Molecular & Biochemical Parasitology 198 (2014) 11–13

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Short technical report

Tetracycline-inducible gene expression system in *Leishmania mexicana*

Natalya Kraeva [a], Aygul Ishemgulova [a], Julius Lukeš [b,c,d], Vyacheslav Yurchenko [a,b,*]

[a] Life Science Research Centre, Faculty of Science, University of Ostrava, 710 00 Ostrava, Czech Republic
[b] Biology Centre, Institute of Parasitology, Czech Academy of Sciences, 370 05 České Budějovice (Budweis), Czech Republic
[c] Faculty of Sciences, University of South Bohemia, 370 05 České Budějovice (Budweis), Czech Republic
[d] Canadian Institute for Advanced Research, Toronto, ON M5G 1Z8, Canada

ARTICLE INFO

Article history:
Received 12 October 2014
Received in revised form
10 November 2014
Accepted 14 November 2014
Available online 22 November 2014

Keywords:
Leishmania mexicana
Gene expression
Tet-inducible system

ABSTRACT

Here we present a T7-driven, tetracycline-inducible system for protein expression in human pathogen *Leishmania mexicana*. The gene expression in this strain is tightly regulated and dose- and time-dependent. This system can be widely used by the parasitology community to analyze effects of genes of interest on biology, physiology and virulence of parasites causing cutaneous leishmaniases.

The genus *Leishmania* unites parasitic protozoa of the family Trypanosomatidae causing leishmaniases, several closely related diseases that affect human and animal populations mainly in the tropical and subtropical regions. The clinical manifestations vary from spontaneously healing skin lesions to progressive and potentially fatal visceral infections. Leishmaniases represent a global health problem with over 350 million people at risk and an annual incidence rate of 2–10 million worldwide. In the last decade, the genomes of several *Leishmania* species have been sequenced [1,2]. The information these studies have amassed needs to be analyzed in functional assays. Yet at the moment, there is a rather limited set of tools available to the research community to achieve this goal. Historically, heterologous or homologous gene expression driven by endogenous RNA polymerases was assayed after conventional knock-ins *via* homologous recombination [3]. Two major draw-backs of such a system are the absence of the controllable regulation and its intrinsic inability to deal with toxic products. The majority of the available conditional gene expression systems in *Leishmania* still relies on endogenous promoters and enzymes [4,5]. The alternative approach utilizes foreign polymerases, such as those derived from T3 or T7 bacteriophages [6]. In Trypanosomatidae, transcription and RNA processing are uncoupled allowing bypassing of the RNA capping requirement and therefore proficient expression driven by heterologous enzymes. One of the most efficient expression systems based on such a principle was established in a reptile parasite *Leishmania tarentolae*, which allowed overexpression to a level reaching 1% of total cellular protein [7]. While being widely used for eukaryotic protein production (marketed under the name LEXSY by Jena Bioscience GmbH), this system has a limited value for basic and translational research. Most importantly, *L. tarentolae* is not pathogenic for humans. Its life cycle and transmission mechanisms are poorly understood and there is no reliable experimental system to investigate biology of this species *in vitro* [8]. A similar system developed in *L. infantum* (*L. chagasi* [9]) has proven to be restricted to studies of visceral leishmaniases only [10].

To overcome these deficiencies we established a T7 polymerase-dependent Tetracycline (Tet)-inducible expression system in *Leishmania mexicana* (strain MNYC/BZ/62/M379), hereafter called *L. mexicana*, a causative agent of human cutaneous leishmaniasis [11]. The choice was determined by the following factors: (1) medical and biological importance; (2) available genomic information; (3) ease of cultivation; (4) established conditions for *in vitro* differentiation into metacyclic promastigotes and amastigotes and (5) established experimental macrophage infection and mouse models [12,13]. It is also worth noting that in many instances *L. mexicana* surpassed the model organism *L. major* in its adaptability and tolerance to genetic manipulations [14].

