Identification and in vitro deoxynucleotidylation of the terminal protein of the linear plasmid pAL1 of Arthrobacter nitroguajacolicus Rü61a

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Abstract

The plasmid pAL1 of Arthrobacter nitroguajacolicus Rü61a is a linear replicon, characterized by inverted terminal repeats and terminal proteins (TPs) covalently bound to its 5'-ends. Previous sequence analysis and predictions of possible secondary structures formed by telomeric 3'-overhangs indicated significant differences of the 'left' and 'right' telomere of pAL1, raising the question of whether each terminus is recognized by a specific protein. The genes pAL1.102 and pAL1.103, located close to a terminus, code for possible DNA-binding proteins; however, only the pORF102 protein encoded by pAL1.102 shows a weak similarity to known TPs of Streptomyces linear replicons. pORF102, purified from recombinant A. nitroguajacolicus Rü61a as a fusion with maltose-binding protein (MBP), was specifically associated with terminal pAL1 DNA, whereas MBP-pORF103 was devoid of DNA, suggesting that pORF102 represents the protein attached to both ends of the linear plasmid. In electrophoretic mobility shift assays, the MBP-pORF102 protein was not capable of specifically recognizing telomeric DNA sequences. Consistent with its proposed role as a protein primer in DNA synthesis, pORF102 was deoxynucleotidyated in vitro with dCMP, complementary to the 3'-ends (...GCAGG) of pAL1.

Introduction

Linear plasmids are widespread among streptomyces and also occur in a number of rhodococci and other Actinobacteria (Chater & Kinashi, 2007; Chen, 2007; Fetzner et al., 2007). Typical features of the linear replicons of Streptomyces spp. are inverted terminal repeats of various lengths and terminal proteins (TPs) attached to each 5'-end (Sakaguchi, 1990). Their replication is initiated bidirectionally from an internal origin, resulting in single-stranded gaps at the ends of replication intermediates (Chang & Cohen, 1994; Chang et al., 1996). DNA synthesis to fill in the recessed 5'-ends is assumed to be primed by the hydroxyl group of an amino acid residue of the TP, and so as a consequence, the TP remains covalently linked to the 5'-ends (Qin & Cohen, 1998; Bao & Cohen, 2001; Yang et al., 2002, 2006). Both TP and a telomere-associated protein (Tap), which is presumed to recruit and position TP to the telomere (Bao & Cohen, 2003), are necessary for the propagation of Streptomyces replicons in their linear form. The Streptomyces telomere complex besides TP and Tap was found to contain DNA polymerase I and DNA topoisomerase I proteins (Bao & Cohen, 2004); however, it is not clear which polymerase is involved in end patching of Streptomyces replicons, as PolI is not essential (Huang & Chen, 2008). Because centrally located origins were detected not only on Streptomyces linear replicons but also on pRHL3 of Rhodococcus sp. RHA1 (Warren et al., 2004) and pCLP of Mycobacterium celatum (Picardeau et al., 2000), actinomycetal linear plasmids may share a similar mode of DNA replication.

The 113-kb plasmid pAL1 of Arthrobacter nitroguajacolicus Rü61a, which codes for the degradation of 2-methylquinoline, is so far the only described linear replicon within the genus Arthrobacter. Its termini contain the inverted repeat sequence 5'-CCTGC ... GCAGG-3', and its 5'-ends are covalently capped with protein (Overhage et al., 2005; Parchat et al., 2007). Our previous sequence analysis of pAL1 and predictions of possible secondary structures
formed by potential telomeric 3’-overhangs indicated significant differences of the ‘left’ and ‘right’ terminus of pAL1, raising the question of whether each terminus of pAL1 is recognized, or even capped, by a specific protein (Parschat et al., 2007). Rhodococcal plasmids pHG201 and pHG205 are other examples of actinomycete linear plasmids that do not show striking homology between their ‘left’ and ‘right’ telomere sequences (Kalkus et al., 1998), but their TPs have not been described. In contrast, the ends of *Streptomyces* linear replicons usually contain well-conserved terminal palindromic sequences (Zhang et al., 2006).

