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Leishmania: Responding to environmental signals and challenges without regulated transcription

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ABSTRACT

Here we describe the non-canonical control of gene expression in *Leishmania*, a single-cell parasite that is responsible for one of the major neglected tropical diseases. We discuss the lack of regulated RNA synthesis, the post-transcriptional gene regulation including RNA stability and regulated translation. We also show that genetic adaptations such as mosaic aneuploidy, gene copy number variations and DNA sequence polymorphisms are important means for overcoming drug challenge and environmental diversity. These mechanisms are discussed in the context of the unique flow of genetic information found in *Leishmania* and related protists.

Contents

1. Introduction

Infections by parasites of the genus *Leishmania* manifest in three main forms: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL) and visceral leishmaniasis (VL), which

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together are counted among the most important neglected tropical diseases [1]. Leishmaniasis is endemic in large parts of the tropical and subtropical regions of the World and poverty-related [2]. The genus *Leishmania* is part of the order Trypanosomatida and of the early-branching phylum Euglenozoa [3,4]. *Leishmania* spp. are closely related to other important pathogens, such as the causative agents of human sleeping sickness, *Trypanosoma brucei* ssp, and *T. cruzi*, the aetiologic agent of Chagas disease.

There are over 20 human-pathogenic species of *Leishmania*, divided into sub-genera, of which *Leishmania* and *Viannia* [5,6] are the most prominent. All leishmaniae undergo a biphasic life cycle with two main morphologically distinct stages. In their vectors, sandflies such as *Phlebotomus* spp. and *Lutzomyia* spp., leishmaniae proliferate rapidly as elongated, flagellated promastigotes within the midgut of females, which require mammalian blood for fertility. These promastigotes undergo small morphological changes during the insect stage, emerging as infective, highly motile forms that upon injection into the mammalian skin during the next sandfly blood meal end up in phagocytic, antigen-presenting cells (APCs). Within those, they undergo a critical differentiation into ovoid, aflagellated, non-motile amastigotes that reside and proliferate inside the phagosomes of macrophages and other APCs. By shedding protein-loaded exosomes [7], they can modulate macrophage activity and thus ensure their survival.

Proliferating amastigotes will destroy the host cell, escape and infect new APCs. The ensuing immune reaction involves influx of various immune cells, inflammatory responses, tissue swelling and destruction, either restricted to the infection site (CL), spreading through the lymphatic system (diffuse CL, MCL), or afflicting the major lymphatic organs (spleen, liver, bone marrow) in VL. The latter is almost invariably lethal in untreated cases or when therapeutic options fail. The manifestation of *Leishmania* infections in CL, MCL or VL is mostly preordained by the infecting species, but the host immune status has also a considerable influence on the outcome. In recent years, it has become increasingly clear that not all *Leishmania* infections cause a symptomatic disease and that parasites can persist in humans in subclinical infections for years [8]. These subclinical cases are thought to constitute an important reservoir for anthroponotic *Leishmania* species but may exacerbate upon permanent or temporary immune suppression.

Conversion from the insect stage to the mammalian stage requires *Leishmania* to change its gene expression. While gene expression in most eukaryotes is controlled mainly at the level of transcription initiation, by *cis*-acting promoters and *trans*-acting transcription factors [9], *Leishmania* spp. and the entire order Trypanosomatida lack gene-specific, regulated transcription by RNA Polymerase II, missing both canonical promoter elements and genes for cognate transcription factors [10]. Rather, *Leishmania* chromosomes comprise of large polycistronic transcription units (PTUs) of functionally unrelated genes [11,12]. The regions between the PTUs are called strand switch regions where transcription is initiated and terminated [13,14] (Fig. 1). However, episomal DNA, e.g. plasmid- or cosmid-based transgenes, are efficiently transcribed [15,16], without harbouring strand switch region DNA. This argues for additional, unspecific initiation of RNA synthesis. The polycistronic pre-mRNAs are processed into mature mRNAs by coupled *trans*-splicing of a leader RNA and polyadenylation [17]. With very few exceptions, *Leishmania* genes lack introns and *cis*-splicing [18], eliminating alternative splicing as a means of gene regulation.

