Ribosomes - The Bio Monitors of Protein Synthesis

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Abstract:

Protein synthesis is the final part of gene expression and is necessary for vital functioning of system. The three parts initiation, elongation and termination are equally important also control of any part leads to entire fidelity of the process. Stalk of ribosome is necessary for translocation of ribosome from A site to P site and also for interaction of tRNA with ribosome. tRNA can acts as structural component of ribosome and head swivelling and use of tRNA hybrids had made the study of translocation of ribosome as possible and finally post translational modifications of proteins proven to be necessary for control of protein synthesis.

Key words: *Ribosome, LUCA, Head swivelling, Rocker switch, Mitoribosomes*

I. INTRODUCTION

Ribosomes are the complexes of RNA and protein. As we know RNA was the biomolecule evolved first in the living world and probably DNA replication was evolved next as the LUCA (Last Universal Common Ancestor) comprises the imprint of translation machinery and believed to evolve before LUCA (George E. Fox (2010). 50s subunit of bacteria and archea comprising 23S rRNA contributes major part of P- site and A-site with some part in 30s subunit but E- site was contributed by both 50s and 30s subunits. Translation begins by assembly of ribosome subunits and ends by dissociation of them. In between mRNA acts as bridge between tRNA and ribosome and also acts as a template. Whereas tRNA carries the amino acid with its CCA end and adds them to the growing chain. Peptidyl transferase reaction mainly takes place in 50s subunit where as decoding takes place in 30s subunit through interaction of anticodon arm of tRNA and mRNA. The ribosome moves in the 5’- 3’ direction and the peptide bond formation occurs through 3’-5’ direction and finally release of the synthesised protein occurs at the E- site.

Translocation of ribosome a part of elongation is mediated by rotation of head of 30s subunit mediated by two hinge regions in 16 s rRNA and the antibiotic spectinomycin inhibits translation by binding to hinge region 2 (Mohan et al., (2014). Translocation of ribosome is mediated by EF-G by regulation of moving helices comprising of rRNA segments in contact with tRNA, inter subunit segments and helices 28, 32 and 34 of 30S subunit (Maxim Paci and George E. Fox (2015). Recent study on ribosome structure has shown that ribosomes organise in to three conformational polysomes with naked RNA and the ribosomes in polysomes interact like cliques which depend on translational functionality of the cell (Viero et al., (2015). The present review mainly focuses on ribosome structure and functionality of ribosome and translation updates.

II. CRYSTAL STRUCTURE OF RIBOSOME

Recent studies on ribosome structure, and understanding of protein synthesis resulted in great revolution. Determination of crystal structure of 2.4 A0 structure of 50S subunit of archea Haloarcula marismortuii comprising of L1stalk, L11- RNA region and the L7/L12 stalk is majorly required for protein translocation and some of the stem loop regions that contacts 30S subunit was found to be disordered. Later on comparison of 50 S subunit of Deinococcus radioduarans at 3.1A0 resolution has reported similarity to the disordered structures of Haloarcula (V. Ramakrishnan (2002).

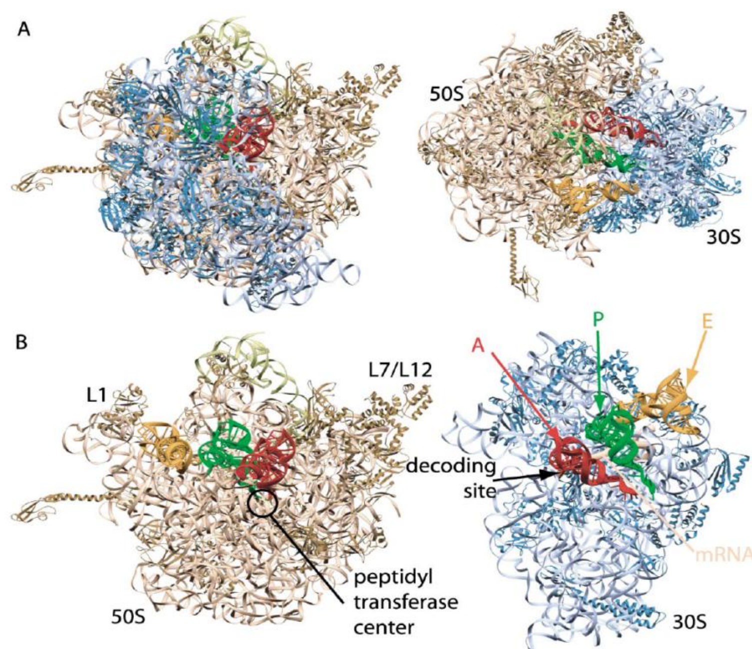

Figure: 1 Crystal structure of Ribosome of E.coli. From V. Ramakrishnan (2002)

