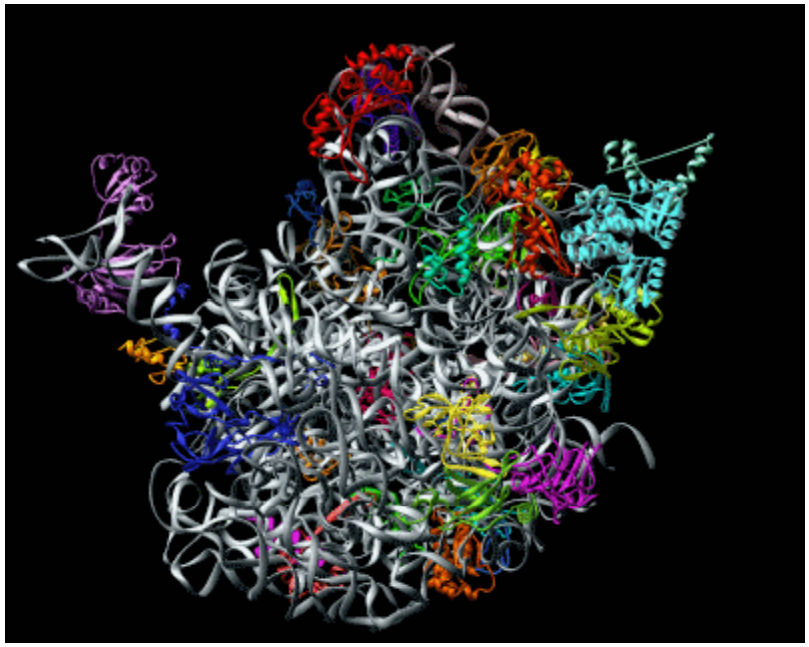
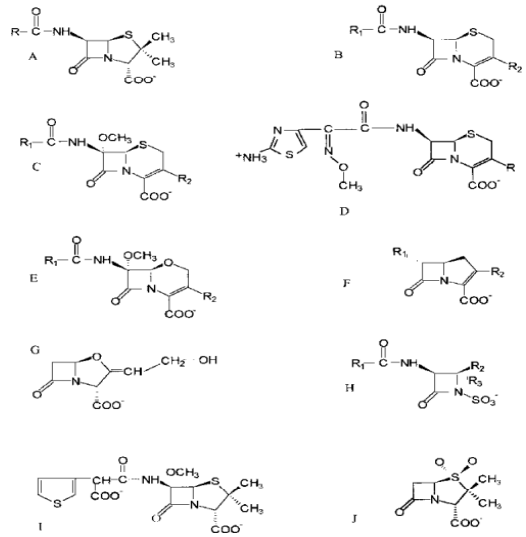
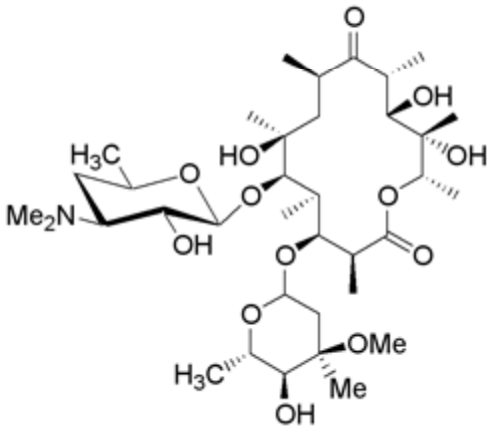
Ribosomes and Antibiotics





www.biochemj.org/bj/330/0581/bj3300581.htm



Professor Vassie Ware
Bioscience in the 21st Century
November 10, 2010

PERSPECTIVE

- **Widespread use of antibiotics after WWII to improve global health**

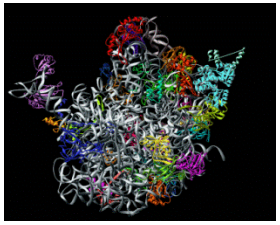
- **Increasing antibiotic resistance in bacterial pathogens coupled with a lag in the development of additional antibiotics by pharmaceutical companies poses an escalating problem in the 21st century**

  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)

