Biochemistry and Biophysics Reports 8 (2016) 200–206

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Cloning, recombinant production and crystallographic structure of Proliferating Cell Nuclear Antigen from radioresistant archaeon *Thermococcus gammatolerans*

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ARTICLE INFO

Article history:
Received 19 April 2016
Received in revised form
23 July 2016
Accepted 1 August 2016
Available online 11 August 2016

Keywords:
Thermoccocus gammatolerans
PCNA
DNA metabolism
Gamma radiation

ABSTRACT

Thermococcus gammatolerans is a strictly anaerobic; hyperthermophilicarchaeon belongs to the order *Thermococcales* in the phylum *Euryarchaeota*. It was extracted from a hydrothermal vent from the Guaymas Basin (Gulf of California, Mexico). Different studies show that *T. gammatolerans* is one of the most radioresistant organisms known amongst the *archaea*. This makes it a unique model to study adaptations to the environment and to study DNA repair mechanisms in an organism able to tolerate harsh conditions. A key protein in these mechanisms is the Proliferation Cell Nuclear Antigen (PCNA). Its function is focused on their ability to slide along the DNA duplex and coordinating the activities of proteins mainly related to DNA edition and processing. Analysis of *archaeal* proteins have proven to be enormously fruitful because much of the information obtained from them can be extrapolated to eukaryotic systems, and PCNA is no exception. Here we report the cloning, recombinant expression and crystallographic structure of PCNA from *T. gammatolerans* (TgPCNA).

Contents

1. Introduction

The mechanisms involved in DNA metabolism are essential to preserve genome integrity in every organism. In these mechanisms are present multiple highly ordered protein complexes such as one that catalyses a specific incision to remove 5′ flaps in double-stranded DNA substrates and those involving chromatin remodeling [1,2]. A key protein in these complexes is the Proliferation Cell Nuclear Antigen (PCNA) [3]. A protein evolutionarily highly conserved, which was initially identified as an autoantigen in patients with systemic lupus erythematosus [4]. Depending on

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http://dx.doi.org/10.1016/j.bbrep.2016.08.004

the life domain, sliding clamps can be organized, in solution and crystal structures, forming dimers or trimers. Whereas in bacteria is assembled as a dimer (so-called β-clamp), in *archaea* and eukaryotes PCNA assembles into trimeric structure, wherein each monomer consists of two domains topologically identical [5–7].

All the PCNA are structurally almost identical, sharing a pseudo-hexameric shape when their three-dimensional structures are superposed. In eukaryotes and *archaea*, each PCNA monomer is composed of two structurally similar domains, with low amino acid sequence identity (8–22% of identity) [8]. These two domains are connected by a large Interdomain Connector Loop (IDCL) whose primary function is the interaction with PIP-Box (or PCNA Interaction Peptide). The inner diameter of trimeric PCNA from eukaryotes and dimeric β-clamp from bacteria, in general, is approximately 35 Å, diameter sufficient to accommodate a DNA duplex [9–11].

The ring formed in trimeric PCNA has two faces with important structural and functional differences. Only one of them, so-called C side (termed so because the C-termini of the PCNA monomers protrudes from this face) has been associated with protein-protein interactions with multiple enzymes [12]. Furthermore, the surface charge distribution is asymmetric: while the outer surface of PCNA ring is negatively charged, typically the inner surface is positively charged due to nine lysine and arginine residues within each monomer [6]. Therefore favoring interactions with the negatively charged sugar-phosphate backbone of DNA [13]. However, in the halobacteria, *Haloferaxvolcanii* it has been proposed a different mechanism for PCNA-DNA interactions, mediated by cations which can also coordinate the interaction with DNA [14].

PCNA itself lacks enzymatic activity; its function is focused on their ability to slide along the DNA duplex and coordinating the activities of several other proteins, mainly related to DNA edition and processing. So far, most interactions characterized in *archaeal* PCNA are involved in DNA replication and repair, principally with replicative DNA polymerases [15], DNA ligases [16], nucleases [2] and helicases [17]. Most of the interactions with PCNA occur via the PIP-box motif, a short peptide with a consensus sequence QXXΦXXΩΩ [11,15,18]. Where Φ is a moderately hydrophobic amino acid (Ile/Leu/Met) and Ω an aromatic amino acid (typically Phe/Trp) [19].

Interestingly within the *archaeal* kingdom, *Euryarchaeota* and *Thaumarchaeota* contain only one gene encoding PCNA, which is expressed typically as a homotrimer. While *Crenarchaeota* have three genes that assemble into heterotrimeric protein structures [20]. Studies of *archaeal* proteins have proven to be enormously fruitful because much of the information obtained from them can be extrapolated to eukaryotic systems [21], and PCNA is no exception. Also, their three-dimensional structures have highlighted its complexity in terms of adaptation to extreme environments, where large numbers of *Achaea* live [14,19].

T. gammatolerans is a strictly anaerobic; hyperthermophilicarchaeon belongs to the order *Thermococcales* in the phylum *Euryarchaeota*. Its growth occurs between 328–368 K, being their optimum growing temperature 361 K. It was extracted from a hydrothermal vent from the Guaymas Basin (Gulf of California, Mexico). As its name implies, this microorganism can survive after been exposed to high doses of gamma radiation. Withstands up to 5 kGy with no detectable lethality [22,23].

