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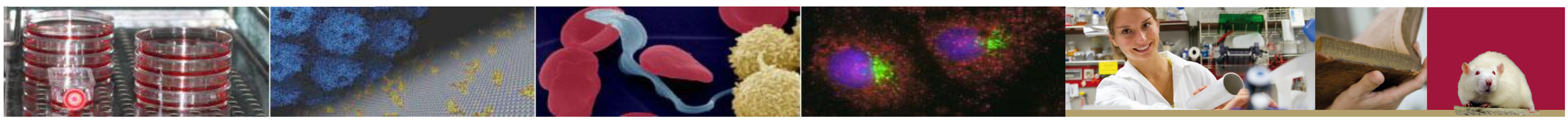

Characterization of endonuclease G from Trypanosoma brucei

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Introduction

Trypanosoma brucei is an unicellular parasite and the causative agent of human African trypanosomiasis (HAT) or sleeping sickness. In mammals, slender form trypanosomes are the proliferative population. They regulate their cell density by quorum sensing and are capable to differentiate into a non-dividing stumpy form. Figarella et al. showed that this stumpy form produces PGD_2, which acts as mediator to induce a caspase-independent apoptotic cell death. This altruistic mechanism prolongs the survival of the host.
In *Leishmania*, *Toxoplasma*, and *Trypanosoma* some features of apoptosis similar to those seen in multicellular organisms have been observed. These include permeabilization of the mitochondrial outer membrane, which leads to the release of cytochrome c and other cell-death activating factors like endonuclease G (EndoG) into the cytosol. Flow cytometry analysis shows an increase of intracellular ROS, loss of mitochondrial transmembrane potential, condensation of nuclear chromatin, fragmentation of genomic DNA and phosphatidylserine exposure. EndoG is one of the most abundant nucleases in eukaryotic cells. It is encoded in the nucleus and imported to the mitochondrial intermembrane space. During apoptosis, it is translocated to the nucleus to cleave single- and double-stranded DNA. Here we want to study the role of TbEndoG during apoptosis in *T. brucei*.

Endo G expression and activity assay

Putative TbEndoG (Tb427.8.4040) was cloned into pProExHTa (Invitrogen) and expressed in BL21(DE3) bacterial cells with 1mM IPTG for 4h at 37° C. Because most of the expressed protein was found in inclusion bodies, cells were lysed in 1% lauroylsarcosine and the cleared lysate was loaded on a Ni–NTA column to be purified according to the manufacturer's instructions (Qiagen). After refolding, the His-tag was cleaved with ProTEV (Promega).

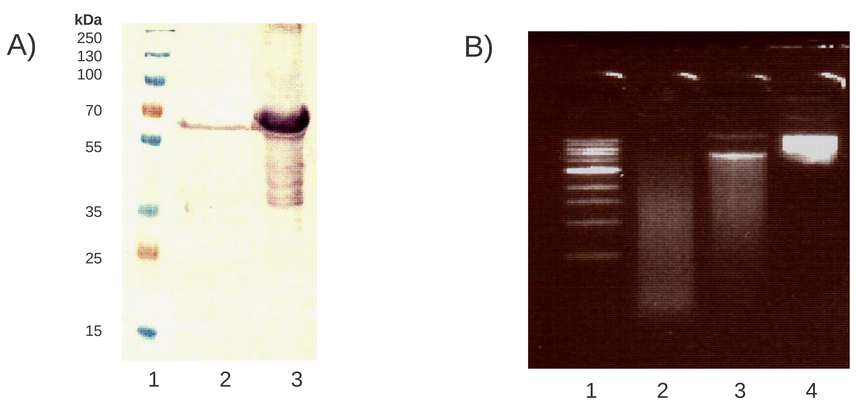

Figure 1. Western blot (α-6xHis-antibody) **and activity assay**

A) **1:** Protein marker
2: TbEndoG after Ni-NTA column purification
3: after isolation from inclusion bodies

B) 1µg DNA was incubated for 1 h at 37° C with 200ng recombinant TbEndoG in 20mM HEPES, 3mM $MgCl_2$, 0,1% Triton X-100, pH 7.5 and applied on a 1.5% (w/v) agarose .
1: DNA marker **2:** w/o His-tag
3: with His-Tag **4:** gDNA

Flow cytometry

All cell lines were induced with 5µg/ml tetracycline and incubated 24h with 10µM PGD_2. Fluorescent markers were added 30 min before FCM analysis (BD FacsCant).