The differentiation from the non-pathogenic promastigote form of the insect host to the pathogenic amastigote stage is induced by factors that are not yet fully understood. It is known, however, that for a range of *Leishmania* spp., an acidic pH of 5.5 and a temperature increase to 33–37 °C are the major triggers for differentiation even under axenic culture conditions [19,20]. Conversion into amastigotes is crucial for survival within the mammalian host and thus plays an important role in the pathogenicity of *Leishmania* spp.. The pivotal role of heat shock proteins in differentiation and adaption was proposed early and has been extensively studied in *Leishmania* [21].

In the following, we shall discuss the ways by which *Leishmania* can respond to environmental stimuli using post-transcriptional gene regulation and adapt to environmental challenges by constitutive genetic diversity and selection.

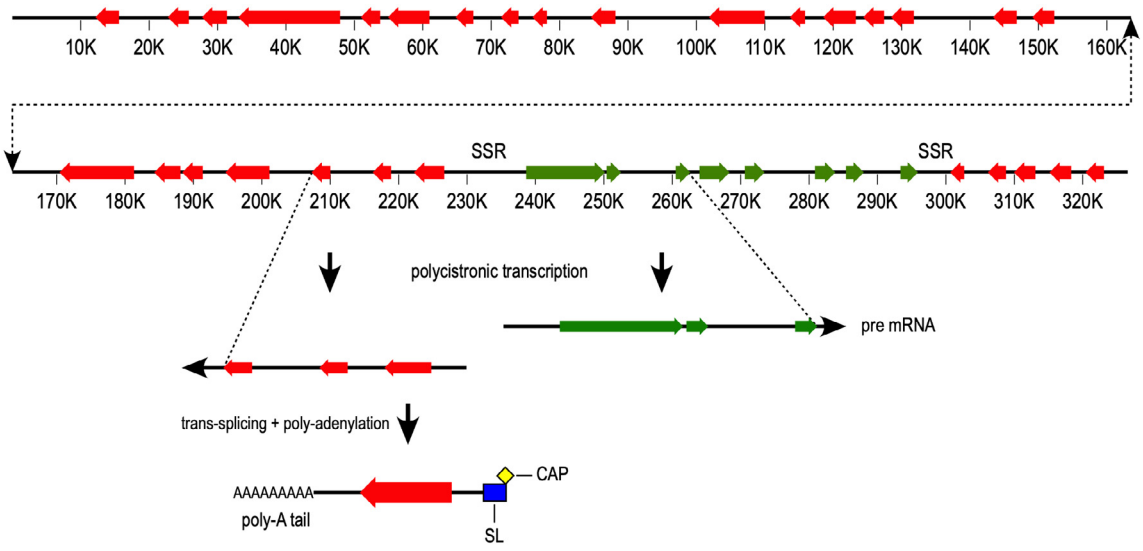

Fig. 1. Polycistronic transcription and RNA processing in *Leishmania*. The figure shows the distribution of coding sequences (CDSs) on the *L. donovani* chromosome 2. Arrows in red signify upper strand CDSs, green arrows indicate lower strand CDSs. Polycistronic transcripts are generated and processed into mature, monocistronic mRNA by *trans*-splicing of a 39-nucleotide spliced leader RNA (SL) and coupled poly-adenylation (poly A). The SL also contributes the CAP structure (yellow diamond) to the mRNA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Post-transcriptional responses to environmental signals

2.1. Post transcriptional regulation

The response to environmental stimuli is mediated by post-transcriptional regulation of gene expression in *Leishmania* spp., a fact that was demonstrated by nuclear run-on experiments and using the universal cellular heat shock response as model [22,23]. Nuclear run-on and microarray studies showed that *Leishmania* genes are constitutively transcribed, consistent with the lack of gene-specific transcription regulation. However, a global down regulation of RNA synthesis was observed for axenic amastigotes when compared with promastigotes [24,25]. The stage differentiation process itself is associated with a globally increased protein synthesis [26], but also with specific increases for amastigote stage-specific proteins [27].

2.2. The role of mRNA stability and translation efficiency

While early research focused mainly on mRNA levels of selected genes, later proteome analysis and system-wide RNA abundance studies revealed that the regulation of *Leishmania* gene expression occurs on levels other than RNA synthesis, i.e. RNA processing and stability [28]. Even then, changes of mRNA abundance do not necessarily translate into corresponding protein synthesis rates or abundance, hinting at translation efficiency and protein half life as additional targets of regulation [26,29–33].