Currently, it has been identified several organisms with acceptable resistance to ionizing radiation [24,25]. However, different studies show that *T. gammatolerans* is one of the most radioresistant organisms known amongst the *archaea* [23]. This makes it a unique model to study adaptations to the environment and to study DNA repair mechanisms in an organism able to tolerate harsh conditions. Moreover, since *archaea* and eukaryotes have many homologous proteins between each other, the findings could be extrapolated from *archaea* to eukaryotes [26]. On the other hand, to date, only six crystallographic structures from *archaeal* PCNA have been deposited in the PDB.

All of the above highlights the importance of studying fundamental proteins such as PCNA in microorganism adapted to live in extreme conditions such as *T. gammatolerans*. A search in its genome, shows that *T. gammatolerans* only possesses a homolog gene PCNA, which is consistent with other *Euryarchaeota*[14,27]. Here we report the cloning, recombinant expression and crystallization of PCNA from *T. gammatolerans* (TgPCNA).

2. Materials and methods

2.1. Cloning of full-length TgPCNA

The open reading frame of 750 bp coding TgPCNA (accession code Tgam_1046) was PCR-amplified from genomic DNA from *T. gammatolerans* which was a generous gift from Dr. Patrick Forterre (Institut de GénétiqueetMicrobiologie, Université Paris-Sud). The PCR primers were 5′-ggtcggaattc**CATATG**CCGTTTGAGATCGTTTTTG-3′ (forward) and 5′-ggttgg**GGATCC**TCACTCCTCAACGCGGGGAGC-3′ (reverse). These primers contained restriction sites for *Nde*I and BamHI restriction enzymes respectively (the recognition sequences are shown in bold). The purified PCR product was digested and ligated into a pCold-I, resulting in the recombinant vector pCold-I+TgPCNA to overproduce the protein in fusion with an N-terminal hexahistidine tag. Bacterial strain for DNA propagation and cloning was DH5α. Positive clones were confirmed by automated DNA sequencing using local facilities (Unidad de Síntesis y SecuenciacióndelIBt-UNAM). For DNA transformation standard molecular biology protocols were used [28]. Plasmid was purified using the GeneJET™ Plasmid Miniprep kit (Thermo Fisher Scientific, Waltham, MA, United States).

2.2. Expression and purification of TgPCNA

E. coli BL21 Star (DE3) was transformed with the pCold-I+TgPCNA plasmid according with the manufacturer protocol (TakaraBio, Shiga, Japan) and plated onto an agar plate supplemented with ampicilin100 $\mu g\ mL^{-1}$ and incubated at 310 K for 12 h. A single colony was used to inoculate 100 mL of a seed stage medium (glycerol 5 $g\ L^{-1}$; peptone from casein 5 $g\ L^{-1}$; yeast extract 5 $g\ L^{-1}$; NaCl 5 $g\ L^{-1}$; ampicillin 100 $\mu g\ mL^{-1}$), incubated at 310 K and 200 rev min^{-1} for 12 h. Seeding medium was used to inoculate the production medium (glycerol 10 $g\ L^{-1}$; peptone from casein 10 $g\ L^{-1}$; M9-Salts 5x solution 200 $mL\ L^{-1}$, 0.01 mM $MgSO_4$, 0.0005 mM $CaCl_2$, ampicillin 100 $\mu g\ mL^{-1}$). The M9-Salts solution ($Na_2HPO_4 \cdot 2H_2O$ 42.5 $g\ L^{-1}$; KH_2PO_4 15 $g\ L^{-1}$; NaCl 2.5 $g\ L^{-1}$; NH_4Cl 5.0 $g\ L^{-1}$) was prepared and autoclaved separately. Inoculated production medium was incubated at 310 K and 200 rev min^{-1} until it reached an optical density (OD_{600})of 0.5. Heterologous expression was induced by adding isopropyl β-d-1-thiogalactopyranoside (IPTG) at a final concentration of 0.1 m*M*, incubated at 288 K and 200 rev min^{-1} for 14 h [29].

The cell culture was centrifuged in 250 mL flask at 6000 rev min^{-1} for 15 min. The cell pellet was resuspended in 50 mL of lysis buffer *A*(50 mM Tris-HCl pH 7.5, 400 mM NaCl) and mixed with 1 mg mL^{-1} of lysozyme (Sigma-Aldrich, St Louis MO, USA). After incubation by 30 min in slow agitation, PMSF was added until a final concentration of 1 mM. Cells were lysed by sonication*using Sonics Vibra Cell* (Cole-Parmer Ultrasonic processor) at amplitude of 40% for 5 min. The lysate was centrifuged at 15,000 rev min^{-1} for 30 min at 277 K. The supernatant was filtered through a 0.45 μm pore size filter, the remaining supernatant was loaded onto a Sepharose High Performance column™

