bacterial resistance.**



# ***General Lecture Outline***

1. General information about antibiotics and their targets
2. Bacterial antibiotic resistance
3. Ribosomes as evolutionarily conserved nanomachines required to make proteins
4. Why study ribosome structure? Why study ribosomes from different species?
5. How are ribosomes manufactured in bacteria and eukaryotic cells?
6. Bacterial ribosomes as targets for antibiotics

# Antibiotics

- **Natural or synthetic compounds that either kill (bactericidal) or inhibit growth (bacteriostatic) of bacteria (or other microorganisms)**
- **Antibiotics may be classified in several ways. Most common classification schemes are based on chemical structure of the antibiotic**

# Antibacterial agents, suitable for therapy:

## Natural –

*Derived from natural sources such as fungi and soil bacteria.*

*Penicillin as the classic example, derived from the fungus *Penicillium**

*Pharmaceutical industry produces penicillin from cultures of*

**Penicillium chrysogenum* that are adapted for high yield*

*Others: many aminoglycosides from soil bacteria (e.g., streptomycin)*

## Semi-synthetic -

*Natural products that have been chemically modified to improve effectiveness of the product or to reduce side effects, etc*

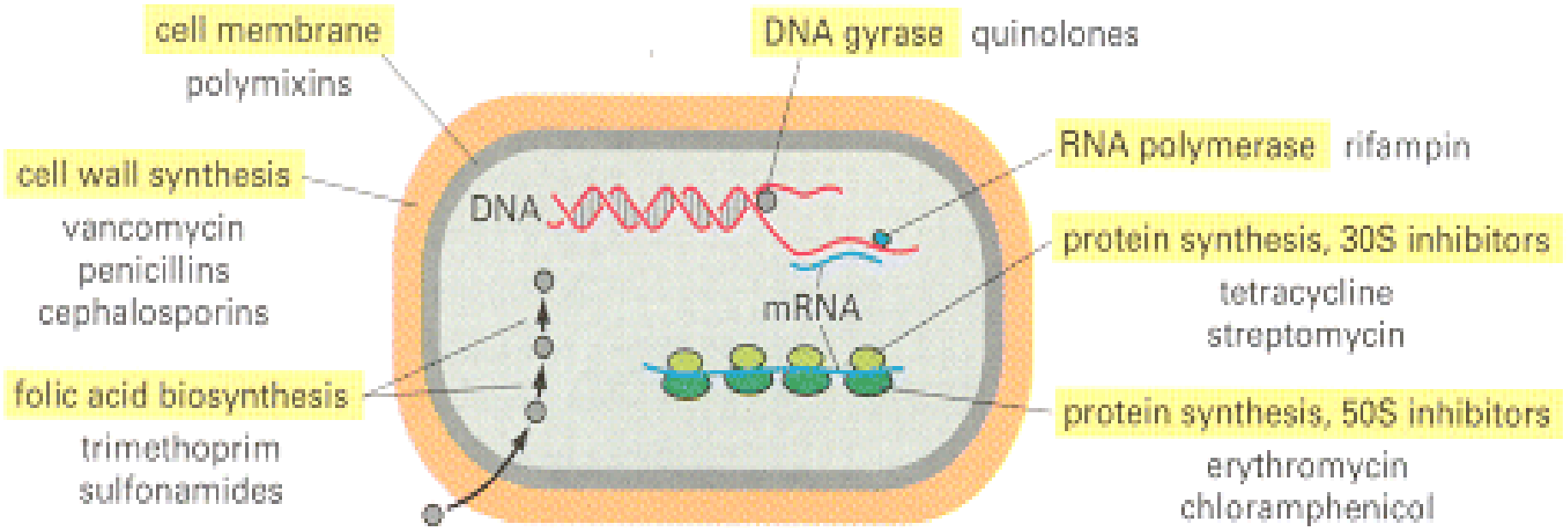
*Examples include the  $\beta$ -lactams ampicillin, amoxicillin, etc, derived from fungi*

## Completely synthetic –

Products are synthesized completely in the laboratory

Sulfa drugs, folic acid analogs are examples

# Antibiotic Targets in Bacterial Cells



# Resistance to Antibiotics

## Intrinsic resistance

Some bacteria are naturally more resistant to certain classes of antibiotics than others (e.g., Gram positive bacteria are more resistant than Gram negative bacteria to polymyxins – a class of antibiotics that behave as detergents and cause leakiness of the cell membrane)

## Acquired resistance

Bacteria acquire resistance to antibiotics for which they were previously susceptible. For example, in 10 years' time between 1985 and 1995, the percentage of ampicillin-resistant *Shigella* (causes intestinal illness) grew from 32% to 67%!

# How do bacteria acquire resistance?

Bacteria acquire genes that encode proteins that shield or protect them from the effects of the antibiotic.

