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^{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^{Met} ternary complex and promotes their binding to the 40S ribosomal subunit during 43S preinitiation complex (PIC) assembly (Valaˇ sek 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; Valaˇ sek et al., 2004).

In budding yeast, eIF3 is composed of five essential subunits (a/Tif32, b/Prf1, c/Nilp1, 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; Valaˇ sek et al., 2001), while the eIF3b C-terminal domain (CTD) is necessary for the interaction with eIF3i and eIF3g (Asano et al., 1998; Hermannová 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; Hermannová 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 central axis, resulting in a multibladed, doughnut-like propeller architecture (Stirnimann 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 “1N + 3C 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 (Stirnimann 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 cteIF3b WD40 propeller reveals several noncanonical folding properties. It uses an unusual “3N + 1C,innermost Velcro” closure type and possesses a central
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 eIF3b 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 cefIF3b 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 multwavelength 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 cefIF3b 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 “1N + 3C Velcro” closure of the β-propeller fold (Xu and Min, 2011), the WD40 repeats of cefIF3b apply a “3N + 1C” 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 cefIF3b WD40 propeller is that the central channel resembles a circular truncated cone (Figures 1B and 1C). The

Figure 1. Overall Structure of cefIF3b-WD40 (A) Schematic domain organization of cefIF3b. The boundaries of the domains are indicated. (B) Top, side, and bottom views of the overall structure of cefIF3b-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 cefIF3b 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.
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

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Values for the data in the highest resolution shell are shown in parentheses.  
$^a$Anomalous correlation and mean anomalous difference (SigAno), calculated with XSCE.  
$^b$R$_{free}$ = $\sum_{\text{test}} |F_{\text{obs}}| - |F_{\text{calc}}|/\sum_{\text{test}} |F_{\text{obs}}|$, where “Test” is a test set of about 5% of the total reflections randomly chosen and set aside before refinement for the complex.  
$^c$Wilson B factor was estimated with phenix.refine package.
conserved surface areas of cteIF3b-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 \(\beta\)-propeller fold can also be found in proteins other than cteIF3b 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 cteIF3b is only 20%. The secondary structure prediction of eIF2A assigns 36 \(\beta\) strands, exactly matching the strands number of a nine-bladed \(\beta\)-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 \(\beta\)-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 \(\beta\)-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 cteIF3 resembles mammalian eIF3. Like its yeast counterpart, cteIF3 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 \(\beta\)-propeller protein eIF3i was fitted into a doughnut-like density below the shoulder of the 40S subunit;
Structure of eIF3b

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 cteIF3b and their mutual interaction studied by cosedimentation experiments. Upon the addition of cteIF3b, we noted the appearance of an additional ~86 kDa protein in the pellet, which is absent in purified 40S subunits, indicating a stable association. Under these experimental conditions, unbound eIF3b remained in the supernatant (Figure 4E). In addition, cteIF3b<sub>167-670</sub> (containing the WD40 domain plus an ~30-residue C-terminal extension) also binds to the 40S 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 cteIF3b and N-terminally truncated cteIF3b<sub>167-C</sub> bind to glutathione S-transferase (GST)-tagged ctrpS9e (Figure 4F), while cteIF3b<sub>167-670</sub> and cteIF3b<sub>167-704</sub> fail to do so (Figure S6B). This implies that the 42 C-terminal residues of cteIF3b are crucial for the interaction with ctrpS9e. Importantly, the interaction of cteIF3b-CTD is not a prerequisite for binding to the 40S ribosomal subunit, as cteIF3b<sub>167-670</sub> is still capable of this association (Figure S6A). Thus, interactions with other parts of the 40S ribosome (e.g., the rRNA ES6<sub>5</sub>-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 cteIF3b and ctrpS9e affects the binding of cteIF3i and cteIF3g to cteIF3b. 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 cteIF3b apparently also contributes to the formation of the ctrpS9e-elf3b<sub>gi</sub> complex, as the N-terminally truncated cteIF3b<sub>167-C</sub> exhibits a lower affinity to eIF3g<sub>i</sub> (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
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 unravelled 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 eIF3b 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 (Petterson 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 (Petterson et al., 2004). The voxel size and contour level were set to 2.24 Å and 0.019 on the object scale, respectively. Nonconserved loops, which are present in cteIF3b-WD40 but not in the rabbit eIF3b, were removed prior to fitting. cteIF3b-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 cteIF3b
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 eIF3b133 or eIF3b167–670 (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 Sepharose (GE Healthcare Life Sciences) beads and subsequently mixed

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Structure of eIF3b

AUTHOR CONTRIBUTIONS

Y.L., B.K., and R.F. designed the experiments. Y.L. performed the protein expression, purification, and crystallization of cteIF3b. 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 cteIF3b 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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Supplemental Information

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Inventory of Supplemental Information

Supplemental Data

Figure S1, related to Figure 1, showing the SDS-PAGE of full-length cteIF3b, cteIF3b WD40 domain and dissolved crystals, reflecting that the crystallized fragment only contains the WD40 domain of eIF3b.