* Corresponding author at: Life Science Research Centre, University of Ostrava, Chittussiho 10, 710 00, Ostrava, Czech Republic. Tel.: +420 597092326; fax: +420 597092382.
E-mail address: vyacheslav.yurchenko@osu.cz (V. Yurchenko).

http://dx.doi.org/10.1016/j.molbiopara.2014.11.002
0166-6851/

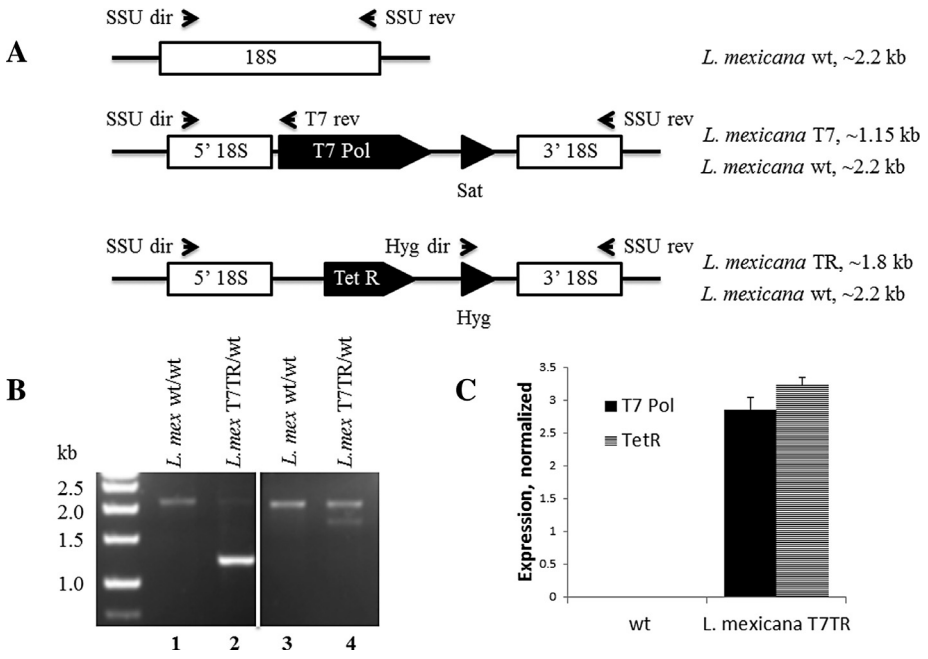

Fig. 1. Establishing the *Leishmania mexicana* T7TR system. (A) Schematic representation of the 18S rRNA loci of the wild type and transgenic *L. mexicana* lines. Arrows indicate primers used to check integration. The sizes of the expected PCR products are indicated. (B) PCR validation of the correct integration of the transgenes into the 18S ribosomal locus using primers SSU dir – T7 rev – SSU rev (lanes 1 and 2 to control T7 polymerase integration), and SSU dir–Hyg dir–SSU rev (lanes 3 and 4 to control Tet repressor integration). Primer sequences are available upon request from authors. (C) qPCR confirmation of the T7 polymerase and Tet repressor gene expression. Quantitative PCR was done and analyzed as described before and normalized to 18S ribosomal RNA [17]. Primer sequences are available upon request from authors.

