CHAPTER 1: Introduction

1.1. Ribosome: A protein synthesis factory

The translation of the mRNA is taking place on the ribosome. The bacterial ribosome is a compact 2.5 MDa ribonucleoprotein complex with a relative sedimentation coefficient of 70S and a diameter of 200-250 Å. All ribosome consists of two ribosomal subunits. The 16S rRNA (1,542 nt), and 21 ribosomal proteins (numbered from S1 to S21) compose the small (30S) subunit in *Escherichia coli* ribosomes. The large (50S) subunit comprises two rRNA molecules, i.e. the 23S (2,904 nt) and 5S rRNA (120 nt), and 33 ribosomal proteins (L1, L2···) (Figure 1.1). The rRNA of both subunits makes approximately two thirds by weight (Moore P., 2002; Ramakrishnan and Moore, 2001). Eukaryotic ribosomes contain more components and are significantly larger than prokaryotic ones, however eukaryotic ribosomes resemble in both architecture and function the prokaryotic homologues (Dube *et al*., 1998; Ramakrishnan and Moore, 2001).

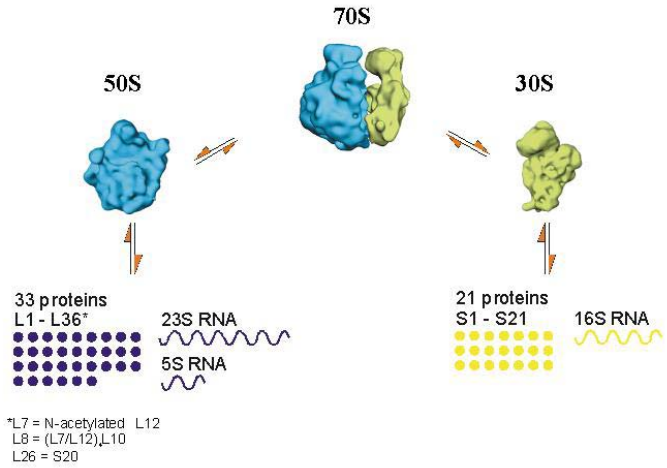

Figure 1.1 Ribosome composition of Escherichia coli

Ribosomes are major components of the cell. In *E. coli*, during rapid growth, ribosomes constitute approximately 50% of the total dry cell mass (Jinks *et al*., 1984; Jinks and Nomura, 1987)

The ribosome travels along the mRNA reading the message and synthesizing a protein in a codon-specific manner. A tRNA molecule serves as adapter molecule for the decoding of the genetic information encoded in the mRNA. Initially, two binding sites for tRNAs were proposed for the ribosome (Lipmann, 1963; Watson, 1963; Watson, 1964). The two sites of this model are the ‘’A’’ site (for aminoacyl-tRNA or acceptor site) and a ‘’P’’ site (for peptidyl-tRNA). However, functional studies at the beginning of 80’s (Grajevskaja *et al*., 1982; Lill *et al*., 1984; Rheinberger and

Nierhaus, 1980; Rheinberger *et al*., 1981) have demonstrated a third tRNA binding site, the ‘’E’’ site (E for exit) from which deacylated tRNA leaves the ribosome. The E site could be confirmed by neutron scattering, cryoelectron microscopy and X-rays diffraction studies (Wadzack *et al*., 1997; Nierhaus *et al*., 1998; Agrawal *et al*., 2000; Yusupov *et al*., 2001). This third ribosomal binding site has been found on ribosomes of all kingdoms and seems to be a universal feature of ribosomes (for review see Blaha and Nierhaus, 2001). However disagreement exists on several points concerning the importance of the E site (for more details see Burkhardt *et al*., 1998; Wilson *et al*., 2002).

Protein synthesis can be divided in three functional phases: (a) initiation, (b) elongation, and (c) termination (Figure 1.2).

