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Short communication

Inactivation of the cytosolic and mitochondrial serine hydroxymethyl transferase genes in *Leishmania major*

Gaétan Roy[a], Marc Ouellette[a,b,*]

[a] Centre de Recherche en Infectiologie de l'Université Laval et Centre de Recherche du CHU de Québec, Canada
[b] Département de Microbiologie, Infectiologie et Immunologie, Faculté de Médecine, Université Laval, Québec, QC, Canada

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ABSTRACT

Leishmania has two serine hydroxylmethyl transferase (*SHMT*) genes, one coding for a cytosolic and the other for a mitochondrial enzyme. *Trypanosoma cruzi* has only the gene coding for the cytosolic enzyme and *Trypanosoma brucei* has no *SHMT*. We tested whether these genes were dispensable for growth in *Leishmania major*. By gene inactivation we succeeded in generating three cells lines one without the cytosolic *cSHMT*, one without the mitochondrial *mSHMT*, and finally one *L. major* line without any *SHMT*. SHMT is thus dispensable for growth of Leishmania in rich medium. The ability of the various *shmt* null mutants to grow in defined medium was tested and the growth of the *shmt* null mutant was dependent on the presence of serine. Overall this work has shown that SHMT is dispensable for Leishmania growth but it may be necessary when growing in environments poor in serine.

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Studies on folate metabolism in the protozoon parasite Leishmania have highlighted a number of interesting features that could ultimately be exploited for interventions [1,2]. Leishmania are folate auxotrophs and depend on a series of Folate Biopterin Transporters (FBT) for the uptake of folates [3,4], but also biopterin [5,6] and *S*-adenosyl methionine [7]. Several other FBTs are encoded in the genome but their substrates are unknown [8]. Folate derivatives are involved in a series of one-carbon (C1) metabolism reactions and the activation of the C1 units are dependent on the formation of 5,10-methylene-tetrahydrofolate (methylene-THF) by either serine hydroxymethyl transferase (SHMT) or the glycine-cleavage complex (GCC) [9]. Methylene-THF is the main C1 intermediate and is required for the synthesis of thymidine via dihydrofolate reductase-thymidylate synthase (DHFR-TS) [2]. Methylene-THF can also be converted to other active reduced folates with the essential 10-formyltetrahydrofolate that is involved in the formylation of the initiator methionyl-tRNA in the mitochondria [10].

Folate metabolism is compartmentalised in Leishmania [2] and Leishmania has two SHMT orthologues that were shown experimentally to locate to the cytosol and the mitochondria [11]. While Leishmania has two *SHMT* genes, *Trypanosoma cruzi* has only the one coding for the cytosolic enzyme and *Trypanosoma brucei* has no *SHMT* gene [9]. There is evidence that SHMT and the GCC are redundant and if this was the case it may be possible to generate a Leishmania cell without its *SHMT* genes. Inactivation of both the cytosolic and mitochondrial SHMT genes of *Leishmania major* was tested experimentally.

Constructs were made for inactivating the two genes that are encoded on chromosome 14 for the cytosolic cSHMT and on chromosome 28 for the mitochondrial mSHMT. These constructs were made by a PCR fusion strategy that we have described previously[12] The PCR fragments of the 5′ and 3′UTR of *cSHMT* were fused to hygromycin phosphotransferase (*HYG)*, while the 5′ and 3′UTR of *mSHMT* were fused to either the puromycin *N*-acetyltransferase *(PUR)* or neomycin phophotransferase *(NEO)* markers. A list of the primers used for the generation of these inactivation cassettes can be found in Table S1. We succeeded in inactivating both alleles of the *cSHMT* gene with the *HYG* casssette, possibly by loss of heterozygocity [13]. The *HYG* deletion cassette was electroporated in *L. major* LV39 and the DNA of cloned transfectants growing in the presence of hygromycin B (600 μg/ml) was isolated. The *L. major* strains were grown in SDM-79 medium [14] a rich medium with high concentration of folates, serine and glycine. When the DNA derived from wild-type cells is digested with XhoI and hybridized with a *cSHMT* 5′UTR probe it recognised a 2.9 kb band (Fig. 1A and B, lane 1, upper panel). This band is absent in the *HYG/HYG cshmt* null mutant and instead the expected 3.2 kb band hybridized to the 5′UTR probe (Fig. 1B, lane 2, upper panel). The same 3.2 kb band hybridized with an *HYG* probe (not shown). Hybridization with the

* Corresponding author at: Centre de Recherche en Infectiologie, 2705 Boul. Laurier, Québec, QC G1 V 4G2, Canada. Fax: +1 418 654 2715.
E-mail address: Marc.Ouellette@crchul.ulaval.ca (M. Ouellette).