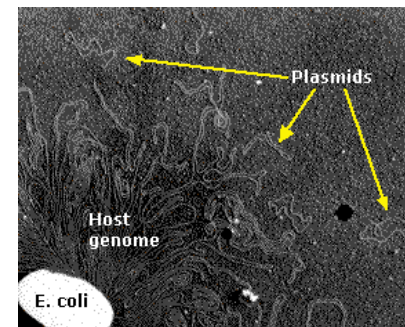
These genes may have arisen by **mutation** of existing genes  
OR

they may have been acquired from other resistant bacteria through the **transfer of genetic information between bacteria**

Antibiotic resistance genes are often carried on **plasmids**



[www.life.uiuc.edu](http://www.life.uiuc.edu)

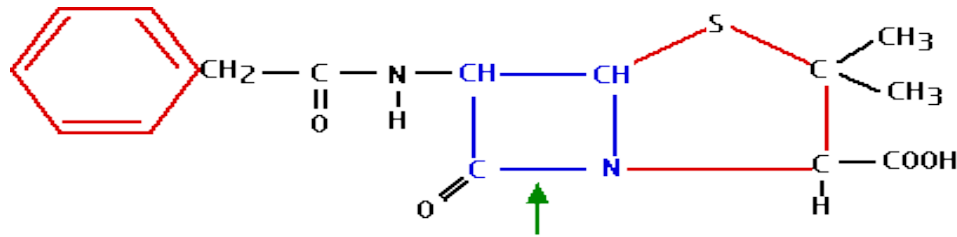


[users.rcn.com](http://users.rcn.com)



# “Clever tricks” by bacteria to inactivate antibiotics:

1. **Synthesis of enzymes that breakdown the antibiotic:**  
Penicillinase (a type of  $\beta$ -lactamase, breaks the  $\beta$ -lactam ring, thereby destroying the antibiotic). Other enzyme types are also prevalent (e.g., cephalosporinases)

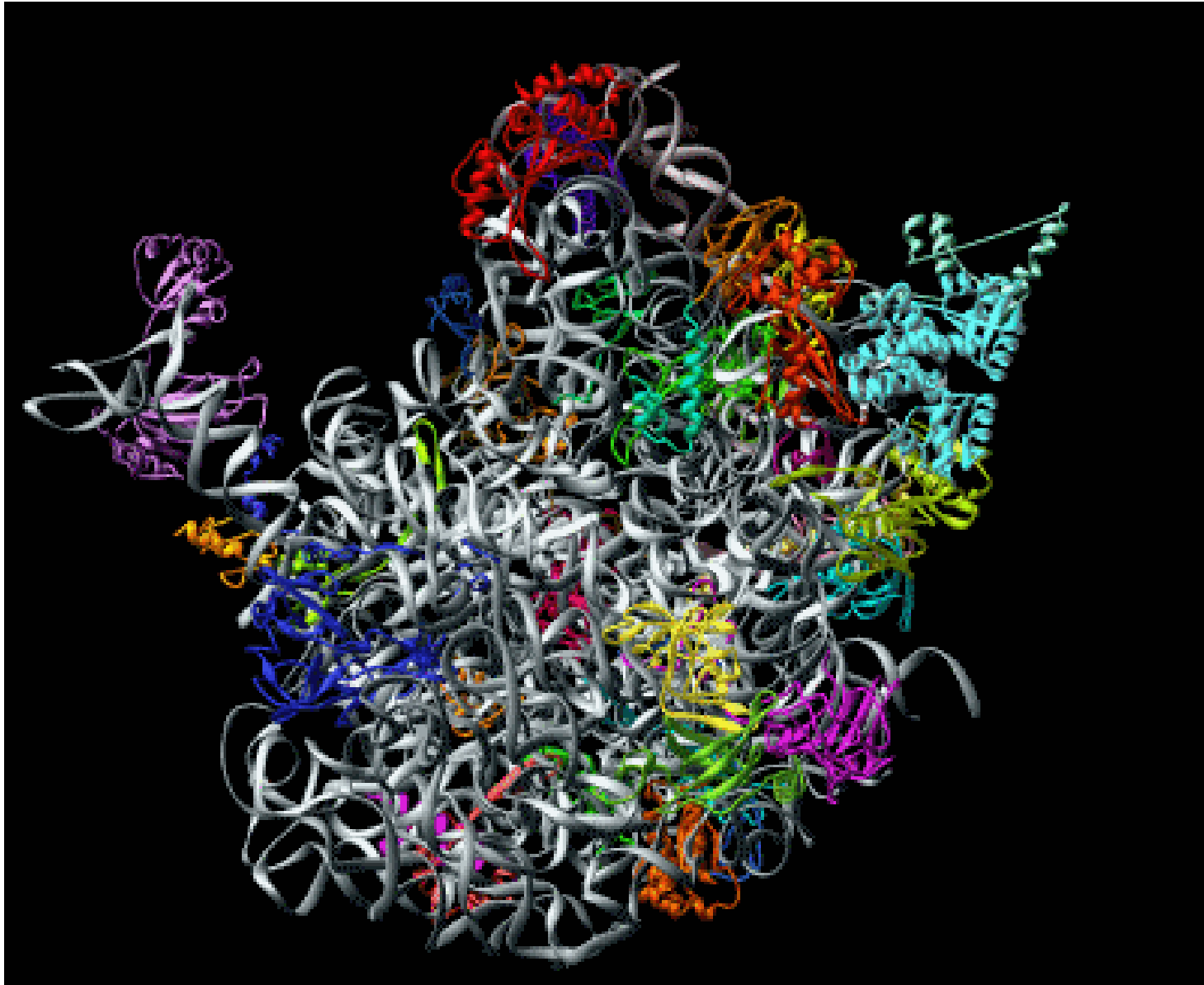


**Penicillin G**

2. **Modification of their own enzymes that would normally be targets of the antibiotic (e.g., DNA gyrase)**

