Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Purification, crystallization and preliminary X-ray diffraction analysis of *Methanococcus jannaschii* TATA box-binding protein (TBP)

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Received 10 August 2004
Accepted 24 September 2004

TATA box-binding protein (TBP) from *Methanococcus jannaschii* has been crystallized by the hanging-drop vapour-diffusion method using PEG MME 2000 as a precipitant. The crystal belongs to space group $P2_1$, with unit-cell parameters a = 53.2, b = 55.5, c = 123.4 Å, α = 90.0, β = 91.0, γ = 90.0°, and contains four molecules in the asymmetric unit. A data set was collected to 1.9 Å resolution using synchrotron radiation. A molecular-replacement solution was found using the structure of TBP from *Sulfolobus acidocaldarius* as a model. Crystallographic refinement is in progress.

1. Introduction

TATA box-binding protein (TBP) plays a central role in gene expression *via* its interactions with RNA polymerase and gene-specific transcription factors in eukaryotes and archaea (Roeder, 1996; Woychik & Hampsey, 2002; Reeve, 2003). As TBP is one of the common components of eukaryotic and archaeal transcription initiation, investigating the biochemical and biological function of TBP in more detail is the most attractive strategy to understand the unity and diversity of the mechanism of transcription initiation among eukaryotes and archaea.

The first eukaryotic TBP was purified and cloned from *Saccharomyces cerevisiae* and its structure–function relationship has been investigated (Horikoshi *et al.*, 1989*a*,*b*, 1990, 1992; Lee *et al.*, 1991; Yamamoto *et al.*, 1992; Poon *et al.*, 1993; Kim *et al.*, 1994). The isolation of TBP from various species, including *Schizosaccharomyces pombe* (Hoffmann, Horikoshi *et al.*, 1990), *Arabidopsis thaliana* (Gasch *et al.*, 1990), *Drosophila melanogaster* (Muhich *et al.*, 1990) and *Homo sapiens* (Hoffmann, Sinn *et al.*, 1990), revealed the evolutionary conservation and diversification of its structural and functional properties. The N-terminal tail region of TBP diverges in size and sequence in different species, suggesting that the region might be related to species-specific regulatory-factor interactions. The core region, which corresponds to the DNA-binding and transcription-activating domains, is highly evolutionarily conserved and divided into two directly repeated regions of about 30% sequence identity.

The tertiary structure of the core region of eukaryotic TBP has been solved for three species: *A. thaliana* (Nikolov *et al.*, 1992; Patikoglou *et al.*, 1999), *S. cerevisiae* (Chasman *et al.*, 1993; Kim *et al.*, 1993) and *H. sapiens* (Nikolov *et al.*, 1996; Juo *et al.*, 1996). The structures indicated that TBP has a highly symmetric α/β structure $(\beta\alpha\beta_4\alpha)_2$ which contains a unique DNA-binding fold (a curved antiparallel β-sheet) resembling a molecular 'saddle' that sits on the DNA.

Archaeal TBP was purified, cloned and analyzed from *Pyrococcus woesei* (Marsh *et al.*, 1994; Rowlands *et al.*, 1994) and suggested that the transcription systems of eukaryotes and archaea are fundamentally homologous (Wettach *et al.*, 1995). The tertiary structures of *P. woesei* and *Sulfolobus acidocaldarius* TBPs revealed that the structural folds of archaeal TBPs are quite similar to those of eukaryotic TBPs, whereas their surface properties are quite different (DeDecker *et al.*, 1996; Kosa *et al.*, 1997; Littlefield *et al.*, 1999; Koike *et al.*, 2004). The surface-charge distributions of eukaryotic TBPs are strongly basic, whereas those of *P. woesei* and *S. acidocaldarius* TBPs are comparatively neutral. This implies that *P. woesei* and *S. acidocaldarius* TBPs have distinct interacting factors from eukaryotic TBPs.

