

Translation Initiation Factor eIF3b Contains a Nine-Bladed β -Propeller and Interacts with the 40S Ribosomal Subunit

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SUMMARY

The multisubunit eukaryotic translation initiation factor 3, among which the subunit b (eIF3b) is a major scaffold protein, plays essential roles in protein synthesis. Here, we report the crystal structure of the WD40 domain of *Chaetomium thermophilum* eIF3b, revealing a nine-bladed β -propeller fold. Sequence analysis indicates that this propeller architecture is common to all eIF3b orthologs. Revisiting the cryo-electron microscopy (cryo-EM) map of the 43S preinitiation complex suggests an interaction of the eIF3b with the 40S ribosomal subunit involving the ribosomal protein S9e and the 18S rRNA. This model is strongly supported by the direct binding of eIF3b to 40S ribosomes and to the isolated ribosomal protein rpS9e in vitro.

INTRODUCTION

In eukaryotes, the elongation-competent ribosome is assembled during the initiation phase on the mRNA start codon with the Met-tRNA_i^{Met} in its P site. The fidelity of this critical step is ensured by a set of at least 12 different auxiliary protein factors, called eukaryotic translation initiation factors (eIFs) (Jackson et al., 2010). The largest among these factors is the multisubunit complex eIF3, which is involved in almost all steps of the initiation process (Hinnebusch, 2006). It associates with eIF1, eIF5, and the eIF2·GTP·Met-tRNA_i^{Met} ternary complex and promotes their binding to the 40S ribosomal subunit during 43S preinitiation complex (PIC) assembly (Valásek et al., 2002, 2003). Moreover, eIF3 assists the subsequent recruitment of mRNA to the 43S PIC (Jivotovskaya et al., 2006; Korneeva et al., 2000) and mutation studies in yeast indicated an involvement of eIF3 in scanning and the stringent start codon selection (Chiu et al., 2010; Valásek et al., 2004).

In budding yeast, eIF3 is composed of five essential subunits (a/Tif32, b/Prt1, c/Nip1, i/Tif34, and g/Tif35), all of which have corresponding orthologs in the significantly larger eIF3 complex of other eukaryotes (13 subunits [a–m] in mammals), and the nonessential substoichiometric subunit Hcr1/eIF3j (Asano et al., 1998; Hinnebusch, 2006). Within the eIF3 complex,

eIF3b plays a central role as scaffold protein that provides an interaction platform for the other core subunits. eIF3b is an ~90 kDa protein that has been predicted to fold into three independent domains (Marintchev and Wagner, 2004) (Figure 1A). The N-terminal domain (NTD) of eIF3b contains a structurally canonical RNA recognition motif (RRM) and is responsible for the binding to eIF3a and eIF3j (ElAntak et al., 2007; Valásek et al., 2001), while the eIF3b C-terminal domain (CTD) is necessary for the interaction with eIF3i and eIF3g (Asano et al., 1998; Herrmannová et al., 2012). The NTD and CTD are connected by the central WD40 domain, which was predicted to fold most likely into two seven-bladed β -propellers (Marintchev and Wagner, 2004). So far, structural information about eIF3b is limited to the N-terminal RRM domain (~100 amino acids) and an ~40 residue α helix of the CTD (ElAntak et al., 2007; Herrmannová et al., 2012; Khoshnevis et al., 2010). In contrast, neither functional nor structural information is available for the central WD40 repeats, which form ~65% of the protein and are highly conserved from yeast to humans.

The WD40 repeat is a small motif of ~40 amino acid residues usually forming a four-stranded antiparallel β sheet and often containing the eponymous Trp-Asp (WD) dipeptide at the C terminus (Xu and Min, 2011). Tandem WD40 repeats are radially arranged around a center axis, resulting in a multibladed, doughnut-like propeller architecture (Stirnemann et al., 2010). In most cases, the last blade of a propeller is composed of the N-terminal β strand situated in the outermost shell of the propeller and the last three C-terminal strands, creating a “1_N + 3_C Velcro” closure that stabilizes the radial folding (Xu and Min, 2011). WD40 domains are involved in a wide range of cellular functions, commonly by serving as a rigid scaffold to facilitate protein-protein interactions (Stirnemann et al., 2010; Xu and Min, 2011). Consistent with their diversity in sequence length and composition, WD40 propellers vary considerably in their number of blades. To date, four- to ten-bladed WD40 β -propellers have been structurally identified, among which the seven-bladed propellers are the most abundant in the Protein Data Bank (PDB), followed by six and eight repeats (Chen et al., 2011).

