International Journal for Parasitology 41 (2011) 405–416

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Poly(ADP-ribose) polymerase plays a differential role in DNA damage-response and cell death pathways in *Trypanosoma cruzi*

Salomé C. Vilchez Larrea [a], Guillermo D. Alonso [a,b], Mariana Schlesinger [a], Héctor N. Torres [a,b], Mirtha M. Flawiá [a,b], Silvia H. Fernández Villamil [a,c,*]

[a] *Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Consejo Nacional de Investigaciones Científicas y Técnicas, Vuelta de Obligado 2490, Ciudad Autónoma de Buenos Aires, 1428 Buenos Aires, Argentina*
[b] *Departamento de Fisiología, Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina*
[c] *Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina*

ARTICLE INFO

Article history:
Received 26 October 2010
Accepted 5 November 2010
Available online 23 December 2010

Keywords:
Trypanosoma cruzi
PARP
DNA repair
PARG
Cell death

ABSTRACT

Poly(ADP-ribosyl)ation is a post-translational modification of proteins. Poly(ADP-ribose) polymerase (PARP) and poly(ADP-ribose) glycohydrolase (PARG) are the enzymes responsible for poly(ADP-ribose) (PAR) polymer metabolism and are present in most higher eukaryotes. The best understood role of PARP is the maintenance of genomic integrity either via promotion of DNA repair at low levels of genotoxic stress or via promotion of cell death at higher levels of damage. The unicellular eukaryote *Trypanosoma cruzi*, as opposed to humans and other organisms, has only one PARP (TcPARP) and one PARG (TcPARG). In the present study we show that under different DNA-damaging agents (H_2O_2 or UV-C radiation) TcPARP is activated and translocated from the cytosol to the nucleus, while TcPARG always shows a nuclear localisation. Parasites in the presence of PARP or PARG inhibitors, as well as parasites over-expressing either TcPARP or TcPARG, suggested that PAR metabolism could be involved in different phases of cell growth, even in the absence of DNA damage. We also believe that we provide the first reported evidence that different proteins could be poly(ADP-ribosyl)ated in response to different stimuli, leading to different cell death pathways.

1. Introduction

In order to ensure accurate transmission of genetic information in dividing cells, it is important that specific biochemical pathways maintain genomic integrity. Organisms have evolved to efficiently respond to DNA insults that result from either endogenous sources, such as cellular metabolic processes, or exogenous sources such as environmental factors. Among endogenous sources of DNA damage, metabolites capable of causing alkylation or oxidation of nucleotides and mismatches of DNA bases occurring during replication are the most common. Exogenous sources of DNA damage include ionising radiation (IR), ultraviolet radiation (UV) and chemical agents. Damaged DNA that is not properly repaired can lead to genomic instability, apoptosis or senescence, which can greatly affect the development of the organism. Therefore, it is essential for cells to efficiently respond to DNA damage through coordinated and integrated DNA-damage checkpoints, signalling and DNA-repair pathways (Satoh and Lindahl, 1992; D'Amours et al., 1999; Szabó, 2000; Assuncao Guimaraes and Linden, 2004; Lockshin and Zakeri, 2004a; Burkle, 2005; Malanga and Althaus, 2005; Hassa et al., 2006; Jeggo and Lobrich, 2006a,b; O'Driscoll and Jeggo, 2006; Schreiber et al., 2006). The recognition of the damage by specific proteins is the first step in this pathway. PARP-1, which is one of the most characterised DNA break-sensor enzymes, rapidly binds and becomes activated by DNA strand breaks (sbDNA), and subsequently modifies itself and other target proteins with branched chains of poly(ADP-ribose) (PAR) of up to several hundred ADP-ribose units. The binding as well as the activity of PARP-1 at the sbDNA is transient, because poly(ADP-ribosyl)ated PARP-1 rapidly dissociates from DNA and PAR polymer is degraded mainly by poly(ADP-ribose) glycohydrolase (PARG) (D'Amours et al., 1999; Bonicalzi et al., 2005). Damage detection, transduction of signals emanating from DNA interruptions and signal amplification by PAR formation are the main characteristics of the enzymatic activity attributed to PARP (Satoh and Lindahl, 1992; de Murcia and Menissier de Murcia, 1994; Shall, 1995; Maruta et al., 1997; Burkle, 2001; Ziegler and Oei, 2001; Diefenbach and Burkle, 2005; Malanga and Althaus, 2005; Gagne et al., 2006; Hakem, 2008; Woodhouse and Dianov, 2008). In living