(GE Healthcare, Little Chalfont, United Kingdom) charged with Ni^{2+}, previously and equilibrated with buffer *A*. TgPCNA was eluted with a 0–100%imidazole gradient in buffer *B* (50 mM Tris-HCl pH 7.5, 400 mM NaCl and 500 mM imidazole) using an ÄKTA Prime FPLC system (GE Healthcare, Little Chalfont, United Kingdom). Fractions corresponding to the largest peak were pooled and dialyzed against buffer *C* (50 mM Tris-HCl pH 7.5, 400 mM NaCl, 2 mM EDTA and 5 mM DTT), using a dialysis tubing cellulose membrane with molecular weight cut-off of 12,400 Da (Sigma-Aldrich, St Louis MO, USA) at 277 K for 12 h. Dialyzed TgPCNA was concentrated to 2 mL using an Amicon ultracentrifuge device with a 10,000 Da molecular weight cut-off (Millipore Corporation, Billerica, MA, USA). A second purification step was performed using a HiLoad 26/600 Superdex 200 pg gel filtration column (GE Healthcare, Little Chalfont, United Kingdom) previously equilibrated with buffer *C* and calibrated with molecular mass standards. The molecular weight markers were Aldolase (158,000 Da), Conalbumin(75,000 Da), Carbonic Anhydrase (29,000 Da) and Ribonuclease A (13,700 Da). Fractions with the highest protein concentration and purity were concentrated and analyzed by SDS-PAGE at 12%.

2.3. Protein crystallization

Protein concentration was measured by Bradford (Bio-Rad, Hercules, CA, USA) assay using bovine serum albumin dilutions as standards [30], densitometric analysis of proteins on SDS-PAGE and by absorbance at 280 nm. Initial protein crystallization screening was performed using a Mosquito LCP robot (TTP LabTech, Cambridge, United Kingdom) by sitting-drop vapor-diffusion method. The experiments were carried out using 96-well sitting drop iQ plate (TTP LabTech, Cambridge, United Kingdom) against Index, Crystal Screen, Crystal Screen II kits (Hampton Research, Aliso Viejo, CA, USA), Wizard Classic crystallization screen series I–IV (Rigaku Reagents, Bainbridge Island, WA, USA), and the conditions previously described to crystallize PCNA from *Pyrococcusfuriosus* [27]. The crystallization conditions were optimized using microbatch and sitting-drop vapor-diffusion methods. In brief 1 μL of TgPCNA at 15 mg mL^{-1} in buffer *C* (see above) was mixed with 1 μL of reservoir solution. All crystallization experiments were performed at 291 K.

2.4. X-ray data collection and processing

Crystals were soaked in a cryoprotectant solution consisting of its mother liquor where water where exchanged by 20% of Glycerol, in order to maintained the remaining component contend, and mounted in a rayon cryo-loop and immediately flash-cooled in liquid nitrogen. Diffraction data were collected at the beamline 14–1 from the Stanford Synchrotron Radiation Light source, SSRL, Menlo Park, USA. Diffraction data were processed with XDS and scaled using XSCALE [37]. According to the unit cell parameters indexed in XDS, space group and the molecular mass of each TgPCNA monomer, the packing of the crystal was analyzed using the Matthews Probability Calculator Server (http://www.ruppweb.org/mattprob/default.html) and proposing 2 TgPCNA molecules in the asymmetric unit (AU). The structure was successfully determined by molecular replacement, in space group P 3 and with 2 copies in the AU. MR was performed with Phaser [38] and using the coordinates of PCNA1 from Thermococcus kodakaraensis tk0535 as a search model (PDB entry 3lx1 [20], with a sequence identity of 92% with TgPCNA). The TGPCNA coordinates were refined up to 2.8 Å resolution using a combination of REFMAC5 from the CCP4 suite [39], Phenix [40] and manual modeling using the molecular-graphics program Coot [41]. In all cases, due the low data parameter / diffraction data ratio, restrained Non-Crystallographic Symmetries (NCS) were applied during the refinement process. Figures were generated using CCP4mg [42]. The atomic coordinates and structure factors have been deposited in the PDB as entry 5a6d, and a summary of data collection and refinement statistics are given in Table 1.

Table 1
Data-collection statistics.

Beamline	SSRL 14–1
Wavelength (Å)	1.18
Resolution (Å)	49.86–2.8 (2.9–2.8)
Space Group	*P*3
Temperature (K)	100
Detector	MARCCD
Unit-cell parameters (Å, °)	a=92.669, b=92.669, c=63.632, α=β=90, γ=120
Solvent content (%)	56.13
Unique Reflections	15,022 (1521)
$I/\sigma(I)$	15.75 (2.64)
Average redundancy	3.2
Data completeness (%)	99.69 (99.87)
R_{merge} (%)	0.06311 (0.5523)
Refinement	–
R factor (%)	0.1883
R_{free} (%)	0.2379
R.m.s.d. bond distance (Å)	0.011
R.m.s.d. bond angle (°)	1.51
Average *B* factor (Å)	70.7
Macromolecules	70.7
Ramachandran plot (%)	–
Core	93
Disallowed	0.82
No. of protein atoms	3898
No. of solvent atoms	0
PDB code	5a6d

Crystallographic data and data-collection statistics of *T. gammatolerans* PCNA. Values in parentheses are for the highest-resolution shell.