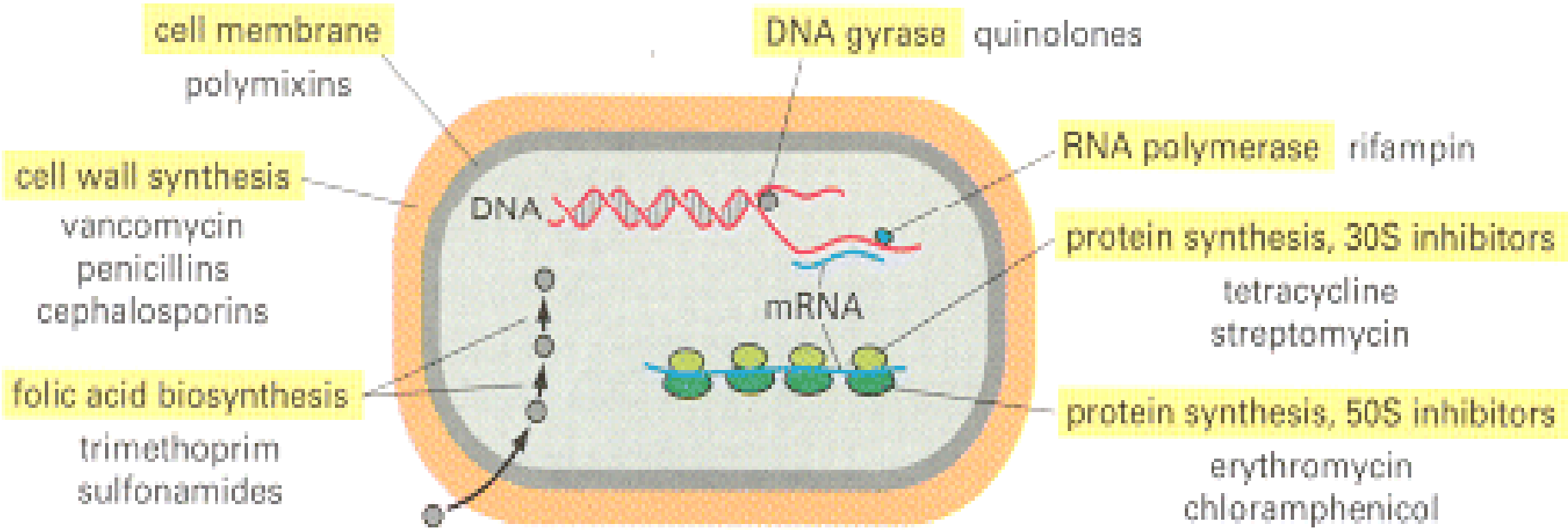
Semi-synthetic -

Natural products that have been chemically modified to improve effectiveness of the product or to reduce side effects, etc
Examples include the β-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



Molecular Biology of the Cell by Alberts *et al*. 2002

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 polymixins – a class of antibiotics that behave as detergents and cause leakiness of the cell membrane)

Acquired resistance

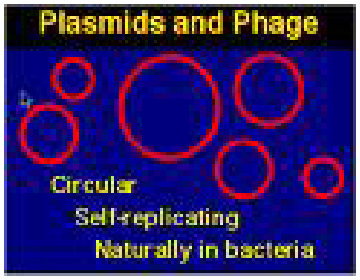
Bacteria acquire resistance to antibiotics for which they were previously susceptible. For example, in 10 years' time between 1985 and 1995,the percentage of ampicillin-resistant *Shigella* (causes intestinal illness) grew from 32% to 67%!

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**



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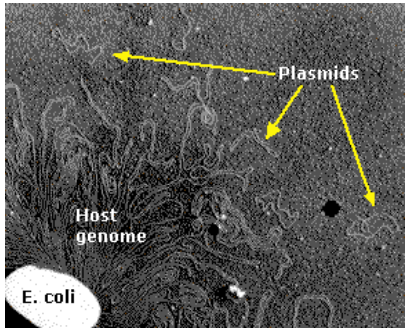
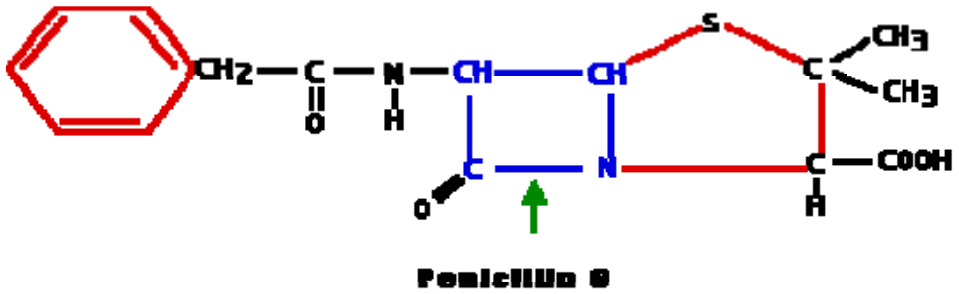


users.rcn.com

“Clever tricks” by bacteria to inactivate antibiotics:

1. Synthesis of enzymes that breakdown the antibiotic: Penicillinase (a type of β-lactamase, breaks the β-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)

