Available online at www.sciencedirect.com
ScienceDirect
Plant Physiology and Biochemistry 45 (2007) 113–118
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Short communication

The elongation factor 1A: A novel regulator in the DNA replication/repair protein network in wheat cells?

Magali Toueille [a,1], Bruno Saint-Jean [b,1], Michel Castroviejo [c], Jean-Pierre Benedetto [c,*]

[a] Institute of Veterinary Biochemistry and Molecular Biology, University of Zurich -Irchel, Winthertherstrasse 190, 8057 Zurich, Switzerland
[b] Glycobiologie et Transport Chez les Végétaux, UMR 6037, Université de Rouen, Faculté des Sciences et Techniques, 1, rue Thomas Becket, 76821 Mont-Saint-Aignan cedex, France
[c] Laboratoire Réplication et Expression des génomes Eucaryotes et Rétroviraux, Université Bordeaux 2-CNRS (UMR 5097), Bât 3A, 146, rue Léo Saignat, 33076 Bordeaux cedex, France

Received 28 August 2006; accepted 15 January 2007
Available online 20 January 2007

Abstract

Proliferating cell nuclear antigen (PCNA) is a DNA sliding clamp interacting with multiple partners in DNA transactions such as DNA replication/repair and recombination as well as chromatin assembly. We previously detected and purified by chromatographic procedures a 31 kDa PCNA from cultured wheat cells (*Triticum monococcum L*). Here we report the complete sequence of the wheat 31 kDa PCNA showing a very high aminoacid identity with its plant counterparts (maize and rice). This recombinant PCNA has been used as a bait in an affinity chromatography procedure, in order to capture PCNA interacting proteins. We detected by liquid chromatography, tandem mass spectrometry and search in plant protein databases, several specific bands from wheat cell lysates in fractions bound to wheat PCNA-affinity column. One of them is the wheat elongation factor 1A. Its putative regulatory role in DNA replication/repair is discussed.

Keywords: PCNA; DNA replication; Plant; Translation factor; Proteomic; Proteasome; Plant

1. Introduction

Eukaryotic DNA replication/repair, recombination and chromatin remodelling, as well as cell cycle control are processed by a network of proteins cooperating accurately. A pivotal protein in these processes is the proliferating cell nuclear antigen (PCNA) which belongs to the sliding clamp family [9,13]. PCNA has been described at first as a processivity factor of the replicative DNA polymerase delta in eukaryotic DNA replication [2]. More recently, the identification of many PCNA interacting proteins from animals and yeasts showed the diverse spectrum of PCNA functions in DNA metabolism (for reviews see refs. [12,19,21]).

Although present in plant cells, data concerning PCNA are scarce [4,24,25]. However, PCNA polypeptide sequence has been shown to be very highly conserved among plants [17]. In a previous work performed in our laboratory [20], we detected two wheat PCNA polypeptides (a short highly expressed 31 kDa type and a long low expressed 37 kDa one). A comparative analysis of their partial nucleotide sequences showed that the 31 kDa PCNA represented the classical PCNA. Here we present the complete coding sequence of this PCNA showing a very high degree of amino-acid identity with other plant PCNAs. Expressed as a recombinant protein, this PCNA has been used as bait in order to capture wheat factors by a PCNA affinity column. In this report, we identified by mass spectrometry and protein databases search a new PCNA- binding protein with a very high reliability score: elongation factor 1A (EF1A). Its putative role in the regulation of DNA replication/repair is proposed.

* Corresponding author. Tel.: +33 5 5757 1758; fax: +33 5 5757 1766.
E-mail address: jean-pierre.benedetto@reger.u-borbeaux2.fr (J.-P. Benedetto).
[1] M. Toueille and B. Saint-Jean contributed equally to this work and should be considered as first authors.

0981-9428/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.plaphy.2007.01.006

2. Results

2.1. Wheat PCNA cDNA analysis

As PCNA appears highly conserved in plants at both amino acid and nucleotide levels, we designed two sets of degenerated nested PCR primers fitting with the nucleotide sequence of rice and maize PCNA. In a previous work [20], we cloned two PCNA 3′ nucleotide sequences from wheat cell total RNA. These two different PCNAs named PCNA 1 and PCNA 2 differ by a shorter 3′ untranslated region and at amino acid level by an extra heptapeptide sequence and at six different amino acid positions. Using another couple of PCR primers (see Section 4) we obtained the complete coding sequence of the highly expressed wheat short PCNA 1. Alignment of the nucleotide and amino acid sequence of this wheat PCNA with rice and maize PCNA showed a 93% amino-acid identity with punctual differences interspersed all along the amino-acid sequence (Fig. 1). The size, the amino-acid sequence and a high expression in the dividing wheat cell culture indicate that the 31 kDa polypeptide represents the ‘‘typical’’ wheat PCNA.

2.2. Proteomic analysis

The complete cDNA sequence of PCNA 1 fused to a His-tag sequence at its 3′ end has been expressed using a bacterial vector (see Section 4). The pool of the soluble proteins containing the recombinant PCNA has been injected on a nickel affinity column in the presence of 30 mM imidazole to reduce the non-specific interactions. The retained fraction was eluted with 0.25 M imidazole. After injection on a mono Q column, the retained proteins were eluted at 0.5 M NaCl (see Section 4). The PCNA purification scheme is indicated in Fig. 2. Five mg of the highly purified recombinant PCNA were used to prepare a NHS-Hitrap PCNA affinity column. Most of the purified PCNA covalently bound to the column. In parallel to this PCNA affinity column, we prepared an *E. coli* cell extract column as well as the classical BSA column, both used as negative controls.

The S100 lysate from wheat cells was loaded on the PCNA affinity column (Fig. 3). The retained fractions were eluted by salt step and analysed by SDS-PAGE electrophoresis. The bands were analysed after trypsin digestion followed by mass spectra analysis (see Section 4). Fraction F1 represents the pool of proteins retained on the PCNA affinity column and eluted at 600 mM NaCl. However, the non-retained fraction may contain some PCNA interacting proteins if all the sites are saturated by major PCNA interacting proteins. For this reason, the non-retained fraction was subsequently injected on a second PCNA affinity column. In order to fractionate the PCNA interacting factors, we eluted the retained fractions by two steps (300 mM NaCl (F2) and 600 mM NaCl (F3)). The Fig. 3 summarizes this affinity purification/mass spectrometry approach.

Analysis of the retained proteins using protein profiling by shot gun proteomics allowed us to identify the PCNA interacting proteins presented in Table 1. The major factor identified was the translation elongation factor, EF1A. Other isolated factors, such as the chromatin assembly (CAF1), nucleosome restructuring (NAP, chromatin remodelling complex ATPase) and DNA replication licensing (MCM) factors are involved in DNA transactions. The chaperones HSP 80, HSP70 and their co-factors Dnaj and DnaK, as well as the 14-3-3 protein kinase regulator, were also identified as PCNA interacting proteins. Several of these factors were already identified as PCNA interactors [19,21]. Moreover Table 1 only presents the proteins that were found to interact exclusively with the PCNA-affinity column and not with the BSA and *E. coli* extract control columns, thus confirming the specificity of the identified PCNA interacting proteins. We also identified a band corresponding to PCNA, which can be explained by its ability to form a trimer rather than by leakage from the column, as no PCNA was released from the column by washing at 1 M NaCl. The presence of the wheat homolog of ribosomal proteins PO and S3 can be related to their extra-ribosomal functions [22], as the human 60S ribosomal protein PO and 40S ribosomal S3 were shown to exert an AP endonuclease activity in DNA repair [8,10].

The major band among the proteins retained by the PCNA column detected in SDS-PAGE at the first round (F1) was the 52 kDa wheat elongation factor EF1A. The presence of this major protein was assessed by four experiments and was in each case absent from the BSA column and from the *E. coli extract* columns

3. Discussion

Expressed with a histidine tag, the wheat recombinant PCNA 1 has been purified by IMAC and ion exchange chromatography. Bound covalently on a column, this PCNA retained specifically some wheat cell factors involved in DNA transactions such as CAF-1 and NAP which mediate assembly of spaced nucleosomal arrays with DNA, core histones and ATP. It retained also factors involved in cell cycle control (CDC48, DNA replication licensing factor, HSP70).

Although pertinent to detect new associated factors in the protein complex interacting with a PCNA affinity-column, our approach did not reveal some expected partners known as PCNA interacting proteins such as subunits of the replicative DNA polymerase delta. This can be explained by the lack of data concerning DNA polymerases in the wheat DNA sequence database. Moreover a review listing methods by which PCNA interacting proteins in eukarya were shown, indicates that different partners have been detected by different methods (yeast-two hybrid, GST-pull-down, far western-blot, Co-immunoprecipitation, PCNA affinity-column…) [21]. Our major finding concerns the capture of the wheat elongation factor 1A with a very high reliability mass score. In view of the data presented in this manuscript, we propose that EF1A may be involved in the DNA replication fork where the sliding clamp PCNA play a pivotal role. It should be noted that this factor has been captured by a yeast two-hybrid strategy using as bait the wheat homolog of the large Human RF-C subunit [18]. What could be the role of EF1A in the context of DNA replication fork? Some recent published data may give