Figure S2, related to Figure 2, showing the multiple sequence alignment of eIF3b orthologs.

Figure S3, related to Figure 2, displaying the electrostatic potential of conserved surface areas of cteIF3b-WD40.

Figure S4, related to Figure 3, showing the interaction studies between the eIF3b,i,g subcomplex and eIF3c using analytical SEC. No eIF3b,i,g,c complex was observed.

Figure S5, related to Figure 4, comparing the best fit of eIF3b-WD40 (blue) and eIF3i (yellow, PDB code: 3ZWL)

Figure S6, related to Figure 4, showing an eIF3b truncation (eIF3b<sub>167-670</sub>) is able to bind to the 40S, but eIF3b<sub>167-670</sub>, eIF3b<sub>167-704</sub>-3i complex, eIF3i, eIF3g cannot interact with rpS9e.

Supplemental Experimental Procedures

Supplemental References
Figure S1, related to Figure 1. SDS-PAGE of full-length cteIF3b, cteIF3b WD40 domain and dissolved crystals.

The molecular weight of full-length cteIF3b and its WD40 domain are ~90 kDa and ~55 kDa, respectively. The size of the crystallized fragment is the same as cteIF3b WD40 domain.
Figure S2, related to Figure 2. Multiple sequence alignment of eIF3b orthologs.

Amino acid sequences of eIF3b from Chaetomium thermophilum (ct), Oryctolagus cuniculus
(oc), Homo sapiens (hs), Mus musculus (mm), Schizosaccharomyces pombe (sp), and Saccharomyces cerevisiae (sc) are aligned using ClustalW2 (www.ebi.ac.uk/Tools/msa/clustalw2/, Chenna et al., 2003). Identical residues are highlighted with blue background and conserved residues with cyan background. Secondary structural elements (β-strands: arrows; α-helices: rounded rectangles; loops: lines) are indicated above the alignment (residues that were not built in the structure are represented as dashed lines). The WD40 domain of eIF3b is colored as in Fig 1B, while the N-terminal RRM domain and the C-terminal α-helix, which are not present in our structure, are colored light gray.
Figure S3, related to Figure 3. Electrostatic potential of conserved surface areas of cteIF3b-WD40.

The electrostatic surface potential of cteIF3b-WD40 colored from red (-5kT/e) through white (0kT/e) to blue (+5kT/e). Areas containing conserved surface residues are encircled and labeled based on blade numbers or propeller side. The orientations are similar to Fig 2C.
Figure S4, related to Figure 3. Interaction studies between the cteIF3b,i,g subcomplex and cteIF3c using analytical SEC.

Overlay of the analytical SEC profiles of the cteIF3b-i-g subcomplex (dotted black), cteIF3c (dashed gray) and the cteIF3b-i-g subcomplex together with subunit c (solid dark). In case of cteIF3b,i,g + cteIF3c, two overlapping peaks are observed; the main peak at a higher elution volume corresponds to eIF3c alone, whereas the smaller peak at a lower elution volume (forming the shoulder of the eIF3c peak) corresponds to the cteIF3b,i,g complex. A shift to a lower elution volume does not occur, indicating that the cteIF3b,i,g,c complex is not formed.
Figure S5, related to Figure 4. Comparison of the best fit of eIF3b-WD40 (blue) and eIF3i (yellow, PDB code: 3ZWL) into the density map of rabbit 43S initiation complex (grey, EMDB ID: 5658). In figure 5A the density assigned to DHX29 located above the doughnut-like density was removed for clarity.
Figure S6, related to Figure 4. Interaction assays between eIF3b truncations and the 40S as well as rpS9e

