

## RNA-Binding Proteins in *Escherichia coli*

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### Abstract

In *Escherichia coli* RNA-binding proteins (RBPs) have a fundamental relationship with gene expression. All the RBPs are combined with uniquely structured domains that target specific arrangements of RNA sequences and/or structures. The interaction between the RBPs and the RNAs generates ribonucleoprotein complexes (RNP). The significant diversity of those domains in *Escherichia coli* allows the cells to employ them in varied physiological pathways. In *Escherichia coli*, RBPs control many cytosolic events including protein translation, mRNA decay, ribosomal, and transfer RNA maturations for gene regulation as well as viral infections, down/upshifting temperature, altered osmotic pressure, reduced nutrient response among other stimuli. Therefore, Understanding the specificity of RBPs and their various functional domains toward their target RNA would introduce valuable tools for biological research as well as potential therapeutic strategies. In this review, we address the well and newly characterized RBPs, but not metabolic enzymes, that *E. coli* express including examples of different stress conditions.

**Keywords:** RNA Binding; RNase; Metabolic Enzymes; CRISPER; Ffh

### Introduction

A significant proportion of the genome in bacteria is dedicated to the production of various forms of RNA-binding proteins (RBPs) that are required for cellular RNA activities. Single- or double-stranded RNAs are always found associated with one or more proteins in the form of a ribonucleoprotein (RNP) complex [1-3]. For many years, research has been analyzing various RNA-protein interactions to understand and characterize in a systematic way how RBP targets a specific RNA from a mixture of structurally varied RNAs. However, this process still cannot be generalized [1]. The basic structure of RBPs is modular in nature, consisting of domains for RNA binding, domains for protein interaction and domains for sub-cellular targeting [2]. The RNA binding domain contains an RNA recognition motif (RRM) that is usually present in multiple copies within the RNA binding domain [1]. This domain is responsible for targeting a specific RNA and the affinity for binding of that RNA to the protein. It is now clear that RBPs tend to combine multiple copies of a similar RNA-binding domain for a higher avidity to a specific RNA target [1,2]. In some cases, RNA binding domains can be highly specific in recognizing the binding sites within the RNA pseudoknot structures, while in others, RNA binding domains exhibit high affinity to simple RNA hairpins and/or short RNA sequences [4]. In these cases, the RBP's main and side chains form hydrogen bonds with the target RNA which has been shown to contribute to protein-RNA sequence specific binding. The RRM N- and C- terminus may play a role toward different and unique RNA structures as in the case of clustered regularly-interspaced short palindromic repeats, or CRISPR [1,2].

RBPs control many cytosolic events including protein translation, ribosomal RNA (rRNA) assembly, transfer RNA (tRNA) maturation, and gene regulation [1,2,4]. In *E. coli*, RBPs are primarily associated with mRNAs to control all cytosolic processes involved in mRNA metabolism including maturation, transport, localization, and stability to ensure efficient protein synthesis and mRNA decay [3,4]. RBPs

also show a specificity in *E. coli* for a specific untranslated region (UTR) of mRNA in conditions affecting cellular proliferation, viral infections, down/upshifting temperature, altered osmotic pressure, and nutrient stress among other stimuli [3,5]. Furthermore, RBPs are also involved in the metabolism of other types of RNAs that are known as untranslated or small RNAs (sRNAs) [5]. They are abundant in the cytoplasm with diverse structural, catalytic, and regulatory functions [5,6]. These sRNAs are smaller in size than the rest of the cellular RNAs and exist in various structural folding patterns [6]. They are normally non-coding, functional RNAs that cooperate primarily through base pairing with mRNAs to maintain mRNA stability, since their half-life in *E. coli* ranges from 2 to 25 minutes; sRNAs also regulate the translation of certain mRNAs [5,6].

Understanding RNA target selection and RNA-protein specificity would be key to generating new tools and applications in manipulating RNA and potentially generating new therapeutics. The mechanism of RNA folding to generate secondary and tertiary structures remains to be obscure, although it is believed that RNAs require cytosolic proteins to generate those structure and achieve their final fold [1,3,7]. Our detailed knowledge of DNA-protein interactions has created many important tools for DNA manipulations. Some examples include the zinc-finger (ZF), TALNT, and CRISPER/Cas9. This has been very difficult in work involving RNA because the AU/GU sequence specificity makes RNA-protein binding prediction difficult. In this review we will highlight some of the structural features of the well characterized RBPs in *E. coli* in order to develop more hypotheses related to the specificity and predictability of protein-RNA interactions.

### Host factor (Hfq) in *E. coli*

In the 1960s, the discovery of the thermostable Host Factor (Hfq) in *E. coli* renewed interest in RNA research. Hfq, also known as (HF-1), is a monomeric polypeptide that is 102 amino acids long [8-10]. The *hfq* gene in *E. coli* is part of an operon that regulates different genes including the *hflKC* genes (ATP-dependent protease regulators) [10-12]. The complexity of this operon is regarded in some studies as a super-operon because it contains a minimum of three heat-shock promoters ( $\sigma^{32}$ -dependent) and four other promoters ( $\sigma^{70}$ -dependent) [11,13]. The function of the heat-shock promoters is to maintain a proper concentration of Hfq within the cell during stress that normally would be between  $1.6 \times 10^4$  and  $5 \times 10^4$  molecules [11,13].

Hfq performs a variety of functions in *E. coli*, including nitrogen fixation, cell virulence, and modification of mRNA structure [14,15]. *E. coli* Hfq is also capable of interacting with different types of RNAs, including sRNAs [12,13]. The chromosomal DNA of *E. coli* expresses over 50 members of sRNAs. These sRNAs have neither a specific function in protein coding nor do they participate in other functions like rRNAs or tRNAs [13]. However, many of the sRNAs play an important role in post-translational regulation through the activity of Hfq to control the targeted mRNAs after transcription [9]. Therefore, in the case of sRNAs, Hfq facilitates the pairing of target mRNAs with their respective sRNAs; implicating two possible roles played by Hfq in stimulating the pairing [13]. The first one is that the binding of Hfq to a specific target might alter or stabilize the structure of the RNA to make the complementary sequences available for the purpose of sRNA pairing. The second one involves the hydrophobic interaction between two Hfq hexamers after each is bound to their respective RNA [16,17].

Hfq appears to have the ability to regulate the rate of its own gene expression through the reduction of its mRNA stability [9]. This clearly indicates the existence of an auto-regulated feedback mechanism that regulates Hfq synthesis. Hfq was originally identified as a host factor required for the successful replication of the Q $\beta$  bacteriophage (Q $\beta$ ) in *E. coli* [11]. Hfq binds to two different sites on the Q $\beta$  RNA around ~4200 nt to initiate maximal replication [9,10]. Research has shown that the maximal replication is generated by the RNA loops that are created when Hfq is bound to the two sites on Q $\beta$  RNA [9,10]. *In vitro* studies showed that the Hfq hexamer under certain conditions (0.2 M NH<sub>4</sub>SO<sub>4</sub> solution) gives a selective binding to specific sequences that are A/U-rich [10].

Finally, some studies have shown that Hfq is also directly engaged in alteration of *ompA* mRNA, a highly stable mRNA that encodes major outer membrane proteins of *E. coli* [10,18]. The stability of this transcript is inversely related to the rate of *E. coli* growth [19]. The cleavage efficiency at 5' UTR specifies the changes in the *ompA* mRNA turnover that are induced by the changes in rate of growth [19]. Research has also shown that Hfq, in the slow-growing cell extracts, is associated with 5' UTR of *ompA* mRNA and played a vital role in controlling its stability [19,20]. This specific region contains a stem-loop structure that operates as a stabilizer as well as recognition sites for RNase [20]. During the stage of exponential growth, when translation occurs, the 30S ribosomal subunit associates with *ompA* mRNA to protect it from degradation [19,20]. During the stationary phase, Hfq competes with the 30S ribosomal subunit to prevent a ternary initiation-complex formation.