The gene product of *pAL1.102* is the only protein exhibiting a weak similarity to known (*Streptomyces*) TPs; however, due to the low sequence similarity, its annotation as a ‘putative terminal protein’ was tentative (Parschat et al., 2007). As a first step toward characterizing the telomere complex of pAL1, we identified the protein attached to both termini of pAL1 and demonstrated its specific deoxynucleotidyltransferase activity in vitro.

**Materials and methods**

**Bacterial strains, media, and growth conditions**

The strains and plasmids used in this study are listed in Table 1. For isolation of total DNA, *A. nitroguajacolicus* was grown in a mineral salts medium (Parschat et al., 2003) on 8 mM sodium benzoate at 30 °C. *Arthrobacter nitroguajacolicus* Ru61a [pAL1, pART2malE-ORF102 or pART2malE-ORF103] was cultivated in a mineral salts medium supplemented with 4 mM 4-hydroxyquinoline and 140 μg mL⁻¹ kanamycin. Cells were harvested by centrifugation at an OD₆₀₀ nm of approximately 2.5. *Escherichia coli* DH5α clones containing derivatives of pMal-c2x or pART2 were grown in lysogeny broth (LB) (Sambrook & Russell, 2001) at 37 °C in the presence of 100 μg mL⁻¹ ampicillin or 50 μg mL⁻¹ kanamycin, respectively. For the synthesis of fusion proteins of maltose-binding protein (MBP) and the protein encoded by *pAL1.102* (termed pORF102), *E. coli* K12 ER2508 [pLysSRARE] harboring pMal-c2x-ORF102 was grown in LB with ampicillin (100 μg mL⁻¹), chloramphenicol (34 μg mL⁻¹), and auto induction solutions ‘5052’ and ‘M’ (Studier, 2005) at 30 °C. Cells were harvested by centrifugation at an OD₆₀₀ nm of ~5 and stored at −80 °C before use.

**DNA isolation and gene cloning**

Total DNA of *A. nitroguajacolicus* Ru61a [pAL1] was isolated according to Rainey et al. (1996). Plasmid DNA was isolated using the E.Z.N.A. Plasmid Miniprep kit (Peqlab, Erlangen, Germany). Gel extraction of DNA fragments from agarose gels was performed with the Perfectprep gel cleanup kit (Eppendorf, Hamburg, Germany). For cloning purposes, DNA fragments were purified using the High Pure PCR Product Purification kit (Roche Diagnostics GmbH, Mannheim, Germany). Standard protocols were used for agarose gel electrophoresis, restriction digestion, and DNA ligation (Sambrook & Russell, 2001). ORF102 and ORF103 of pAL1 were amplified by PCR using Phusion™ Hot Start High-Fidelity DNA Polymerase (Finnzymes Oy, Espoo, Finland), using total DNA of *A. nitroguajacolicus* Ru61a [pAL1] as the