Archaeal TBPs can be divided into two groups, I and II: group I archaeal TBPs have a strong phylogenetic relationship to eukaryotic TBPs (Fig. 1*a*) and an acidic C-terminal tail region, while group II archaeal TBPs do not (Fig. 1*b*). Additionally, the ratio of acidic residues among all the charged residues in the core region tends to be different: it is 30–35% in eukaryotic TBPs, 42–51% in group I archaeal TBPs and 55–64% in group II archaeal TBPs. These results indicate that the surface properties of eukaryotic, group I archaeal and group II archaeal TBP are quite different. *Archaeoglobus fulgidus* and *Nanoarchaeum equitans* TBPs, which belong to group II, are the only exceptions and show lower acidicities than other members of group II (49 and 47%, respectively), although the

numbers of acidic residues are similar to those of other members of group II. There are species-specific basic residues on the first α-helix in the core region of *A. fulgidus* TBP (Fig. 1*b*) and in the N- and C-terminal tail regions and the DNA-binding region of *N. equitans* TBP (Fig. 1*b*). This suggests that there might be distinct species-specific TBP-interacting factors interacting *via* these basic residues.

P. woesei and *S. acidocaldarius* TBPs, the structures of which have been solved, are members of group I (DeDecker *et al.*, 1996; Kosa *et al.*, 1997; Littlefield *et al.*, 1999; Koike *et al.*, 2004). Although the tertiary structure of group II archaeal TBPs is not yet known, it is likely that group II archaeal TBPs may have different surface properties and interacting factors from group I archaeal TBPs. In fact, unlike eukaryotic and group I archaeal TBPs, *M. jannaschii* TBP, a group II archaeal TBP, did not show a significant TATA box binding activity (Ouhammouch *et al.*, 2003). Hence, this suggests that *M. jannaschii* TBP should have a distinct action from eukaryotic and group I archaeal TBP in accordance with the difference in surface properties. To analyze the conservation and diversification of the mechanism of action of TBP at the atomic level, we have initiated structural and functional studies of *M. jannaschii* TBP. Here, we report the purification, crystallization and preliminary crystallographic analysis of *M. jannaschii* TBP.