* Corresponding author at: Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Consejo Nacional de Investigaciones Científicas y Técnicas, Vuelta de Obligado 2490, Ciudad Autónoma de Buenos Aires, 1428 Buenos Aires, Argentina. Tel.: +54 11 4783 2871; fax: +54 11 4786 8578.
E-mail addresses: s.villamil@ingebi.conicet.gov.ar, villamil@dna.uba.ar (S.H. Fernández Villamil).

0020-7519/$36.00
doi:10.1016/j.ijpara.2010.11.008

cells, different DNA-repair pathways, including (i) direct reversal, (ii) mismatch repair (MMR), (iii) nucleotide excision repair (NER), (iv) base excision repair (BER), (v) homologous recombination (HR) and (vi) non-homologous end joining (NHEJ), are present (Hakem, 2008). It is important to point out that while the last two mentioned pathways are involved in DNA double-strand break (DSB) repair, the others are crucial during the DNA single-strand break (SSB) repair. The role of PARP-1 has been extensively documented during the restoration of genomic material integrity in both cases (Dantzer et al., 1999; Leppard et al., 2003; Schreiber et al., 2006; Caldecott, 2008; Hakem, 2008; Shrivastav et al., 2008; Woodhouse et al., 2008).

Trypanosoma cruzi, the causative agent of American trypanosomiasis, is a protozoan parasite of humans, as well as of other mammals, which affects people primarily in tropical and subtropical developing countries, especially in South America. The analysis of the complete genome of the trypanosomatids (*T. cruzi, T. brucei* and *Leishmania major*) demonstrates that some of the predicted genes for DNA repair are present in these parasites (Berriman et al., 2005; El-Sayed et al., 2005). However, only a few have been studied in depth. DNA DSB repair has been clearly shown to be important for *T. brucei*, as this process contributes to genetic changes that are critical to the immune evasion mechanism (termed antigenic variation), which allows these parasites to survive in the face of host immunity (Barry and McCulloch, 2001; Ulbert et al., 2002; Donelson, 2003; Stockdale et al., 2008; Boothroyd et al., 2009). In addition, some of the key factors of DSB and MMR repair mechanisms have been analysed in *T. brucei* and the related kinetoplastid *L. major* (McCulloch and Barry, 1999; McKean et al., 2001; Robinson et al., 2002). The presence of functional nuclear MMR has been reported in *T. cruzi* and *T. brucei* (Augusto-Pinto et al., 2001, 2003; Bell et al., 2004; Machado-Silva et al., 2008).

We have previously characterised PARP from *T. cruzi* (TcPARP) as a 65-kDa protein which contains three of the typical PARP-1 domains, but lacks the Zinc-finger DNA binding domain and the carboxyl-terminal domain of the breast cancer gene 1 (BRCT domain) (Fernandez Villamil et al., 2008). The high percentage of basic amino acids in the amino-terminal portion of the protein is a potential DNA binding site responsible for the modulation of its activity. Exposure of *T. cruzi* epimastigotes to DNA-damaging agents leads to a drastic increase in the levels of PAR in the nucleus, as shown in our earlier report (Fernandez Villamil et al., 2008), thus confirming PAR synthesis in vivo and suggesting a physiological role of PARP in trypanosomatid DNA repair signalling. These results have opened a new field to be explored in the biology of *T. cruzi*.

PARP activity has also been reported to be an active player in cell death processes, in apoptosis as well as in necrosis (Yu et al., 2002, 2006; Ame et al., 2004; Andrabi et al., 2006; Schreiber et al., 2006; Cohausz et al., 2008; Cohausz and Althaus, 2009). Although cell death mechanisms in trypanosomatids have not been thoroughly unveiled, Menna-Barreto et al. (2009) have reported that the treatment of *T. cruzi* with different drugs produces ultrastructural alterations indicative of different cell death pathways.

