General introduction

1. The ribosome

During protein synthesis the ribosome translates the genetic information encoded in the messenger RNA (mRNA) into the amino acid sequence of a protein. The ribosome, an ancient ribonucleo-protein particle whose structural core and fundamental mechanism of action are conserved among all forms of life (Hill 1990) consists of two subunits. Based on their sedimentation behavior in sucrose gradients, the prokaryotic subunits were termed 30S and 50S, which associate to form the complete 70S ribosome. Each subunit consists of about two thirds (mass ratio) RNA and one-third protein, and has 3 different tRNA binding sites: A (for aminoacyl), which is important for accepting the incoming amionoacylated tRNA; P (for peptidyl), which binds the tRNA with the nascent polypeptide chain; and E (for exit), which mediates the release of the deacylated tRNA after peptidyl bond formation. The small 30S subunit consists of the 16S rRNA (1542 nucleotides) and 21 proteins (S1 - S21), whereas the large 50S subunit is composed of the 23S rRNA (2904 nucleotides), the 5S rRNA (120 nucleotides) and 33 proteins (L1 - L33).

The large subunit can easily be recognized by its crown-shape (**Figure 1A**) formed by the L1 arm, the central protuberance (CP) harboring the 5 S rRNA, and the L7/L12 stalk. The small subunit (**Figure 1B**) is marked by six distinct structural features that are described as head, beak, shoulder, body,

platform and spur.

2. The cycle of protein synthesis

The process of protein biosynthesis can be divided into four functionally distinct steps: initiation, elongation, termination and recycling that are outlined in **Figure 2** and will each be described in the following paragraphs for prokaryotes in great detail.

2.1 Initiation

In prokaryotes three initiation factors, IF1, -2 and -3 are required to ensure the formation of the initiator complex. The initiator complex consists of the 30S subunit bound to mRNA, and the initiator tRNA which codes for the amino-acid formyl Methionine (fMet $tRNA^{fMet}$), assembled at the start codon (AUG) of the mRNA which is located at the P site of the small subunit (**Figure 2, step 1**) (Gualerzi and Pon 1990), (Brock, Szkaradkiewicz et al. 1998), (Gualerzi, Brandi et al. 2000).

Formation of the initiator complex requires prior dissociation of the 30S subunit from the 50S subunit, which is mediated by IF3 (Grunberg-Manago, Dessen et al. 1975). Upon dissociation of the ribosomal subunits, IF3 binds to the small 30S subunit, thereby inhibiting re-association of the large subunit. Subsequently, IF2-GTP (Guanosine-5'-triphosphate) brings along fMet $tRNA^{fMet}$ to the start codon at the P site. IF2 and IF3 cooperate in ensuring that only the initiator tRNA binds to the ribosomal P site (Canonaco, Calogero et al. 1986),

(Wu and RajBhandary 1997) and that it specifically interacts with the start codon AUG (Meinnel, Sacerdot et al. 1999).

It has been postulated that IF1 could assist IF3 in dissociating the two subunits (Grunberg-Manago, Dessen et al. 1975), (Dottavio-Martin, Suttle et al. 1979) and that the factor blocks tRNA binding to the A site (Brock, Szkaradkiewicz et al. 1998), (Moazed, Samaha et al. 1995). The crystal structure by Carter et al. (Carter, Clemons et al. 2001) revealed that IF1 actually binds to the ribosomal A site in line with the idea that it may inhibit tRNA binding to this site. Following dissociation of IF3 and IF1 from the initiator complex, the 50S subunit can bind to the 30S thus triggering hydrolysis of the GTP bound to IF2 which results in the release of the factor from the ribosome. The 70S ribosome carrying the initiator tRNA at its P site (**Figure 2, step 2**) is now ready to enter the elongation cycle.

2.2 Elongation

During the course of one elongation cycle (**Figure 2, step 3 - 9**) the nascent polypeptide chain is elongated by one amino acid. The cycle starts with the binding of elongation factor–Tu (EF-Tu), which in complex with GTP, brings an aminoacyl - tRNA to the A site in a process called decoding (**Figure 2, step 4 and 9**). Correct codon - anticodon interaction triggers GTP hydrolysis by EF-Tu (Rodnina, Pape et al. 1996), inducing a conformational change (Berchtold, Reshetnikova et al. 1993), (Kjeldgaard, Nissen et al. 1993) that leads to the dissociation of EF-Tu from the ribosome (**Figure 2, step 5**). After peptide bond

formation in the PTC (peptidyl tranferase center) of the 50S subunit (Kaziro 1978), the newly formed peptidyl – tRNA is translocated from the A- to the P site and the deacylated tRNA is translocated from the P- to the E site. This process is accompanied by the movement of the mRNA by one codon (**Figure 2, step 6 - 7**). Translocation is catalyzed by elongation factor–G (EF-G) in complex with GTP. The factor binds to the ribosomal A site and hydrolyzes GTP during translocation and prior to its dissociation from the ribosome (**Figure 2, step 8**) (Ishitsuka, Kuriki et al. 1970), (Rodnina, Savelsbergh et al. 1997). The pre-translocation (PRE) (**Figure 2, step 6**) and post - translocation (POST) (**Figure 2, step 7**) states constitute the two main conformational and functional states of the ribosome within the elongation cycle. In the PRE-state, the A- and P site are occupied, whereas EF-G-dependent translocation results in occupation of the P- and E sites in the POST-state. Elongation proceeds until a stop codon appears at the A site that triggers the termination process of protein biosynthesis.

2.3 Termination

When a stop codon on the mRNA is reached, it is recognized by the release factors. There are 2 classes of release factors (RF) involved in termination. Class I release factors, composed of RF1 and RF2, bind to the stop codon at the A site and catalyze polypeptide release from the P site ((Scolnick, Tompkins et al. 1968) and reviewed in (Poole and Tate 2000)). Both factors are capable of recognizing the stop codon UAA. In addition, RF1 binds to the codon UAG, whereas RF2 recognizes the codon UGA (**Figure 2 -10**). RF3, the only

member of the class II release factors, is a G-protein that hydrolyses GTP leading to the dissociation of the class I RF (Pavlov, Freistroffer et al. 1997), (Arkov, Freistroffer et al. 1998), (Grentzmann, Kelly et al. 1998). A post - termination complex is left, consisting of the 70S ribosome, bound mRNA, and a deacylated tRNA at the P site.