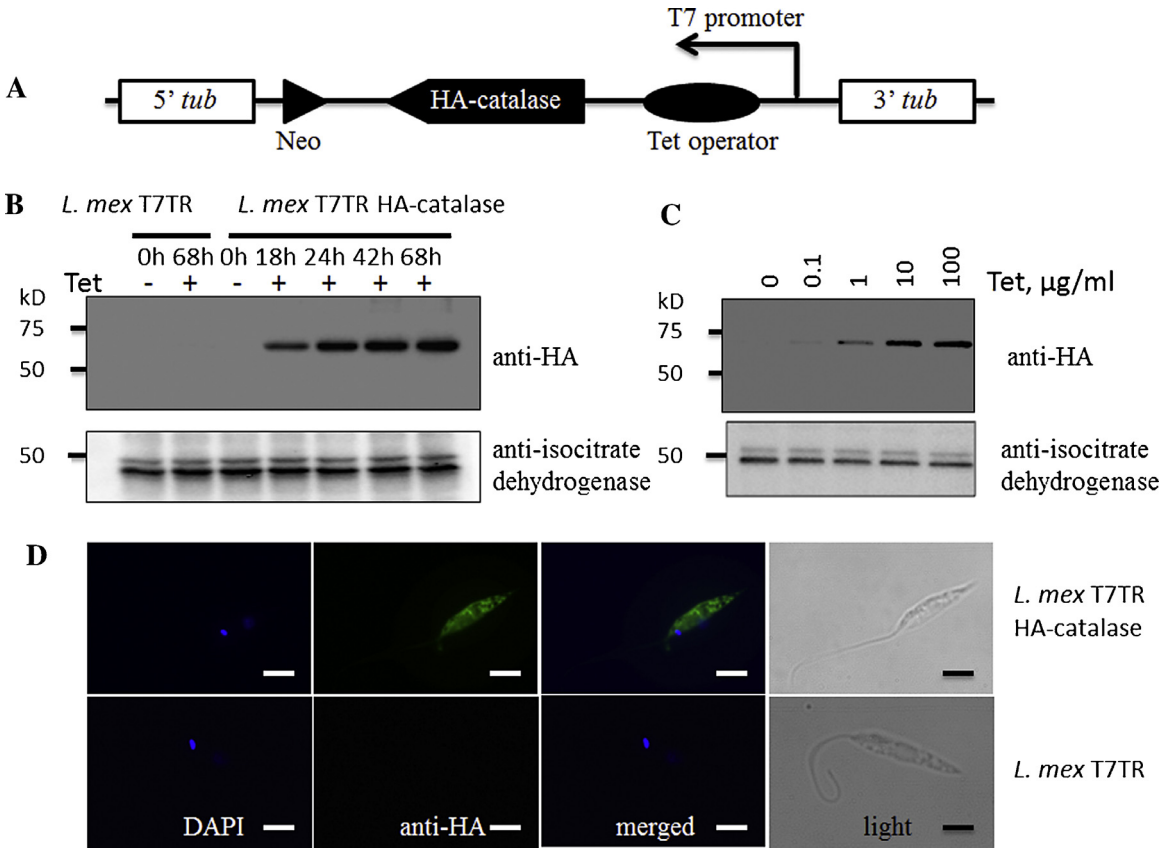

Fig. 2. Expression of the heterologous genes in *L. mexicana* T7TR. (A) Schematic representation of the tubulin locus of the transgenic *L. mexicana* T7TR. Please note anti-sense orientation of the T7 promoter – Tet repressor – gene of interest cassette. The map of the integration plasmid pTUBneoF4 HA-catalase is presented in Suppl. Fig. 1. (B) The time course of HA_3-tagged catalase expression monitored by western blotting with rabbit polyclonal anti-HA antibodies (Sigma–Aldrich, St. Louis, USA). Rabbit polyclonal anti-isocitrate dehydrogenase antibodies were used for loading control. (C) Protein expression is dose-dependent. HA_3-tagged catalase expression was monitored as in 2B. (D) Immuno-fluorescent microscopy with anti-HA antibodies detects localization of the protein of interest expressed in *L. mexicana* T7TR 12 h after induction with tetracycline. Scale bar 5 µm.

Firstly, the transgenic *L. mexicana* strain with integrated genes for nuclear localization signal-tagged T7 polymerase and Tet-repressor (*L. mexicana* T7TR) was established (Fig. 1). This was achieved using pF4T7polNLS1.4sat and pF4TR1.4hyg plasmids described before and selection in the presence of hygromycin B and nourseothricin at final concentration of 100 μg/ml each [7]. Both constructs were integrated into the fairly conserved in *Leishmania* 18S ribosomal RNA gene locus (Fig. 1A and B) and their expression was monitored by quantitative PCR (Fig. 1C).

To validate this system, we modified the integration vector pTUBneoF4rev [7] by replacing the *L. tarentolae* tubulin arms of homology with corresponding sequences of *L. mexicana*. To monitor the heterologous expression, an HA_3-tagged gene encoding catalase from *Leptomonas pyrrhocoris* H10 has been cloned into this vector [15] (Fig. 2A). This gene is absent in all *Leishmania* species analyzed to date [16]. The anti-sense orientation of the T7 promoter–Tet repressor–gene of interest cassette ensures low background expression in an un-induced state. The time course of the inducible expression of the HA_3-tagged catalase is presented in Fig. 2B, which shows a detectable expression of the protein as early as 18 hrs after induction with Tet. The expression is dose-dependent and can be detected after induction with as low as 1 μg/ml of the drug (Fig. 2C). Consistent with previous reports, higher doses of Tet (over 20 μg/ml) led to considerable cell death (data not shown and [7]). Importantly, our system can also be used to monitor protein localization and abundance by immunofluorescent microscopy (Fig. 2D).

In summary, here we present a T7-driven, Tet-inducible system for protein expression in human pathogen *Leishmania mexicana*. The gene expression in *L. mexicana* T7TR is tightly regulated and dose- and time-dependent. We believe that such a system can be widely used by the parasitology community to study effects of genes of interest on biology, physiology and virulence of *Leishmania*. It is freely available from authors upon request.

Conflict of interest

Authors declare no conflict of interest.