RNA half life as a post-transcriptional regulatory mechanism was shown for resistance to antimony, which differs in various *Leishmania* species and correlates with the stability of the mRNA coding for aquaglyceroporin (AQP1) and is mediated by the 3′-untranslated region (3′ UTR) [34]. RNA stability is regulated by non-long terminal repeat retrotransposons in the 3′ UTR. In *L. major*, two families of these Short Interspersed DEgenerated Retroposons (SIDER) could be identified, LmSIDER1 and LmSIDER2. For SIDER2 it was demonstrated that it destabilises the mRNA and even leads to an mRNA decay [35,36], while the SIDER1 family can regulate mRNA translation in a stage-specific manner [37].

2.3. Ribosome profiling shows a regulated translation in Leishmania

RNA-Seq analysis is a powerful tool to obtain a genome-wide view of mRNA abundance patterns and is often seen as the equivalent to genome-wide gene expression analysis. However, in *Leishmania* and the related *Trypanosoma* spp., inducible mRNA abundance and corresponding protein synthesis/abundance show only a limited correlation [26,33,38–40]. To study the effect of environmental triggers on gene expression in the parasite, the steady-state level of proteins or translation rates can be measured. The former can be done by mass spectrometry-based proteomics [26,27,41] and the latter is achieved by ribosome profiling analysis [42] or a combined metabolic labeling/mass-spectrometry strategy [43]. The combination of ribosome profiling and RNA-Seq facilitates not only correlation studies of mRNA abundance and translation, but also gives a measure of the relative translation efficiencies of mRNAs in response to environmental triggers. Ribosome profiling analyses in *L. donovani*, combined with RNA-Seq, showed that inhibition of HSP90, while having no global impact on gene expression, changes steady state levels for many mRNAs and causes increased or decreased protein synthesis rates for <10% of the proteome. RNA abundance variations correlate poorly with changes to translation rates [33]. Among the proteins that show increased synthesis upon HSP90 inhibition are many that are known as markers of early amastigote differentiation, while induced morphological changes are reminiscent of *in vitro* stage conversion [16,26,33,44].

2.4. Translation factors

While no canonical transcription factors are involved in controlling gene expression in *Leishmania*, translation factors may play important roles. Axenically induced amastigote differentiation coincided with reduced overall translation and phosphorylation of translation factor eIF2α [45]. Reduction of protein synthesis in amastigotes may reflect the adaptation to the reduced proliferation rate observed for the intracellular stage [46]. Furthermore, it is known for eukaryotes that stress causes a switch from cap-dependent to cap-independent translation. *Leishmania* spp. can adapt their translation machinery to environmental stress by a special cap structure (cap-4) and associated cap-binding protein eIF4E [47]. There are several factors, e.g. Leish4E-IP, that bind to eIF4E and thus mediate the switch from cap-dependent to alternative translation initiation mechanisms [48]. Apart from these findings, very little is known about translation factors and their role in the regulation of inducible gene expression in *Leishmania*.

2.5. Post-translational modifications

Post-translational modifications (PTMs) are also involved in the regulation of gene expression patterns by influencing the function and stability of proteins through phosphorylation, acetylation, methylation, and glycosylation. High throughput liquid chromatography/tandem mass spectrometry (LC-MS/MS) methodologies were used to study the prevalence of PTMs during axenic differentiation into amastigotes and showed altered abundance of modified proteins [49–51]. MAP kinases (MAPK), for instance, play a major role in altering gene expression profiles. They are highly conserved serine/threonine-specific protein kinases in all eukaryotes and are important in signal transduction cascades. By phosphorylating their substrates, MAPKs regulate critical cellular functions in *Leishmania* affecting cell viability, parasitic life cycle control, morphology and drug resistance [52–59]. Phosphorylation of HSP70 and HSP90 by the *L. donovani* MAP kinase 1 is thought to affect the stability of heat shock proteins and their functions during the life cycle [60].

Casein kinases also play crucial roles in overcoming environmental adversity. They are exported to the host cell cytoplasm via exosomes and interact with parasite chaperones and host proteins [61–64,94].

3. Genetic diversity and selection under environmental challenge

The lack of gene regulation at the transcription level and the absence of *cis*-splicing in *Leishmania*, the Trypanosomatida and possibly all Euglenozoa sets these organisms apart from other eukaryotic phyla [10,28,65,66]. Although *Leishmania* chromosomes are divided into gene arrays that are transcribed in a polycistronic mode [11,13,67], those arrays do not constitute operons of jointly regulated, functionally related genes. Nevertheless, *Leishmania* populations are able to adapt to environmental adversity.

