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Methods

Protein preparation

E. coli BL21 Rosetta cells (Novagen) were used for expressing *Ec*TBP and *Ec*TBP^{K103E} (pET28 (Novagen)) or for co-expressing *Ec*TBP and *Ec*Mot1^{NTD} (residues 1–779) (pET-DUET (Novagen)). Purification of proteins was done by Ni^{2+}-affinity chromatography (QIAGEN) using a high salt buffer at pH 8. Further purification of *Ec*TBP was achieved by anion exchange chromatography (HiTrap SP HP, GE Healthcare). For crystallization of *Ec*TBP, the His-Tag was removed by TEV protease digestion. Final purification of all proteins was performed by size exclusion chromatography (Superdex S200, GE Healthcare). Purification of *Ec*TBP and *Ec*Mot1$^{NTD\Delta latch}$ (Δlatch = Δ96–132) or selenomethionine labelled *Ec*TBP and *EcM*ot1^{NTD} was performed accordingly.

Sequences encoding for full-length *Ec*Mot1 (residues 1–1275), *Ec*Mot1$^{\Delta CT}$ (residues 1–1016), *Ec*Mot1^{NTD} (residues 1–779) and *Ec*Mot1$^{\Delta latch}$ (Δ96–132) including an N-terminal His_{10}-tag were cloned into pFBDM transfer vector (Invitrogen). *Ec*Mot1^{E912Q} (Walker B mutant) was generated by site-directed mutagenesis of pFBDM-*Ec*Mot1. Transposition of the coding sequence into MultiBac baculoviral DNA was performed in *E. coli* DH10MultiBac^{Cre} cells[31]. Isolated bacmid DNA was used for transfection of *Trichoplusia ni* High Five^{TM} insect cells (Invitrogen) to produce baculovirus for large scale infections. Purification of proteins was done by Ni^{2+}-affinity chromatography (QIAGEN) using buffer containing 50 mM Mes pH 6.5, 200 mM NaCl, 12.5/300 mM Imidazole and 10 mM ß-mercaptoethanol

(*Ec*Mot1^{NTD}/*Ec*Mot1$^{NTD\Delta latch}$) or 50 mM Tris pH 7.5, 400 mM NaCl, 10 mM ß-mercaptoethanol, 12.5/300 mM Imidazole and 10% Glycerol (v/v) (*Ec*Mot1/*Ec*Mot1$^{\Delta CT}$/*Ec*Mot1$^{\Delta latch}$). Further purification was achieved by ion exchange chromatography (HiTrap Q HP, GE Healthcare). For crystallization of the complex, *Ec*TBP was added in access amounts to *Ec*Mot1. Final purification of the proteins was done by size exclusion chromatography (Superdex S200, GE Healthcare). The preparation of *Ec*Mot1^{BeF}:*Ec*TBP (*Ec*Mot1 with ADP-BeF_3^-) was performed as described[32].

Crystallization

Proteins were crystallized by hanging drop vapour diffusion at 18°C in a mixture of 1 µl protein (10 mg/ml *Ec*TBP and 5 mg/ml *Ec*Mot1^{NTD}:*Ec*TBP) and 1 µl precipitant (0.1 M Mes pH 6.5, 2 M NaCl, 4% Acetone for *Ec*TBP; 50 mM Mes pH 6, 200 mM Ammoniumacetate, 5% MPD, 4% Peg 3350, 200 mM NDSB-201 for *Ec*Mot1^{NTD}:*Ec*TBP). Crystals were cryoprotected with 1,2-Ethanediol (*Ec*TBP) or 2,3-Butanediol (*Ec*Mot1^{NTD}:*Ec*TBP) and flash frozen in liquid nitrogen.

Structure determination

All data were processed with XDS[33]. The structure of *Ec*TBP was solved by molecular replacement using Phaser[34] and yeast TBP (1TBP) as a model. The structure of *Ec*Mot1^{NTD}:*Ec*TBP was solved by a single anomalous dispersion experiment using SeMet data in combination with molecular replacement using *Ec*TBP structure as a partial model (Phaser[34]). Heavy atom sites were obtained with SHARP[35] and initial automatic model building was performed with Buccaneer[36]. Model building and refinement was conducted in Coot[37] and PHENIX[38], respectively. Figures were prepared in Pymol[39] or Chimera[40].

Footprinting assays

Footprinting assays were performed as described previously[41]. Reactions contained 20 nM *Ec*TBP, 30 nM *Ec*Mot1, and 50 mM ATP as indicated. Following incubation of TBP and DNA together for 20 min at 37° C, Mot1 was added with or without ATP for 5 min prior to DNase I digestion and sample processing.

ATPase assay

The rates of ATP hydrolysis were measured as described previously[41] in buffer containing 4 mM Tris-Cl pH 8, 60 mM KCl, 5 mM $MgCl_2$, 4% Glycerol (v/v), 100 mg/ml BSA, and 1 mM DTT at 22° C.

