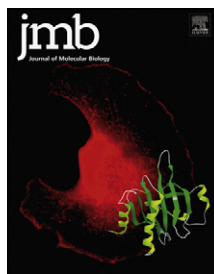

The Intersubunit Bridge B1b of the Bacterial Ribosome Facilitates Initiation of Protein Synthesis and Maintenance of Translational Fidelity

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Abstract

In bacteria, ribosomal subunits are connected via 12 intersubunit bridges involving RNA–RNA, RNA–protein, and protein–protein interactions. The only protein–protein bridge in the ribosome is ribosomal intersubunit bridge 1b (B1b), which is mainly formed by the bacterial protein L31 (bL31) and connects the head domain of 30S subunit and the central protuberance of the 50S subunit. It is known to be the most dynamic intersubunit bridge. Here, we have evaluated the role of bL31 and thereby the bridge B1b in the working cycle of the ribosome. First, bL31-deficient ribosomes are severely compromised in their ability to ensure translational fidelity particularly in reading frame maintenance *in vivo*. Second, in the absence of bL31, the rate of initiation is significantly reduced both *in vivo* and *in vitro.* Third, polysome profile and subunit reassociation assays demonstrate that bL31 is important for stabilizing subunit joining *in vivo* and *in vitro*. Together, our results demonstrate that bL31 is important for determining translational fidelity and stabilizing subunit association. We conclude that the only protein–protein intersubunit bridge of the bacterial ribosome facilitates translation initiation and is essential for maintaining the reading frame of mRNA translation.
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Introduction

There are altogether 12 intersubunit bridges in the bacterial ribosome [1], as determined by the crystal structure of the *Thermus thermophilus* ribosome [1,2]. Both ribosomal RNA (rRNA) and ribosomal proteins (r-proteins) contribute to these molecular contacts: there are six RNA–RNA bridges, five RNA–protein bridges, and one protein–protein bridge. The bridges with a protein component are rather localized into the periphery of the ribosome, that is, relatively distant from its functional centers as compared with the RNA–RNA bridges. In addition, bridges with a protein component are shown to be dynamic during the ribosomal working cycle to facilitate subunit moving with respect to each other [3].

The head domain of the 30S subunit and the central protuberance of the 50S subunit are connected via two intersubunit bridges: ribosomal intersubunit bridges 1a and 1b (B1a and B1b) [2]. The B1a bridge is composed of the universal small subunit protein S13 (uS13) and helix 38 of 23S rRNA [1]. Bridge B1b, the only protein–protein bridge in the bacterial ribosome, is composed of uS13, universal ribosomal large subunit protein 5 (uL5), and bacterial L31 (bL31) [1] (Fig. 1a and b). Owing to its dynamic nature [4] and relatively loose binding to the ribosome [5], the determination of bL31 as an intersubunit bridge component was first achieved putatively [6] and confirmed later when bL31 was discovered to be a part of the protein network around the A-site tRNA [7]. More specifically, the C terminus of bL31 makes intersubunit contacts with uS13, while the N terminus of bL31 is situated on the central protuberance, where it contacts uL5 and 5S rRNA [7]. During translocation, bridge B1a is disrupted [8], whereas bridge B1b remains intact [9]. However, it is rearranged so that bL31 comes in contact with universal ribosomal small subunit protein 19 (uS19), universal ribosomal small subunit protein 14, and h42 of 16S rRNA [4]. Taken together, bL31 acts as a linker between other B1b proteins (uS13, uL5).

Based on the contacts with uS13 and uS19 whose C termini reach P- and A-site tRNAs, respectively, bL31 has been proposed to be involved in proofreading tRNA to discriminate between cognate and near-cognate tRNA [7]. In addition, as a dynamic