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Source or references</th>
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<tr>
<td><em>A. nitroguajacolicus</em> Ru61a</td>
<td>Soil isolate; wild type; pAL1</td>
<td>Overhage et al. (2005)</td>
</tr>
<tr>
<td><em>E. coli</em> DH5x</td>
<td>supE44, ΔlacU169, (Φ80lacZAM15), hsdR17, recA1, endA1, gyrA96, thi-1, relA1, F⁻ F₉ ara-14 leuB6 thiA2 Δ(lac-pro) yeqY1 lon-1 miniTn10(Tet) glnV44 gaiK2 rpsL20(Str) xyl-5 mtl-5 Δ(malE) zyc::Tn5(Kan)Δ(mrcC-mrr) Δ8101</td>
<td>Novagen, New England Biolabs</td>
</tr>
<tr>
<td><em>E. coli</em> K12 ER2508</td>
<td>This work</td>
<td></td>
</tr>
<tr>
<td>Plasmid</td>
<td>plysSRARE</td>
<td>Cam³; encodes tRNAs for rare codons AGG, AGA, AUU, CUA, CCC, GGA, T7 lysozyme; 7393 bp</td>
</tr>
<tr>
<td>pMal-c2x</td>
<td>lacO, lacI, M13, and pBR322 replicon, malE, Amp³, 6646 bp</td>
<td>New England Biolabs</td>
</tr>
<tr>
<td>pMal-c2x-ORF102</td>
<td>ORF102 (630 bp) of pAL1 (nt 111108–111740) inserted in to the EcoRI/PstI site; 7258 bp</td>
<td>This work</td>
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<tr>
<td>pART2</td>
<td>ColE1 and pCG100 replicon, Kan³, 8 × His, 4634 bp</td>
<td>Sandu et al. (2005)</td>
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<tr>
<td>pART2malE</td>
<td>1115-bp PCR fragment (nt 1478–2628 of pMal-c2x) inserted in to the BamHI/Dral site of pART2; 5784 bp</td>
<td>This work</td>
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<tr>
<td>pART2malE-ORF102</td>
<td>ORF102 (630 bp) of pAL1 (nt 111108–111740) inserted in to the SgrS/XbaI site of pART2malE; 6388 bp</td>
<td>This work</td>
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<tr>
<td>pART2malE-ORF103</td>
<td>ORF103 (621 bp) of pAL1 (nt 112360–111737); inserted in to the SgrS/XbaI site of pART2malE; 6379 bp</td>
<td>This work</td>
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Nucleotide positions of pAL1 correspond to GenBank entry AM286278.
template and the primer sets listed in Supporting Information, Table S1. PCR products were subsequently ligated into pMal-c2x or pART2malE-ORF102. Competent *E. coli* and *A. nitroguajacolicus* Rüt61a [pPAL1] cells were generated as described by Hanahan (1983) and Gartemann & Eichenlaub (2001), respectively. All plasmid inserts and flanking regions were verified by sequencing (GATC Biotech AG, Konstanz, Germany).

**Preparation of covalent protein–DNA complexes and identification of DNA bound to TP**

For the preparation of covalent complexes of telomeric pAL1-DNA and MBP-pORF102 or MBP-pORF103, frozen cells of *A. nitroguajacolicus* Rüt61a [pAL1, pART2malE-ORF102] or *A. nitroguajacolicus* Rüt61a [pAL1, pART2malE-ORF103] were thawed in 20 mM Tris/HCl buffer containing 400 mM NaCl, 1 mM EDTA (pH 7.4) (buffer A), and 1 mM phenylmethylsulfonyl fluoride. After incubation for 30 min with 2 mg mL$^{-1}$ lysozyme, crude extracts containing soluble proteins were prepared by sonication, followed by centrifugation. Supernatants were applied to an amylose column (5 mL bed volume), equilibrated in buffer A. After washing with the same buffer, MBP fusions were eluted with buffer A containing 20 mM maltose. Eluates were loaded to a GF/C Whatman glass microfiber filter (Whatman International Ltd, Kent, UK) (Coombs & Pearson, 1978). The immobilized complexes were washed four times with 1 M NaCl and eluted with 0.5% sodium dodecyl sulfate (SDS), 0.1 M NaCl in water (Bao & Cohen, 2001). Eluted complexes were precipitated twice with 0.1 volume of 3 M sodium acetate and 2.5 volumes of ethanol. Precipitates were redissolved in sterile water. In order to identify the DNA attached to MBP-pORF102, MBP-pORF103, or both, the redissolved complexes were used as templates in PCR reactions with GoTaq$^\text{TM}$ Green DNA polymerase (Promega GmbH, Mannheim, Germany). The primer pairs for amplification of terminal regions of pAL1, an internal segment of pAL1, and a region of the chromosome of *A. nitroguajacolicus* Rüt61a [pPAL1] are listed in Table S1.