Starting in the 1980s, researchers discovered that certain *Leishmania* genes were present in more than one copy per haploid set of chromosomes [68–72], and the copy numbers often varied between species and parasite isolates. This already hinted at gene copy number variation (CNV) as a mechanism of genetic adaption.

The advent of reverse genetics in *Leishmania* research [73,74] provided more puzzles. Double-allelic gene replacement by homologous recombination using different selection marker genes for

both alleles often left detectable copies of the genes of interest (GOI), in spite of a successful and verified gene replacement and marker gene insertion [74,75].

The availability of deep sequencing technology in recent years has greatly expanded our knowledge of the structure and dynamics of *Leishmania* genomes. Parasite populations selected under virtually any sort of pressure, e.g. drug challenge, temperature stress, pH milieu, oxidative stress, but also loss-of-function mutations with growth defects, can be subjected to whole genome sequencing (WGS) alongside the pre-selection parental strain. The resulting sequence read densities can then be analysed for ploidy changes, gene CNVs, and single nucleotide polymorphisms (SNPs) [76].

3.1. Aneuploidy and mosaic aneuploidy

Individual chromosome ploidy in *Leishmania* is highly variable within populations [77–79]. Between one and up to six sister chromosomes may occur. Chromosome ploidy can be assessed from the relative NGS read alignment density, normalised to a reference strain [76] (Fig. 2A). Intermediate fold values can indicate mosaic aneuploidy, e.g. variances within the population, or partial chromosome duplication events. Distinction between these possibilities is done by plotting read alignment density against the length of the chromosome: even read alignment density indicates whole chromosome/partial population changes (Fig. 2B), while localised