### Carbon storage regulatory system (Csr)

In order to be responsive to environmental changes, several processes are used by *E. coli*. The carbon storage regulatory system (Csr) is one of the systems that provides a gene control pathway, linking complex global regulatory networks [21]. Base pairing with mRNAs allows efficient functioning of most sRNAs, like CsrB RNA for example, which is a sRNA that acts through confiscating an RNA-binding regulatory protein of *E. coli* [21,22]. Most cellular activities such as carbon metabolism, extracellular product generation, cell motility, formation of biofilm, sensing of quorum and/or pathogenesis are controlled and regulated by the global Csr regulatory circuits [22]. The Csr family encompasses active members that spread widely among eubacteria for global regulatory circuits [22,23]. A homodimeric RBP (CsrA) is the main element of the Csr system and the homologous repressor of secondary metabolites (RsmA) systems [21,24].

CsrA is a 61-amino acid long dimeric protein that is a crucial element in activating glycolysis, acetate metabolism, and biosynthesis of flagellum in *E. coli*. It suppresses gluconeogenesis, biosynthesis of glycogen and catabolism, and formation of biofilm [21]. CsrA inhibits the regulation of important enzymes in carbohydrate metabolism such as glycogen synthesis, glycogen branching enzyme, glycogen phosphorylase, and ADP-glucose pyrophosphorylase. This takes place by binding to a specific sequence inside the coding area that allows CsrA to destabilize the target mRNAs including the ribosome-binding site, arrests mRNA translation, and leads to its degradation [25,26]. Studies indicated that CsrA targets different sites on each of the mRNA targets, but those sites appear to contain the same nucleotide sequence (GGA). In other studies, SELEX (systematic evolution of ligands by exponential enrichment) confirmed that a 100% recognition and binding of CsrA occurs on a RNA hairpin that contains the sequence "RUACARGGAUGU" motif where the GGA sequence is located in the loop [23,27]. On the other hand, CsrB and CsrC are two untranslated RNAs that regulate intracellular levels of CsrA [27]. Among all known cases of *E. coli*, CsrB and CsrC are capable of deactivating CsrA by binding to CsrA in multiple protein dimers [21,22,27]. Therefore, a highly repetitive sequence component is presented in the loops of predicted CsrB/C hairpins and mediates CsrA binding to both CsrB and CsrC.

CsrB is a 366 nt sRNA that contains ~22 CsrA binding sites and estimates show that this sRNA is able to sequester ~9 CsrA dimers [24,27]. CsrC is the smaller sRNA with 245 nt and is the cooperative partner [23,26]. Although both CsrB and CsrC do not share structural similarity, both are activated by CsrA [23,26]. The levels of CsrB/C are main determinants of cellular activity of CsrA and are co-expressed with CsrA in equilibrium between CsrB/C and CsrA-controlled mRNAs [27,29]. In order to form a bridge known as a "bridge complex", CsrA dimer binds to the surface of both CsrB and CsrC RNAs [27,29]. On the other hand, as CsrA's expression level is controlled research shows that the expression of CsrB and CsrC is also controlled by RNases [29]. More recently CsrD has been added to this family of small regulatory RNAs. CsrD is yet to be characterized and is currently classified as an undefined regulator, although it is thought to be a direct target of CsrA [21,27].

### 6S RNA

The 184 nt 6S RNA is a noncoding RNA that is part of an RNA polymerase (RNAP) protein complex in *E. coli* [30,31]. The direct interaction of 6S RNA, explicitly long-lived complexes, and the housekeeping multi-subunit enzyme RNAP coordinate a number of physiological activities [32,33]. In one study, the *E. coli* 6S RNA was proposed to act as a template for RNA polymerase leading to the *de novo* generation of sRNAs [34]. Using various sigma factors, RNAP mediates gene expression, however, most of the sigma factors are controlled by environmental changes [32]. Thus far *E. coli* has been shown to express up to seven different characterized  $\sigma$  subunits. These include the  $\sigma^{70}$  which is an important growth factor,  $\sigma^S$  which is expressed during stationary phase of growth and other stress conditions, and five other  $\sigma$  subunits that are utilized under different environmental conditions [31]. In the late stationary phase in *E. coli*, 6S RNA is abundant (reaching ~10 000 copies per cell) [35,36]. At certain  $\sigma^{70}$ -promoters, transcription of  $\sigma^S$ -dependent genes is up-regulated along with 6S RNA-dependent down regulation of transcription triggered by the stationary phase and other stress responses in *E. coli* [32,35,37].

RNAP is composed of  $\alpha$ ,  $\beta$ , and  $\beta'$  subunits together representing the core RNAP that is responsible for the elongation stage of the transcription [36]. However, the transcriptional initiation and the promoter recognition is controlled by the holoenzyme that is composed of the core and  $\sigma$  subunits [36,37]. Studies show that the core RNAP binding affinity depends on the specific  $\sigma$  subunit involved because each  $\sigma$  subunit differs in its binding specificity to RNAP during gene regulation [35,37]. Studies also indicate that the secondary structure is significantly important for interactions between 6S RNA and  $\sigma^{70}$ -RNA polymerase [30,34,35].

Open complex is an area that surrounds the start site of transcription where the DNA promoter is melted at the time of transcriptional initiation [38]. In fact, 6S RNA is present near the DNA binding subunit as 6S RNA can be cross-linked to the  $\sigma^{70}$  subunit within RNAP [35,38,39]. Recently, it has been demonstrated that 6S RNA might bind inside the active site of RNAP [40]. Indeed, as 6S RNA can act as a template in synthesis of RNA that leads to the production of about 14 - 20 nucleotides, it takes active participation in the active site. It also associates with transcription of a DNA promoter template that is bound in the active site of RNAP, blocking the accessibility of any DNA promoter to the RNAP's active site [40,41].

## RNA Degradosome

### RNase E

*E. coli* expresses a 1061 amino-acid-residues protein RNase E, which is considered to be the largest among *E. coli* proteins [42]. RNase E is a 5'-3' endoribonuclease that is composed of two distinct domains each having distinct roles - the N-terminal homotetramer domain (NTD) contains 530 residues, and the C-terminal RNA degradosome domain (CTD) contains 531 residues [42]. RNase E is an arginine-rich protein where the arginine rich region is located between residues 601 - 700; a region that facilitates RNase E binding to substrate RNA. The residues between 701 and 1601 is a segment for the enzymatic interaction including polynucleotide phosphorylase (PNPase) and ATP-dependent helicase (RhlB) [42,43]. The first 395 amino acids are indicated as single-strand-specific endonuclease. In addition, CTD is not involved in the process of any catalytic activity.

The multifunctional RNase E is required for cellular viability as well as in different cellular events such as processing tRNA, catalytic RNA present in RNase P, 9S ribosomal RNA, and transfer-messenger RNA (tmRNA) that helps in the release of decayed ribosomes [44]. One of its main roles is regulating the number of transcripts of enzymes involved in glycolysis [44,45]. Interestingly, RNase E targets its own mRNA and this auto-regulation is crucial in balancing its enzymatic activity as well as the activity of all of its targets [46].