Here, we report the crystal structure of the eIF3b WD40 β -propeller from *Chaetomium thermophilum* at 2.7 Å resolution, revealing an unforeseen nine-bladed propeller. Besides its nine-bladed architecture, the ctelF3b WD40 propeller reveals several noncanonical folding properties. It uses an unusual “3_N + 1_{C,innermost} Velcro” closure type and possesses a central

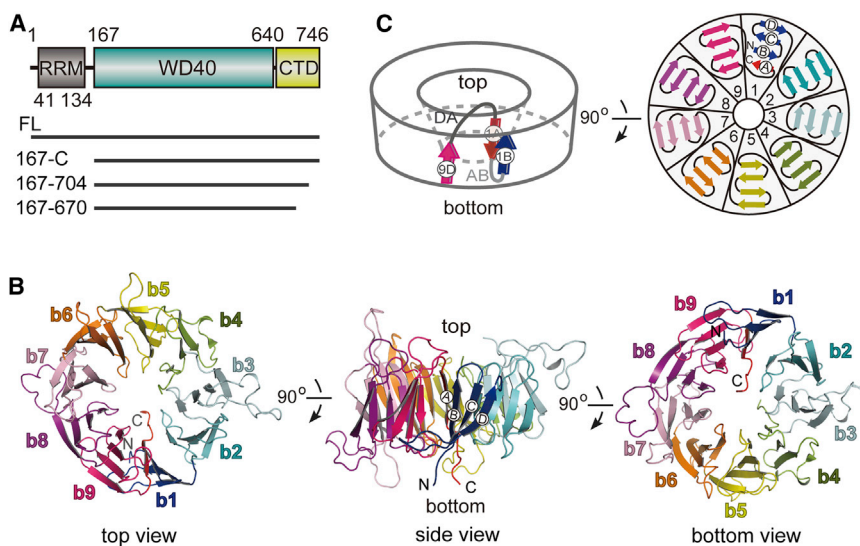


Figure 1. Overall Structure of *ctelF3b*-WD40

(A) Schematic domain organization of *ctelF3b*. The boundaries of the domains are indicated.

(B) Top, side, and bottom views of the overall structure of *ctelF3b*-WD40 in cartoon representation. Blades 1 to 9 are colored individually and N termini (N) as well as C termini (C) are labeled.

(C) Topology plot of the *ctelF3b* β -propeller fold. The left and right drawings are shown in orientations similar to the side and bottom views in (B), respectively.

See also Figure S1.

channel exhibiting the shape of a circular truncated cone rather than the common cylindrical form. On the basis of its unique shape, we were able to unambiguously fit the *ctelF3b* WD40 structure into the recently published cryo-EM map of the 43S PIC in a position previously assigned to the seven-bladed β -propeller protein eIF3i (Hashem et al., 2013). Because of this location in the shoulder region of the 40S ribosomal subunit, eIF3b is expected to be in close proximity to the ribosomal protein S9e. Consistently, we observed a stable interaction between eIF3b and the isolated protein rps9e as well as with the 40S ribosomal subunit by *in vitro* binding studies. Hence, our data strongly argue for a direct involvement of eIF3b in the recruitment of eIF3 to the 40S ribosomal subunit and 43S PIC assembly.

RESULTS AND DISCUSSION

Overall Structure

Crystallization of full-length *ctelF3b* yielded crystals containing only the WD40 domain because of proteolytic cleavage during the crystallization process (Figure S1 available online). The crystal structure was solved *de novo* by means of multiwavelength anomalous dispersion (MAD) and refined at a resolution of 2.7 Å (Table 1). It comprises residues 165 to 638, with one region (loop residues 299 to 311) not defined in the electron density map.

Surprisingly, in contrast to the prediction of two tandem propellers (Marintchev and Wagner, 2004), the WD40 domain of *ctelF3b* actually adopts a single β -propeller fold composed of nine blades (Figure 1B). The nine blades are pseudosymmetrically arranged in sequential order around a central channel. Each blade of the propeller is formed by a β sheet consisting of four antiparallel strands, designated A, B, C, and D from the inside to the outside of the propeller. Different from the common “ $1_N + 3_C$ Velcro” closure of the β -propeller fold (Xu and Min, 2011), the WD40 repeats of *ctelF3b* apply a “ $3_N + 1_C$ ” arrangement whereby the last C-terminal β strand is positioned as the innermost strand of the first blade (Figures 1B and 1C). Another unusual feature of the nine-bladed *ctelF3b* WD40 propeller is that the central channel resembles a circular truncated cone (Figures 1B and 1C). The

diameter of the channel on the “bottom” side is ~ 22.4 Å, which is further decreased to ~ 15.6 Å by the conserved AB loops, compared with the increased average diameter of 29.5 Å at the “top.” The outermost shape of the *ctelF3b* propeller is almost cylindrical, as revealed by the overall diameters of 51.5 Å and 53.6 Å for the bottom and top parts of the propeller, respectively. When compared with seven- and eight-bladed propellers, the additional blade(s) of the *ctelF3b* WD40 expand the propeller’s dimensions, volume, and accessible surface area. Taken together, this might be of functional relevance for eIF3b’s role as a central scaffold protein within eIF3 and its simultaneous association with proteins and rRNA of the 40S ribosomal subunit (see below).