Two independent structures of 30S subunit from the Planck's group showed the RNA and protein components of the two independent structures. But it is very difficult to predict the tRNA and mRNA inter molecular interactions and with the P and E sites. In addition to the study of interactions by these two structures the structures of 50S subunit that is disordered in Haloarcula was found to be organised in the combined subunits of 70S ribosome.

III. INITIATION

Initiation in E.coli begins with binding of ribosome 16S rRNA to the shine dalgarno sequence which is complementary to the 3' end of the RNA. IF3 binds with the 30S subunit and prevents the premature binding of 50S and 30S subunits. IF3 found to increase the affinity for initiator tRNA where as IF2 binds with aminoacyl site of tRNA and occupies A site of the ribosome. IF3 binds to E site of the ribosome and IF1 prevents the addition of initiator tRNA to the A site and found to be present at neck regions of 30S subunit. The GTPase activity of IF2 is not required either for initiator tRNA binding in the P-site or for the release of IF2. The location of IF2 is not precise but the GTPase domain was found in the vicinity of the interacting regions of elongation factors Tu and G (EF-Tu and EF-G).

IV. ELONGATION

Elongation begins by binding of EF-Tu-GTP-tRNA ternary complex to the A-site of the ribosome through selection of correct match between the mRNA codon and tRNA anticodon. The binding of cognate tRNA provides both steric hindrance and kinetic proofreading to the near tRNA and helps in correct match of the previous step. It also helps in dissociation of unoccupied tRNA in the E-site. Three conserved residues of ribosome namely G530, A1492 and A1493 of 16S rRNA are foot printed by Asite tRNA and required for viability of E.coli. Actually A1492 and A1493 are the conserved residues that forms helix loop, the binding site for the amino glycosides like paramomycin which resembles with that of cognate tRNA. Binding of cognate tRNA causes conformational change in A1492 and A1493 leads to flipping in G530 residues and results in codon-anticodon interaction in minor groove of the first base pair extending to second codon-anticodon helix. The no specificity in third base pair leads to formulation of wobble hypothesis with respect to t RNA and mRNA. The GTP hydrolysis triggers the release of secondary complex EF-Tu.GDP from the A site. Peptide bond formation occurs in A-site and the ribosome translocates by one codon by using GTP and the unoccupied tRNA in the P site moves to E site. The ribosome is ready for next cycle of amino acid addition.

Translocation requires EF-G-GTP. It is already known fact that hydrolysis of GTP is required for translocation but now the hydrolysis of tRNA from A site is sufficient to move the ribosome by one codon as confirmed by the experiments that use GTP analogs that doesn't undergo hydrolysis. GTP hydrolysis is only required for removal of EF-G.

V. TERMINATION

Protein synthesis continues until A site of ribosome occupies a stop codon. Two termination

factors namely RF1 and RF2 are required to recognise the termination codons. Both recognise UAA where as UGA was recognised by RF1 and UAG were recognised by RF2. RF3 is the GTPase that binds to RF1/2 complex and causes release of RF1 and RF2 by GTP hydrolysis. Now the ribosome is with mRNA and deacylated tRNA in P-site. These must be dissembled in order to begin another round of protein synthesis by ribosome. Ribosome Release Factor (RRF) and EF-G is necessary for dissociation of deacylated tRNA from the P-site through GTP hydrolysis and IF3 is required for dissociation of deacylated tRNA.

