Available online at www.agrifarming.org

DOI: http://dx.doi.org/10.18782/2582-7146.218

ISSN: 2582 – 7146

Curr. Rese. Agri. Far. (2023) *4*(5), 40-47

Review Article

Current Research in Agriculture and Farming

Peer-Reviewed, Refereed, Open Access Journal

Inside the Ribosomal Tunnel: Life of the Nascent Polypeptide Chain

Himangana Das, Munmi Phukon and Ratna Kalita*

Department of Agricultural Biotechnology, Assam Agricultural University, Jorhat-785013, (Assam), India

*Corresponding Author E-mail: ratna.kalita@aau.ac.in

Received: 15.06.2023 | Revised: 29.07.2023 | Accepted: 13.08.2023

ABSTRACT

The ribosome, one of the largest molecular machines in living cells, is in charge of protein synthesis. Ribosomes are the birth place of proteins in living cells. It is an RNA-protein complex. The ribosomal small subunit is mainly responsible for decoding the genetic information carried on messenger RNA (mRNA) while the large subunit elongates the nascent protein chain by catalyzing the formation of peptide bonds. During the synthesis the nascent chain migrates through a tunnel in the large subunit, the so-called exit tunnel, to exit the ribosome. For a long time the exit tunnel was considered to be a passive conduction channel for the nascent protein to migrate through, however, an increasing number of studies have shown that the exit tunnel is actually involved in many co-translational activities of the nascent peptides, such as folding of the nascent chain inside the exit tunnel, translation stalling of certain peptide and antibiotic binding and resistance. Detailed insights into the architecture of the tunnel have been obtained from X-ray and cryo-EM structures of prokaryotic and eukaryotic ribosomes. Protein biogenesis factors are thought to bind to NC not before they exit the ribosomal exit tunnel, one such factor involvement with NC is the Nascent Chain Association Complex. This review provides an insight and understanding about the functionality of the Ribosome tunnel and its association with the nascent peptide chain.

Keywords: *Ribosome, Ribosome Exit Tunnel, NAC complex, Nascent polypeptide chain.*

INTRODUCTION

The living systems genetic science is encapsulated in the genome sequences i.e., their DNA (deoxyribonucleic acid). (Watson & Crick, 1993). A larger portion of these genomic sequences codes for a large number of functional proteins which carries out many functional tasks in all living organisms. The information stored in these DNA sequences are made available by transcribing these genes into mRNAs (messenger RNAs) by the process of transcription which is then translated into amino acid sequences which encodes the proteins in an organism. The RNA polymerase is an essential functional component present in all the organisms which plays a role in transcription of genetic material in to the messenger RNA and the translation of these generated mRNA is carried out by the ribosome.

Cite this article: Das, H., Phukon, M., & Kalita, R. (2023). Inside the Ribosomal Tunnel: Life of the Nascent Polypeptide Chain, *Curr. Rese. Agri. Far. 4*(5), 40-47. doi: http://dx.doi.org/10.18782/2582-7146.218

The Ribosomes are ribonucleoprotein complexes which consist of two different subunits: one large and one small. These subunits are composed of protein termed as ribosomal proteins and ribosomal rRNA (STRUCTURE AND FUNCTION OF THE RIBOSOME, 2009). These ribosomal proteins and rRNA differ in composition for both the subunits also these subunits are different in composition for eukaryotes and prokaryotes (Fig. 1). Even with the difference in their compositions for both the domains of life its function remains same i.e. in the process of amino acid peptide chain synthesis. The polypeptide synthesis happens through the process of translation from the mRNA which comprises of three following ways: Initiation, Elongation and Termination. The mRNA goes and sits at the binding site of the ribosome and the synthesis of the peptide chain starts. The binding site consists of three sites the E, P and A site.

