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Regulation and spatial organization of PCNA in *Trypanosoma brucei*

Doris Kaufmann [a,2,3], Alwine Gassen [a,3], Andreas Maiser [b], Heinrich Leonhardt [b], Christian J. Janzen [a,1,*]

[a] University of Munich (LMU), Department Biology I, Genetics, Großhaderner Str. 2-4, 82152 Martinsried, Germany
[b] University of Munich (LMU), Department Biology II, Großhaderner Str. 2-4, 82152 Martinsried, Germany

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ABSTRACT

As in most eukaryotic cells, replication is regulated by a conserved group of proteins in the early-diverged parasite *Trypanosoma brucei*. Only a few components of the replication machinery have been described in this parasite and regulation, sub-nuclear localization and timing of replication are not well understood. We characterized the proliferating cell nuclear antigen in *T. brucei* (TbPCNA) to establish a spatial and temporal marker for replication. Interestingly, PCNA distribution and regulation is different compared to the closely related parasites *Trypanosoma cruzi* and *Leishmania donovani*. TbPCNA foci are clearly detectable during S phase of the cell cycle but in contrast to *T. cruzi* they are not preferentially located at the nuclear periphery. Furthermore, PCNA seems to be degraded when cells enter G2 phase in *T. brucei* suggesting different modes of replication regulation or functions of PCNA in these closely related eukaryotes.

1. Introduction

One of the most conserved biological processes is the accurate duplication and segregation of the genome in order to pass on the entire genetic information to the daughter cell before cell division. To ensure only one duplication per cell cycle, a carefully regulated course of events has to be spatially and temporally coordinated.

To enable DNA replication, the pre-replicative complex has to assemble at defined DNA sites, the origins of replication. The components of the pre-replicative complex are conserved among many eukaryotes and include the origin recognition complex (Orc1-6), cell division cycle 6 (Cdc6), the replication factor Cdt1 and the mini-chromosome maintenance proteins (Mcm2-7). After licensing of the origin additional factors are recruited and full activation is achieved by unwinding of DNA, formation of the replication fork and association of replicative DNA polymerases (reviewed in [1]). A central component of the replication machinery is the proliferating cell nuclear antigen (PCNA), a cofactor of DNA polymerase that encircles DNA and maintains association of polymerases to DNA. PCNA is a member of the family of DNA sliding clamps and forms homotrimeric rings, which are able to slide in both directions along the DNA. The proteins provide replicative polymerases with the high processivity necessary for efficient duplication of the genome. Furthermore, PCNA interacts as moving platform with many different associated factors often containing a conserved PCNA-interacting protein (PIP) motif. Through these interactions PCNA is also involved in other important cellular processes such as DNA repair, chromatin assembly and cell cycle control (reviewed in [2,3]).

DNA replication takes place in distinct areas in the mammalian nucleus, the replication foci, where DNA is associated with the replication machinery [4–6]. PCNA was the first protein shown to co-localize with incorporated 5-bromodeoxyuridine (BrdU) at replication foci [7]. By exploiting the periodic appearance in S phase cells, PCNA is widely used as a cellular marker for replication sites and has been used to study the dynamics of DNA replication factories in living cells [8].

Many orthologues of conserved eukaryotic cell cycle regulators are also present in *Trypanosoma brucei*, the causative agent of African trypanosomiasis. However, some mechanisms and structures seem to be unique to trypanosomes. For example replication and segregation of the kinetoplast, which contains the mitochondrial DNA, precedes that of the nucleus. Thus, the configuration and morphology of kinetoplasts and nuclei has been used as a marker for the approximate position of individual cells in the cell cycle [9]. To improve classification a subsequent study used quantification of the DNA content of cells in different cell cycle stages [10]. It revealed that many cells with one nucleus and an elongated kinetoplast (1NeK) were still in S phase, which was contradictory to previous studies that classified 1NeK as G2 phase cells [9]. In summary, the analysis of morphology or configuration of nuclei and kinetoplasts provides an approximate classification of different cell cycle stages, a precise definition of S phase cells is, how-

* Corresponding author.
E-mail address: christian.janzen@uni-wuerzburg.de (C.J. Janzen).
[1] Current address: Department of Cell and Developmental Biology, Biocenter, University of Wuerzburg, Am Hubland, 97074 Wuerzburg, Germany.
[2] Current address: Walter Brendel Centre of Experimental Medicine (LMU), Schillerstr. 44, 80336 Munich, Germany.
[3] These authors contributed equally to this work.

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ever, not possible. Thus, there is need for a nuclear DNA replication marker to learn more about the spatial organization and regulation of replication in *T. brucei*.

Only a few studies have been performed to investigate replication in trypanosomatids. In *Trypanosoma cruzi*, an Orc1 homologue (Orc1/Cdc6) seems to be constitutively expressed and is associated with chromatin throughout the cell cycle [11]. Replication sites were identified by incorporation of BrdU in the nuclear periphery at the beginning of S phase. Later in the cell cycle, these sites were observed in the interior of the nucleus due to chromosome rearrangement or movement [12]. The temporal and spatial distribution of TcPCNA seems to be different compared to higher eukaryotes [13]. TcPCNA is constrained to the nuclear periphery during S phase where it co-localizes in distinguishable foci with TcOrc1/Cdc6. During all other phases of the cell cycle it is visible in a dispersed pattern throughout the nucleus [13]. In *Leishmania* PCNA can be detected primarily in the nucleus throughout the cell cycle although expression levels and pattern seem to vary [14,15]. Maximum expression was observed in G1 and S phase with subnuclear foci in S phase and a more diffuse pattern in G2/M phase and after mitosis suggesting that replication factories similar to higher eukaryotes exist.