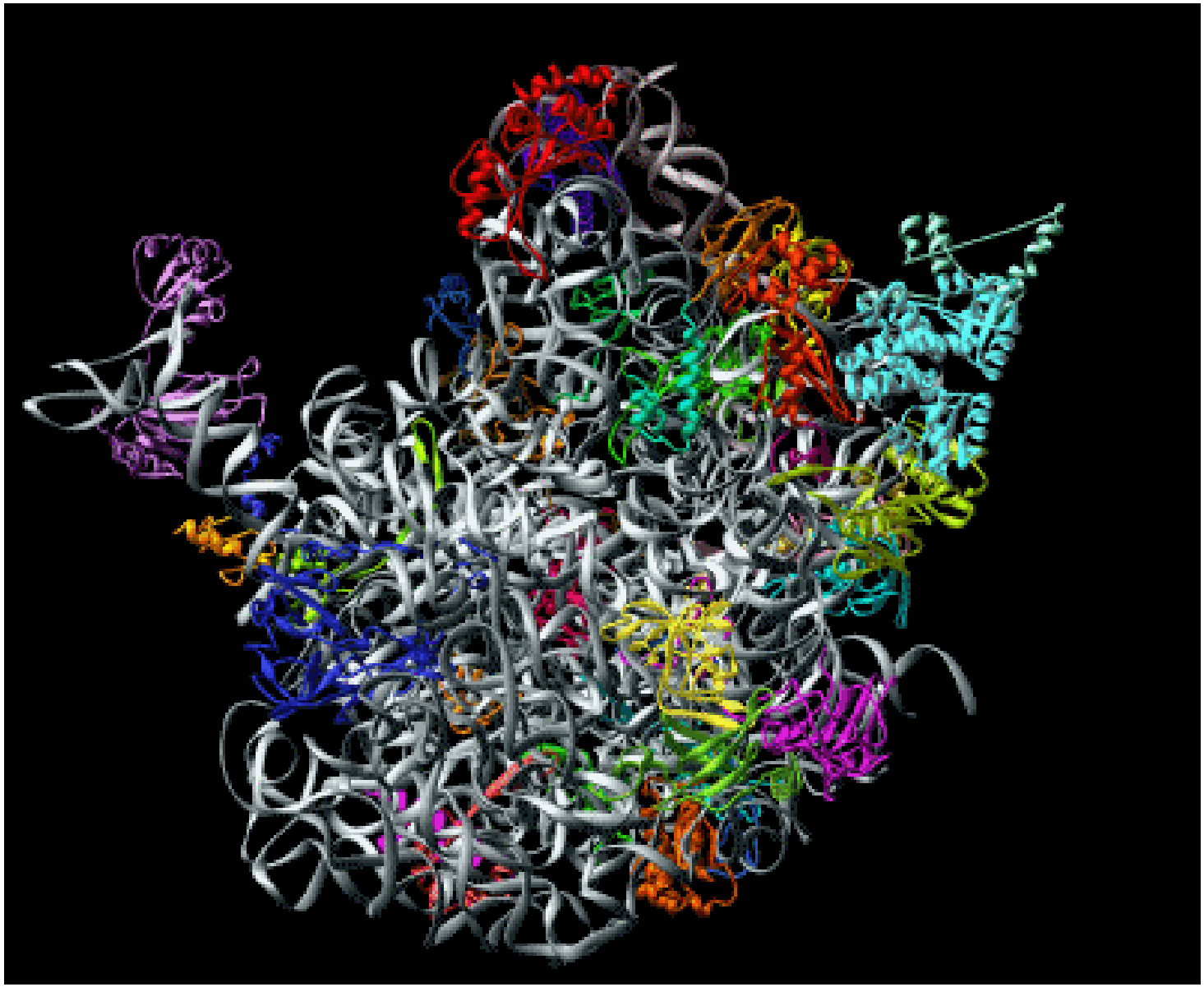
Clever tricks, continued:

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

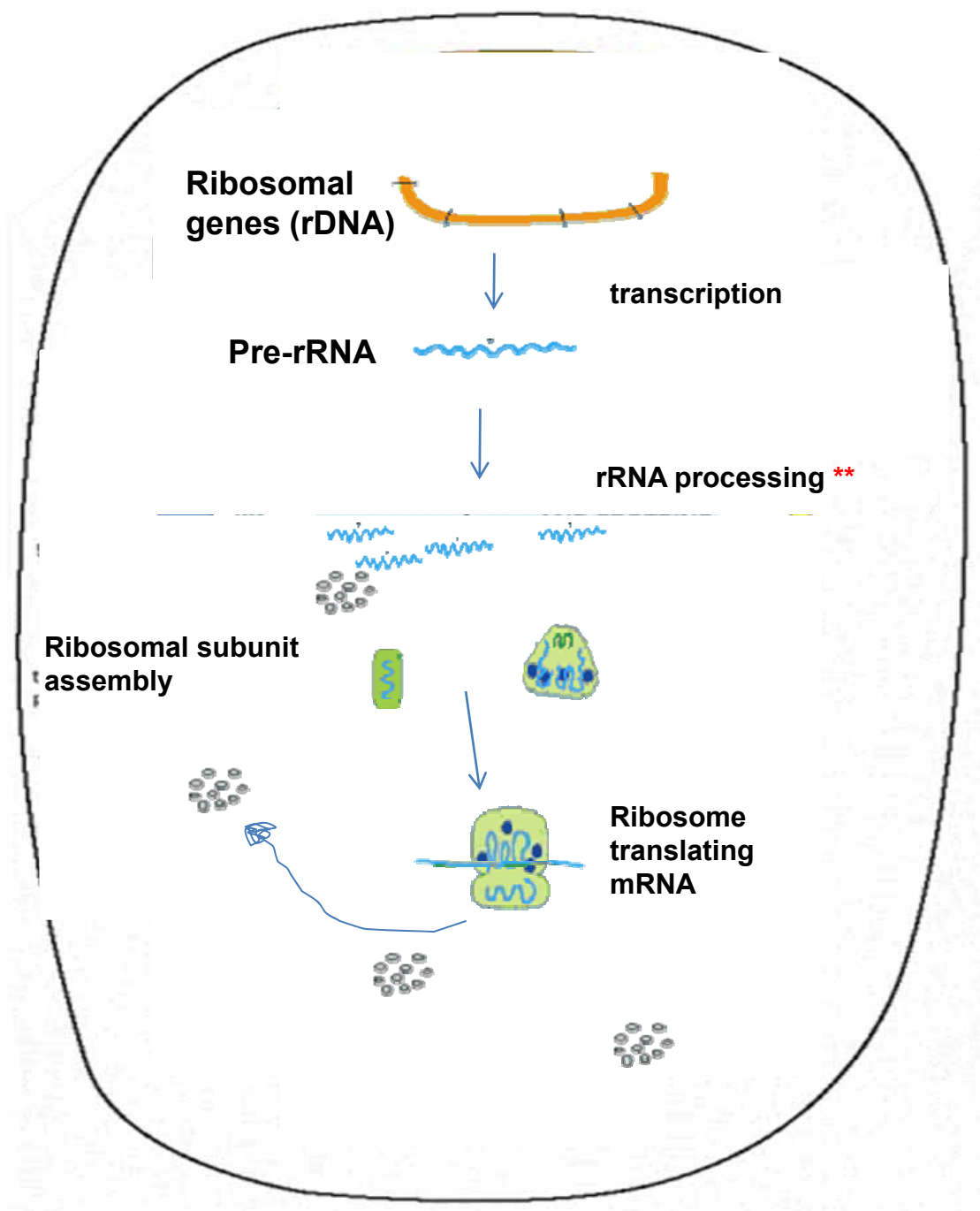
Yale University New Haven, CT

Ada E. Yonath

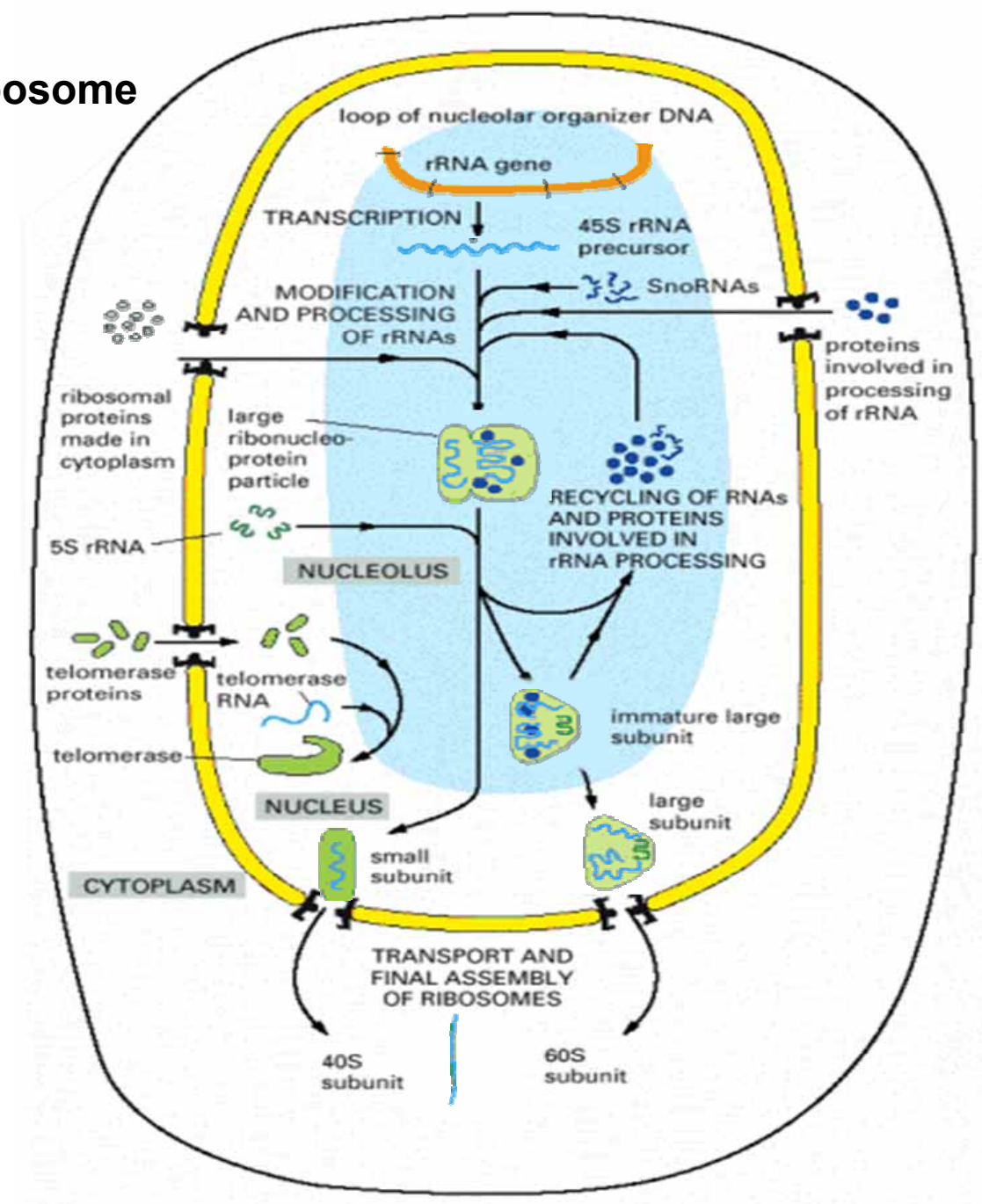
Weizmann Institute of Science Rehovot, Israel