Acknowledgements

We would like to thank members of our laboratories for helpful discussions and technical assistance. Special thanks to P. Changmai, D. Faktorová, E. Horáková (Biology Centre, České Budějovice) for their assistance in the initial stages of the project and with immunofluorescent microscopy, K. Alexandrov (Institute for Molecular Bioscience, Brisbane) for sharing plasmids pF4T7polNLS1.4sat, pF4TR1.4hyg, and pTUBneoF4rev, and P. Volf (Charles University, Prague) for the strain of *L. mexicana* used in this work. We acknowledge the use of research infrastructure that has received funding from the EU 7th Framework Programme, grant agreement No. 316304. This work was supported by Bioglobe grant CZ.1.07/2.3.00/30.0032 and Praemium Academiae award to J.L., and the Czech Science Foundation (P506/13/24983S) to V.Y. who is also supported by the funds of the Moravskoslezský Kraj research initiative. N.K. and A.I. received grant SGS25/PrF/2014 from the University of Ostrava.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molbiopara.2014.11.002.

References

[1] Choi J, El-Sayed NM. Functional genomics of trypanosomatids. Parasite Immunol 2012;34:72–9.
[2] Leishmania: after the genome. Wymondham: Caister Academic Press; 2008. p. 306.
[3] Clayton CE, Ha S, Rusche L, Hartmann C, Beverley SM. Tests of heterologous promoters and intergenic regions in *Leishmania major*. Mol Biochem Parasitol 2000;105:163–7.
[4] Madeira da Silva L, Owens KL, Murta SM, Beverley SM. Regulated expression of the *Leishmania major* surface virulence factor lipophosphoglycan using conditionally destabilized fusion proteins. Proc Natl Acad Sci U S A 2009;106:7583–8.
[5] Yan S, Myler PJ, Stuart K. Tetracycline regulated gene expression in *Leishmania donovani*. Mol Biochem Parasitol 2001;112:61–9.
[6] Wirtz E, Hartmann C, Clayton C. Gene expression mediated by bacteriophage T3 and T7 RNA polymerases in transgenic trypanosomes. Nucleic Acids Res 1994;22:3887–94.
[7] Kushnir S, Gase K, Breitling R, Alexandrov K. Development of an inducible protein expression system based on the protozoan host *Leishmania tarentolae*. Protein Expres Purif 2005;42:37–46 (see http://images.webofknowledge.com/WOK46/help/WOS/P_abrvjt.html).
[8] Bates PA. Transmission of *Leishmania* metacyclic promastigotes by phlebotomine sand flies. Int J Parasitol 2007;37:1097–106.
[9] Lukeš J, Mauricio IL, Schonian G, Dujardin JC, Soteriadou K, Dedet JP, et al. Evolutionary and geographical history of the *Leishmania donovani* complex with a revision of current taxonomy. Proc Natl Acad Sci U S A 2007;104:9375–80.
[10] Yao C, Luo J, Hsiao CH, Donelson JE, Wilson ME. *Leishmania chagasi*: a tetracycline-inducible cell line driven by T7 RNA polymerase. Exp Parasitol 2007;116:205–13.
[11] Lainson R, Strangways-Dixon J. The epidemiology of dermal leishmaniasis in British Honduras: Part II: reservoir – hosts of *Leishmania mexicana* among the forest rodents. Trans R Soc Trop Med Hyg 1964;58:136–53.
[12] Rogers MB, Hilley JD, Dickens NJ, Wilkes J, Bates PA, Depledge DP, et al. Chromosome and gene copy number variation allow major structural change between species and strains of *Leishmania*. Genome Res 2011;21:2129–42.
[13] Bates PA. Complete developmental cycle of *Leishmania mexicana* in axenic culture. Parasitology 1994;108(Pt 1):1–9.
[14] Castanys-Munoz E, Brown E, Coombs GH, Mottram JC. *Leishmania mexicana* metacaspase is a negative regulator of amastigote proliferation in mammalian cells. Cell Death Dis 2012;3:e385.
[15] Votýpka J, Klepetková H, Yurchenko VY, Horák A, Lukeš J, Maslov DA. Cosmopolitan distribution of a trypanosomatid *Leptomonas pyrrhocoris*. Protist 2012;163:616–31.
[16] Deponte M, Rahlfs S, Becker K. Peroxiredoxin systems of protozoal parasites. Subcell Biochem 2007;44:219–29.
[17] Záhonová K, Hadariová L, Vacula R, Yurchenko V, Eliáš M, Krajčovič J, et al. A small portion of plastid transcripts is polyadenylated in the flagellate *Euglena gracilis*. FEBS Lett 2014;588:783–8.