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Short Communication

Deletion of *Pol IA* alleles increases sensitivity to hydrogen peroxide in the parasitic protozoan *Trypanosoma brucei*

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***Trypanosoma brucei* is the etiological agent of African Trypanosomiasis. *T. brucei* possesses biologically unique mitochondrial DNA called kinetoplast DNA (kDNA). Genetic studies provide strong evidence that kDNA is essential. Understanding kDNA maintenance may yield promising drug targets. RNA interference (RNAi) studies of mitochrondrial DNA polymerase IA (*POL IA*) did not show a growth phenotype under normal conditions. In this study we used a classical genetics approach and deleted both alleles of *POL IA*. Comparison of the growth phenotype of the *POL IA* double mutant to wild-type indicates that *POL IA* is required for the oxidative stress response in *T. brucei*.**

Key words: African sleeping sickness, kDNA, DNA repair, *POL IA*, *Trypanosoma brucei*.

INTRODUCTION

African Trypanosomiasis (sleeping sickness) is a devastating disease caused by the protozoan parasite, *Trypanosoma brucei*. There is no vaccine against trypanosomiasis and current therapies rely mostly on drugs developed over 60 years ago that are difficult to administer and often toxic. These organisms have a biphasic life cycle, requiring both a mammalian host and an insect (tsetse fly) vector (Shlomai, 2004; Burchmore et al., 2002). Trypanosomatids are interesting organisms both because of their impact on global human health as well as their unique evolutionary positions as one of the earliest diverging eukaryotic lineages that contains a mitochondrion. *T. brucei* and related trypanosomes have a number of unique biological properties, but their most distinctive feature is their mitochondrial DNA called kinetoplast DNA (kDNA). Unlike any DNA structure in nature, kDNA is a network containing thousands of catenated DNA molecules (maxicircles and minicircles). Recent genetic studies provide strong evidence that kDNA is essential (Klingbeil et al., 2002). Therefore, understanding the processes of kDNA replication and repair are crucial aspects of trypanosome biology.

While most other eukaryotes have a single mitochondrial DNA polymerase (pol), pol gamma, at least six trypanosomal polymerases have been identified that localize to the mitochondrion. Four of these mitochondrial pols are related to bacterial DNA polymerase I. Genetic studies using RNA interference (RNAi) indicate that POL IB, POL IC, and POL ID are essential for kDNA replication. In contrast very little is known about repair of kDNA in trypanosomes. The RNAi studies of *POL IA* under normal conditions did not cause a growth phenol-type even though the *POL IA* mRNA was degraded. In addition localization studies of POL IA using a Green Flourescent Protein (GFP) tag showed dual localization of POL IA to both the mitochondrion and nucleus (Klingbeil et al., 2002). This dual localization pattern was not observed for POL IB-1D. Evidence from localization studies of other oxidative stress responsive proteins including mammalian p53 show multi-compartmental localization (Erster and Ute, 2005). We therefore hypothesized that given the lack of RNAi phenotype under normal growth conditions and its dual localization pattern, *POL IA* might be a repair polymerase.

RESULTS AND DISCUSSION

Both alleles of the *POL IA* gene were targeted for deletion via homologous recombination as previously described (Bell et al., 2004). Vectors containing a different drug selection marker were used to disrupt each of the *POL IA* alleles (Bangs et al., 1996). Initial verification of the absence of *POL IA* was performed using a PCR