3. Results and discussion

TgPCNA contains 249 amino acids residues and is annotated in the NCBI GenBank with the identification number ACS33548.1. An extensive search was conducted in the genome database from *T. gammatolerans* to identify duplicated PCNA genes as in the case of *Thermococcus kodakaraensis* [20]. Our search shows only one gene encoding a protein homologous to PCNA. To date, only six PCNAs of *archaea* have been deposited in the PDB. An alignment of primary sequences using Expresso T-coffee server [31] (Fig. 1) revealing that TgPCNA has a high percent of identity with other *archaeal* PCNA. As displayed by colors on the alignment, indicating the relative reliability of the various sections (color code: blue and green, unreliable; yellow, low reliability; pink, highly reliable portion of the alignment) [32]. Amino acid sequence of TgPCNA depicts several residues and motifs well conserved. In particular, the Asp^{41} that appears to stimulate *archaeal* family B polymerases and FEN-1 in homologous PCNA [19]. As well as the sequence $Leu^{243}Ala^{244}Pro^{245}Arg^{246}$ near to N-terminal, responsible for proper folding of PCNA [33–35]. Interestingly the less conserved region is near the IDCL (yellow boxes) and Backside loop (gaps) which might suggest that this is the most divergent region where the species-specific interactions can occur and probably it is also, the region with major structural changes.

Several *E. coli* strains were tested to over-express TgPCNA, and the best results were obtained with strain BL21 Star (DE3). The expression of recombinant TgPCNA did not show any significant adverse effects in the *E. coli* expression host. Approximately 50% of the heterologous induced TgPCNA was soluble in a lysis buffer. Histidine-tagged TgPCNA remain bound to the nickel column even after extensive washes. After first purification step, TgPCNA was