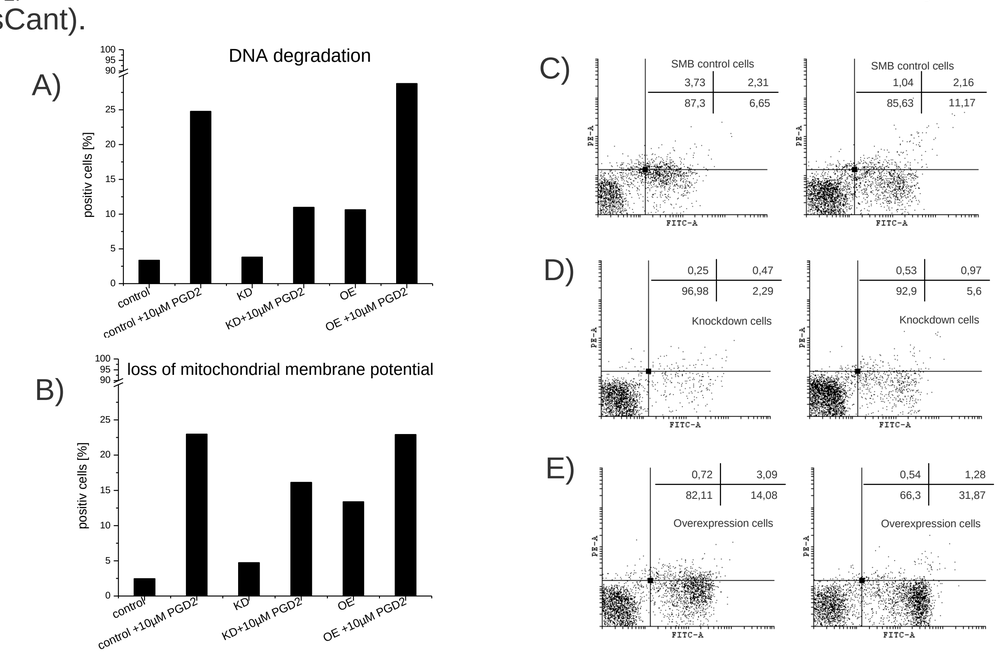

Figure 3.

SMB, KD and OE cells were treated with 10 µM PGD_2, washed twice in PBS and resuspended in Ringer solution.

A) DNA degradation (20µg/ml propidium iodide)
B) mitochondrial membrane potential (10µM TMRE)
C-E) Annexin-V (green FITC-A channel) / PI (red PE-A channel) double staining for apoptotic/necrotic cells.
Left: uninduced
Right: induced cells with 10µM PGD2

Endo G knockdown and overexpression

T. brucei has two copies of EndoG with 99% identical nucleotide and protein sequences. Part of the homologue sequence was cloned into p2T7 to create an inducible knockdown, pLEW100v5Hyg was used for overexpression.

Both vectors were linearized with NotI and electroporated into single marker bloodstream cells (T7RNAP::TETR::NEO). Recombinant clones were selected with 2µg/ml G418 and 2.5µg/ml hygromycin in HMI-9 medium.

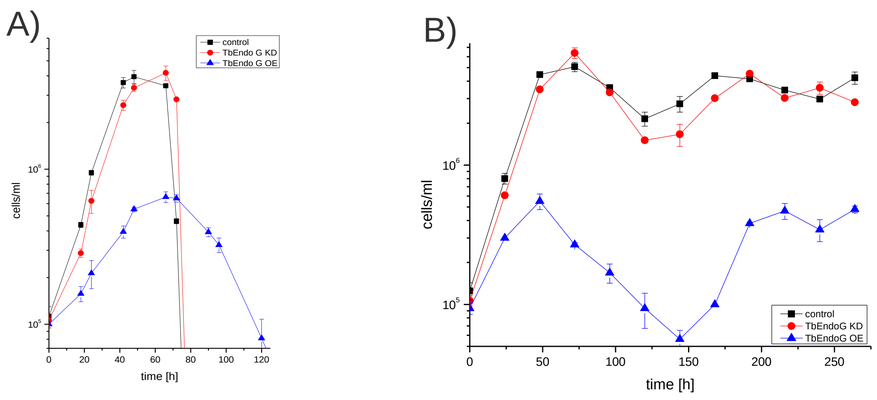

Figure 2: Growth curve of SMB control cells, TbEndoG KD and OE cells
Cells were induced by adding 5 µg/ml tetracycline every 24h.

A) no medium exchange.
B) with daily medium exchange.

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