Analytic Gel Filtration

Analytical size exclusion experiments were performed on Ettan LC system (GE Healthcare, Superose 12 PC 3.2/30) according to the manufacturer's instructions (50 mM Hepes pH 8/50 mM Mes pH 6.5, 200 mM NaCl and 2 mM DTT)

Electrophoretic mobility shift assays (EMSAs)

These assays employed a radiolabeled fragment of the Adenovirus Major Late Promoter[41]. Typically, <1 nM DNA was incubated with 15-20 nM *Ec*TBP for 20 min at 37° C in the same buffer as was used for ATPase assays, then 30 nM Mot1 (or a Mot1 mutant) was added with or without 50 mM ATP for 5 min prior to loading on a gel as previously described[42].

Dissociation kinetic assays

Kinetic analysis of the dissociation reaction was performed by addition of ATP to pre-formed ternary complexes under the conditions used in the experiment shown in Figure 2e. Reactions contained radiolabeled DNA (<1 nM), 20 nM *Ec*TBP and either 30 nM WT *Ec*Mot1 or 30 nM *Ec*Mot1$^{\Delta latch}$. *Ec*TBP was incubated with the radiolabeled DNA template for 20 min, followed by addition of *Ec*Mot1 or *Ec*Mot1$^{\Delta latch}$ for 10 min. ATP was added to 100 μM for 2 to 20 min, and reaction products were resolved at the indicated times on nondenaturing gels. To quantify the extent of complex dissociation at each time point, the free DNA band was quantified and expressed as a proportion of the free DNA present in reactions with no added protein. The results are expressed as the average +/- standard error associated with two independent experiments.

FeBABE cleavage assays

FeBABE-mediated protein cleavage have been described[43,44]. The yeast system was used to take advantage of an antibody raised to the C-terminus of yeast Mot1[45].

Static light scattering

For molecular weight determination of proteins samples (2-4 mg/ml, with 40 mM Hepes pH 8, 200 mM NaCl, 2 mM DTT as a running buffer) by static light scattering we used a combination of a Viscotek 270 detector and an Viscotek VE-3580 refractive index monitor connected to a microscale HPLC system (AEKTAmicro, GE Healthcare) equipped with an analytical size exclusion column Superdex S200 15/150 GL (GE Healthcare). Data analysis was performed using the OmniSEC-Software (Viscotek) using BSA (Thermo Fisher) as a reference for calibration. The chromatographs of the size exclusion monitored by UV-absorption at 280 nm and the subsequent refractive index and light scattering chromatographs all showed a single prominent peak indicating a homogenous sample. Plots of the determined

molecular weight vs. elution volumes for the evaluated peaks all showed stable molecular weights for the chosen peak areas.

Dynamic light scattering

Dynamic light scattering was measured using a Viscotek/Malvern Instruments 802DLS system. Protein samples (1 mg/ml in size exclusion buffer) were centrifuged and the supernatant was measured at 20° C using fluorescence cuvettes. At least 10 autocorrelation curves per sample were recorded, averaged and evaluated using the OmniSIZE Software and the mass model for globular proteins. All samples showed intensity distributions indicating a homogenous sample with a single peak at the given hydrodynamic radius.

Electron microscopy

3.5 µl (10-30 µg/ml) of freshly prepared protein sample was applied to pre-coated Quantifoil holey carbon supported grids and negatively stained. Data was collected under low dose conditions at a nominal magnification of 90,000 X and a nominal defocus of – 0.9 µm using an Eagle 2048 x 2048 pixel CCD camera (FEI Company, Eindhoven, Netherlands) with a resolution of 30 µm/pixel (3.31 Å/pixel object scale). 5518 (*Ec*Mot1^{BeF}:*Ec*TBP), 7737 (*Ec*Mot1$^{\Delta CT}$:*Ec*TBP; C-terminally truncated *Ec*Mot1) and 12558 (*Ec*Mot1^{E912Q} Walker mutant) particles were picked using boxer[46]. Initial image processing was done using IMAGIC-5[47]. The images were normalized, filtered at the first zero without CTF correction and centred by iteratively aligning them to their rotationally averaged sum. Initial class averages were obtained by 2-3 rounds of multivariate statistical analysis followed by multi-reference alignment using homogenous classes as references. The datasets were classified into 10-20 images per class. A low resolution density map was created by angular reconstitution

and used as an initial model for projection matching in EMAN 1.9[46]. The models underwent between 8 and 24 rounds of refinement at an angular increment of up to 5 degrees until angular assignment was stable. The final reconstructions comprised approx. 90% of the original dataset. All visualization and rigid body fittings were carried out using the UCSF Chimera package[40]. Surface representations show density rendered at a threshold accounting for the expected molecular mass of the complexes: *Ec*Mot1$^{\Delta CT}$:*Ec*TBP (140 kDa; 170226 A^2), *Ec*Mot1^{E912Q} (145 kDa; 172366 A^2), *Ec*Mot1^{BeF}:*Ec*TBP (169 kDa; 198984 A^2). Crystal structures and density maps were merged in VMD using MDFF as described[48]. Difference densities were created by subtracting maps from one another that were rendered at a threshold accounting for the expected molecular mass of the complex.

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