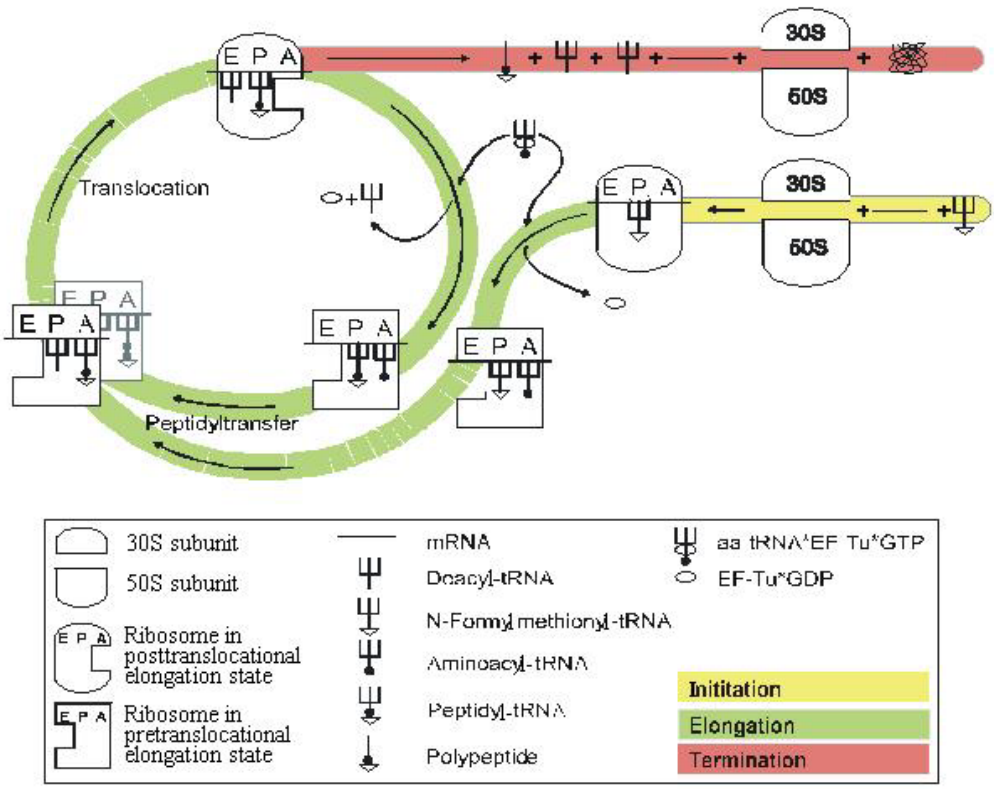

Figure 1.2 The functional phases of the ribosome during protein synthesis

1.1.1. Initiation

During the initiation phase, the small ribosomal subunit in conjunction with fMet-$tRNA_f^{Met}$ forms an initiation complex with an mRNA, having the initiator codon AUG and the fMet-$tRNA_f^{Met}$ at the P site. The purine-rich Shine Dalgarno sequence (SD) preceding the AUG is complementary to the 3'-end of the 16S rRNA (antiSD) sequence (Gualerzi and Pon, 1990) and helps to find the initiator AUG. Three factors assist in the initiation step, termed initiation factors (IF's): IF1, IF2 and IF3. IF1 closely mimics the tRNA anticodon stem loop, and correspondingly it has been found to bind to the ribosomal A site (Carter *et al*., 2001; Dahlquist and Puglisi, 2000). On the other hand, IF3 has been found to interact with 30S ribosomes and interferes with the E site tRNA binding (Dallas and Noller, 2001). IF1 and IF3 thus might improve the selection of the correct codon AUG to exclusively the P site by blocking the access to the adjacent sites A and E, respectively. IF2 is a G-protein analog to the elongation factor EF-Tu. It assists in fMet-$tRNA_f^{Met}$ binding to the ribosomal P-site (Lockwood *et al*., 1971) and is stimulated by IF1 (perhaps through direct interaction). Hydrolysis of the GTP is necessary for the translation initiation and triggers the release of IF2 from the ribosome after the association of the large ribosomal subunit (Luchin *et al*., 1999).

IF3 is a discriminator factor ensuring selection of correct tRNA for the initiation, i.e., fMet-$tRNA_f^{Met}$, since it destabilizes non-cognate initiation complexes that form at non-canonical codons (Gualerzi and Pon, 1990; La Teana *et al*., 1996; Meinnel *et al*., 1999). Furthermore IF3 acts in dissociation of 70S complex prior the correct initiation (Blumberg *et al*., 1979). It follows, that IF3 in addition to its "anti-association" functions fulfills another role during recycling of the ribosomes, namely it triggers the release of the deacylated tRNA from the P site after subunit dissociation (Karimi *et al*., 1999).