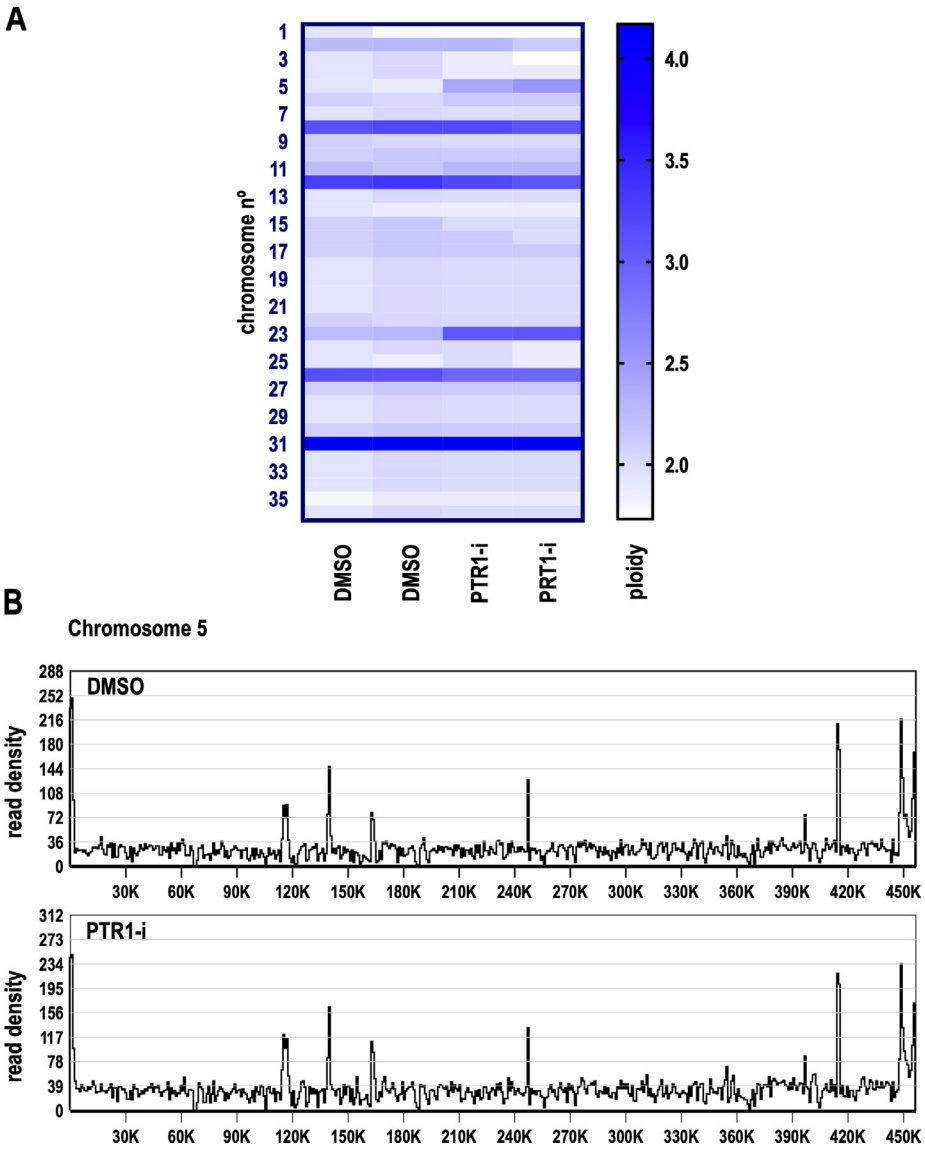

Fig. 2. Constitutive and selected aneuploidy in *Leishmania donovani* selected under IC_{50} of a pteridin reductase 1 inhibitor (PTR1-i) or an equivalent dose of solvent (DMSO) for 40 days. (A) NGS read alignment densities were established for each chromosome using the Bowtie2 algorithm, normalised against the overall average read density set at 2, and displayed as heat map. Note that i) chromosome 31 is constitutively tetrasomic, ii)chromosomes 8, 12, and 24 are constitutively trisomic, and chromosome 23 shows trisomy after PTR1-i selection. In addition, chromosome 5 shows an intermediate ploidy indicating mosaic aneuploidy or partial chromosome amplification. (B) Sequence read alignment over chromosome 5 for DMSO- (upper panel) or PTR1-i-selected (lower panel) shows equal relative read distribution, indicating mosaic aneuploidy for chromosome 5 in the PTR1-i-selected population.

read alignment density increases may hint at partial chromosome/whole population events.

Aneuploidy has been studied extensively in the context of drug resistance. Decades of use of pentavalent antimony-based drugs in endemic regions have led to wide-spread resistance, rendering these drugs almost obsolete in certain areas [80–83]. The advances of systems biology strategies in *Leishmania* spp. confirmed initial proposals [84] linking antimony resistance with aneuploidy patterns specific for resistant parasites [85–87]. However, mosaic aneuploidy also plays a role in the adaption to vector and host as distinct patterns of ploidy are observed for vector- and host-derived leishmaniae [86], but also for *in vitro* cultivated cells [86,88,89].

Even more telling is a recent finding comparing somy patterns of identical *L. donovani* strains obtained from cultivated parasites or directly from clinical biopsies. This approach skips the *in vitro* cultivation of parasites and the concomitant karyotypic diversification prior to whole genome sequencing. While cultured parasites showed a wide variety of somy patterns, ploidies were remarkably similar for bone-marrow-derived parasite genomes [90], in keeping with comparable selective pressures within the human hosts.

In multicellular organisms, aneuploidy is known to have severe consequences, including tumour formation and chromosome instability [91–93]. Yet, the leishmaniae seem to cope well with genome plasticity. Firstly, aneuploidy is reversed quickly once the benefits of a supernumerary chromosome expire. Secondly, over expression due to additional gene copies appears to be limited at the protein level [86,94]. Regulated translation may therefore play a role in ameliorating the effects of aneuploidy.

3.2. Gene CNVs

As mentioned before, gene CNVs were observed for *Leishmania* species and strains, often affecting heat shock gene arrays [12,44,68–72], which can be assumed to mediate tolerance against various physical and chemical stresses. Indeed, increased abundances of HSP90 or HSP70 appear to protect *Leishmania* spp. against antimony-containing drugs [95–97]. Drug selection pressure can also lead to increased copy numbers for genes encoding ABC transporter proteins [98]. The prevalent mechanism for gene amplification was shown to depend on the SIDERs that are distributed over the *Leishmania* genomes. In addition to their roles in mRNA stability, they can be found flanking amplified intrachromosomal and episomal genes and gene clusters, suggesting a role of such repeats in the formation of linear and circular episomes [94,99]. Such amplifications occur frequently and stochastically within a given population and, like mosaic aneuploidy, contribute to the constitutive genetic diversity within *Leishmania* isolates [85–87]. In fact, these fluctuations of gene copy numbers are known to account for a majority of genetic adaptations [100]. However, while gene dosage affects the abundance of the corresponding RNA(s) proportionally, their effect on protein abundance during natural gene copy number variation [86] and targeted over expression [94,101] is extenuated, likely due to modulated translation. Still, increased gene copy numbers lead to increased expression.

