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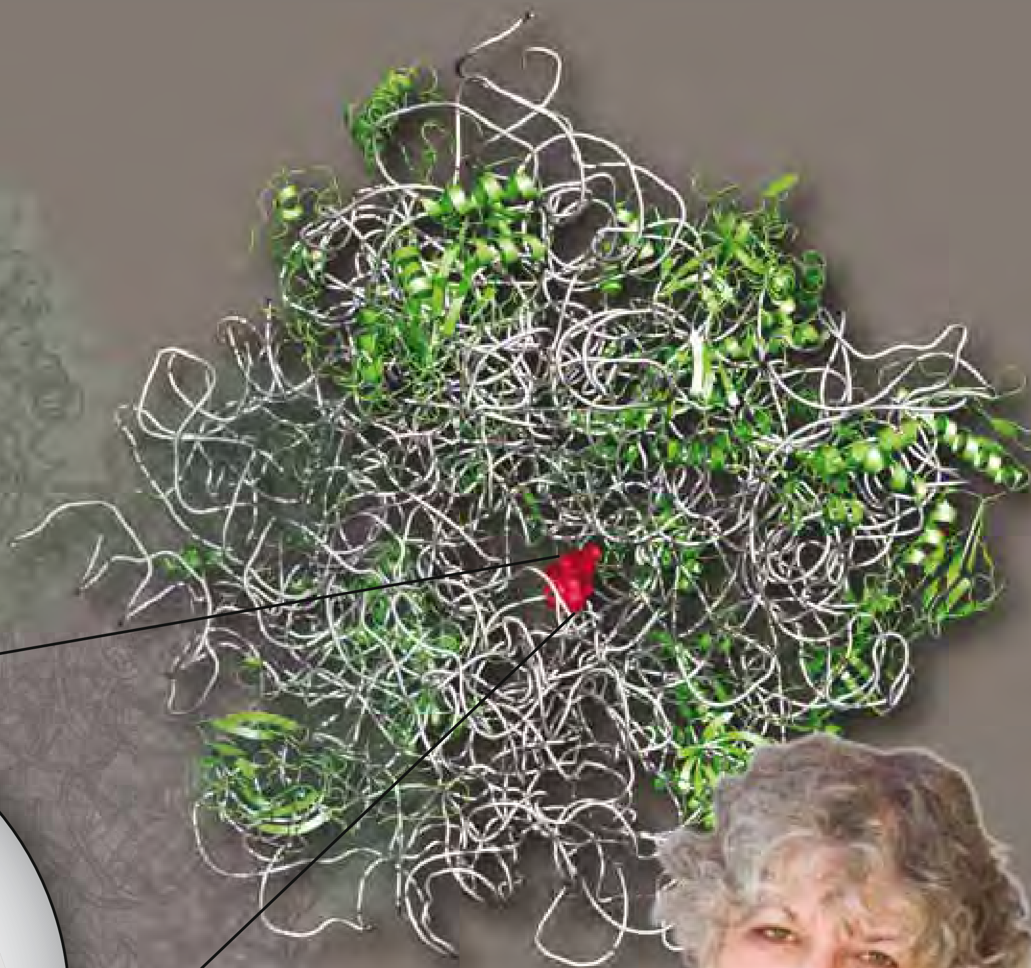
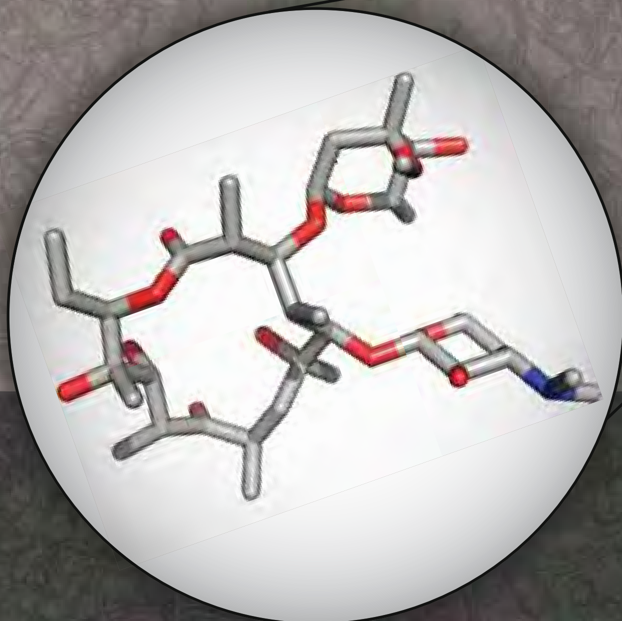




Prof. Ada Yonath of the Weizmann Institute of Science has been awarded the 2009 Nobel Prize in Chemistry

Letter from the editor

Prof. Matityahu Fridkin
Editor-in-chief

The Bulletin of the Israel Chemical Society extend its heartiest and warmest congratulations to Prof. Ada Yonath of the Department of Structural Biology of the Weizmann Institute of Science, Laureate of the 2009 Nobel Prize in Chemistry. The Prize was awarded for her outstanding achievements in deciphering the secrets of the ribosomal "machinery", one of the most intricate and mysterious systems in biology. The relevant press release of the Weizmann Institute of Science is included in this issue.

The continuous increase in antibiotic resistance among pathogenic bacterial strains poses a major health threat. The design and development of novel advanced drugs is thus urgently needed. Since the ribosome represents a common drug target of many antibiotic drugs, understanding its structure has immediate consequences on drug discovery. The article by Dr. Anat Bashan, Prof. Ada Yonath and colleagues summarizes the relevant implications.

The major problem with the currently used anti-HIV drugs is the high viral mutations, which results in the emergence of drug-resistant virus strains. To cope with this problem it is important to identify new targets and new approaches for drug design. The article of Dr. Zvi Hayouka, Prof. Assaf Friedler and colleagues suggests a novel concept towards these goals.