**Purification of the MBP-pORF102 fusion protein**

For the preparation of the MBP-pORF102 fusion protein, 5 g of frozen cells of *E. coli* K12 ER2508 [pLysSRARE, pMal-c2x-ORF102] were thawed in buffer A. After incubation for 30 min and addition of 1 mM MgCl$_2$ and 10 U mL$^{-1}$ benzonase, crude extracts were prepared by sonication and centrifugation as described above. The eluate from subsequent amylose affinity chromatography (performed as described above) was applied on HiTrap$^\text{TM}$ Desalting columns (4 × 5 mL, GE Healthcare, Munich, Germany) equilibrated in buffer B, consisting of 50 mM Tris/HCl (pH 8.0). The desalted eluate was loaded onto a UnoQ column (6 mL bed volume, Bio-Rad Laboratories, Munich, Germany) equilibrated in the same buffer. After a washing step and a linear gradient (10 mL) from 0 to 450 mM NaCl in buffer B, MBP-pORF102 was eluted by applying a gradient (10 mL) from 450 to 490 mM NaCl in buffer B. For use in control experiments, MBP that elutes from UnoQ within the first gradient was collected. Proteins were concentrated by ultrafiltration (Vivaspin 20, molecular weight cutoff 10 kDa; Sartorius AG, Göttingen, Germany) in buffer A, supplemented with 10% glycerol.

**Detection of fusion proteins**

Protein concentrations were measured using the bicinechonic acid method (Smith *et al.*, 1985). Proteins separated in SDS-polyacrylamide gels (Laemmli, 1970) were stained with ethyl violet and zircon (Choi *et al.*, 2002). Transfer of proteins from polyacrylamide gels to polyvinylidene fluoride membranes was performed according to the protocol of Qiagen (QIAexpress protocol; Qiagen GmbH, Hilden, Germany). MBP-fusion proteins were detected using primary anti-MBP antibodies (anti-MBP antiserum from rabbit; New England Biolabs), secondary antibodies (anti-rabbit horseradish alkaline phosphatase-conjugated IgG from goat; Sigma-Aldrich Chemie GmbH, Munich, Germany), and p-nitrotetrazolium blue and 5-bromo-4-chloro-3-indolyl phosphate (QIAexpress protocol; Qiagen GmbH).

**Electrophoretic mobility shift assays (EMSAs)**

Terminal pAL1 DNA [GenBank accession no. AM286278, nucleotide (nt) 1–285 and nt 112710–112992], an internal region of pAL1 (nt 3045–3328), and a 251-bp stretch of chromosomal DNA were amplified by PCR with Phusion$^\text{TM}$ Hot Start High-Fidelity DNA Polymerase (Finnzymes Oy), using total DNA of *A. nitroguajacolicus* Rüt61a [pAL1] as the template (for primer pairs, see Table S1). After purification of the digoxigenin end-labelled PCR products (High Pure PCR Product Purification kit; Roche Diagnostics GmbH), single-stranded DNA (ssDNA) was generated by denaturation at 99 °C and subsequent cooling in liquid nitrogen. Samples of MBP-pORF102 purified as described above were washed by ultrafiltration in binding buffer (10 mM Tris/HCl, 80 mM NaCl, 1 mM EDTA, 10 mM DTT, 5% glycerol, 0.005% Triton X114, pH 8.0). Protein and target DNA were incubated on ice for 1 h and subsequently mixed with binding buffer additionally containing 15% Ficoll$^\text{TM}$ 400 and 0.02% bromophenol blue. After incubation for another 15 min on ice, the DNA–protein complexes were separated on prerun native polyacrylamide gels (5% acrylamide) in ice-cold 22.5 mM Tris, 22.5 mM boric acid, and 0.5 mM EDTA (pH 8.0) at 100 V and 15 mA for 1 h. Southern blotting onto nylon membranes (Parablot NY plus; Macherey & Nagel, Düren, Germany) and colorimetric
detection with p-nitro tetrazolium blue and 5-bromo-4-chloro-3-indolyl phosphate were carried out following the Digoxigenin System User’s Guide for Filter Hybridization (Roche Molecular Biochemicals, 1995).