(A) Co-sedimentation of eIF3b\textsuperscript{167-670} and 40S ribosomes. eIF3b\textsuperscript{167-670} co-sediments with 40S (Lanes 2-3) and excess eIF3b\textsuperscript{167-670} remains in the supernatant (Lanes 2''-3''). Lane 1 and Lanes 2'-3' show controls of 40S alone and protein, respectively. (B) Interaction study between eIF3b truncations, eIF3i, eIF3g and rpS9e by GST pull-down assays. Lanes 1-5 show
the single proteins as reference. Lanes 12-14 show the elution fractions of the GST pull-down after the removal of unbound proteins (Lanes 6-8) and extensive washing (Lanes 9-11). rpS9eΔ corresponds to rpS9e1-183. No binding of eIF3b167-670 or eIF3b167-704-eIF3i complex to rpS9e can be detected. A weak binding between eIF3i and eIF3g complex and rpS9e could be detected (lane 12).
Supplemental Experimental Procedures

Cloning, protein expression and purification

Genes encoding eIF3b, a, c, i, and g were amplified by PCR from Chaetomium thermophilum genomic DNA, and the ctrpS9e gene from cDNA. Each gene was cloned into the pGEX-6P-1 vector (GE Healthcare), with the exception of eIF3a, which was cloned into the pET-28b vector (Merck), following the In-Fusion HD cloning kit user manual (Clontech). The ORFs of eIF3b\textsuperscript{167-670} and eIF3b\textsuperscript{167-704} were subcloned into the pGEX-6P-1 vector (GE Healthcare), while eIF3b\textsuperscript{167-C} into a modified pETDuet-1 vector. The GST fusion proteins (containing a Precission protease cleavage site), eIF3a with C-terminal His\textsubscript{6}-tag and eIF3b\textsuperscript{167-C} with N-terminal His\textsubscript{6}-tag were individually expressed in Escherichia coli strain BL21 (DE3) grown in 2×YT medium. Target protein expression was induced by addition of isopropyl β-D-thiogalactopyranoside (IPTG) to a final concentration of 0.25 mM at an OD\textsubscript{600} of ~1.0. Cells were harvested after incubation overnight at 16 °C by centrifugation (4,800 xg, 20 min, 4 °C; Beckman) and resuspended in lysis buffer (25 mM HEPES/NaOH, pH 7.5, 500 mM NaCl, 5% glycerol and 5 mM β-mercaptoethanol (β-ME)). A microfluidizer (Microfluidics, Newton, US) was used to rupture the bacterial cells and the cell debris was separated via centrifugation (30,000 xg, 35 min, 4 °C). For the GST fusion proteins the supernatant was applied to a GSTrap column (GE Healthcare) equilibrated in lysis buffer. After sample loading and extensive washing with lysis buffer, the column was equilibrated in a low salt buffer (25 mM HEPES/NaOH, pH 7.5, 100 mM NaCl, 5% glycerol and 5 mM β-ME). The GST fusion proteins were eluted with the low salt buffer containing additional 30
mM reduced glutathione. The GST-tag was cleaved by Prescision protease at 4 °C overnight at a 1:100 mass ratio of protease to fusion protein.

For further purification of full-length eIF3b and eIF3g, respectively, the protein was loaded onto an ion exchange column (GE healthcare) (Source 30Q for eIF3b; SP sepharose for eIF3g) equilibrated in buffer A (25 mM HEPES/NaOH, pH 7.5, 100 mM NaCl, 5% glycerol and 5 mM β-ME) and the target protein was eluted applying a linear gradient to buffer B (25 mM HEPES/NaOH, pH 7.5, 1 M NaCl, 5% glycerol and 5 mM β-ME).

For the eIF3a-His₆ protein the supernatant after cell rupture and centrifugation was applied to an IMAC column (His60 Ni superflow, Clontech). After sample loading and extensive washing with lysis buffer, the column was equilibrated in a low salt buffer (25 mM HEPES/NaOH, pH 7.5, 200 mM NaCl, 5% glycerol and 5 mM β-ME). eIF3a-His₆ was eluted with the low salt buffer containing additional 400 mM imidazole. Subsequently eIF3a-His₆ was loaded onto a HiTrap Heparin HP column (GE healthcare) equilibrated in buffer A (20 mM HEPES/NaOH, pH 7.3, 200 mM NaCl, 5% glycerol and 5 mM β-ME) and was eluted with a linear gradient to buffer B (20 mM HEPES/NaOH, pH 7.5, 1 M NaCl, 10% glycerol and 5 mM β-ME).