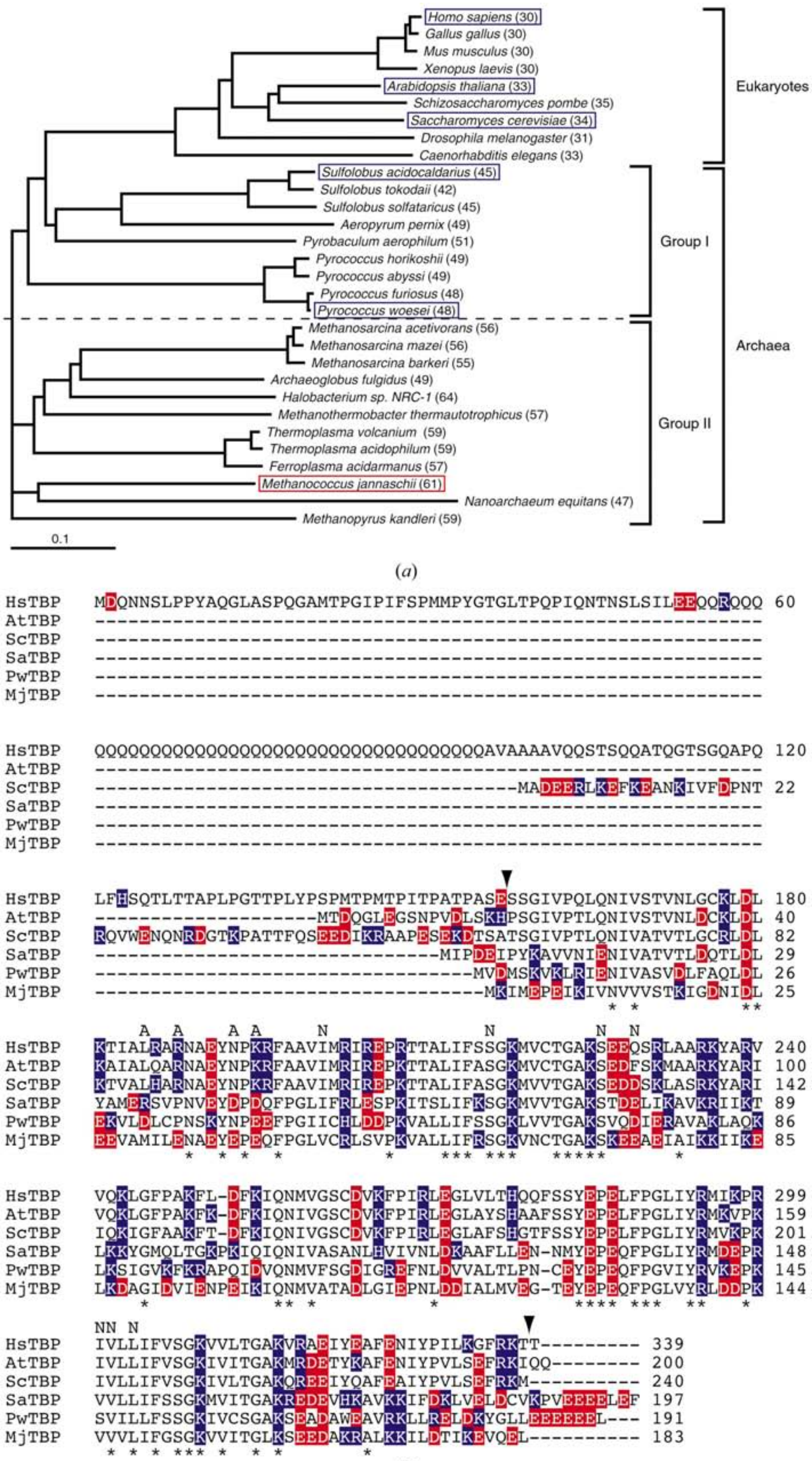
Homo sapiens (30)
Gallus gallus (30)
Mus musculus (30)
Xenopus laevis (30)
Arabidopsis thaliana (33)
Schizosaccharomyces pombe (35)
Saccharomyces cerevisiae (34)
Drosophila melanogaster (31)
Caenorhabditis elegans (33)
Sulfolobus acidocaldarius (45)
Sulfolobus tokodaii (42)
Sulfolobus solfataricus (45)
Aeropyrum pernix (49)
Pyrobaculum aerophilum (51)
Pyrococcus horikoshii (49)
Pyrococcus abyssi (49)
Pyrococcus furiosus (48)
Pyrococcus woesei (48)
Methanosarcina acetivorans (56)
Methanosarcina mazei (56)
Methanosarcina barkeri (55)
Archaeoglobus fulgidus (49)
Halobacterium sp. NRC-1 (64)
Methanothermobacter thermautotrophicus (57)
Thermoplasma volcanium (59)
Thermoplasma acidophilum (59)
Ferroplasma acidarmanus (57)
Methanococcus jannaschii (61)
Nanoarchaeum equitans (47)
Methanopyrus kandleri (59)
Eukaryotes
Group I
Archaea
Group II
0.1
(a)
HsTBP MDQNNSLPPYAQGLASPQGAMTPGIPIFSPMMPYGTGLTPQPIQNTNSLSILEEQQRQQQ 60
HsTBP QQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQQAVAAAAVQQSTSQQATQGTSGQAPQ 120
ScTBP MADEERLKEFKEANKIVFDPNT 22
HsTBP LFHSQTLTTAPLPGTTPLYPSPMTPMTPITPATPASESSGIVPQLQNIVSTVNLGCKLDL 180
AtTBP MTDQGLEGSNPVDLSKHPSGIVPTLQNIVSTVNLDCKLDL 40
ScTBP RQVWENQNRDGTKPATTFQSEEDIKRAAPESEKDTSATSGIVPTLQNIVATVTLGCRLDL 82
SaTBP MIPDEIPYKAVVNIENIVATVTLDQTLDL 29
PwTBP MVDMSKVKLRIENIVASVDLFAQLDL 26
MjTBP MKIMEPEIKIVNVVVSTKIGDNIDL 25
HsTBP KTIALRARNAEYNPKRFAAVIMRIREPRTTALIFSSGKMVCTGAKSEEQSRLAARKYARV 240
AtTBP KAIALQARNAEYNPKRFAAVIMRIREPKTTALIFASGKMVCTGAKSEDFSKMAARKYARI 100
ScTBP KTVALHARNAEYNPKRFAAVIMRIREPKTTALIFASGKMVVTGAKSEDDSKLASRKYARI 142
SaTBP YAMERSVPNVEYDPDQFPGLIFRLESPKITSLIFKSGKMVVTGAKSTDELIKAVKRIIKT 89
PwTBP EKVLDLCPNSKYNPEEFPGIICHLDDPKVALLIFSSGKLVVTGAKSVQDIERAVAKLAQK 86
MjTBP EEVAMILENAEYEPEQFPGLVCRLSVPKVALLIFRSGKVNCTGAKSKEEAEIAIKKIIKE 85
HsTBP VQKLGFPAKFL-DFKIQNMVGSCDVKFPIRLEGLVLTHQQFSSYEPELFPGLIYRMIKPR 299
AtTBP VQKLGFPAKFK-DFKIQNIVGSCDVKFPIRLEGLAYSHAAFSSYEPELFPGLIYRMKVPK 159
ScTBP IQKIGFAAKFT-DFKIQNIVGSCDVKFPIRLEGLAFSHGTFSSYEPELFPGLIYRMVKPK 201
SaTBP LKKYGMQLTGKPKIQIQNIVASANLHVIVNLDKAAFLLEN-NMYEPEQFPGLIYRMDEPR 148
PwTBP LKSIGVKFKRAPQIDVQNMVFSGDIGREFNLDVVALTLPN-CEYEPEQFPGVIYRVKEPK 145
MjTBP LKDAGIDVIENPEIKIQNMVATADLGIEPNLDDIALMVEG-TEYEPEQFPGLVYRLDDPK 144
HsTBP IVLLIFVSGKVVLTGAKVRAEIYEAFENIYPILKGFRKTT 339
AtTBP IVLLIFVSGKIVITGAKMRDETYKAFENIYPVLSEFRKIQQ 200
ScTBP IVLLIFVSGKIVLTGAKQREEIYQAFEAIYPVLSEFRKM 240
SaTBP VVLLIFSSGKMVITGAKREDEVHKAVKKIFDKLVELDCVKPVEEEELEF 197
PwTBP SVILLFSSGKIVCSGAKSEADAWEAVRKLLRELDKYGLLEEEEEEL 191
MjTBP VVVLIFGSGKVVITGLKSEEDAKRALKKILDTIKEVQEL 183
(b)