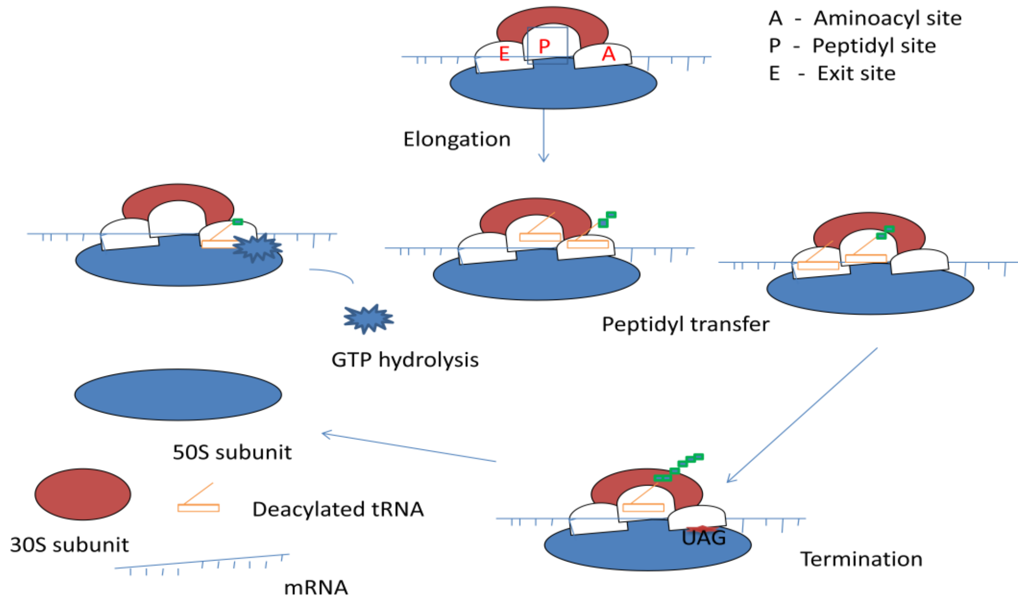

Figure: 2 Protein synthesis in prokaryotes. Where UAG represents termination codon and the process is briefly explained here. The process begins by addition of 1st amino acid to the initiation codon followed by peptidyl transfer and the process continues up to the ribosome encounters termination codon.

Termination of translation by release of RF-1 and RF-2 with the help of RF-3:

After recognition of stop codons by RF1/RF2 in the Asite leads to termination of translation followed by peptide cleavage. The release of RF1 requires RF3 which is a G- protein. In elongation factor EF-TU the GDP to GTP exchange is facilitated by EF-Ts where as in case of EF-G it is by the protein itself where as incase of RF3 ribosome in complex with RF1. RF3 exist in GDP bound form in the cytoplasm and encounter of RF3-GDP with ribosome – RF1 leads to exchange of GDP with GTP which causes conformational change in ribosome and release of RF1 followed by GTP hydrolysis and release of RF3 in the form of RF3-GDP in to cytoplasm (Pallesen et al., (2013).

Binding of RF3- GDP involves interaction of RF3 with ribosomal protein L12. Helix α7 of RF3 interacts with α4 and α5 of L12 CTD. Further L12 CTD interacts with L11 NTD after interacting with RF3. The domain 1 of RF3 must rotate away from domain 3 inorder to achieve open conformation. The change in conformation of ribosome involves rotation of ribosome subunits 30S and 50S after hydrolysis of GTPase of EF-G.

