Crystal Structure of Hypusine-Containing Translation Factor eIF5A Bound to a Rotated Eukaryotic Ribosome

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Abstract

Eukaryotic translation initiation factor eIF5A promotes protein synthesis by resolving polyproline-induced ribosomal stalling. Here, we report a 3.25-Å resolution crystal structure of eIF5A bound to the yeast 80S ribosome. The structure reveals a previously unseen conformation of an eIF5A–ribosome complex and highlights a possible functional link between conformational changes of the ribosome during protein synthesis and the eIF5A–ribosome association.

Introduction

The universally conserved translation factor eIF5A in eukaryotes and its bacterial homolog EF-P help ribosomes produce proteins containing three or more consecutive proline residues by resolving polyproline-induced stalling [1–3]. In eukaryotes, eIF5A activity depends on its unusual post-translationally modified residue hypusine, which is thought to augment the rate of peptide bond formation in the ribosomal catalytic center [4–6]. Structural studies of EF-P bound to the bacterial 70S ribosome from *Thermus thermophilus* and of eIF5A bound to the eukaryotic 80S ribosome from yeast showed that both EF-P and eIF5A bind the ribosome in the vicinity of the ribosomal catalytic center [1,7,8], positioning the critical hypusine residue (in the eIF5A structure) to contact the acceptor stem of the P-site tRNA [8]. Although these studies provided mechanistic insights into EF-P/eIF5A activity, in both structural studies the ribosome was in the same classical state conformation. Therefore, these studies left unexplored how eIF5A activity is related to ribosome dynamics during protein synthesis. In fact, during translation, the ribosomal subunits make rotation-like motions, which serve to coordinate tRNA and mRNA translocation between the active sites of the ribosome [9,10]. It is unknown whether changes in the ribosome conformation alter the structure the eIF5A-binding site and possibly facilitate eIF5A recruitment or release from the ribosome.

Structure of the eukaryotic ribosome bound to hypusinated eIF5A

Our interest in eIF5A arose from crystallographic studies of the eukaryotic ribosome. Curiously, we observed that 80S ribosomes purified from glucose-starved yeast cells carry two additional proteins—stress-related factor Stm1 and eIF5A (Table S1) [11]. While Stm1 appeared in the electron density map, eIF5A was absent, possibly due to its dissociation during extensive post-crystallization treatments of the crystals (see Materials and Methods in Sup. Data). In this study, we modified the post-crystallization

treatments and supplemented the soaking solutions with recombinant eIF5A. These changes have enabled us to determine the structure of hypusinated eIF5A from *Saccharomyces cerevisiae* bound to the yeast 80S ribosome at 3.25 Å resolution (I/σ = 1, Table 1; Fig. 1a).

The electron density map revealed hypusinated eIF5A bound to one of two 80S ribosomes in the asymmetric unit of the crystal (Fig. 1a). Although our crystal structure lacks a P-site tRNA, we found that eIF5A occupies the same position as was observed in the 3.9 Å cryo-EM structure of the 80S ribosome complex with A- and P-site tRNAs [8] (Fig. 2a and S1). In this position, eIF5A binds adjacent to the P site, where it interacts with the intersubunit interface and two dynamic features of the large ribosomal subunit—the L1-stalk and helix H69 of the 25S rRNA (Fig. 1b). In addition, eIF5A forms several eukaryote-specific contacts with the large ribosomal subunit (Fig. S1). As in the cryo-EM structure of the 80S ribosome/eIF5A complex [8], the hypusine residue is located in direct vicinity of the ribosomal P site. The hypusine residue adopts a sharply bent conformation in which its positively charged κ-amino group is directed toward the CCA-end of the P-site tRNA, pointing between the 2′- and 3′-OH groups on the C75 ribose (Fig. S2). This conformation of hypusine is similar to the hypusine conformation in the cryo-EM structure of the 80S ribosome/eIF5A complex [8], although the κ-amino group is slightly shifted toward residue A2808 of the 25S rRNA in the cryo-EM structure (Fig. 1c and S2). This subtle difference might possibly result from the presence of the tRNA in the P site or might partially reflect the coordinate error in both structures. Although it remains unclear how hypusine stimulates the rate of protein synthesis, our structure illustrates that the hypusine position in the P site of the ribosome is determined predominantly by its interaction with the ribosome, rather than by interactions with the tRNA bound to the ribosomal P site.

In a parallel experiment, we determined the structure of unmodified eIF5A from *S. cerevisiae* bound to the yeast 80S ribosome at 3.35 Å resolution (I/σ = 1, Table 1). The eIF5A lacking hypusine occupied the same position as hypusinated eIF5A in the eIF5A/80S ribosome complex; however, the unmodified eIF5A showed only partial occupancy in the binding site (Table 1). This partial occupancy is consistent with previous observations showing that, in addition to its essential role in promoting protein synthesis [1,6,12], hypusine stabilizes eIF5A association with the ribosome [13,14].

Table 1. Data collection and refinement statistics

	80S/eIF5A–Hyp51	80S/eIF5A–Lys51
Data collection		
No. of crystals	3	2
Space group	$P2_1$	$P2_1$
Unit cell		
a, *b*, *c* (Å)	435.63/286.45/303.41	438.23/289.33/305.47
α = β = 90°, γ(°)	98.95	98.92
Resolution	189.16–3.15 (3.25–3.15)	190.48–3.25 (3.35–3.25)
R_{meas}	36.0 (212.0)	43.4 (235.6)
I/σI	6.54 (1.03)	6.38 (0.98)
$CC_{1/2}$	99.4 (39.6)	99.5 (35.5)
Resolution at $CC_{1/2}$ = 0.5 (Å)	3.25	3.35
Completeness (%)	99.9 (99.7)	99.9 (99.9)
Redundancy	9.1	8.2
Refinement		
Resolution	189.16–3.15	190.48–3.25
No. reflections	1,261,726	1,176,370
R_{work}/R_{free}	25.56/26.37	25.43/29.54
No. of atoms	407,909	407,903
Protein	184,678	185,006
RNA and ions	223,231	220,897
B-factors		
Protein	85.5	103.9
RNA and ions	73.5	98.2
r.m.s. deviations		
Bond lengths (Å)	0.006	0.006
Bond angles (Å)	1.039	1.016
eIF5A-occupancy refinement (%)	97	88
PDB ID	5dat	5dc3

Values in parenthesis show statistics of the highest resolution shell.