1.4 Recycling

In concert with EF-G the Ribosome Recycling Factor (RRF) disassembles the post - termination complex. Together they dissociate the ribosomal subunits and remove the mRNA and the deacylated tRNA from the 30S subunit (Hirashima and Kaji 1972), (Ogawa and Kaji 1975), (Karimi, Pavlov et al. 1999), (Janosi, Hara et al. 1996), (Kaji, Kiel et al. 2001) (**Figure 2, step 11**). The detailed mechanism for this process remains unclear though different models have been proposed.

Due to RRF's overall shape, resembling that of a tRNA, a structural and functional mimicry between the two molecules has been suggested (Selmer, Al-Karadaghi et al. 1999). In this model, RRF would bind to the A site from which it would be translocated with the help of EF-G / GTP to the P site, resulting in release of the deacylated tRNA from the E site and the dissociation of the subunits. Although structural results (Lancaster, Kiel et al. 2002), (Agrawal, Sharma et al. 2004) contradict the "molecular mimicry" hypothesis by showing that RRF's binding position is nearly orthogonal to that one of a tRNA, there is

still evidence that the recycling factor dissociates from the 70S ribosome after translocation by EF-G (Kiel, Raj et al. 2003).

3. Monosomes & polysomes

Throughout the translation cycle the ribosome is associated with about 40 nucleotides (nt) of the mRNA template (which usually exceeds 300 nt) as it moves forward the termination codon in a 5' to 3' direction. Multiple ribosomes can translate along one mRNA molecule, which is often found to be polycistronic in prokaryotes, thus forming the so-called polyribosome or polysome. Polysomes were found to be a form of actively translating ribosomes both in prokayotes and eukaryotes, explaining the high amount of ribosomal RNA (~80%) compared to mRNA (~5%) in the cell (taking out of (Spirin 1999)). **Figure 3A and 3B** show a schematic and electron microscopy image of polysomes. Next to polysomes there exists a pool of single 70S ribosomes that do not take part in the translation pool and are called monosomes.

Ribosomal subunits (30S and 50S), monosomes (70S), disomes (100S, consisting of two 70S molecules) and polysomes can be separated on sucrose gradients as shown in **Figure 3C**.

4. Importance of metal ions in ribosome structure and function

In general, monovalent cations and magnesium ions are essential for the

folding and stability of nucleic acid (Pyle 2002). A significant structural insight into how metal ions stabilize the RNA's tertiary structure was provided by the x-ray structure of the P4-P6 domain of the group I intron (Cate, Gooding et al. 1996), revealing that Mg^{2+} binds mainly in the major groove of RNA helices (Cate and Doudna 1996).

It is well known that the activity of ribosomes in inhibited by the depletion of certain cations, especially NH_4^+ and Mg^{2+}. Removal of magnesium ions resulted in the dissociation of the two subunits (Spirin, Kisselev et al. 1963), which was found to be reversible by a study from R.F. Gesteland (Gesteland 1966). A study by A. Zamir (Zamir, Miskin et al.) revealed that the 30S subunit is inactivated by the depletion of monovalent cations, especially NH_4^+, in the presence of high Mg2+ concentration or by the reduction of Mg^{2+} to about 1mM in the presence of appropriate monovalent cations. The 50S subunit can also be inactivated by the depletion of monovalent cations. These studies suggest an important role for cations in maintaining the structural integrity of the ribosome.

5. Structural studies on the ribosome

5.1 The quest for the atomic resolution structures of the ribosome

For almost 20 years people have been trying to solve the crystal-structures of the ribosomal subunits as well as the whole 70S ribosome, which has a mass of over 2.5 million Daltons. In the early 1980s the first crystals were

reported for the 50S and for the 30S subunits from *Bacillis stearothermophilus* (Yonath, Mussig et al. 1980) and the entire 70S ribosome from *Thermus thermophilus* (*T. thermophilus*) (Wittmann, Mussig et al. 1982). Due to technical limitations, however, it was impossible at that time to solve a crystal structure of a molecule with the size of the ribosome. Improvements in area detectors, synchrotron light sources, computers and crystallographic software as well as developments in cryo-cooling and data collection at low temperatures that increased the "in-beam-lifetime" (Hope, Frolow et al. 1989) made it possible to solve these structures almost 20 years later (Ban, Freeborn et al. 1998).

In 1998 the first crystal structure of a ribosomal subunit was solved, the 50S subunit of *Haloarcula marismortuii* (*H. marismortuii*) with a resolution of 9Å (Ban, Freeborn et al. 1998). 1999 was a very exciting year for the ribosome field, Ramakrishnan and co-workers solved the structure of the 30S subunit from *T. thermophilus* to 5.5Å (Clemons, May et al. 1999) and this was followed by a 4.5Å resolution electron density map of the same subunit by the group of A. Yonath (Tocilj, Schluenzen et al. 1999). Within the same year, the structure of the 50S subunit from *H. marismortuii* was improved to 5.5Å by the Steitz group (Ban, Nissen et al. 1999).

Also in 1999, the first crystal structure of the entire 70S ribosome, that of *T. thermophilus* in complex with mRNA and three tRNAs was published by the group of Harry Noller to a resolution of 7.8Å (Cate, Yusupov et al. 1999). These structures, especially the one of the whole 70S ribosome, showed that it was possible to solve structures of such enormous molecules. This observation also

provided evidence for the possibility of solving atomic resolution structures of the ribosome and its subunits in the near future. Already in 2000 the “dream” was fulfilled with a marvelous atomic resolution structure of the 50S subunit of *H. marismortuii* to 2.4Å resolution (Ban, Nissen et al. 2000) and two atomic resolutions structures of the *T. thermophilus* 30S subunit to 3Å (Wimberly, Brodersen et al. 2000) and 3.3Å (Schluenzen, Tocilj et al. 2000).
In 2001 the electron density map of the entire 70S ribosome complexed with mRNA and three tRNAs was improved to 5.5Å resolution (Yusupov, Yusupova et al. 2001).