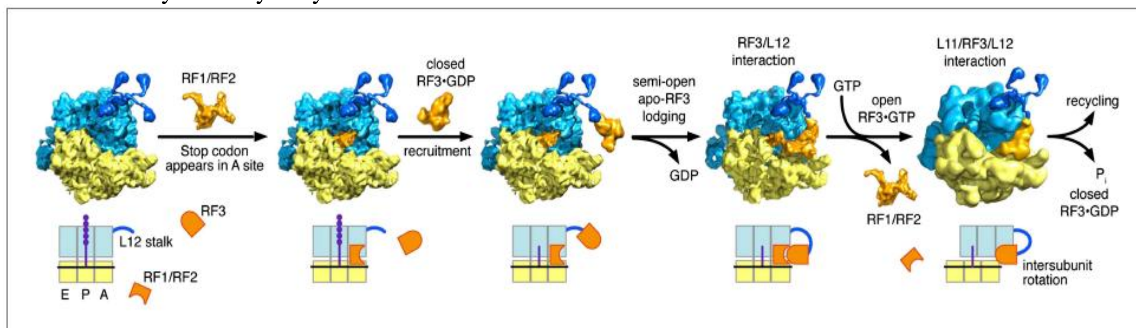

Figure: 3 Schematic representation of termination of translation by RF3. Stop codon appearance in A site leads to binding of RF1/RF2 to the ribosome followed by recruitment of closed RF3-GDP and movement of domain shifts the RF--GDP in to open conformation followed by exchange of GDP in RF3 with GTP which facilitates release of

RF1/RF2 followed by interaction of RF3 with L12 CTD and L11NTD . Recycling of the complex occurs after hydrolysis of Pi. From Pallesen et al., (2013).

VI. MOLECULAR SWITCHING DEPENDS ON L7/L12 PROTEIN IN E.COLI:

Ribosomes are the dynamic particles involved in protein synthesis. The most important structure in ribosome includes stalk that plays an important role in ribosome translocation of ribosome from A site to peptidyl site and is involved in the interaction of tRNA with ribosome. L10 protein is necessary for interaction of L7/L12 with elongation factors. In E.coli the stalk consists of an acidic protein namely L7/L12 present as four copies forming two dimers. The flexible region namely hinge region connects the N-terminal domain and C- terminal domain. The N-terminal domain is necessary for anchoring of the protein to ribosome and found to be also required for dimerisation of the L7/L12 protein where as CTD is required for the interaction of ribosome with translation proteins. Removal of one CTD doesn't have any impact on translation but removal of hinge region lead to reduced mobility and activity of CTD of modified ribosome (Bocharov et al., (2004).

CTDs have restricted internal mobility in which residues Phe 30, Asn 64 of these domains show micro- millisecond conformational change. The dimeric NTD and the CTD tumble similar to that of globular proteins with 152 and 115 residues respectively. In solution the L7/L12 exist as three independent core regions with dimeric NTD and two CTDs as flexible wings attached to the core particle.

In solution the L7 dimer exists as three independent regions linked by hinge region. Both hydrophilic and hydrophobic residues are distributed throughout the region of L7. DNTD and hinge region is less polar compared to the CTD. The DNTD which is necessary for anchoring of ribosome and consists of negative charges where as CTD necessary for interaction with elongation factors consists of both positive and negative charges. CTD helices α4 and α5 is necessary for interaction of ribosome with EF-Tu and Ts (Bocharov et al., (2004).

VII. SWITCHING BETWEEN MT TRNA^{VAL} AND MT TRNA^{PHE} IS A NECESSARY STRUCTURAL COMPONENT OF MITORIBOSOMES:

Normally mitochondrial ribosomes are evolutionarily similar to prokaryotes with 70S ribosomes. Recently it was found that the tRNA substitutes for 5S rRNA in larger subunit of humans and porcine. CryoEM is the technique used by Joanna Rorbach etal., to prove this scenario. In humans and rats mt tRNA^{Val} is incorporated in to the larger subunit compared to Phe. In Porcine it is mt tRNA Phe but in humans when the levels of mt. tRNA Val levels are depleted mt tRNA ^{Phe} is incorporated in to mt ribosomes indicating a switching mechanism between these two tRNAs. Whether this pattern is similar to every eukaryote or restricted to humans and porcine is found now to be specific to the two species but not to be tissue specific (Rorbacha et al., (2016).

In mutations of mt tRNA^{Val} both elongation and overall protein synthesis are affected and use of specific cognate tRNA synthetases and VARS2 induction restores the protein synthesis. The Phe and Val tRNAS are synthesised as 16 S and 12S gene transcripts which undergo post transcriptional modifications and CCA addition occurs prior to incorporation and finally16 S transcripts was cleaved in to Val tRNA after addition to the ribosome. The tRNAs associate with ribosomes in their tertiary structure with L-shape (Rorbacha et al., (2016).

When levels of mt.tRNA^{Val} is depleted Phe tRNA compensates for the structural component of mitoribosomes but how the switch corresponds to correct association of mitoribosomes using two different tRNAs is the major question.

