Molecular & Biochemical Parasitology 193 (2014) 110–113

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Short communication

ORC1/CDC6 and MCM7 distinct associate with chromatin through *Trypanosoma cruzi* life cycle

Simone Calderano[a,c], Patricia Godoy[a], Daiane Soares[a,c], Osvaldo Augusto Sant'Anna[b,c], Sergio Schenkman[d], M. Carolina Elias[a,c,*]

[a] *Laboratório Especial de Ciclo Celular, Instituto Butantan, São Paulo, Brazil*
[b] *Laboratório de Imunoquímica, Instituto Butantan, São Paulo, Brazil*
[c] *Center of Toxins, Immune Response and Cell Signaling – CeTICS, Brazil*
[d] *Departamento de Micro, Imuno e Parasitologia, Universidade Federal de São Paulo, Brazil*

ARTICLE INFO

Article history:
Received 27 December 2013
Received in revised form 17 February 2014
Accepted 17 March 2014
Available online 25 March 2014

Keywords:
DNA replication
ORC1/CDC6
MCM7
Trypanosoma cruzi

ABSTRACT

Trypanosoma cruzi alternates between replicative and non-replicative stages. We analyzed the expression of components of the pre-replication machinery TcORC1/CDC6 and TcMCM7 and their interaction with DNA in all *T. cruzi* stages. TcORC1/CDC6 remains in the nuclear space during all stages of the life cycle and interacts with DNA in the replicative stages; however, it does not bind to DNA in the non-replicative forms. Moreover, TcMCM7 is not present in the non-replicative stages. These data suggest that the lacking of DNA replication during the *T. cruzi* life cycle may be a consequence of the blocking of TcORC1/CDC6–DNA interaction and of the down regulation of the TcMCM7 expression.

Trypanosoma cruzi, the etiological agent of Chagas' disease, is an early divergent protozoon eukaryote. During its life cycle, it alternates between replicative and non-replicative stages in insect and mammalian hosts. In the insect vector, the replicative epimastigote stage differentiates into the non-replicative/infective metacyclic trypomastigote form, and in mammalian hosts, the replicative amastigote differentiates into non-replicative/infective trypomastigote stage. Although *T. cruzi* has been extensively studied since its discovery more than one century ago, the molecular basis for the lack of DNA replication in non-replicative forms remain to be determined.

DNA replication is initiated with the assembly of the pre-replication complex (PRC) at the origins of replication. In eukaryotes, the PRC is composed of an ORC (origin recognition complex), a six-subunit complex (Orc1-6) that recruits Cdc6 to the replication origin in late M-phase, and the MCM (mini-chromosome maintenance) helicase, which is comprised of Mcm2-7 and is loaded by the ORC–Cdc6 complex with Cdt1 onto DNA [1]. During this loading reaction that occurs during the G1 stage, Cdt1 is released from the DNA while MCM encircles double-stranded DNA [2,3]. During S-phase, the MCM helicase is activated by the interaction with Cdc45 and the GINS (from the Japanese go-ichi-ni-san) complex. The assembly of Cdc45 and GINS converts the PRC into a pre-initiation complex. The origins of replication are unwound, and the replicative DNA polymerases as well as the regulatory factors of the replication machinery are recruited, which allows for the establishment of replication forks [1]. The blockage of DNA replication has been studied in detail during the cell cycle, which is when re-replication might be avoided during and after S phase. As the PRC determines the sites where replication will occur, an effective method to block DNA re-replication is through inactivating the pre-replication machinery or by down regulating its expression or activity when DNA replication is initiated. The inactivation of the PRC during and after S phase occurs through different mechanisms in different organisms. In yeast, the ORC subunits are bound to chromatin throughout the cell cycle; however, in mammalian cells, the Orc1 subunit is degraded in S phase by a CDK-dependent polyubiquitination reaction [4], and phosphorylation of Orc2 dissociates the ORC from chromatin [5]. In yeast, Cdc6 is phosphorylated by cyclin-dependent kinases (CDK) and degraded by proteolysis at the onset of the G1-S phase transition, whereas mammalian Cdc6 remains bound to chromatin throughout the cell cycle. In addition, MCM are exported from the nucleus during S phase, G2, and early mitosis in yeast, which prevents the licensing of new origins in non S-phase stages (reviewed in [6]).

