Arabidopsis thaliana proliferating cell nuclear antigen has several potential sumoylation sites

Wojciech Strzalka1,*, Piotr Labecki1, Filip Bartnicki1, Chhavi Aggarwal1, Maria Rapala-Kozik2, Chiharu Tani3,4, Katsunori Tanaka3,4 and Halina Gabrys1

1 Department of Plant Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland
2 Department of Analytical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland
3 Department of Bioscience, Research Center for Environmental Bioscience, School of Science and Technology, Kwansei Gakuin University, 2-1 Gakuen, Sanda 669-1337, Japan
4 Research Center for Environmental Bioscience, School of Science and Technology, Kwansei Gakuin University, 2-1 Gakuen, Sanda 669-1337, Japan

* To whom correspondence should be addressed. E-mail: wojciech.strzalka@uj.edu.pl

Received 19 September 2011; Revised 22 December 2011; Accepted 8 January 2012

Abstract

Proliferating cell nuclear antigen (PCNA) is post-translationally modified in yeast and animal cells. Major studies carried out in the last decade have focused on the role of sumoylated and ubiquitinated PCNA. Using different approaches, an interaction between plant PCNA and SUMO both in vivo and in bacteria has been demonstrated for the first time. In addition, identical sumoylation patterns for both AtPCNA1 and 2 were observed in bacteria. The plant PCNA sumoylation pattern has been shown to differ significantly from that of Saccharomyces cerevisiae. This result contrasts with a common opinion based on previous structural analysis of yeast, human, and plant PCNAs, which treats PCNA as a highly conserved protein even between species. Analyses of AtPCNA post-translational modifications using different SUMO proteins (SUMO1, 2, 3, and 5) revealed similar modification patterns for each tested SUMO protein. Potential target lysine residues that might be sumoylated in vivo were identified on the basis of in bacteria AtPCNA mutational analyses. Taken together, these results clearly show that plant PCNA is post-translationally modified in bacteria and may be sumoylated in a plant cell at various sites. These data open up important new perspectives for further detailed studies on the role of PCNA sumoylation in plant cells.

Key words: Arabidopsis thaliana, PCNA, SUMO.

Introduction

Proliferating cell nuclear antigen (PCNA) is a protein first discovered in eukaryotic cells as an autoantigen in patients suffering from an immunological disorder called systemic lupus erythematosus (Miyachi et al., 1978). Later, it was also found in archaeal cells (Cann et al., 1999). The first basic function attributed to PCNA was connected with the DNA replication process where it was proposed to serve as an accessory factor of DNA polymerase δ, required for the synthesis of a new DNA strand (Tan et al., 1986; Bravo et al., 1987; Prelich et al., 1987). There have been structural studies of yeast (Krishna et al., 1994), human (Gulbis et al., 1996), archaeal (Matsumiya et al., 2001), and, recently, Arabidopsis (Strzalka et al., 2009) PCNAs. These studies provided important data towards a better understanding of the link between the three-dimensional structure of PCNA and its roles in DNA metabolism. Eukaryotic and archaeal PCNAs form trimeric, ring-shaped structures encircling the helical DNA. In a generally accepted model, PCNA with its pseudo-6-fold symmetry is seen as sliding along double-stranded DNA (Krishna et al., 1994; Gulbis et al., 1996;
PCNA, also called a sliding clamp, can serve as a docking site for other proteins involved in DNA metabolism. Further studies on PCNA revealed its crucial role not only in DNA replication but also in DNA repair, cell cycle control, and other processes (Strzalka and Ziemienowicz, 2011). Studies on mammalian PCNA showed that it could show a great impact on the plant, animal, fungal, and archaebacterial DNA code (Vivona and Kelman, 2003). Although the evolutionary process has been shown to stimulate the activity and processivity of two wheat polymerases (Bauer and Burgers, 1988; Ng et al., 1990), as well as the archaeobacterium Pyrococcus furiosus (Waseem et al., 1999). Other important results on PCNA reported in substrate stability, activity, and localization (Gill, 2004). The first crystal structure of sumoylated yeast PCNA was published in 2004 (Novatchkova et al., 2004). The analysis of animal and fungal sumoylated proteins led to the identification of PCNA genes in different species, such as yeast—budding yeast (Bauer and Burgers, 1990) and fission yeast (Waseem et al., 1992); animals—human (Almdredal et al., 1987), rat (Matsumoto et al., 1987), mouse (Yamaguchi et al., 1991), and Drosophila (Yamaguchi et al., 1990); plants—carrot (Hata et al., 1992), maize (Lopez et al., 1995, 1997), rice (Suzuka et al., 1991), common bean (Strzalka and Ziemienowicz, 2007), and runner bean (Strzalka et al., 2010); as well as the archaeabacterium Pyrococcus furiosus (Cann et al., 1999).

Additional experimental evidence supporting the strong evolutionary conservation of PCNA protein was obtained when yeast and Drosophila PCNAs were shown to substitute functionally for mammalian PCNA in DNA replication assays (Bauer and Burgers, 1988; Ng et al., 1990). Moreover, recombinant rice and runner bean PCNA were shown to stimulate the enzymatic activity of human DNA polymerase δ (Matsumoto et al., 1994; Strzalka et al., 2010). Studies on mammalian PCNA showed that it could stimulate the activity and processivity of two wheat δ-like polymerases (Laqul et al., 1993). Other important results highlighting the strong conservation of PCNA described the formation of a stable complex between human p21/WAF-1 and PCNA purified from pea, Arabidopsis, and runner bean (Bull and Lane, 1996; Strzalka et al., 2009, 2010).

Recently, numerous studies have focused on PCNA post-translational modification and its implication for DNA replication and repair. In general, PCNA can form cytosolic-, nuclear-, and chromatin-associated fractions. However, this simple picture of PCNA populations seems to be complicated by the presence of weakly and strongly chromatin-associated PCNA fractions and other different populations of PCNA associated with its post-translational modifications including acetylation, methylation, phosphorylation, sumoylation, and ubiquitination (Stoimenov and Helleday, 2009).

