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Mouse testis–brain RNA-binding protein (TB-RBP): expression, purification and crystal X-ray diffraction

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TB-RBP (testis–brain RNA-binding protein) is a mouse RNA-binding protein that controls the temporal and spatial expression of mRNA. Found most abundantly in brain and male germ cells in the testis, TB-RBP is known to suppress the translation of specific testicular and brain mRNAs as part of cell development. TB-RBP–mRNA complexes can bind microtubules and thereby facilitate RNA translocation. Translin is the human orthologue of TB-RBP which binds to single-stranded ends of DNA sequences in breakpoint regions of chromosomal translocations. TB-RBP/translin has been crystallized in space group $P2_12_12$. The expression, purification, and crystallization of TB-RBP are described as well as preliminary X-ray diffraction data. The multimeric state of TB-RBP is addressed using dynamic light-scattering results.

1. Introduction

During the early phases of spermatogenesis, gene expression is often controlled at the level of transcription. However, transcription ceases midway through spermiogenesis, the haploid phase of spermatogenesis. In the later phases of male germ-cell development, gene expression is controlled at the level of mRNA translation. Stored mRNAs are translationally suppressed until the proteins for which they code are developmentally required.

The testis–brain RNA-binding protein (TB-RBP) is a 228-residue 26 kDa mouse protein initially characterized for its ability to suppress the translation of stored mRNAs by binding to H and Y elements in the 3′ untranslated region (UTR) of a number of testicular and brain mRNAs (Kwon & Hecht, 1991, 1993). The gene coding for TB-RBP has subsequently been cloned and expressed in bacteria (Wu *et al.*, 1997). TB-RBP binds its associated mRNAs to microtubules and moves along reconstructed microtubules (Han, Yiu *et al.*, 1995). In this way, TB-RBP can spatially control mRNA expression. During the haploid phases of spermatogenesis, each spermatid has either an X or Y chromosome. Sex chromosomes contain essential genes, so there is a need to share genetic information between haploid spermatids. TB-RBP crosses through intercellular bridges between developing haploid spermatids and is believed to be involved in maintaining genetic equivalence between spermatids (Morales *et al.*, 1998). TB-RBP is also abundant in brain cells and binds to the 3′ UTR of translationally suppressed and transported brain mRNAs (Han, Gu *et al.*, 1995). The pattern of its cellular localization and its ability to bind microtubules and translationally suppressed mRNAs strongly support its proposed role in the control of mRNA localization and expression in brain and testis (Hecht, 2000).

Translin is the human orthologue of TB-RBP. TB-RBP and translin bind single-stranded DNA (ssDNA) sequences found near breakpoint regions in recombination hot spots (Wu *et al.*, 1997; Aoki *et al.*, 1995). TB-RBP/translin also binds DNA sequences found in regions of gene-translocation events in myeloid leukemia cell lines, where high levels of TB-RBP are found in the nucleus (Aoki *et al.*, 1995). Non-lymphoid cell lines do not have high levels of TB-RBP in the nucleus. However, a shift from cytoplasm to nucleus can be induced by adding DNA-damaging agents to cells (Kasai *et al.*, 1997). The exact nature of the ssDNA-binding ability of TB-RBP/translin is not known. The localization of TB-RBP/translin to the nucleus in pachytene spermatocytes implicates its function in meiotic DNA recombination and repair events (Hecht, 2000).

Structural information will greatly assist in understanding the mechanism of mRNA recognition and protection and also of ssDNA binding. To this end, recombinant TB-RBP has been purified and crystallized. The crystals have been improved so that X-ray diffraction

data are of sufficient quality to begin structure determination.

2. Materials and methods

2.1. Expression and purification

The gene for TB-RBP was previously cloned into a glutathione-*S*-transferase (GST) fusion vector (Wu *et al.*, 1997). Fresh transformants of *Escherichia coli* strain BL21(DE3) were grown in 2YT media at 303 K until the optical density (OD) at 600 nm was between 0.8 and 1.0. Protein expression was induced with 0.3 m*M* IPTG and the cells were incubated at 303 K for an additional 4–6 h. The cells were harvested by centrifugation for 10 min at 3000*g*. Cell pellets were either resuspended in loading buffer (25 m*M* Tris pH 8.0, 300 m*M* NaCl, 1 mM EDTA) for lysis with a French pressure cell or they were stored at 253 K for later use. Lysed cells were spun at 35 000 rev min^{-1} for 45 min to remove cell debris. The supernatant was diluted twofold with loading buffer and passed over a column of glutathione-agarose beads (Sigma) equilibrated in loading buffer. The column was washed extensively with loading buffer ($A_{280} < 0.01$). The loading buffer was then exchanged to a buffer of lower salt concentration (25 m*M* Tris pH 8.0, 150 m*M* NaCl, 1 m*M* EDTA) on the column. The high-salt buffer was desirable for eliminating cellular contaminant proteins during washing steps. However, the high salt concentration caused the protein to precipitate in the subsequent cleavage steps that separate TB-RBP from GST. The column matrix was then transferred to a conical tube containing 40 units of thrombin (Sigma) and thrombin cleavage buffer (25 m*M* Tris pH 8.0, 150 m*M* NaCl, 1 m*M* KCl). The cleavage reaction mixture was gently shaken at room temperature for 16 h and then poured into a fresh glutathione agarose column and allowed to settle. Free TB-RBP was then eluted and collected in fractions at a flow rate of between 0.4 and 0.5 ml min^{-1}. Protein-containing fractions were dialyzed into a storage buffer (10 m*M* Tris pH 7.5, 1 m*M* EDTA) and concentrated to 20–60 mg ml^{-1} (estimated extinction coefficient 0.62 ml cm^{-1} mg^{-1}).