VIII. HEAD SWIVELLING AND USE OF TRNA HYBRIDS SERVE AS MODEL FOR TRANSLOCATION OF RIBOSOME

Previous studies have proved that EF-G is required for translocation along with GTPase activity. Recent work in this aspect include how the tRNA is translocated from P and Asites to E site was made possible by cryo EM and X-ray analysis by using the antibiotic fusidic acid. This antibiotic prevents the conformational changes associated with EF-G induced by GTP hydrolysis. Interaction of domain IV of EF-G with the helix of 16 S rRNA is necessary for movement of mRNA- tRNA complex and head swivelling of ribosome 30S subunit, along with the racheting and unracheting of the ribosome. GTPase activity of EF-G is found to be intrinsic to the ribosome and domain iii region and Switch I region of EF-G and ɤ-phosphate of GTP stabilises the rotated ribosome and GTP hydrolysis promote the unracheting of the platform of 30S subunit with respect to head. The anticodon stem loop region moves in order to interact with P-site and E-site on the 30S subunit and E-site on 50S subunit forming intra hybrids. The binding of CCA end to 50S subunit remain unchanged. So far the tRNA along with EF-G is not catched through any of the technique but now it is possible through experiments of Andreas H Ratke. tRNA in Pre state represents the tRNA before binding of EF-G and POST state represents after the hydrolysis of GTP of EF-G. So, compiling the results of previous section and present tRNA plays an important role as structural component and for the

translocation of the ribosome from one codon to other (Ratje et al., (2010).