Unfortunately, until these days we are still missing an atomic resolution structure of the whole 70S ribosome.

After these, a number of x-ray structures of the 30S, the 50S and the 70S ribosome have been solved within the last 6 years as well as a number of cryo-EM structures of the entire 70S ribosome in complex with EF-G and EF-Tu. All of these structures together with over four decades of biochemical work, provide already a tremendous amount of information of the mechanism of protein biosynthesis.

The following paragraphs summarize the most important findings of these structures.

5.2 X-ray structures of the two ribosomal subunits

The structure of the two ribosomal subunits revealed a few generalities between the two (reviewed in (Ramakrishnan and Moore 2001)). The inter-subunit space of both subunits, in particular the tRNA / mRNA binding regions are made out of RNA and are basically free of protein. In both cases, ribosomal proteins comprise about one third of either subunit and consist of a globular domain placed at the solvent site and long extended tails that fill the gaps within the RNA and stabilize the tertiary structure of the rRNA. The other two thirds of the mass is composed of RNA and determines the shape of both subunits. Surprisingly, RNA secondary structure motifs similar to those found in ribosomal RNA had previously been found in smaller RNA structures. For both subunits, the tertiary structure of the rRNA consists of helical elements that are connected by loops. These interactions are mainly stabilized by minor-groove to minor-groove packing (Cate, Gooding et al. 1996), phosphate backbone and minor-groove interaction, as well as interaction between the minor-groove and adenines (A minor motif (Nissen, Ippolito et al. 2001)).

As opposed to the 30S subunit, where each of the four large secondary structure domains of the 16S rRNA form a morphologically distinct component of the subunit, the 23S rRNA is spread out intricately to form a gigantic 50S structure (see **Figure 4A** for 16S rRNA and **Figure 4B** for 23S rRNA (Ramakrishnan and Moore 2001)).

5.2.1 Atomic resolution structures of the 30S subunit

Several atomic resolution structures of the *T. thermophilus* 30S subunit, all solved at resolutions between 3.0 and 3.3 Å (Wimberly, Brodersen et al. 2000), (Schluenzen, Tocilj et al. 2000), (Ogle, Brodersen et al. 2001), (Carter, Jr et al. 2000), (Ogle, Murphy et al. 2002), (Murphy, Ramakrishnan et al. 2004) shed light on the main function of the 30S subunit, the interaction between the tRNA and mRNA during decoding and the mechanism that ensures the fidelity of translation.

The 3.1 Å structure of the 30S subunit with an A site codon and a tRNA analogue (anticodon stem loop) (Ogle, Brodersen et al. 2001), that was soaked into the crystals, revealed conformational changes occurring within the subunit upon tRNA binding, in which three bases of the 16S rRNA (A1492, A1493, G530) interact with the minor groove of the first two codon - anticodon base-pair. A year later the same group observed that the 30S subunit exists in an “open” and a “closed” conformation (Ogle, Murphy et al. 2002). In the closed form, occurring in the presence of a cognate tRNA, in the presence of a near-cognate tRNA, or when bound to pactamycin (an antibiotic known to induce errors within the decoding site), the head and body were found to enclose the A site tRNA analog. These findings revolutionized the understanding on how the ribosome is able to discriminate between cognate, near-cognate and non-cognate tRNA (Ogle, Carter et al. 2003).

The 30S structure has been also solved in complex with IF1 (Carter, Clemons et al. 2001) to 3.2 Å resolution. This structure the initiation factor binds

to the A site of the 30S lead and leads to global alterations in the conformation of the small subunit.

In addition, several structures of the 30S subunit in complex with antibiotics such as tetracycline, pactamycin, hygromycin B (Brodersen, Clemons et al. 2000), edeine and tetracycline (Pioletti, Schlunzen et al. 2001)) provided a clear picture of how these drugs bind to the subunit and exert their effects on ribosome function.

5.2.2 Atomic resolution structure of the 50S subunit

As in the case of the 30S subunit**,** a number of structures for the 50S subunit were solved within the last few years. These include three structures of the empty 50S subunit (Ban, Nissen et al. 2000), (Bashan, Agmon et al. 2001), (Harms, Schluenzen et al. 2001), several atomic resolution structures of the 50S in complex with antibiotics (Schluenzen, Zarivach et al. 2001), (Schluenzen, Harms et al. 2003), (Berisio, Harms et al. 2003) and low molecular weight substrates (Nissen, Hansen et al. 2000) have been reported.

The high resolution of the 2.4Å structure from *H. marismortui* (Ban, Nissen et al. 2000), (Klein, Moore et al. 2004) made it possible to observe water molecules, metal ions, as well as any base modification with certainty in the electron density. The structure contained most of the 50S subunit, including the central protuberance (CP), the peptidyl-transferase center, as well as the exit tunnel. Unfortunately, regions like the L1 stalk, the L11 region and the L7/L12

stalk, all of which are functionally important regions for the binding of several translation factors and for translocation, appear to be disordered in the structure. However these features were visible in the 3.6 Å *H. marismortui 50S* structure from A. Yonath group (Harms, Schluenzen et al. 2001).

The most significant result yielded by these structures of the 50S subunit was the finding that the peptidyl-transferase center (PTC) consists entirely of rRNA, revealing that the ribosome is indeed a ribozyme. This had already been suspected after the discovery of catalytic RNAs (Kruger, Grabowski et al. 1982), (Guerrier-Takada, Gardiner et al. 1983). In addition it had been supported by biochemical evidence obtained in the early 1980s, which had suggested that nucleotides in the center of domain V of 23S rRNA (U2584 and U2585 in *E.coli*) were involved in the peptidyl transferase activity (Barta, Steiner et al. 1984; Klein, Forman et al. 1984). Yet, the crystal structures gave the first conclusive prove that RNA was indeed at the center of the catalytic site.