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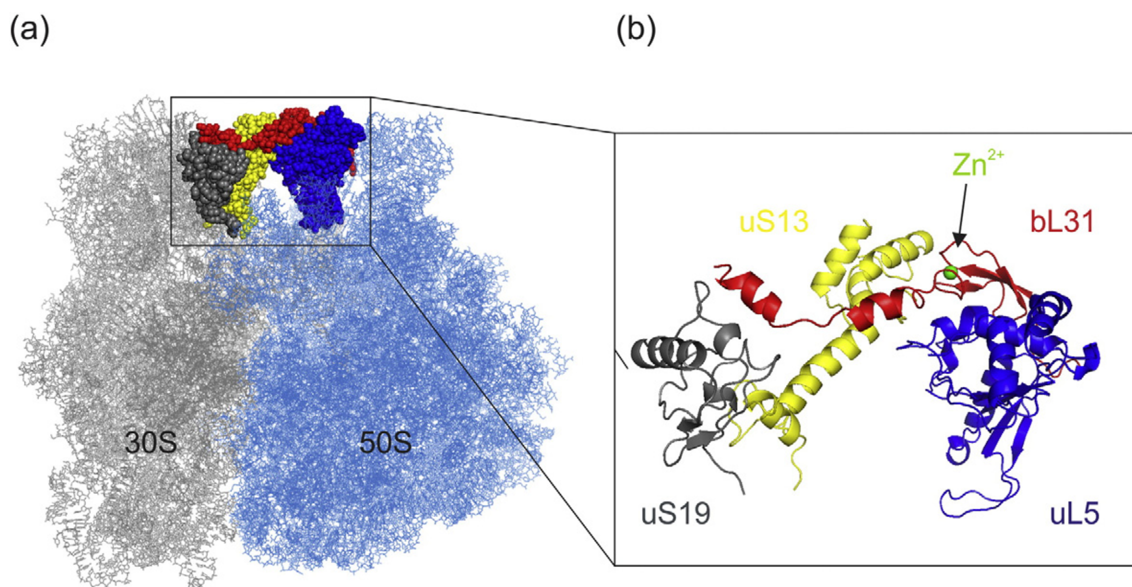

Fig. 1. Ribosomal intersubunit bridge B1b in the pre-translocational 70S ribosome of *Escherichia coli*. (a) The 70S ribosome of *E. coli*. The small subunit (30S) is shown in gray, the large subunit (50S) in light blue. Components of the B1b bridge are uS13 (yellow), uS19 (grey) from the 30S subunit, bL31 (red), and uL5 (dark blue) from the 50S subunit. (b) Closeup view of the B1b intersubunit bridge displaying involved r-proteins colored as in (a); bL31 binds a zinc ion (green sphere). Ribosomal structures were generated with PyMol using coordinates from Ref. [4] (PDB entry 5AFI).

bridge protein, bL31 could contribute to the regulation of 30S head movement during translocation [10]. This notion is supported by the flexible linker region between the N and C terminus of bL31 that adopts different conformations depending on the rotational state of 30S subunit [4].

Ribosomal large subunit protein bL31 is *eo nomine* a bacteria-specific r-protein [11] that has a homolog in yeast mitochondrial ribosome [12] but not in archaea [13] or eukaryotic cytosolic ribosomes [14]. Almost all sequenced bacterial genomes encode bL31, and in many cases, two or more paralogs are present as well [15]. In general, having paralogous genes is not a characteristic feature of bacterial r-protein genes [15]. In *Escherichia coli*, bL31 has two paralogous genes (*rpmE* and *ykgM*) [16] that encode proteins (bL31A 7.8 and bL31B 9.9 kDa, respectively) with 35% identical amino acid sequence. Similar to most of the paralogous r-proteins, bL31 paralogs differ in their zinc binding ability: bL31A binds one zinc ion per molecule, whereas bL31B does not bind zinc [17]. This distinction and the relatively loose binding of bL31 to the ribosome [18] have led to the hypothesis that the Zn-binding paralog could function as a zinc ion reservoir *in vivo* [19].

Previously, the B1b bridge has been studied from the small subunit side by deleting or mutating uS13 protein [20]. Here, we have taken the large subunit perspective, that is, examined the importance of B1b by deleting both paralogs of bL31. Our results show that bL31 and thereby the bridge B1b are necessary for stabilizing ribosomal subunit association *in vivo* and *in vitro*. In addition, bridge B1b is important for maintaining reading frame and reducing nonsense suppression. *In vivo* and *in vitro* experiments demonstrate that the bridge B1b affects the rate of translation initiation, but not the rate of translation elongation. Taken together, we conclude that B1b facilitates translation initiation and is essential for maintaining the reading frame of mRNA translation.

Results

bL31 contributes to optimal growth at physiological temperature and below

To study the functional role of ribosomal intersubunit bridge B1b in the ribosome, we constructed an *E. coli* strain with the genomic deletions of both bL31 paralogous genes (*rpmE* and *ykgM*; MGΔbL31) as specified in Materials and Methods. For control experiments, this strain was transformed with the pBT-bL31 plasmid to constitutively express RpmE (bL31A) *in trans.* The use of bL31A in control experiments is justified, as only this paralog has been identified in the ribosome structure of *E. coli* so far [4]. In addition to this, based on r-protein analysis during different growth phases [18], it was concluded that bL31A is the dominant paralog in ribosomes. Therefore, bL31A is hereafter referred to as bL31.

Generation times of the strains were determined in a rich (2xYT) medium at 30 °C and 37 °C, when

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