Sequence and Structural Conservation of eIF3b

Structure-based sequence alignments of the WD40 repeats of *ctelF3b* reveal several conserved sequence characteristics, notably the Trp-X-Pro (where X represents any amino acid) motif at the end of strands A and the Phe/Tyr- Φ (where Φ stands for a hydrophobic amino acid) segment in strands B, as well as hydrophobic residues in strands D (Figure 2A). However, the Trp-Asp (WD) dipeptide located at the ends of strands C is present only in blades 3 and 8. The Trp residue of the WD motif has been replaced by the aromatic amino acid Tyr (in blades 4 and 9), Phe (in blades 6 and 7), and the hydrophobic Met in blade 5, whereas the Asp has been substituted by other polar residues, with the only exception of a Gly in blade 2 (Figure 2A). The repeating motifs (single blades) within the *ctelF3b* WD40 domain are structurally well conserved, with a root-mean-square deviation (rmsd) ranging from 0.94 Å to 1.57 Å for all $C\alpha$ atoms, whose positions differed less than 3 Å, when superimposing each blade onto blade 1 (Figure 2B).

A multiple-sequence alignment reveals that eIF3b orthologs from different organisms vary considerably in the NTD but are well conserved in the WD40 domain as well as in the C-terminal part of the protein (Figure S2). Secondary structure predictions (using the PSIPRED server; Buchan et al., 2013) of eIF3b orthologs assign 35 or 36 β strands to their WD40 domains, indicating that the nine-bladed WD40 β -propeller fold of *ctelF3b* is not a unique instance but is used universally by eIF3b orthologs. Many of the identical residues among the orthologs are hydrophobic and located in the structural core of the WD propeller (Figure S2), indicating their role in accurate folding and stability. Additionally, two

Table 1. Data Collection and Structure Refinement Statistics

	Se-Met Derivative, MAD			
	Peak	Inflection	Remote	Native
Data Collection				
Wavelength (Å)	0.9798	0.9799	0.9775	0.8266
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$
Resolution (Å)	50–3.25 (3.35–3.25)	50–3.30 (3.40–3.30)	50–3.31 (3.41–3.31)	50–2.72 (2.82–2.72)
Cell dimensions				
a (Å)	108.32	108.31	108.28	108.38
b (Å)	108.32	108.31	108.28	108.38
c (Å)	186.88	186.90	186.84	172.18
Unique reflections	32,904 (2,863)	31,367 (2,719)	30,923 (2,659)	28,259 (2,828)
Redundancy	3.1 (2.9)	3.1 (3.0)	2.7 (2.7)	8.5 (8.6)
Completeness (%)	98.2 (98.9)	98.0 (98.7)	97.9 (98.6)	99.9 (100.0)
R_{merge} (%)	9.2 (73.8)	9.0 (61.0)	9.5 (60.3)	5.7 (61.5)
I/σ (I)	11.67 (1.76)	12.45 (2.13)	10.71 (1.90)	25.67 (3.48)
$CC_{1/2}$	99.7 (66.5)	99.7 (74.5)	99.5 (67.7)	99.9 (92.0)
Phasing				
Anomalous correlation ^a	47 (4)	34 (11)	28 (12)	
SigAno ^a	1.33 (0.78)	1.16 (0.83)	1.08 (0.82)	
FOM after DM	0.573			
Structure Refinement				
Resolution range (Å)				48.47–2.72 (2.82–2.72)
Completeness (%)				100.0 (100.0)
$R_{\text{work}}/R_{\text{free}}$ (%) ^b				20.56/22.98
No. of atoms				
Total				3,819
Protein				3,764
Solvent				51
Ions				4
Rmsd bonds (Å)				0.003
Rmsd angles (°)				0.806
B factor (Å ²)				
Average				74.8
Protein				75.0
Solvent				61.8
Ions				97.0
Wilson B factor (Å ²) ^c				66.87
Solvent content (%)				73
Ramachandran plot				
Favored (%)				95.19
Allowed (%)				4.81
Outliers (%)				0.0

Values for the data in the highest resolution shell are shown in parentheses.

^aAnomalous correlation and mean anomalous difference (SigAno), calculated with XSCALE.

^b $R_{\text{free}} = \frac{\sum_{\text{Test}} |F_{\text{obs}}| - |F_{\text{calc}}|}{\sum_{\text{Test}} |F_{\text{obs}}|}$, where “Test” is a test set of about 5% of the total reflections randomly chosen and set aside before refinement for the complex.

