Extremophiles (2000) 4:357–364

ORIGINAL PAPER

Toshie Iwai · Norio Kurosawa · Yuko H. Itoh
Tadao Horiuchi

Phylogenetic analysis of archaeal PCNA homologues

Received: July 10, 2000 / Accepted: September 26, 2000

Abstract Proliferating cell nuclear antigen (PCNA) is an essential component of the DNA replication and repair machinery in the domain Eucarya. Eukaryotes and euryarchaeotes, which belong to one subdomain of Archaea, possess a single PCNA homologue, whereas two distinct PCNA homologues have been identified from *Sulfolobus solfataricus,* which belongs to the other archaeal subdomain, Crenarchaeota. We have cloned and sequenced two genes of PCNA homologues from the thermoacidophilic crenarchaeon *Sulfurisphaera ohwakuensis*. These genes, referred to as the *Soh* PCNA A gene and the *Soh* PCNA B gene, were found to encode 245 amino acids (aa) (27 kDa) and 248 aa (27 kDa), respectively. In deduced amino acid sequences of both PCNA homologues, the motif L/I-A-P-K/R, implicated in binding of PCNA with replication factor C (RFC), was identified. Phylogenetic analysis of all available archaeal PCNA homologues suggests that crenarchaeal homologues are divided into two groups. Group A consists of *Soh* PCNA A, one of the *S. solfataricus* PCNA homologues, and one of the *Aeropyrum pernix* PCNA homologues. The other crenarchaeal homologues form group B. Crenarchaeal PCNA homologues constitute a monophyletic subfamily. These results suggest that the evolution of crenarchaeal PCNA homologues has been characterized by one or two gene duplication events, which are assumed to have occurred after the split of the crenarchaeal and euryarchaeal lineages.

Key words Phylogeny · PCNA · DNA replication · Archaea · *Sulfurisphaera ohwakuensis*

Communicated by K. Horikoshi

T. Iwai · N. Kurosawa (✉) · Y.H. Itoh · T. Horiuchi
Department of Bioengineering, Faculty of Engineering, Soka University, 1-236 Tangi-cho, Hachioji, Tokyo 192-8577, Japan
Tel. +81-426-91-8175; Fax +81-426-91-9312
e-mail: kurosawa@t.soka.ac.jp

Introduction

In Eucarya, proliferating cell nuclear antigen (PCNA), so named because of its initial discovery as a cell cycle-dependent antigen (Miyachi et al. 1978), is an essential component in the chromosomal replication and the DNA repair system (reviewed by Jónsson and Hübscher 1997; Kelman 1997; Kelman and Hurwitz 1998; Tsurimoto 1998). PCNA acts as the processivity factor or sliding clamp for DNA polymerase δ (Pol δ), an essential replicative enzyme in the eukaryotic cells (Tan et al. 1986; Bravo et al. 1987; Prelich et al. 1987a, b; Wold and Kelly 1988; Müller et al. 1994). In addition, PCNA is also a processivity factor for DNA polymerase ε (Pol ε), another indispensable eukaryotic DNA polymerase (Morrison et al. 1990; Burgers 1991; Lee et al. 1991; Podust and Hübscher 1993).

Archaea, the third domain of life (Woese et al. 1990), resemble the Bacteria in cellular ultrastructure; however, the archaeal DNA replication machinery is similar to that of Eucarya. In the total genome sequences from several archaeal strains, many putative homologues of the eukaryotic replication proteins have been identified (Edgell and Doolittle 1997). The domain of Archaea is divided into two subdomains, Euryarchaeota and Crenarchaeota (Woese et al. 1990). To date, the crenarchaeon *Sulfolobus solfataricus* shows two PCNA homologues (De Felice et al. 1999), whereas euryarchaeote genomes appear to encode only a single PCNA homologue. The identities of amino acid sequences of *S. solfataricus* PCNA homologues with that of euryarchaeal homologues are low (values ranging from 19% to 29%). Moreover, the sequence identity between two *S. solfataricus* PCNA homologues is only 19%. Both homologues were demonstrated to be able to stimulate the polymerization activity of the *S. solfataricus* DNA polymerase B1 (Pisani et al. 1992). It is unclear if *S. solfataricus* PCNA homologues arose by gene duplication after the split of the crenarchaeal and euryarchaeal lineages, or if the present distribution of homologues can be explained by an ancestral gene duplication that occurred before the split of crenarchaeotes and euryarchaeotes, fol-

lowed by the loss of one of the homologues in euryarchaeal lineages.

To study the phylogenetic relationships of archaeal PCNA homologues, we have cloned and sequenced two genes, each encoding distinct putative PCNA homologues, referred to as *Soh* PCNA A and *Soh* PCNA B, from another crenarchaeon, *Sulfurisphaera ohwakuensis*. *S. ohwakuensis* is a thermoacidophilic and facultatively anaerobic archaeon (Kurosawa et al. 1998). It belongs to the order *Sulfolobales* but is rather distantly related to *Sulfolobus solfataricus* phylogenetically. In this article, we describe our phylogenetic analysis, which suggests that the two crenarchaeal PCNA homologues resulted from an event of the early gene duplication that occurred after the split of the crenarchaeal and euryarchaeal lineages.

Materials and methods

Bacterial strains

Sulfurisphaera ohwakuensis strain TA-1^T (IFO15161^T) was grown and its genomic DNA is prepared as previously described (Kurosawa et al. 1998).

DNA probes for screening

Two *Sulfolobus solfataricus* PCNA genes were amplified by PCR using *S. solfataricus* genomic DNA as a template. PCR primers were synthesized according to the sequences previously described (De Felice et al. 1999) as follows: A5′ (5′-GGTTCCATGGCATATGAAAGTAGTTTTAC GATGATGTAAGGGTT) and A3′ (5′-CCTTGGATCC TCAAACTTTTGGAGCTAATAAATAAGTAACT); B5′ (5′-GGTTCCATGGCATATGTTTAAGATTGTTT ACCCTAATGCAAAA) and B3′ (5′-CCTTGGATCCTT ATAACCTTGGCGCTATCCAAAAGATCATGTGACC CCC). PCR products of about 750 bp were used for plaque hybridization as probes.

Plaque hybridization

Genomic DNA library of *Sulfurisphaera ohwakuensis* was constructed in λ ZAP II (Stratagene, La Jolla, CA, USA) according to the protocol of the supplier, and was screened by plaque hybridization with each of the DNA probes described previously. Labeling of probes, hybridization, and detection of signals were performed by using a ECL direct nucleic acid labeling and detection system (Amersham Pharmacia Biotech, Piscataway, NJ, USA) according to the protocol of the supplier.

DNA sequencing

Small restriction fragments of the PCNA genes were subcloned into plasmid Bluescript II SK or KS (Stratagene). DNA sequence analysis was performed on both strands and was carried out by using Thermosequenase^{TM} premixed cycle sequencing kit (Amersham).

Alignments and phylogenetic analysis

The sequences of archaeal and eukaryotic PCNA homologues used in this study are summarized in Table 1. Alignment was created by using the CLUSTAL W program (Higgins and Clustal 1998). Conserved regions that consisted of 122 amino acid positions were picked up and com-

Table 1. Archaeal and eukaryotic proliferating cell nuclear antigens (PCNAs) used in this study

PCNA	Accession no. and/or reference	
Sulfurisphaera ohwakuensis A	AB045089	This paper
Sulfolobus solfataricus A (244 aa)		De Felice et al. 1999
Aeropyrum pernix A (263 aa)		Ishino et al. (in preparation)
Sulfurisphaera ohwakuensis B	AB045090	This paper
Sulfolobus solfataricus B (249 aa)		De Felice et al. 1999
Aeropyrum pernix BI (251 aa)		Ishino et al. (in preparation)
Aeropyrum pernix BII (233 aa)	E72738	Kawarabayasi et al. 1999
Pyrococcus abyssi	G75048	Erauso et al. 1996
Pyrococcus horikoshii	O58398	Kawarabayasi et al. 1998
Pyrococcus furiosus	O73947	Cann et al. 1999
Thermococcus fumicolans	AJ130939	
Methanococcus jannaschii	Q57697	Bult et al. 1996
Methanobacterium thermoautotrophicum	O27367	Smith et al. 1997
Archaeoglobus fulgidus	G69291	Klenk et al. 1997
Homo sapiens	P12004	Almendral et al. 1987
Drosophila melanogaster	P17917	Yamaguchi et al. 1990
Caenorhabditis elegans	O02115	Wilson et al. 1994
Saccharomyces cerevisiae	P15873	Bauer and Burgers 1990

A and B (BI, BII) described with organism names are taxa that we have proposed in this paper
Numbers in parentheses show amino acid residues
aa, amino acids

bined into a final alignment. These regions were used for the phylogenetic analysis, which was performed by using the program package PHYLIP (Felsenstein 1993). Pairwise distances between all the sequences were estimated by Protdist, and the phylogenetic tree was constructed using Neighbor and Protpars. To test the robustness of the phylogenetic tree, the sequence data were sampled 100 times for bootstrap analysis.

Accession numbers for the sequences

The EMBL/DDBJ/GenBank accession numbers for the sequences reported in this paper are AB045089 (A) and AB045090 (B).

Results

Cloning and sequence analysis

We cloned the two genes for PCNA homologues from *Sulfurisphaera ohwakuensis* using *Sulfolobus solfataricus* PCNA genes as probes. *Sulfurisphaera ohwakuensis* PCNA A (*Soh* PCNA A), which showed 61% amino acid identity with one *Sulfolobus solfataricus* PCNA homologue (244 aa), was encoded by 738 bp (245 aa), and its putative molecular mass was 27 kDa. *Sulfurisphaera ohwakuensis* PCNA B (*Soh* PCNA B), which showed 52% amino acid identity with the other *Sulfolobus solfataricus* PCNA homologue (249 aa), was encoded by 747 bp (248 aa) and its putative molecular mass was 27 kDa. No intervening sequence was found in either gene.