* Corresponding author at: Laboratório Especial de Ciclo Celular, Intituto Butantan, São Paulo, Brazil. Tel.: +55 11 3726 1024; fax: +55 11 3726 1024.
E-mail address: carolina.eliassabbaga@butantan.gov.br (M.C. Elias).

http://dx.doi.org/10.1016/j.molbiopara.2014.03.004
0166-6851/

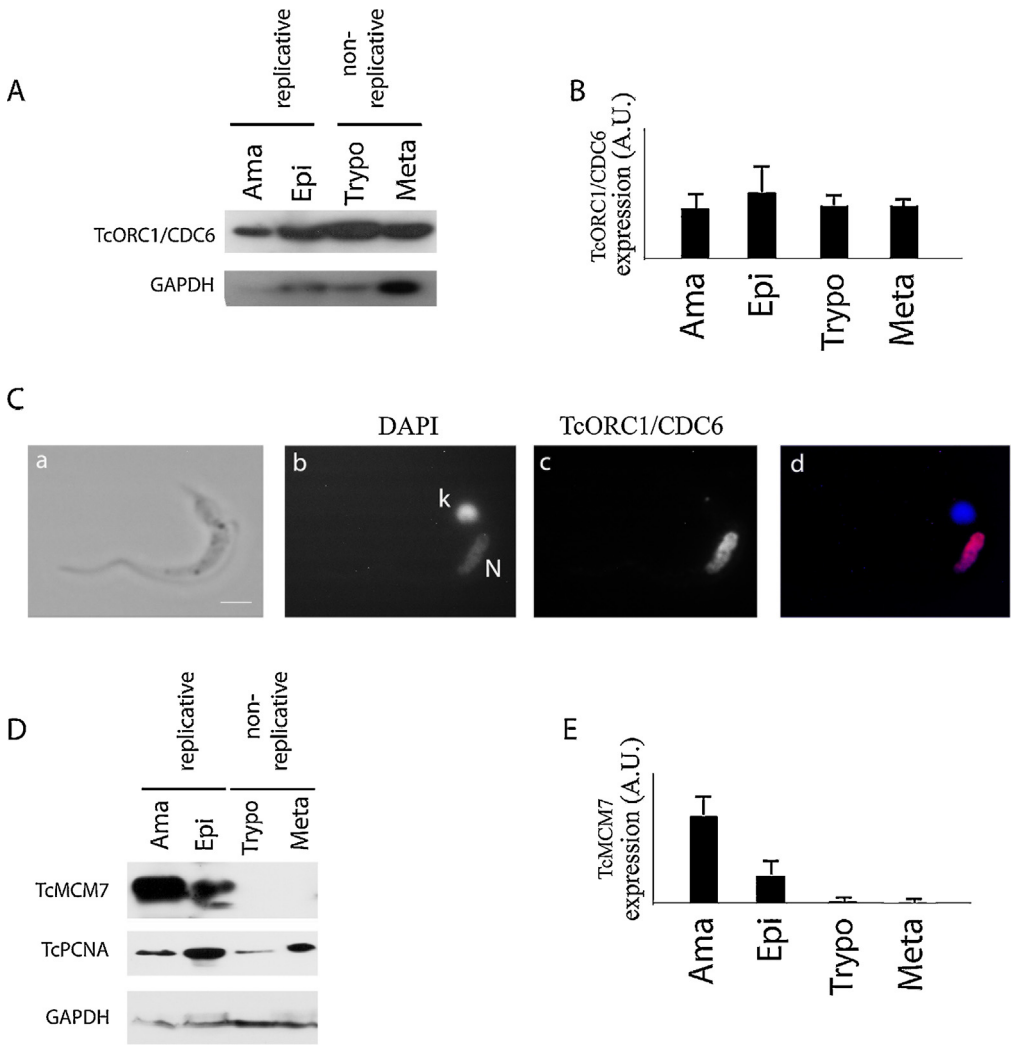

Fig. 1. TcORC1/CDC6, but not TcMCM7, is expressed during the life cycle of *T. cruzi*. Epimastigote forms were maintained in liver-infusion tryptose medium containing 10% fetal bovine serum at 28 °C. Metacyclic-trypomastigotes were obtained by metacyclogenesis. Intracellular amastigotes and trypomastigotes were obtained from infected LLCMK2 cells (American Type Culture Collection; Rockville, MD). Trypomastigotes were collected from the extracellular medium 5–7 days after infection and the intracellular forms were obtained from the 4 days infected cells by scraping the monolayer with a Teflon policeman in a buffer containing 27 mM K_2HPO_4, 8 mM Na_2HPO_4, and 26 mM KH_2PO_4 (pH 7.2). (A) Total proteins from the replicative amastigote (Ama) and epimastigote (Epi) stages and non-replicative trypomastigote (Trypo) and metacyclic trypomastigote (Meta) stages were extracted and submitted to SDS-PAGE analysis, which was followed by Western blotting using anti-rTcORC1/CDC6 [7] and anti-GAPDH antibodies [18]. (B) The intensity of the bands detected in (A) with anti-rTcORC1/CDC6 antibodies was quantified using ImageJ and normalized to GAPDH expression. The graph shows the median and standard deviation from three independent experiments. (C) Non-replicative trypomastigotes were fixed with 2% paraformaldehyde, permeabilized with Triton X-100, incubated with anti-rTcORC1/CDC6 (red) and stained with DAPI (blue). K-kinetoplast, N-nucleus. Bar is 1 μm. (D) Proteins from replicative amastigotes (Ama) and epimastigotes (Epi) as well as non-replicative trypomastigotes (Trypo) and metacyclic trypomastigotes (Meta) were submitted to SDS-PAGE, which was followed by Western blotting using anti-rTcMCM7, anti-rTcPCNA [19] and anti-GAPDH antibodies. (E) The intensity of the bands detected in (D) with anti-rTcMCM7 antibodies was quantified using ImageJ and normalized to GAPDH expression. The graph shows the median and standard deviation from three independent experiments.