Figure 1
(*a*) Phylogenetic tree of eukaryotic and archaeal TBP generated by *ClustalW* (Thompson *et al.*, 1994). Eukaryotic and group I archaeal TBPs and group II archaeal TBPs are divided by a dotted line. TBPs whose tertiary structures have been solved are boxed in blue. *M. jannaschii* TBP is boxed in red. The ratios of acidic residues among all charged residues in the core region are shown in parentheses. (*b*) The amino-acid sequences of TBP from *H. sapiens* (Hs), *A. thaliana* (At), *S. cerevisiae* (Sc), *S. acidocaldarius* (Sa), *P. woesei* (Pw) and *M. jannaschii* (Mj). Asterisks indicate amino acids that are conserved among all six species. The arrowheads indicate the boundaries between the N-terminal tail and core regions and the core and C-terminal tail regions, respectively. 'A' and 'N' indicate species-specific basic residues in *A. fulgidus* and *N. equitans*.

2. Materials and methods

2.1. Protein expression and purification

To overexpress *M. jannaschii* TBP, *Escherichia coli* BL21-CodonPlus(DE3)-RIL (Stratagene) cells were transformed with the pLJ-MJ-TBP recombinant plasmid (Ouhammouch *et al.*, 2003). Transformed cells were grown at 300 K in TBG-M9 medium containing 50 μg ml^{-1} ampicillin and 34 μg ml^{-1} chloramphenicol until OD_{595} reached 0.6–0.8. Overexpression of *M. jannaschii* TBP was induced by the addition of 0.4 m*M* IPTG. After 16 h culture at 291 K, the cells were harvested by centrifugation (3000 rev min^{-1}, 10 min, 277 K), resuspended in buffer containing 20 m*M* Tris–HCl pH 7.4, 10% glycerol,

