Supplementary Materials and Methods

Protein preparation and crystallization

The plasmid for the expression of N-terminally His-tagged *C. thermophilum* (Ct-)eIF5B(517C) (comprising residues 517-1116 that form the G domain, domain II, III and IV) was transformed into *E. coli* BL21 (DE3) cells (Stratagene) by heat shock. Transformed cells were grown in 1 l cultures of 2YT medium at 37 °C to an OD_{600} of 0.6-0.8, followed by the induction of protein expression with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Cells were grown for an additional 18 h at 16 °C before harvesting. The harvested cells were resuspended in buffer A (40 mM Hepes (pH 7.5), 500 mM KCl, 10% glycerol, 30 mM imidazole, 2 mM β-mercaptoethanol), lysed using a Microfluidizer (Microfluidics) and clarified by ultracentrifugation. The supernatant was loaded onto two HisTrap columns (GE Healthcare), equilibrated in buffer A. After the elution of bound proteins with a linear gradient of imidazole (30-300 mM), eIF5B-containing fractions were pooled and desalted in buffer B (20 mM Hepes (pH 7.5), 200 mM KCl, 10% glycerol, 30 mM imidazole and 2 mM β-mercaptoethanol) for subsequent TEV-protease cleavage at 4 °C over night to remove the His-Tag. Uncleaved protein was removed by a second HisTrap in buffer B and the flow-through was pooled and concentrated for the final size exclusion chromatography step on a Superdex 200 column (GE Healthcare), equilibrated in buffer C (10 mM Hepes (pH 7.5), 50 mM KCl, 3 mM $MgCl_2$ and 1 mM DTT). The purified eIF5B was finally pooled and concentrated to 15-20 mg/ml. Other versions of Ct-eIF5B containing residues 517-970 or 517-858 and of *S. cerevisiae* (Sc-) eIF5B containing residues 399-852 were purified according to the same protocol.

For ITC experiments truncated versions of Ct-eIF5B were purified according to the above protocol with the difference that ITC buffer (see below) was used in the size exclusion chromatography step. The concentration of the protein was determined by absorbance at 280 nm (with extinction coefficients for the different Ct-eIF5B constructs: ε_{517C} = 30370 $M^{-1}cm^{-}$

[1], $\varepsilon_{517\text{-}970}$ = 20400 $M^{-1}cm^{-1}$ and $\varepsilon_{517\text{-}852}$ = 14440 $M^{-1}cm^{-1}$). The protein was >95 % pure as judged by SDS-PAGE and free of nucleotides as determined by HPLC.

Crystals of Ct-eIF5B(517C) without nucleotide were obtained after two days at 4 °C *via* sitting-drop vapor diffusion against 100 mM MES (pH 6.8), 12% PEG 20000 and 10 mM Na-lactate. The crystals grow in primitive hexagonal space group $P3_221$ with one molecule per asymmetric unit (unit cell: a = b = 111.47 Å, c = 115.21 Å, α = β = 90°, γ = 120°; diffraction limit = 2.75 Å).

Despite extensive trials we were not able to obtain crystals of the Ct-eIF5B(517C) construct in the GDP- or GTP-bound forms. However in the presence of GTP another type of crystals was obtained after 5 days at 4 °C under the above conditions. These crystals grew in the primitive hexagonal space group $P3_121$ (unit cell: a = b = 98.23 Å, c = 97.42 Å, α = β = 90°, γ = 120°; diffraction limit = 3.2 Å) with one molecule per asymmetric unit comprising only domains III and IV of Ct-eIF5B (residues 870-1116).

Crystals of Ct-eIF5B(517-970) in the presence of 2 mM GDP grew within 4 days at room temperature against 15% PEG 8000 and 0.5 M Li_2SO_4 in primitive orthorhombic space group $P2_12_12_1$ with two protein-GDP complexes per asymmetric unit (unit cell: a = 66.9 Å, b = 72.96 Å, c = 199.23 Å, α = β = γ = 90°; diffraction limit = 2.1 Å).