4. SNPs

SNPs in non-coding or protein-coding sequences may affect expression or function, respectively, of genes and their products. They are usually associated with a loss-of-function phenotype. In the context of drug resistance, SNPs were found in *Leishmania* selected for resistance to the antileishmanial drug miltefosine. Selected parasites carry a plethora of SNPs in the miltefosine transporter gene causing amino acid exchanges or open reading frame shifts [102,103].

Another example for the selection of SNPs involves the *L. major* P46 virulence enhancing protein, which, depending on the geographic origin of *L. major* isolates, shows distinct patterns of SNPs. Defined SNP patterns were indeed selected *in vitro* and in murine hosts, suggesting a role of P46 sequence polymorphism in the adaption to diverse reservoir hosts found in various *L. major*-endemic regions [104].

4.1. Mimicking CNV in the laboratory

Adaptive, extrachromosomal gene amplifications can be mimicked in a laboratory setting by complementation genetics, more specifically by functional cloning from a cosmid library representing a *Leishmania* genome [15,105–109]. Since *Leishmania* genes do not contain introns but need their flanking sequences for proper RNA processing via *trans*-splicing and polyadenylation [110], unaltered, genomic DNA-bearing cosmids contain all the information needed for proportional over expression of several neighbouring gene loci. The compactness of the *Leishmania* genomes (~32–34 M b) contributes to this, with less than 5000 individual cosmid clones covering >99% of the genome [107], and allowing for a genome-wide search for dominant genetic markers using a manageable number of individual transfectants. Using shuttle cosmid vectors, propagation in *Escherichia coli* and *Leishmania* spp. is feasible. Lately, the technology was greatly improved by the use of next generation sequencing (NGS) to map the origin of selected cosmids and to establish the relative preference within a number of selected gene loci [111–113]. This cos-seq strategy can now be employed to identify dominant drug resistance markers for established drugs and for drug leads, improving sustainable drug development [111,114].

5. Conclusions

By dispensing with individually regulated RNA polymerase II transcription, *Leishmania* spp., like all Trypanosomatida, lost an important route to up-regulate the expression of genes needed for overcoming environmental adversity, while allowing them to dispense with cis- and *trans*-acting transcription regulators, thus reducing genome size. The constitutive transcription of most of their genome and the resulting waste of biochemical energy equivalents is compensated in part by the compact arrangement of coding sequences, the lack of *cis*-splicing and introns, and the parasitic life style, utilising the host's energy resources. Generally speaking, obligate parasites such as *Leishmania* spp. live in the stable environments of either sandflies or mammals and are thus shielded against acute environmental changes. Nevertheless, their extreme and constitutive genetic variability as expressed in mosaic aneuploidy, episomal and intrachromosomal gene amplification, a short generation time, and an effective post-transcriptional stress response allows them to adapt — as a population — to environmental challenges in an exemplary fashion.

6. Authors statement

J.G. and J.C. contributed equally to the creation of this manuscript, including conceptualisation, literature research, drafting and finalising of the manuscript. J.C. contributed the Figures.