http://nobelprize.org/nobel_prizes/chemistry/laureates/2009/sci.html

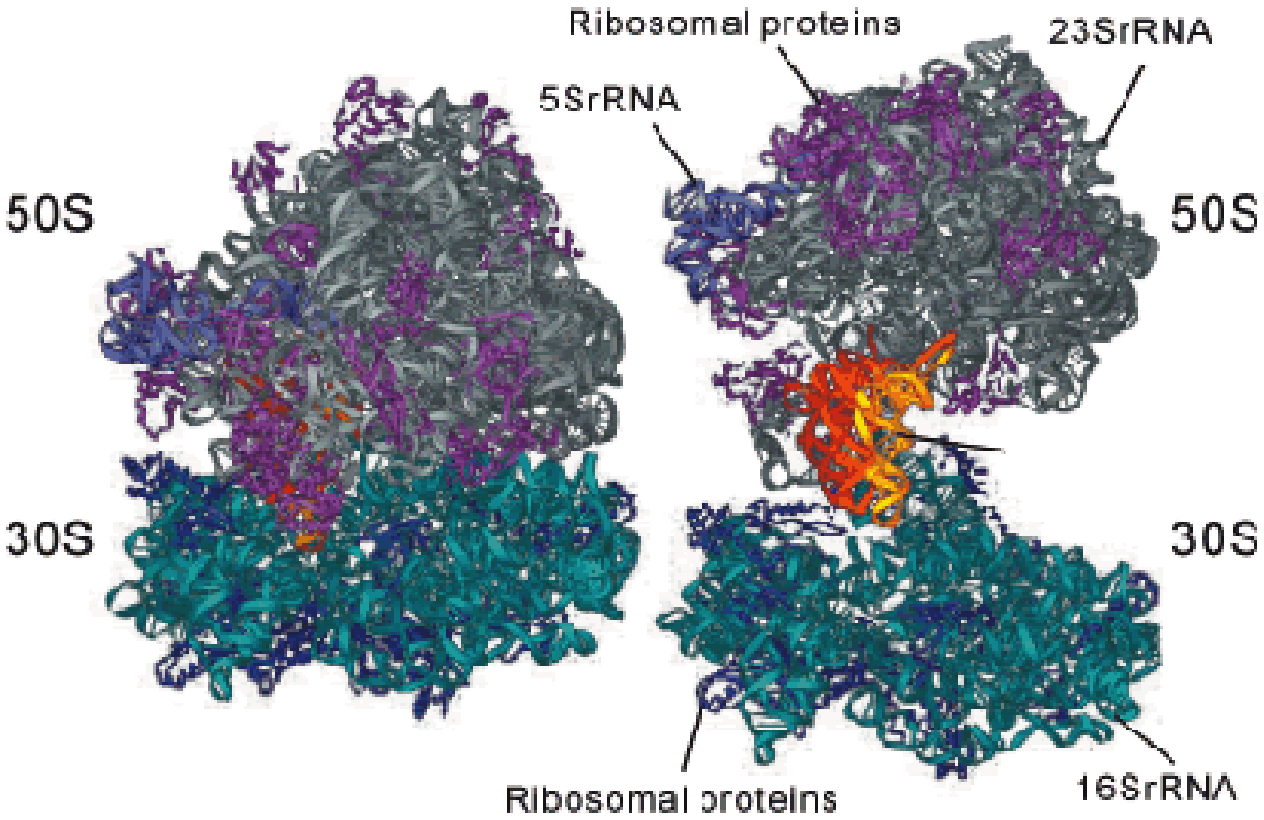
Ribosome Synthesis in Bacteria



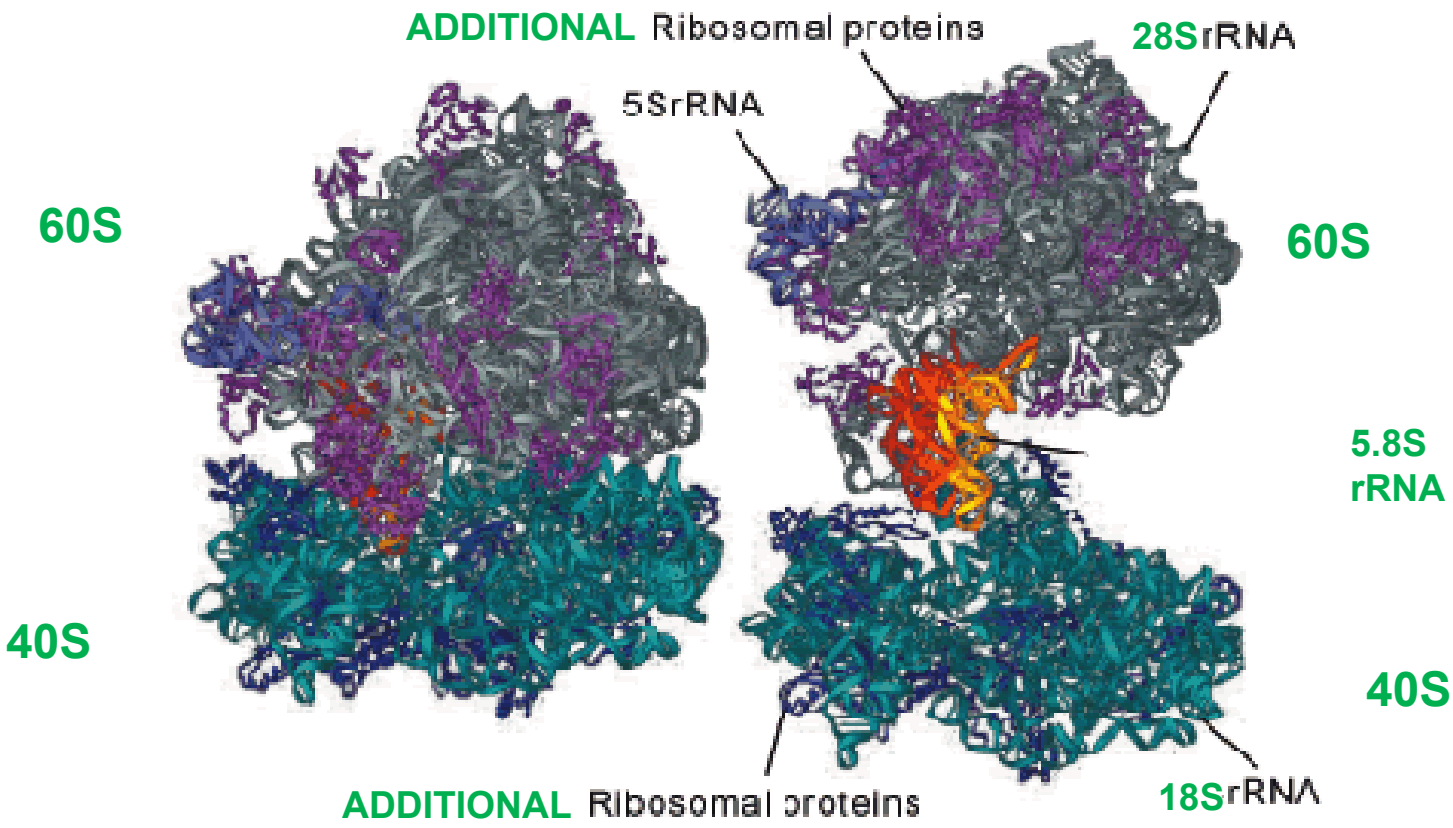
