Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction analysis of the *Saccharomyces cerevisiae* Ran-binding protein Mog1p

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Received 14 October 1999
Accepted 24 December 1999

Mog1p binds the Ras-family GTPase Ran/Gsp1p, which has a central role in nucleocytoplasmic transport and cell-cycle progression. Overexpression of *MOG1* is able to suppress temperature-sensitive *gsp1* mutants in yeast; Δ*mog1* null mutants display temperature-sensitive defects in nuclear trafficking. Orthorhombic crystals of bacterially expressed Mog1p that diffract to beyond 2 Å resolution using synchrotron radiation have been obtained. The crystals have $P2_12_12_1$ symmetry, with unit-cell parameters a = 39.67, b = 51.96, c = 112.23 Å, a Matthews coefficient of 2.44 Å^3 Da^{-1}, an estimated solvent content of 49.5% and one chain in the asymmetric unit.

1. Introduction

Nucleocytoplasmic transport relies on the coordination of both soluble transport factors and components of nuclear pore complexes embedded in the nuclear envelope (reviewed by Görlich, 1998; Ohno *et al.*, 1998; Stewart & Rhodes, 1999). The Ras-family GTPase Ran is central to the transport of macromolecules into and out of the nucleus (Drivas *et al.*, 1990; Melchior *et al.*, 1993; Moore & Blobel, 1992). Ran, like other Ras-family GTPases, cycles between GTP-bound and GDP-bound states. RanGTP is generated by RCC1, the Ran guanine nucleotide-exchange factor (Bischoff & Ponstingl, 1991), while the low intrinsic GTPase activity of Ran is stimulated by RanGAP1 to produce RanGDP (Bischoff *et al.*, 1994). The compartmentalization of these activities, with RCC1 associated with chromatin (Ohtsubo *et al.*, 1989) and RanGAP1 localized to the cytoplasmic face of nuclear pores (Matunis *et al.*, 1996; Mahajan *et al.*, 1997), suggests an enrichment for RanGTP in the nucleus and RanGDP in the cytoplasm. Because the nucleotide state of Ran influences its interactions with other transport factors (reviewed by Avis & Clarke, 1996; Rush *et al.*, 1996; Melchior & Gerace, 1998), the asymmetric distribution of the two nucleotide-bound states is thought to provide directionality for nuclear trafficking (Koepp & Silver, 1996; Görlich *et al.*, 1996; Izaurralde *et al.*, 1997; reviewed by Melchior & Gerace, 1998; Görlich, 1998).

Because of the central role of Ran in nuclear trafficking, proteins that bind to Ran and their complexes have become an area of intense investigation. The structures of importin-β, the carrier molecule that transports cargo into the nucleus (reviewed by Görlich, 1998), RanBP2 and NTF2, which mediates the nuclear import of RanGDP (Ribbeck *et al.*, 1998; Smith *et al.*, 1998), and their complexes with Ran have recently been established (Chingolani *et al.*, 1999; Chook & Blobel, 1999; Stewart *et al.*, 1998; Vetter, Arndt *et al.*, 1999; Vetter, Nowak *et al.*, 1999). A genetic screen for multicopy suppressors of temperature-sensitive alleles of the *S. cerevisiae* Ran homologue Gsp1p identified a novel protein Mog1p which was demonstrated to bind directly to the GTP-bound form of Gsp1p (Oki & Nishimoto, 1998). Although the disruption of *MOG1* is not lethal, the Δ*mog1* strain displays temperature-sensitive defects in signal-mediated protein import (Oki & Nishimoto, 1998). Moreover, many of the *gsp1* mutants suppressed by overexpression of *MOG1* were also suppressed by overexpression of *NTF2*, indicating some overlap of their activities. Therefore, we initiated a structural analysis of Mog1p to complement genetic and biochemical methods in defining its precise role in nucleocytoplasmic transport.

2. Results and discussion

2.1. Expression and crystallization of Mog1p

The yeast *MOG1* gene encodes a 218 amino-acid protein with a calculated molecular mass of 24 307 Da. The open reading frame of *MOG1* from *S. cerevisiae* genomic DNA was amplified by PCR with Vent polymerase (New England Biolabs, Beverly, Massachussetts, USA) and the gel-purified PCR product was cloned into the T7-based expression vector pMW172 (Way *et al.*, 1990) using 5′ *Nde*I and 3′ *Bam*HI sites introduced by the PCR primers. The correct nucleotide sequence of the cloned *MOG1* coding region was confirmed by

Table 1
Data-collection statistics for Mog1p crystals.

	Overall (20.0–1.90 Å)	Highest resolution shell (2.0–1.90 Å)
$R_{\rm merge}$†	0.039	0.143
Completeness (%)	98.5	98.5
Multiplicity	3.5	3.3
No. of measurements	64665	8833
No. of reflections	18664	2679
I/σ	11.5	5.1

† $R_{\rm merge} = \sum_{hkl} \sum_i |I_i(hkl)| - \langle I(hkl)\rangle| \,/\, \sum_{hkl} \sum_i I_i(hkl)$.