500 m*M* KCl, 50 m*M* 2-mercaptoethanol, 1 m*M* PMSF, 20 µg ml^{-1} leupeptin and 20 µg ml^{-1} pepstatin A and then lysed by EmulsiFlex-C5 (Avestin). The cell lysate was centrifuged at 18 500 rev min^{-1} for 30 min at 277 K. The supernatant was then applied to ProBond resin (Invitrogen); after washing the resin, the protein was eluted with buffer containing 20 m*M* Tris–HCl (pH 7.9 at 277 K), 10% glycerol, 250 m*M* NaCl, 50 m*M* 2-mercaptoethanol, 200 m*M* imidazole. For further purification, the eluted protein was concentrated by Centriprep YM-10 (Millipore) and then fractionated by gel filtration with buffer containing 20 m*M* Tris–HCl (pH 7.9 at 277 K), 10% glycerol, 100 m*M* NaCl, 10 m*M* 2-mercaptoethanol on a HiLoad 26/60 Superdex 200 column (Amersham Pharmacia). The pooled *M. jannaschii* TBP protein was concentrated to 20 mg ml^{-1} by Centriprep YM-3 (Millipore). The purity of *M. jannaschii* TBP was examined by SDS–PAGE. The molecular weight of *M. jannaschii* TBP was calculated to be 22 kDa from the sequence and the protein was detected as a molecule of around 22 kDa by SDS–PAGE.

2.2. Crystallization

Crystallization trials were initially performed using the hanging-drop vapour-diffusion method at 293 K. Crystal Screen and Crystal Screen 2 kits (Hampton Research) were used to determine the initial crystallization conditions. The droplet was prepared by mixing 1 µl of 20 mg ml^{-1} protein solution with the components described above with 1 µl reservoir solution. Crystals grew within one month in three conditions containing PEG 400, PEG 4000 and PEG MME 2000. Further screenings to determine the optimal conditions for crystal growth were accomplished by varying the pH, precipitant concentration and volume of solution. Finally, the best large crystals were obtained at 293 K in a drop containing 1.5 µl 20 mg ml^{-1} protein solution [20 m*M* Tris–HCl (pH 7.9 at 277 K), 10% glycerol, 100 m*M* NaCl, 10 m*M* 2-mercaptoethanol] and 1.5 µl reservoir solution (0.2 *M* ammonium sulfate, 0.1 *M* sodium acetate buffer pH 4.6 and 30% PEG MME 2000). Crystals grew to approximate dimensions of 0.2 × 0.1 × 0.03 mm in around one month (Fig. 2).

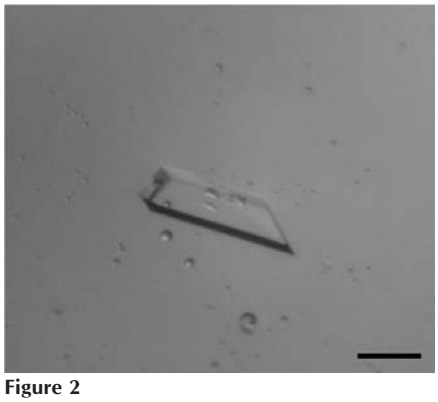

Figure 2
Crystal of *M. jannaschii* TBP. The scale bar indicates 0.1 mm.

2.3. Data collection

Initial inspection of the crystals was performed at 100 K on an R-AXIS IV^{++} imaging-plate system mounted on a Rigaku rotating-anode X-ray generator (FR-D) operated at 50 kV and 60 mA. The crystal diffracted to around 3.5 Å resolution. In order to obtain a better data set, the data were collected at 100 K from a single crystal using an ADSC Quantum 315 CCD detector on beamline BL-5 at the Photon Factory, Tsukuba, Japan. Before freezing the crystal, 2 µl of a cryoprotectant solution was added to the 3 µl droplet solution. The cryoprotectant solution was prepared by mixing 1 g trehalose and 3.3 ml of a solution containing 0.2 *M* ammonium sulfate, 0.1 *M* sodium acetate buffer pH 4.6 and 40% PEG MME 2000 and recovering the supernatant. After 1 min soaking, the crystal was mounted on a CryoLoop (Hampton Research) and flash-frozen in a cold N_2 flow. The wavelength used was 0.97910 Å and the incident beam was collimated to a diameter of 0.2 mm. The crystal-to-detector distance was set to 270 mm.