Eukaryotic Ribosome Synthesis

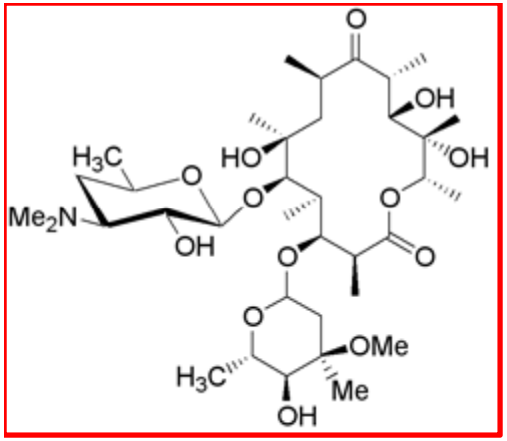


Bacterial ribosome composition

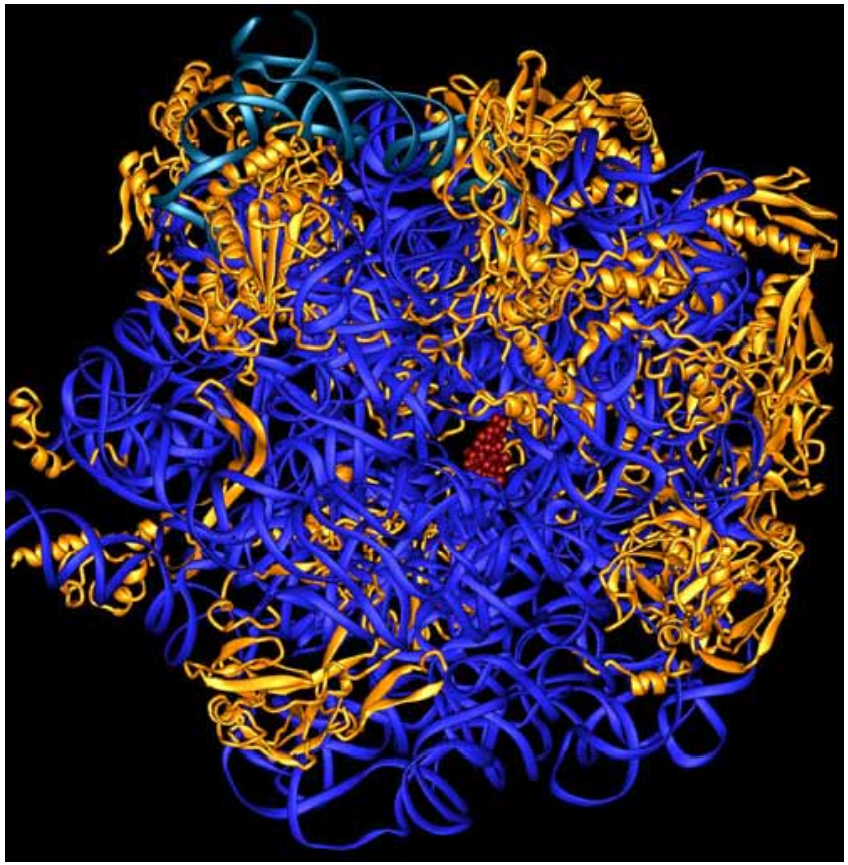


Eukaryotic ribosome composition

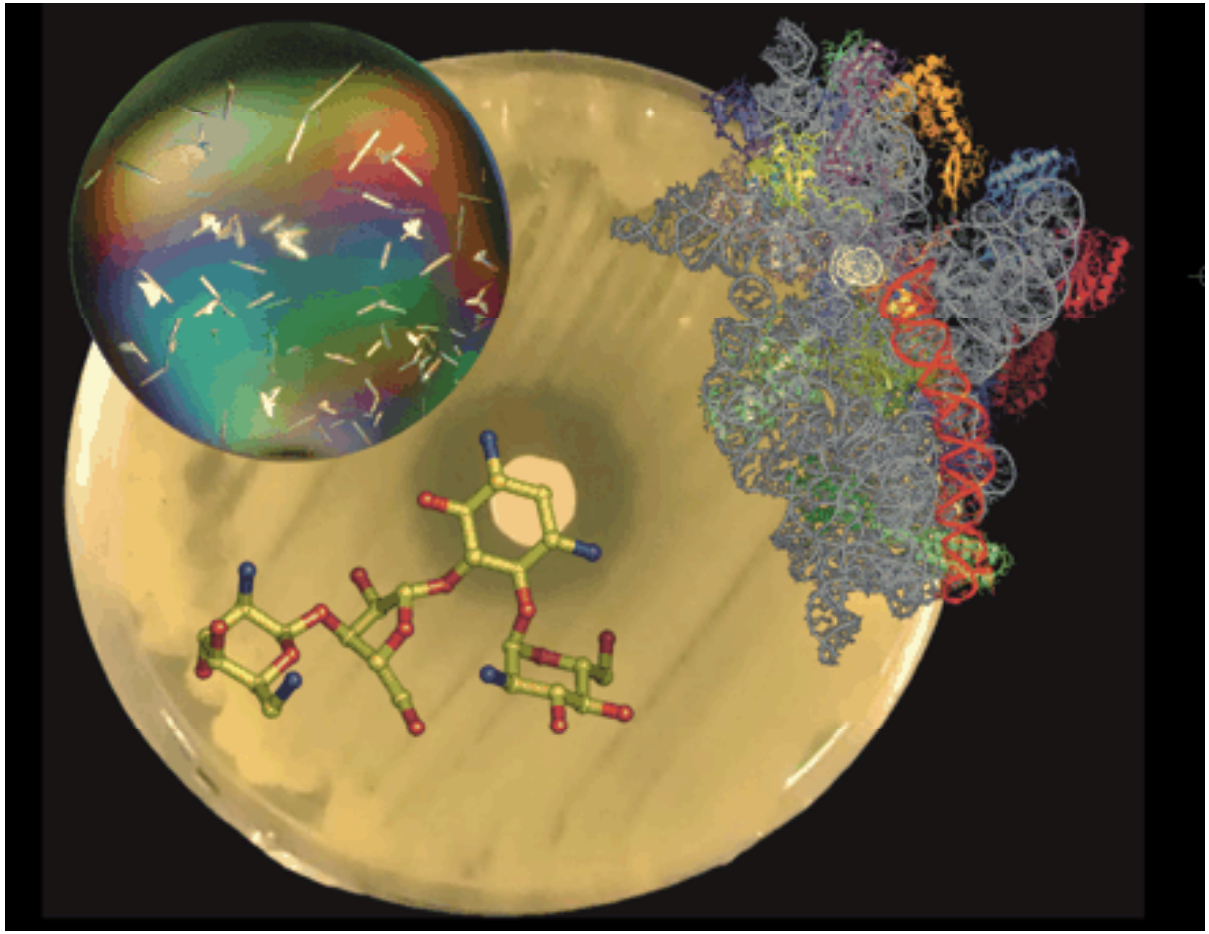




Erythromycin – a macrolide antibiotic that blocks protein synthesis by binding to bacterial ribosomes but not to eukaryotic ribosomes