sequencing. When this construct was transformed into the *Esherichia coli* expression strain BL21 (DE3), Mog1p expression could not be detected in small-scale cultures. However, Mog1p was expressed to high levels in the BL21 (DE3) derivative ENS134. Strain ENS134 carries the *rne-50* mutation, which results in the inactivation of RNAaseE at high temperatures and also overexpresses $tRNA^{Arg5}$, one of the least abundant tRNAs in *E. coli* (Lopez *et al.*, 1994; Iost & Dreyfus, 1995). Thus, an increased stability of transcripts, increased efficiency of translation, or a combination of the two may have contributed to the increased expression of Mog1p in strain ENS134. Large-scale expression of Mog1p was accomplished by inoculating 1 l aliquots of 2XTY medium containing 100 µg ml^{-1} ampicillin with fresh transformants followed by incubation at 310 K overnight. IPTG induction was not required as Mog1p was expressed sufficiently in the presence of basal levels of T7 RNA polymerase. Cultures were harvested by centrifugation at 6000*g* for 10 min at 277 K; the cell pellets from each 1 l culture were resuspended in 10 ml 25% sucrose, 50 m*M* Tris–HCl pH 8.0, 1 m*M* EDTA, 1 m*M* DTT, 0.1 m*M* PMSF and were stored at 253 K.

Bacterially expressed Mog1p was purified to homogeneity by a two-step chromatographic purification scheme that employed ion exchange followed by gel filtration. Pellets were thawed and the cells were lysed at 277 K using a French press. DNA viscosity was reduced with 10 µg ml^{-1} DNAase (Sigma) in the presence of 10 m*M* Mg_2SO_4 and 1 m*M* $MnCl_2$ for 15 min at room temperature. The lysate was clarified by centrifugation at 40 000*g* for 15 min at 277 K and the supernatant was dialysed overnight at 277 K against buffer *A* (20 m*M* Tris–HCl pH 8.0, 1 m*M* EDTA, 1 m*M* DTT, 0.1 m*M* PMSF). After clarification at 40 000*g* for 10 min at 277 K, the sample was applied to a 10 × 2.5 cm DE52 ion-exchange column pre-equilibrated in buffer *A*. The column was washed with at least one column volume of buffer *A* containing 100 m*M* NaCl and Mog1p was then eluted with a 400 ml 100–500 m*M* NaCl gradient. Fractions containing Mog1p were pooled and concentrated using Centricon YM-10 centrifugal filters (Amicon) to an approximate volume of 20 ml. The sample was then applied to a 90 × 2.5 cm Sephacryl S100 gel-filtration column equilibrated in buffer *B* (20 m*M* Tris–HCl pH 7.4, 100 m*M* NaCl, 1 m*M* EDTA, 1 m*M* DTT, 0.1 m*M* PMSF) and eluted at 30 ml h^{-1}. Peak fractions were concentrated to 10–15 mg ml^{-1}, with yields ranging from 30–50 mg of pure Mog1p per litre of culture. The N-terminal sequence of this material, determined using a Procise 494 protein sequencer, was MKIEKAS, which was identical to that predicted. Protein concentration was measured by absorbance at 280 nm using an extinction coefficient of 0.917 for a 1 mg ml^{-1} solution of Mog1p calculated from its amino-acid composition.

Small crystalline needles (approximate dimensions 0.01 × 0.01 × 0.1 mm) were obtained by vapour diffusion at 291 K against 0.2 *M* ammonium acetate, 1 *M* sodium citrate buffer pH 5.6, 30% PEG 4000 after mixing 3 µl drops of 10–12 mg ml^{-1} Mog1p and well buffer. Screening conditions indicated that optimum crystals (measuring up to 0.05 × 0.1 × 1 mm) were obtained from 16–20% PEG 4000, 20% glycerol, 10 m*M* DTT and acetate or MES buffer in the pH range 6.0–6.5 using 5–10 µl hanging or sitting drops. The addition of 0.1–0.25% β-octylglucoside resulted in larger crystals, but completely inhibited nucleation so that it was necessary to streak drops to obtain crystals. Streaking was most effective approximately 12 h after setting up the drops. Crystals appeared within 12 h of seeding and reached full size after approximately one week.

2.2. Data collection and processing

The crystals of Mog1p diffracted to beyond 2.5 Å using laboratory X-ray sources and beyond 2 Å resolution using beamline ID-2 at the European Synchrotron Radiation Source (ESRF, Grenoble). The crystals had orthorhombic symmetry, with unit-cell parameters $a = 39.67$, $b = 51.96$, $c = 112.23$ Å. Characteristic systematic absences along the three principal axes indicated $P2_12_12_1$ symmetry. The calculated Matthews coefficient (Matthews, 1968) was 2.44 Å^3 Da^{-1}, indicating a solvent content of 49.5% and a single Mog1p chain in the asymmetric unit. We collected a 98.5% complete 1.9 Å resolution data set at 100 K from a crystal flash-frozen in a dry nitrogen Cryostream (the glycerol in the crystallization solution provided sufficient cryoprotection) using a MAR 345 image-plate scanner on ESRF beamline ID-2. Table 1 gives data-collection statistics after processing using the *MOSFLM, SCALA* and *TRUNCATE* programs from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994). Because the sequence of Mog1p does not show homology to any other proteins (Oki & Nishimoto, 1998), we are using multiple isomorphous replacement to obtain the structure of Mog1p.

We are most grateful to our colleagues in Cambridge and Grenoble, especially Richard Bayliss, Helen Kent and B. Rasmussen for their assistance and advice and Sew Yeu Peak-Chew for microsequencing the bacterially expressed Mog1p. This work was supported in part by research grant RG0270/1998-M from the Human Frontiers Science Program.

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