A-site	Binding of incoming aminoacyl- tRNA
P-site	Location of peptidyl-tRNA with the associated nascent polypeptide chain
E-site	Deacylated tRNA from P-site binds to this site before leaving the ribosome

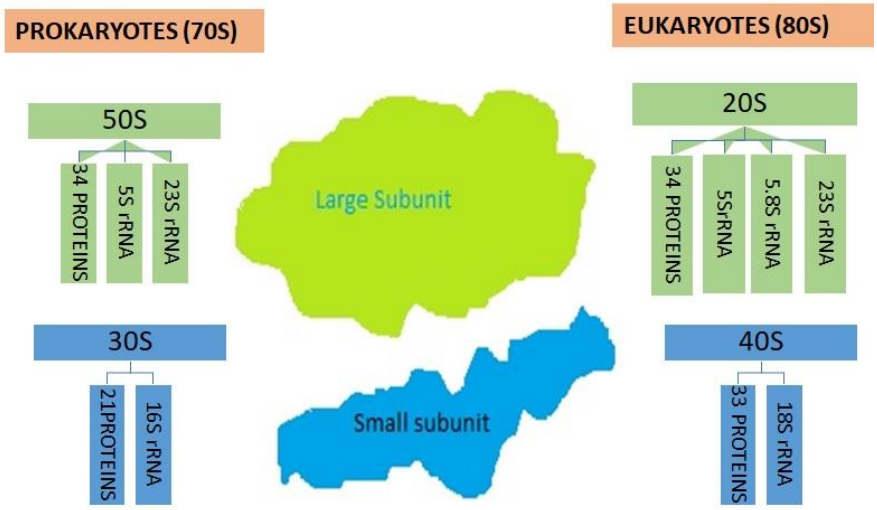

Fig 1: Diagrammatic representation of components of the Ribosome

Formation of the Peptide bond:

The molecular principles of how the ribosome catalyses peptide bond formation at the PTC by transferring the nascent peptide from the P-site peptidyl-tRNA to the A-site aminoacyl-tRNA were rapidly grasped when the 50S ribosomal subunit structure from H. marismortui was acquired at high resolution. Steitz, Moore, and collaborators' previously published structures of the 50S subunits were used in 2005 to develop a mechanistic model for peptidyl-transfer using a molecular computational approach. The authors proposed a network of hydrogen bonds that would endure through the transition state of peptide-bond formation and be pre-organized in the ground state of the peptidyl-transfer reaction. It was empirically demonstrated that the pre-existing network of hydrogen bonds explains why bond production on the ribosome is entropy-driven rather than enthalpy-driven. The extra proton that forms on the amino group of the A-site aminoacyl-tRNA on the ester bond of the P-site tRNA is removed by the 2'OH of the peptide bond, which is a restricted component of a proton shuttling pathway. The proposed method attributed the network of H-bonds that significantly lowers the activation free energy in comparison to the ground state in ribosome catalysed peptide bond formation to ribosomal RNA, namely 2'OH of A2451, as well as a number of water molecules. The 50S subunit complexes with improved resolution (~2.5Å) of the features in

the peptidyl-transfer centre were presented by Steitz and colleagues in the same year.

The proton-shuttle function of 2'OH of A76 in P-site bound peptidyl-tRNA as well as the network of H-bonds involving 23S rRNA bases and water molecules were both confirmed by this crystallographic feat. In conclusion, it can be said that Steitz and colleagues' 50S subunit structures, with the publication serving as the crown gem, played a crucial role in elucidating the mechanism by which ribosomes catalyse peptide bonding.

The Ribosome Exit Tunnel:

The ribosome, a huge macromolecular particle, aids in the synthesis of the NCs, or nascent polypeptide chains, from amino acids. The peptide bonds that are created in the peptidyl-transferase centre (PTC), which is situated in a cleft on the big ribosomal inter-subunit site, bind the amino acids together (Simonovi & Steitz, 2009). The nascent polypeptide interacts with antibodies first on the side of the large subunit that is opposite its subunit interface, according to a paper from 1982 by Bernabeu and Lake. These kinds of findings prompted additional speculations about the existence of a tunnel large enough to hold a developing polypeptide within the larger ribosomal subunit. In 1995, a cryo electron microscopic research unequivocally demonstrated the existence of such an escape tunnel (Frank et al., 1995). Numerous journals have up to this point mentioned the existence of this exit tunnel in their high resolution crystal structures of the big ribosomal subunit and 70S ribosome (Ban et al., 2000; Harms et al., 2000; & Schuwirth et al., 2001). Furthermore, a comparative cross-linking study using the ribosome of E. coli has demonstrated that as peptide length increases, the V, IV, II, III, and II domains of the 23S rRNA, which together form the ribosome tunnel trance and the walls, become increasingly and preferentially cross linked with the nascent peptide. In bacteria, the tunnel is mainly made up of conserved sections of the 23S rRNA and the ribosomal protein segments L4, L22, and L23. In eukaryotes, the area corresponding to the bacteria-specific moieties of L23 overlaps with protein L39e (Kramer et al., 2009). These observations results in accepting the fact that the polypeptide after synthesis do pass through the tunnel (Stade et al., 1994; & Jha & Komar, 2011). The ribosomal tunnel is primarily made of ribosomal RNA (rRNA), but some non-globular ribosomal proteins also contribute to its formation, according to studies on the X-ray structures of bacterial and archaeal ribosomal particles (Nissen et al., 2000; Harms et al., 2001; Schuwirth et al., 2005; Selmer et al., 2006; Lu et al., & Tenson & Ehrenberg, 2002). The tunnel is between 80 and 100 feet long, with a diameter that ranges from 10 feet at its narrowest point to 20 feet at its widest point (the exit location) (Wilson & Beckmann, 2011). Proteins L4 and L22 produce a tunnel constriction in the 50S and 60S subunits that is 30 from the peptidyl transferase centre. Because of insertions in protein L4, the constriction is narrower in eukaryotes (Fig 2) (Javed et al., 2017). Although it is unknown what function these changes between bacteria and eukaryotes serve, it has been hypothesized that the smaller constriction in eukaryotes may prevent some macrolide antibiotics from reaching the peptidyl transferase core. It is believed that the tunnel is how these antibiotics are transported to the binding site. Genetic research has demonstrated that adding six amino acids to E. coli's L4 loop confers on bacterial ribosomes a resistance to larger-size macrolides that is comparable to that of eukaryotes (Kramer et al., 2009; & Wilson & Beckmann, 2011). A polypeptide chain of 30 to 40 amino acids can fit in the tunnel at once (Jha & Komar, 2011). Compact peptide structure formation is not possible in the tunnel (Lu & Deutsch, 2005). According to a report written by Khanh and colleagues, some of the tunnel's major components must be well conserved across species since it serves some important functions (Khanh et al., 2019). Additionally, it has been claimed that there are

likely significant variations in the exit tunnel structure, with the most extreme examples having been seen in mitochondria (Davis et al., 2014; & Chiba, 2014), as well as differences in the translational and co-translational mechanisms between eukarya and bacteria (Khanh et al., 2019) (Amunts et al., 2014).

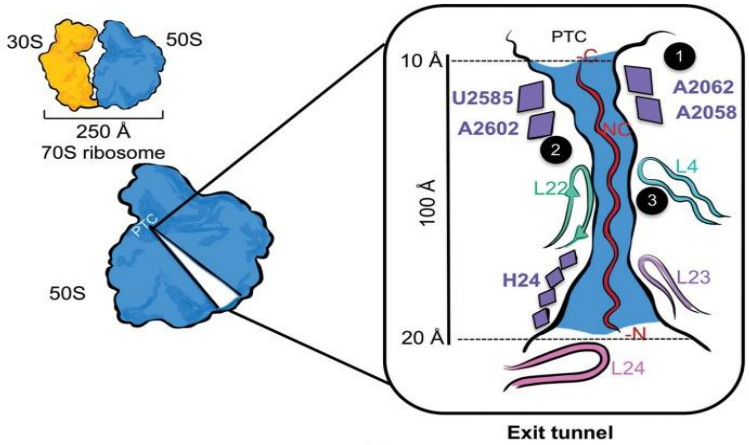

Fig 2: The Cryo- EM visualization of the Peptide Exit tunnel and the nascent polypeptide chain (Javed et al., 2017)

Features and essentiality of the exit tunnel:

The tunnel being uneven in shape and having a compact dimensions it has a multifunctional role in deciding the fate of the nascent polypeptide chain. Originally it was thought that the tunnel has no chemical properties which are capable of facilitating its involvement with the NC but with the progress in studies on the exit tunnel numerous evidences have been gathered about its diverse functional role and its chemical nature (Zimmerman et al., 2014). Earlier it was widely believed that the ribosome tunnel is merely a gateway for the passage of the nascent chain however later it was revealed with many evidences that it actively participates in the nascent chain folding (Jha & Komar, 2011), translation arrest and cellular signaling (Zimmerman et al., 2014). Although tertiary folding of whole protein domains such as folding domains as large as IgG domain is not feasible in the exit tunnel due to its constrict structure the tunnel it is feasible for the formation of small elementary units such as alpha-helixes (Voss et al., 2006). Cryo-EM studies have been used to directly visualize NCs within the ribosomal tunnel including NCs with high alpha-helical propensity (Wilson & Beckmann, 2011). Studies of Fluorescence resonance energy transfer (FRET) has shown that a TM signal anchor (SA) sequence is compacted in a manner consistent with alpha-helix formation in all regions of the ribosomal tunnel (Woolhead et al., 2004). Also in the same study it was seen that upon exiting the tunnel the compaction of TM NC was lost which clearly indicated that the tunnel has a vital role play in the alpha helix conformation stabilization. Also the formation of these helixes inside the tunnel is different for different NCs (Wilson & Beckmann, 2011). Another important job of the ribosome tunnel is in mediating translational regulation. Many of the leader peptides prompt translational stalling to regulate translation of some important downstream genes (Lovett & Rogers, 1996; Tenson & Ehrenberg, 2002; & Wilson & Beckmann, 2011). Studies on chief peptides like *tnaC, secM, mifM, ermCL, and catA86L* have been carried out understand this regulation process like for example stalling during translation of the *tnaC* peptide results in blocking the Rho transcription terminator binding sites by the ribosome leading to translation of the downstream tnaAB genes (Wilson & Beckmann, 2011). Wilson and Beckmann have also stated that all this stalling

of these major peptides requires a specific interaction between some specific residues present in the nascent chain and some components of the exit tunnel. Insight into the eccentric conformations and pathways of the nascent chain regulation in the ribosome tunnel as well as the interactions of the NCs and the ribosome tunnel walls have been done by studying the Cryo-EM structure of the eukaryotic ribosome as well the bacterial ribosome stalled during translation of peptide like AAP & CMV (Bhushan et al., 2010), TnaC (Seidelt et al., 2009), SecM (Bhushan et al., 2011), tnaC (Stel et al., 2021) and VemP (Kolář et al., 2022). Lastly one more function of the ribosome exit tunnel is antibiotic binding and resistance. The exit tunnel consists of high affinity pocket for antibiotics of macrolide, ketolide and streptograminB families. These pockets are situated at the upper portion of the tunnel i.e., below the PTC and above the construction site of the tunnel (Figure 3) (Yonath, 2005). Studies have shown that the Macrolides and ketolides group of antibodies bind to this high-affinity pocket either in vacant or to translating ribosomes, carrying short nascent peptide (Andersson & Kurland, 1987; Tenson et al., 2003; & Allen, 2002). The central macrolactone ring of these drugs forms hydrophobic interactions with the rRNA residues 2057, 2611, and 2058 that form the tunnel wall on the side of the PTC A site (Kannan & Mankin, 2011). Inhibition of protein synthesis by the macrolide antibiotics is done by obstructing the growth of the nascent peptide chain.

CONCLUSION

The development of the peptide chain through the exit tunnel is stopped as the developing peptide approaches the location of drug interaction after the synthesis of the first few amino acids, and the tRNA separates from the ribosome (Otaka & Kaji, 1975; Menninger & Otto, 1982; & Kannan & Mankin, 2011). The tRNA's rate of dissociation is dependent on its length, sequence, and interaction with the peptide exit tunnel, whereas the drug's rate depends on both of its structural and binding specificities.

Acknowledgement

I would like to sincerely thank my co-authors for their support and kind gesture to complete this manuscript in time.

Funding: NIL.

Conflict of Interest:

There is no such evidence of conflict of interest.

Author Contribution

All authors have participated in critically revising of the entire manuscript and approval of the final manuscript.

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