The small ubiquitin-like modifier (SUMO) is one of those peculiar eukaryotic regulatory molecules that can be covalently attached, in a reversible manner, to a target protein and thereby modify its functions. Since the discovery of SUMO, a wealth of new data has been published concerning the functional role of sumoylation. SUMO is known to alter protein–protein interactions that may result in a change in substrate stability, activity, and localization (Gill, 2004). In plants, SUMO has been shown to be involved in stress responses, pathogen defense, abscisic acid signalling, and flower induction (Novatchkova et al., 2004). The genome sequencing project for Arabidopsis thaliana revealed the presence of eight AtSUMO genes and one pseudogene. For Arabidopsis AtSUMO1, 2, and 5 genes expressed sequence tags (ESTs) were found, which provide evidence for the in vivo expression of these genes. In contrast, although AtSUMO4, 6, 7, and 8 genes are believed to be potentially active, their ESTs have not been identified to date. AtSUMO9 is a non-functional pseudogene (Novatchkova et al., 2004). Although there are eight AtSUMO genes in the Arabidopsis genome, only one functional gene encoding a SUMO-conjugating enzyme was found (AtSCE1a, E2) (Novatchkova et al., 2004). For biological activity, the SUMO protein must be post-translationally processed by a SUMO-specific protease which removes the C-terminal tail and creates a mature SUMO with a glycine–glycine (GG) motif at the C-terminus (Novatchkova et al., 2004). Next, mature SUMO is activated by a SUMO-activating enzyme (E1) (composed of two polypeptides AtSAE1 and AtSAE2 in Arabidopsis). The enzymatic steps of SUMO activation proceed via a covalent modification of the SUMO C-terminus (Novatchkova et al., 2004). After activation, a SUMO thioester is transferred from E1 to E2. Next, the conjugated SUMO may be transferred to the ε-amino group of the lysine residue of the target protein either directly or indirectly. The indirect transfer requires the additional formation of an AtSUMO–AtSCE1a substrate and an E3 SUMO ligase ternary complex (Novatchkova et al., 2004). Studies on yeast (Hoeger et al., 2002), Xenopus (Leach and Michael, 2005), and chicken (Arakawa et al., 2006) cells showed that PCNA can be post-translationally sumoylated. PCNA sumoylation was demonstrated to occur in the S phase of the cell cycle (Hoeger et al., 2002; Leach and Michael, 2005). Sumoylation of yeast PCNA occurs predominantly at Lys164 but also, to a lesser extent, at Lys127 located within an interdomain-connecting loop (Hoeger et al., 2002). Xenopus and chicken PCNA have so far been shown to be sumoylated at Lys164 only (Leach and Michael, 2005; Arakawa et al., 2006). Moreover, the sumoylated yeast PCNA was shown to recruit Srs2, a helicase that blocks recombinational repair by disrupting Rad51 nucleoprotein filaments (Papoulis et al., 2005; Pfander et al., 2005).

The first crystal structure of sumoylated yeast PCNA was published in 2011 (Freudenthal et al., 2011). Despite extensive studies on sumoylated and ubiquitinated yeast PCNA, no post-translational modification of plant PCNA
has been reported and characterized to date. In this study, it is demonstrated for the first time that plant PCNA interacts with SUMO in vivo. Using an in bacteria reconstituted plant sumoylation system the sumoylation of Arabidopsis PCNA is characterized in detail and a function for sumoylated PCNA is proposed.

Materials and methods

Plasmids
The construction of plasmids with open reading frames (ORFs) coding for E1 (pACYCDuet-1-His-tag-AtSAE2/AtSAE1b-S-tag) and E2 (pCDEFDuet-1-His-tag-AtSUMO either with the C-terminal GG native motif or mutated to alanine–alanine (AA)/AtSCE1a-S-tag) has been described previously by Okada et al. (2009; Supplementary Fig. S1 available at JXB online). The pQ7070-AtSCE1-His-tag vector was purchased from Professor Stubbe, and its construction has been described by Colby et al. (2006; Supplementary Fig. S1). Also the construction of His-tag-depleted pET29a-c-myc-AtPCNA1 (Supplementary Fig. S1) has been previously described (Strzalka et al., 2009). The vector coding for E3 ligase (AtSiz1) (pMAL-C2-MBP-tag-AtSiz1; Supplementary Fig. S1) was purchased from Professor Nam-Hai Chua. pET28a-His-tag-c-myc-AtPCNA1, AtPCNA2, and ScPCNA were constructed as described below.

AtPCNA1 (AT1G07370), AtPCNA2 (AT2G29570), and ScPCNA (YBR088C) wild-type ORFs containing an N-terminal c-myc tag were amplified using specific primers: AtPCNA1F (5′-CAAGTTTGTACAAAAAAGCAGGCTCCATGTCTAACCCTCA−3′) and AtPCNA1R (5′-ATCTGATGTTGGAGCACGTCTTGTTC-3′) for AtPCNA1F (5′-GGGGATACTTTGTACAAGAAAGCTGGGTCTTCTGGTG-3′) and AtPCNA1R (5′-GGGGATCCTTATTCTGGTTTGGTGTCTTC-3′); AtPCNA2F (5′-GGGATCATATGGGACACGTCTTCTGGTTC-3′) and AtPCNA2R (5′-CCGGATCCTTTGTACAAAGAAACAACTTTGTACAG-3′); and ScPCNAF (5′-GGGATCATATGGGACACGTCTTCTGGTTC-3′) and ScPCNA R (5′-CCGGATCCTTTGTACAAAGAAACAACTTTGTACAG-3′), respectively. The PCRs were carried out in a 100 μl volume, containing 1× PCR buffer [20 mM TRIS-HCl, 2 mM MgSO4, 10 mM KCl, 10 mM (NH4)2SO4, 0.1% (v/v) Triton X-100, bovine serum albumin (BSA) 0.1 mg ml−1, pH 8.8], 0.2 mM dNTPs, 2.5 U of DNA polymerase (Fermentas) and 2 μM of each primer, and 1 ng of template plasmids described previously (Colby et al., 2006; Strzalka et al., 2009). The preliminary denaturation step was performed at 94 °C for 5 min. Subsequently, 30 cycles of amplification were performed with denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, DNA synthesis at 72 °C for 2 min, followed by incubation at 72 °C for 5 min in a thermocycler (Eppendorf). The resulting PCR products were purified, digested with either BamHI and NotI or BamHI and XhoI restriction enzymes (Fermentas) for AtPCNAS and ScPCNA, respectively, ligated into the pET28a expression vector carrying an N-terminal His-tag using T4 DNA ligase (Fermentas), and sequenced. Mutagenized AtPCNA1 variants (AtPCNA1K254R, AtPCNA1K166R, and their single lysine reverted mutants) were synthesized by GeneScript USA Inc. The agrobacterial binary expression vector pET29a-c-myc-AtPCNA1, or directly pET28a-His-tag-c-myc-AtPCNA1, AtPCNA2, and ScPCNA were constructed using Gateway technology (Invitrogen). AtPCNA1, AtPCNA2 and AtSUMO3 ORFs were amplified as described previously using a set of specific primers AtPCNA1GWF (5′-GGGGACAAGGTGTTTGACCGTCAGC-3′) and AtPCNA1GWR (5′-GGGGACACCTTTTTGA-3′), AtPCNA2GWF (5′-GGGGACACCTTTTTGA-3′), and AtSUMO3GWF (5′-GGGGACACCTTTTTGA-3′) and AtPCNA1GWR (5′-GGGGACACCTTTTTGA-3′).

Bimolecular fluorescence complementation (BiFC) analysis
Wild-type Nicotiana benthamiana was grown in commercial soil under long-day conditions (16 h light/8 h dark cycles) at 23 °C. For BiFC screening, all binary plasmids [carrying AtPCNA1_GFP, AtPCNA1_CtermGFP, AtPCNA2_GFP, AtPCNA2_CtermGFP, GFP_AtSUMO3(GG/AA), and NtermGFP_AtSUMO3 fusions] were transferred into Agrobacterium tumefaciens strain C58 using a freeze–thaw method. The Agrobacterium strains obtained were used to infiltrate tobacco leaves for transient expression according to the protocol described by Yang et al. (2000) with slight modifications. The transformed Agrobacterium strains were grown for 2 d at 28 °C in YEB medium supplemented with appropriate antibiotics (gentamicin 20 mg l−1, spectomycin 100 mg l−1, and rifampicin 100 mg l−1). After incubation, Agrobacterium cells were harvested by centrifugation and resuspended in agro-infiltration buffer (10 mM MES, 10 mM MgCl2, 100 μM aceto-syringone, pH 5.6). The final OD600 of the culture was adjusted to 0.5 using the agro-infiltration buffer. For co-expression studies, 500 μl of each bacterial culture (with OD600 adjusted to 0.5) were mixed and incubated at room temperature for 2–5 h. The final inoculum was infiltrated into the lower epidermis of tobacco leaves. The infiltrated area was assayed 3–5 d after infiltration using a Zeiss LSM 510 META laser scanning microscope. At least three infiltrated leaves were analysed per combination.

In bacteria reconstitution of the PCNA sumoylation system
Escherichia coli BL21 (DE3) cells were transformed with plasmids pACYCDUET-1 encoding AtE1 (AtSAE1b and AtSAE2), followed by pCDEFDuet-1 encoding AtE2 (AtSCE1a) and AtSUMO1, 2, 3, or 5 (GG or AA variant). Either pMAL-C2X encoding AtE3 ligase (AtSiz1) followed by pET28a-c-myc-AtPCNA1, or directly pET28a-His-tag-c-myc-AtPCNA1, AtPCNA2, and ScPCNA were introduced into the prepared competent cells carrying AtE1, AtE2, and AtSUMO ORFs. The expression of all proteins was tested by western blotting (data not shown). The transformed cells were grown at 37 °C in LB medium containing chloramphenicol (25 mg l−1), kanamycin (50 mg l−1), and spectinomycin (100 mg l−1), and in the presence of a plasmid with AtSiz1, the medium was supplemented with ampicillin (100 mg l−1). When the culture reached an OD95 of 0.6, the temperature was decreased to 20 °C and protein overexpression was induced using 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG). After 20 h of induction, cells were harvested by centrifugation (5000 g for 15 min at 4 °C), re-suspended in 1 ml of the protein extraction buffer (50 mM NaH2PO4, 300 mM NaCl, and 10 mM imidazole, pH 8.0) containing protease inhibitor cocktail for poly(histidine)-tagged proteins (Sigma), and sonicated.
(5 min, 5 s pulses, 10 s intervals, 4 °C). All subsequent isolation steps were performed at 4 °C. The sonicated cells were centrifuged at 35 000 g for 20 min. After centrifugation, the cell lysate was mixed with 20 μl of Ni-NTA agarose (Qiagen) equilibrated previously with the extraction buffer and the mixture was incubated for 1 h with constant rotation. After incubation, the unbound proteins were removed by a wash with an extraction buffer containing 20 mM imidazole (1 ml, three times for 5 min). The bound proteins were eluted with 20 μl of an extraction buffer containing 250 mM imidazole and then mixed with an SDS–PAGE loading buffer, denatured at 100 °C for 5 min, and kept at −20 °C until use.

Western blotting analysis of PCNA post-translational modification
A 40 μl aliquot of sumoylated AtPCNA1 (c-myc-AtPCNA1–His-AtSUMO3) purified from *E. coli* cells on nickel and p21-peptide columns for liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis, and 10 μl of a His-c-myc-At/ScPCNA sample isolated from *E. coli* cells overexpressing the plant enzymes (AtE1, AtE2, and AtSUMO) were separated by 12% (v/v) SDS–PAGE and electrotransferred onto a PVDF membrane (0.2 μm) (Millipore), as described previously (Towbin *et al.*, 1979). All subsequent steps were performed at room temperature. After the transfer, the membrane was washed three times for 5 min in 1× phosphate-buffered saline (PBS) supplemented with 0.5% Tween-20 (v/v) (PBS-T) and blocked with PBS-T containing 5% (w/v) fat-free milk (PBS-TB) for 30 min. The membrane was incubated for 2 h with a monoclonal anti-c-myc antibody (Sigma, dilution 1:20 000). After washing in PBS-TB, the membrane was incubated for 1 h with a goat anti-mouse IgG–horseradish peroxidase conjugate (Pierce, dilution 1:20 000). After several washes in PBS-T, chemiluminescent detection was performed using a Super Signal West Pico Chemiluminescent substrate (Pierce) with the help of a Multi Spectral Imaging System (UVP).

**Purification of c-myc-AtPCNA1–His-AtSUMO3 conjugate**
Proteins encoded by plasmids carrying AtE1 (AtSAE1b and AtSAE2), AtE2 (AtSCE1a), AtSUMO3, and His-tag-depleted AtPCNA1 were overexpressed in *E. coli* BL21(DE3) cell culture and purified on a 1 ml Ni-NTA column as described above. The eluted proteins were dialysed against 1× PBS with 0.1% (v/v) Triton X-100 and loaded onto a 100 μl p21-peptide column prepared by coupling biotinylated synthetic p21-peptide (139GRKRRQTSMTDFYHSKRRLIFS160, synthesized by the Protein Analysis service unit at FMI, Basel) to streptavidin–agarose beads (Pierce) (Ball and Lane, 1996; Strzalka *et al.*, 1979). All subsequent isolation steps were performed at 4 °C. After centrifugation, the cell lysate was loaded onto an Ni-NTA agarose (Qiagen) column equilibrated with extraction buffer, washed with extraction buffer containing 20 mM imidazole, and eluted with the extraction buffer containing 250 mM imidazole. The eluted proteins were dialysed against a storage buffer [50 mM TRIS, 200 mM NaCl, 0.2 mM EDTA, and 10% glycerol (v/v), pH 8.0] then frozen in liquid nitrogen and stored at −80 °C until use.

**Surface plasmon resonance analysis**
The experiments were performed using a BIACORE 3000 system (GE Healthcare) in a running buffer [10 mM HEPES, 150 mM NaCl, 10 mM MgCl$_2$, and 0.005% surfactant (v/v), pH 7.5]. The AtSCE1a protein (0.1 μg in 50 μl) was immobilized onto a CM5 sensor chip (GE Healthcare) by using the standard amine-coupling method (the manufacturer’s protocol). Different concentrations of purified AtPCNA protein solutions (60 μl) were injected at a flow rate of 30 μl min$^{-1}$ at 25 °C. The association and dissociation of AtPCNAs and the AtSCE1a proteins were indicated as shifts in resonance units during the injection and after the change to the running buffer. Dissociation and association rate constants ($k_{\text{diss}}$ and $k_{\text{ass}}$) were obtained with a simple Langmir model (1:1) with drifting baseline, using BIAevaluation 2.1 software (GE Healthcare), and $K_D$ values were calculated as the ratios of these values.

**Results**

**In vivo interaction of Arabidopsis PCNA and SUMO**

*Nicotiana benthamiana* was chosen to study the *in vivo* interaction between *Arabidopsis* PCNA and SUMO using BIFC analysis. A mixture of *A. tumefaciens* carrying plasmids with an N-terminal GFP fragment fused to AtSUMO3 (NtermGFP_AtSUMO3) and AtPCNA fused to a C-terminal GFP fragment (AtPCNA1_CtermGFP or AtPCNA2_CtermGFP) was infiltrated into epidermal cells of *N. benthamiana*. Confocal microscopy analysis of transiently transformed leaves showed GFP fluorescence in the samples where AtPCNA_CtermGFP and NtermGFP_AtSUMO3 were carry out three successive scans consisting of the following: first, a full-scan MS over the range 450–2000 m/z, and then data-dependent scans of the two most abundant ions obtained in the first scan. Automatic MS/MS spectra were obtained from the highest peak in each scan by setting a relative collision energy of 35% and an exclusion time of 15 min for molecules in the same m/z value range. Molecular masses of the resulting peptides were checked against the non-redundant NCBI database using the MASCOT program with an additional mass corresponding to the conjugated SUMO peptide.

**Purification of the AtPCNA1, AtPCNA2, and AtSCE1a proteins**

*Escherichia coli* BL21(DE3) cells were transformed using either pET28a-His-tag-c-myc-AtPCNA1, pET28a-His-tag-c-myc-AtPCNA2, or pQE70AtSCE1a-His-tag vector. The cells carrying the introduced construct were vigorously shaken at 37 °C in LB medium containing either kanamycin (50 mg l$^{-1}$) for AtPCNA1 and 2 or ampicillin (100 mg l$^{-1}$) for AtSCE1a. The cells were grown until the culture reached an OD$_{600}$ of 0.6. Next, the temperature was decreased to 20 °C and the overexpression of recombinant proteins was performed for 20 h using 0.2 mM IPTG, followed by centrifugation (5000 g for 15 min at 4 °C). The centrifuged cells were resuspended in 50 μl of an extraction buffer (see above) containing a protease inhibitor cocktail for poly(histidine)-tagged proteins (Sigma), and sonicated (50 min, 5 s pulses, 10 s intervals). All subsequent procedures were performed at 4 °C. The cells were centrifuged at 35 000 g for 30 min. After centrifugation, the cell lysate was equilibrated with 1 ml of Ni-NTA agarose beads (Qiagen) (Qiagen), as described previously (Towbin *et al.*, 1979). All subsequent steps were performed at room temperature. After the transfer, the membrane was washed three times for 5 min in 1× phosphate-buffered saline (PBS) supplemented with 0.5% Tween-20 (v/v) (PBS-T) and blocked with PBS-T containing 5% (w/v) fat-free milk (PBS-TB) for 30 min. The membrane was incubated for 2 h with a monoclonal anti-c-myc antibody (Sigma, dilution 1:20 000). After washing in PBS-TB, the membrane was incubated for 1 h with a goat anti-mouse IgG–horseradish peroxidase conjugate (Pierce, dilution 1:20 000). After several washes in PBS-T, chemiluminescent detection was performed using a Super Signal West Pico Chemiluminescent substrate (Pierce) with the help of a Multi Spectral Imaging System (UVP).

**Analysis of sumoylated peptides by LC-MS/MS**
The protein sample AtPCNA1 conjugated with AtSUMO3 and purified subsequently on Ni-NTA and p21-peptide columns was separated by 12% (v/v) SDS–PAGE. The substrate proteins, with or without SUMO, were excised from the gel, reduced with 10 mM dithiothreitol (DTT), alkylated with 55 mM iodoacetamide, and digested with 10 μg l$^{-1}$ modified trypsin (Promega) at 37 °C for 16 h. After in-gel digestion, the peptides were extracted with 5% (v/v) formic acid and 50% (v/v) acetonitrile, dried under a vacuum, and dissolved in 2% (v/v) acetonitrile and 0.1% (v/v) formic acid. The digested peptides were fractionated by C18 reverse-phase chromatography (NanoFritor nLC; Hitachi High-Technologies Corporation, Tokyo, Japan) coupled directly to a Linear Ion Trap-TOF Mass Spectrometer (NanoFritor LD; Hitachi High-Technologies Corporation, Tokyo, Japan) with a Picotip EMIT-TER (NEW OBJECTIVE, USA). The ion trap was programmed to
overexpressed, and in samples expressing AtPCNA1, AtPCNA2, and AtSUMO3 fused with full-length GFP (Fig. 1). No fluorescence signal was observed in the control samples (NtermGFP_AtSUMO3, AtPCNA1_CtermGFP, and AtPCNA2_CtermGFP). The result of this analysis confirmed that plant PCNA interacts with SUMO in vivo in both the cytoplasm and the nucleus.

In bacteria analysis of Arabidopsis PCNA sumoylation

An Arabidopsis sumoylation system reconstituted in bacteria (a heterologous system, in vivo), as previously described (Okada et al., 2009), was employed to test the post-translational modification of AtPCNA proteins by AtSUMO. E1 (AtSAE1b and AtSAE2), E2 (AtSCE1a), and one of AtSUMO1, 2, 3, or 5 (GG or AA C-terminal motif variant) were simultaneously overexpressed with either His-c-myc-AtPCNA1 and 2 wild type (WT) or His-c-myc-ScPCNA WT in E. coli host cells (Supplementary Table S1 at JXB online). His-c-myc-PCNAs were isolated on nickel mini-columns. A comparative western blotting analysis of AtPCNA sumoylation patterns was performed using anti-c-myc antibodies. Overexpression of AtSUMO with GG and AA C-terminal motifs resulted in an expected substantial difference between the observed AtPCNA sumoylation patterns (Fig. 2A, B). When AtSUMO GG variants were used, two identical sumoylation products characterized by different molecular masses were observed for both AtPCNA1 and AtPCNA2. The first (low molecular mass) detected product of the post-translational modification had a molecular mass between 43 kDa and 55 kDa (Fig. 2A, B, lanes 1–4). An additional, second (high molecular mass) detected product had a slightly higher molecular mass of between 55 kDa and 72 kDa. All identified sumoylation patterns were identical; that is, the sumoylation products of AtPCNA1 and AtPCNA2 were identical not only within the group of products for each AtPCNA separately, but also for both AtPCNAs (Fig. 2A, B, lanes 1–4). No sumoylation of either AtPCNA1 or 2 was detected in the samples containing AtSUMO with AA C-terminal motifs in contrast to AtSUMO variants with GG C-terminal motifs (Fig. 2A, B, lanes 5–8). To conclude, the in bacteria analysis of AtPCNA1 and AtPCNA2 sumoylation did not show any differences in sumoylation pattern.

An in bacteria Arabidopsis sumoylation system was also used to compare AtPCNA1 and ScPCNA post-translational modification patterns. The PCNA proteins were overexpressed and purified as already described. A western blotting analysis of sumoylated plant and yeast PCNA...
samples showed significant differences in sumoylation patterns (Fig. 2C, lanes 1–8). In contrast to Arabidopsis PCNA, where two different sumoylation products were observed, three post-translationally sumoylated forms were detected in yeast when ScPCNA was overexpressed with AtSUMO1, 2, and 5 (Fig. 2C, lanes 1, 2, and 4). When AtSUMO3 was expressed with yeast PCNA, two types of modifications were observed (Fig. 2C, lane 3). An ScPCNA post-translational modification with a low molecular mass (43–55 kDa) was present in all four tested AtSUMO proteins (AtSUMO1, 2, 3, and 5). The size of this product corresponded well to the low molecular mass AtPCNA–AtSUMO conjugate (Fig. 2C, lanes 1–8). An ScPCNA sumoylation product located just above the low molecular mass (43–55 kDa) product was detected only for AtSUMO3 (Fig. 2C, lane 3). Middle and high molecular mass ScPCNA sumoylation products were observed only for AtSUMO1, 2, and 5, having a molecular mass between 55–72 kDa and 72–95 kDa, respectively. The size of the middle ScPCNA sumoylation product corresponded well to the high molecular mass AtPCNA post-translational modification product (55–72 kDa; Fig. 2C, lanes 1 and 2, and 4–8) while the high molecular mass ScPCNA modification product was not observed in AtPCNA1s when tested with AtSUMO1, 2, 3, and 5 (Fig. 2C, lanes 5–8). To conclude, the comparison of AtPCNA1 and ScPCNA sumoylation patterns in the bacteria system showed that these two proteins are sumoylated differently by the same set of enzymes.

Identification of the sumoylation target in Arabidopsis PCNA

The amino acid sequences of AtPCNA1 and AtPCNA2 were probed for potential sumoylation sites using SUMOsp 2.0 (Ren et al., 2009) and a SUMO plot provided by Abgent. The SUMOsp 2.0 software predicted two putative sumoylation residues for each AtPCNA. Lys200 and Lys254 were proposed for AtPCNA1, and Lys254 and Lys262 for AtPCNA2 (Table 1). In contrast, the SUMO plot suggested Lys91, Lys190, Lys200, and Lys254 as putative sumoylation targets for AtPCNA1 while Lys91, Lys190, and Lys254 were suggested for AtPCNA2 (Table1). In order to assess experimentally in bacteria the AtPCNA lysine residue(s) modified by AtSUMO, four elements, E1,
E2, an AtSUMO3 (GG) variant (which gives a short branch after trypsin digestion), and a His-tag-depleted c-myc-AtPCNA1, were overexpressed in *E. coli* cells. This was followed by purification of the c-myc-AtPCNA1–His-AtSUMO3 conjugate, first using a nickel column, and then a p21-peptide column with an affinity for PCNA. Western blotting analysis and Coomassie staining showed the presence of AtPCNA1, both unmodified and modified by AtSUMO3 (Fig. 3A, lanes 1 and 2). The c-myc-AtPCNA1–His-AtSUMO3 conjugate, which was detected after Coomassie staining (molecular mass between 43 kDa and 55 kDa), was subjected to trypsin digestion followed by LC-MS/MS analysis. The trypsin digestion of covalently attached AtSUMO3 produces a specific tag of five amino acid residues (AMSGG), which remains with the target lysine after digestion. An LC-MS/MS analysis gave signals representing >60% of the expected peptides produced by a cleavage of non-branched AtPCNA1 and 36% of AtSUMO3. The analysis revealed a putative modification for only one out of 16 lysine residues in AtPCNA1. The detected mass peaks point to the presence of a sumoylated branched AtPCNA1 peptide containing Lys254 as a potential sumoylation site for AtPCNA1. To verify the results of previous in silico and LC-MS/MS analyses, the AtPCNA1K254R mutant was tested (Fig. 3C). After substitution of Lys254 with arginine, no high molecular mass AtPCNA1 sumoylation product (55–72 kDa) (Fig. 3C, lane 3) was detectable. The observed result suggests that the product with a molecular mass of 55–72 kDa was formed as a consequence of AtPCNA1 monomer sumoylation at two different sites. To study in detail the observed phenomenon, western blotting analysis was again used to test the sumoylation of the AtPCNA1K1-16/R mutant with all lysine residues changed to arginine along with 16 single AtPCNA1K1-16/R revertants, where each arginine, one by one, was converted back into lysine (Fig. 4). The only modification product detected in 16 sumoylated AtPCNA1K1-16/R single reverted mutants had a molecular mass of between 43 kDa and 55 kDa. The second product of high molecular mass (55–72 kDa) observed in WT AtPCNA1 (Fig. 4, lane 1) was absent in the revertants. This result confirmed the sumoylation of Lys254 identified earlier by LC-MS/MS. Additionally, five other residues, Lys13, Lys14, Lys20, Lys217, and Lys240, were found to be sumoylated in bacteria in the tested mutated AtPCNA1 variants (Fig. 4, lanes 3, 4, 5, 16, 17, 18, 19).

### Table 1. Prediction of sumoylation sites with computational analyses

The lysine residues which were predicted as sumoylation sites with low (L) and high (H) scores are indicated.

<table>
<thead>
<tr>
<th>Position of lysine residues</th>
<th>Surrounding amino acids</th>
<th>SUMOsp 2.0 prediction software</th>
<th>SUMO plot prediction software</th>
</tr>
</thead>
<tbody>
<tr>
<td>AtPCNA1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys91</td>
<td>ITIKADD</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Lys190</td>
<td>TVDKPED</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>Lys200</td>
<td>IEKMEPV</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Lys254</td>
<td>LAPKIEE</td>
<td>H</td>
<td>L</td>
</tr>
<tr>
<td>AtPCNA2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys91</td>
<td>ITIKADD</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Lys190</td>
<td>TVDKPED</td>
<td>L</td>
<td></td>
</tr>
<tr>
<td>Lys254</td>
<td>LAPKIEE</td>
<td>H</td>
<td>L</td>
</tr>
<tr>
<td>Lys262</td>
<td>EDTKPE</td>
<td>H</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 3.** Analysis and confirmation of the c-myc-AtPCNA1 WT–His-AtSUMO3 conjugate sumoylation site. (A) The c-myc-AtPCNA1 WT–His-AtSUMO3 conjugate was purified using Ni-NTA followed by a p21-peptide column. Next the sample was separated on a 12% polyacrylamide gel and subjected either to Coomassie Brilliant Blue (CBB) staining (lane 1) or to western blotting (WB) using anti-c-myc antibodies. (B) The structure of the branched c-myc-AtPCNA1 WT–His-AtSUMO3 peptide identified by LC-MS/MS. (C) The AtPCNA1 WT and K254/R mutant were overexpressed in *E. coli* BL21(DE3) cells together with AtSUMO3 (GG) and (AA) variants, followed by purification. Next the samples were separated on a 12% polyacrylamide gel and subjected to western blotting (WB) using anti-c-myc antibodies for sumoylation analysis. Lane 1, AtPCNA1 WT, AtSUMO3 (AA); lane 2, AtPCNA1 WT, AtSUMO3 (GG); lane 3, AtPCNA1K254R mutant, AtSUMO3 (GG). The positions of molecular mass markers are indicated.
neither Lys200 predicted by SUMOsp 2.0, nor Lys91, Lys190, and Lys200 predicted by the SUMO plot software for AtPCNA1 were found to be sumoylated.

Interaction of AtSCE1a and AtPCNAs

Surface plasmon resonance (SPR) analysis was applied to compare the strengths of the interaction between AtPCNA and AtSCE1a that can directly transfer AtSUMO proteins onto a target protein. AtSCE1a was immobilized on a chip and then various concentrations of either AtPCNA1 or 2 were passed over the chip (Fig. 5). The $K_D$ values for the AtPCNA1 and AtPCNA2 interactions with AtSCE1a were calculated using the simple binding Langmuir model (1:1) with a drifting baseline. The calculated $K_D$ values were similar for AtPCNA1 $K_D=2.47 \times 10^{-8}$ M (Fig. 5A) and AtPCNA2 $K_D=9.33 \times 10^{-8}$ M (Fig. 5B). The $K_D$ value for AtPCNA2 was four times higher than the $K_D$ for AtPCNA1. The obtained $K_D$ values may suggest that when both AtPCNA1 and AtPCNA2 are present in the cell, AtPCNA1 might be sumoylated more efficiently than AtPCNA2 due to a higher affinity of AtSEC1a for AtPCNA1.

Discussion

PCNA is an important eukaryotic DNA replication factor. To date, detailed studies of PCNA have been performed mainly on yeast and animal models due to their simplicity. PCNA is generally considered to have the same function and properties in different species of fungi, animals, and plants. Previous structural studies of Arabidopsis PCNA (Strzalka et al., 2009) confirmed its typical three-dimensional structure with a trimeric ring conformation, which is very similar to the structures presented before for yeast, human, and archeal PCNAs (Krishna et al., 1994; Gulbis et al., 1996; Matsumiya et al., 2001). Although differences in the structure of the PCNA from different species are subtle, the results suggest that these minor variations lead to different biological properties of the PCNA from unrelated species. In this study, it was tested whether plant PCNA can be post-translationally modified by SUMO, as already described for yeast (Hoeghe et al., 2002), Xenopus (Leach et al., 2005), and chicken (Arakawa et al., 2006). Previously experiments were carried...
out to try to determine the post-translational modification of plant PCNA using either leaves of green pea or root tips of germinating runner bean embryos as PCNA sources. However, these attempts were unsuccessful (unpublished data). With the present experimental approach, it has been successfully shown that plant PCNA interacts with SUMO under in vivo conditions (Fig. 1). In vivo analysis of the AtSUMO3 (AA) variant which cannot covalently modify the target protein clearly shows that interaction between plant PCNA and SUMO may occur through non-covalent binding in plant cells (Supplementary Fig. S2 at JXB online). This is not surprising in the light of the currently available data where non-covalent interactions between SUMO and other proteins were described (Hecker et al., 2006; Geiss-Friedlander and Melchior, 2007; Kerscher, 2007). It was found here that AtPCNA interacts with SUMO not only in the nucleus but also in the cytoplasm. This result is interesting in the light of current opinion, according to which PCNA is known to play an important role in the nucleus but not in the cytoplasm. It is not known whether PCNA might be sumoylated in the cytoplasm and next imported to the nucleus, or modified in the nucleus independently of cytoplasmic sumoylation. It cannot be ruled out that interaction of AtSUMO with AtPCNA in the cytoplasm may have a function different from that in the nucleus. Moreover, it is also not known how SUMO affects the three-dimensional structure of plant PCNA. The only crystal structure of sumoylated PCNA was presented for yeast, where SUMO seems to occupy a position on the back face of the PCNA ring which is proposed to be a site of regulation. That site can be easily modified without disrupting the reactions ongoing on the front of PCNA, for example normal DNA replication (Freudenthal et al., 2011).

In bacteria analysis of AtPCNA sumoylation

Using an in bacteria reconstituted Arabidopsis sumoylation system described previously (Okada et al., 2009), in this study it was shown that published data concerning the sumoylation of S. cerevisiae PCNA cannot be considered directly applicable to the PCNA from other species, especially plants. A comparison of AtPCNA1 and ScPCNA post-translational modification patterns clearly showed that Arabidopsis PCNA had an altered sumoylation pattern in comparison with yeast PCNA (Fig. 2C). The results of the present ScPCNA studies were partly consistent with data previously presented, when purified recombinant proteins of the Arabidopsis sumoylation system were tested in vitro with yeast PCNA (Colby et al., 2006). In contrast to the present results, several ScPCNA sumoylation products were detected when AtSUMO1 and AtSUMO2 were used (Colby et al., 2006). Their amount gradually decreased as a function of an increasing number of self-sumoylated SUMO molecules attached to ScPCNA. For AtSUMO3, only one modification product was reported (Colby et al., 2006).

Once it had been had proved that plant PCNA could be sumoylated in bacteria, the focus moved to AtPCNA1, and the lysine residue(s) modified by SUMO was identified. Although earlier studies of yeast PCNA showed that Lys127 and Lys164 (Hoege et al., 2002; Pfander et al., 2005; Colby et al., 2006) are post-translationally sumoylated, this information was not of use with regard to AtPCNA. This is caused by the presence of a glycine residue instead of lysine at position 127 of the AtPCNA1 and 2 amino acid sequences. Moreover, Lys164 was excluded as a major modification target, based on data from yeast PCNA studies, where Lys164 (conserved between plant, fungi, and animal PCNAs) was shown to require SUMO E3 ligase (ScSiz1) for efficient sumoylation (Pfander et al., 2005). Using bioinformatic tools (SUMOsp 2.0 and SUMO plot software), the amino acid sequences of A. thaliana PCNA1 and PCNA2 proteins were analysed (Table 1) along with PCNA sequences from other plant species (data not shown). Taking into account: (i) in silico analysis; (ii) the presence of Lys254 in the consensus sequence for SUMO modification (ΨKXE/D); and (iii) data from LC-MS/MS, the sumoylation of the AtPCNA1K254R mutant was tested, and only one modification product (43–55 kDa) was revealed (Fig. 3C). This result indicated that Lys254 was not the only modification site. Although other single variants of AtPCNA1 WT with mutated target sites were not tested, it also cannot be excluded that the lack of other target lysine residues may have an impact on the modification observed between 55 kDa and 72 kDa. It is also noteworthy that both sumoylation products (43–55 kDa and 55–72 kDa) were present in an AtPCNA1 WT variant with Lys164 mutated into arginine (data not shown). It cannot be excluded that the mutation of Lys254 might have changed the affinity of the AtSCE1a–AtSUMO conjugate for the target residue, causing inefficient transfer of AtSUMO from AtSCE1a to the other target lysine. Moreover, if the sumoylation of AtPCNA target residues is a sequential rather than a random process, it cannot be ruled out that Lys254 is not a priority target. Taking into account that the specificity of post-translational modification might be influenced by SUMO E3 ligase, it is possible that this enzyme is necessary for efficient modification of the AtPCNA monomer at more than one site.

To identify the other AtPCNA1 target lysine residues, all AtPCNA1 lysine residues were mutated into arginine and single reverted mutants were constructed. The result of this experiment confirmed that Lys254 was indeed sumoylated. However, unexpectedly, five additional residues were found to be sumoylated in bacteria (Fig. 4). Comparing the identified sumoylated target lysine residues with the results of in silico analysis, it can be concluded that the power of algorithms such as those applied to the identification of putative sumoylation residues in plant proteins is still far from expectations. However, it cannot be ruled out that the proposed lysine residues may additionally require a specific E3 ligase for their modification. Looking at the structure of the AtPCNA1 monomer (Fig. 6A), Lys13, Lys14, and Lys20 are located in the N-terminal domain of AtPCNA1, in the same inner α-helix. Three of the other identified lysine residues are located in the C-terminal domain of AtPCNA1. Lys217 is situated in the helical region, Lys240 in the
β-sheet, and Lys254 is near the C-terminal tail. In contrast to Lys127 and Lys164 of ScPCNA, which are located outside of the PCNA ring (Fig. 6B), Lys13, Lys14, Lys20, and Lys217 of AtPCNA1 are inside the ring in the DNA contact region, whereas Lys240 is close to the loop connecting two β-sheets, and Lys254 is outside of the structure. After in bacteria analysis an attempt was made to confirm sumoylation of identified AtPCNA1 lysine residues in plant cells. Due to a detection sensitivity limit, in vivo sumoylation of selected residues could not be confirmed by western blotting.

**Characteristics of AtPCNA sumoylation products**

Analysing the sumoylation patterns of AtPCNA1 and 2, two modification products were observed (Fig. 2A, B). One working hypothesis suggested that the observed products were AtPCNA monomers sumoylated at different positions. Such a phenomenon has been described for yeast PCNA which, when sumoylated at Lys127, migrated on SDS–PAGE with a different molecular mass in comparison with ScPCNA (pdb: 2ZVV). (B) *Saccharomyces cerevisiae* PCNA (pdb: 1PLQ). The monomers are coloured in green. The interdomain connecting loop is coloured dark blue, and the C-terminus is red. Identified sumoylated target lysine residues are coloured pink. The structures were prepared using PyMol software.

![Fig. 6. PCNA monomer structures with depicted identified sumoylation lysine residues. (A) Arabidopsis thaliana PCNA1 (pdb: 2ZVV). (B) Saccharomyces cerevisiae PCNA (pdb: 1PLQ). The monomers are coloured in green. The interdomain connecting loop is coloured dark blue, and the C-terminus is red. Identified sumoylated target lysine residues are coloured pink. The structures were prepared using PyMol software.](https://academic.oup.com/jxb/article-abstract/63/8/2971/728369/2980-Strzalka-et-al)

In bacteria studies did not reveal any impact of AtSiz1 on AtPCNA1 sumoylation (data not shown) in accordance with unpublished results reported previously by Colby and co-workers for *S. cerevisiae* PCNA (Colby et al., 2006).

**AtPCNA–AtE2 interaction analysis**

Finally, using SPR, the affinity of AtSCE1a for AtPCNA1 and AtPCNA2 was analysed. For both interactions the Langmuir binding model (1:1) with a drifting baseline was used, as in previous experiments, where an analysis of the interaction between human or plant PCNA and human p21 was performed (Oku et al., 1998; Strzalka et al., 2009). The lower $K_D$ value for AtPCNA1 in comparison with AtPCNA2 may suggest that AtPCNA1 could be more efficiently sumoylated than AtPCNA2 in the plant cell. Although the model used for $K_D$ calculation was supported by Biacore software, the data presented should be treated with caution. A possible interaction of the AtSCE1a enzyme with six different lysine residues in AtPCNA1 significantly complicates the model for calculating the $K_D$ value. The fact that the affinity of the AtSCE1a enzyme for AtPCNA was evaluated is not satisfactory. In future, the affinity for each of the six sumoylated lysine residues should be assessed. This may provide crucial information on a possible in vivo AtPCNA sumoylation scenario. It is plausible that there is a difference in the priority of sumoylation between...
identified lysine targets which might be dependent on the physiological status of the plant cell and on the presence of SUMO E3 ligase.

Role of AtPCNA sumoylation

The data show that in bacteria Arabidopsis PCNA has several potential sumoylation lysine residues. This finding is very intriguing and raises two questions. (i) How might AtSUMO regulate the activity of AtPCNAs? (ii) What is the role of AtPCNA interaction with SUMO in vivo? Sumoylation of lysine residues located inside the PCNA ring which are in close contact with DNA suggests interesting new mechanisms of plant PCNA activity regulation which have never been described. Sumoylation of identified residues may affect binding of PCNA to DNA. Such a phenomenon was shown for sumoylated p53 (Wu and Chiang, 2009). Other in vitro studies of Sanchez-Duran and co-workers have shown that sumoylation of plant PCNA is impaired by Tomato golden mosaic virus AL1 protein which is necessary for virus replication (unpublished results, personal communication). These data suggest that SUMO may also affect PCNA binding to DNA. It is suspected that sumoylation of plant PCNA among other functions may be associated with the regulation of DNA replication and repair. The sumoylated residues identified in the present study (Lys13, Lys14, Lys20, and Lys217) are located within the regions which were shown to interact with cyclin D1 and D3 in mouse PCNA (Matsuoka et al., 1994). It cannot be ruled out that sumoylation of these residues has an impact on the interaction between plant PCNA and cyclin Ds. The results from human fibroblast studies showed that microinjection of cyclin D1 antisense accelerated DNA repair, whereas overexpression of cyclin D1 prevented DNA repair, relocation of PCNA after DNA damage, and entrance of fibroblasts into S phase. In contrast, co-expression of PCNA and cyclin D1 restored the ability of cells to repair their DNA (Pagano et al., 1994).

Conclusions

The sumoylation machinery seems to be conserved among different species. This has been shown for the Arabidopsis sumoylation system that could modify ScPCNA at the physiological Lys127 position (Colby et al., 2006). It is intriguing whether the yeast sumoylation system could modify plant PCNA, given the same post-translational modification pattern as that observed for the plant system. Using the present system, it was shown that yeast PCNA cannot serve as a good model for plant PCNA studies, taking into account: (i) the lack of Lys127 in plant PCNA; (ii) different plant PCNA target lysine residues; and (iii) different sumoylation patterns. An identical sumoylation pattern was observed for AtPCNA1 and AtPCNA2 which may suggest equivalent roles for these two proteins in the cellular processes that require sumoylated PCNA. The data presented are just the beginning of plant PCNA sumoylation studies and will require further investigations. Some of them may differ from in vivo results due the fact that the nature of the constructed AtPCNA mutants is not known. For example, it is not known: (i) if mutations of lysine residues to arginine change the PCNA structure; (ii) whether lysine residues in AtPCNA WT may influence each other; and (iii) whether mutations may have an impact on the sumoylation of lysine residues that have not been identified in the present assay as target residues. This will not be simple to resolve, due to the lack of a proper experimental system for such analysis. New tools should be developed to overcome this problem in the future.

Supplementary data

Supplementary data are available at JXB online.

Figure S1. Schematic drawing of the plasmids used in the study.

Figure S2. Control for the BiFC experimental setting.

Table S1. Analysis of sumoylation–overexpression scheme.

Acknowledgements

The project was supported by the Polish Ministry of Science and Higher Education (project no. N N301 474438). FB and CA were supported by the Polish Ministry of Science and Higher Education (project Iuventus Plus, no. IP 2010 039570). WS was supported by the Polish National Centre for Research and Development (project no. LIDER/28/54/ L-2/10/NCBR/2011). CT and KT were supported by a Support Project to Assist Private Universities in Developing Bases for Research by the Ministry of Education, Culture, Sports, Science and Technology. The Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University is the beneficiary of structural funds from the European Union: grant no. POIG.02.01.00-12-064/08. We express our gratitude to Professor Stuible for the pQE70AtSCE1a-His-tag plasmid and Professor Nam-Hai Chua for pMAL-C2X-MBP-tag-AtSiz1 plasmid support. We would like to acknowledge the excellent technical assistance of Jolanta Dluzewska and Maria Pilarska.

References


Hoege C, Pfander B, Moldovan GL, Pyrowolakis G, Jentsch S. 2002. RAD6-dependent DNA repair is linked to modification of PCNA by ubiquitin and SUMO. Nature 419, 135–141.