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References

[1] WHO. Integrating neglected tropical diseases into global health and development: fourth WHO report on neglected tropical diseases. 2017.
[2] Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS ONE 2012;7:. https://doi.org/10.1371/journal.pone.0035671e35671.
[3] Simpson AG, Gill EE, Callahan HA, Litaker RW, Roger AJ. Early evolution within kinetoplastids (euglenozoa), and the late emergence of trypanosomatids. Protist 2004;155:407–22. https://doi.org/10.1078/1434461042650389.
[4] Vesteg M, Hadariova L, Horvath A, Estrano CE, Schwartzbach SD, Krajcovic J. Comparative molecular cell biology of phototrophic euglenids and parasitic trypanosomatids sheds light on the ancestor of Euglenozoa. Biol Rev Camb Philos Soc 2019;94:1701–21. https://doi.org/10.1111/brv.12523.
[5] Banuls AL, Hide M, Prugnolle F. Leishmania and the leishmaniases: a parasite genetic update and advances in taxonomy, epidemiology and pathogenicity in humans. Adv Parasitol 2007;64:1–109. https://doi.org/10.1016/S0065-308X(06)64001-3.
[6] Burza S, Croft SL, Boelaert M. Leishmaniasis. Lancet 2018;392:951–70. https://doi.org/10.1016/S0140-6736(18)31204-2.
[7] Silverman JM, Reiner NE. Leishmania exosomes deliver preemptive strikes to create an environment permissive for early infection. Front Cell Infect Microbiol 2011;1:26. https://doi.org/10.3389/fcimb.2011.00026.
[8] Singh OP, Hasker E, Sacks D, Boelaert M, Sundar S. Asymptomatic leishmania infection: a new challenge for Leishmania control. Clin Infect Dis 2014;58:1424–9. https://doi.org/10.1093/cid/ciu102.
[9] Lee TI, Young RA. Transcription of eukaryotic protein-coding genes. Annu Rev Genet 2000;34:77–137. https://doi.org/10.1146/annurev.genet.34.1.77.
[10] Clayton CE. Life without transcriptional control? From fly to man and back again. EMBO J 2002;21:1881–8.
[11] Ivens AC, Peacock CS, Worthey EA, Murphy L, Aggarwal G, Berriman M, et al. The genome of the kinetoplastid parasite, Leishmania major. Science 2005;309:436–42.
[12] Peacock, C.S.; Seeger, K.; Harris, D.; Murphy, L.; Ruiz, J.C.; Quail, M.A.; Peters, N.; Adlem, E.; Tivey, A.; Aslett, M., et al. Comparative genomic analysis of three Leishmania species that cause diverse human disease. Nat Genet 2007, 39, 839-847, doi:ng2053 [pii] 10.1038/ng2053.
[13] Martinez-Calvillo, S.; Yan, S.; Nguyen, D.; Fox, M.; Stuart, K.; Myler, P.J. Transcription of Leishmania major Friedlin chromosome 1 initiates in both directions within a single region. Mol Cell 2003, 11, 1291-1299, doi: S1097276503001436 [pii].]
[14] Martinez-Calvillo S, Nguyen D, Stuart K, Myler PJ. Transcription initiation and termination on Leishmania major chromosome 3. Eukaryot Cell 2004;3:506–17. https://doi.org/10.1128/ec.3.2.506-517.2004.
[15] Choudhury K, Zander D, Kube M, Reinhardt R, Clos J. Identification of a Leishmania infantum gene mediating resistance to miltefosine and SbIII. Int J Parasitol 2008;38:1411–23.
[16] Hombach A, Ommen G, Chrobak M, Clos J. The Hsp90-Sti1 interaction is critical for*Leishmania donovani* proliferation in both life cycle stages. Cell Microbiol 2013;15:585–600. https://doi.org/10.1111/cmi.12057.
[17] Liang XH, Haritan A, Uliel S, Michaeli S. Trans and cis splicing in trypanosomatids: mechanism, factors, and regulation. Eukaryot Cell 2003;2:830–40. https://doi.org/10.1128/ec.2.5.830-840.2003.
[18] Lynn MA, McMaster WR. Leishmania: conserved evolution–diverse diseases. Trends Parasitol 2008;24:103–5. https://doi.org/10.1016/j.pt.2007.11.006.
[19] Saar Y, Ransford A, Waldman E, Mazareb S, Amin-Spector S, Plumblee J, et al. Characterization of developmentally-regulated activities in axenic amastigotes of Leishmania donovani. Mol Biochem Parasitol 1998;95:9–20. https://doi.org/10.1016/s0166-6851(98)00062-0.
[20] Barak E, Amin-Spector S, Gerliak E, Goyard S, Holland N, Zilberstein D. Differentiation of Leishmania donovani in host-free system: analysis of signal perception and response. Mol Biochem Parasitol 2005;141:99–108. https://doi.org/10.1016/j.molbiopara.2005.02.004.