**In vitro deoxynucleotidylation of MBP-pORF102**

Specific deoxynucleotidylation of the pORF102 protein was demonstrated in an *in vitro* assay. Each reaction mixture in a total volume of 20 μL contained 0.4 μM purified MBP-pORF102 protein, 0.33 mg mL⁻¹ crude extract (soluble proteins) of *A. nitroguajacolicus* Ru61a [pAL1], 0.1 μM MBP-pORF101 fusion protein (unpublished data), 1 mM ATP, 1 μM of DNA template ‘left70’ (see Table S1), and different [α-³²P]dNTPs (0.33 μM, 111 TBq mmol⁻¹; Perkin-Elmer, Rodgau-Jügesheim, Germany) in 35 mM Tris/HCl (pH 8), 72 mM KCl, 5 mM MgCl₂, 5 mM DTT. The samples were incubated for 16 h at 30°C. In controls, MBP-pORF102 and MBP-pORF101 were replaced by equimolar amounts of MBP, prepared from the same genetic background as MBP-pORF102 and MBP-pORF101, respectively, by chromatography on amylose resin as described above. The controls were incubated in the presence of all [α-³²P]-labelled dNTPs (0.33 μM each). After treatment with 0.5 U μL⁻¹ DNase I at 30°C for 1 h, samples were separated in a 10% SDS-polyacrylamide gel and radiolabelled proteins were detected using a phosphoimager (PharosFX Plus, Bio-Rad Laboratories).

**Results and discussion**

The pORF102 protein encoded by *pAL1.102* is associated with telomeric pAL1 DNA

Based on the observation that pAL1, even after proteinase K or SDS treatment, is insensitive to 5’-exonuclease, but sensitive to 3’-exonuclease, we previously concluded that it has proteins covalently attached to its 5’-ends (Overhage et al., 2005). The gene product of *pAL1.102* exhibits a weak similarity to TPs of *Streptomyces* linear replicons (Fig. 1), for example 24% identity of amino acid (aa) 57–199 to a corresponding region (aa 39–178) of TpgCL1, and is thus a possible candidate for the 5’-TP of pAL1. However, considering the marked differences in the secondary structures predicted for potential 3’-overhangs of the termini of pAL1 (Parschat et al., 2007), it was conceivable that each of the telomeres of pAL1 interacts with its own TP. The protein encoded by *pAL1.103* does not show similarity to known TPs, but like pORF102 and TPs of *Streptomyces* linear replicons, it has a high theoretical pI value and is conserved in rhodococcal linear replicons (Parschat et al., 2007). We therefore tested the hypothesis that it might act as a second TP.

If *A. nitroguajacolicus* Ru61a during replication of pAL1 is able to use an MBP–TP fusion as the *in vivo* primer for DNA replication at the telomere, identification of the DNA linked to the purified fusion protein allows for assignment of the TP to the respective terminus. Pursuing such an approach, MBP-pORF102 and MBP-pORF103 were prepared from *A. nitroguajacolicus* Ru61a [pAL1, pART2malE-ORF102] and *A. nitroguajacolicus* Ru61a [pAL1, pART2malE-ORF103], respectively (Fig. 2a). The preparation after amylase affinity chromatography involved binding of protein complexes to a glass filter, washing steps with salt, treatment with SDS to disrupt noncovalent interactions, and precipitation of protein–DNA complexes. Whereas amplification of terminal DNA was not possible with the preparations of MBP-pORF103, PCR reactions performed with the MBP-pORF102 complex as the template resulted in specific products representing both termini of pAL1 (Fig. 2b). Because control PCR analyses using primers for amplification of non telomeric DNA failed to yield products in either case (Fig. 2b), nonspecific adsorption of DNA to MBP-pORF102 can be excluded. Thus, the protein encoded by *pAL1.102* is proposed to represent the TP bound to each 5’-end of pAL1.