## **Clever tricks, continued:**

- 3. Synthesis of “pumps” inserted into the cell membrane to remove the antibiotic from the interior of the cell**
- 4. Addition of chemical groups onto the target so that the antibiotic does not recognize the target. (e.g., erythromycin resistance)**
- 5. Modification of the antibiotic so that it no longer recognizes its target (e.g., kanamycin resistance)**
- 6. Modification of the peptidoglycan cell wall to avoid the antibiotic effect**





# The Nobel Prize in Chemistry 2009

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



**Venkatraman  
Ramakrishnan**

**MRC Laboratory  
of Molecular Biology  
Cambridge, UK**



**Thomas A. Steitz**

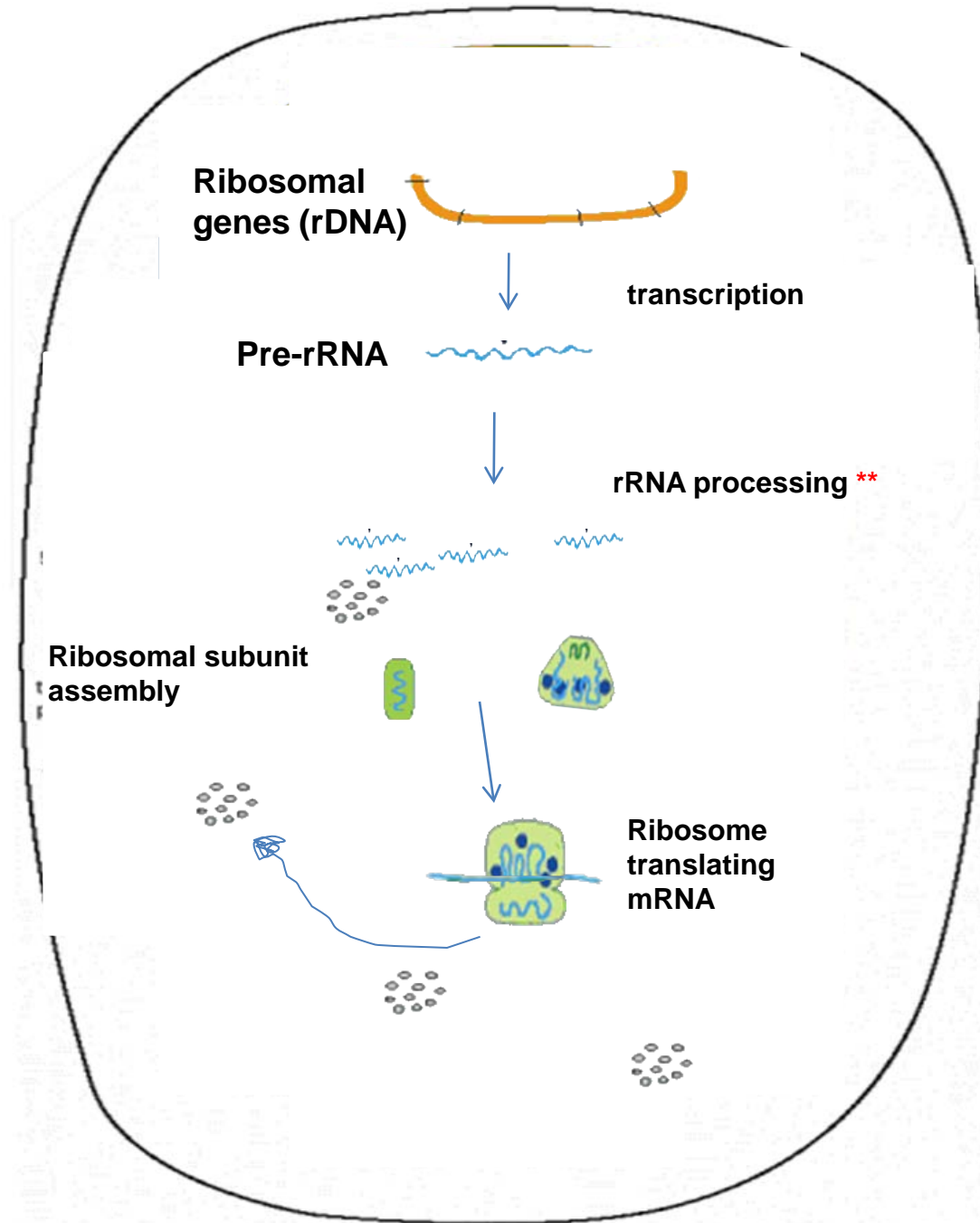
**Yale University  
New Haven, CT**



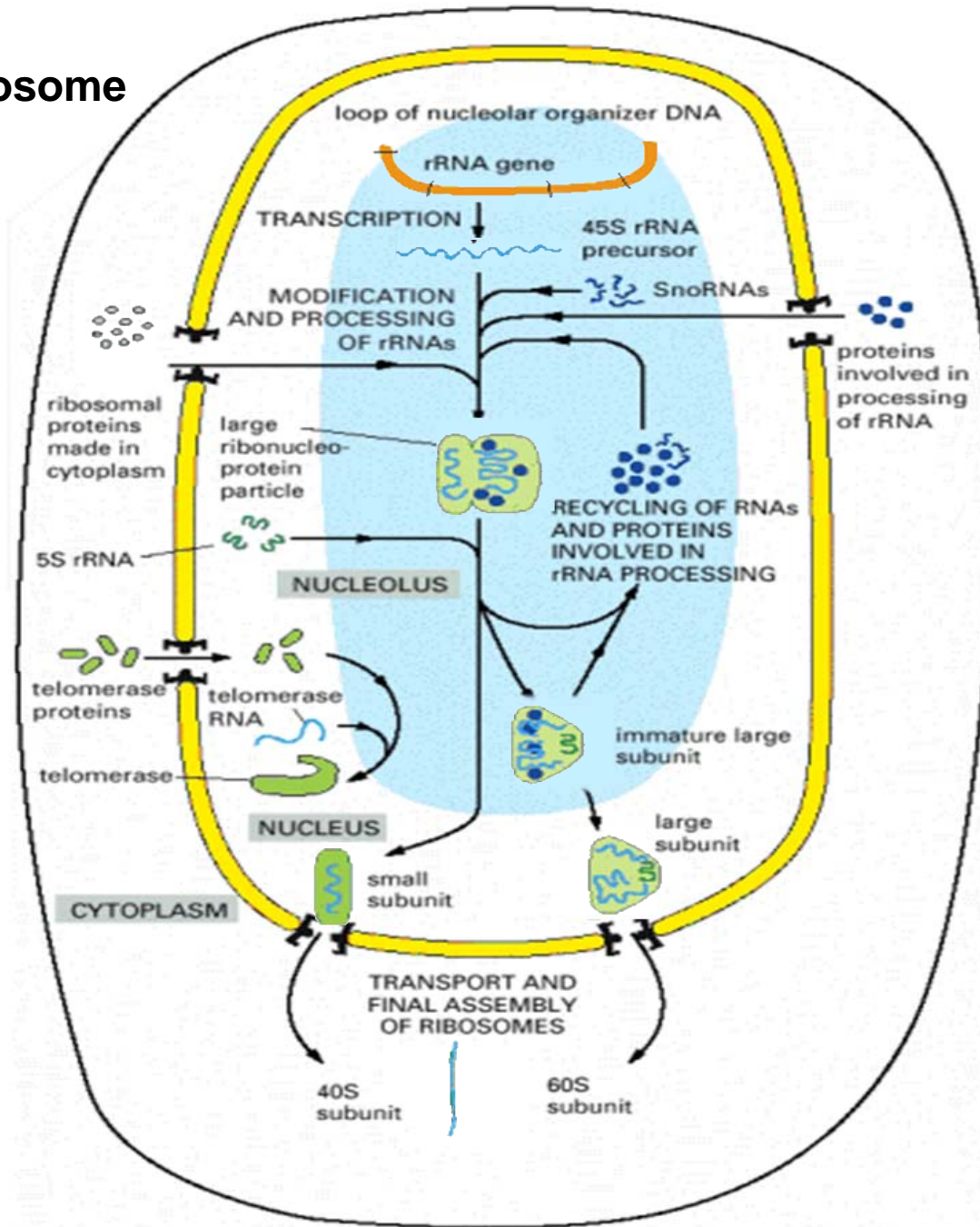
**Ada E. Yonath**

**Weizmann Institute  
of Science  
Rehovot, Israel**