The delicate balance between basic academic research, in particular in its early stages, and the industrial R&D, is discussed in a "case study" by Dr. Revital Green.

The highlights of the 75th ICS meeting, held in February at the David Intercontinental Hotel in Tel-Aviv, are outlined by Profs Ehud Keinan, Doron Shabat and Shmuel Carmeli, Chairman of the Organizing Committee.

Profs. Lia Addadi and Steve Weiner, winners of the 2009 ICS Prize of Excellence, summarize their studies in an article "On amorphons and crystalline phases in biomeneralization – one hundred years to answer a question".

Prof. Hanoch Senderowitz and Drs. Galia Blum and Micha Fridman provide highlights of the 8th Meeting of the Medicinal Chemistry Section of the Israel Chemical Society, held in March 16th 2010 in the Weizmann Institute of Science.

Dr. Bob Weintraub, Director of the Libraries at the Sami Shamoon College of Engineering, brings here a fabulous story on the Zionist Charles Dreyfus and his activities in the field of Yellow Dye.

We intend to publish the next issue of Chemistry in Israel early next year, which will be the International Year of Chemistry. We wish to thank very much all authors of this issue and those who will contribute to the future issues of this important bulletin.

Prof. Ada Yonath of the Weizmann Institute of Science has been awarded the 2009 Nobel Prize in Chemistry

The Weizmann Institute of Science congratulates Prof. Ada Yonath on receiving the 2009 Nobel Prize in chemistry and is proud of her scientific achievements. We are delighted that the Nobel Prize committee has recognized the significance of Prof. Ada Yonath's scientific research and awarded her this important prize.

Prof. Yonath's research is driven by curiosity and ambition to better understand the world and our place within it. This research aims high: to understand one of the most complicated "machines" of the biological system.

In the late 1970s, Prof. Yonath decided, when she was a young student at the Weizmann Institute, to take on the challenge of answering one of the key questions concerning the activities of live cells: to decipher the structure and mechanism of action of ribosomes – the cell's protein factories. This was the beginning of a long scientific journey that has lasted decades, and which required courage and devotion from the start. The journey began in a modest laboratory with a modest budget, and with the years, increased to tens of researchers under the guidance of Prof. Yonath.

This basic research, which began in the attempt to understand one of the principles of nature, eventually led to the understanding of how a number of antibiotics function, something that is likely to aid in the development of more advanced and effective antibiotics. This discovery will hopefully also help in the struggle against antibiotic-resistant bacteria, a problem recognized as one of the most central medical challenges of the 21st century.

Prof. Yonath can be considered a model of scientific vision, courage in choosing a significant scientific question, and devotion in realizing the goal to its end – which will hopefully broaden knowledge for the benefit of humanity.

Beyond the Basics

"People called me a dreamer," says Prof. Ada Yonath of the Structural Biology Department, recalling her decision to undertake research on ribosomes – the cell's protein factories. Solving the ribosome's structure would give scientists unprecedented insight into how the genetic code is translated into proteins; by the late 1970s, however, top scientific teams around the world had already tried and failed to get these complex structures of protein and RNA to take on a crystalline form that could be studied. Dreamer or not, it was hard work that brought results: Yonath and colleagues made a staggering 25,000 attempts before they succeeded in creating the first ribosome crystals, in 1980.

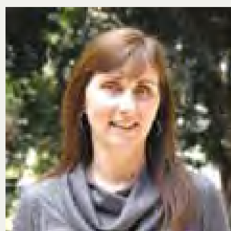
And their work was just beginning. Over the next 20 years, Yonath and her colleagues would continue to improve their technique. In 2000, teams at Weizmann and the Max Planck Institute in Hamburg, Germany – both headed by Yonath – solved, for the first time, the complete spatial structure of both subunits of a bacterial ribosome. Science magazine counted this achievement among the ten most important scientific developments of that year. The next year, Yonath's teams revealed exactly how certain antibiotics are able to eliminate pathogenic bacteria by binding to their ribosomes, preventing them from producing crucial proteins.

Yonath's studies, which have stimulated intensive research worldwide, have now gone beyond the basic structure. She has revealed in detail how the genetic information is decoded, how the ribosome's inherent flexibility contributes to antibiotic selectivity and the secrets of cross-resistance to various antibiotic families. Her findings are crucial for developing advanced antibiotics.

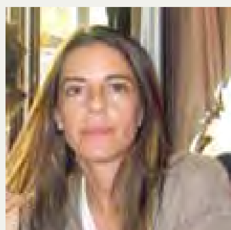
Prof. Ada Yonath's research is supported by the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly. Prof. Yonath is the Martin S. and Helen Kimmel Professor of Structural Biology.

The ribosome as drug target: lessons from 3D structures

Anat Bashan, Ella Zimmerman, Mattew Belousoff, Haim Rozenberg ,Chen Davidovich, Itai Wekselman, Tal Shapira, Miri Krupkin and Ada Yonath

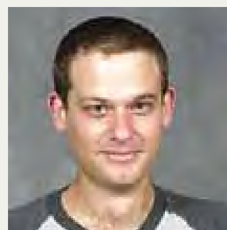


Dr. Anat Bashan earned her B.Sc. degree in chemistry at the Hebrew University, Jerusalem in 1987, and received her M.Sc.(1989) and Ph.D. (1995) degrees at the Department of Structural Biology of the Weizmann Institute of Science, Israel. Between 1995 and 1998 she was a postdoctoral fellow at the Weizmann Institute, and since then she has been a staff scientist in the group of Prof. Ada Yonath at the Department of Structural Biology, Weizmann Institute. Her research activities are focused on structure and function of ribosomal components and the mode of action of ribosomal antibiotics.



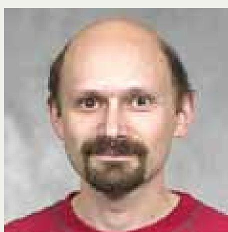
Dr. Ella Zimmerman earned her B.Sc. degree in chemistry at Tel Aviv University in 1991, and received her M.Sc. (1995) and Ph.D. (2002) degrees at the Department of Structural Biology of the Weizmann Institute for Science, Israel. Between 2002 and 2006 she was a postdoctoral fellow at the Weizmann Institute, and since then she has been a staff scientist in the group of Prof. Ada Yonath at the Department of Structural Biology, Weizmann Institute. Her research activities are focused on structure and function of ribosomal components and the mode of action of ribosomal antibiotics, and in developing delicate methods for analyzing biological structures by electron microscopy.

Dr. Matthew Belousoff earned his B.Sc (Hons) in Chemistry/Mathematics at Monash University (Australia) where he completed his Ph.D. studies at the School of Chemistry in 2008. Currently he is a post-doctoral fellow at the Weizmann Institute in the group of Prof. Ada Yonath working on synergistic pairs of ribosomal interfering antibiotics.



Mr. Chen Davidovich is a Ph.D. student in the research group of Prof. Ada Yonath, at the Weizmann Institute of Science, studying ribosomal antibiotics and resistance to them, and ribosome evolution. He earned his B.Sc. degree in Biotechnology Engineering at Ben-Gurion University in 2004. Currently he is an Adams Fellow of the Israel Academy of Sciences and Humanities.

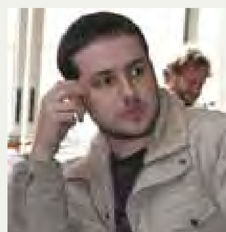
Mr. Itai Wekselman is a Ph.D. student in the research group of Prof. Ada Yonath, at the Weizmann Institute of Science, studying the resistance to antibiotics targeting the ribosome. He earned his B.Sc. degree in Biology at Tel- Aviv University in 2005 and his M.Sc. in Chemistry at the Weizmann Institute of Science, in 2008.



Dr. Haim Rozenberg earned his B.Sc. (1988) and M.Sc. (1989) degrees in physics and DEA (1990) of Molecular Biophysics at the Universitée de Jussieu, Paris, France. In 1999, he received his Ph.D. degree at the Department of Structural Biology of the Weizmann Institute of Science, Israel. From 1999-2001, he was a postdoctoral fellow at the X-ray Crystallography Laboratory of the Weizmann Institute. Since then, he has been a staff scientist in the groups of Prof. A. Yonath and Z. Shakked at the Department of Structural Biology, Weizmann Institute of Science. His research activities are focused on ribosome crystals handling and data collection with Prof. Yonath and on structure and function of the tumor-suppressor protein p53 and its complexes with DNA with Prof. Shakked.

Ms. Miri Krupkin received a B.Sc. in Chemistry from Bar-Ilan University in 2008. She is currently working on her M.Sc. thesis in the lab of professor Ada Yonath at the Weizmann Institute of Science. She is studying the structure and function of ribosomal particles, the mechanisms of ribosomal antibiotics and the origin of the contemporary ribosome.

Dr. Ada Yonath is a structural biologist who in using X-ray crystallography. She earned her B.Sc. (1962) and M.Sc. (1964) degrees in chemistry at the Hebrew University in Jerusalem and her Ph.D. (1968) at the Weizmann Institute of Science. She conducted her postdoctoral studies at Carnegie Mellon University and at the Massachusetts Institute of Technology and in 1970 she established the first protein crystallography laboratory in Israel. She pioneered ribosomal crystallography in 1980 and together with her coworkers, some of which are coauthors of this manuscript, determined the high resolution structure of the small ribosomal subunit from Thermus thermophilus (2000) and of the large ribosomal subunit from Deinococcus radiodurans (2001). She is the Martin S. and Helen Kimmel Professor of Structural Biology at the Weizmann Inst. of Science, the Director of the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly, a member of the Israeli, the European and the US National Academies of Sciences, and a 2009 Nobel Prize Laureate in Chemistry.



Mr. Tal shapira is a M.Sc. Student at the department of structural biology at the Weizmann Institute. He earned his B.Sc. in chemistry and biology from Tel-Aviv University. He is currently focusing on studies of ribosomal antibiotics, and crystallization of antibiotics-ribosome complexes.

ABSTRACT

The increase in antibiotic resistance among pathogenic bacterial strains poses a significant health threat. Therefore, improvement of existing antibiotics and the design of advanced drugs are urgently needed. The ribosome is the drug target for many antibiotic families. Antibiotics bind at functionally active centers, some of which are highly conserved. In several cases the ribosome utilizes its inherent functional flexibility to trigger induced fit mechanisms by remote interactions, facilitating antibiotic synergism as well as reshaping improper binding pockets. These interactions lead to antibiotic selectivity even for antibiotics that bind to conserved functional regions, as less conserved nucleotides reside in proximity to the binding pocket. Exploitation of the diverse properties of antibiotics binding and benefiting from the detailed structural information that keeps emerging, should result in significant improvement of current antibiotic treatment.

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Introduction

Ribosomes, the universal cellular riboprotein assemblies, are the nano-machines which translate the genetic code into proteins. The translation process requires a complex apparatus composed of many components. Among them the ribosome is the key player, as it provides the framework for the proper positioning of all other components participating in the peptide bond and nascent chain elongation. Ribosomes operate continuously, in each living cell, since the constant programmed cell death implies constant proteins degradation and requires simultaneous production of proteins. Hundreds of thousands of ribosomes are present in typical mammalian cells. Fast replicating cells, e.g. liver cells, may contain a few millions ribosomes. Even bacterial cells may contain up to 100,000 ribosomes during their log period.

Within the framework of living cells, ribosomes are giant assemblies, composed of many different proteins (r-proteins) and long ribosomal RNA (rRNA) chains. The ratio of rRNA to r-proteins (~2:1) is maintained throughout evolution, with the exception of mammalian mitochondrial ribosome (mitoribosome) where almost half of the bacterial rRNA is replaced by r-proteins (consequently in mitoribosome the ratio of RNA to proteins is ~1/1). All ribosomes are composed of two unequal subunits (Table 1). In prokaryotes, the small subunit, denoted as 30S, contains an RNA chain (16S) of about 1500 nucleotides and 20–21 different proteins, whereas the large subunit (called 50S in prokaryotes) has two RNA chains (23S and 5S RNA) of about 3000 nucleotides in total, and 31–35 different proteins (see more details in Table 1). In all organisms the two subunits exist independently and associate only for forming functionally active ribosomes.

The process of mRNA-encoded protein synthesis requires a complex apparatus composed of the ribosome, transfer RNA molecules (tRNA) and accessory protein factors. The mRNA chains are produced by the transcription of the segments of the DNA that should be translated. The mRNA chains carry the genetic information to the ribosomes, while tRNA molecules deliver the cognate amino acids. For increasing efficiency, a large number of ribosomes act simultaneously on a single transcript as polymerases synthesizing proteins by one-at-a-time addition of amino acids to a growing peptide chain. While translocating along the mRNA template, at ribosomes produce proteins on a continuous basis an incredible rate (namely >15 new peptide bonds per second in prokaryotes and 2-5 new peptide bonds per second in eukaryotes). While the elongation of the nascent chain proceeds, the two subunits perform cooperatively. The small subunit provides the path along which the mRNA progresses, the decoding center and the mechanism controlling translation fidelity while the large subunit provides the site for the main ribosomal catalytic function, polymerization of the amino acids and the protein exit tunnel (Figure 1).

The recent availability of the over two dozens of crystal structures of bacterial ribosome and their complexes (see below) have enabled a quantum leap in the understanding of the machinery of protein biosynthesis. These structures have shown that in each of the two subunits the ribosomal proteins are entangled within the complex rRNA conformation, thus maintaining a striking dynamic architecture that is ingeniously designed for their functions: precise decoding; substrate mediated peptide-bond formation and efficient polymerase activity. This review will focus on the ribosome as

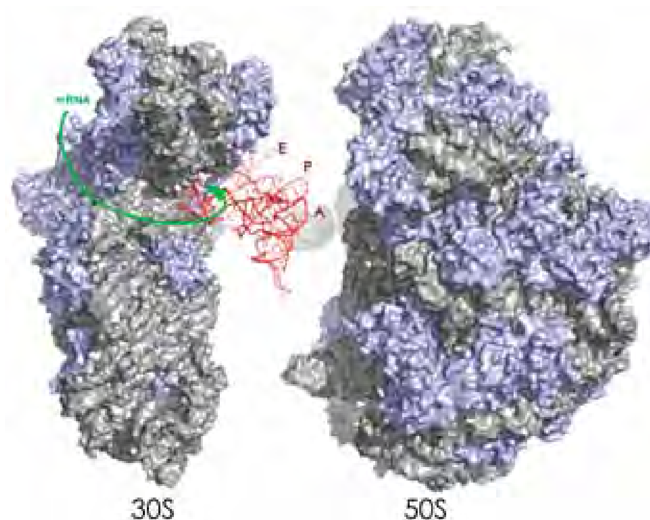

Figure1: The interface view of the crystal structures of the two ribosomal subunits, 50S from D. radiodurans and 30S from T. thermophilus. rRNA is colored grey and r-proteins are colored light blue. A, P and E site tRNA molecules as bound to the T70S subunit (PDB code 2WDM) were superimposed on the D50S structure and mRNA path is marked.

a target for antibiotics and will attempt to answer the question: “Can structures lead to improved antibiotics?”

Ribosome structure can lead to improved antibiotics

The intensive research on ribosomes has some practical aspects; one of them has clinical relevance. Owing to its key role in life cycle, namely in producing the proteins required for vitality, the ribosome is one of the major targets in the cell for antibiotics. The increasing incidence of antibiotic resistance and toxicity creates serious problems in modern medicine; combating resistance to antibiotics has been a major clinical concern in recent years. The vast amount of structural data on ribosomal antibiotics accumulated recently may supply critical information to overcome current barriers faced by antibiotics.

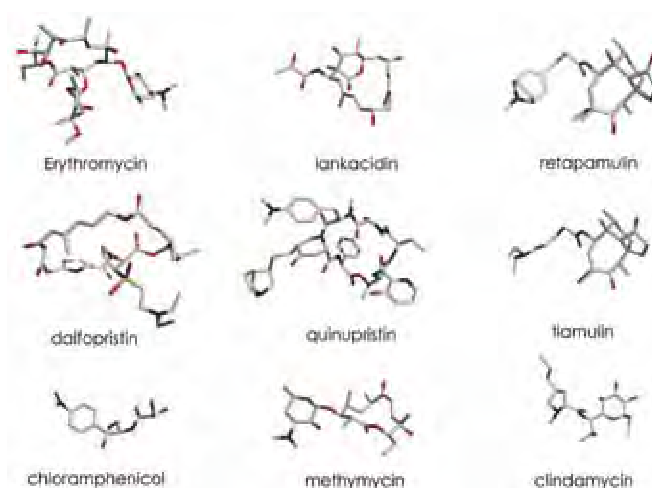

Figure 2: 3D structure of Antibiotic molecules that inhibit the large ribosomal subunit activity, represented in CPK colors.