3. Results

Examination of diffraction data from *M. jannaschii* TBP crystals revealed that the crystals diffract to beyond 1.9 Å resolution and belong to space group $P2_1$, with unit-cell parameters a = 53.2, b = 55.5, c = 123.4 Å, α = 90.0, β = 91.0, γ = 90.0°. The diffraction data were processed and scaled using the programs *MOSFLM* and *SCALA*, respectively (Collaborative Computational Project, Number 4, 1994) (Fig. 3). Data-collection statistics are summarized in Table 1. A value of 2.1 Å^3 Da^{-1} for the Matthews coefficient and a solvent content of 40.3% were obtained assuming the presence of four molecules in the asymmetric unit. This was confirmed by a Patterson self-rotation map which was calculated with the program *POLARRFN* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). The self-rotation map suggests that there are two non-crystallographic twofold axes.

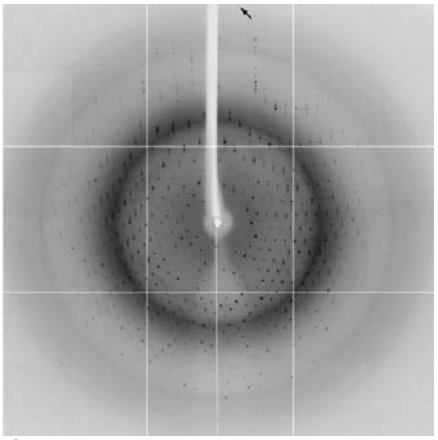

Figure 3
A 1° oscillation diffraction pattern from a crystal of *M. jannaschii* TBP. The arrowhead indicates 2.0 Å resolution.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the last shell.

Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 53.2, b = 55.5, c = 123.4, α = 90.0, β = 91.0, γ = 90.0
Resolution range (Å)	62.00–1.90 (2.00–1.90)
No. of measured reflections	208543
No. of unique reflections	57094
$R_{\rm merge}$† (%)	3.9 (35.0)
Completeness (%)	99.9 (99.9)
Average $I/\sigma(I)$	9.2 (2.1)

† $R_{\rm merge} = \sum|I(\mathbf{h}) - \langle I(\mathbf{h})\rangle|/\sum I(\mathbf{h})$, where $I(\mathbf{h})$ is the observed intensity and $\langle I(\mathbf{h})\rangle$ is the mean intensity of reflection **h** over all measurements of $I(\mathbf{h})$.

Molecular replacement (MR) was performed with the program *MOLREP* (Vagin & Teplyakov, 1997) using all data in the resolution range 49.2–3.3 Å. The crystal structure of TBP from *S. acidocaldarius* (PDB code 1mp9; Koike *et al.*, 2004), with a sequence identity of 49%, was used as a search model. Initial attempts to solve the structure by MR using the single-subunit coordinates from 1mp9 were unsuccessful. When the protruding parts of the N-terminal and C-terminal amino acids (residues 5–13 and 185–197 of chain *A* and residues 3–13 and 185–191 of chain *B*) were removed from the coordinates and a dimeric model was applied as a search model, a reasonable solution was obtained for the two dimeric TBPs in the asymmetric unit. There were no unfavourable molecular contacts observed in the crystal packing. The crystal packing shows that there are two pseudo-twofold axes in the asymmetric unit, which seem to cause the two peaks found in the self-rotation map. After several cycles of rigid-body and simulated-annealing refinement at 2.5 Å resolution using the program *CNS*

(Brünger *et al.*, 1998), the R_{cryst} and R_{free} factors fell to 33.6 and 37.2%, respectively. Further crystallographic refinement is in progress.

We thank Dr E. P. Geiduschek for the pLJ-MJ-TBP plasmid, Drs S. Wakatsuki, M. Suzuki, N. Igarashi and N. Matsugaki of the Photon Factory for their kind help in intensity data collection, which was performed under the approval of the Photon Factory (proposal Nos. 02G316, 02G311 and 03G122), and K. Hasegawa and Drs A. Kimura and B. Padmanabhan for comments on the manuscript. This work was supported in part by Grants-in-Aid for Science Research from the Ministry of Education, Science, Sports and Culture of Japan, the New Energy and Industrial Technology Development Organization (NEDO) and the Exploratory Research for Advanced Technology (ERATO) of the Japan Science and Technology Corporation (JST).

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