^cWilson B factor was estimated with phenix.refine package.

clusters of surface residues are highly conserved from yeast to humans (Figure 2C), suggesting a potential role in binding other proteins and/or the other eIF3b domains. The first conserved surface region is located on the side of the propeller and consists of

residues mainly belonging to blades 5 and 6; the second conserved area is situated primarily on the top of the propeller, involving residues from the AB loops of blades 9, 1, 2, and 3 and the D strands of blades 1 and 2 (Figure 2C). Interestingly, both

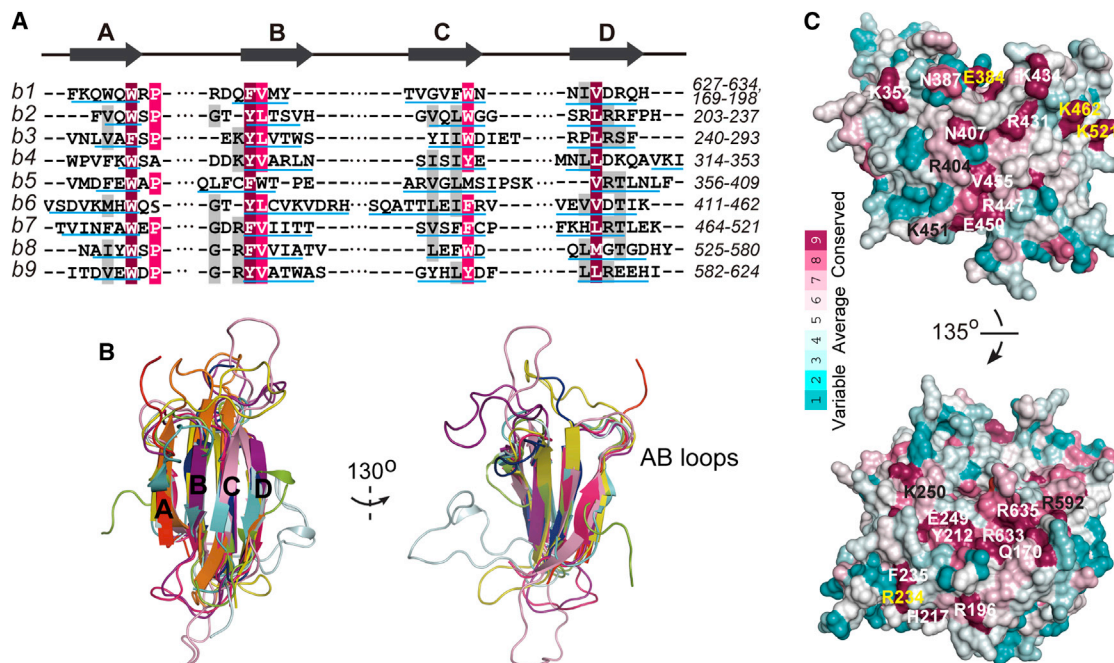


Figure 2. Sequence and Structural Conservation of eIF3b

(A) Sequence alignment of the nine β sheets of *ctelF3b*-WD40. Residues are colored according to the conservation pattern (highly conserved: dark red; conserved: pink). Residues forming the β strands are underlined, and arrows on the top indicate the positions of strands A to D.

(B) Superposition of the nine β -propeller blades of *ctelF3b*. The blades are colored as in Figure 1B. In the image on the right, the long β sheet 6 was omitted for clarity reasons.

(C) Surface representation of *ctelF3b*-WD40 with sequence conservation represented in the color-coding bar, ranging from cyan (variable) to purple (conserved). The conservation scores were generated by ConSurf (<http://consurf.tau.ac.il>) using the sequence alignment of eIF3b-WD40 as shown in Figure S2. Highly conserved surface residues are labeled.

conserved surface areas of *ctelF3b*-WD40 mainly contain hydrophobic and positively charged residues, which could be involved in RNA binding (Figure S3).

To address the question of whether the nine-bladed WD40 β -propeller fold can also be found in proteins other than eIF3b orthologs, we searched the National Center for Biotechnology Information Conserved Domain Database (Marchler-Bauer et al., 2011). We identified eIF2A, an alternative initiator tRNA-binding protein involved in internal ribosome entry site-mediated translation initiation (Reineke et al., 2011), as a potential candidate, even though the sequence identity between the WD40 domains of eIF2A and eIF3b is only 20%. The secondary structure prediction of eIF2A assigns 36 β strands, exactly matching the strands number of a nine-bladed β -propeller. While this manuscript was under revision, the crystal structure of eIF2A became available (PDB accession number 3WJ9; Kashiwagi et al., 2014), revealing an almost identical nine-bladed β -propeller fold, which is reflected by an rmsd of 1.79 Å for 300 common $C\alpha$ atoms of the eIF2A and eIF3b structures. However, the high degree of sequence degeneracy among WD40 domains makes the prediction of the correct blade number and domain boundaries and therefore of other nine-bladed β -propeller very difficult.

eIF3b Serves as the Central Scaffold Subunit for the eIF3 Core Complex in *C. thermophilum*

The genome of *C. thermophilum* contains 13 genes encoding eIF3 subunits, suggesting that *ctelF3* resembles mammalian

eIF3. Like its yeast counterpart, *ctelF3* contains a stable five-subunit core, as shown by means of analytical size-exclusion chromatography (SEC) (Figure 3A). The conservation between yeast and *C. thermophilum* is also reflected by the existence of two distinct subcomplexes containing eIF3b, which consist of eIF3a,b,c and eIF3b,g,i respectively (Figures 3B and 3C). However, in contrast to yeast eIF3 (Khoshnevis et al., 2012), a stable eIF3b-c-i-g subcomplex could not be reconstituted in vitro from *C. thermophilum* proteins (Figure S4). This indicates that the interaction between eIF3c and the eIF3b-i-g subcomplex is either not universal and therefore might not be essential for the assembly of the eIF3 complex or depends on the presence of additional subunits of eIF3 in *C. thermophilum*.

Localization of eIF3b on the 40S Ribosomal Subunit

It has previously been demonstrated by both biochemical studies and cryo-EM reconstructions that eIF3 associates with the solvent-exposed side of the 40S ribosomal subunit. Biochemical investigations suggested that the eIF3 core is located in the head and shoulder regions of the 40S, while the cryo-EM structures indicated that the major part of eIF3 is anchored underneath the platform of the 40S (Hashem et al., 2013; Siridechadilok et al., 2005; Valásek, 2012). In the cryo-EM map of the rabbit 43S PIC bound to helicase DHX29 (Electron Microscopy Data Bank [EMDB] accession number 5658), the seven-bladed β -propeller protein eIF3i was fitted into a doughnut-like density below the shoulder of the 40S subunit;

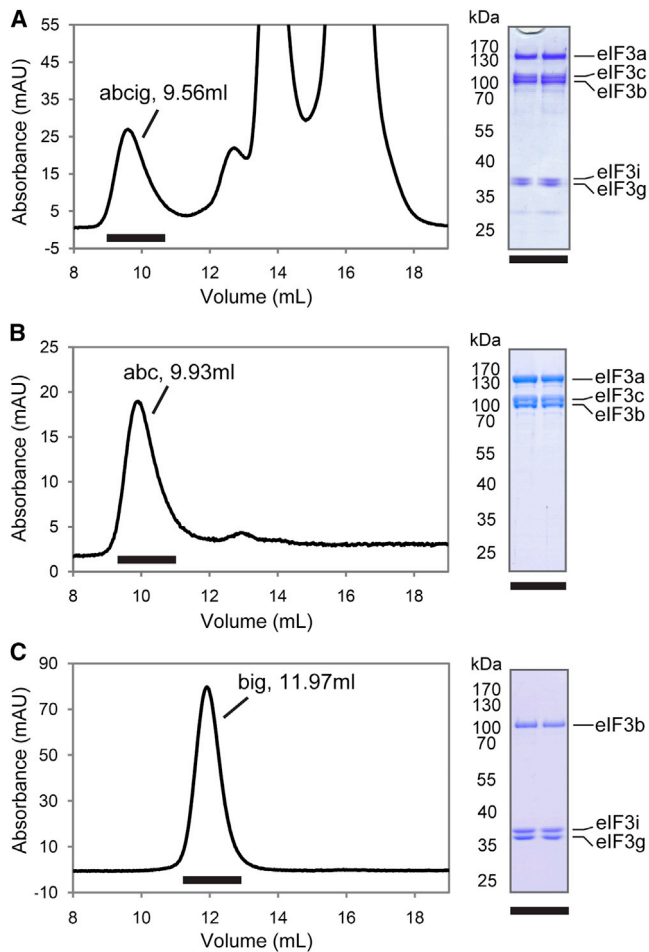


Figure 3. Analytical SEC of ctelF3 Subcomplexes

Analytical SEC profiles of the five-component ctelF3 core (A), the ctelF3a-b-c subcomplex (B), and the ctelF3b-i-g subcomplex (C), respectively. The corresponding SDS-PAGE gels are shown in the right panel.

however, the possibility that this density originated from the β -propeller of eIF3b could not be excluded (Figure 4A) (Hashem et al., 2013). Notably, this cryo-EM segment definitely possesses a channel with a wide top and narrow bottom. As aforementioned, the WD40 domain of eIF3b harbors a channel with similar geometric parameters, which reaches a consensus with the cryo-EM density on the axle outline (Figures 1B, 4B, and 4C). By contrast, eIF3i holds a channel that has a similar width on both sides (PDB accession number 3ZWL). Additionally, the overall size of eIF3i is too small to fully explain this segmented EM density (Figure S5). Because of the lack of technical details, we could not repeat the fitting of eIF3i to the doughnut-like density and reach a similar result as reported (Hashem et al., 2013). Our fit of eIF3i using UCSF Chimera 1.8 (Pettersen et al., 2004) resulted in a cross-correlation coefficient (CCC) of 0.52 and 38.5% atom outliers. The fitting statistics significantly improved when the WD40 propeller (without the nonconserved loops) of ctelF3b was positioned in the doughnut-like density (Figure S5), with a corresponding CCC of 0.91 and only 17% of the atoms outside the density. On the basis of our fit, the surface formed