Antibiotics are compounds used in clinical medicine for treating bacterial infections selectively by inhibiting the biological function of the bacterial ribosomes and not that of the host (Figure 2). More than 40% of the useful antibiotics interfere with the biosynthetic machinery and most of them target the ribosomes at distinct locations within functionally relevant sites. These act by diverse mechanism, many of them were revealed by analysis of crystallographic results or structural data based biochemical studies (for review see Auerbach *et al.*, 2004; Yonath and Bashan 2004; Yonath, 2005a; Tenson and Mankin, 2006; Poehlsgaard and Douthwaite, 2005; Bottger, 2006; 2007; Mankin, 2001, 2006, 2008). Numerous structural, biochemical and genetic studies provided indispensable information that illustrated the basic mechanisms of ribosomal antibiotics activity and synergism; provided the structural basis for antibiotic resistance and enlightened the principles of antibiotic selectivity, namely the discrimination between the pathogens and host, the key for therapeutical usefulness.

By its nature, X-ray crystallography should be the method of choice for investigating ribosome-antibiotics interactions. However, since X-ray crystallography requires diffracting crystals, and since so far no ribosomes from pathogenic bacteria have been crystallized, currently the crystallographic studies of antibiotics that bind the large ribosomal subunit are confined to the currently available crystals of suitable pathogen models. Currently available are high-resolution structures of antibiotic complexes with the whole ribosome or the small ribosomal subunits from the eubacterium *Thermus thermophilus* and of the large ribosomal subunits from eubacterium *Deinococcus radiodurans* (D50S), two species the ribosomes of which are suitable to serve as pathogen models. On the other hand, complexes of antibiotics with the large ribosomal subunit from the Dead Sea archaeon *Haloarcula marismortui* (H50S) that resembles eukaryotes in respect to antibiotics binding site, have been obtained.

The structures of the large ribosomal subunit D50S complexed with various antibiotics determined so far (Schluenzen *et al.*, 2001, 2004; Berisio *et al.*, 2003a and b; Auerbach *et al*., 2004; Yonath and Bashan 2004; Harms *et al.*, 2004; Pyetan *et al.*, 2007; Yonath, 2005; Davidovich *et al.*, 2007, 2008; Vazquez-Laslop *et al.*, 2008; Auerbach *et al.*, 2009 ,2010) revealed common traits: binding sites that are composed primarily of rRNA and coincide with functionally critical centers of the ribosome. Furthermore, comparisons between these structures demonstrated that members of antibiotic families possessing common chemical elements with minute differences might bind to ribosomal pockets in significantly different modes, and that the nature of seemingly identical mechanisms of drug resistance may be dominated by the antibiotics’ chemical properties. Among the modes of action of ribosomal antibiotics, are interfering with substrate binding at the PTC (Figure

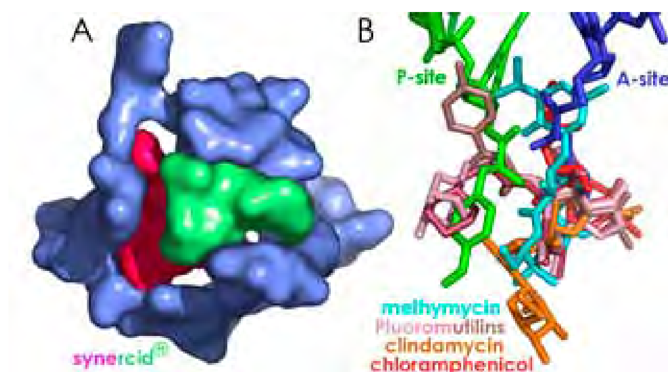

Figure 3: Antibiotics blocking the nascent peptide tunnel entrance and the PTC. **A.** The clinically used drug synercid is blocking both the PTC and the tunnel entrance using its two componants: quinupristin (in green) and dalfopristin (in pink). **B.** antibiotic molecules bound at the PTC are blocking the binding site of the A and/or P-site tRNA substrates.

3B), or blocking the protein exit tunnel (Figure 3A).

A major issue concerning the clinical usefulness of ribosomal antibiotics is their selectivity; their capabilities in the discrimination between the ribosomes of the eubacterial pathogens and those of eukaryotes. As mentioned above, although prokaryotic and eukaryotic ribosomes differ in size (~ 2.4 and 4 Mega daltons, respectively), their functional regions, which are the targets for the antibiotics, are highly conserved. Therefore the imperative distinction between eubacterial pathogens and mammals, the key for antibiotic usefulness, is achieved generally, albeit not exclusively, by subtle structural difference within the antibiotics binding pockets of the prokaryotic and eukaryotic ribosomes. A striking example for discrimination between pathogens and humans is the immense influence of the minute difference in at the rRNA position 2058 where an adenine of the eubacteria is replaced by a guanine in eukaryotes. This small difference was found to govern the binding of macrolides, a prominent antibiotics family (Lai and Weisblum, 1971) that obstructs the progression of the nascent proteins within the tunnel. However, although 2058 identity determines the antibiotic affinity, this analysis showed that the mere binding of the antibiotics is not sufficient for obtaining efficient therapeutical effectiveness. Comparisons between crystal structures of antibiotics bound to the eubacterial large ribosomal subunit, D50S to structures of complexes of the large ribosomal subunit from H50S, an archaeon sharing properties with eukaryotes, which required either extremely high antibiotics concentrations (Hansen *et al*., 2002a, 2003) or G2058A mutations, to facilitates macrolides/ketolides binding (Tu *et al*., 2005), indicated the significance of additional structural elements (Yonath and Bashan, 2004) of the binding pocket, which dictate inhibitory activity. Similar observations were made by mutagenesis in the yeast Saccharomyces cerevisiae at a position equivalent to *E. coli* A2058, which allows erythromycin binding but does not confer erythromycin susceptibility (Bommakanti *et al*., 2008).