Cleavage site preference for RNase E is in A/U rich segments in single strand RNA [42,47]. The relation of RNase E and additional RNA-processing enzymes that are present in the complex having various kinds of RNA degradosome components makes the function of RNase E present in *E. coli* smoother [43,47]. RNase E is 5'-end-dependent that demonstrates a specificity for RNAs with 5'-monophosphate ends. PNPase, glycolytic enzyme enolase and RhlB are the basic elements of this complex that participate in 3'-5' degradation pathway [42,44]. Recently, research has showed that the NTD of RNase E contains a 'pocket' that employs the 5'-monophosphate group of RNA substrate that prepares the active site for cleavage [43]. According to Callaghan A, *et al.* forecast of composition analysis revealed that the CTD is mainly unstructured, but it contains four small sections ranging from 10 to 80 residues that have high structural inclination (RISP or regions of increased structural propensity) [46,47].

### RNase G

In *E. coli*, Ribonuclease G (RNase G) is an 5'-3' or 5'-end dependent endoribonuclease that targets RNA for maturation and degradation, since it is an important mechanism to control gene expression [48]. In 1999, studies renamed CafA to be RNase G that shares almost 50% of RNase E catalytic domain topology at the first 470 residues [49]. RNase G is 489 amino-acid-residues protein, and in *E. coli* the *rng* gene codes for RNase G that belongs to a family called (RNase E/G) named after RNase E and RNase G [50]. Research indicated that intracellular RNase G expression level is very limited, which suggests that its absence does not actively affect *E. coli* growth rate as RNase E activity [51]. Although both RNases show some structural similarities, they may have distinctive roles within *E. coli* [49,51]. RNase E requires both N- and C-terminal domains for its activity, whereas RNase G does not need the C-terminal for its activity [50]. RNase G and RNase E show the ability to cleave the same RNAs, but at different recognition sites from each other [50]. Research also showed that RNase G enrolls major intracellular involvements in the maturation and degradation of RNAs including 16S rRNA and *adhE* mRNA respectively [48,51]. It recognizes and cleaves the substrate at ssRNA 5' monophosphate group that is AU-rich [50,51]. RNase G is considered to be newly discovered and the research is ongoing to identify the possible structural and functional roles.

### RNase I

In *E. coli*, Ribonuclease I (RNase I) is considered to be the nonspecific endoribonuclease because it does not require any divalent cations and cleaves all phosphodiester bonds in the rRNA [52,53]. Its enzymatic activity generates 3'-monophosphate and 5'-hydroxyl of a single-stranded RNA. This 357 amino-acid containing endonuclease was the first to be extensively characterized to degrade any RNA but not DNA, despite the fact that it binds to DNA [52]. Research indicated that RNase I has a tendency of recognizing substrates and cleaving at any one of the four homopolymers site of the RNAs (poly (A), poly (U), poly (G) poly(C)) [53,54]. Studies showed that RNase I plays a role in bacterial immune systems by degrading any invading foreign RNA as well as degrading RNAs under specific conditions for bacterial survival such as temperature elevation, lack of proper nutrition, and exposure to different ions during cell growth [52].

It is believed that RNase I is a family of different enzymes that carry identical enzymatic activities in degrading stable RNAs but are quite distinct in their biological structures [53]. Studies showed that RNase I is involved in many applications, primarily, RNase I found in *E. coli* has a huge involvement in ribosomal profiling that includes initiation, elongation and termination based on the observation that RNase I mostly interacted with the 30S ribosomal subunit [53,54]. It is believed that RNase I is localized in the periplasmic space near the bacterial surface [52]. Recently, it has been implicated in many eukaryotic applications, such as a biomarker for the pancreatic adenocarcinoma cases.

### RNase III

In *E. coli*, maturation and elimination of RNAs is a crucial step and mostly requires the involvement of Ribonuclease H (RNase III) [55]. RNase III is an endoribonuclease that cleaves a larger double-stranded RNA (dsRNA) into fragments (12 and 15 bp) containing 3'-end overhang composed of two nucleotides [56]. RNase III forms a homodimer that is about 25 kDa each, the dimerization occurs between the ribonuclease domains [55]. Research shows that the C-terminus is responsible for binding to the dsRNA and between the N-terminus and C-terminus domains of RNase III; about ~7 amino acids linker connects both domains [57]. Both domains contribute evenly in recognizing and hydrolyzing the substrate of each RNA strand of the dsRNA [55,56]. RNase III(E38A). Early studies showed that *rnc* is the gene for RNase III that is located between *purL* and *nadB* genes in the chromosomal DNA of *E. coli* [55].

In fact, RNase III is considered to be a family of three classes. Class I, which represents eubacteria, archaeobacteria, and yeast, is composed of a single ribonuclease domain and a dsRNA-binding domain [56]. Class II represents eukaryotic including Drosha family, and class III, which represents enzymes that are related to the Dicer family [56,57]. Members of both classes essentially share the same structural topology, which differ from class I by having two ribonuclease domains instead of one. Class III is the largest because it carries two extra domains which are a DEAD box helicase domain and a PAZ domain [56]. The class I of RNase III is involved in the maturation process of RNAs including rRNA and snRNA but not miRNA because miRNA is class II specialty. Class III RNase III contains enzymes that mostly interact with RNAi [56,57].

In *E. coli*, the RNA binding domain of RNase III contains a signature motif in a shape of  $\alpha\beta\beta\alpha$  fold which gives a high affinity to dsRNAs [56,58]. The RNase III-dsRNA interaction includes other factors that work as site-specific substrates including four bp of Proximal Box (PB) and two bp of the Distal Box (DB) [57]. Those factors showed *in vitro* study that they have the ability of higher specificity targeting RNase III to the cleavage site [57]. The catalytic domain of RNase III in *E. coli* contains the amino acid sequence (ERLEFLGD) known as signature motif to form the active site of the enzyme with the addition of divalent cation ( $Mn^{2+}$ ) [56,58]. Research involving *in vivo* experiments showed that RNase III cleaves a dsRNA at specific site and manner requiring proper recognition by PB and DB for efficient functionality [55,56,58]. RNase III(E38A).

### RNase P

In *E. coli*, Ribonuclease P (RNase P) is a site specific endoribonuclease that is 119 amino-acid-residue protein [59]. It is involved mainly in the biosynthesis and maturation of tRNA from the precursor tRNA (ptRNA) pathway [59,60]. RNase P is also involved in other RNA-protein interaction including 4.5S RNA, tmRNA, and some small phage RNAs to generate 5'-monophosphates and 3'-hydroxyls products [61]. RNase P is characterized as one-protein and one-RNA subunit that is simpler than ribosomal profiling because a ribosome is also considered to be a ribonucleoprotein (RNP) [59]. Studies showed that RNase P requires 30S prRNA as a cofactor to cleave tRNA at the 5'-end. The substrate is composed of multiple elements including a -CCA sequence at 3'-end on the pre-tRNAs for an efficient cleavage as well as tRNA maturation [60,61]. On the other hand, studies showed that the absence of a -CCA sequence from the tRNA showed no major effects on the maturation of tRNA [62]. In *E. coli*, RNase P is characterized as an enzyme that has the ability of recognizing and cleaving a substrate that is composed of 4 bp and connected by a tetra loop with a single-stranded CCAC sequence at the 3'-end and a short 5' overhang, which is considered to be the smallest substrate [62].

The catalytic mechanism of RNase P requires divalent ions in the form of  $Mg^{++}$  [59]. Although the divalent ions are not highly important for the catalytic activity, they are important for the correct RNA folding [59,60]. In *E. coli*, the proper catalytic activity of RNase P on RNA structural molecules is generated by two domains (C and S domains) [59,60,62]. The S domain involved in the recognition of pre-tRNA substrate through TCC stem-loop, D-loop, and the acceptor stem allow the C domain to bind and therefore initiate the enzymatic activity [59,60,62]. The 5' leader sequence recognizes the substrate and also functions by enhancing divalent ions activity as a cofactor in the active site while RNase interacts with the 3' terminal sequence (-CCA) of pre-tRNA [62]. In fact, pre-tRNA in *E. coli* does not necessarily carry the -CCA sequence but the -CCA sequence is always added post transcriptionally for the RNase P activity [62]. The -CCA sequence is not involved in the recognition process, but rather in the cleavage process. In addition, it is believed that there is cooperative cleavage activities between RNase P and RNase E because studies showed that RNase E creates a substrate that is recognized by RNase P at the 3'-end of mRNAs [60-62].

## RNase H

Ribonuclease H (RNase H) is a 5'-3' endoribonuclease that recognizes and degrades only the RNA strand of RNA/DNA hybrids in the presence of divalent cation [63]. RNase H actively cleaves RNA and creates products of 3'-hydroxyl and 5'-phosphate. This prevents any unnecessary *E. coli* chromosomal replication at different sites than the *oriC* gene site. It is also used in many molecular biological applications RT-PCR [64]. RNase H in *E. coli* is a family of two types, RNase HI and RNase HII [63-65]

*E. coli* express RNase HI by about 95% and 5% of RNase HII [66]. RNase HI is 5' - 3' endoribonuclease and 155 amino-acid-residue protein that is encoded by *rnhA* gene [67,68]. It recognizes certain ribonucleotides to start its catalytic and cleavage activity on RNA substrate in the presence of  $Mg^{2+}$  [67,68]. RNase HI generates 5'-phosphate of a ssRNA cleavage, but when the RNA forms a duplex with the DNA it would be impossible for RNase HI to cleave all the RNA's phosphodiester bonds [66,67]. RNase HI distinguishes between two different substrates - RNA primers and R-loops [67]. RNA primers are normally created while DNA is synthesized and the R-loops are generated during the annealing processes of RNA/DNA hybridization [67]. Although the exact physiological functionality of RNase HI has not been determined yet, it is believed that it is involved in DNA replication and repair. In fact, research showed that RNase HI actively reacts on stable transcripts that is associated with R-loops [63,67]. The C-terminal amino acid sequence comparison between RNase HI and other enzymes showed that RNase HI may act as a retroviral reverse transcriptase (RTs) due to its structural similarity, which suggests a functional similarity as well [63,67]. In addition, RNase HI of *E. coli* and RNase HI of *T. thermophilus* express almost an identical structural topology with a few structural shifts that are suggested to be due to the mismatched amino acids in the hydrophobic core [67]. The absence of RNase HI in *E. coli* would cause DNA replication suspension, on the other hand, the complete loss of RNase HI functionality is important to regain RecBCD functionality [67]. RecBCD is an enzyme that has the ability of initiating a DNA damage repair during replication, oxidation or radiation affects, or through other stress effects.

Although, *E. coli* utilizes RNase HI more than RNase HII, RNase HII is thought to be more universal of the RNase H family. RNase HII is a 5' - 3' endoribonuclease that is encoded by *rnhB* gene in *E. coli* and is composed of 198 amino-acid-residues [66]. RNase HII recognizes RNA substrate from the DNA substrate in the duplex and cleaves the RNA strand through single ribonucleotides [64]. Research suggests that RNase HII acts as a monomer in *E. coli* that prefers  $Mn^{2+}$  over  $Mg^{2+}$  for its activity [64]. Although the specific functionality of RNase HII has not been determined yet, the absence of RNase HII effective activity is involved in physiological and biological processes that are crucial for the cell growth. It is important to mention that RNase HI and HII may have similar functionality, as *in vivo* studies showed that both have the ability of recognizing the same substrate.

## The most important exoribonucleolytic RNases in *E. coli*

### RNase PH

In 1988, RNase PH was discovered due to its phosphorolytic activity against natural precursor tRNAs and rRNA analogues [69]. In contrast, RNase E digests at the 5'-end during the processing of tRNA precursors. This means RNase PH utilizes inorganic phosphate instead of water as a nucleophile to enhance the catalytic activity of RNA degradation [69]. *E. coli* RNase PH is a 3'-5' exoribonucleases which trims residues downstream of the -CCA series in the tRNAs and rRNAs analogues [69,71]. Based on the function of other exo-RNases during the processing of RNA, the action of exo-RNases assimilates structured domains [69,71]. It is required for eliminating the series of precursors because base-paired residues are near the 3' ends of mature tRNAs and rRNAs [69,71]. Therefore, it is one of the most effective exo-RNases that has the ability to digest close to structured regions. RNase PH also engages up to 40 free nucleoside diphosphate residues on the 3' terminus of RNA molecules.

RNase PH is a 238 amino-acid-residue protein, and is of great importance for cellular viability [69]. The active RNase PH forms a homo-hexameric complex structure, composed of three dimers of ribonuclease PH which are RNA-binding domains (KH and S1) and  $\alpha$ -helical domain [69,72]. RNase PH carry helix-turn-helix motifs that allow the nonspecific binding to DNA. RNase PH is encoded by a *rph* gene that show a great number of RNase PH homologues in some microorganisms such as RNase PH-like proteins, hence a conserved role of this enzyme in RNA metabolism has become apparent [72]. Research indicates that any mutation to RNase PH results in growth temperature-

sensitivity [69]. Research also suggested that RNase PH in some cases is considered to be an important growth factor, particularly in cells that lack exoribonucleases such as RNases II, D, BN [69-71].

### Polynucleotide Phosphorylase (PNPase)

In *E. coli*, PNPase consumes phosphate in order to phosphorylate RNAs of single-stranded polyribonucleotides processively and produce nucleoside diphosphates in the form of cleavage products [73,74]. In 1951, PNPase was characterized as a bi-functional, nuclease possessing exoribonucleolytic and endoribonucleolytic activities on RNA [74,75]. The activity produces polyadenylated mRNAs in *E. coli*, which signals degradative enzyme in the process of a rapid exoribonucleolytic degradation [74,75]. PNPase is a cytoplasmic protein in *E. coli* containing a small domain that has the ability to interact with the degradosome enzyme (RNase E) [76]. The physical interaction that is involved between the glycolytic enzyme (enolase) and RNase E requires a phosphosugar stress agent upon the communication between RNA degradosome and central metabolism [75,77]. PNPase participates in the quality control of ribosomal RNA, the decay of bulk RNA, the turnover of small regulatory RNA, and cold shock response in *E. coli*. Although the main involvement of the enzyme is RNA's processive phosphorolytic degradation, it is also engaged in ribonucleoside diphosphates' reverse polymerization [75,77].

PNPase of *E. coli* is a homotrimer composed of 711 amino-acid-residues, the N-terminus contains an  $\alpha$ -helix domain and the C-terminus contains domains responsible for RNA binding [77]. Both RNA-binding domains (KH and S1) are highly reactive to RNAs [75,78]. PNPase reduces its enzymatic activity by the elimination of any one or both RNA-binding domains [74,77]. According to Nurmohamad S (2009), the activity of PNPase is physiologically influenced by citrate in significantly different levels of concentrations [78]. PNPase's vestigial active site is a potential allosteric pocket that reacts to metal-free citrate. Studies show that ATP inhibits *E. coli* PNPase activity which leads to an emphasis on the linkage between RNA degradative machines and central metabolism for cell viability.

### RNase BN (RNase Z)

To remove the -CCA residues from precursor tRNA, *E. coli* makes use of RNase BN. The removal of (-CCA) specific sequences from tRNA is required for its functionality [79]. RNase BN is a 305 amino-acid-residue protein that has the capability of being an exoribonuclease as well as an endoribonuclease in the cellular cytoplasmic compartment [80]. Therefore, tRNA precursors maturation activity are initiated and simply inhibited according to the presence of RNase BN. RNase BN trims (-CCA) from tRNA precursors depending on the nature of the substrate, and the divalent cation on tRNA precursor 3'-terminal structure. That means RNase BN can be activated as exoribonuclease in the presence of the  $\text{Co}^{2+}$  cofactor whereas  $\text{Mg}^{2+}$  cofactor stimulates the activity of endoribonuclease activity of RNase BN [80]. RNase BN enzymatic activities occur through a very narrow catalytic channel that can only fit a single-stranded RNA [79]. At the ends of the transcripts, the channel possesses an exoribonuclease enzymatic activity only under certain conditions [79,81]. For instance, exoribonuclease enzymatic activities are more often involved in degradation processes where endoribonucleases tend to participate in the maturation of the transcripts.

In some cases, RNase BN can also act as an endoribonuclease on tRNA precursors that contains no -CCA sequence [80]. For those tRNA precursors, RNase BN endonucleolytic activity takes place after a subsequent addition of -CCA sequence to tRNA precursor by tRNA nucleotidyltransferase [80,81]. Although, the effective elimination and re-addition of the necessary residues is controlled by RNase BN enzymatic activity in some strains of *E. coli*, RNase BN homologues (known as RNase Z) are often responsible for the maturation of tRNA precursors present in other strains and organisms [81,82]. The *Escherichia coli* homolog of RNase Z, was previously shown to act as both a distributive exoribonuclease and an endoribonuclease on model RNA substrates and to be inhibited by the presence of a 3'-terminal CCA sequence. Here, we examined the mode of action of RNase BN on bacteriophage and bacterial tRNA precursors, particularly in light of a recent report suggesting that RNase BN removes CCA sequences (Takaku, H., and Nashimoto, M. (2008). RNase Z is encoded by the *elaC* gene which is now known as (*rbn*), because *elaC* and *elaD* are the two genes of an operon that is a part of the gene that encodes RNase BN [79,82] which cleaves after the discriminator nucleotide to allow addition of the universal -CCA sequence. In some eubacteria, such as *Escherichia coli*, the -CCA sequence is encoded in all known tRNA genes. Nevertheless, an RNase Z homologue (RNase BN. RNase Z was initially characterized as a zinc phosphodiesterase, but studies originally conducted in 1983 on bacteriophage T4 tRNA precursors that have no-CCA sequence proved that the functionality of RNase Z in *E. coli* is as important as RNase BN for tRNA maturation [80]

## RNase D

RNase D is one of the *E. coli* exoribonucleases that was discovered for the first time to possess a high level specificity of exoribonucleases on denatured tRNAs [83]. RNase D is a 3'-5' exoribonuclease that is composed of 375 amino-acid-residues which contribute in the maturation process of several RNAs such as tRNA and sRNA [83]. Taking no crucial action on RNA homopolymers, -CCA sequence is considered to be the most active of RNase D substrates even if tRNA-like substrates lack all or certain portions of the tRNA [84]. In addition, RNase D requires the presence of the divalent metal ions (e.g.,  $Mg^{2+}$ ,  $Mn^{2+}$  or  $Co^{2+}$ ) for its activity. As a result this hydrolytic enzyme becomes a crucial component for cell viability as it acts as a supportive enzyme [84,85]

RNase D is related to a superfamily that includes exonuclease for DNA and RNA, for example, RNase T and oligoribonuclease of *E. coli* [84]. The superfamily, called (DEDD), is based on four invariant acidic residues. Those residues are responsible for forming the catalytic center and regulating the common catalytic process that is shared by all the members of DEDD exonuclease superfamily [84]. The DEDD superfamily is divided into two smaller groups based on the richness of histidine and tyrosine [84,85]. The Histidine (His) rich is called (DEDDh) and the Tyrosine (Tyr) rich is known as (DEDDy) [84,86]. It is believed that RNase D fall into DEDDy due to its similarities to Werner Syndrome protein (WRN) [85]. WRN is a helicase that carries DEDDy domain and the nucleic acid binding domain HRDC (helicase and RNase D C-terminal) [84,87]. According to studies, a funnel-shaped ring architecture that is formed by HRDC and DEDDy domains play a vital role in RNase D exonuclease activity [83,84,88]

## RNase T

In *E. coli*, RNase T (RNT) is a 215 amino-acid-residue protein that plays a significant role during the final stage of RNAs' maturation such as, 5S and 23S rRNAs [54,89]. RNT is a hydrolytic enzyme that is also involved in the elimination and regeneration of the turnover tRNA -CCA sequence end as well as DNA repair [89-91]. RNT is a 3'-5' single strand-specific exoribonuclease that utilizes  $Mg^{2+}$  or  $Mn^{2+}$  as the cofactor to start its activity [90]. This defines its belonging to the DEDD superfamily that contains acidic residues who are highly attracted to ionic metals; in particular, RNT is a member of DEDDh subfamily [89].

The structural feature possessed by RNase T substrates is a double stranded stem with a 3base overhang at the 3' end [89]. The homodimer formation of RNase T is extremely important for its activity as studies showed that RNase T binds up to 10 residues, but the last 4 residues are mostly acidic residues and are the bait of the catalytic center formation [92]. Studies showed that forming a functional RNT active site means that binding and catalysis substrates have to be recognized by the two subunits of RNT homodimer [89,90]. Substrates normally form secondary structure with a 3'-end free nucleotides to present stable and double-stranded stem [90,91]. The presence of the free 3'-end allows the nucleic acid-binding sequence (NBS) fragment to be more accessible to the site since studies indicated that NBS fragment is necessary for binding activity [90]. Studies identified that the NBS segment is rich in Arg/Lys residues which indicates RNT is a highly negatively charged enzyme [89]. In fact, RNT appears to be significantly slow in enzymatic activity before approaching a nucleic acid structure but once the first elimination of RNA's nucleotide takes place from the unwinding structure, the enzymatic activity increases [89,93]. Furthermore, research showed that RNase T is the only enzyme that has the ability of trimming the unwound double helix and creating a blunt-ended DNA duplex [92].

## RNase II

The presence of RNase II is everywhere in *E. coli* cytoplasm to control the process of cell development [94]. A 644 amino-acid-residue protein, this 3'-5' exoribonuclease has the ability of binding to RNAs and performs its enzymatic activity by cleaving single-strand RNA into one nucleotide each time in the presence of a divalent cation element [95,96]. The production of 5' monophosphates results from the hydrolyses activity of RNase II on RNAs [97,98] *E. coli's* RNase II consists of a catalytic domain and three RNA-binding domains [94,97]. The N-terminal begins with an  $\alpha$ -helix along with two consecutive anti-parallel  $\beta$ -barrels having five-strands recognized as cold-shock domains CSD1 and CSD2 [99]. Although the CSD2 does not have sequence motifs for RNPI and RNPII, it plays an important role in binding of RNA [99]. A third RNA-binding domain is present on carboxy terminus; this third RNA is a usual S1 RNA-binding fold.

Anchor and catalytic domains are connected by a flexible, intermediate area of the RNA 6 - 8 nucleotides chain where the piece of RNA interacts [100] Between the two CSDs and the S1 domain a deep gap is situated in the anchor region, which is also the place where

nucleotides 1 - 5 found at the 5'-end of the RNA fragment reside. During 'recruitment' of RNA, the function of CSD1 and CSD2 is controlled by most canonical oligonucleotide-binding (OB) motifs found in CSD1 and CSD2. Considering longer and more-structured RNAs, there is a possibility of the certain extent site of 'recruitment' to be replaced with the anchor site [100-102]. Upon RNA binding, catalytic activity takes place at the 3'-end of the substrate. Specificity of RNase II occurs with the help of motif III in relations with the phosphate backbone of motif IV [100,101]. Due to the steric obstacle at the opening there is a limitation in the admittance to the catalytic pocket of sRNA. The incapability of RNase II in degradation of double strand RNA (dsRNA) allows other family members to involve their enzymatic activity in degradation the dsRNA such as RNase R or multi-protein complexes [101,103].

### RNase R

RNase R is a 3'-5' exoribonuclease that is composed of 813 amino-acid residues [104,105]. In *E. coli*, RNase R has varied intracellular hydrolytic and physiological activities including RNAs' degradation and maturation, protein expression, *trans*-translation process, and during any environmental stress condition [104,106,107]. RNase R falls under the RNR exoribonuclease superfamily that shows the ability of digesting molecules with secondary structures during the absence of a helicase activity [106]. RNase R recognizes and binds to 3'-end single-stranded free nucleotides [104,105]. Despite the fact that RNase R is still not quite understood structurally, most studies that are related to RNase R are based on understanding RNase II since both belong to RNR superfamily [104,108]. RNase R expresses a catalytic domain that is covered by CSD1 and CSD2 at the N-terminus as RNA binding domains and S1 domain at the C-terminus [104].

The exposure of *E. coli* to various environmental stresses includes cold shock, growth in minimal media, inactive phase, and starvation for phosphate, carbon, or nitrogen causing a gradual elevation of RNase R levels [109]. Various RNase R regulation in response to any stress event shows the ability of *E. coli* in having a significant physiological functionality [109]. Although RNase R is increased during stress conditions, understanding the mechanisms of RNase R regulation is essential [108,110]. In the experiment of *E. coli*  $\Delta$ RNase R showed that presence or the absence of RNase R has no effects on *E. coli* growth which indicates an overlapping functionality with other RNases [105,108,110]. According to research, in the stationary phase the *rnr* expression actually decreases because of the stabilization of the *rnr* mRNA [107]. Another study suggests that there are no relation between the reduction in the amount of *rnr* transcript in the stationary phase and the elevation of RNase R protein, hence, there may be differences of RNase R expression during various stress conditions [111].

### Cold shock proteins family

*E. coli* carries an adaptive mechanism that is activated for cell survival during any exposure to a new environment or to a sudden temperature shifting [112]. In a case of cold-shock adaptation, there are a number of proteins that are induced during stress. These proteins fall into two categories: class I proteins present during temperature downshifting from 37°C, and class II proteins maintain the same level of expression even during temperature downshifting [113,114]. We will be focusing on class I to understand RBP activities during temperature downshifting.

Class I in *E. coli* is controlled by CspA, a 7.4 kDa protein that is the main protein of the cold shock inducible family. About 10% of all the expressed proteins during the adaptation phase are contributed by CspA [115]. Proteins in the CspA family range from CspA to CspI as members of the family in *E. coli* [115]. The Csp family is responsive to different stresses in the environment, for instance, CspA, CspB, CspG and CspI respond to cold-shock while CspD responds to a deprivation of nutrients [116]. Some of the *E. coli* family proteins are cold shock inducible, for example, CspA, CspB, CspG and CspI while some are not such as, CspC and CspE that *E. coli* express at 37°C. However, the regulation of these proteins is different; CspB and CspG complement CspA gene expression whereas CspI is a cold shock inducible gene expression [113,116]. CspA, CspB, CspG and CspI possess one common unique expression feature which is a very long and highly conserved 5' untranslated region (5'-UTR) [113,116]. 5'-UTR contains the cold box which is also unique and composed of an eleven base sequence by cold shock gene [117]. The cold box is the site where the transcription pauses and also prevents the expression of CspA. Research suggest that CspA controls its gene expression automatically by reducing the transcription process [114,118]. During the DNA transcription, the RNA polymerase diverts from the cold box on the CspA mRNA caused by a drop in temperature. However, during the adoption phase, the concentration of CspA in the cells increases which initiates the binding of CspA's mRNA by CspA. This attenuates the transcription process

due to the destabilization of the RNA polymerase's complex for elongation. When 5'-UTR is produced in excess at a temperature of 15°C, the response to cold-shock is induced slowly which prolongs the manufacture of CspA, CspB and CspG [114,116,118].

Although the mechanism used in CspA regulation stabilizes the CspA mRNA dramatically, the stabilization process is very brief and when the cells adapt to the low temperature, it disappears [112,119]. According to research, the CspA promoter is highly active at 37°C even though the CspA mRNA is unstable [115,120]. Lpp is a constitutive promoter for the main outer membrane lipoprotein under which CspA can be induced in cold stress events [114]. CspA mRNA is rich in A/T residues that has a sequence located in the initiation codon fourteen bases downstream complementary to the 16s rRNA's penultimate stem [118]. The sequence of a downstream box (DB) facilitates the translation before initiation complex formation through the 16s rRNA's binding [112,118]. The hybridization, in turn, supports the translation initiation which basically is defective at a temperature of 15°C [118,120]. On the other hand, it has been thought that during a sudden downshifting, ribosomes remain physiologically non-functional to mRNAs particles but, their functionality is maintained toward only mRNAs for cold shock proteins [117,118]. Smartly, during the adoption phase, ribosomal factors that are cold-shock, for example, RbfA and CsdA, are produced which are in turn acquired by the cold-un-adapted ribosomes to be converted into cold-adapted ribosomes that allow them to translate the non-cold-shock mRNAs at the presence of translational factors [113]. Translational factors such as IF-2 are expressed by *E. coli* during any sudden temperature shifting to initiate tRNA (fMet-tRNA) binding to the 30S subunit. As a result, a proper utilization of an increased number of ribosomal subunits (30S, 50S) to overcome intracellular protein syntheses processes and maintain bacterial growth and survival during low-temperature exposure occurs [113]. In fact, other studies suggest that at low temperatures, CspA tends to lower RNA binding affinity, thus lowering specificity in sequence and its ability in destabilizing RNA's secondary structures into being more susceptible to RNases, and makes it function as an RNA chaperone [116,121]. This function is significant in the sense that at low temperatures, it makes the mRNAs' translation efficient with effective transcriptional processes [116,121].

### Rho factor

A Rho factor is highly utilized in *E. coli* during the termination of transcription process by RNA polymerase [122]. Rho is a 46.8 kDa homohexamer protein, a product of a 419 amino-acid-polypeptide that is able to enhance cells viability. Rho is a universal protein that most eubacteria utilize [123]. In *E. coli*, Rho has been significantly involved in the exploration of the auxiliary proteins that have a regulatory role in the termination of RNA polymerases from transcripts. Rho factor is RNA/DNA helicase or translocase that also acts as a molecule and gets its energy by hydrolyzing ATP through its RNA-dependent ATPase activity to ensure that RNA polymerase is dissociated from DNA template and releases mRNA [124,125]. Transcriptional termination of the downstream gene can be caused by Rho in an operon because in *E. coli* transcription and translation occur spontaneously which leads to block the downstream gene expression. Rho protein implicates releasing such terminated transcripts when several Rho-dependent termination factors like Nus A, B and C interact with and play a major role in gene expression [126,127].

The functional domains in Rho promoter consist of three parts [122]. The first part is Q-loop and R-loop with a Rho secondary RNA binding site. The second one is P-loop that is highly conserved among RecA family of ATPase and is associated with ATP binding and ATPase activity of Rho. The third one is a core RNA binding domain, known as the primary RNA binding site of Rho, it binds to single stranded RNA and DNA molecule that is held responsible for the tethering of Rho within *E. coli*. Despite the fact that R-loop has implications in binding both RNA and ATP, Q-loop extends toward the Rho ring center to be used strictly for RNA binding. A possible explanation as to how RNA-binding couples ATP hydrolysis is suggested by how close the R-loop is to P-loop, or to the ATP binding domain.

### Nus-Factors

The Nus transcription-factors are termed N-utilization substances because they are originally identified as part of the *E. coli* phage  $\lambda$  N-protein-controlled anti-termination system [128,129]. There are four different important Nus present within *E. coli* cells (Nus A, B, E and G) but their presence are varied and depending on the different external signals [130].

### NusA

NusA in *E. coli* is formed of 495 amino-acid residues, it is essentially involved in transcriptional termination, elongation and anti-termination processes, for example, the termination of Rho-dependent transcription and the anti-termination of phage  $\lambda$  N-mediated

transcription [129,131]. It is also significantly engaged in the cellular responses for DNA damaging by stimulating the nucleotide repairing processes along with re-transcription activity as well as its interaction with the hairpin structure of a nascent RNA to increase lifetimes [132]. Studies showed that  $\sigma$  factor competes with NusA in interacting with RNA polymerase (RNAP), a mediator of RNA synthesis [129,133]. NusA starts its enzymatic activity with RNAP once RNAP complex binds to the nascent RNA during the elongation phase [129,133]. The N-terminus domain of NusA binds to three RNA-binding subdomains, S1 and two K homolog domains, to form an RNA binding domain. S1 is 63 amino acids in length, KH1 is 74 amino acids in length and KH2 is 67 amino acids in length [129,133]. Despite the fact that these subdomains show different functions and ability of binding to single-stranded RNA, their structural topology shares the same domain; (SKK) the ssRNA binding domain [129,130]. The C-terminus contains a double homologues acidic domain (AD) responsible for protein-protein interaction [129]. AD forms an intramolecular, interdomain complex that masks the RNA binding domain of NusA from any interaction with single stranded RNA [133,134]. In fact, a study by Li, *et al.* [135], showed that NusA over-expression plays a role in *E. coli* resistance and protein synthesis during heat shock stress.

### NusB

In *E. coli*, NusB is composed of 139 amino-acid-residues that usually operates during gene transcription [129]. NusB forms a heterodimerization structure which extensively enhances its affinity and interaction with RNA [129,136]. NusB dimerize with NusE, which is one of the Nus family members, and binds specifically to *boxA* RNA at the nut site in order to activate the anti-termination process. The nut site composed of *boxA* in a form of twelve nucleotides together as a single stranded RNA and *boxB* that is represented as a stem looped structure [136,137]. Researchers have established that this connection is slightly-aligned in the functional elongation complex [136,138]. Anti-termination process for example in  $\lambda$  phage is considered to be a collaboration process between N protein and Nus factors to modify RNA polymerase in order to terminate the binding from the nut site on the transcribed RNA [137,139]. NusB maintains helical structures in the form of sub-domains in both the N- and the C-terminal [136]. The sub-domains were thought to be interacting with ssRNA, but studies showed that RNA structure binds to  $\beta$ -sheet in which NusB does not express exposed, positively charged or aromatic residues for RNA binding [129,136]. Since NusB binds to RNA in the presence of NusE, NusE might be involved in the recognition processes by alerting NusB structure for RNA or binding to a part of RNA [129,136,140].

### NusE

NusE in *E. coli* is a 103 amino acids long protein that folds into four anti-parallel beta-sheets bound to two  $\alpha$ -helices [129]. Research showed that NusE is the operational active part in the complex with NusB [139]. Crystal structural showed that two helical bundles of NusB interact with  $\alpha_1$  and  $\beta_2$  of NusE through a bridge without any structural alterations [136]. NusE not only dimerize with NusB and plays a significant role in transcriptional anti-termination, it is also known to interact with NusG and enhance read-through of terminators on the lambda phage chromosomal DNA in the presence of N protein. NusE also takes a part with 30S ribosomal subunit protein as a transcriptional elongation component to support NusG capacity level during transcription and translation [129,136,140]. Research showed that NusE facilitates the interaction of NusG with RNA polymerase (RNAP) while it binds to 30S ribosomal subunit. Although NusE enhances the ribosomal degradation, it is involved in the protein translation process as part of the ribosome [129,140]. NusE establishes a strong binding to NusB that allows NusB to direct the heterodimer complex to be the major section of the EC [139].

### NusG

In *E. coli*, NusG is a crucial element for physiological and intercellular viability of the cell. It is composed of 181 amino-acid-residues that forms a dual-domain protein which is important in RNA polymerase (RNAP) regulation, rRNA assistance and anti-terminator for lambda N protein [129,141]. The N-terminus of NusG contains four  $\beta$ -sheets covered by three  $\alpha$ -helices that allows the interaction between the N-terminus, a 116 amino-acid and a 65 amino-acid NusG C-terminus [141,142]. It is believed that the NusG utilizes the hydrophobic side on its C-terminus to interact with the RNAP during its attachment to NusE [140,141]. Other studies suggested that the NusG N-terminus also has the ability of binding to RNAP and enhances the transcriptional elongation rate [143]. Thus, transcription elongation, termination and anti-termination are NusG major functions [140,143]. NusG is capable of improving the rate of transcription elongation by RNAP during the disappearance of Rho factor [129]. However, once Rho factor appears, it interacts with the RNAP to reduce the complex susceptibility to the termination activity [140,143]. This analysis suggests that translation elongation rates may significantly be affected once NusG is depleted [129,140]. Thus, transcription and  $\rho$ -dependent termination are supported by translation coupling.

### BglG protein

In *E. coli*, BglG protein regulates *bgl* operon through inactivating dual transcriptional terminators known as the ribonucleic anti-terminator (RAT) to facilitate transcription elongation [144]. The first terminator is within the signal sequence region of *bglG* gene and the second one is downstream *bglG* in the intercistronic region of *bglG-bglF* [145]. BglG is 278 amino acids long, contains an RNA-binding protein specific sequence and has the ability of interacting with 32 nucleotides target sequence [145]. Its involved in regulating BglF (aryl- $\beta$ -glycoside transporter) and other essential proteins for the use of aryl- $\beta$ -glucoside sugars [144]. BglG is controlled negatively by BglF because *bglF* gene is a transporter leading to the PTS phosphoenolpyruvate (PEP)-dependent phosphotransferase system [146]. The Phosphoryl groups found within the PTS are transported from PEP through HPr and enzyme I (EI), both of which are general phosphotransferases to enzymes II (EII), which finally phosphorylate its enzyme substrate. In the absence of  $\beta$ -glucosides, the BglF is engaged in transferring phosphoryl groups to the BglG instead of the sugar, and the reverse process occurs when the substrate is present; sending BglG to the cell membrane [146]. Additionally, BglF-catalyzed phosphorylation/phosphorylation actually activates the dimer/monomer transition of BglG.

The structural component of BglG family consists of three domains [147]. The RNA binding domain and two phosphoenolpyruvate-dependent phosphotransferase system (PTS) regulatory domains (PRD1 and PRD2) [146]. Both PRD 1 and 2 contain histidine residues that are highly important for PTS phosphorylation activities [146,148]. Collective research show that in *E. coli*, enzymeI (EI) phosphorylates the histidine on PRD1 regulatory domain but the histidine in PRD2 is phosphorylated by HPr to maintain PTS anti-terminator activity [146,148]. In recent studies, it was suggested that the structural arrangement of terminal complex in conjunction with HPr and EI leads to BglG activation without the physical movement of phosphoryl group [146,148,149]. However, in some cases HPr is not required for the anti-terminator activities because of BglG direct interaction [146,147]. Research also indicated that BglG dimer requires HPr and EI to be released and recruited to the cell pole for its stability [145,146]

### Signal Recognition Particle

*E. coli* expresses transportation systems to ensure an efficient fidelity of localization to all newly synthesized proteins [150]. In *E. coli*, signal recognition particles (SRPs) are universally conserved as to mediate the co-translation of proteins within the cell and directs protein trafficking to the plasma membrane, or to be secreted out of the cell. SRP is a ribonucleoprotein complex that contains an RNA [151,152]. The complex in *E. coli* consists of the SRP protein Fifty-four homolog (Ffh), and 4.5 SRP RNA. Ffh (a 453 amino acid residues) is formed by three domains, the first domain is the N domain (the alpha -helical domain), the second domain is the G domain (the RNA binding domain), and the final domain is the M domain (the alpha -helical domain). Most secretory proteins are engaged with a guide polypeptide chain called signal sequence [153,154].

The signal sequence consists of N-terminal hydrophobic amino-acids, between 9 and 12 large hydrophobic amino acids, and recognized by SRP complex [153]. Those residues can be a transient or permanent portion to the final formation of the protein [153,154]. Researchers believe that the signal sequence polypeptide determines the final localization of the newly synthesized protein across or integration into the plasma membrane of the cell [155,156]. Upon the SRP's recognition to the signal peptide, it forms a complex that binds to the nascent N-terminal sequence as it emerges from the ribosomal complex [156,157]. Then directs the complex to SRP membrane receptor (SR), (FtsY is a combination of three domains A, N and G domain), where the conformational changes between SRP and SR complex occur for the movement toward the protein-conducting channel (SecYEG a translocation machinery). As the interaction between the ribosome and SecYEG translocon occurs, the dissociation of SRP and SR from the nascent polypeptide spontaneously occurs by the GTP hydrolysis activity [152,156]. This activity is mainly due to interaction of FtsY with *E. coli* membrane phospholipids [152,156]. Although, the interaction between the nascent polypeptide and the SRP is not fully understood, research indicates that the SRP RNA cooperates with one or more SRP proteins to facilitate the binding and releasing of the signal sequence from the SRP complex [156,158].

### CRISPR-Cas

In 1987, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) was discovered in *E. coli* [159]. It is an RNA-guided immunity system that protects its host from any foreign genomic invaders [160]. The system works as an adaptive immune system that attacks the genomic invader with the expression of a short guide CRISPR RNAs (crRNAs) [161]. These crRNAs are derived from the tran-

scription of CRISPR arrays and production of pre-CRISPR RNAs (pre-crRNAs) [161]. Pre-crRNAs carry unique single repeat, spacer units which are driven from sub-sequence (proto-spacers) [161,162]. The pre-crRNAs hybridize with complementary sequences on the target sequence of the invader and starts the degradation process [161-163]. However, the degradation process is significantly achieved in the presence of specific Cas endonuclease proteins (CRISPR/cas).

CRISPR/cas complex consists of a leader RNA sequence and Cas protein binding at specific site of CRISPR sequence [164]. Cas is a functional protein that is formed by several domains including helicase, nuclease, polymerase, and polynucleotide binding proteins. Cas is a significantly important element as it plays a crucial role in CRISPR propagation and function as well as its responsibility in engaging a newly acquired spacer unit between the leader and CRISPR sequence and taking a part in blocking the foreign invasion by diverse repertoire elements [161,163]. The leader is about 500 bp non-coding AT-rich sequence located upfront the CRISPR sequence which harbors the promoter to generate the pre-crRNAs array [161,165].

CRISPR loci are widely distributed between the prokaryotic genome where about 40% - 70% are present in eubacteria [166]. Most of loci are contained between 21 and 48 direct DNA repeats base pair which are incorporated with about 26 to 72 base pair of non-repetitive similar in length spacers. The locus of each CRISPR is recognized by the frequency of the repeat sequence within the locus but the spacer is a unique sequence [166,167]. The spacer sequence acts as an adaptive immune response provider to new or repetitive infections [167,168]. Therefore, during the degradation process a new spacer from the invader genomic material is injected into the CRISPR array. Although CRISPR-Cas system remains mysterious, so far studies indicate that most *E. coli* strains carry two different CRISPR loci that fall under CRISPR-Cas I-E subtype [165]. However, just one strain of *E. coli* carries two subtypes of CRISPR-Cas system (CRISPR-Cas I-E and CRISPR-Cas I-F) [165,168]. CRISPR-Cas I-E is divided into two loci CRISPR-I and CRISPR-II, whereas CRISPR-Cas I-F locus is named as CRISPR-III. Each locus expresses different arrays with a site of *cas*-genes for CRISPR activity [165,168].

### Metabolic enzymes

The list of metabolic enzymes capable of binding DNA or RNA continues to grow since the early discoveries of their dual functionality. These are typically single domain molecules that bind a specific target RNA molecule or a specific DNA sequence. The nucleic acid binding feature of these enzymes gives them a role in regulating gene expression level. They are involved in a number of metabolic pathways and include aconitases, enzymes of the glycolytic and pentose cycles, the TCA cycle, fatty acid metabolism, thymidylate synthesis cycle, and others. In a review of these enzymes, Cieřła J [170] listed 20 different metabolic enzymes with dual functionality in binding DNA/RNA. The lists includes metabolic enzymes from both eukaryotes and prokaryotes.

In *E. coli*, perhaps the best characterized of such enzymes are the aconitase and the methyl transferase enzyme KsgA. The aconitase from *E. coli* is an enzyme that catalyzes the conversion of citrate to isocitrate. The protein is approximately 97 kDa in size and contains an essential but redox-inactive [4Fe-4S] centre [171]. In *E. coli*, two differentially regulated aconitases (AcnA and AcnB) have been reported [171]. Direct binding of mRNA by *E. coli* aconitases was reported by Tang and Guest [172]. The 16S rRNA methyl transferase KsgA is a 28 kDa protein that dimethylates two adjacent adenosines near the 3' terminus of the 16S rRNA [169]. It has been shown by RNA-protein equilibrium electrophoresis that KsgA represses its own synthesis through binding to its own mRNA [169].

### Conclusion and Perspectives

In this article, we have addressed the existing knowledge regarding the RBPs in *E. coli* cell. RBPs particles serve a varied range of functions and moderate different cellular mechanisms and metabolism to maintain *E. coli*'s survival including-translation initiation, transcriptional terminations, anti-terminations, translational repression, post-transcription, RNA maturation, transportation, degradations, and stability.

A number of active RBPs have been identified including Hfq, CSR, CSP, RNA degradosome, Nus-Factors, BglG, CRISPR, SRP and Rho-Factor. These RBPs apply different mechanisms to generate a high affinity to most of RNAs in order to form RNP. The role of RNPs show a great specificity in controlling many of the cytosolic events associated with RNA metabolism.

Some of the RBPs have received a great amount of attention, but others are still not fully understood, therefore, further research is also required to understand the mechanism of RBP-RNA's interactions to some other RBPs such as SRP. Moreover, RBPs appear to have a huge

role in mediating sRNAs including OxyS RNA, and RyhB RNA. Since sRNAs have only recently been discovered and show a huge affinity to RBPs, further investigation of newly identified sRNAs will lead to a better understanding of how *E. coli* utilizes this type of RNAs in response to physiological and environmental changes. This knowledge would aid in discovering new members and may create opportunities for alternative antimicrobial strategies simply by blocking the expression or the function of RBPs.

### Conflict of Interest

The work does not have financial and/or non-financial competing interest. The authors declare that there is no conflict of interest.

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