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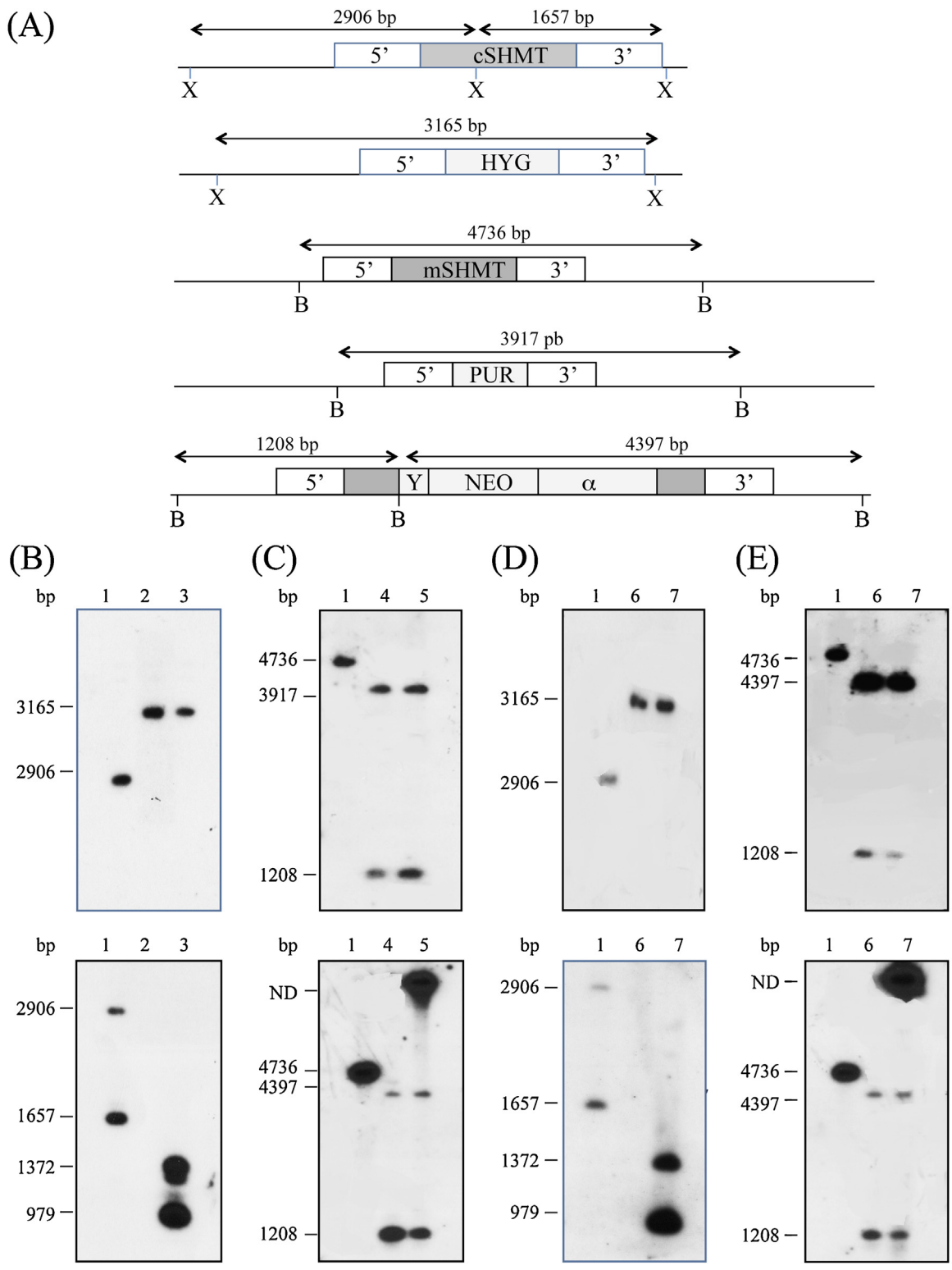

Fig. 1. Inactivation of the *Leishmania major* cytosolic and mitochondrial serine hydroxymethyl transferase (*SHMT*) genes. (A) Schematic map of the loci encoding the cytosolic *cSHMT* and the mitochondrial *mSHMT* and the integration of the cassette leading to gene inactivation. Relevant XhoI (X) and BglII (B) restriction sites and predicted size are shown. (B) Inactivation of *cSHMT*. The *HYG* gene deletion cassette was made using a fusion PCR method [7,12]. The linear cassettes were introduced by electroporation. The DNA of the cells were isolated using DNAzol, digested with XhoI and hybridized to PCR generated probes recognizing the 5′UTR (upper panel) or the open reading frame (lower panel) of *cSHMT*. (C) Inactivation of *mSHMT*. The *PUR* gene deletion cassette was made using oligonucleotides using a fusion PCR method. A Y*NEOα* expression cassette, where *NEO* is flanked by a stretch of pyrimidine (Y) and the intergenic region of the α-tubulin gene (α) was inserted in the *mSHMT* gene and this gene disruption cassette was introduced in Leishmania by electroporation. The DNA of the transfectants were digested with BglII and hybridized to PCR generated probes recognizing the 5′UTR (upper panel) or the open reading frame (lower panel) of *mSHMT*. (D and E) Generation of a double *cSHMT* and *mSHMT* null mutant. The *HYG/HYG cshmt* mutant was sequentially electroporated with the *PUR* and *NEO* cassettes. The DNA of the cells were isolated using DNAzol, digested with XhoI (D) and BglII (E) and hybridized to PCR generated probes recognizing the 5′UTR (upper panel) or the open reading frame (lower panel) of either *cSHMT* (D) or *mSHMT* (E). (1) *L. major* LV39; (2) *HYG/HYG cshmt* null mutant; (3) *HYG/HYG cshmt* null mutant with an episomal pSPα*ZEO-cSHMT*; (4) *PUR/NEO mshmt* null mutant; (5) *PUR/NEO mshmt* null mutant with an episomal pSPα*ZEO-mSHMT*; (6) *HYG/HYG/PUR/NEO cshmt/mshmt* null mutant; (7) *HYG/HYG/PUR/NEO cshmt/mshmt* null mutant with an episomal pSPα*ZEO-cSHMT* and pSPα*BLAS-mSHMT*.

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