The fine details of binding, resistance and selectivity of other members of the macrolide family and its offsprings, the azalides and ketolides, addresses additional issues, such as the sequence specificity that determines the susceptibility and the fitness cost of the ketolides (Pfister, *et al*., 2005). Another intriguing issue, which led to continuous expansion of research and to new insights, relates to the nature of the contributions of two ribosomal proteins, namely L4 and L22, to macrolides resistance. These two proteins line the exit tunnel at its constriction, and do not interact directly with most of the members of the macrolides family, yet several types of mutations at their tip acquire resistance to them (Gregory and Dahlberg, 1999; Davydova et al., 2002; Lawrence et al., 2008; Berisio *et al*., 2006; Zaman *et al*., 2007; Moore and Sauer, 2008), presumably by perturbing the rRNA structure at the tunnel walls (Gregory and Dahlberg, 1999; Lawrence *et al*., 2008).

Attempts aimed at alleviating the resistance problem include the development of synergetic antibiotics. An example is the very potent family of the streptogramins, a two-component antibiotics drug family, each of which is a rather weak drug. The impressive synergetic effect of this family can be understood by examining the mechanism exploited by the rather recent antibiotic drug, Synercid® (that is composed of 2 componants; dalfopristin and quinupristin). This mechanism is based on the binding of one of the components to the PTC that causes a dramatic alteration in the orientation of the very flexible nucleotide, U2585, which plays a principal role in the A- to P-site rotatory motion, and the fixation of the altered orientation by the second compound that binds at the tunnel entrance (Harms *et al.*, 2004;

Yonath and Bashan, 2004) (Fig 2a). Another pair of new potential synergistic drug was investigated lately in our laboratory. Upon study of the structure of lanakacidin, an antibiotic that is produced by *Streptomyces rochei*, it was found to bind the PTC at the large ribosomal subunit (Auerbach *et al*., 2010). This same species also produces lankamycin, a macrolide that we recently showed is a weak protein synthesis inhibitor that seems to bind at the tunnel entrance. Biochemical work showed that there is a synergistic effect between the two drugs and crystallographic work on this new synergistic pair is underway. We anticipate that this crystallographic work will guide improved structure-based drug design to achieve a more effective antibiotic synergism.

Even subtle differences, such as the identity of nucleotide 2058 (A in eubacteria, G elsewhere) hardly exist in the PTC. Therefore, obtaining selectivity in antibiotics binding to the PTC, the core catalytic center of the ribosome, is more complex. Nevertheless, some of the antibiotics bind to the PTC of eubacterial ribosomes with high affinity and great specificity, without significant effect on the eukaryotic hosts. The crystal structures of ribosomal complexes with antibiotics indicated that the PTC provides binding sites to several clinically useful antibiotics. This super family of PTC antibiotics can shed light on general as well as specific properties of the interactions of the members of this family with their binding pockets in the PTC. This super family includes phenicols, lincosamides, pleuromutilins, streptograminsA, oxazolidinones and lankacidins. Although basically all PTC antibiotics act by blocking part or the entire PTC, they utilize different binding modes and consequently they possess various inhibitory mechanisms. Thus, chloramphenicol was found to hamper the binding of the A-site tRNA, like (Schluenzen *et al*., 2001), the pleuromutilins, linezolid and streptograminsA occupy both the A- and the P-site tRNAs (Harms *et al*., 2004; Hansen *et al*., 2003; Ippolito *et al*., 2008), and clindamycin, methymycin and lankacidin interfere with the peptide bond formation (Schluenzen *et al*., 2001, Auerbach *et al.* 2009, 2010).

Contribution of several PTC flexible nucleotides to productive binding was also observed by investigating the mode of action of the pleuromutilin family, which revealed a unique inhibitory mechanism alongside novel selectivity and resistance strategies. In particular, the elaborate pleuromutilins binding mode demonstrates how selectivity and resistance are acquired despite almost full conservation (Davidovich *et al*., 2007, 2008; Schluenzen *et al*., 2004). As all nucleotides in the immediate vicinity of the binding site are highly conserved, pleuromutilins selectivity is determined by nucleotides that are not located in the immediate vicinity of the antibiotic binding site, hence are less conserved. Thus, pleuromutilins binding triggers an induced-fit mechanism by exploiting the flexibility of the rRNA nucleotides residing in and around the PTC, as well as a network of interactions with less conserved remote nucleotides, hence allowing for drug selectivity (Davidovich *et al*., 2007; 2008). A key player in this mechanism is nucleotide 2504 that defines part of the binding surface and was observed in different conformations in crystal structures of large ribosomal particles from bacteria and archaea. This results in different network of interactions between this nucleotide and less conserved nucleotides that vary between eubacteria to archaea and eukaryotes. This mechanism for selectivity that was first observed for pleuromutilin antibiotics (Davidovich *et al*., 2007), suggested to determine selectivity to other PTC antibiotics based on a comparative study (Davidovich *et al*., 2008) and was later reconfirmed by crystal structures of the archaeal large ribosomal subunit with PTC antibiotics (Gurel *et al*., 2009). In particular, this family exploits the remote interactions that affect the positioning of the extremely flexible nucleotide U2506, as well as of U2585 that participate in navigating and anchoring the rotatory motion of the tRNA 3' end from A to P-site. These interactions partially evacuate the binding region and at the same time tighten the binding pocket on the bound antibiotic molecule. As mutations within the PTC should be lethal, resistance to pleuromutilins requires mutations or modifications of nucleotides residing either in PTC components with identity that is less crucial for ribosome function, or in the PTC environs rather than within the core of the binding pocket, therefore should occur in a relatively slow pace. Remarkably, these crystallographic studies led the way in attempts to produce advanced compounds (Lolk *et al*., 2008). Indeed, cross resistance was detected between all PTC antibiotics, regardless of their mode of binding, and the nucleotides mediating it are residing only on one side of the PTC, similar or in close proximity to those acquiring resistance to the pleuromutilins.