Crystals of Ct-eIF5B(517-970) in the presence of 2 mM GTP grew over night at room temperature against 100 mM Hepes (pH 7), 13% PEG 4000 and 100 mM sodium acetate in primitive monoclinic space group $P2_1$ with two protein-GTP complexes per asymmetric unit (unit cell: a = 55.4 Å, b = 114.83 Å, c = 65.85 Å, α = 90°, β = 102.3°, γ = 90°; diffraction limit = 1.87 Å).

Crystals of the nucleotide free form of Sc-eIF5B(399-852) grew after 2 days at 4 °C against 20% ethylene glycol, 5% PEG 3350 and 20 mM $MgCl_2$ in primitive tetragonal space group P41 with two molecules per asymmetric unit (unit cell: a = b = 118.01 Å, c = 77.51 Å, α = β = γ = 90°; diffraction limit = 1.83 Å). The data were twinned with a twin fraction of 18% (twin law h, -k, -l).

Finally, crystals of the GDP-bound form of Sc-eIF5B(399-852) could be obtained over night at 10 °C against 8% PEG 8000 and 0.37 M Li_2SO_4 in primitive orthorhombic space group $P2_12_12_1$ with two protein-GDP complexes per asymmetric unit (unit cell: a = 73.56 Å, b = 119.46 Å, c = 120.73 Å, α = β = γ = 90°; diffraction limit = 3.02 Å).

X-ray data collection, structure determination and refinement

For the structure of Ct-eIF5B·GTP X-ray diffraction data were collected at BL 14.1 (HZB, BESSY, Berlin) (Mueller et al, 2012). All other structures were solved using X-ray diffraction data collected at P13 beamline (EMBL, PETRA III, Hamburg). The structure of free Ct-eIF5B(517C) was solved by molecular replacement using the program PHASER (McCoy et al, 2007) using the atomic coordinates of the archeal ortholog of eIF5B from *M. thermoautotrophicum* (PDB: 1G7R) with the individual domains as independent search models. The structure was refined using the program PHENIX (Adams et al, 2010). The other structures where solved by means of molecular replacement using the individual domains of the newly determined structure as search model as described below (Data collection and refinement statistics are summarized in Table I).

The second structure obtained from Ct-eIF5B(517C), this time in the presence of GTP, was solved using domains I to IV of Ct-eIF5B(517C) as independent search models. A solution was found only for domains III and IV and the final model after refinement comprises residues 870 to 1116 (domains III and IV). A Coomassie Blue-stained SDS-PAGE gel of dissolved crystals confirmed that domains I and II are missing, probably due to a proteolytic event during crystallization.

The structures of Ct-eIF5B(517-970) in the GDP- and GTP-bound state and of free Sc-eIF5B(399-852) were solved using domains I to III of Ct-eIF5B(517C) as independent search models. The resulting models of Ct-eIF5B(517-970) in complex with either GDP or GTP contains two copies of Ct-eIF5B(517-970), each bound to a Mg^{2+} ion and GDP or GTP, respectively. In the final model of Ct-eIF5B(517-970)·GTP residues 517-859 are resolved in the electron density, however the entire domain III (residues 860-970) is missing, probably

due to a proteolytic event during crystallization as indicated by a Coomassie Blue-stained SDS-PAGE gel of dissolved crystals. The final model of the nucleotide-free form of Sc-eIF5B(399-852) contains two copies of Sc-eIF5B(399-852).

Finally, the structure of Sc-eIF5B·GDP was solved using domains I to III of free Sc-eIF5B(399-852) as independent search models. The final model contains two Sc-eIF5B·GDP complexes in the asymmetric unit.