by highly conserved residues of blades 5 and 6 of the propeller is oriented toward the ribosomal protein S9e (rpS9e) and the rRNA helix h16 (Figures 4B and 4C). The EM density also indicates a possible interaction between the rRNA expansion segment ES6^S-hA and blade 9 and/or conserved basic residues C-terminally adjacent to the WD40 domain (Figure 4D; Figure S2).

The position of eIF3b directly below the shoulder of the 40S is in good agreement with previous studies, as eIF3j, which interacts with eIF3b-RRM via its NTD, has been proposed to bind to the 40S mRNA entry channel adjacent to the shoulder of the 40S subunit (ElAntak et al., 2007; Elantak et al., 2010; Fraser et al., 2007; Nielsen et al., 2006).

Interaction between eIF3b and the 40S Ribosomal Subunit

The interpretation of the EM map described above suggests a direct interaction of eIF3b with the 40S ribosomal subunit. Thus far, no biochemical evidence exists for such an interaction, and therefore we sought a biochemical validation. For this, purified *C. thermophilum* 40S ribosomal subunits were incubated with increasing amounts of ctelF3b and their mutual interaction studied by cosedimentation experiments. Upon the addition of ctelF3b, we noted the appearance of an additional ~ 86 kDa protein in the pellet, which is absent in purified ct40S subunits, indicating a stable association. Under these experimental conditions, unbound eIF3b remained in the supernatant (Figure 4E). In addition, ctelF3b¹⁶⁷⁻⁶⁷⁰ (containing the WD40 domain plus an ~ 30 -residue C-terminal extension) also binds to the ct40S subunit (Figure S6A), suggesting that the N-terminal RRM domain is dispensable for binding.

Our interpretation of the 43S PIC EM 3D reconstruction places eIF3b next to the ribosomal protein rpS9e, which could be verified by pull-down assays. Both full-length ctelF3b and N-terminally truncated ctelF3b^{167-C} bind to glutathione S-transferase (GST)-tagged ctrpS9e (Figure 4F), while ctelF3b¹⁶⁷⁻⁶⁷⁰ and ctelF3b¹⁶⁷⁻⁷⁰⁴ fail to do so (Figure S6B). This implies that the 42 C-terminal residues of ctelF3b are crucial for the interaction with ctrpS9e. Importantly, the interaction of ctelF3b-CTD is not a prerequisite for binding to the ct40S ribosomal subunit, as ctelF3b¹⁶⁷⁻⁶⁷⁰ is still capable of this association (Figure S6A). Thus, interactions with other parts of the ct40S ribosome (e.g., the rRNA ES6^S-hA) are sufficient for stable association.

Because the eIF3b-CTD also contains binding sites for eIF3i and eIF3g, we further tested whether the interaction between ctelF3b and ctrpS9e affects the binding of ctelF3i and ctelF3g to ctelF3b. The observed quaternary complex (Figure 4F) suggests that these intermolecular interactions are not mutually exclusive. Consistently, the rpS9e binding site of eIF3b is located C-terminally to the α helix, which interacts with the eIF3i β -propeller (Herrmannová et al., 2012). Interestingly, the NTD of ctelF3b apparently also contributes to the formation of the ctrpS9e-eIF3b,g,i complex, as the N-terminally truncated ctelF3b^{167-C} exhibits a lower affinity to eIF3g,i (Figure 4F).

With regard to the 43S PIC/DHX29 EM map, we expect the C-terminal 42 residues of eIF3b to fill some of the noninterpreted density between rpS9e and the β -propeller. There is also some unassigned density protruding from the β -propeller (Figure 4B). Because this density is adjacent to both the N and C termini of