Attempts to overcome antibiotics resistance and increase their selectivity are going on currently (e.g. Yassin *et al.*, 2005; Wilson *et al.*, 2005; Bottger, 2007). These studies exploit several strategies, including the insertions of moieties that should compensate for the lost interactions of the resistant strains, benefiting from synergism of known or novel compounds possessing inhibitory properties of various levels of potency, and reviving "forgotten" antibiotics families (such as the lankacidin) (Auerbach et al., 2010). Furthermore, most eubacteria belonging to specific families utilize similar structural principles for selectivity and resistance, comprehending the factors allowing for selectivity should provide powerful tools to understand many of the mechanisms exploited for acquiring resistance. Therefore, the lessons learned from ribosome crystallography concerning combating resistance to antibiotics targeting the ribosome, are rather optimistic, as these studies have opened new paths for antibiotics improvement.

There are many revelations pertinent to antibiotic drug development that high resolution structures have provided. Firstly there is significant variability (even between drugs of the same family) of their binding modes. Secondly, there are common as well as species unique nucleobases that confer resistance. Thirdly, remote interactions in rRNA are responsible for certain induced fit binding, enabling species discrimination (even in highly conserved regions of rRNA). Combined with the identification of deleterious mutations in rRNA, there is considerable scope to exploit this knowledge for structural improvement of existing compounds as well as yielding exciting prospects for de novo drug design.

Table 1: Ribosome composition and available crystal structures

Prokaryotic ribosome: Sedimentation coefficient: 70S
small subunit: 30S
One rRNA molecule (16S with ~1600 nucleotides)
~ 20 different proteins, called S1-S21

large subunit: 50S
Two rRNA molecules (5S and 23S, with 120 and 2900 nucleotides, respectively)
~ 30 different proteins, called L1-L31, among which only L7/L12 is present in more than a single copy

Eukaryotic ribosomes: Sedimentation coefficient: 80S
small subunit: 40S
One rRNA molecule (18S with 1,900 nucleotides)
~ 30 different proteins, called S1-S33

large subunit: 60S
Three rRNA molecules (5S, 5.8S and 28S, with ~120, 160 and 4,700 nucleotides, respectively)
~ 50 different proteins, called L1-L50

Acknowledgments

Thanks are due to all members of the ribosome group at the Weizmann Institute for their experimental efforts and illuminating discussion. Support was provided by the US National Inst. of Health (GM34360), and the Kimmelman Center for Macromolecular Assemblies. CD is supported by the Adams Fellowship Program of the Israel Academy of Sciences and Humanities. AY holds the Martin and Helen Kimmel Professorial Chair.

The currently available crystal structures are of native and complexed ribosomal subunits from two eubacteria and one archaeon with substrate-analogs or inhibitors (including antibiotics). These are the small ribosomal subunit from *Thermus thermophilus* (called T30S), the large subunit from *Deinococcus radiodurans* (called D50S), and the large ribosomal subunit of the archaeon *Haloarcula marismortui* (called H50S). Also available are structures of assembled ribosome complexed with their substrates from *Thermus thermophilus* (called T70S) and of empty ribosomes from *E. coli* (called E70S).

REFERENCES

Auerbach, T., Bashan, A., and Yonath, A. (2004). Ribosomal antibiotics: structural basis for resistance, synergism and selectivity. Trends Biotechnol 22, 570-576.

Yonath, A., and Bashan, A. (2004). Ribosomal Crystallography: Initiation, Peptide Bond Formation, and Amino Acid Polymerization are Hampered by Antibiotics. Annu Rev Microbiol 58, 233-251.

Yonath, A. (2005a). Antibiotics targeting ribosomes: resistance, selectivity, synergism, and cellular regulation. Annu Rev Biochem 74, 649-679.

Tenson, T., and Mankin, A. (2006). Antibiotics and the ribosome. Mol Microbiol 59, 1664-1677.

Poehlsgaard, J., and Douthwaite, S. (2005). The bacterial ribosome as a target for antibiotics. Nat Rev Microbiol 3, 870-881.

Bottger, E. C. (2006). The ribosome as a drug target. Trends Biotechnol 24, 145-147.

Bottger, E. C. (2007). Antimicrobial agents targeting the ribosome: the issue of selectivity and toxicity - lessons to be learned. Cell Mol Life Sci 64, 791-795.

Mankin, A. S. (2001). Ribosomal antibiotics. Mol Biologia 35, 509-520.

Mankin, A. S. (2006). Nascent peptide in the "birth canal" of the ribosome. Trends Biochem Sci 31, 11-13.

Mankin, A. S. (2008). Macrolide myths. Curr Opin Microbiol 11, 414-421.

Schluenzen, F., Zarivach, R., Harms, J., Bashan, A., Tocilj, A., Albrecht, R., Yonath, A., and Franceschi, F. (2001). Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. Nature 413, 814-821.

Schluenzen, F., Pyetan, E., Fucini, P., Yonath, A., and Harms, J. (2004). Inhibition of peptide bond formation by pleuromutilins: the structure of the 50S ribosomal subunit from Deinococcus radiodurans in complex with tiamulin. Mol Microbiol 54, 1287-1294.

Berisio, R., Schluenzen, F., Harms, J., Bashan, A., Auerbach, T., Baram, D., and Yonath, A. (2003a). Structural insight into the role of the ribosomal tunnel in cellular regulation. Nat Struct Biol 10, 366-370.

Berisio, R., Harms, J., Schluenzen, F., Zarivach, R., Hansen, H. A., Fucini, P., and Yonath, A. (2003b). Structural insight into the antibiotic action of telithromycin against resistant mutants. J Bacteriol 185, 4276-4279.

Harms, J., Schluenzen, F., Fucini, P., Bartels, H., and Yonath, A. (2004). Alterations at the peptidyl transferase centre of the ribosome induced by the synergistic action of the streptogramins dalfopristin and quinupristin. BMC Biol 2, 4;1-10.

Pyetan, E., Baram, D., Auerbach-Nevo, T., and Yonath, A. (2007). Chemical parameters influencing fine-tuning in the binding of macrolide antibiotics to the ribosomal tunnel. Pure Appl Chem 79, 955-968.