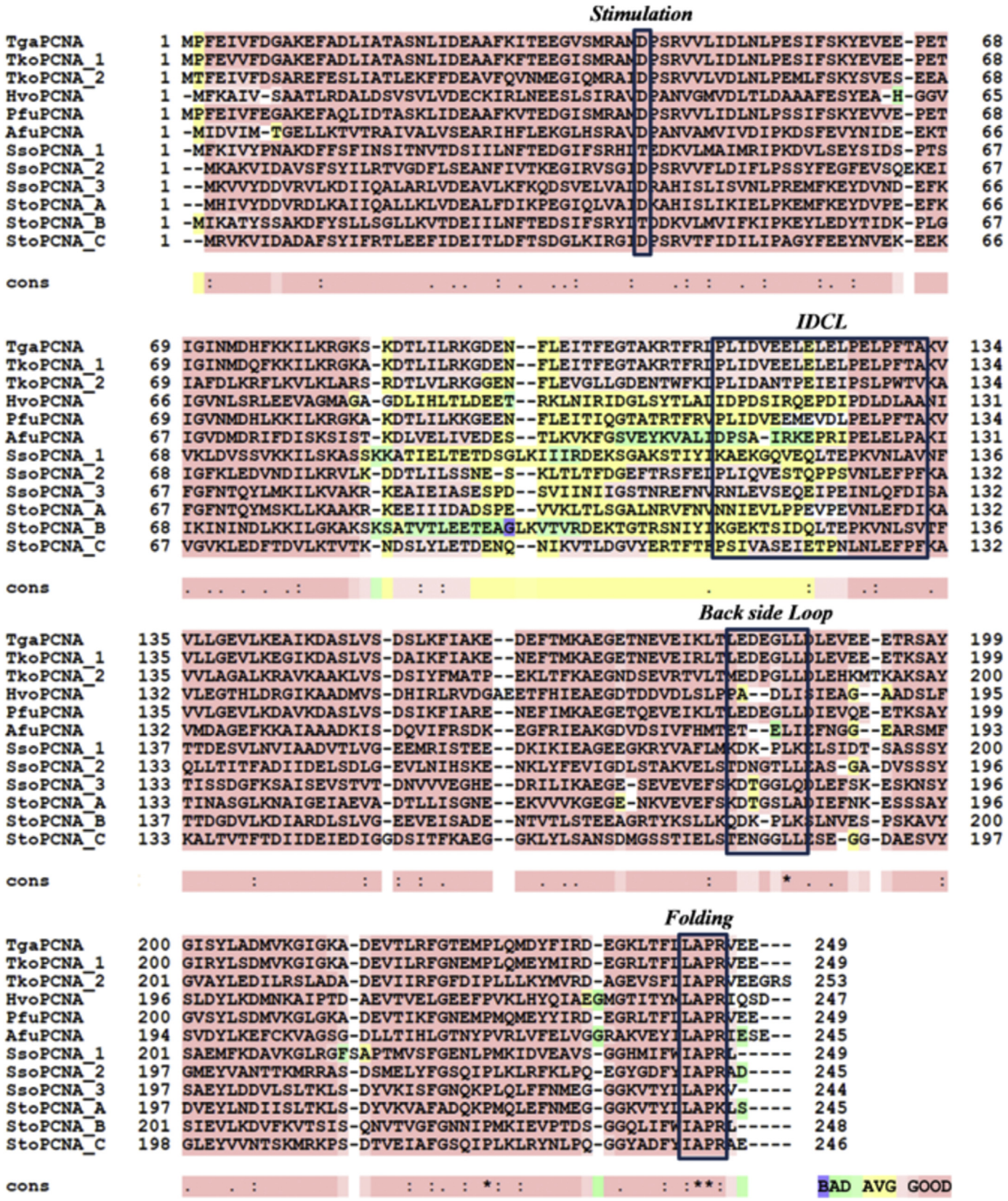

Fig. 1. Amino-acid sequence alignment of TgPCNA with other *archaeal* PCNA. Residues and structural elements present in PCNA are in blue boxes. (Tga: *Thermococcusgammatolerans*, GenBank accession number ACS33548.1; Tko: *Thermococcuskodakarensi*, BAD84724.1 and BAD84771.1; Hvo: *Haloferaxvolcanii*, ADE03802.1; Pfu: *Pyrococcusfuriosus*, BAA33020.2; Afu: *Archaeoglobusfulgidus*, AAB90899.1; Sso: *Sulfolobussolfataricus*, AAK40726.1, AAK41309.1 and AAK40734.1; Sto: *Sulfolubustokodaii*, BAK54261.1, BAK54265.1 and BAK54417.1).

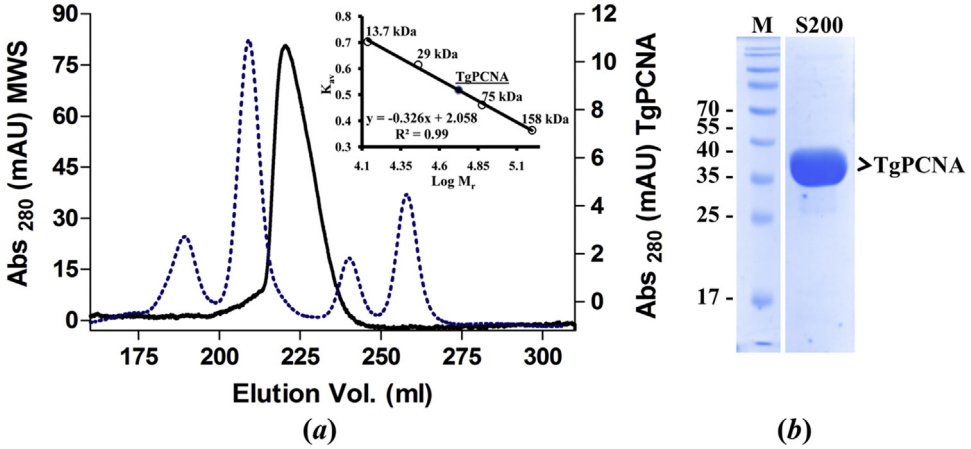

Fig. 2. TgPCNA is a monomer according to gel filtration.(*a*) Purified TgPCNA applied to a HiLoad 26/600 Superdex 200 pg column depicting it oligomeric state by gel filtration. (*b*) 12% SDS–PAGE of TgPCNA after size-exclusion chromatography.

approximately 85% pure (Data no shown). However a second purification step applying the sample in a HiLoad 26/600 Superdex 200 pg gel filtration column was included (Fig. 2). This second purification step was used to increase TgPCNA purity before crystallization and determining the molecular weight. As a result we identified an anomalous migration in the recombinant expressed TgPCNA, which was confirmed by elution profile (Fig. 2a) and SDS-PAGE (Fig. 2b). By gel filtration, the molecular mass was approximately 52,000 Da, which is slightly larger than the theoretical mass but closer to the monomeric TgPCNA. Similar behaviors have been observed previously and it is speculated that may be due to the acidic nature of TgPCNA with a theoretical pI of 4.5 [36].

Protein droplets consisting of 1 μL PCNA (14 mg mL^{-1} in 50 mM Tris-HCl pH 7.5, 400 mM NaCl, 2 mM EDTA and 5 mM DTT) were mixed with 1 μL precipitant solution. First, crystals were obtained by using the sitting-drop vapor-diffusion method. TgPCNA crystallized under different conditions, but best crystal growth was observed under condition No. 42 of Wizard Classic crystallization screen III (30% v/v PEG 4000, 200 mM Li_2SO_4 and 100 mM Tri-HCl pH 8.5) and under conditions previously described by Matsumiya [27]. Optimization matrices were designed and performed for both conditions. Optimization matrix for condition No. 42 evaluated how changes in PEG (10–35% w/v) and protein (10–16 mg mL^{-1}) concentration affect crystal growing, while matrix for Matsumiya condition evaluated changes in protein (10–16 mg mL^{-1}), ammonium sulfate (2.4–2.8 M) and MPD (5–10%) concentrations, as well as buffer pH (5.2–5.8). After 3 months crystals were collected, cryocooled and diffracted at the SSRL. The best data set was obtained from Matsumiya condition matrix, where prism-like crystal was obtained. The approximate dimensions of the crystal were 0.50 × 0.25 × 0.25 mm (Fig. 3). In brief, 1 μL of TgPCNA at 10 mg $mL^{-1}$was mixed with 1 μL precipitant solution consisting in 2.8 M ammonium sulfate, 7.5% MPD and 100 mM citrate buffer pH 5.2.