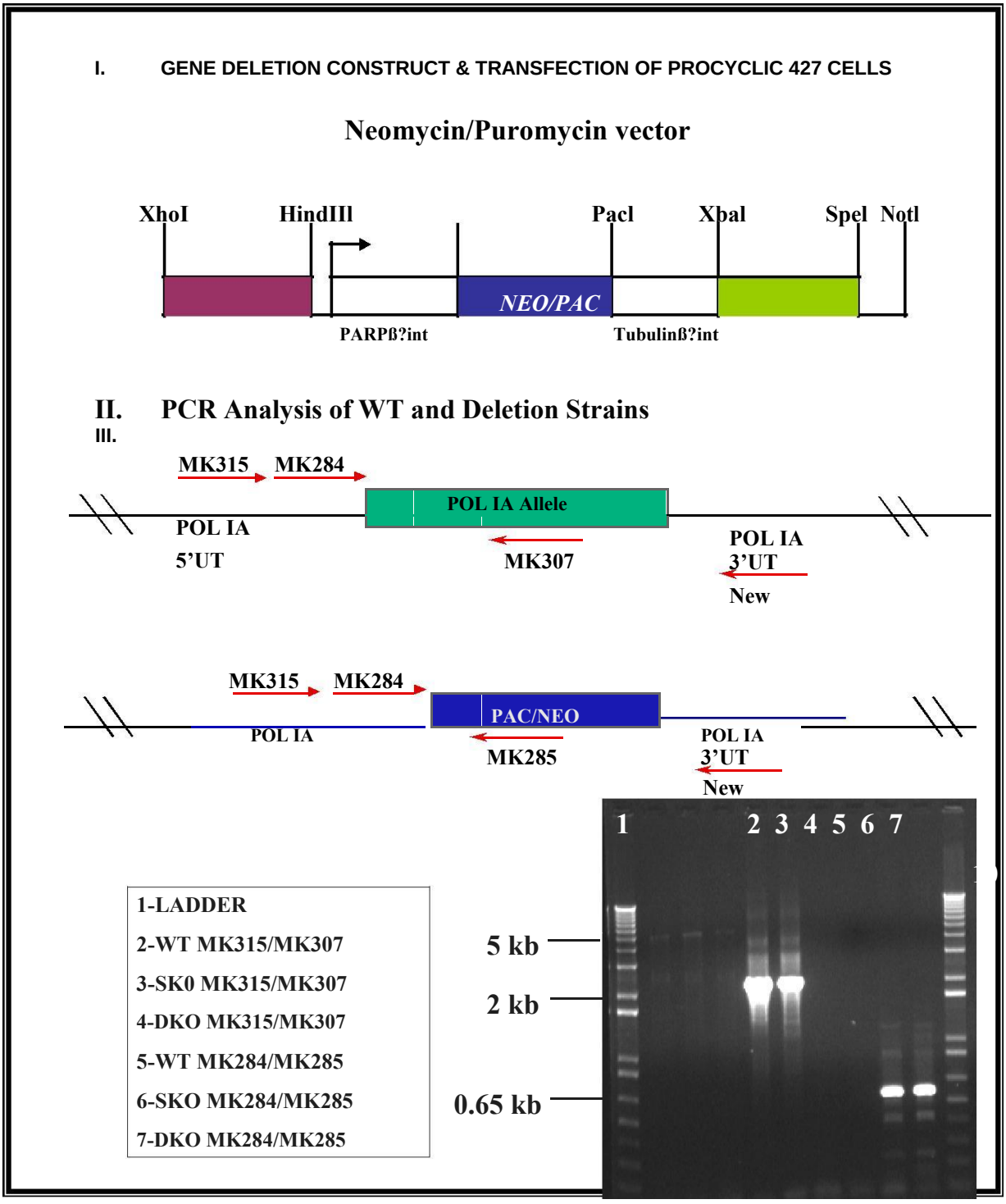

Figure 1. Deletion, transfection and verification of POL IA mutant Strains.

based methods with primers that annealed internal to the *POL IA* gene or drug resistance cassette, and external to the cloning region (Figure 1) . Construction of the single and double deletion strains allowed us to assay their growth phenotype and compare it to our wild- type strain after exposure to non-lethal doses of the DNA damaging agent hydrogen peroxide. Our results were supportive of our hypothesis. Deletion of one or both alleles of the *POL IA* gene resulted in a loss of fitness when the *POL IA* mutant strains were grown in the presence of non-lethal doses of hydrogen peroxide (Figure 2).

These results are the summary of an initial study to

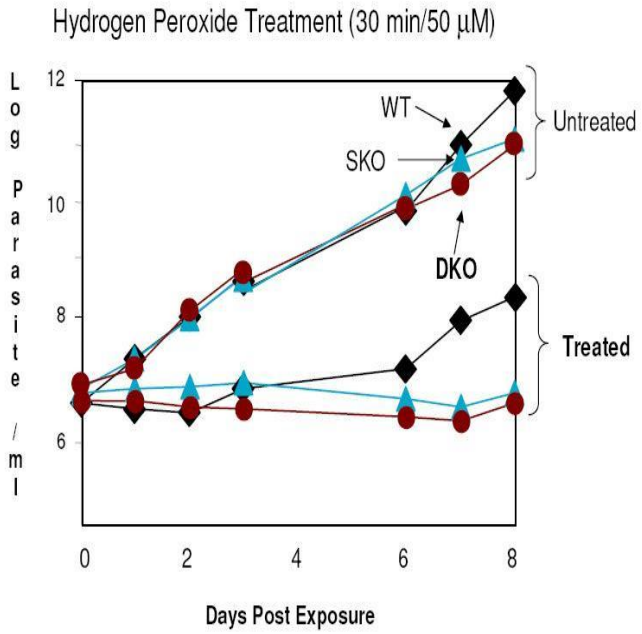

Figure 2. *POL IA* mutants are hypersensitive to oxidative stress.