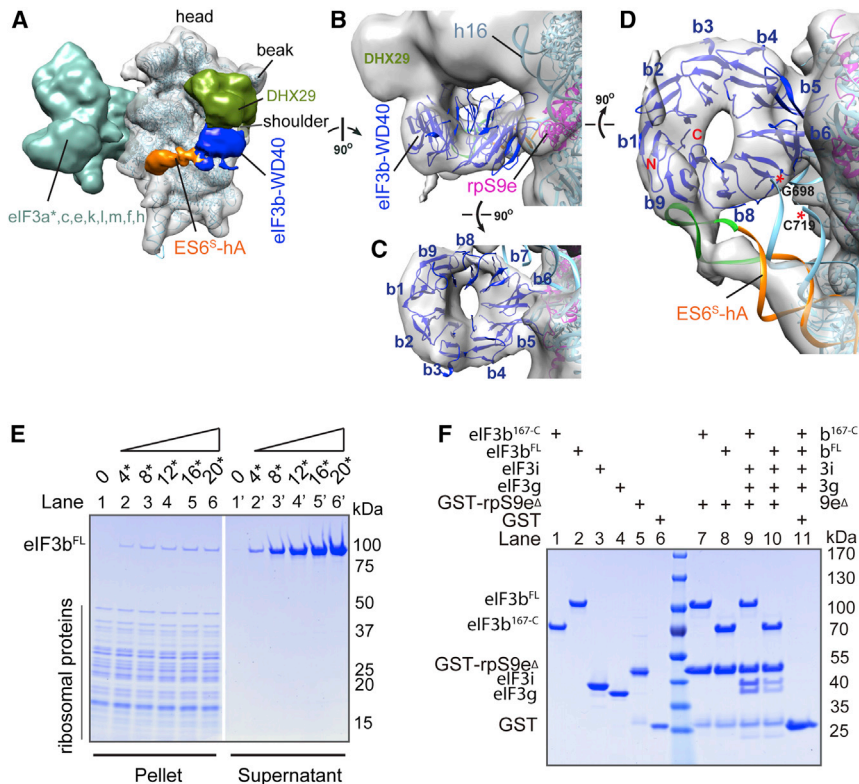


Figure 4. Interaction of eIF3b with the 40S Ribosomal Subunit

(A–D) Localization of eIF3b on the 40S ribosome. The nonconserved loop insertions of ctelF3b-WD40, which are absent in rabbit eIF3b, were removed before fitting. In (C) and (D), the density assigned to DHX29 was removed for clarity.

(A) Overall view of the position of eIF3b-WD40 β -propeller on the 40S ribosomal subunit (EMDB accession number 5658). eIF3b-WD40 (blue) is located below the shoulder of the 40S and is disconnected from the eIF3 subcomplex containing the subunits a*, c, e, f, h, k, l, and m (cyan). The asterisk marks a truncated version of eIF3a.

(B) Detail side view of eIF3b-WD40 localization. The rpS9e and the rRNA helix h16 are indicated.

(C) Top view of eIF3b-WD40 showing the characteristic conical central channel.

(D) Structural model of a possible interaction between the eIF3b WD40 propeller and the 18S rRNA expansion segment ES6^S-hA. The rRNA helix was manually fitted into the EM density portion using the crystal structure of the 40S ribosome (PDB accession number 4KZZ; Lomakin and Steitz, 2013). Nineteen nucleotides of ES6^S-hA, which are missing in the crystal structure, have been modeled (colored green) to illustrate their potential extension.

(E) Binding of ctelF3b to ct40S ribosomes. Incubation of increasing amounts of ctelF3b leads

to the cosedimentation with ct40S subunits (lanes 2–6), while excess ctelF3b remains in the supernatant (lanes 2'–6'). Lanes 1 and 1' represent ct40S in the absence of ctelF3b.

(F) Interaction of ctelF3b and ctrpS9e by GST pull-down assay. Lanes 1 to 6 show the isolated proteins as a reference. Lanes 7 to 11 show the elution fractions of the GST pull-down after the removal of unbound proteins and extensive washing. rpS9e^A corresponds to ctrpS9e^{1–183}.

the β -propeller (Figure 4D), it could either correspond to one of the α helices predicted between the β -propeller and the C-terminal 42 residues or belong to the linker connecting the eIF3b NTD. However, an unambiguous localization of the missing NTD and CTD is currently not feasible and would be highly speculative.

Structure of eIF3

In the EM structure of the 43S PIC/DHX29 complex, the position of the eIF3b WD40 domain appears to be rather distant to the complex of the eight eIF3 subunits a*, c, e, f, h, k, l, and m (Figure 4A). However, the structure of this octameric complex mainly represents a core formed by the PCI/MPN domains (proteasome, COP9, initiation factor 3/Mpr1, Pad1 N-terminal), whereas other domains of these subunits are not defined in the EM map. This is consistent with previous EM structures of full eIF3, which have unraveled the structure of the octameric PCI/MPN complex. However, other domains and the subunits b, d, g, and i are also missing in these EM structures, most likely because of the high flexibility and dynamics of isolated eIF3 (Querol-Audi et al., 2013; Sun et al., 2011). Furthermore, in the 43S PIC/DHX29 complex, eIF3a is lacking some 600 C-terminal residues and is therefore denoted as a*. Thus, the missing and flexible parts of eIF3 subunits would be sufficient to fill the gap between the PCI/MPN core and the eIF3b WD40 domain.

In summary, our studies support a direct binding of eIF3b to the 40S ribosomal subunit, which suggests an essential role of

the central scaffold subunit eIF3b in the formation of the 43S PIC and might provide a structural basis for dissecting the mechanisms of the assembly of 43S and 48S PIC.