The aim of this work was to explore whether PARP in *T. cruzi* is involved in different DNA-repair pathways, probably involving several different proteins. In addition we attempted to demonstrate that TcPARP is connected with diverse cell death mechanisms.

2. Materials and methods

2.1. Materials

All restriction endonucleases and Taq DNA Polymerase were from New England Biolabs Inc., Beverly, MA, USA. Bacto-tryptose and liver infusion were from Difco Laboratories, Detroit, MI, USA. All other reagents were purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.2. Parasites cultures and cell extracts

Trypanosoma cruzi epimastigote forms (CL Brener strain) were cultured at 28 °C for 7 days in liver infusion tryptose (LIT) medium (5 g L^{-1} liver infusion, 5 g L^{-1} bacto-tryptose, 68 mM NaCl, 5.3 mM KCl, 22 mM Na_2HPO_4, 0.2% (w/v) glucose and 0.002% (w/v) hemin) supplemented with 10% (v/v) FCS, 100 U mL^{-1} penicillin and 100 mg L^{-1} streptomycin. Cell viability was assessed by direct microscopic examination. Cells were harvested by centrifugation at 750*g* and 4 °C, washed three times with PBS and resuspended in buffer (50 mM Tris–HCl, pH 8.0, 1.0 mM EDTA, 10% (v/v) glycerol, 10 mM 2-mercaptoethanol, containing protease inhibitors: 1 μg mL^{-1} *trans*-epoxysuccinyl-L-leucylamido(4-guanidino) butane (E-64), 1 mM pepstatine A, 1 mM phenylmethylsulfonylfluoride (PMSF), and 0.1 mM Na-tosyl-L-lysine chloro-methyl ketone (TLCK)). Cells were lysed in an Ultrasonic Processor Model W385 Sonicator (Heat Systems-Ultrasonic Inc. Plainview, IL, New York, USA) and the whole extract obtained was used as a protein source for Western blot analysis (Fernandez Villamil et al., 2008).

2.3. Antibody preparation

Antisera against each TcPARP and TcPARG were obtained by respective immunization of female BALB/c strain mice via i.p. injection of 100 μg of recombinant protein plus 0.1 mL of incomplete Freund's adjuvant. After two subsequent injections given 15 days apart, mice were bled and sera obtained. Antibodies were tested for titer and cross-reactivity using the recombinant protein and *T. cruzi* extracts. Appropriate measures were taken to minimise pain or discomfort of the experimental animals used in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.4. SDS–PAGE and Western blot analysis

For Western blot analysis, the protein in the whole cell lysate was quantified using the Bradford method, 35 μg of protein were electrophoresed on 10% SDS–PAGE gel and transferred to Amersham Hybond-ECL nitrocellulose membrane (GE healthcare, Little Chalfont, Buckinghamshire, UK), according to the manufacturer's instructions. Immunodetection of PAR polymer was carried out using a 1:5000 dilution of rabbit polyclonal antibody directed against the PAR polymer (BD Biosciences, San Jose, CA, USA), followed by 1:6000 anti-rabbit horseradish peroxidase (HRP) conjugated antibody.

2.5. PAR and DNA damage immunodetection

Epimastigotes of *T. cruzi* (1.10^7 cells mL^{-1}) were resuspended in PBS – 2% glucose and exposed to the indicated treatment with genotoxic agents. The parasites were fixed for 25 min with 3.8% (w/v) formaldehyde in PBS at 4 °C, permeabilized with fresh PBS – 0.1% Triton X-100 and blocked for 1 h at room temperature with 5% (w/v) BSA in PBS. PAR polymer was detected with 1:200 rabbit polyclonal PAR antibody followed by 1:600 anti-rabbit Cy3 conjugated antibody. The nucleus was stained with DAPI. DNA damage immunodetection after treatment with hydrogen peroxide (H_2O_2) or UV-C light was performed with the DeadEnd™ Fluorometric TUNEL System (Promega, Madison, WI, USA) following the instruction manual. Slides were viewed with a confocal microscope Nikon C1 (Nikon Instruments Inc., Melville, NY, USA).