From the 3.0 - 3.2 Å 50S structures in complex with peptidyl-transferase substrates (Nissen, Hansen et al. 2000), a model for the peptidyl-transfer was proposed, in which a single adenine base, A2451 of 23S rRNA, serves as a general acid-base catalyst to facilitate peptide transfer. Although this model was disproved later on by chemical probing and mutagenesis analyses (Bayfield, Thompson et al. 2004), it was the first attempt to describe the mechanism of peptide bond formation in atomic detail.

In addition the 50S structure revealed more details about the polypeptide exit tunnel that emanates right below the petidyl transferase center and ends at

the back of the subunit (Nissen, Hansen et al. 2000). The length of the tunnel is about 100Å with a diameter varying between 10 – 20 Å, suggesting that protein folding beyond formation of _-helices within the ribosome seem unlikely. The structure also suggested an answer to the question why no proteins would stick to the tunnel wall, and in fact proposed that the polypeptide chain would be passively sliding through the ribosome (Nissen, Hansen et al. 2000).

5.3 X-ray structures of the 70S ribosome

In addition to the atomic resolution structures of the ribosomal subunits a total of three 70S ribosome structures from *T. thermophilus* in complex with tRNAs, tRNA analoges (anticodon stem loops) and mRNA were solved up to a resolution of 5.5Å (Cate, Yusupov et al. 1999), (Yusupov, Yusupova et al. 2001), (Yusupova, Yusupov et al. 2001). In addition, two *E. coli* 70S ribosome structures were determined a wild type ribosome solved to 9.5Å and a hyper-accurate mutant solved to 8.7 Å (Vila-Sanjurjo, Ridgeway et al. 2003).

The *T. thermophilus* structures showed conformational changes when compared to the atomic resolution structures of the subunits, probably due to subunit association. For example, important features of the 23S rRNA, like the L1 arm and the L11 RNAs, disordered in the atomic resolution of the 50S subunit alone, were visible in the 70S complex. In addition, molecular components involved in the inter-subunit bridges could be identified in greatest detail so far (Yusupov, Yusupova et al. 2001). Moreover, the path of mRNA through the ribosome could be seen for the first time at a resolution of 7Å (Yusupova,

Yusupov et al. 2001). Interestingly, the crystal packing in this structure revealed a possible way for how multiple ribosomes might interact with one mRNA molecule in polysomes. By placing the E site of one ribosome directly adjacent to the A site of the next ribosome within the crystal lattice.

The 70S structures obtained in our laboratory from *E. coli* ribosomes (Vila-Sanjurjo, Ridgeway et al. 2003) showed that it is possible to crystallize ribosomes from this species that was biochemically and genetically best characterized. In addition, the structures revealed that mRNA decoding was primarily coupled to the movement of the 30S body and did not involve conformational changes at an rRNA helical switch near the decoding center of the small subunit as had been proposed (Lodmell and Dahlberg 1997). One of the structures solved by our group was that of a hyper-accurate ribosome carrying a single mutation in protein S12, known to affect the fidelity of translation. This structure showed that the maintenance of the genetic code does not involve conformational changes at an rRNA helical switch near the decoding center of the small subunit as had been proposed (Vila-Sanjurjo, Ridgeway et al. 2003).

5.4 Cryo-Electron Microscopy (EM) structures of ribosomal complexes

Most of the cryo-EM studies were performed on complexes of *E. coli* 70S ribosome with EF-G (Stark, Orlova et al. 1997), (Agrawal, Penczek et al. 1998), (Agrawal, Heagle et al. 1999), (Stark, Rodnina et al. 2000), (Frank and Agrawal 2000), (Valle, Zavialov et al. 2003), and EF-Tu (Stark, Rodnina et al. 1997), (Stark, Rodnina et al. 2000), (Stark, Rodnina et al. 2002), (Valle, Zavialov et al.

2003). Although the resolution of these structures ranges from 15A to near 8A they have enlightened us enormously on the process of translocation and decoding. Cryo-EM is so far the only method that allows the study of the most elusive ribosomal complexes.

5.4.1 Translocation as seen by cryo-EM

With the help of antibiotic fusidic acid as well as non - cleavable GTP analogs Frank and co-workers were able to take snapshots of pre - and post - translocational complexes of ribosomes and EF-G at 10Å (Valle, Zavialov et al. 2003). Significant conformational changes involving both the elongation factor and the 70S ribosome were revealed. The binding of EF-G to the pre-translocational ribosome leads to a ratchet-like movement of the 30S with respect to the 50S in the direction of mRNA movement. The rotation is reversed after translocation of the tRNAs has occurred.

The ratchet-like model will be described in further detail in the introduction part of chapter II.

5.4.2 Decoding as seen by cryo-EM

As a result of 9 Å cryo-EM study of the interaction of the EF-Tu/GTP/aatRNA ternary complex a model for the incorporation of aminoacyl-tRNA into the ribosome was proposed (Valle, Zavialov et al. 2003). In this model, the delivery of the aminoacyl-tRNA by EF-Tu to the A site, is coupled to a

conformational change of the GTP-activating center within the 50S which, in turn, triggers GTP hydrolysis on the factor that leads to its dissociation from the ribosome.

6. Antibiotics and the ribosome

Of particular interest are the effects of antibiotics on the prokaryotic ribosome. The ribosome is, in fact, a major target for a variety of antibiotics, combating such diseases as tuberculosis and hospital acquired infections, but little is known about the specific binding sites of these antibiotics on the intact 70S ribosome. However as resistance in bacteria to known antibiotics spreads, there is a desperate need for new ways to combat infections. Many cases of antibiotic resistance in clinical strains for example can be linked to alterations of specific nucleotides of the 23S rRNA of the 50S subunit within the peptidyl transferase center (Vester 2001).

In addition to the medical relevance resulting from studies of antibiotics bound to the ribosome, antibiotics have also been heavily used in order to understand the mechanism of protein synthesis. In addition to the antibiotics that have been seen in the recent crystal structures (summarized under “5. Structural studies on the ribosome” in this thesis), which were also used in biochemical assays, here are a few more examples of antibiotics that have been used. Streptomycin leads to misreading of the mRNA, which revealed that the ribosome is capable in maintaining the fidelity of the genetic code (Davies, Gilbert et al. 1964). Another example is the use of colicin E3 that is leading to cleavage of the 16S rRNA, resulting in complete inactivation of the 30S subunit

(Konisky and Nomura 1967), (Bowman, Dahlberg et al. 1971). This served as one evidence that RNA and not protein is the effector of ribosome function.