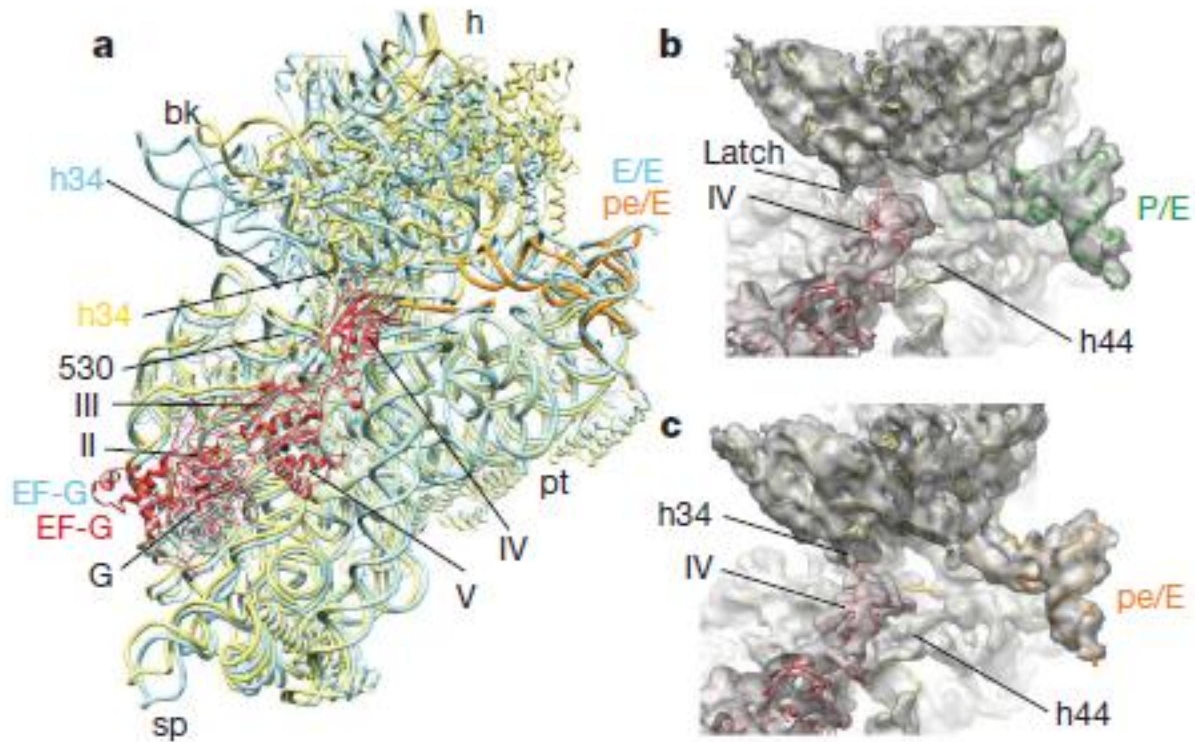

Figure: 4 Stabilisation of head swivelling in 30S subunit of ribosome by EF-G. Where h34 is the helix 34 of the 30S subunit and I, II, III, IV are the domains of EF- G and Pe/E and E/E is the hybrid forms between P and E sites of ribosome. Latch is nothing but the opening of the m-RNA channel after interaction of IV domain of EF-G and helix 34 of 16S rRNA of 30S subunit. From Ratje et al.,(2010).

IX. L3 FUNCTIONS AS ROCKER SWITCH IN COORDINATING LARGER SUBUNITS OF RIBOSOME

Majorly rRNA of ribosomes serves most important functions but some of proteins in ribosome also contribute majorly to the structural conformation and functionality of ribosome. One of the proteins includes L3 necessary for peptidyl transfer, aa- tRNA addition drug resistance, maintenance of translational frame, elongation factor binding, translocation by EF-G and also serve as binding site for ribosomal inhibitory protein. L3 domains penetrates deeper in to ribosome core and tip of which contains Trp and acts as W- finger that extends in to A-site and act as integral part of active site of Peptidyl transfer reaction (Meskauskas and Dinman (2008). aa-tRNA addition to Asite protects against base changes caused by the chemical base analogues proved by development of mutants with non-functional L3 protein. L3 protein acts as piston pump regulating the closing and opening of the gate of the A-site. Addition of aa-tRNA leads to movement of helix 90-92 to helix 89 and away from the sarcin – Ricin loop. After binding of aa- tRNA at A-site, leads to closed conformation of it, which is in open conformation earlier. Anisomycin is one of the inhibitor that acts as inhibitor of aa-tRNA binding which can be used to study the aa-tRNA protection against base analogues (Meskauskas and Dinman (2008).

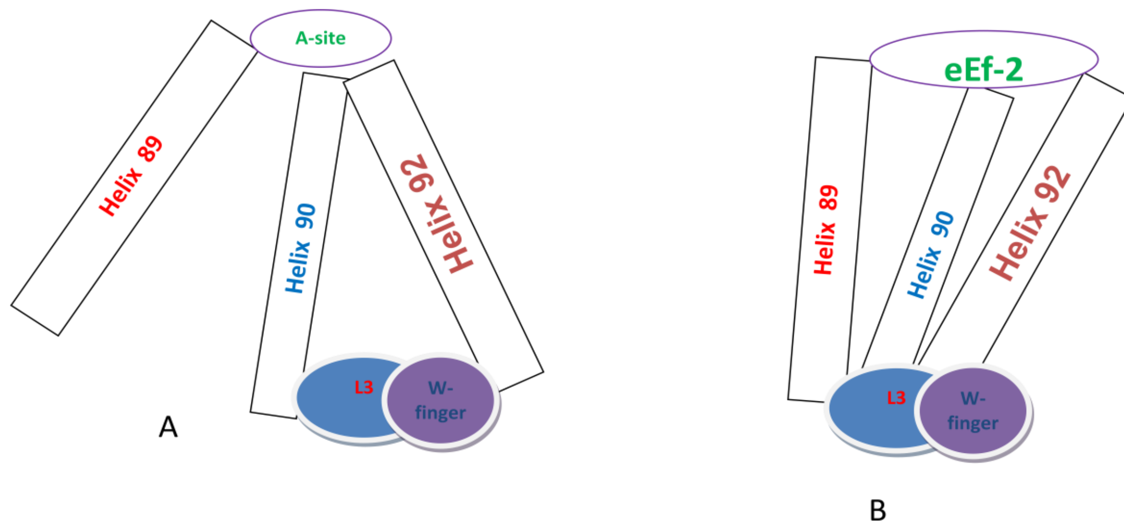

Figure: 5 rocker switch mechanism of l3 protein. (a) is the open conformation and (b) is the closed conformation after aa-trna addition. In open confirmation the helix 89, 90 and 92 are separate where as in closed conformation helix 90, 92 move to helix 89 and w-finger of l3 protein is required for this mechanism.