EXPERIMENTAL PROCEDURES

Cloning, Expression, Protein Purification, and Crystallization

C. thermophilum eIF3a, b, c, i, g, and rpS9e genes were amplified from genomic DNA or cDNA and cloned into the pGEX-6P-1 (GE Healthcare) or pET28b (Merck) vector for expression in *Escherichia coli* cells. Proteins were purified using GST-affinity or immobilized metal affinity chromatography, ion exchange, and gel filtration chromatography. Both native and selenomethionine (Se-Met) derivative crystals were obtained at 20°C using the sitting drop vapor diffusion method with reservoir solution containing 15% polyethylene glycol 20k, 0.08 M MnCl₂, and 0.1 M MES buffer (pH 6.5). Details regarding the cloning, expression, purification, and crystallization are described in Supplemental Experimental Procedures.

X-Ray Data Processing and Structure Determination

X-ray diffraction data of a native and a Se-Met ctelF3b crystal were processed and scaled using XDS (Kabsch, 2010) up to resolutions of 2.72 Å and 3.30 Å, respectively (Table 1). Crystals belong to space group P4₃2₁2. The Matthews coefficient (4.6 Å³/Da) suggested the presence of one protein molecule in the asymmetric unit corresponding to a solvent content of 73%. The crystal structure was solved by means of three-wavelength Se-Met MAD combined with automatic model building. Final refinement was performed with PHENIX (Adams et al., 2010). Figures showing crystal structures were made with PyMOL (<http://www.pymol.org>) or UCSF Chimera 1.8 (Pettersen et al., 2004). Details are described in Supplemental Experimental Procedures.

Fitting of eIF3b-WD40 into the EM Map

Fitting of the eIF3b crystal structure into the cryo-EM map of the 43S PIC+DHX29 from rabbit (EMDB accession number 5658) was done using UCSF Chimera 1.8 (Pettersen et al., 2004). The voxel size and contour level were set to 2.245 Å and 0.019 on the object scale, respectively. Nonconserved loops, which are present in ctelF3b-WD40 but not in the rabbit eIF3b, were removed prior to fitting. ctelF3b-WD40 was fitted in a local six-dimensional search as a rigid-body into the doughnut-like EM density portion using the fit to map algorithm as implemented in Chimera. A CCC defining the agreement of a simulated map of the crystal structure (resolution 10 Å) and the EM map as well as the respective number of outlier atoms relative to all atoms were calculated, and the fit with highest CCC was accepted. In order to validate the model further, fitting was repeated using the program package Sculptor 2.1.1_r1 (Birmanns et al., 2011), obtaining virtually identical results.

Purification of *C. thermophilum* 40S Ribosomal Subunit and Interaction Studies with ctelF3b

C. thermophilum cultivation and purification of 80S ribosomes were performed as described (Amlacher et al., 2011; Leidig et al., 2013). ct80S ribosomes were dissociated into subunits using puromycin and separated on a sucrose density gradient (Blobel and Sabatini, 1971). Fractions containing pure ct40S ribosomes were pooled. Interaction with eIF3b^{FL} or eIF3b^{167–670} (using 4, 8, 12, 16, 20, or 5, 15-fold molar excess) were tested by sedimentation through 30% sucrose cushions in an MLA-130 rotor (Beckman) run at 100,000 rpm for 2 hr at 4°C. Supernatants were carefully decanted, and the pellets were resuspended. Both sucrose cushions and resuspension buffers were in 50 mM BisTris (pH 6.8), 50 mM KCl, 10 mM MgCl₂, and 5 mM dithiothreitol.

GST Pull-Down Assays

Fifty micrograms of GST fusion protein was immobilized on glutathione Sepharose (GE Healthcare Life Sciences) beads and subsequently mixed with a 2-fold molar excess of the nontagged protein. After 30 min of incubation and extensive washing, the proteins were eluted and analyzed by SDS-PAGE (for details, see Supplemental Experimental Procedures).

ACCESSION NUMBERS

Atomic coordinates and structure factors have been deposited in the PDB (accession number 4NOX).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and six figures and can be found with this article online at <http://dx.doi.org/10.1016/j.str.2014.03.010>.

AUTHOR CONTRIBUTIONS

Y.L., B.K., and R.F. designed the experiments. Y.L. performed the protein expression, purification, and crystallization of ctelF3b. Y.L. and P.N. performed crystal structure analysis. Y.L., S.S., and A.C. prepared 40S ribosomes and performed sedimentation studies. Y.L. performed interaction studies with rpS9e. Y.L. and B.K. performed the in vitro reconstitution of ctelF3 subcomplexes. Y.L. and T.M. performed the interpretation of the EM map. Y.L., B.K., T.M., P.N., S.S., A.C., and R.F. wrote the manuscript.

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