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Review

Eukaryotic ribosomal protein S3: A constituent of translational machinery and an extraribosomal player in various cellular processes

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ABSTRACT

Ribosomal proteins from the S3 family are universal components of small ribosomal subunits in all three domains of life. In eukaryotes, ribosomal protein S3e (rpS3e) is one of 33 proteins of small subunit of the ribosome. It functions not only within the ribosome participating in translation but also as an extraribosomal player involved in a number of vitally important cellular events. RpS3e is directly implicated in translation initiation via participation in rearrangements of the small subunit structure occurring upon the binding of initiation factors eIF1 and eIF1A, which opens the ribosomal mRNA binding channel for incoming mRNA and allows scanning. Being located at the mRNA entry site of the ribosome, rpS3e is suggested to interact with mRNA part downstream of the codon at the decoding site and it could be implicated in helicase activity of the ribosome by analogy to its bacterial counterpart rpS3p. Extraribosomal functions of rpS3e are mainly based on its ability to bind to nucleic acids, although protein–protein interactions take place too. As an independent player, rpS3e is involved in DNA repair, selective gene regulation via implication in NF-κB signaling pathway, inducing apoptosis, control of expression of the own gene at the translation level and molecular interactions affecting half-life of the protein. Involvement of rpS3e in various cellular processes is mediated by specific mechanisms utilizing post-translational modifications of the protein. Here, we present accumulated to date information and current ideas concerning functions of rpS3e as a constituent of translational machinery and of the free protein as a key player in various events of the cell life.

1. Introduction

Now it is well known that ribosomal proteins can function not only as constituents of the ribosome, large ribonucleoprotein complex responsible for translation of mRNAs, but also have so called extraribosomal functions related to their implication in a number of events of the cell life besides translation (reviewed in Ref. [1]). In other words, these proteins can be involved in various cellular processes in a free state, i.e., beyond the ribosome. In particular, several ribosomal proteins have been found to participate in DNA repair, induction of apoptosis, suppression of tumors, cell growth and proliferation regulation, pre-mRNA splicing and so on. One of ribosomal proteins that have bright “individualities” related to their own distinctly recognized functions within the ribosome and to their specific extraribosomal roles is ribosomal protein (rp) S3 from the small (40S) subunit of the eukaryotic ribosome (rpS3e).

The main peculiar features of rpS3e in the ribosome concern its implication in translation initiation via participation in rearrangements of the 40S subunit structure induced by binding of the subunit to initiation factors, which promotes subsequent recognition of the start codon and interaction with mRNA (see, for example, Ref. [2]). The most well studied extraribosomal function of rpS3e relates to its enzymatic activities in DNA repair [3–5]. Besides, rpS3e is implicated in the nuclear factor-kappa B (NF-κB) signaling pathway as a component of NF-κB complex regulating transcription of genes involved in a variety of cellular functions including immune responses and cellular proliferation [6]. In addition, rpS3e participates in regulation of its own level in the cell and interacts with various proteins, which directly affects cell survival [7–9]. RpS3e is shown to be involved in inducing apoptosis and is proposed to play a central role in regulating numerous aspects of host–pathogen interactions, for example, in pro-inflammatory signaling during bacterial infection; its important role in antagonizing cancer development is also discussed

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(reviewed in Ref. [10]). Remarkably, molecular mechanisms of involvement of rpS3e into the mentioned events of the cell life are closely concerned with and directed by specific post-translational modifications such as phosphorylation, methylation and sumoylation that regulate extra-ribosomal functions of this protein under different conditions. In this review, we accumulate and discuss currently known data and ideas concerning roles of rpS3e in the small ribosomal subunit structure and translation process and its several extra-ribosomal functions displayed at various events of the cell life (namely, DNA repair, specific gene regulation and apoptosis), as well as mechanisms providing these functions and molecular interactions affecting level of rpS3e in the cell.

2. RpS3e as mRNA gate keeper and eIF's mate in translation

Functions of rpS3e and of its prokaryotic counterpart rpS3p in translation can be well understood taking into account various biochemical data and atomic structures of prokaryotic and eukaryotic ribosomes known from various studies performed with the use of X-ray crystallography (e.g., see Refs. [11–17]) and cryo-electron microscopy (cryo-EM) of ribosomes and their complexes with various ligands (e.g., see Refs. [18–20]). A peculiar common property of proteins from the rpS3 family is that they are key components of the small ribosomal subunit site where mRNA enters the ribosome (entry to the mRNA binding channel or the mRNA entry site). For all that, involvement of eukaryotic members of the rpS3 family in translation has specific features, some of which have no analogy with those typical for the involvement in this process of their bacterial counterparts. This is displayed in a number of eukaryote-specific contacts of rpS3e with other components of the 40S subunit, interactions of the protein with eukaryote-specific initiation factors and participation of rpS3e in specific rearrangements of the 40S structure crucial for translation initiation.

2.1. RpS3e structure and its specific contacts with other 40S ribosomal components

RpS3e has counterparts (rpS3p) in archaea and bacteria; lengths of proteins from the rpS3 family generally are in the range of 218–250 amino acid residues (with exception for several archaeal proteins, which have specific long C-terminal extensions) (Fig. 1). Proteins from the rpS3 family contribute significantly to the architecture of the beak region at the head of the small ribosomal subunit, mainly at its solvent side [11–20] (Fig. 2). Amino acid sequences of rpS3e are practically identical in mammals and have 70–80% identity between lower and higher eukaryotes with main differences in the very C-terminal part. Extent of sequence similarity between eukaryotic and prokaryotic members of the rpS3 family is drastically lower than that among eukaryotes (Fig. 1); nevertheless, structures of bacterial and eukaryotic rpS3 in the small ribosomal subunits share remarkable degree of similarity (Fig. 3). The conserved structural core of rpS3e in the ribosome, which is stabilized by interactions with other ribosomal proteins and the small subunit rRNA, comprises a major portion of the protein, leaving variable parts at the very N-terminal and C-terminal tails (Fig. 3) whose sequences are practically dissimilar in prokaryotes and eukaryotes (Fig. 1). It is worth mentioning here that in Ref. [15] the whole rpS3e structure was considered as conserved, while in Ref. [16] the C-terminal tail of rpS3e was regarded as eukaryote/archaea-specific. The conserved core of proteins from the rpS3 family consists of two well defined domains, N-terminal and C-terminal ones, each containing a four-stranded β-sheet stacked against two helices (Fig. 3). The major part of the N-terminal domain consists of so called K homology (KH) domain (a widespread protein RNA-binding domain that was first identified in the human heterogeneous nuclear ribonucleoprotein K) including two parallel helices packed against three β-strands (Fig. 3) [14]. Interactions of rpS3e with the small subunit rRNA and neighboring ribosomal proteins in the 40S subunit head comprise the conserved contacts (analogous to those of rpS3p in the 30S subunit [14]) and eukaryote-specific ones [15,16]. The C-terminal domain of the protein interacts with 18S rRNA fragments belonging to the conserved core of the small subunit rRNA, namely, with helix h34 in the head and h18 in the body (Fig. 2); these interactions are similar to those of rpS3p in the 30S subunit [14]. The N-terminal domain does not interact with the 18S rRNA and forms contacts with rpS20e and rpS29e at the subunit head [15,16], which resemble contacts of rpS3p with rpS10p and rpS14p [14], the counterparts of rpS20e and rpS29e, respectively. Besides, rpS3e makes a number of eukaryote-specific intra-ribosomal contacts. In particular, its long C-terminal tail interacts with eukaryote-specific protein RACK1, while the N-terminal domain makes multiple contacts with the beak protein rpS10e that has no bacterial counterparts too [15,16]. Exact roles of these interactions in translation remain, to our knowledge, unknown. Several intra-ribosomal interactions of rpS3e that are directly implicated in translation initiation are discussed below in the Section 2.3.

2.2. Interactions of rpS3e with mRNA at the mRNA entry site and possible implication in the ribosomal helicase activity

Suggestion that rpS3e is located at the mRNA entry site has been inferred from cryo-EM and X-ray data with mRNA-free 40S subunits and 80S ribosomes [15–21] by their comparison with atomic models of bacterial ribosomal complexes containing mRNA [13]. The latter study showed that the 70S ribosome interacts with mRNA stretch comprising about 30 nucleotides, and the A and the P site codons are in the middle part of this stretch, and that rpS3p interacts with mRNA nucleotides in positions +11 to +15 with respect to the first nucleotide of the P site codon. Positioning of rpS3e at the downstream mRNA channel has been experimentally proved in site-directed cross-linking studies with human ribosomes exploiting mRNA analogs bearing cross-linkers at the 3′-termini [22,23]. Namely, rpS3 was the main target of cross-linking of mRNA analogs in human 80S ribosomal complexes where mRNA analogs were fixed on the ribosome by P site-bound $tRNA^{Phe}$, so that mRNA nucleotides bearing the cross-linker were in positions +9 or +12 relative to the first nucleotide of the P site codon [22]. These data provided direct evidence that rpS3e is a keystone component of the mRNA entry site at the mammalian 40S subunit. Notably, derivatives of various short oligoribo- and deoxyribonucleotides were able to cross-link to rpS3e not only in the complexes where mRNA was fixed on the ribosome by interaction with cognate tRNA at the P site, but also in their labile binary complexes with ribosomes obtained without tRNA [23]. The latter demonstrated that rpS3e within the 40S subunit has affinity to unstructured single stranded nucleic acids. This affinity is likely caused by the KH domain, which can interact with single stranded RNA [24] and DNA [25] and thereby implicate the protein in various extra-ribosomal functions (will be discussed below in Section 3). It has been hypothesized that the ability of rpS3e to attract unstructured RNAs could be utilized by the 40S subunit at the very first step of translation initiation, and a labile nature of this initial transitory RNAs binding could prevent programming of 40S ribosomal subunits with incorrect messengers incapable of participating in the subsequent initiation steps [23] that are in eukaryotes drastically more complicated than in bacteria.

It is known that in the bacterial ribosome rpS3p (together with rpS4p) is responsible for the ribosomal helicase activity, which makes possible translation of mRNAs having extensive secondary