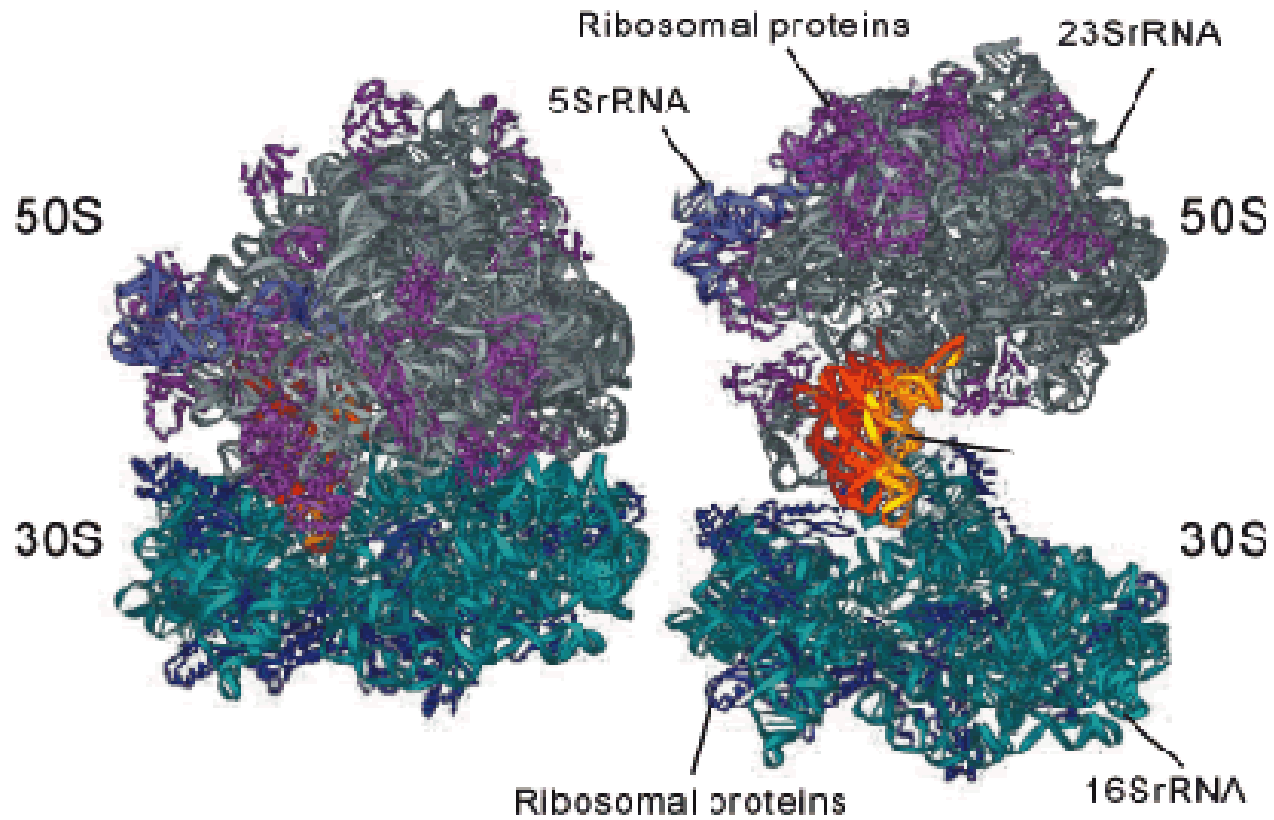
# Ribosome Synthesis in Bacteria



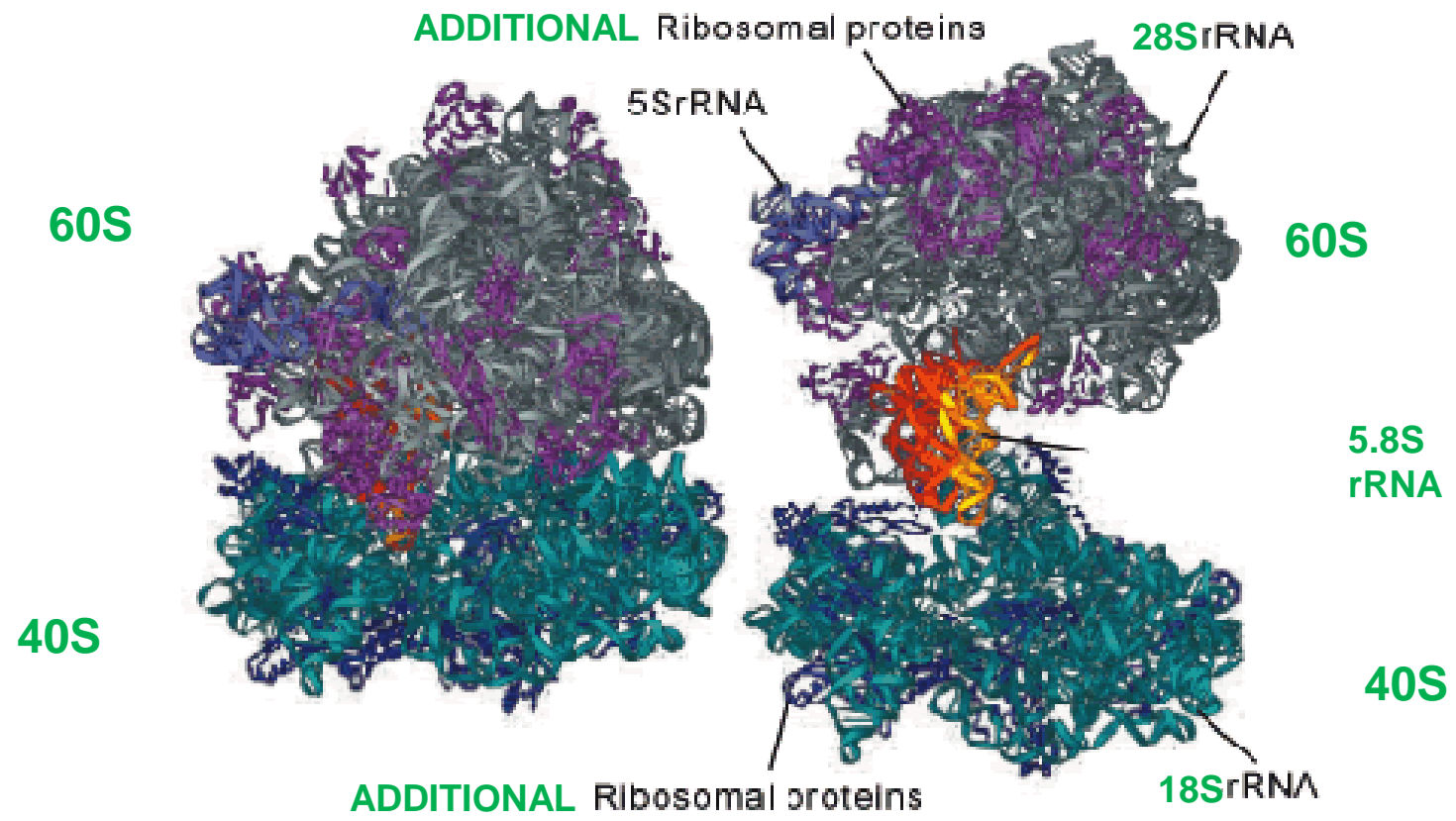
# Eukaryotic Ribosome Synthesis



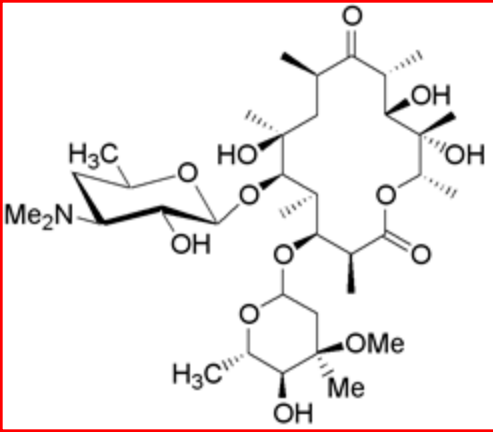
# Bacterial ribosome composition



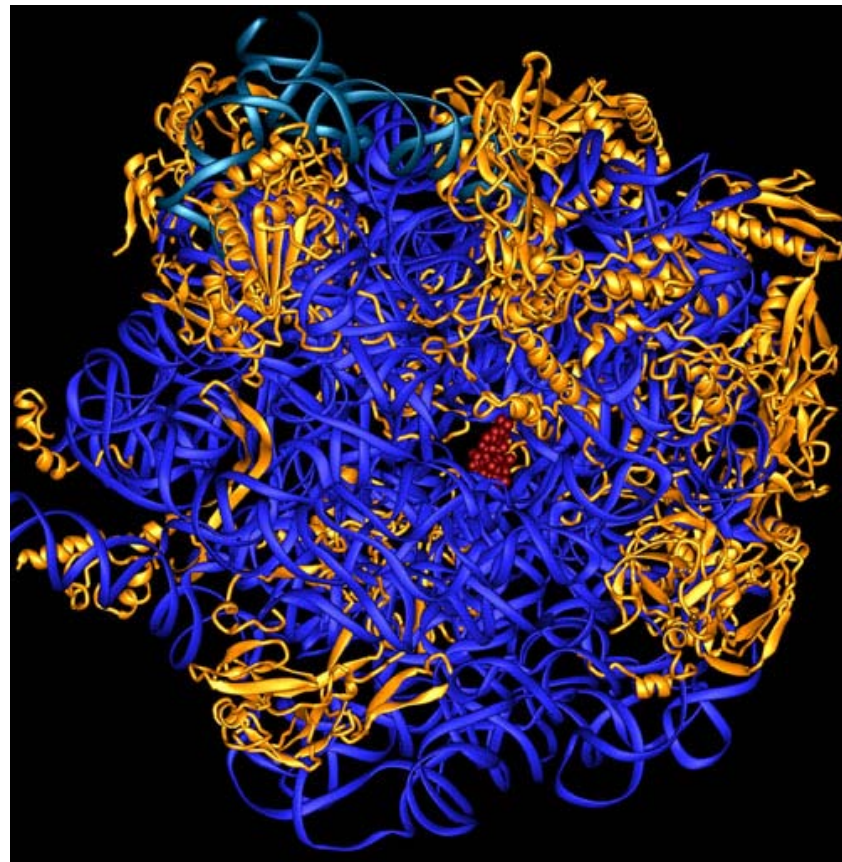
# Eukaryotic ribosome composition



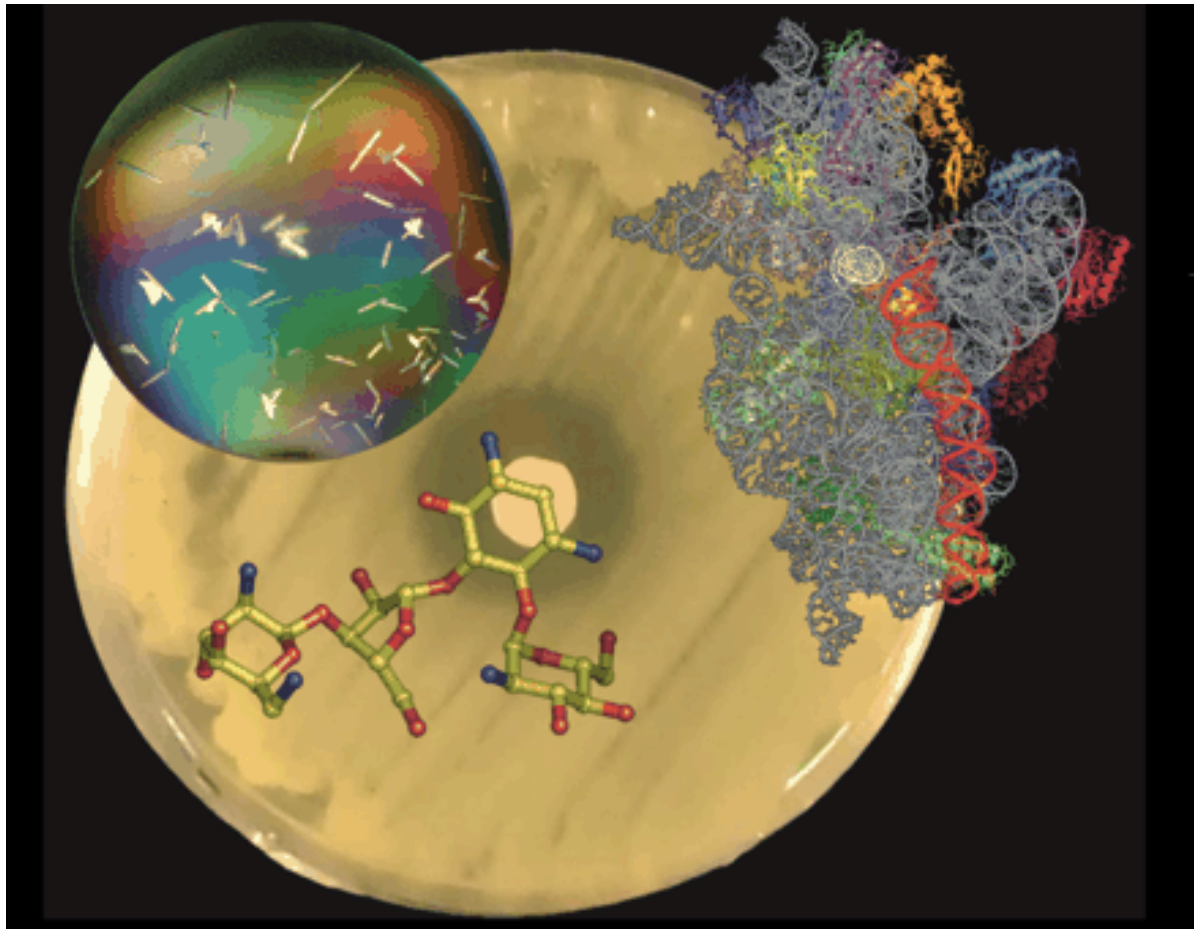




**Erythromycin – a macrolide antibiotic that blocks protein synthesis by binding to bacterial ribosomes but not to eukaryotic ribosomes**



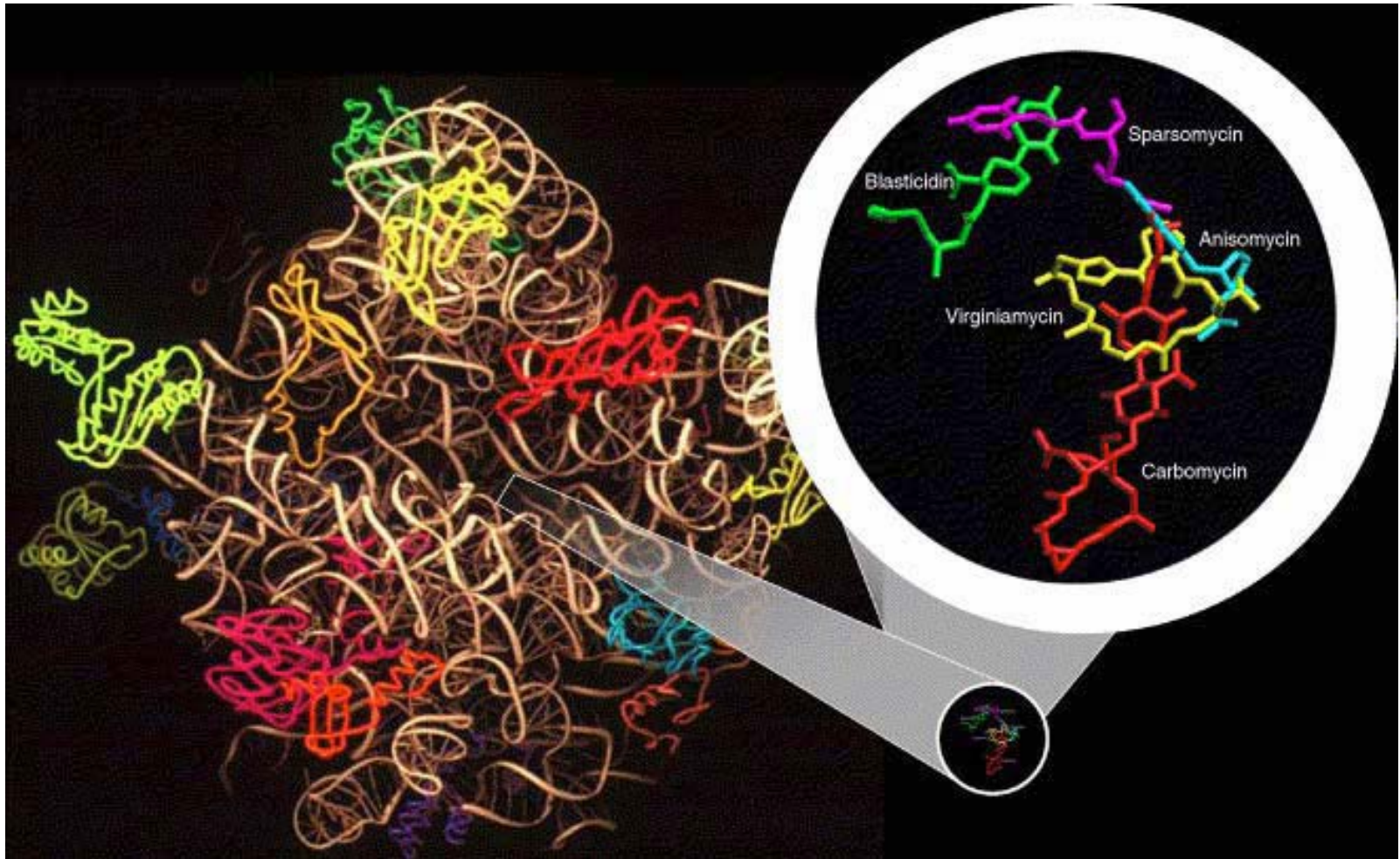
[www.molgen.mpg.de](http://www.molgen.mpg.de)



**The aminoglycoside antibiotic paromomycin binds to the small ribosomal subunit and blocks protein synthesis.**

[nar.oxfordjournals.org/.../issue1/cover.dtl](http://nar.oxfordjournals.org/.../issue1/cover.dtl)

# Antibiotics Targeting the Large Ribosomal Subunit of Bacteria



[http://nobelprize.org/nobel\\_prizes/chemistry/laureates/2009/sci.html](http://nobelprize.org/nobel_prizes/chemistry/laureates/2009/sci.html)

# Classes of Antibiotics Affecting the Small Ribosomal Subunit in Bacteria

**Table 1a – Available structures of antibiotics targeting the small ribosomal subunit (30S)**

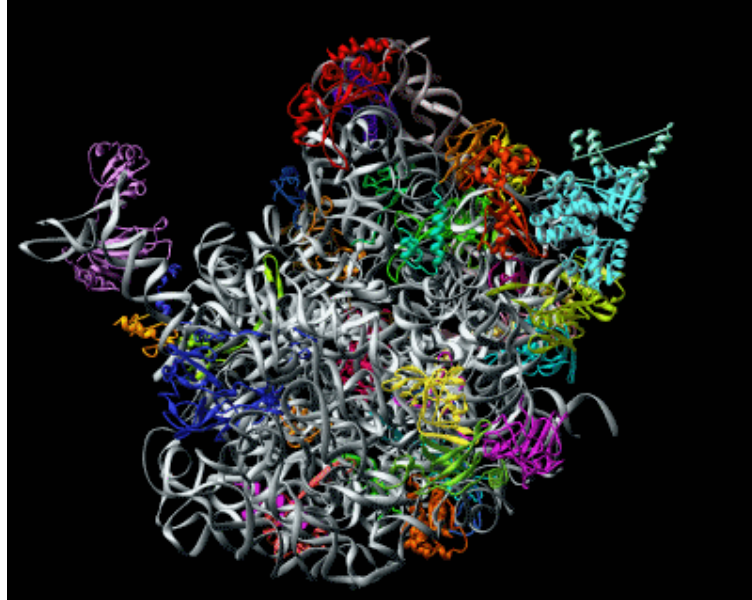
| Proposed mechanism of action               | Antibiotic class | Antibiotic    | Refs. | PDB ID | System used for structural determination |
|--|------------------|---------------|-------|--------|--|
| Bind to A- or P-sites and affect decoding. | Aminoglycosides  | Apramycin     | [66]  | 1YRJ   | RNA fragment                             |
|  |                  | Geneticin     | [67]  | 1MWL   | RNA fragment                             |
|  |                  | Hygromycin B  | [68]  | 1HNZ   | <i>T. thermophilus</i>                   |
|  |                  | Paromomycin   | [26]  | 1FJG   | <i>T. thermophilus</i>                   |
|  |                  | Paromomycin   | [48]  | 1IBK   | <i>T. thermophilus</i>                   |
|  |                  | Paromomycin   | [25]  | 1J7T   | RNA fragment                             |
|  |                  | Tobramycin    | [50]  | 1LC4   | RNA fragment                             |
|  |                  | Streptomycin  | [26]  | 1FJG   | <i>T. thermophilus</i>                   |
| Block binding of A-site tRNA               | Tetracyclines    | Tetracycline  | [68]  | 1HNW   | <i>T. thermophilus</i>                   |
|  |                  | Tetracycline  | [69]  | 1I97   | <i>T. thermophilus</i>                   |
| Inhibit translocation                      | Various          | Edeine        | [69]  | 1I95   | <i>T. thermophilus</i>                   |
|  |                  | Pactamycin    | [68]  | 1HNX   | <i>T. thermophilus</i>                   |
|  |                  | Spectinomycin | [26]  | 1FJG   | <i>T. thermophilus</i>                   |

**Table 1b – Available structures of antibiotics targeting the large ribosomal subunit (50S)**

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

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.

## **SUMMARY:**



[www.molgen.mpg.de](http://www.molgen.mpg.de)

**Bacterial antibiotic resistance is an increasingly serious global health problem**

**Development of new generations of antibiotics becomes increasingly important**

**Ribosomes (as essential complexes for making proteins in all cells)  
are one of many antibiotic targets**

**Ribosomes have many evolutionarily conserved features but  
important structural differences exist between bacterial  
and eukaryotic ribosomes**

**Ribosome structural differences between organisms can be  
exploited as potential targets in drug development**