Alignment of archaeal and eukaryotic PCNA homologues

The amino acid sequences of all available archaeal and selected eukaryotic PCNA homologues were used in this study (Table 1). The alignment of the amino acid sequences is shown in Fig. 1. The identities among these sequences are summarized in Table 2. Identity between *Soh* PCNA A and *Soh* PCNA B was very low (19%); similarly, identity between *Sso* PCNA homologues and that among *Ape* PCNA homologues was only 20% and ranging from 23% to 29%, respectively. The primary structure identity between *Soh* PCNA A and one *Sso* PCNA homologue (244 aa) and that between *Soh* PCNA B and the other *Sso* PCNA homologue (249 aa) were medium values (61% and 52%, respectively), although identities between each of three *Ape* PCNA homologues and each of the crenarchaeal PCNA homologues were low (ranging from 17% to 29%). The percentages of identities among euryarchaeal homologues ranged from 25% to 93%. The sequence identities between archaeal and eukaryotic PCNA homologues appeared to be lower (from 14% to 29%). However, the highly conserved L/I-A-P-K/R motifs, which were demonstrated to be critical amino acid residues for the functional interaction of human PCNA with the replication factor C (RFC), was generally found in the C-terminal regions of the primary structures of PCNA homologues. In the putative amino acid sequences of *Soh* PCNA A and PCNA B, L/I-A-P-K/R motifs were identified at the predicted positions (Fig. 1). The loop between βD-2 and βE-2 of eukaryotic PCNA was demonstrated to be important for the interaction with DNA polymerase ε (Krishna et al. 1994; Gulbis et al. 1996). In this loop of archaeal PCNA homologues, a conspicuous gap is present.

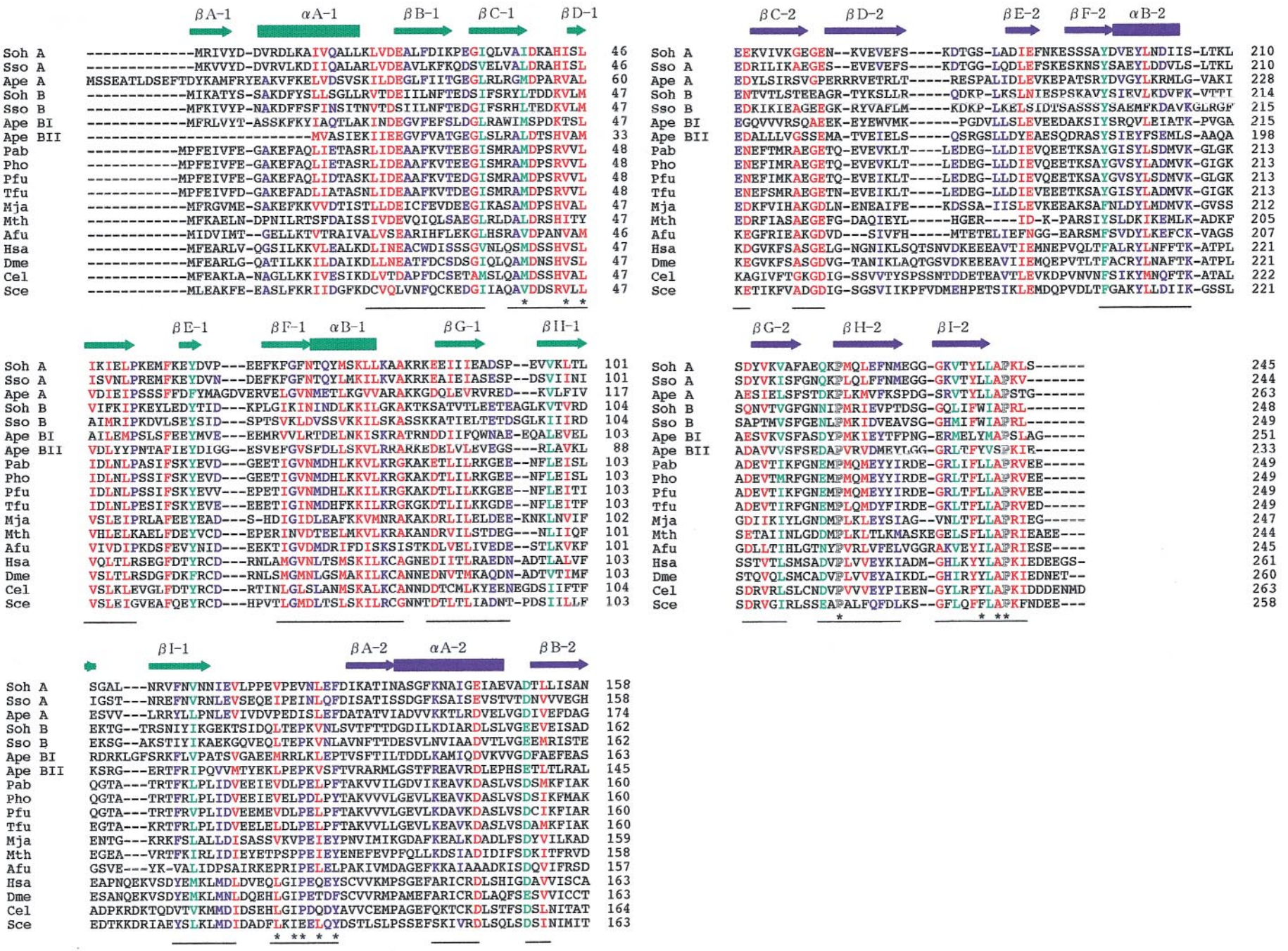
Phylogenetic analysis

To analyze the phylogenetic relationships among the archaeal PCNA homologues, a phylogenetic tree was constructed using a data set that contained all available archaeal PCNA sequences, and eukaryotic PCNA homologues as outgroup sequences (Fig. 2). Based on the alignment of amino acid sequences, conserved regions (122 positions) were picked up and used for this analysis.

The crenarchaeal PCNA homologues are split into two groups. We propose calling one group A and the other group B. Group A is composed of one *Sulfurisphaera ohwakuensis* PCNA homologue (accession number, AB045089), *Sulfolobus solfataricus* (*Sso* PCNA A, 244 aa), and one *Aeropyrum pernix* homologue (*Ape* PCNA A, 263 aa). Group B consists of another *Sulfurisphaera ohwakuensis* (accession number, AB045090), *Sulfolobus solfataricus* (*Sso* PCNA B, 249 aa), and two *Aeropyrum pernix* (*Ape* PCNA BI, 251 aa, and *Ape* PCNA BII, 233 aa). *Soh* PCNA A and *Sso* PCNA A, and *Soh* PCNA B, *Sso* PCNA B, and *Ape* PCNA BI, were clustered with high bootstrap values (100% and 93%, respectively). Nevertheless, *Ape* PCNA A and *Ape* PCNA BII were grouped with group A and group B with only 29% and 48% of bootstrap values, respectively. Crenarchaeal PCNA homologues form a monophyletic group, although the bootstrap value supporting this topology was very low (22%).

Discussion

In the total genome sequences of several archaeal strains, many homologous proteins necessary for eukaryotic DNA replication have been identified (Edgell and Doolittle 1997). These data suggest that the archaeal DNA replication system is similar not to that of Bacteria but to the eukaryotic system. However, there are some differences between crenarchaeal and euryarchaeal DNA replication mechanisms because there are disparities in the DNA polymerase sets of each subdomain. Crenarchaeota has at least two family B DNA polymerases, whereas euryarchaeotes have a single family B DNA polymerase and a family D DNA polymerase (Cann et al. 1998; Ishino et al. 1998; Cann and Ishino 1999). Family D DNA polymerase is a heterodimeric enzyme composed of a small subunit that shows some similarity to the small subunit of eukaryotic


βA-1 αA-1 βB-1 βC-1 βD-1
βC-2 βD-2 βE-2 βF-2 αB-2
βE-1 βF-1 αB-1 βG-1 βII-1
βG-2 βH-2 βI-2
βI-1 βA-2 αA-2 βB-2
Soh A
Sso A
Ape A
Soh B
Sso B
Ape BI
Ape BII
Pab
Pho
Pfu
Tfu
Mja
Mth
Afu
Hsa
Dme
Cel
Sce

DNA polymerase δ, and a large subunit which is not similar to any known DNA polymerases. Recently, two PCNA homologues were identified in the genome sequences of the crenarchaeon *Sulfolobus solfataricus*, whereas euryarchaeote genomes appear to encode only a single PCNA homologue. Both *S. solfataricus* PCNA homologues were demonstrated to be able to stimulate the polymerization activity of its DNA polymerase B1 (De Felice et al. 1999).

There are three possibilities for the evolution of two *Sulfolobus solfataricus* PCNA homologues: (i) *S. solfataricus* PCNA homologues arose independently of other archaeal homologues by gene duplication in the evolutionary process, (ii) two homologues arose independently of euryarchaeal homologues by gene duplication after the split of the crenarchaeal and euryarchaeal lineages, or (iii) the present distribution of homologues can be explained by an ancestral gene duplication that occurred before the split of crenarchaeotes and euryarchaeotes, followed by loss of one homologue in the euryarchaeal lineage. To clarify the evolutionary process of the archaeal PCNA homologues, we have attempted to clone and sequence two genes from another crenarchaeon, *Sulfurisphaera ohwakuensis*, and have analyzed phylogenetic relationships of the archaeal PCNA homologues. The two genes of PCNA homologues from *S. ohwakuensis* were referred to as *Soh* PCNA A and *Soh* PCNA B. Further, three genes of PCNA homologues have been found from the thermophilic crenarchaeon *Aeropyrum pernix* (Kawarabayasi et al. 1999; Cann and Ishino 1999). Our phylogenetic analysis, using all the

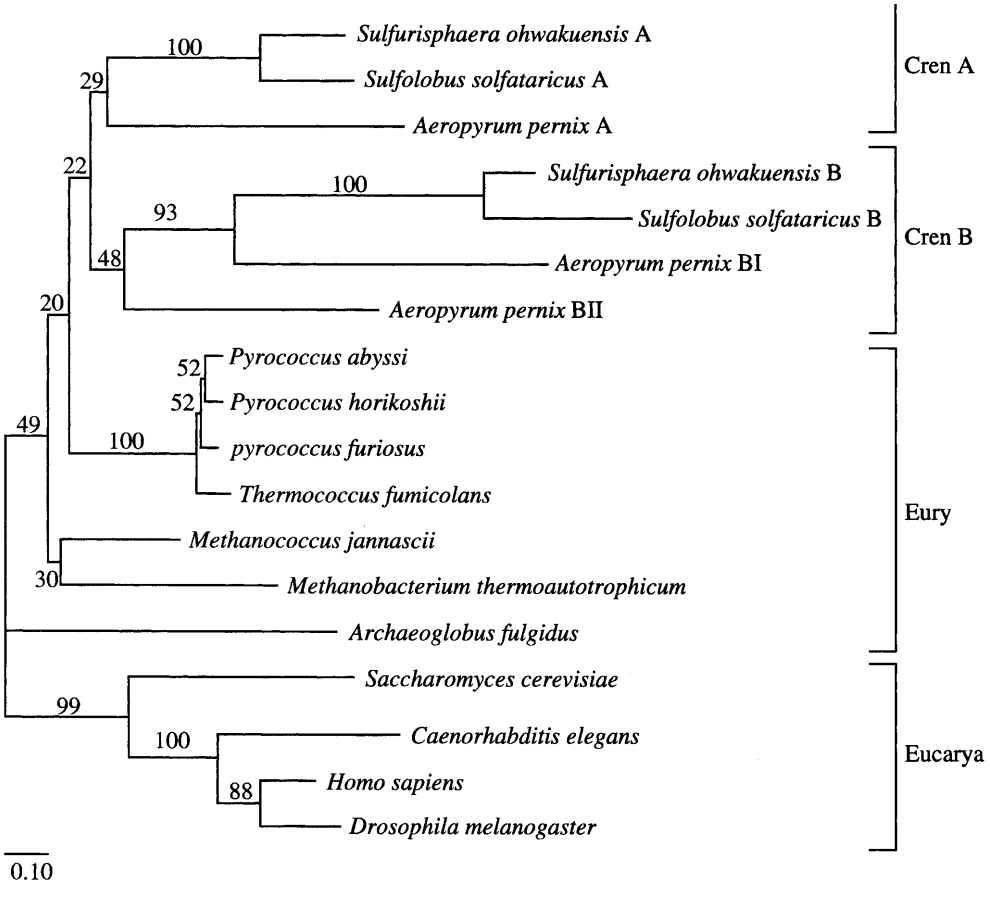


Fig. 2. Phylogenetic tree of archaeal and eukaryotic PCNAs constructed by the neighbor-joining method. Bootstrap probabilities (in percentages) are given *above* the internal branches

Fig. 1. Amino acid alignment of PCNA homologues from archaeal and eukaryotic species. *Soh*, *Sulfurisphaera ohwakuensis*; *Sso*, *Sulfolobus solfataricus*; *Ape*, *Aeropyrum pernix*; *Pab*, *Pyrococcus abyssi*; *Pho*, *Pyrococcus horikoshii*; *Pfu*, *Pyococcus furiosus*; *Tfu*, *Thermococcus fumicolans*; *Mja*, *Methanococcus jannaschii*; *Mth*, *Methanobacterium thermoautotrophicum*; *Afu*, *Archaeoglobus fulgidus*; *Hsa*, *Homo sapiens*; *Dme*, *Drosophila melanogaster*; *Cel*, *Caenorhabditis elegans*; *Sce*, *Saccharomyces cerevisiae*. A and B (BI, BII) are the same as shown in Table 1. Amino acid residues that are identical (*white letter*) and similar (*green letters*) in all sequences, similar in ≥80% of sequences (*red letters*), or similar in 70%–80% of sequences (*purple letters*) are shown. Similar amino acids are grouped as AG, LIMV, YFW, DEQN, KRH, and ST. The positions of the α-helices and β-sheets of *S. cerevisiae* PCNA are shown by *rectangles* and *arrows*, respectively; *green* and *purple* indicate domains 1 and 2 of *S. cerevisiae* PCNA monomer, respectively (Krishna et al. 1994). *Asterisks* indicate amino acids corresponding to those forming the hydrophobic pocket in human PCNA (Gulbis et al. 1996). The regions using phylogenetic analysis are *underlined*

Table 2. Sequence comparison of archaeal and eukaryotic PCNAs

	Organism	Amino acid identity (%)																			
Crenarchaeota	*Soh* A	–																			
	Sso A	61	–																		
	Ape A	27	26	–																	
	Soh B	19	23	21	–																
	Sso B	17	20	17	52	–															
	Ape BI	26	26	27	22	23	–														
	Ape BII	27	24	29	23	21	23	–													
Euryarchaeota	*Pab*	31	32	31	25	21	28	30	–												
	Pho	31	30	33	24	21	28	30	93	–											
	Pfu	32	32	32	25	22	29	30	89	90	–										
	Tfu	30	32	31	23	22	27	31	84	85	85	–									
	Mja	26	25	31	22	20	27	25	43	43	41	42	–								
	Mth	23	24	23	18	20	21	26	30	30	32	29	35	–							
	Afu	22	23	23	18	18	19	22	25	25	25	26	31	25	–						
Eucarya	*Hsa*	24	22	19	19	14	16	22	23	24	24	25	25	28	23	–					
	Dine	23	20	19	18	14	14	21	22	22	24	25	24	24	22	71	–				
	Cel	21	21	15	16	15	16	23	23	24	23	22	27	24	21	48	48				
	Sce	19	21	18	19	17	14	18	25	26	25	25	29	22	22	36	36	40	–		
		Soh A	*Sso* A	*Ape* A	*Soh* B	*Sso* B	*Ape* BI	*Ape* BII	*Pab*	*Pho*	*Pfu*	*Tfu*	*Mja*	*Mth*	*Afu*	*Hsa*	*Dme*	*Cel*	*Sce*		

Abbreviations are as defined in Fig. 1
A and B (BI, BII) are as shown in Table 1

archaeal PCNA sequences, suggested that these three PCNA homologues were grouped into PCNA A and PCNA B, respectively.

The highly conserved L/I-A-P-K/R motifs, which were demonstrated to be critical amino acid residues for the functional interaction of human PCNA with the replication factor C (RFC), was identified in the amino acid sequences of archaeal homologues. However, in all the three *A. pernix* PCNA homologues, this motif is less conserved. One- and two-amino-acid substitutions were identified in the *Ape* PCNA BI and PCNA BII, and PCNA A, respectively.

PCNA has been identified as a target not only for replicative DNA polymerase but also for the cell cycle checkpoint protein p21 (Chen et al. 1995; Luo et al. 1995), the replication endonuclease Fen1 (Li et al. 1995; Chen et al. 1996; Wu et al. 1996), DNA (cytosine) methyltransferase (MCMT) (Chuang et al. 1997), and the DNA repair endonuclease XPG (Gary et al. 1997). In the primary structures of these proteins, a consensus motif called the PIP box, which is important for binding to PCNA, has been found (Warbrick et al. 1997; Warbrick 1998). Study of the crystal structure of the human PCNA complexed with a 22-residue peptide derived from the cell cycle checkpoint protein p21 showed that a hydrophobic pocket that is formed by Met^{40}, Val^{45}, Leu^{47}, Leu^{126}, $Ileu^{128}$, Pro^{129}, Tyr^{133}, Pro^{234}, Tyr^{250}, Ala^{252}, and Pro^{253} of human PCNA interacts with a hydrophobic cavity formed by Met^{147}, Phe^{150}, and Tyr^{151} of the PIP box in human p21 (Gulbis et al. 1996). In all the archaeal PCNA homologues, 8 of 11 amino acids corresponding to those forming the hydrophobic pocket in human PCNA are conserved or substituted with similar, hydrophobic, or aromatic residues. These amino acids of archaeal PCNA homologues seemed to be able to form the hydrophobic pocket.

The phylogenetic analysis showed that two *Sulfurisphaera ohwakuensis* PCNA homologues did not branch together, and that two *Sulfolobus solfataricus* and three *Aeropyrum pernix* homologues also did not. Crenarchaeal PCNA homologues were divided into two groups, one referred to as group A and the other as group B, and the clustering topology of the phylogenetic tree suggests that crenarchaeal A and B are paralogues. However, the phylogenetic tree constructed by parsimony showed that *Ape* PCNA BII is branched with *Ape* PCNA A with a very low bootstrap value, 26% (data not shown). It is a possibility that *Ape* PCNA BII belongs to group A.

In conclusion, it is assumed that the presence of at least two PCNA homologues is a general feature of Crenarchaeota, and that these homologues arose by an early gene duplication(s). The phylogenetic analysis suggested that crenarchaeal PCNA homologues arose independently of euryarchaeal homologues by gene duplication after the split of the crenarchaeal and euryarchaeal lineages, although it cannot be ruled out that an ancestral gene duplication occurred before the split of crenarchaeotes and euryarchaeotes, and then one paralogue was lost in the euryarchaeal lineage immediately after the split of the crenarchaeal and euryarchaeal lineages.

References

Almendral JM, Huebsch D, Blundell PA, Macdonald-Bravo H, Bravo R (1987) Cloning and sequence of the human nuclear protein cyclin: homology with DNA-binding proteins. Proc Natl Acad Sci USA 84:1575–1579

Bauer GA, Burgers PM (1990) Molecular cloning, structure and expression of the yeast proliferating cell nuclear antigen gene. Nucleic Acids Res 18:261–265

Bravo R, Frank R, Blundell PA, Macdonald-Bravo H (1987) Cyclin/PCNA is the auxiliary protein of DNA polymerase-δ. Nature (Lond) 326:515–517

Bult CJ, White O, Olsen GJ, Zhou L, Fleischmann RD, Sutton GG, Blake JA, FitzGerald LM, Clayton RA, Gocayne JD, Kerlavage AR, Dougherty BA, Tomb JF, Adams MD, Reich CI, Overbeek R, Kirkness EF, Weinstock KG, Merrick JM, Glodek A, Scott JL, Geoghagen NSM, Weidman JF, Fuhrmann JL, Nguyen D, Utterback TR, Kelley JM, Peterson JD, Sadow PW, Hanna MC, Cotton MD, Roberts KM, Hurst MA, Kaine BP, Borodovsky M, Klenk H-P, Fraser CM, Smith HO, Woese CR, Venter JC (1996) Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. Science 273:1058–1073

Burgers PM (1991) *Saccharomyces cerevisiae* replication factor C. II. Formation and activity of complexes with the proliferating cell nuclear antigen and with DNA polymerases δ and ε. J Biol Chem 266:22698–22706

Cann IK, Ishino Y (1999) Archaeal DNA replication: identifying the pieces to solve a puzzle. Genetics 152:1249–1267

Cann IK, Komori K, Toh H, Kanai S, Ishino Y (1998) A heterodimeric DNA polymerase: evidence that members of Euryarchaeota possess a distinct DNA polymerase. Proc Natl Acad Sci USA 95:14250–14255

Cann IK, Ishino S, Hayashi I, Komori K, Toh H, Morikawa K, Ishino Y (1999) Functional interactions of a homolog of proliferating cell nuclear antigen with DNA polymerases in *Archaea*. J Bacteriol 181:6591–6599

Chen J, Jackson PK, Kirschner MW, Dutta A (1995) Separate domains of p21 involved in the inhibition of Cdk kinase and PCNA. Nature (Lond) 374:386–388

Chen J, Chen S, Saha P, Dutta A (1996) $p21^{Cip1/Waf1}$ disrupts the recruitment of human Fen1 by proliferating-cell nuclear antigen into the DNA replication complex. Proc Natl Acad Sci USA 93:11597–11602

Chuang LS, Ian H-I, Koh T-W, Ng H-H, Xu G, Li BF (1997) Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for $p21^{WAF1}$. Science 277:1996–2000

De Felice M, Sensen CW, Charlebois RL, Rossi M, Pisani FM (1999) Two DNA polymerase sliding clamps from the thermophilic archaeon *Sulfolobus solfataricus*. J Mol Biol 291:47–57

Edgell DR, Doolittle WF (1997) Archaea and the origin(s) of DNA replication proteins. Cell 89:995–998

Erauso G, Marsin S, Benbouzid-Rollet N, Baucher M-F, Barbeyron T, Zivanovic Y, Prieur D, Forterre P (1996) Sequence of plasmid pGT5 from the archaeon *Pyrococcus abyssi*: evidence for rolling-circle replication in a hyperthermophile. J Bacteriol 178:3232–3237

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author, Department of Genetics, University of Washington, Seattle.

Gary R, Ludwig DL, Cornelius HL, MacInnes MA, Park MS (1997) The DNA repair endonuclease XPG binds to proliferating cell nuclear antigen (PCNA) and shares sequence elements with the PCNA-binding regions of FEN-1 and cyclin-dependent kinase inhibitor p21. J Biol Chem 272:24522–24529

Gulbis JM, Kelman Z, Hurwitz J, O'Donnell M, Kuriyan J (1996) Structure of the C-terminal region of $p21^{WAF1/CIP1}$ complexed with human PCNA. Cell 87:297–306

Higgins D, Clustal W (1998) Multiple Sequence Alignment (5 February 1998, copyright date) [Online]. http://www.genome.ad.jp/SIT/CLUSTALW.html

Ishino Y, Komori K, Cann IK, Koga Y (1998) A novel DNA polymerase family found in *Archaea*. J Bacteriol 180:2232–2236

Jónsson ZO, Hübscher U (1997) Proliferating cell nuclear antigen: more than a clamp for DNA polymerases. Bioessays 19:967–975

Kawarabayasi Y, Sawada M, Horikawa H, Haikawa Y, Hino Y, Yamamoto S, Sekine M, Baba S, Kosugi H, Hosoyama A, Nagai Y, Sakai M, Ogura K, Otsuka R, Nakazawa H, Takamiya M, Ohfuku Y, Funahashi T, Tanaka T, Kudoh Y, Yamazaki J, Kushida N, Oguchi A, Aoki K, Yoshizawa T, Nakamura Y, Robb FT, Horikoshi K, Masuchi Y, Shizuya H, Kikuchi H (1998) Complete sequence and gene organization of the genome of a hyper-thermophilic archaebacterium, *Pyrococcus horikoshii* OT3. DNA Res 5:55–76

Kawarabayasi Y, Hino Y, Horikawa H, Yamazaki S, Haikawa Y, Jinno K, Takahashi M, Sekine M, Baba S, Ankai A, Kosugi H, Hosoyama A, Fukui S, Nagai Y, Nishijima K, Nakazawa H, Takamiya M, Masuda S, Funahashi T, Tanaka T, Kudoh Y, Yamazaki J, Kushida N, Oguchi A, Aoki K, Kubota K, Nakamura Y, Nomura N, Sako Y, Kikuchi H (1999) Complete genome sequence of an aerobic hyper-thermophilic crenarchaeon, *Aeropyrum pernix* K1. DNA Res 6:83–101, 145–152

Kelman Z (1997) PCNA: structure, functions and interactions. Oncogene 14:629–640

Kelman Z, Hurwitz J (1998) Protein-PCNA interactions: a DNA-scanning mechanism? Trends Biochem Sci 23:236–238

Klenk H-P, Clayton RA, Tomb J-F, White O, Nelson KE, Ketchum KA, Dodson RJ, Gwinn M, Hickey EK, Peterson JD, Richardson DL, Kerlavage AR, Graham DE, Kyrpides NC, Fleischmann RD, Quackenbush J, Lee NH, Sutton GG, Gill S, Kirkness EF, Dougherty BA, McKenney K, Adams MD, Loftus B, Peterson S, Reich CI, McNeil LK, Badger JH, Glodek A, Zhou L, Overbeek R, Gocayne JD, Weidman JF, McDonald L, Utterback T, Cotton MD, Spriggs T, Artiach P, Kaine BP, Sykes SM, Sadow PW, D'Andrea KP, Bowman C, Fujii C, Garland SA, Mason TM, Olsen GJ, Fraser CM, Smith HO, Woese CR, Venter JC (1997) The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. Nature (Lond) 390:364–370

Krishna TS, Kong X-P, Gary S, Burgers PM, Kuriyan J (1994) Crystal structure of the eukaryotic DNA polymerase processivity factor PCNA. Cell 79:1233–1243

Kurosawa N, Itoh YH, Iwai T, Sugai A, Uda I, Kimura N, Horiuchi T, Itoh T (1998) *Sulfurisphaera ohwakuensis* gen. nov., sp. nov., a novel extremely thermophilic acidophile of the order *Sulfolobales*. Int J Syst Bacteriol 48:451–456

Lee S-H, Pan Z-Q, Kwong AD, Burgers PM, Hurwitz J (1991) Synthesis of DNA by DNA polymerase ε *in vitro*. J Biol Chem 266:22707–22717

Li X, Li J, Harrington J, Lieber MR, Burgers PM (1995) Lagging strand DNA synthesis at the eukaryotic replication fork involves binding and stimulation of FEN-1 by proliferating cell nuclear antigen. J Biol Chem 270:22109–22112

Luo Y, Hurwitz J, Massagué J (1995) Cell-cycle inhibition by independent CDK and PCNA binding domains in $p21^{Cip1}$. Nature (Lond) 375:159–161

Miyachi K, Fritzler MJ, Tan EM (1978) Autoantibody to a nuclear antigen in proliferating cells. J Immunol 121:2228–2234

Morrison A, Araki H, Clark AB, Hamatake RK, Sugino A (1990) A third essential DNA polymerase in *S. cerevisiae*. Cell 62:1143–1151

Müller F, Seo Y-S, Hurwitz J (1994) Replication of bovine papillomavirus type 1 origin-containing DNA in crude extracts and with purified proteins. J Biol Chem 269:17086–17094

Pisani FM, Martino CD, Rossi M (1992) A DNA polymerase from the archaeon *Sulfolobus solfataricus* shows sequence similarity to family B DNA polymerases. Nucleic Acids Res 20:2711–2716

Podust VN, Hübscher U (1993) Lagging strand DNA synthesis by calf thymus DNA polymerases α, β, δ and ε in the presence of auxiliary proteins. Nucleic Acids Res 21:841–846

Prelich G, Kostura M, Marshak DR, Mathews MB, Stillman B (1987a) The cell-cycle regulated proliferating cell nuclear antigen is required for SV40 DNA replication *in vitro*. Nature (Lond) 326:471–475

Prelich G, Tan C-K, Kostura M, Mathews MB, So AG, Downey KM, Stillman B (1987b) Functional identity of proliferating cell nuclear antigen and a DNA polymerase-δ auxiliary protein. Nature (Lond) 326:517–520

Smith DR, Doucette-Stamm LA, Deloughery C, Lee H, Dubois J, Aldredge T, Bashirzadeh R, Blakely D, Cook R, Gilbert K, Harrison D, Hoang L, Keagle P, Lumm W, Pothier B, Qiu D, Spadafora R, Vicaire R, Wang Y, Wierzbowski J, Gibson R, Jiwani N, Caruso A, Bush D, Safer H, Patwell D, Prabhakar S, McDougall S, Shimer G, Goyal A, Pietrokovski S, Church GM, Daniels CJ, Mao J, Rice P,

Nölling J, Reeve JN (1997) Complete genome sequence of *Methanobacterium thermoautotrophicum* DH: functional analysis and comparative genomics. J Bacteriol 179:7135–7155

Tan C-K, Castillo C, So AG, Downey KM (1986) An auxiliary protein for DNA polymerase-δ from fetal calf thymus. J Biol Chem 261:12310–12316

Tsurimoto T (1998) PCNA, a multifunctional ring on DNA. Biochim Biophys Acta 1443:23–39

Waga S, Stillman B (1998) The DNA replication fork in eukaryotic cells. Annu Rev Biochem 67:721–751

Warbrick E (1998) PCNA binding through a conserved motif. Bioessays 20:195–199

Warbrick E, Lane DP, Glover DM, Cox LS (1997) Homologous regions of Fen1 and p21Cip1 compete for binding to the same site on PCNA: a potential mechanism to coordinate DNA replication and repair. Oncogene 14:2313–2321

Wilson R, Ainscough R, Anderson K, Baynes C, Berks M, Bonfield J, Burton J, Connell M, Copsey T, Cooper J, Coulson A, Craxton M, Dear S, Du Z, Durbin R, Favello A, Fraser A, Fulton L, Gardner A, Green P, Hawkins T, Hillier L, Jier M, Johnston L, Jones M, Kershaw J, Kirsten J, Laisster N, Latreille P, Lightning J, Lloyd C, Mortimore B, O'Callaghan M, Parsons J, Percy C, Rifken L, Roopra A, Saunders D, Shownkeen R, Sims M, Smaldon N, Smith A, Smith M, Sonnhammer E, Staden R, Sulston J, Thierry-Mieg J, Thomas K, Vaudin M, Vaughan K, Waterston R, Watson A, Weinstock L, Wilkinson-Sproat J, Wohldman P (1994) 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. Nature (Lond) 368:32–38

Wold MS, Kelly T (1988) Purification and characterization of replication protein A, a cellular protein required for *in vitro* replication of simian virus 40 DNA. Proc Natl Acad Sci USA 85:2523–2527

Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci USA 87:4576–4579

Wu X, Li J, Li X, Hsieh C-L, Burgers PM, Lieber MR (1996) Processing of branched DNA intermediates by a complex of human FEN-1 and PCNA. Nucleic Acids Res 24:2036–2043

Yamaguchi M, Nishida Y, Moriuchi T, Hirose F, Hui C-C, Suzuki Y, Matsukage A (1990) *Drosophila* proliferating cell nuclear antigen (cyclin) gene: structure, expression during development, and specific binding of homeodomain proteins to its 5'-flanking region. Mol Cell Biol 10:872–879