Although a large number of atomic resolution structures of the ribosomal subunits in complex with different antibiotics have been solved (Carter, Jr et al. 2000), (Brodersen, Clemons et al. 2000), (Hansen, Ippolito et al. 2002), (Schluenzen, Zarivach et al. 2001), (Berisio, Harms et al. 2003), (Pioletti, Schlunzen et al. 2001), as mentioned briefly in the previous paragraph, the interaction of a drug with the complete 70S ribosome would physiological be more relevant.

Atomic resolution structures of the intact *E. coli* 70S ribosome alone and in complex with antibiotics will serve as a model to study and design new drugs in the challenge against bacterial resistance.

7. The ribosome during cold shock

Adaptation to environmental stress, such as changes in temperature, is the assumption for all living organisms to survive. An increase in temperature results in the expression of a subset of proteins, the so called heat-shock proteins that are highly conserved from bacteria to mammals (Craig 1985). In contrast, a conserved set of cold shock proteins in all forms of life has not been identified until now. However, most organisms share a common cold-shock response (Cavicchioli, Thomas et al. 2000), (Fujita 1999), (Graumann and Marahiel 1994), (Guy 1999), (Yamanaka 1999), including alterations in membrane fluidity (Klein, Weber et al. 1999), (Weber, Klein et al. 2001), metabolism (Jones, VanBogelen et al. 1987), (Graumann, Schroeder et al. 1996)

and most importantly changes in the ribosome (Jones and Inouye 1996), (Jones, Mitta et al. 1996). A temperature downshift results in the accumulation of 70S monosomes and a decrease in polysomes during the acclimation phase (Broeze, Solomon et al. 1978) accompanied with an increased expression of cold shock proteins (CSP) whereas the expression of non-cold shock proteins (non-CSP) is blocked. After cold adaptation ribosomes resume protein synthesis of non-CSP proteins resulting in the recovery of polysomes (Jones and Inouye 1996).

In bacteria, a conserved set of about 25 CSPs is expressed in the cold due to increased stability of their mRNAs (Goldenberg, Azar et al. 1997), (Brandi, Pietroni et al. 1996). Three control elements for post-transcriptional regulation could be identified so far. First, the cold shock mRNAs are preferably translated due to cis-acting elements located in their 5' UTR (untranslated region). These elements, which are most likely secondary or tertiary structure motifs rather than instead of primary sequence motifs, were found to be essential at least in the case of the major cold shock protein CspA (Giuliodori, Brandi et al. 2004).

The second control element for the post-transcriptional regulation of CSPs is an increased expression of trans-acting elements, such as CspA. CspA functions as a RNA chaperone most likely preventing the formation of secondary structures in mRNA for efficient translation (Jiang, Hou et al. 1997).

The third and probably most important control element is the increased expression of IF1, -2 and –3. Since the relative stochiometry of the factors is not altered during cold shock, the net result is an imbalance of the initiation factor/ribosome ratio (Giuliodori, Brandi et al. 2004). IF 3 is the most important trans-acting element because of its capability to select in favor of cold-shock mRNAs in the cold. In fact, non cold-shock mRNAs are insensitive to IF3 (Giuliodori, Brandi et al. 2004).

Thesis overview

This thesis is divided into two main chapters:

In chapter I ("Structural basis for the control of translation initiation during stress") a new pathway of translation regulation during cold shock is discussed. Structural and biochemical assays characterized the function of Protein Y, an *E .coli* stress response protein that is required for on the adaptation of ribosomes during cold shock (Vila-Sanjurjo, Schuwirth et al. 2004). This chapter also includes work done by a former post-doc in the laboratory Antón Vila-Sanjurjo, as stated in the different sections.

Chapter II ("Towards atomic resolution structures of the *E. coli* ribosome") summarizes recent results obtained with a new crystal form of the complete *E. coli* 70S ribosome that diffracts up to atomic resolution. Within the asymmetric unit, two ribosomes were observed in two different conformations that may represent the ribosomal movement during translocation.

Additionally, the antibiotic kasugamcyin and the ribosome recycling factor (RRF) have been soaked into this new crystal form and datasets were collected to resolutions of 3.5Å and 4.5Å, respectively.

The 3.5 Å structure can be seen as an assumption for x-ray structures of ribosomal complexes that will ultimately lead to a complete movie of the translation cycle at atomic resolution.

Figures

Figure 1: General features of the ribosomal subunits

The RNA of the subunits is shown in magenta and proteins are shown in green.

A The large 50S subunit viewed from the subunit – interface reveals a crown - shape that consists of the L1 arm (L1), the central protuberance (CP) harboring the 5S rRNA and the L7/L12 stalk (L7/L12).

B The 30S subunit, viewed from the subunit-interface, can be portioned into the head, platform, body, spur, shoulder (s) and beak.