The trypanosome PRC contains TcORC1/CDC6 (annotated as ORC1 – TcCLB.508239.10), which is a molecule homologous to Orc1 and Cdc6, which associates with DNA during the entire cell cycle of *T. cruzi* [7]. A highly divergent ORC4 was characterized in *Trypanosoma brucei*, and its ortholog was found in *T. cruzi* [8]. Orc2-3, Orc5-6, Cdc6 and Cdt1 were not found in the *T. cruzi* genome database. All MCM helicase subunits, MCM2 to MCM7, are annotated in the *T. cruzi* genome database (tritrypdb.org). Blasting MCMs from *T. cruzi S. cerevisiae* genome and blasting back *S. cerevisiae* Mcms against *T. cruzi* genome it was possible to find MCMs homologous to all *S. cerevisiae* MCM subunits (Fig. S1A). *T. cruzi* MCM helicase subunits contain the conserved P-loop containing nucleoside triphosphate hydrolase and the AAA+ ATPase domain identified by InterProScan5 tool (Fig. S1B). These functional motifs are all involved in nucleotide binding and hydrolysis and the presence of multiple MCMs strong suggests that these organisms use a heterohexamer helicase as expected for organisms from eukaryotic group. In fact, MCM2-7 were identified in the related kinetoplastid *T. brucei*, where inter-MCM subunit interactions have been examined and are consistent with such an arrangement [8].

Supplementary Fig. S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molbiopara.2014.03.004.

Because *T. cruzi* alternates between replicative and non-replicative stages, we asked if TcORC1/CDC6 and TcMCM, components of PRC and PCNA that is a marker of replication machinery [9] were present exclusively in dividing forms. We used the Y strain of *T. cruzi*. We extracted total protein content from all stages of *T. cruzi* life cycle. As shown in Fig. 1A and 1B TcORC1/CDC6 was found similarly expressed in all stages. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control. In addition, TcORC1/CDC6 is present in the nuclear