determine if *Trypanosoma brucei POL IA* might be involved in DNA repair and maintenance of kDNA. The preliminary evidence suggests that the absence of even a single *POL IA* allele is sufficient to case a severe growth defect in *T. brucei* when cells are under oxidative stress. *Trypanosoma brucei* does not appear to possess a catalase enzyme that would normally function in hydrogen peroxide detoxification. Hydrogen peroxide toxicity has also been implicated as one of the main factors resulting in the extreme susceptibility of the bovine form of *T. brucei* to human serum (Lueder and Margaret, 1996). The pathways of T. brucei DNA repair have yet to be fully elucidated, especially in regards to the role(s) of mitochondrial DNA polymerases that may be required for repair of the essential and unique trypanosomatid kDNA network. Therefore, future studies of how POL IA might function in kDNA maintenance and whether it also interacts with other known DNA repair enzymes will potentially provide further evidence of a vital role for *POL IA* in *T. brucei* proliferation.

A 750 bp PCR fragment of *POL IA* (-644 to + 106) was cloned into the XhoI/HindIII sites of the pXS2 vector (5), upstream of the neomycin (NEO) or Puromycin (*PAC*) resistance cassette. A 753bp PCR fragment of the *POL IA* 3' untranslated region (3'UTR) was cloned into the XbaI/SpeI restriction sites downstream of the *NEO* or *PAC* cassette. These new vectors were named pKONEO-IA amd pKOPAC -IA. The vectors also contain a unique NotI restriction site used for linearization prior to transfection into Tb427 cells.

We linearized 12.5 µg of the pKONEO-IA vector with NotI and transfected into Tb427 wild type cells. Transfectants were selected with G418 (50 µg/ml) after 48 h. Stable cell lines were maintained with 50 µg/ml G418. To replace the second *POL IA* allele we linearized 12.5 µg of the pKOPAC -IA vector with NotI and transfected into the Tb427 *POL IA* : NEO single knockout cell line. Transfectants were selected with puromycin (1 µg/ml). Stable cell populations maintained with 1 µg/ml puromycin and 50 µg/ml G418. II. PCR analysis with primer pairs MK315/MK307, and MK284/MK285 was conducted using genomic DNA from the wild type (WT), single knockout (SKO), and double knockout (DKO) strains to verify correct integration of the constructs. MK315 anneals to a region of the POL IA 5'UTR upstream of the site used to generate the PCR fragment for cloning into the pKONEO/PAC vectors. The expected size of the PCR product using MK315/MK307 in WT cell line is ~2.2 kb and ~0.65 kb using MK284/MK285 in the knockout strains. PCR analysis shows bands of the appropriate size range in the three strains.

Untreated cells were grown in SDM-79 tissue culture media with the appropriate drugs. Cell counts were taken every day using a Z2 series Coulter Counter. Cells were maintained at densities between 5.5×10^5 - 1.0×10^7 cells/ml. Culture were also exposed to hydrogen peroxide (50 mM) for 30 min and allowed to recover for seven days. Closed squares are WT, closed triangles are single knockout cell lines and closed circles are double knockout cell lines. These data indicate that under oxidative conditions the knockout cell populations have a growth-impaired phenotype. The graph shown is a representative experiment. The above growth assays were repeated a minimum of three times on clonal cell lines and the results showed the same growth patterns.

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REFERENCES

Shlomai J (2004). Curr. Molecular Med. 4: 623-647.
Burchmore R, Ogbunude P, Enanga B, Barrett M (2002). Curr. Phar. Des. 8: 257-267.
Klingbeil M (2002). Molecular Cell 10: 175-186.
Erster S, Ute Moll M (2005). Biochem. Biophys. Res. Commun. 10: 331(3): 843-50.
Bell J, Harvey T, Sims A, McColloch R (2004). Mol. Micro. 51: 159-173.
Bangs J (1996). J. Biochem. 271:18387-18393.
Lueder DV, Margaret AP (1996). J. Biol. Chem. 271: 17485-17490.