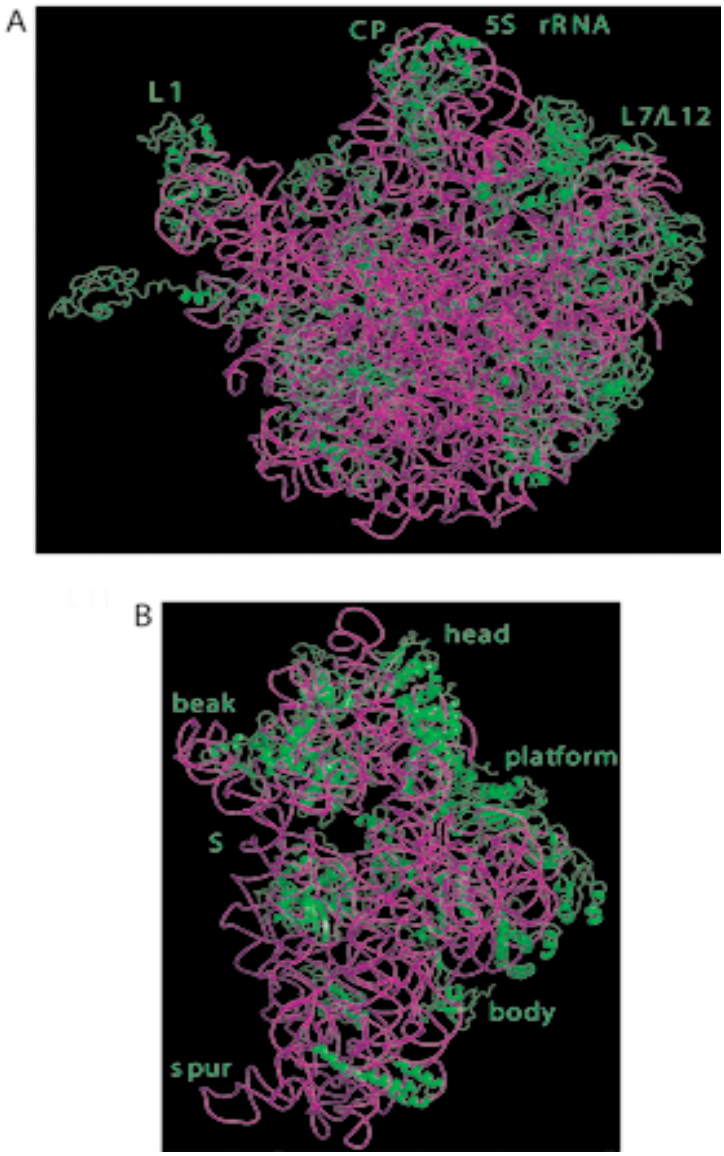

A
CP
5S rRNA
L1
L7/L12
B
head
beak
platform
S
body
spur

Figure 2: The translation cycle

(1) – (4) initiator tRNA fMet $tRNA^{fMet}$ is shown in green. **(5) – (9)** deacylated $tRNA^{fMet}$ shown in green, **(4) – (9)** aminoacylated/peptidyl tRNA shown in magenta, **(9)** aminoacylated tRNA shown in yellow, **(3) – (11)** show the 70S cut open looking into the intersubunit–cavity. EF-G is shown in brown, EF-Tu in purple, RF3 in pink.

(1) 30S–Initiation-complex, **(2)** 70S–Initiation-complex, **(3)** shows **(2)** cut open, **(4)** decoding, involving EF-Tu /GTP, **(5)** peptide bond formation takes place between A and P site tRNA, detailed reaction is shown in extra panel, **(6)** EF-G / GTP bound to pre-translocation complex, **(7)** EF-G/GTP bound to post-translocation complex, **(8)** GTP hydrolysis by EF-G releases the factor, **(9)** decoding as in **(4), (10)** termination involving RF1 and RF3, **(11)** recycling involving RRF and EF-G/GTP.

Individual figures are used from a movie by Joachim Frank.

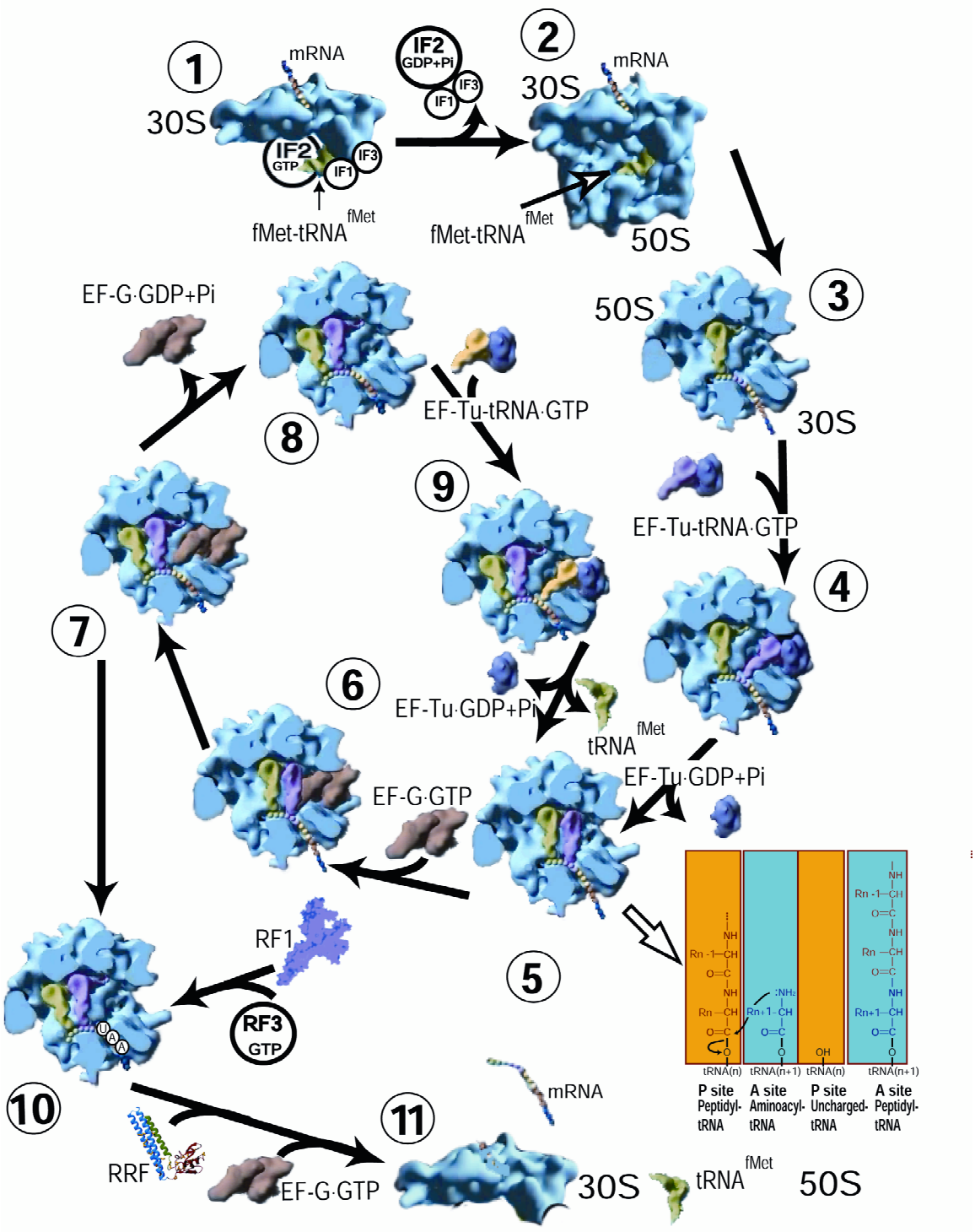

1
mRNA
30S
IF2
GTP
IF3
IF1
fMet-tRNA fMet
IF2
GDP+Pi
IF3
IF1
2
mRNA
30S
fMet-tRNA fMet
50S
3
50S
30S
EF-Tu-tRNA·GTP
4
EF-Tu·GDP+Pi
tRNA fMet
5
6
EF-Tu·GDP+Pi
EF-G·GTP
7
8
EF-G·GDP+Pi
9
EF-Tu-tRNA·GTP
RF1
RF3
GTP
U A A
10
RRF
11
EF-G·GTP
mRNA
30S
tRNA fMet
50S
tRNA(n)
tRNA(n+1)
tRNA(n)
tRNA(n+1)
P site Peptidyl-tRNA
A site Aminoacyl-tRNA
P site Uncharged-tRNA
A site Peptidyl-tRNA

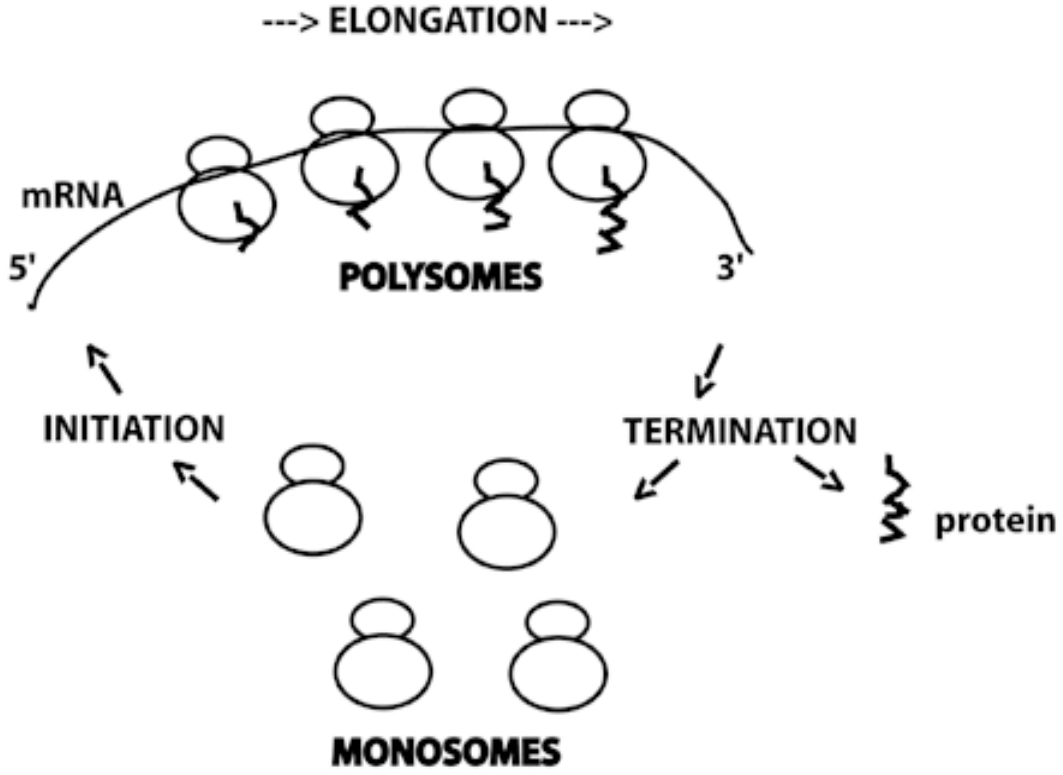
Figure 3: **Polysomes & Monosomes**

A Schematic view of polysomes and monosomes in the cell.

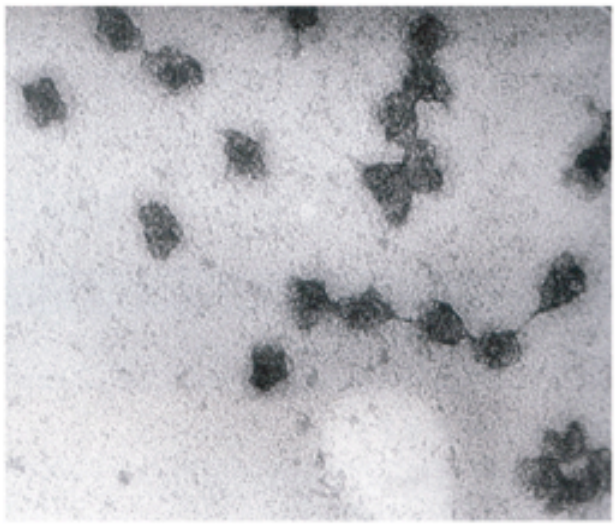
B Protein synthesis occurs on polysomes. Photograph was taken out of Benjamin Lewis' "Genes VI".

C Ribosome profile separates ribosomal subunits, monosome (70S), disomes (100S) and polysomes.