Final purification of the proteins (eIF3b, eIF3b₁⁶⁷-₇⁰⁰, eIF3b₁⁶⁷-C, c, i, g, rpS9e and eIF3b₁⁶⁷-₇⁰₄-eIF3i complex as well as eIF3a-b-c complex) was achieved by a Superdex 200 (26/60) gel filtration column (GE Healthcare) in 10 mM HEPES/NaOH, pH 7.5, 150 mM NaCl, 2% glycerol and 2 mM DTT. Protein-containing fractions were pooled, concentrated, flash-frozen in liquid nitrogen and stored at -80 °C.
The selenomethionine (SeMet) substituted cteIF3b was expressed in the methionine auxotroph *E. coli* strain B834 (DE3). The cells were initially grown in M9 mineral medium supplied with 50 mg·L⁻¹ methionine until an OD₆₀₀ of ~0.6 was reached. The medium was then removed by centrifugation (2,500 xg, 15 min, 4 °C) and the cells were resuspended in new M9 medium, which was supplemented with 50 mg·L⁻¹ selenomethionine after residual methionine was depleted. Target protein expression was induced by addition of IPTG to a final concentration of 0.25 mM. The purification of selenomethionine labeled cteIF3b was analogous to the wild type protein purification.

**Crystallization and X-ray data collection**

Initial crystallization screening for full-length cteIF3b (12 mg·ml⁻¹) was performed using the sitting drop vapor diffusion method with droplet compositions of 0.25µl : 0.25µl or 0.25µl : 0.125µl for protein and reservoir solutions, respectively. In total, 1056 commercially available conditions at 4 °C and 20 °C have been tested. Bipyramidal crystals were obtained at 20 °C from both droplets from JBScreen Nuc-Pro HTS (Jena Bioscience) with 15% (w/v) PEG 20k, 0.08 M MnCl₂, 0.1 M MES, pH 6.5 after approximately 30 days. Crystals of SeMet derivative protein were grown under the same conditions. Prior to X-ray data collection, crystals were transferred into the cryoprotectant buffer (crystallization condition supplemented with 23% (v/v) of ethylene glycol) for 5 seconds and then plunged into liquid nitrogen. A native diffraction dataset was collected from a single crystal at 100K at beamline P13 at PETRA III, DESY (Hamburg, Germany), while the MAD datasets of Se-Met substituted protein crystals were collected at wavelengths of 0.9798 Å (peak), 0.9799 Å
Structure determination and refinement

Data were processed and scaled using the programs XDS and XSCALE (Kabsch, 2010a, b) to a final resolution of 2.72 Å and 3.30 Å for native and Se-Met derivative cteIF3b, respectively. Using the Se-MAD peak, inflection and remote diffraction datasets, six (out of seven expected) selenium atoms were found using the program SHELXD (Sheldrick, 2008) and initial phases were calculated at 4 Å resolution. Se positions were further refined using SHARP (Vonrhein et al., 2007) followed by density modification using the program Solomon (Abrahams and Leslie, 1996). An initial poly-alanine model was built by ARP/wARP (Langer et al., 2008) and refined against Se-Met data with Refmac5 (Morris et al., 2004; Perrakis et al., 1999), which was used for manual rebuilding and sequence assignment in Coot (Emsley et al., 2010). Due to the lack of isomorphism between Se-Met and native crystals (the c axis differs in length by 14.7 Å, see Supplementary Table S1), this initial model of the cteIF3b WD40 domain was positioned by molecular replacement into the unit cell of the native crystal. Further model optimization and completion has been performed against the native data. Prior to structural refinement, randomly selected 5% test set of the reflections were set aside for the calculation of $R_{free}$ as a quality monitor (Brunger, 1992, 1993). Refinement was performed with the PHENIX package (Adams et al., 2010). The electrostatic surface potential was calculated with PDB2PQR (Dolinsky et al., 2007) and displayed using the APBS plugin of the PyMol software.
Analytical size exclusion chromatography

An analytical Superdex 200 (10/300) column (GE healthcare) was used for the *in vitro* reconstitution of eIF3 subcomplexes. The buffer contained 25 mM HEPES/NaOH, pH 7.5, 150 mM NaCl, 5% glycerol and 2.5 mM β-ME. In each case, ~50 µg protein in a volume of 400 µl was injected on the column at a flow rate of 0.5 ml·min⁻¹.

GST pull-down assays

50 µg GST fusion protein was mixed with two-fold molar excess of non-tagged protein in a buffer containing 25 mM HEPES/NaOH, pH 7.5, 100 mM NaCl, 5% glycerol and 2.5 mM β-ME and incubated for 30 min with 100 µl glutathione beads. After washing four times with 1 mL buffer, bound protein was eluted with the same buffer containing additional 30 mM reduced glutathione.
Supplemental References