X. RIBOSOME REGULATES GENE EXPRESSION THROUGH ITS STRUCTURE AND HETEROGENEITY

Heterogeneity in rRNA and posttranslational modification of ribosomal proteins and interaction of ribosomes with certain mRNA sequences known to constitute specialised ribosomes that over all regulates the translation mechanism. rRNA is found to be involved in peptidyl transferase activity and in turn the rRNA folding in nucleoli requires ribosomal proteins. This clearly explains that ribosomal proteins are involved in enzymatic role of ribosome. Ribosomal proteins are encoded by nearly equal to 8000 but most of them are pseudo genes. In yeast and others the ribosomal proteins are synthesised by more than one gene but in mammals these proteins are synthesised by a single gene (Shifeng Xue and Maria Barna (2012).

The proteins are known to be tissue specific in most cases. For example in A. thaliana RPS5A is mostly expressed in dividing cells where as RPS5B is expressed during differentiation of cells. So, the ribosome machinery is unique to each tissue in context of tissue dependent function and necessicity. In addition to these, post translational modification of ribosomal proteins plays an important role in translation regulation. RPs undergoes modifications like phosphorylation, acetylation, methylation and glycosylation at hydroxyl group of serine residues. The enzyme O - Glycosyl transferase found to be associated with increase in peaks, in 60S and 80 S ribosomes clearly suggesting its role in ribosome biogenesis. In case of RPS6 deletion of five phosphorylated residues at 5' tract oligo pyrimidine (TOP) resulted in increase of protein synthesis. In addition to these modifications ubiquitination of RPl28 protein is more during S phase but it is decreased during G1 phase. RPl28 protein is found to associate with peptidyl transferase centre and has positive effect on translation.

Associated factors of ribosome also play a diverse role in fidelity of protein synthesis and ribosome biogenesis. In D. melanogaster the proapoptotic protein Reaper inhibits cap dependent translation by binding to 40S subunit and preventing AUG recognition by disrupting scanning through 48S complex. RACK1 the receptor of protein kinase C is involved in phosphorylation of initiation factor eIF6 and causing its release from the ribosome. It is also associated in miRNA dependent gene regulation and also the binding factor of integrin receptor. Ribosome associated factors also regulate functions other than translation. For example mTORC2 associates with ribosomes and regulate cell growth and metabolism.

Heterogeneity of rRNA also plays an important role in translation regulation. For example the plasmodium consists of two subsets of rRNA one during its sporozoite stage of lifecycle in mosquito and the other during life cycle in humans. During translation ribosomes scan over the 5'UTR sequence and initiation of protein synthesis occurs with the help of initiation factors in cap dependent manner. In stress conditions normally cap dependent translation is repressed in that cases the IRES elements present at 5', initiate translation without any requirement for initiation factors.

Upstream ORFs are necessary and found to be present in many human and mouse transcripts. uORFs found to repress the main frame ORF. Ribosomes that associate with uORFS near cognate non AUG codons has shown to have positive impact on translation of main frame ORF. So, totally the ribosome structural

components (proteins) play an important role in regulation of translation on whole.