Davidovich, C., Bashan, A., Auerbach-Nevo, T., Yaggie, R. D., Gontarek, R. R., and Yonath, A. (2007). Induced-fit tightens pleuromutilins binding to ribosomes and remote interactions enable their selectivity. Proc Natl Acad Sci U S A 104, 4291-4296.

Davidovich, C., Bashan A., and Yonath, A., (2008) Structural basis for cross resistance to ribosomal PTC antibiotics, Proc Natl Acad Sci U S A, 105, 20665-20670.

Vazquez-Laslop, N., Thum, C., and Mankin, A. S. (2008). Molecular mechanism of drug-dependent ribosome stalling. Mol Cell 30, 190-202.

Auerbach, T., Mermershtain, I, Davidovich, C., Bashan A., Belousoff M., Wekselman I., Zimmerman, E., Xiong L., Arakawa K., Kinashi H., Mankin A.S., and Yonath A., Proc Natl Acad Sci U S A 2010, 11.

Auerbach T., Mermershtain I., Bashan A., Davidovich C., Rozenberg H., Sherman D.H., and Yonath A. , Biotechnolog, 84, 24-35 (2009).

Lai, C. J., and Weisblum, B. (1971). Altered methylation of ribosomal RNA in an erythromycin-resistant strain of Staphylococcus aureus. Proc Natl Acad Sci U S A 68, 856-860.

Hansen, J. L., Ippolito, J. A., Ban, N., Nissen, P., Moore, P. B., and Steitz, T. A. (2002a). The structures of four macrolide antibiotics bound to the large ribosomal subunit. Mol Cell 10, 117-128.

Hansen, J. L., Moore, P. B., and Steitz, T. A. (2003). Structures of five antibiotics bound at the peptidyl transferase center of the large ribosomal subunit. J Mol Biol 330, 1061-1075.

Tu, D., Blaha, G., Moore, P. B., and Steitz, T. A. (2005). Structures of MLSBK Antibiotics Bound to Mutated Large Ribosomal Subunits Provide a Structural Explanation for Resistance. Cell 121, 257-270.

Bommakanti, A. S., Lindahl, L., and Zengel, J. M. (2008). Mutation from guanine to adenine in 25S rRNA at the position equivalent to E. coli A2058 does not confer erythromycin sensitivity in Sacchromyces cerevisae. RNA 14, 460-464.

Pfister, P., Corti, N., Hobbie, S., Bruell, C., Zarivach, R., Yonath, A., and Bottger, E. C. (2005). 23S rRNA base pair 2057-2611 determines ketolide susceptibility and fitness cost of the macrolide resistance mutation 2058A->G. Proc Natl Acad Sci U S A 102, 5180-5185.

Gregory, S. T., and Dahlberg, A. E. (1999). Erythromycin resistance mutations in ribosomal proteins L22 and L4 perturb the higher order structure of 23 S ribosomal RNA. J Mol Biol 289, 827-834.

Davydova, N., Streltsov, V., Wilce, M., Liljas, A., and Garber, M. (2002). L22 ribosomal protein and effect of its mutation on ribosome resistance to erythromycin. J Mol Biol 322, 635-644.

Lawrence, M., Lindahl, L., and Zengel, J. M. (2008). Effects on translation pausing of alterations in protein and RNA components of the ribosome exit tunnel. J Bacteriol 190, 5862-5869.

Berisio, R., Corti, N., Pfister, P., Yonath, A., and Bottger, E. C. (2006). 23S rRNA 2058A->G Alteration Mediates Ketolide Resistance in Combination with Deletion in L22. Antimicrob Agents Chemother 50, 3816-3823.

Zaman, S., Fitzpatrick, M., Lindahl, L., and Zengel, J. (2007). Novel mutations in ribosomal proteins L4 and L22 that confer erythromycin resistance in Escherichia coli. Mol Microbiol 66, 1039-1050.

Moore, S. D., and Sauer, R. T. (2008). Revisiting the mechanism of macrolide-antibiotic resistance mediated by ribosomal protein L22. Proc Natl Acad Sci U S A 105, 18261-18266.

Ippolito, J. A., Kanyo, Z. F., Wang, D., Franceschi, F. J., Moore, P. B., Steitz, T. A., and Duffy, E. M. (2008). Crystal Structure of the Oxazolidinone Antibiotic Linezolid Bound to the 50S Ribosomal Subunit. J Med Chem 51, 3353-3356.

Schluenzen, F., Zarivach, R., Harms, J., Bashan, A., Tocilj, A., Albrecht, R., Yonath, A., and Franceschi, F. (2001). Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. Nature 413, 814-821.

Lolk, L., Pohlsgaard, J., Jepsen, A. S., Hansen, L. H., Nielsen, H., Steffansen, S. I., Sparving, L., Nielsen, A. B., Vester, B., and Nielsen, P. (2008). A Click Chemistry Approach to Pleuromutilin Conjugates with Nucleosides or Acyclic Nucleoside Derivatives and Their Binding to the Bacterial Ribosome. J Med Chem 51, 4957-4967.

Yassin, A., Fredrick, K., and Mankin, A. S. (2005). Deleterious mutations in small subunit ribosomal RNA identify functional sites and potential targets for antibiotics. Proc Natl Acad Sci U S A 102, 16620-16625.

Wilson, D. N., Harms, J. M., Nierhaus, K. H., Schlunzen, F., and Fucini, P. (2005a). Species-specific antibiotic-ribosome interactions: implications for drug development. Biol Chem 386, 1239-1252.

Gürel, G., Blaha, G., Moore, P. B., Steitz, T. A. (2009) U2504 determines the species specificity of the A-site cleft antibiotics: the structures of tiamulin, homoharringtonine, and bruceantin bound to the ribosome. J Mol Biol. 29;389(1):146-56.