**Purified MBP-pORF102 binds unspecifically to ssDNA in vitro**

To address the question of whether pORF102 specifically recognizes telomeric DNA, we aimed to produce recombinant protein in *E. coli* for use in EMSA. All attempts to prepare hexahistidine-tagged pORF102 or fusions of pORF102 with a chitin-binding domain failed, because all proteins precipitated with the insoluble fraction of cell extracts (data not shown). An N-terminal fusion with MBP yielded soluble protein, which could be purified to near electrophoretic homogeneity (Fig. 3a). Because cleavage of MBP-pORF102 with factor Xa protease and the subsequent attempt to remove MBP by affinity chromatography again resulted in loss of soluble protein, EMSAs were performed with the fusion protein. Migration of ssDNA was retarded by MBP-pORF102 (Fig. 3b), whereas the mobility of double-stranded DNA was not affected by an up to 1000-fold molar excess of protein (not shown). However, the shift in retardation with increasing protein concentrations suggests non-stoichiometric binding of pORF102 to the ssDNA, and interaction of the fusion protein with ssDNA representing an internal coding sequence of pAL1 indicated that the MBP-pORF102 protein was not able to specifically recognize telomeric DNA sequences (Fig. 3b). However, it cannot be excluded that recognition fails because the conformation of ssDNA under the experimental conditions differs from the native *in vivo* conformation of telomeric 3’-overhangs of pAL1 or because the MBP fusion (which, as shown above,
Fig. 1. Alignment of the amino acid sequences of pORF102 and TPs of Streptomyces linear replicons. The alignment was performed using the CLUSTALW2 algorithm (Larkin et al., 2007). pORF102, protein encoded by pAL1.102 of the linear plasmid pAL1 of Arthrobacter nitroguajacolicus Ru ë 61a (GenBank accession no. CAL09957); TpgSLP2, putative terminal protein of plasmid SLP2 of Streptomyces lividans (AAL10510); TpgC/L, terminal proteins of linear replicons of S. coelicolor/ S. lividans (AAL05041/AAL05040); TpgCL1, putative terminal protein of pSCL2 of S. clavuligerus (AAQ93595). Residues conserved throughout are marked with an asterisk, while residues marked with a colon and dot indicate conserved and semi-conserved substitutions, respectively. The box indicates a significantly conserved segment.

Fig. 2. Identification of pORF102 as the TP associated with both ends of pAL1. (a) Purification of MBP-pORF102 and MBP-pORF103 from Arthrobacter nitroguajacolicus Ru ë 61a [pAL1, pART2malE-ORF102 or pART2malE-ORF103]. SDS-polyacrylamide gel with an overall acrylamide concentration of 10.8% (w/v) and a cross-linker concentration of 2.6% (w/v) in the separating gel, after ethyl violet/zincon staining (A), and corresponding Western blot detected with anti-MBP antiserum (B). Lanes M, protein standard (molecular masses of 130, 100, 70, 55, and 35 kDa); 1, proteins after elution with 0.5% SDS from a glass filter; 2, after ethanol precipitation. The molecular mass of MBP-pORF102 and MBP-pORF103 is 64.1 and 64.0 kDa, respectively. The protein at about 40 kDa that detects in (B) probably represents MBP of A. nitroguajacolicus Ru ë 61a. (b) Identification by PCR of DNA attached to the purified fusion proteins (MBP-pORF102, MBP-pORF103). Agarose gel (2%) of PCR amplificates; 0.1 mg of MBP-pORF102 (see a), or 0.1 mg of MBP-pORF103 (see a), or 0.1 ng of total DNA from A. nitroguajacolicus Ru ë 61a served as templates for each PCR reaction. Primer pairs specific for amplification of the following DNA segments were used: Lane 1, left terminus of pAL1 (nt 1–285, 285-bp product); lane 2, right terminus of pAL1 (nt 112710–112992, 283-bp product); 3, internal coding region of pAL1 (nt 3045–3328, 284-bp product); 4, a chromosomally located coding region (251-bp product). For primer sequences, see Table S1.
did not prevent Arthrobacter from using MBP-pORF102 for in vivo replication of pAL1) impedes specific in vitro DNA binding. In this context, it is noteworthy that binding of the terminal protein TpgL of Streptomyces lividans to ssDNA corresponding to the 3'-overhang of plasmid pSLA2 telomeres also showed little specificity (Bao & Cohen, 2003). Similar to what was observed in the Streptomyces system, recruitment of pORF102 to the termini of pAL1 might require additional proteins.