Isothermal Titration Calorimetry

The thermodynamic parameters of eIF5B binding to GDP or GTP were measured by means of ITC using a MicroCal VP-ITC instrument (GE Healthcare). Experiments were carried out in ITC buffer (30 mM Hepes pH 7.5, 100 mM KCl, 10% glycerol, 4 mM β-mercaptoethanol, 0.01% tween20, 2.5 mM $MgCl_2$) at six different temperatures (5, 10, 15, 20, 25 and 30 °C). 14-µl aliquots of 200-400 µM ligand were injected into the 1.42 ml cell containing 10-30 µM eIF5B. The heat of dilution was measured by injecting the ligand into the buffer solution without protein; the values were then subtracted from the heat of the individual binding reactions to obtain the effective heat of binding. The final titration curves were fitted using the 'Origin' based MicroCal software, assuming one binding site per protein molecule. For each isotherm the binding stoichiometry (N), enthalpy changes (ΔH) and the association constants (K_a), were obtained by a nonlinear regression fitting procedure. These directly measured values were then used to estimate the Gibbs energy (ΔG) from the relation $\Delta G = - R \cdot T \cdot \ln K_a$ and the entropy changes (ΔS) through $\Delta G = \Delta H - T \cdot \Delta S$.

To investigate the influence of the Mg^{2+} ion on the binding of GDP to eIF5B, experiments were repeated in a modified ITC buffer, containing 0.25 mM EDTA instead of $MgCl_2$ (Mg^{2+} was omitted also from the buffers used during purification).

In order to estimate the change in heat capacity (ΔC_p) upon complex formation, the measured ΔH values were plotted against the temperature (Jelesarov & Bosshard, 1999; Prabhu & Sharp, 2005). Depending on whether a linear or non-linear temperature dependency was applicable for ΔH, the data were fitted either to a linear or a second-order

polynomial function. In the first case, the slope of the fitted line directly represents the ΔC_p of the binding reaction, whereas in the second case the ΔC_p for a given temperature can be calculated from the first derivative of the polynomial function.

ΔC_p can be used as an estimate for the change in solvent-accessible surface area (ΔASA) upon complex formation as it was found to be proportional to the size of the area which is either exposed to or excluded from the aqueous environment during the binding event (Connelly & Thomson, 1992; Mitkevich et al, 2006; Murphy & Freire, 1992; Paleskava et al, 2012; Perozzo et al, 2004; Spolar & Record, 1994). ΔC_p and ΔASA are connected by the empirically determined relation $\Delta C_p = \Delta c_{ap} \cdot \Delta ASA_{ap} + \Delta c_p \cdot \Delta ASA_p$ where Δc_{ap} and Δc_p are the area coefficients in $cal \cdot K^{-1} \cdot (mole \cdot Å^2)^{-1}$ for the contributions of apolar or polar side chains to ΔASA, respectively (Perozzo et al, 2004). Studies on the dissolution of model compounds and protein unfolding suggest a negative contribution due to the burial of apolar surfaces (ΔASA_{ap}) and a positive contribution upon burial of polar surfaces (ΔASA_p) with values for Δc_{ap} and Δc_p of 0.45 and -0.26, respectively (Murphy et al, 1992; Murphy & Freire, 1992; Perozzo et al, 2004). Since binding of guanine nucleotides can be expected to involve both, apolar and polar residues, a treatment of the obtained ΔC_p values solely based on nonpolar contributions according to Connelly et al. (1992) would be insufficient. At the same time, the differential treatment by calculating the individual contributions of apolar and polar groups to the total surface area often proved to be inaccurate, especially in cases where the interactions did not conform to a rigid-body binding model (Faergeman et al, 1996; Frisch et al, 1997; Holdgate et al, 1997; Schrift et al, 2006). We therefore use two values for the area coefficients to estimate the total surface area upon ligand binding: $\Delta c_{max} = 0.24$ ($= 0.7 \cdot \Delta c_{ap} + 0.3 \cdot \Delta c_p$) as the upper limit case, assuming ~70% apolar and ~30% polar groups contributing to the total ΔASA (calculated from the crystal structures of Ct-eIF5B in its apo, GDP and GTP forms using the program AREAIMOL from the CCP4 Program Suite 6.3.0) and $\Delta c_{min} = 0.45$ as the lower limit case in which all involved residues are apolar.

Supplementary References

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