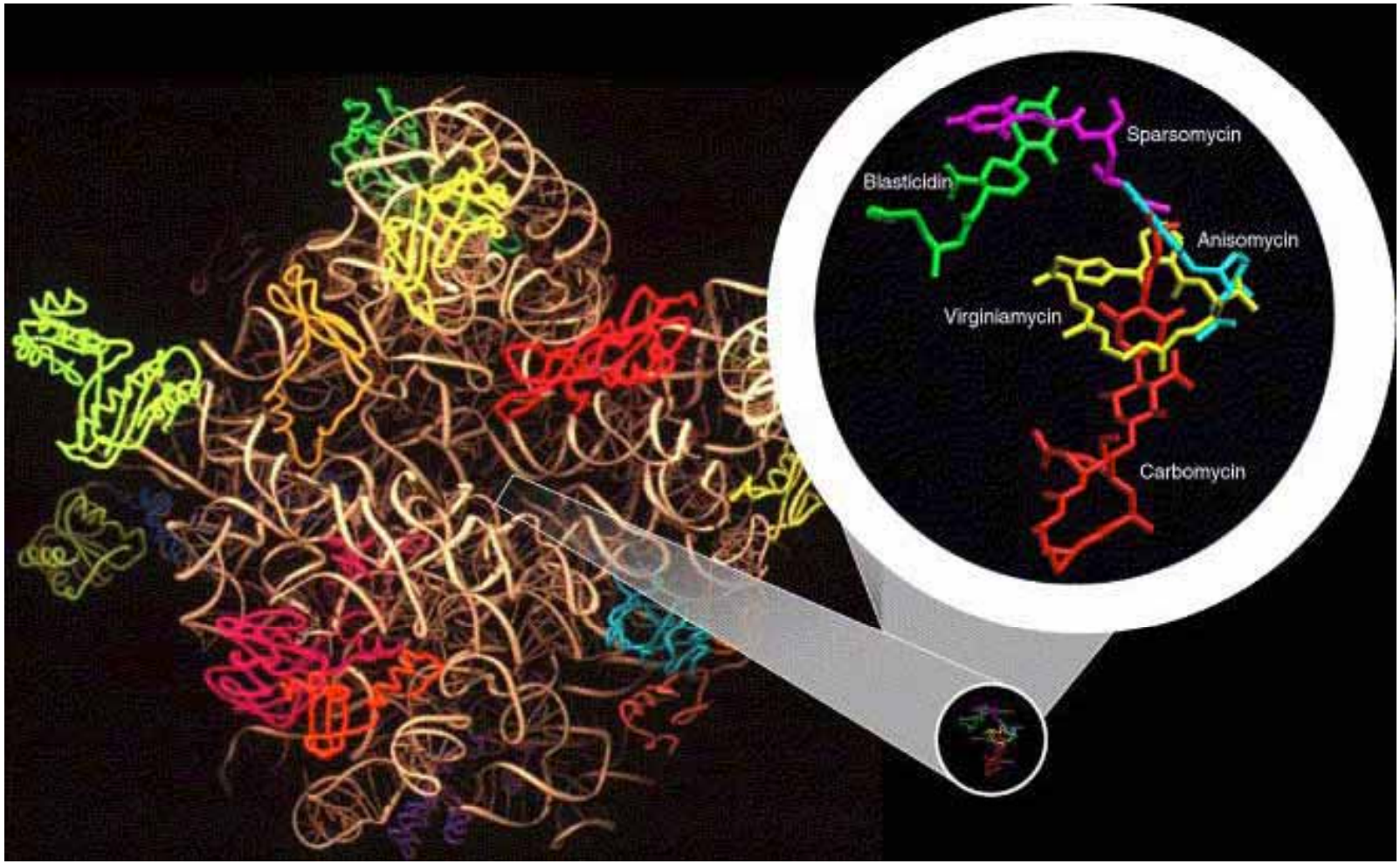
www.molgen.mpg.de



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

nar.oxfordjournals.org/.../issue1/cover.dtl

Antibiotics Targeting the Large Ribosomal Subunit of Bacteria



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	*T. thermophilus*
		Paromomycin	[26]	1FJG	*T. thermophilus*
		Paromomycin	[48]	1IBK	*T. thermophilus*
		Paromomycin	[25]	1J7T	RNA fragment
		Tobramycin	[50]	1LC4	RNA fragment
		Streptomycin	[26]	1FJG	*T. thermophilus*
Block binding of A-site tRNA	Tetracyclines	Tetracycline	[68]	1HNW	T. thermophilus
		Tetracycline	[69]	1I97	*T. thermophilus*
Inhibit translocation	Various	Edeine	[69]	1I95	*T. thermophilus*
		Pactamycin	[68]	1HNX	*T. thermophilus*
		Spectinomycin	[26]	1FJG	*T. thermophilus*

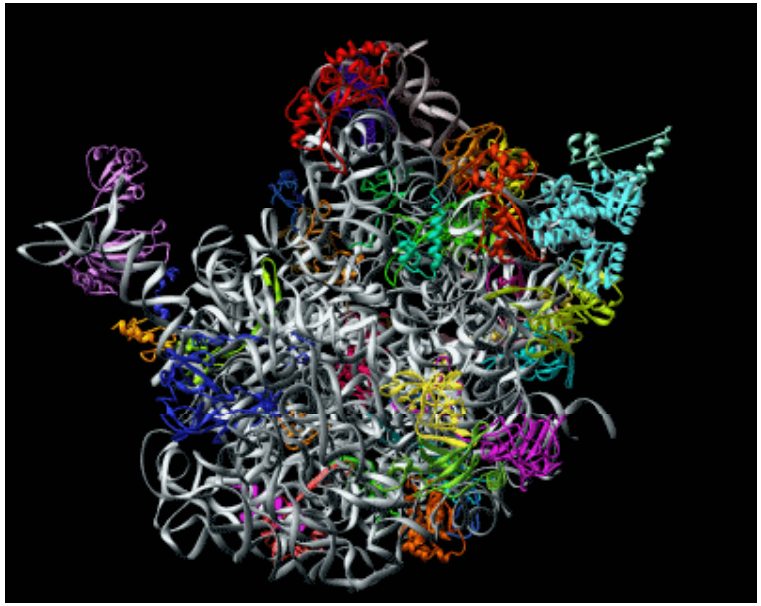
http://nobelprize.org/nobel_prizes/chemistry/laureates/2009/sci.html

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	*H. marismortui*
		Azithromycin	[71]	1NWY	*D. radiodurans*
		Azithromycin	[19]	1YHQ	*H. marismortui* (G2058A)
		Erythromycin	[72]	1JZY	*D. radiodurans*
		Carbomycin	[70]	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]	1OND	*D. radiodurans*
		Tylosin	[70]	1K9M	*H. marismortui*
	Ketolides	ABT-773	[71]	1NWX	*D. radiodurans*
		Telithromycin	[74,79]	1P9X	*D. radiodurans*
		Telithromycin	[19]	1YIJ	*H. marismortui* (G2058A)
	Streptogramins	Dalfopristin	[75]	1SM1	*D. radiodurans*
		Quinupristin	[75]	1SM1	*D. radiodurans*
		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.

SUMMARY:



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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