**In vitro deoxynucleotidylation of MBP-pORF102**

To investigate whether pORF102 can act as a replication priming protein, we used an in vitro deoxynucleotidylation assay, which contained an ssDNA template representing the 3'-terminal 70 nucleotides of the 'left' end of pAL1, purified MBP-pORF102 protein, a crude extract of A. nitroguajacolicus Rü61a, MBP-pORF101 fusion protein that exhibits DNA polymerase activity (unpublished data), ATP, and different [α-32P]dNTPs in a Mg2+-containing buffer. As shown in Fig. 4, dCMP was specifically incorporated into the 64.1-kDa MBP-pORF102 protein. The deoxynucleotidylation was not detected in the absence of pORF102 or pORF101 (Fig. 4), or in the absence of crude extract, ATP, or Mg2+ (data not shown). When the single-stranded 'left70' DNA was omitted from the reaction, dNMP incorporation into pORF102-MBP likewise was not observed (not shown), indicating that the reaction requires a DNA template. Specific dCMP incorporation, complementary to the 3'-end of the S. lividans chromosome, was also reported for the TP of S. coelicolor (Yang et al., 2006). The specificity for dCMP incorporation into pORF102 leaves the possibility that either the first or the second nucleotide of the 3'-end of pAL1 (.. GCAGG-3') may serve as a template for the deoxynucleotidylation reaction.

**Conclusion**

In this study, we identified the gene product of pAL1.102 as a protein that is associated with both termini of the linear Arthrobacter plasmid pAL1. The proposed TP – at least when fused to MBP to ensure solubility – was not capable of specifically recognizing telomeric pAL1 DNA in vitro. However, in an in vitro deoxynucleotidylation assay, the pORF102 protein specifically incorporated dCMP, complementary to the 3'-ends of pAL1. This is consistent with its presumed role as a protein primer in DNA replication.

**Acknowledgements**

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Fig. 4. In vitro deoxynucleotidylation of pORF102 with [α-32P]dCMP. MBP-pORF102 protein was incubated with the DNA template representing 70 nucleotides of the ‘left’ 3′-end of pAL1 (‘left70’, see Table S1), crude extract of Arthrobacter nitroguajacolicus Rü61a, MBP-pORF101 protein, and ATP in the presence of different [α-32P]dNTPs for 16 h at 30 °C. After DNAse I treatment, proteins were separated in a 10% SDS-polyacrylamide gel, and radiolabelled proteins were detected using a phosphoimager. Prestained marker proteins were visualized using the same imaging system. Lane M, protein standard (molecular masses of 250, 130, 100, 70, 55, 35, and 27 kDa). Lanes C, G, A, T, samples after incubation with [α-32P]dCTP, [α-32P]dGTP, [α-32P]dATP, and [α-32P]dTTP, respectively. N, all four [α-32P]dNTPs in control assays, with MBP-pORF102 or MBP-pORF101 replaced by MBP.

References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Primers and ssDNA template used in this study.

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