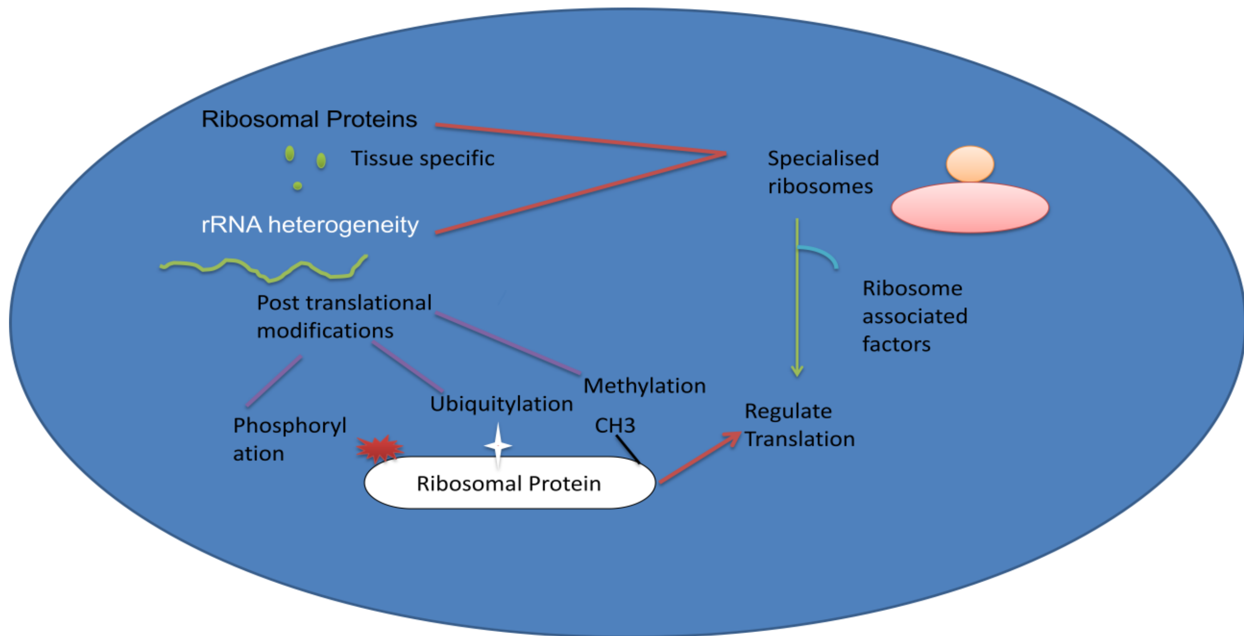

Figure: 6 over all view of Ribosome structural components in regulation of Translation. Tissue specific ribosomal proteins along with r RNA heterogeneity with posttranslational modification of proteins regulate translation by forming specialised ribosomes. The ribosome associate with some of sequences like 5'UTR on mRNA is required for regulation of protein synthesis.

XI. KSG A AS A CHECKPOINT FOR RIBOSOME BIOGENESIS IN PROKARYOTES

For ribosome biogenesis ATP dependent RNA helicases, Chaperones and ribosomal factors that depends on GTP hydrolysis and recently KsgA protein which methylates 16S r RNA is found to be required for ribosome biogenesis. KsgA found to associate with the smaller subunit and prevents the larger subunit to associate with it. It also prevents the initiation of translation unless and until the two subunits associate. KsgA protein dimethylates A1518 and A1519 of the 16S rRNA of the smaller subunit and these are found to be the universally conserved methylation sites on the smaller subunit. Other methylation sites like U2552 in larger subunit and G996 was the other conserved methylated sites in smaller subunit and requires S7 and S19. So, KsgA protein can be targeted to assess the ribosome biogenesis invitro and to combat the diseases by prokaryotes (Chand S. Mangat and Eric D. Brown (2008).

XII. DISCUSSION

Ribosomes are the one of the major components of protein synthesis although mRNA, tRNA and translation proteins are equally important. Head swivelling and formation of cliques is necessary for ribosome movement on the mRNA and to polysome configuration. Polysomes appear as beads of strings similar to the nucleosome of DNA. Ribosomes now and then fall off from the string leading to differentiation between actively synthesising and silent ribosomes. In addition to helices, domains and tRNA in ribosomes, proteins also play a major role in protein synthesis as ribosome structural components and also as initiation and elongation factors. Proteins like ksg control ribosome biogenesis through methylation of 16s rRNA. Mitoribosomes with tRNA as structural component proves the evolutionarily RNA has first place compared to protein. Post translational modifications like methylation, phosphorylation and ubiquitylation also regulate translation as those may reduce accessibility of the required proteins during translation. Proteins contribute for rRNA heterogeneity and form specialised ribosomes which may associate with ribosomal factors and regulate protein synthesis. tRNA mainly acts as adaptor molecule between the mRNA and ribosome and incorporates amino acids in to growing chain. mtoR mainly signals the ribosome biogenesis and as initiator of protein synthesis. Like this the whole system organises each step in controlled manner to achieve the final outcome the protein.

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