[21] Kröber-Boncardo C, Grünebast J, Clos J. Heat shock proteins in leishmania parasites. In: Asea A, editor. Heat Shock Proteins. Dordrecht: Springer:; 2020. https://doi.org/10.1007/7515_2020_27.
[22] Brandau S, Dresel A, Clos J. High constitutive levels of heat-shock proteins in human-pathogenic parasites of the genus Leishmania. Biochem J 1995;310(Pt 1):225–32. https://doi.org/10.1042/bj3100225.
[23] Quijada L, Soto M, Alonso C, Requena JM. Analysis of post-transcriptional regulation operating on transcription products of the tandemly linked Leishmania infantum hsp70 genes. J Biol Chem 1997;272:4493–9. https://doi.org/10.1074/jbc.272.7.4493.
[24] Leifso K, Cohen-Freue G, Dogra N, Murray A, McMaster WR. Genomic and proteomic expression analysis of Leishmania promastigote and amastigote life stages: the Leishmania genome is constitutively expressed. Mol Biochem Parasitol 2007;152:35–46. https://doi.org/10.1016/j.molbiopara.2006.11.009.
[25] Alcolea PJ, Alonso A, Gomez MJ, Moreno I, Dominguez M, Parro V, et al. Transcriptomics throughout the life cycle of Leishmania infantum: high down-regulation rate in the amastigote stage. Int J Parasitol 2010;40:1497–516. https://doi.org/10.1016/j.ijpara.2010.05.013.
[26] Lahav T, Sivam D, Volpin H, Ronen M, Tsigankov P, Green A, et al. Multiple levels of gene regulation mediate differentiation of the intracellular pathogen Leishmania. FASEB J 2011;25:515–25. https://doi.org/10.1096/fj.10-157529.
[27] Rosenzweig, D.; Smith, D.; Opperdoes, F.; Stern, S.; Olafson, R.W.; Zilberstein, D. Retooling Leishmania metabolism: from sand fly gut to human macrophage. FASEB J 2008, 22, 590-602, doi:fj.07-9254com [pii] 10.1096/fj.07-9254com.
[28] Clayton C, Shapira M. Post-transcriptional regulation of gene expression in trypanosomes and leishmanias. Mol Biochem Parasitol 2007;156:93–101. https://doi.org/10.1016/j.molbiopara.2007.07.007.
[29] Charest H, Zhang WW, Matlashewski G. The developmental expression of Leishmania donovani A2 amastigote-specific genes is post-transcriptionally mediated and involves elements located in the 3'-untranslated region. J Biol Chem 1996;271:17081–90. https://doi.org/10.1074/jbc.271.29.17081.
[30] Yao C, Leidal KG, Brittingham A, Tarr DE, Donelson JE, Wilson ME. Biosynthesis of the major surface protease GP63 of Leishmania chagasi. Mol Biochem Parasitol 2002;121:119–28. https://doi.org/10.1016/s0166-6851(02)00030-0.
[31] Decuypere S, Vanaerschot M, Brunker K, Imamura H, Muller S, Khanal B, et al. Molecular mechanisms of drug resistance in natural Leishmania populations vary with genetic background. PLoS Negl Trop Dis 2012;6:. https://doi.org/10.1371/journal.pntd.0001514e1514.
[32] Azevedo A, Toledo JS, Defina T, Pedrosa AL, Cruz AK. Leishmania major phosphoglycerate kinase transcript and protein stability contributes to differences in isoform expression levels. Exp Parasitol 2015;159:222–6. https://doi.org/10.1016/j.exppara.2015.09.008.
[33] Bifeld, E.; Lorenzen, S.; Bartsch, K.; Vasquez, J.J.; Siegel, T.N.; Clos, J. Ribosome Profiling Reveals HSP90 Inhibitor Effects on Stage-Specific Protein Synthesis in Leishmania donovani. mSystems 2018, 3, doi:10.1128/mSystems.00214-18.
[34] Mandal G, Mandal S, Sharma M, Charret KS, Papadopoulou B, Bhattacharjee H, et al. Species-specific antimonial sensitivity in Leishmania is driven by post-transcriptional regulation of AQP1. PLoS Negl Trop Dis 2015;9:. https://doi.org/10.1371/journal.pntd.0003500e0003500.
[35] Bringaud F, Muller M, Cerqueira GC, Smith M, Rochette A, El-Sayed NM, et al. Members of a large retroposon family are determinants of post-transcriptional gene expression in Leishmania. PLoS Pathog 2007;3:1291–307. https://doi.org/10.1371/journal.ppat.0030136.
[36] Azizi H, Dumas C, Papadopoulou B. The Pumilio-domain protein PUF6 contributes to SIDER2 retroposon-mediated mRNA decay in Leishmania. RNA 2017;23:1874–85. https://doi.org/10.1261/rna.062950.117.
[37] Boucher N, Wu Y, