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Arabidopsis thaliana

Proliferating cell nuclear antigen 1 and 2 possibly form homo- and hetero-trimeric complexes in the plant cell

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The proliferating cell nuclear antigen (PCNA) is a key component of the eukaryotic DNA replication machinery. It also plays an important role in DNA repair mechanisms. Despite the intense scientific research on yeast and human PCNA, information describing the function of this protein in plants is still very limited. In the previous study *Arabidopsis* PCNA2 but not PCNA1 was proposed to be functionally important in DNA polymerase η-dependent postreplication repair. In addition to the above study, PCNA2 but not PCNA1 was also shown to be necessary for *Arabidopsis* DNA polymerase λ-dependent oxidative DNA damage bypass. Taking into account the reported differences between PCNA1 and PCNA2, we tested the idea of a possible cooperation between PCNA1 and PCNA2 in the plant cell. In a bimolecular fluorescence complementation assay an interaction between PCNA1 and PCNA2 was observed in the nucleus, as well as in the cytoplasm. This finding, together with our previous results, indicates that PCNA1 and PCNA2 may cooperate in planta by forming homo- and heterotrimeric rings. The observed interaction might be relevant when distinct functions for PCNA1 and PCNA2 are considered.

The proliferating cell nuclear antigen (PCNA) is a conserved protein encoded in the genomes of archaebacteria and eukaryotic organisms.[1] The lethal phenotype of *pcna* knockouts indicates the significance of this protein in yeast, animals and plants. PCNA is an important component of the DNA replication machinery. It forms a pseudo-6-fold symmetry ring around DNA and acts as a DNA polymerase δ processivity factor.[2-5] The gene sequences coding for PCNA have been identified and reported for diverse species, including human,[6] mouse,[7] yeast,[8] rice,[9] common bean[10] and runner bean.[11] Moreover, in some of them, for instance Drosophila,[12,13] *Arabidopsis*[14] and maize,[15,16] two *pcna* genes were discovered. The structural similarities and conservation between PCNA proteins from different eukaryotes including yeast,[5] human[17] and *Arabidopsis*[18] were confirmed in crystallographic studies.

Thirty years of PCNA research resulted in the identification of a great number of PCNA interacting proteins, which also include enzymes involved in post-translational modifications of PCNA.[19-22] One of the broadly studied PCNA post-translational modifications is ubiquitination.[20,22] The stalled replication forks induce PCNA ubiquitination at lysine 164 and activate postreplication repair.[23] PCNA monoubiquitination leads to error-prone translesion synthesis, and polyubiquitination to error-free template switch combined with recombination.[23] In our recent study, using a ubiquitination assay, we observed a similar ubiquitination pattern at lysine 164 for *Arabidopsis* PCNA1 and PCNA2.[22] Based on the fact that PCNA1 and PCNA2 demonstrate 96.6% identity at the amino acid level, this result was not surprising. However, the high similarity between PCNA1 and PCNA2 brings up the question of their functional relevance in the cell. Interestingly, the data from Anderson and coworkers[24] pointed to the functional difference between *Arabidopsis* PCNA proteins in the context of plant postreplication repair. Using a yeast system they have shown that PCNA2, but not PCNA1, could functionally interact with the *Arabidopsis* translesion DNA polymerase η. To explain the observed differences it was proposed that the ubiquitination of PCNA1 at lysine 164 might be inhibited.[24] However, this suggestion was not confirmed in our *Arabidopsis* PCNA ubiquitination studies.[22] In addition to Anderson's report, *Arabidopsis* DNA polymerase λ, together with PCNA2 but not PCNA1, was also shown to be required for oxidative DNA damage bypass.[25] Taking into account the reported differences between PCNA1 and PCNA2[24,25] we applied a bimolecular fluorescence complementation assay to examine whether the two proteins can interact in the plant cell. Such cooperation could combine different features of PCNA1 and PCNA2 by the formation of a heterotrimeric ring. Selected combinations of PCNA1, PCNA2 and CycA1 (BiFC control) fused with full length, N-terminal or C-terminal GFP fragments were transiently overexpressed in *Nicotiana benthamiana* epidermal cells.[21,26] The results of this

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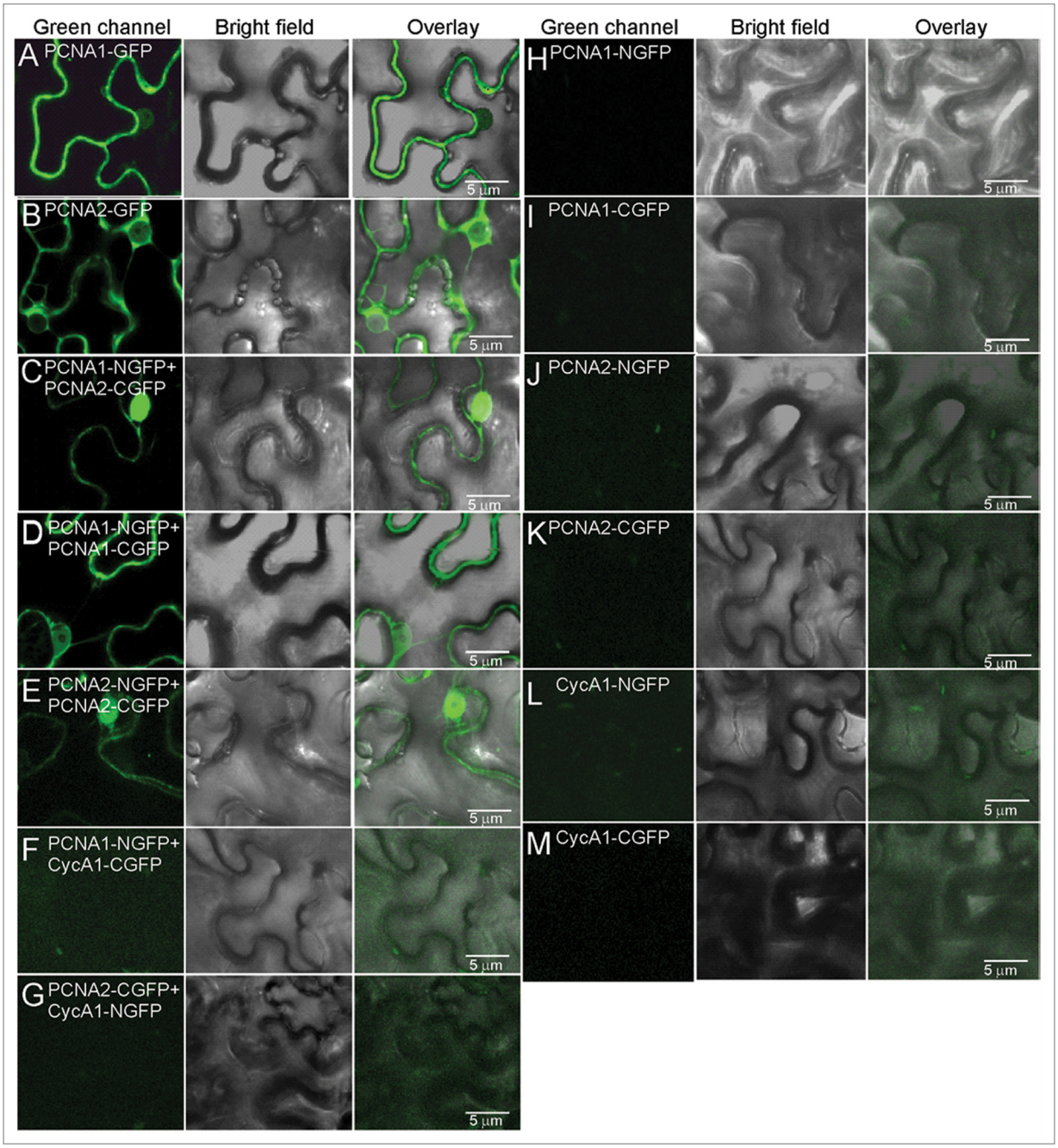

Figure 1. Analysis of PCNA1 and PCNA2 interaction using a bimolecular fluorescence complementation assay. Confocal images showing transient expression of: (**A**) PCNA1-GFP, (**B**) PCNA2-GFP, (**C**) split PCNA1-NGFP and PCNA2-CGFP, (**D**) split PCNA1-NGFP and PCNA1-CGFP, (**E**) split PCNA2-NGFP and PCNA2-CGFP, (**F**) split PCNA1-NGFP and CycA1-CGFP, (**G**) split PCNA2-CGFP and CycA1-NGFP, (**H**) PCNA1-NGFP, (**I**) PCNA1-CGFP, (**J**) PCNA2-NGFP, (**K**) PCNA2-CGFP, (**L**) CycA1-NGFP and (**M**) CycA1-CGFP in epidermal cells of *Nicotiana benthamiana*. Bar = 5 μm.

experiment showed an interaction between PCNA1-PCNA1, PCNA2-PCNA2 and most interestingly also between PCNA1-PCNA2, but not between PCNA1/2 and CycA1 (**Fig. 1**). The fluorescence signal was observed both in the nucleus and the cytoplasm. Based on the current knowledge which restricts the role of PCNA only to the nuclear compartment, the interaction observed in the cytoplasm might result from inefficient transport of PCNA to the nucleus or the overexpression of PCNA. The detected interaction between PCNA1 and PCNA2 in *N. benthamiana* cells is in agreement with the results of our previous study where we showed that recombinant PCNA1 and PCNA2 were able to form a heterotrimer.[18]

In conclusion, the presented data support our idea that PCNA1 and PCNA2 heterotrimerization/cooperation can occur in the plant nucleus. This finding seems to be relevant especially when differential roles of PCNA1 and PCNA2 are considered. Due to the fact that our understanding of PCNA-dependent mechanisms in *Arabidopsis* is still very limited, a *pcna1* and *pcna2*

knockout analysis would be advisable to study in detail the functional relevance of these two genes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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