The TGPCNA coordinates were refined up to 2.8 Å resolution using a combination of REFMAC5 from the CCP4 suite [39], Phenix [40] and manual modeling using the molecular-graphics program Coot [41] (Fig. 4a). In all cases, due the low data parameter/diffraction data ratio, restrained Non-Crystallographic Symmetries (NCS) were applied during the refinement process. Figures were generated using CCP4mg [42]. The atomic coordinates and structure factors have been deposited in the PDB as entry 5a6d, and a summary of data collection and refinement statistics are given in Table 1. The structure was then superposed with other PCNA, deposited at the PDB, to look for structural differences within them. The structures selected for this comparation belong to the organisms: *T. kodakarensis* (5dai); *Pyrococcus furiosus* (1iz5); *Haloferax volcanii* (3ifv); *Drosophila melanogaster* (4hk1); *Arabidopsis thaliana* (2zvv); *Homo sapiens* (5e0u); *Archaeoglobus fulgidus* (1rxz); *Sulfolobus sulfataricus* (2ijx). The r.m.s.d. was: 5dai, 0.85; 3lx1, 0.88; 1iz5, 0.88; iz4, 1.25; 3ifv, 1.35; 4hk1, 1.38; 2zvv, 1.43; 5e0u, 1.47; 1rxz, 1.51; 1rwz, 1.51; 2ijx, 1.71(Fig. 4b).

The superposition of coordinates from phylogenetically close and distant organisms yield to low rmsd values, between 0.88 Å for *T. kodakarensis* and 1.71 Å for *S. sulfataricus*. PCNAs respectively.

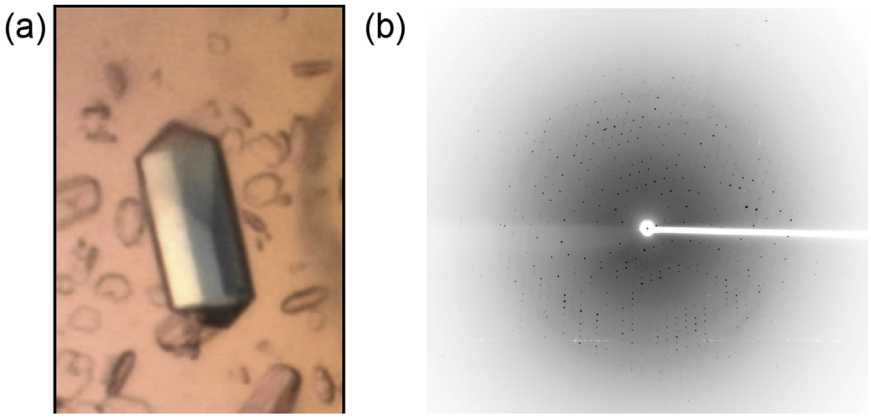

Fig. 3. TgPCNA crystal obtained in the precipitant solution of 2.8 M ammonium sulfate, 7.5% MPD and 100 mM citrate buffer pH 5.2 with approximate dimensions of 0.50 × 0.25 × 0.25 mm (a). X-ray diffraction pattern recorded at SSRL from TgPCNA crystal with visible diffraction to 2.8 Å resolution (b).

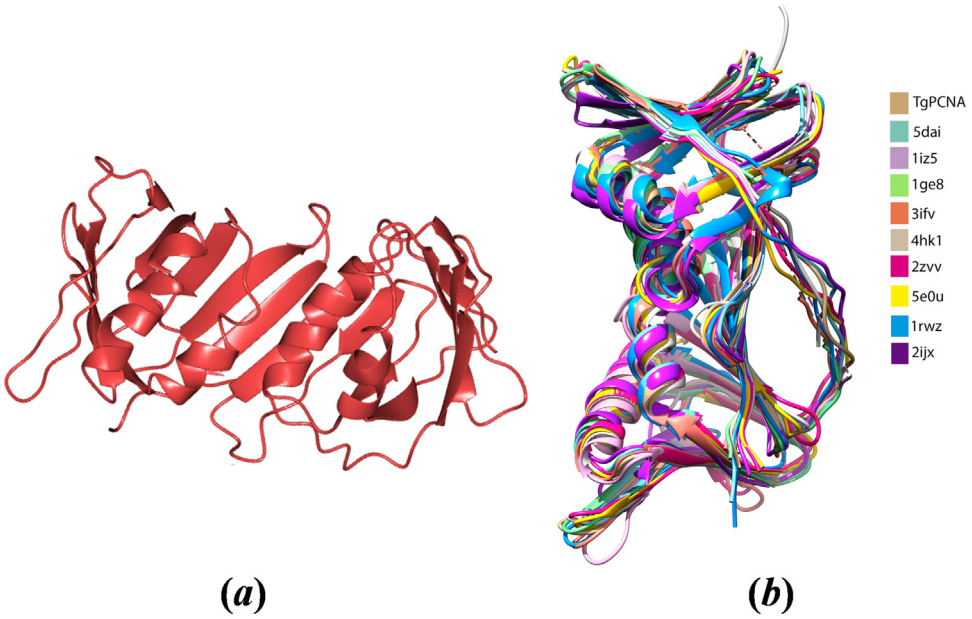

Fig. 4. TgPCNA structure determinated from the crystal. The structure was determinated at 2.8 Å resolution (a). Multiple overlapping PCNA to look for structural differences within them. The structures selected for this comparation belong to the organisms: *T. kodakarensis* (5dai); *Pyrococcus furiosus* (1iz5); *Haloferax volcanii* (3ifv); *Drosophila melanogaster* (4hk1); *Arabidopsis thaliana* (2zvv); *Homo sapiens* (5e0u); *Archaeoglobus fulgidus* (1rxz); *Sulfolobus sulfataricus* (2ijx). The r.m.s.d. was: 5dai, 0.85; 3lx1, 0.88; 1iz5, 0.88; iz4, 1.25; 3ifv, 1.35; 4hk1, 1.38; 2zvv, 1.43; 5e0u, 1.47; 1rxz, 1.51; 1rwz, 1.51; 2ijx, 1.71 (b).

Unfortunately, there were no clear differences in the coordinates which allow us to speculate if TgPCNA is intrinsically resistant to the radiation and thus be a key factor for cell survival after irradiation.

Acknowledgments

CSCF is thankful to the Postdoctoral fellowship from Dirección General de Asuntos del Personal Académico (DGAPA) of the Universidad Nacional Autónoma de México (UNAM). AAVL is supported by a master fellowship from Consejo Nacional de Ciencia y Tecnología Grant 384419 (CONACYT-México). This article was partially supported by the Grant 247842 PDCPN201401 (CONACYT-México). We are grateful to Dr. Patrick Forterre and Dr. Aurore Gorlas from Institut de Génétique et Microbiologie, Université Paris-Sud for kindly providing genomic DNA. We also thank Dr. Adelaida Díaz Vilchis, Jazmín Sánchez Pérez and Sonia P. Rojas-Trejo for their valuable assistance in protein crystallization setup.

Appendix A. Transparency document

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.08.004.

References

[1] S.N. Naryzhny, H. Zhao, H. Lee, Proliferating cell nuclear antigen (PCNA) may function as a double homotrimer complex in the mammalian cell, J. Biol. Chem. 14 (2005) 13888–13894.
[2] T.D. Craggs, R.D. Hutton, A. Brenlla, et al., Single-molecule characterization of Fen1 and Fen1/PCNA complexes acting on flap substrates, Nucleic Acids Res. 42 (2014) 1857–1872.
[3] G. Ghosal, J. Chen, DNA damage tolerance: a double-edged sword guarding the genome, Transl. Cancer Res. 2 (2013) 107–129.
[4] K. Miyachi, M.J. Fritzler, E.M. Tan, Autoantibody to a nuclear antigen in proliferating cells, J. Immunol. 121 (1978) 2228–2234.
[5] P.T. Stukenberg, P.S. Studwell-Vaughan, M. O'Donnell, Mechanism of the sliding beta-clamp of DNA polymerase III holoenzyme, J. Biol. Chem. 15 (1991) 11328–11334.
[6] T.S. Krishna, X.P. Kong, S. Gary, et al., Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA, Cell 79 (1994) 1233–1243.
[7] N. Yao, J. Turner, Z. Kelman, et al., Clamp loading, unloading and intrinsic stability of the PCNA, beta and gp45 sliding clamps of human, E. coli and T4 replicases, Genes Cells 1 (1996) 101–113.
[8] M. Hedglin, R. Kumar, S.J. Benkovic, Replication clamps and clamp loaders, Cold Spring Harb. Perspect. Biol. 5 (2013) a010165.
[9] C.S. Cardona-Felix, S. Lara-Gonzalez, L.G. Brieba, Structure and biochemical characterization of proliferating cellular nuclear antigen from a parasitic protozoon, Acta Cryst. D: Biol. Cryst. 67 (2011) 497–505.
[10] J. Fang, J.R. Engen, P.J. Beuning, Escherichia coli processivity clamp β from DNA polymerase III is dynamic in solution, Biochemistry 50 (2011) 5958–5968.
[11] M. Pan, L.M. Kelman, Z. Kelman, The archaeal PCNA proteins, Biochem. Soc. Trans. 39 (2011) 20–24.
[12] Z.O. Jónsson, R. Hindges, U. Hübscher, Regulation of DNA replication and repair proteins through interaction with the front side of proliferating cell nuclear antigen, EMBO J. 17 (1998) 2412–2425.
[13] G. Xing, V. Hlinkova, H. Ling, Purification, crystallization and preliminary diffraction studies of the Sulfolobus solfataricus PCNA proteins in different oligomeric forms, Cryst. Growth Des. 7 (2007) 2202–2205.
[14] J.A. Winter, P. Christofi, S. Morroll, et al., The crystal structure of Haloferax volcanii proliferating cell nuclear antigen reveals unique surface charge characteristics due to halophilic adaptation, BMC Struct. Biol. 9 (2009) 55.
[15] L. Cubonová, T. Richardson, B.W. Burkhart, et al., Archaeal DNA polymerase D but not DNA polymerase B is required for genome replication in Thermococcus kodakarensis, J. Bacteriol. 195 (2013) 2322–2328.
[16] K. Mayanagi, S. Kiyonari, H. Nishida, et al., Architecture of the DNA polymerase B-proliferating cell nuclear antigen (PCNA)-DNA ternary complex, Proc. Natl. Acad. Sci. USA 108 (2011) 1845–1849.
[17] A. Bacquin, C. Pouvelle, N. Siaud, et al., The helicase FBH1 is tightly regulated by PCNA via CRL4(Cdt2)-mediated proteolysis in human cells, Nucleic Acids Res. 41 (2013) 6501–6513.
[18] K. Tori, M. Kimizu, S. Ishino, et al., DNA polymerases BI and D from the hyperthermophilic archaeon Pyrococcus furiosus both bind to proliferating cell nuclear antigen with their C-terminal PIP-box motifs, J. Bacteriol. 189 (2007) 5652–5657.
[19] J.A. Winter, K.A. Bunting, Rings in the extreme: PCNA interactions and adaptations in the archaea, Archaea 2012 (2012) 951010.
[20] J.E. Ladner, M. Pan, J. Hurwitz, et al., Crystal structures of two active proliferating cell nuclear antigens (PCNAs) encoded by Thermococcus kodakaraensis, Proc. Natl. Acad. Sci. USA 108 (2011) 2711–2716.
[21] Z. Li, T.J. Santangelo, L. Cuboňová, et al., Affinity purification of an archaeal DNA replication protein network, MBio 1 (2010) e00221–10.
[22] E. Jolivet, S. L'Haridon, E. Corre, et al., Thermococcus gammatolerans sp. nov., a hyperthermophilic archaeon from a deep-sea hydrothermal vent that resists ionizing radiation, Int. J. Syst. Evol. Microbiol. 53 (2003) 847–851.
[23] Y. Zivanovic, J. Armengaud, A. Lagorce, et al., Genome analysis and genome-wide proteomics of Thermococcus gammatolerans, the most radioresistant organism known amongst the Archaea, Genome Biol. 10 (2009) R70.

[24] M.M. Cox, J.R. Battista, Deinococcus radiodurans – the consummate survivor, Nat. Rev. Microbiol. 3 (2005) 882–892.
[25] D.D. Horikawa, T. Sakashita, C. Katagiri, et al., Radiation tolerance in the tardigrade Milnesium tardigradum, Int J. Radiat. Biol. 82 (2006) 843–848.
[26] A. Tapias, C. Leplat, F. Confalonieri, Recovery of ionizing-radiation damage after high doses of gamma ray in the hyperthermophilic archaeon Thermococcus gammatolerans, Extremophiles 13 (2009) 333–343.
[27] S. Matsumiya, Y. Ishino, K. Morikawa, Crystal structure of an archaeal DNA sliding clamp: proliferating cell nuclear antigen from Pyrococcus furiosus, Protein Sci. 10 (2001) 17–23.
[28] J. Sambrook, D.W. Russel, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, 2001.
[29] O. Spadiut, G. Posch, R. Ludwig, et al., Evaluation of different expression systems for the heterologous expression of pyranose 2-oxidase from Trametes multicolor in E. coli, Micro. Cell Fact. 9 (2010) 9–14.
[30] M.M. Bradford, Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding, Anal. Biochem. 72 (1976) 248e254.
[31] F. Armougom, S. Moretti, O. Poirot, et al., Expresso: automatic incorporation of structural information in multiple sequence alignments using 3D-Coffee, Nucleic Acids Res. 1 (2006) W604–W608.
[32] O. Poirot, K. Suhre, C. Abergel, et al., 3DCoffee@igs: a web server for combining sequences and structures into a multiple sequence alignment, Nucleic Acids Res. 32 (2004) W37–W40.
[33] S.N. Naryzhny, Proliferating cell nuclear antigen: a proteomics view, Cell Mol. Life Sci. 65 (2008) 3789–37808.
[34] W. Strzalka, A. Ziemienowicz, Proliferating cell nuclear antigen (PCNA): a key factor in DNA replication and cell cycle regulation, Ann. Bot. 107 (2011) 1127–1140.
[35] M. Oke, S. Manal, Z.S. Handam, PCNA Structure and Interactions Partner Proteins, Molecular Life Sciences, Springer Science Business Media, New York, 2014.
[36] A. Matagne, B. Joris, J.M. Frère, Anomalous behaviour of a protein during SDS/PAGE corrected by chemical modification of carboxylic groups, Biochem. J. 280 (1991) 553–556.
[37] W. Kabsch, XDS, Acta Crystallogr. Sect. D: Biol. Crystallogr. 66 (2010) 125–132.
[38] A.J. McCoy, R.W. Grosse-Kunstleve, P.D. Adams, et al., Phaser crystallographic software, J. Appl. Cryst. 40 (2007) 658–674.
[39] G.N. Murshudov, A.A. Vagin, E.J. Dodson, Refinement of macromolecular structures by the maximum-likelihood method, Acta Crystallogr. Sect. D: Biol. Crystallogr. 53 (1997) 240–255.
[40] P.D. Adams, P.V. Afonine, G. Bunkóczi, et al., PHENIX: a comprehensive Python-based system for macromolecular structure solution, Acta Crystallogr. Sect. D: Biol. Crystallogr. 66 (2010) 213–221.
[41] P. Emsley, K. Cowtan, Coot: model-building tools for molecular graphics, Acta Crystallogr. Sect. D: Biol. Crystallogr. 60 (2004) 2126–2132.
[42] S. McNicholas, E. Potterton, K.S. Wilson, M.E.M. Noble, Presenting your structures: the CCP4mg molecular-graphics software, Acta Crystallogr. Sect. D: Biol. Crystallogr. 67 (2011) 386–394.