The initiation phase is completed, when the 50S ribosomal subunit associates with the 30S and the fMet-$tRNA_f^{Met}$ located in the ribosomal P site, forming the 70S initiation complex or Pi complex (Pi for initiation).

1.1.2. Elongation

1.1.2.1. General description

The elongation cycle is responsible for the growth of the nascent polypeptide chain. Once the 70S initiation complex has been formed, an empty A-site binds an aminoacyl-tRNA (aa-tRNA) that has a complementary anti-codon to the mRNA codon at the same site. A PRE-translocational or PRE-state is formed in this way with a peptidyl-tRNA (fMet-tRNA) at the P site and an aminoacyl-tRNA at the A site. Peptide bound formation occurs between the α-amino group of the aminoacyl-tRNA and the carbonyl group of the peptidyl-tRNA at the P site leaving the PRE state with a deacylated tRNA at the P site and a peptidyl-tRNA at the A site. Finally, the mRNA: $tRNA_2$ complex is shifted one codon towards the 3' (downstream) direction to make the A site free for next tRNA. This movement on the ribosome is called translocation, during which deacylated tRNA is moved from the P to the E site and peptidyl-tRNA from the A to the P site, forming a POST-translocational or POST state. The elongation cycle is repeated as long as a "sense" codon is shifted into the A site upon a translocation step. Thus, throughout the elongation cycle a ribosome oscillates between PRE and POST states.

Two elongation factors are involved in the elongation cycle. Ternary complex formed by an aminoacyl-tRNA together with an elongation factor EF-Tu, a G protein, and one molecule of GTP (aa-tRNA·EF-Tu·GTP) binds to the pre-A site with a high affinity of about 10^8 M^{-1} (Schilling-Bartetzko *et al*., 1992b). After ternary complex has located the aa-tRNA in the correct place, the GTPase center on EF-Tu is activated. The resulting EF-Tu·GDP dissociates from aa-tRNA and the ribosome, since it has a low affinity for the aa-tRNA. EF-Tu·GDP is regenerated to EF-Tu·GTP by the nucleotide exchange factor EF-Ts. The aa-tRNA is accommodated into the proper A site and is ready to accept the peptidyl-residue from the adjacent peptidyl-tRNA at the P site *via* a peptide-bond. After peptide-bond formation a deacylated tRNA is at the P site and the peptidyl-tRNA, prolonged by one aminoacyl residue, at the A site. This complex is a pre-translocational state or briefly PRE state ready to be translocated to the POST state.

The second factor EF-G is, like EF-Tu, also a G-protein, with the difference that EF-G does not have any nucleotide exchange factor like EF-Ts. The substrate for EF-G function is the PRE state. EF-G·GTP provokes translocation moving the

mRNA·$tRNA_2$ complex by three nucleotides in the ribosome (Beyer *et al*., 1994). After that the GTPase center of the factor is activated by the ribosome and EF-G·GDP dissociates from the ribosome. EF-G lowers the activation energy barrier of about 120 kJ/mol between the PRE and POST states of the ribosome (Schilling-Bartetzko *et al*., 1992a), although in the absence of EF-G ribosomes can perform translocation at ~1000-fold lower rates (Bergemann and Nierhaus, 1983; Gavrilova *et al*., 1976).

1.1.2.2. Models for the elongation cycle

Three models have been proposed to explain how the elongation cycle proceeds.

According to the "Hybrid-Site Model" (Moazed and Noller, 1989) translocation moves the tRNA parts bound to 50S subunits after peptide-bond formation, whereas the tRNA parts bound to 30S subunits follow only during the translocation reaction. This mechanism generates hybrid states for the two tRNAs at the PRE state after peptide bound formation and before translocation, for example P/E and A/P sites. A peptidyl-tRNA is thought to be at the A/P sites, if the tRNA is still at the A site of the 30S subunit, but has moved on the 50S subunits to the P site (Moazed and Noller, 1989; Wilson and Noller, 1998). EF-G dependent translocation moves the states mentioned above to the E/E and P/P, respectively (Noller *et al*., 2002).

The basis of the second model, the allosteric three-site model, is based on the observation that A and E-sites are allosterically coupled through negative cooperativity: when aa-tRNA binds to the A-site, the affinity of deacylated tRNA to the E-site drops and tRNA leaves the ribosome. In contrast, when the deacylated tRNA occupies the E site, the affinity of the A-site is low which enables the ribosome to select the correct ternary complex (Geigenmüller and Nierhaus, 1990). This model also incorporates the finding that deacylated tRNA at the E-site undergoes codon-anticodon interaction.

The α-ε model is an extension of the allosteric three site model. This model incorporates data regarding the tRNA contact patterns on the ribosome. PRE-state ribosomal complexes exhibit different contact patterns for A and P site tRNAs. However, following translocation thus forming the respective POST-complex, the contact patterns of the A-site tRNA before translocation and the P-site tRNA after translocation did not change. The interpretation was that micro-topography on the ribosome did not change during translocation. These results argue for a movable

ribosomal domain that binds tightly the tRNAs at A and P sites and moves them to the P and E sites during the translocation reaction (Dabrowski *et al*., 1995; Dabrowski *et al*., 1998). This domain contains two tRNA binding regions that have been termed " α" for the tRNA contact pattern displayed at the A and P site before and after translocation, respectively; and "ε" for the tRNA contact pattern present at the P site before translocation and at the E site after. It follows that at the A site only α can appear and at the E site only ε (reviewed in Wilson *et al*., 2002).

1.1.3. Termination

1.1.3.1. General description

The synthesis of the polypeptide chain continues until a stop codon (UAA, UAG or UGA) is invading the A site. Proteins factors, called release factors (RF), are in charge of releasing the nascent polypeptide chain from the ribosomes and recycling the ribosomes for the next initiation. Two classes of release factors are distinguished: Class I do not consume energy and are specific decoding factors that are responsible for the hydrolysis of the peptidyl-tRNA. RF1 and RF2 belong to this group, the factors recognize UAG and UGA respectively; both RF's overlap in the recognition of the termination codon UAA. Class I RF's promote hydrolysis of the ester bond between the polypeptide and the P site tRNA on the ribosome. RF1 and RF2 genes (*prfA* and *prfB*) have been shown to have a high similarity at the amino acid level (Caskey *et al*., 1984; Craigen *et al*., 1985; Weiss *et al*., 1984). In eukaryotes and in archea only a single class I factor, eRF1 (and aRF1) have been identified that recognizes all three-stop codons (reviewed by Wilson *et al*., 2002).

The Class II release factors are non-decoding and energy consuming factor. The RF3 belongs to this class. It stimulates the termination process in a GTP dependent manner.

RF3 in *E. coli* is not essential; knockout strains of its gene *prfC* gene are viable. The main function of RF3 is to support removal of the class I RF's from the ribosome using GTP hydrolysis, once the peptide hydrolysis has taken place (Freistroffer *et al*., 1997; Zavialov *et al*., 2001). In other words, the post-termination complex is the substrate for RF3 that stimulates the recycling of RF1 and RF2 (Freistroffer *et al*., 1997).

1.1.3.2. How is termination achieved?

The mechanism how class I decoding factors work is not yet clear. One possibility is that RF's recognize the stop codons directly. This model is strongly supported by crosslinking experiments of RF2 with a piece of mRNA near to the decoding center (Brown and Tate, 1994). Murgola has suggested a second possibility, where the RF1/RF2 binding site on the 50S ribosomal subunit plays a role in the transmission of a signal from the termination codon to the peptidyl-transferase center, the ‘’hydrolytic center’’ in this case. This idea arose from results employing mutants with mutations in the ‘’GTPase associate center’’ (the 1070 region of 23S rRNA) that are defective in the polypeptide termination (Arkov *et al*., 2000; Goringer *et al*., 1991; Murgola *et al*., 1989; Murgola *et al*., 1988)**.**

More recently a new hypothesis has been proposed; it assumes that highly conserved tRNA-like hairpin structures of helices 69 (domain IV) and 89 (domain V) of the 23S rRNA play a role in termination (Ivanov V *et al*., 2001). These helices contain anticodon hairpins with triplets complementary to stop codons. According to this model, RF's could recognize the RNA-RNA duplex (mRNA: rRNA). Until now there is no experimental evidence available that supports this hypothesis.

In contrast, there is evidence that the universally conserved A2602 of the ribosomal peptidyl transferase center is involved in translation termination. Ribosomes carrying a mutation at 2602 position were severely affected in the peptide-release reaction, whereas normal levels of peptide-bond formation were retained (N. Polacek, personal commnication).

On the other hand, Nakamura and co-workers, based in the RF-tRNA mimicry hypothesis (Ito *et al*., 1998a; Ito *et al*., 1998b), have identified a "tripeptide anticodons" that seems to be essential for the stop-codon recognition. In the case of RF1, the tripeptide sequence suggested was Pro- (Ala)-Thr (P (A) T aa position: 188-190), whereas for RF2 it was Ser-Pro-Phe (SPF: aa position 205-207) (Ito *et al*., 2000).

Another set of information derived from the crystal structure of the eukaryotic release factor 1 (eRF1) has provided some clues about how the termination mechanism might occur. The structure in a form of ‘’Y’’ shape of eRF1 resembles a tRNA, although the similarity is not reaching the same level as that seen with RRF (Connell and Nierhaus, 2000). Interestingly, one of the tips of eRF1, which could correspond to the CCA-3’ of the tRNA, contains a universal GGQ motif (GlyGlyGln),

which is proposed to interact with the peptidyl-transferase center (Frolova *et al*., 1999). Furthermore, a tetrapeptide located at the N-terminal domain of eRF1 (Asn-Ile-Lys-Ser = NIKS), might be able to interact with the stop codons. The GGQ motif and the anticodon-like site are separated by a distance of 80 Å, similar to the distance found between the acceptor stem and the anticodon stem loop in a tRNA molecule (75 Å; Connell and Nierhaus, 2000).

Song et al., have suggested that the glutamine residue of the conserved GGQ sequence in eRF1 guides an H_2O molecule into the hydrophobic pocket of the peptidyl-transferase center. This transfer would allow a nucleophilic attack on the ester bond of the peptidyl-tRNA by the oxygen atom of a water molecule during termination, replacing the attack of the α-amino group of the newly selected aminoacyl-tRNA during an elongation cycle (Song *et al*., 2000).

More recently, the crystal structure of *E. coli* RF2 has been determined at 1.8 Å resolution. The protein consists of four domains and biochemical important residues are exposed in loops on the surface of the molecule. The tripeptide SPF motif (RF2 specific motif) is located in a loop distant from the N-terminal domain (domain 2). On the other hand, the conserved GGQ motif is in another loop 23 Å apart from the SPF motif (domain 3; Vestergaard *et al*., 2001). This observation seems to eliminate the possibility that the functional groups of RF2 can be located at the decoding center and at the peptidyl transferase center simultaneously. However, cryo-electron microscopy study of termination complexes on ribosomes carrying the decoding factor bound have shown that RF2 is an open conformation when bound to the ribosome, allowing GGQ to reach the peptidyl transferase center while still allowing interactions between the SPF-motif and a stop codon (Joachim Frank, unpublished results).

Finally, the termination sites an mRNAs are biased in the bases around the stop codons. The efficiency in the termination process is strongly modulated by the base at the +4 position (+1 is the first nucleotide of the A-site codon), in the order of strength: U > G > C > A (Pavlov *et al*., 1998; Poole *et al*., 1995).

1.1.3.3. Recycling

After the oligopeptide cleavage of the P-site peptidyl-tRNA by the class I RF's has occurred, the ribosome is found in a post termination complex, i.e., with RF1 or RF2 located in the ribosomal A site, and one deacylated tRNA bound at the P site

and probably another one at the E site. RF3 in a GDP form (Zavialov *et al*., 2001) binds to the post termination complex and nucleotide exchange on the ribosome promotes the dissociation of the decoding factors from the ribosome. Subsequent hydrolysis of the GTP on RF3 triggers its dissociation after it has accelerated the removal of the decoding RF's from ribosomes (Freistroffer *et al*., 1997).

Once the decoding factors and the RF3 have left the ribosome, the RRF (Ribosome Recycling Factor) mediates the ribosome recycling. The presence and importance of RRF has been demonstrated since many years (Hirashima and Kaji, 1970; Hirashima and Kaji, 1972). In *E. coli* the gene encoding the RRF, *frr*, is essential for bacterial growth (Janosi *et al*., 1994), but it is not found in eukaryotes. RRF acts together with EF-G, and both factors catalyze the breakdown of polysomes (Janosi *et al*., 1996). Although it is unclear how these proteins promote the splitting of the 70S ribosomes into their subunits, GTP hydrolysis on EF-G is necessary (Janosi *et al*., 1996; Karimi *et al*., 1999).

Two models have been proposed to explain RRF functions. One of these takes advantage of the RRF tRNA mimicry hypothesis derived from the crystal structure of RRF from *Thermotoga maritima* at 2.55 Å resolution. The similarity of RRF with a tRNA molecule is compelling, it has practically the same dimensions (Selmer *et al*., 1999).

Kaji suggests that RRF binds the ribosomal A site and by means of EF-G action translocates it from the A-site to the P-site and thus moving the deacylated tRNA from the P to the E site site. In other words, EF-G promotes translocation in the same way as during the elongation cycle (Hirokawa *et al*., 2002; Selmer *et al*., 1999). Although the RRF structure is similar to a tRNA, the expected binding of RRF to the ribosomal A site would be codon independent and no contact with the PTF should exist so that the RRF does not mimic the CCA end of a tRNA. Indirect evidence seems to support these assumptions (Hirokawa *et al*., 2002).

An alternative hypothesis comes from the Ehrenberg group. They proposed that RRF and EF-G split directly the ribosome into its subunits and deacylated tRNA remains on the 30S subunit in a complex with the mRNA. Then the release of the deacylated tRNA from the 30S subunit is catalyzed by the initiation factor 3 (IF3) (Karimi *et al*., 1999). Note that according to this model EF-G has a new unknown function and is not used to promote a translocation reaction. This model seemsto be supported from hydroxyl radical mapping experiments, where a position of RRF was

deduced that was transversely going across A and P sites on the large ribosomal subunit (Lancaster *et al*., 2002).

1.2. Translational errors and two tRNAs on the ribosome

During protein synthesis the ribosome produces errors. These errors have been classified as: (1) processivity errors; (2) missense errors and (3) loss of the correct reading frame (frameshifting). A processivity error is defined as the release (drop-off) of a prematurely short peptidyl-tRNA from the ribosome. Premature termination is also a cause of processivity error. Release of the peptidyl-tRNA from the ribosome could occur, if a sense codon is mis-recognized as a termination signal, although false stop constitutes a very low proportion of processivity errors (Jorgensen and Kurland, 1990). A missense error results from the incorporation of an incorrect aminoacyl residue into the nascent peptide. Generally this kind of mistake is not harmful, i.e., most amino acid substitutions do not eliminate protein function, since most often the cognate amino acid is misread by a chemically similar one due to the organization of the genetic code. In contrast, a shift in the reading frame generates truncated and usually non-functional proteins resulting in the loss of genetic information.

How does the ribosome avoid these kinds of errors, and what is the relation between ribosomal translational errors and codon-anticodon interaction at the E site?

As mentioned before, during the elongation cycle the ribosome oscillates between two major states: the PRE and the POST states. An important consequence associated with these states relates to the accuracy of the translational process and the maintenance of the correct reading frame. During the elongation cycle there are, at all times, two tRNAs bound on the ribosome, i.e. in P and A sites in the PRE-state and in E and P sites in the POST state.

According to the allosteric three-site model, the negative allostery between the E and A sites reduce the chance for non-cognate tRNAs to bind to the ribosomal A site (Geigenmüller and Nierhaus, 1990; Nierhaus, 1990). An important signal for the ribosome to adopt a POST state is obviously codon-anticodon interaction at the ribosomal E site, since a near-cognate tRNA at the E site does not reduce the error of aa-tRNA selection at the A site (Geigenmüller and Nierhaus, 1990). Additionally, crystal structure analyses have shown that the E-site tRNA makes extensive contact

with the small subunit (Yusupov *et al*., 2001) and POST complexes with tRNAs bound to E and P sites can be isolated by centrifugation through sucrose cushion without loss of deacylated tRNA from the E site (Wadzack *et al*., 1997) proving that the tRNA at the E site is bound in a stable fashion.

The fact that there are at all times two tRNAs bound on the ribosome during the elongation cycle guarantees maintenance of the correct reading frame. It has been demonstrated that the number of nucleotides at the anticodon (three or four) determines the number of nucleotides of the codon (three or four) and the corresponding movement of three or four nucleotides during translocation and thus the reading frame. It follows that tRNAs play an important role in defining the reading frame (Atkins J. A., 2000). Studies on frameshift mutations (*sufD41*), tRNA suppressors ($tRNA^{Gly}$, $tRNA^{Pro}$, $tRNA^{Ser}$ isoacceptors) and insertion elements, support the idea that anticodon-codon base pairing influences maintaining the reading frame (Atkins J. A., 2000). Most interesting is that apparently -1 and +1 frameshifting is produced by peptidyl-tRNA slippage (Farabaugh and Björk, 1999; Qian *et al*., 1998), which implies a tRNA anticodon detachment and re-formation of their respective mRNA codon. The conclusion from these observations is that losing one of the two tRNAs present on the ribosome could lead to a loss of the correct reading frame of the translation and thus loss of the genetic information. Indeed a ribosome carrying a peptidyl-tRNA will slide along the mRNA if a ‘’hungry’’ codon is located at the A site, for example, if codons are starved of their correct (cognate) aa-tRNA, e.g., AAG for $tRNA^{Lys}$ (Lindsley and Gallant, 1993).

An ideal model to study the problem at issue would be one that enables us to evaluate both events at the E site, namely taking influence on the selection of an aminoacyl-tRNa at the A site maintaining the reading frame. Such a system we found in the programmed +1 frameshifting that occurs during translation of the bacterial release factor 2 (RF2) protein in *E. coli*.

1.3. Mechanism of genetic expression of RF2 protein: an autoregulatory mechanism

RF2 protein is regulated by a programmed +1 frameshifting mechanism, i.e., a change in the reading frame downstream by one nucleotide. Furthermore, programmed frameshifting form part of an auto regulatory mechanism. In the case of *E. coli* release factor 2 mRNA; the codon number 26 is an UGA stop codon, which is

recognized by RF2 (Baranov PV *et al*., 2002). When the concentration of RF2 in the cell is sufficiently high, stop recognition takes place and the translation finishes after the synthesis of a non-functional 25-mer peptide, which is rapidly degraded. However, when there is a shortage of RF2 in the cell, a +1 frameshift occurs enabling the translation of the complete RF2 protein. This frameshift can occur with an astonishingly efficiency of up to 100 % (Donly *et al*., 1990), whereas during normal translation the error frequency for frameshift is not higher than 1 case in 30,000 amino acid incorporations (Jorgensen and Kurland, 1990), i.e., the frameshift on the RF2 mRNA occurs with a frequency that is more than four orders of magnitude larger than normal.

Several features have been identified that contribute to this efficiency. Frameshifting is facilitated because (i) ribosomes translate slowly at the UGA codon (Adamski *et al*., 1993; Craigen and Caskey, 1986), (ii) a G:U wobble base pair on the oligopeptidyl-tRNA^{Leu} at the P site exist. This base pair is weak facilitating the slippage from the initial frame, (iii) a perfect realigned with the new aminoacyl-tRNA Asp-tRNA in the new frame is acquired after the frameshifting (Curran, 1993) and, (iv) a Shine-Dalgarno sequence precedes the UGA stop codon complementary to the antiSD sequence of the 3' end of 16S rRNA (nucleotides: 1534-1540, *E. coli* numbering; Weiss *et al*., 1988).

It is already known that the SD: antiSD interaction on the RF2 mRNA enhances frameshifting (Weiss *et al*., 1988), however the mechanism by which this interaction stimulates frameshifting is not known. In this thesis the mechanism of the highly efficient frameshift is dissected, and we demonstrate that the SD: antiSD interaction enhances the frameshifting by causing the release of the deacylated tRNA from the ribosomal E site (Márquez *et al*., 2002). A novel *in vitro* translation system was developed that allowed measuring both the efficiency of frameshifting and the extent of the tRNA^{Tyr} release at the E site. The results obtained in this thesis demonstrate that the presence of a tRNA at the E site and probably codon-anticodon interaction at this site is instrumental for maintaining the reading frame, and that this dependence is exploited for the feed-back regulation of the translation of the RF2 mRNA.