2.2. Crystallization

TB-RBP was diluted to 5 mg ml^{-1} with storage buffer and screened using the Hampton Research and Emerald Bioscreen sparse-matrix kits by the hanging-drop vapor-diffusion method. Attempts were made to optimize conditions that yielded crystals. These conditions were also modified using Hampton Research Additive and Detergent screens as well as various cryogenic compounds. The crystals used in data collection were grown from 0.9 *M* NH_4SO_4, 100 m*M* sodium acetate pH 4.8. 9 µl of this precipitating solution were added to 1 µl of 20 mg ml^{-1} TB-RBP on a plastic cover slip. The cover slip was placed over 400 µl well solution. 1 µl of 0.1 m*M* *n*-tetradecyl-β-D-maltoside or 0.33 m*M* *n*-tridecyl-β-D-maltoside was sometimes added to the growth drop to reduce the amount of precipitate.

2.3. Dynamic light scattering

A DynaPro-801 molecular-sizing detector (Protein Solutions, Charlottesville, VA) was used to analyze the dynamic light-scattering characteristics of TB-RBP solutions. Samples were passed through a 0.2 µm filter (Whatman) into a quartz cuvette for exposure to laser light. *DYNAMICS* software was used to calculate the hydrodynamic radii of solution particles and to estimate particle molecular weights and regularize scattering data.

2.4. Data collection and processing

Crystals were transferred to a cryogenic solution (10% PEG 4000, 30% glycerol, 100 m*M* sodium acetate pH 4.8) to prepare them for liquid-nitrogen cryocooling. Diffraction intensity data were collected on an ADSC Quantum 4 CCD detector at the X12B beamline of the National Synchrotron Light Source, Brookhaven National Laboratory. The wavelength of the beamline was 0.9794 Å. The data were processed using *DENZO* and *SCALEPACK* from the *HKL* suite (Otwinowski & Minor, 1997).

3. Results and discussion

On average, 7 mg of TB-RBP were purified per liter of bacterial culture. The protein was stable in storage buffer for longer than three months.

The best crystals of TB-RBP were grown from 0.9 *M* NH_4SO_4, 100 m*M* sodium acetate pH 4.8 (Fig. 1). These crystals also grew in the presence of the detergents *n*-tetradecyl-β-D-maltoside and *n*-tridecyl-β-D-maltoside. Adding these detergents tended to reduce the amount of protein precipitate in growth drops. The crystal dimensions were typically 0.6 × 0.25 × 0.25 mm. The crystals diffracted to 2.65 Å on NSLS beamline X12B.

The crystal diffraction data were indexed as orthorhombic space group $P2_12_12$, with unit-cell parameters a = 97.2, b = 135.5, c = 92.2 Å. The R_{merge} after scaling was 4.5%. Other crystal data are given in Table 1. A reasonable Matthews parameter of 2.9 Å^3 Da^{-1} is calculated assuming that there are four 26 kDa monomers of TB-RBP in the asymmetric unit. Crystallographic symmetry may reveal higher molecular symmetry for the protein complex.

Table 1
Crystal parameters and data statistics.

Values in parentheses are for the last resolution shell.

Space group	$P2_12_12$
Unit-cell parameters (Å)	a = 97.2, b = 135.5, c = 92.2
Resolution (Å)	2.65
Wavelength (Å)	0.9794
R_{merge} (°)	4.5 (45.7)
$I/\sigma(I)$	29.7 (2.9)
Completeness (%)	95.3 (98.1)
Unique reflections	34219
Redundancy	5.6

Light-scattering experiments were performed with solutions of TB-RBP in order to assess its polydispersity and molecular size. With TB-RBP at 2 or 4 mg ml^{-1} in 10 m*M* Tris pH 7.5, the largest portion of the scattering mass (>99%) was estimated to have a hydrodynamic radius of around 4.7 nm. Given a spherical approximation, this corresponds to a molecule of 125 kDa, consistent with a tetramer of TB-RBP. Larger aggregates were also detected (20–30 nm particles), but they accounted for a very small portion of the scattering matter (<1%). Adding *n*-tetradecyl-β-D-maltoside or *n*-tridecyl-β-D-maltoside to the protein solution did not change the hydrodynamic radius of the dominant particle, but the amount of large aggregates contributing to the scattering matter did decrease (<0.2%). Aoki *et al.* (1999) reported mouse TB-RBP/translin particles to be 8.6 nm in diameter from electron-microscopic studies. It is certain that the particle detected by light scattering is multimeric, but it is not clear exactly how many monomers are involved.

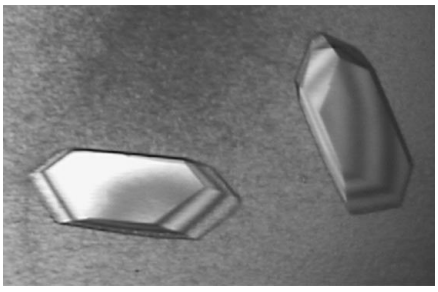

Figure 1
Orthorhombic crystals of TB-RBP. Both of the crystals are approximately 0.5 × 0.25 × 0.25 mm.

There may be an equilibrium between tetramers and octamers in solution.

In summary, we have a useful system for the expression, purification and crystallization of TB-RBP/translin. Solving the structure of TB-RBP/translin will hopefully answer questions about the RNA-binding mode and the multimeric assembly.

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