In *T. brucei*, re-localization of chromosomes during S phase has not been observed. Visualization of replication has only been achieved by BrdU incorporation, which could distort localization due to the harsh protocol and the temporal appearance of replication sites cannot easily be addressed because of long BrdU labeling periods.

Recently, Dang and colleagues characterized many components of the regulatory complex at origins of replication in *T. brucei* [16], however, PCNA was not included.

Therefore, we exploited TbPCNA as a tool to investigate the localization and exact time frame of DNA replication in the nucleus of a single cell. Unexpectedly, we observed differences in distribution and regulation of TbPCNA compared to the closely related parasites *T. cruzi* and *Leishmania donovani*. In *T. brucei* PCNA is clearly detectable during S phase of the cell cycle, however, it seems to be degraded when cells enter G2 phase, which is in contrast to the situation found in *T. cruzi* and *Leishmania*. In addition, using high-resolution microscopy, we observed that replication sites are not restricted to the nuclear periphery. These findings establish TbPCNA as a suitable marker for replication in *T. brucei* and point to different mechanisms of replication regulation that evolved within the group of trypanosomatids.

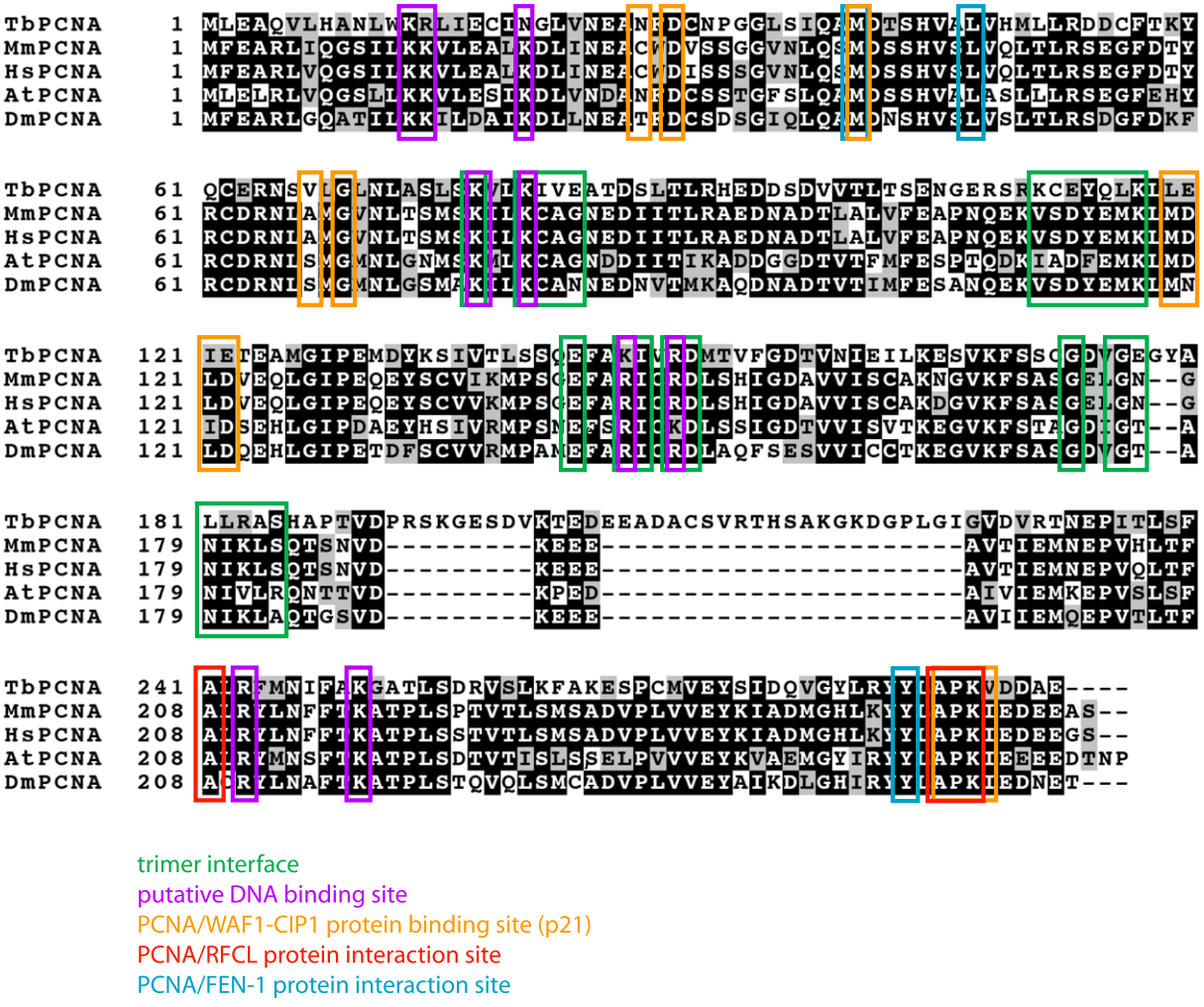

Fig. 1. Amino acid alignment of PCNA from *T. brucei* (TbPCNA, Tb09.160.3710) with PCNA from other organisms. Mm: *M. musculus*, Hs: *H. sapiens*, At: *A. thaliana*, Dm: *D. melanogaster*. Black background indicates sequence identity, gray background indicates sequence similarity. Colored boxes mark conserved domains. Motifs important for trimer interface (green), DNA binding (purple) and interaction with known proteins such as WAF1-CIP1 (orange), RFCL (red) and FEN-1 (blue) are shown. Alignments and shading were done with ClustalW2 (http://www.ebi.ac.uk/Tools) and Boxshade 3.21 (http://www.ch.embnet.org). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)