A


---> ELONGATION --->
mRNA
5'
POLYSOMES
3'
INITIATION
TERMINATION
protein
MONOSOMES

B



C

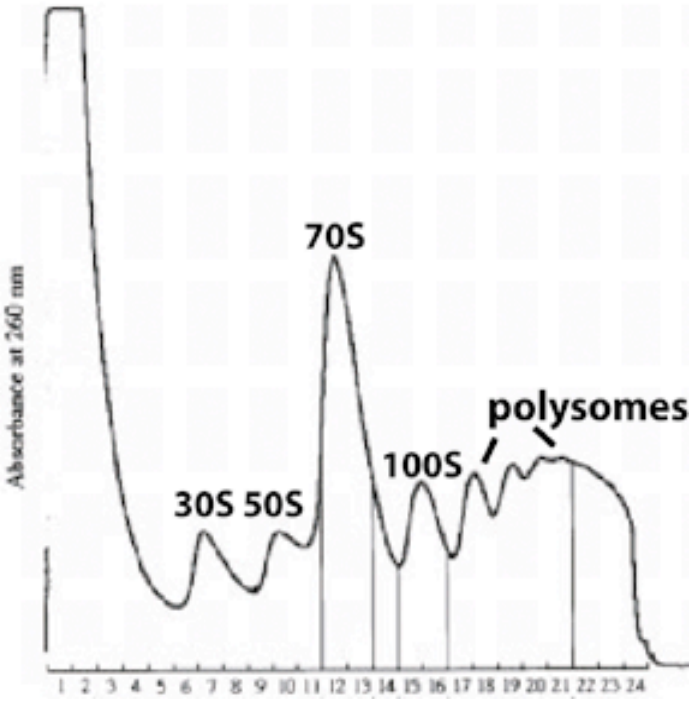
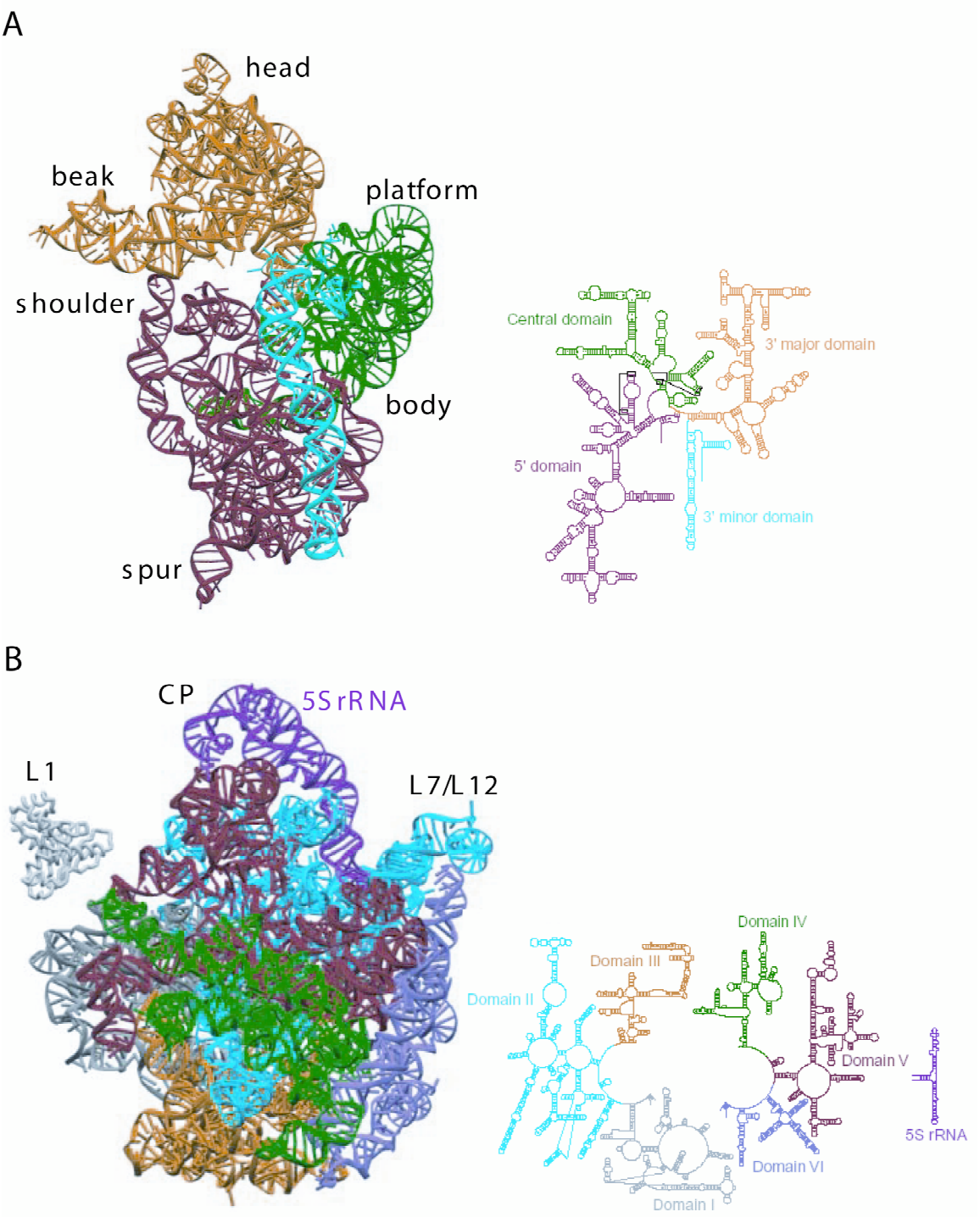

Absorbance at 260 nm
70S
30S 50S
100S
polysomes
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Figure 4: Secondary and tertiary structure of 16S rRNA and 23S rRNA

A Tertiary structure of the 16S rRNA on the left, secondary structure on the right (taken from Figure 3 in (Ramakrishnan and Moore 2001)); central domain in green, 5' domain in red, 3' minor domain in blue, 3' major domain in yellow.

B Tertiary structure of the 23S rRNA on the left, secondary structure on the right (taken from Figure 3 in (Ramakrishnan and Moore 2001)); domain I in yellow, domain II in blue, domain III in grey, domain IV in green, domain V in red, domain VI in purple. 5S rRNA is shown in pink.


A
head
beak
platform
shoulder
body
spur
Central domain
3' major domain
5' domain
3' minor domain
B
CP
5S rRNA
L1
L7/L12
Domain IV
Domain III
Domain II
Domain V
5S rRNA
Domain VI
Domain I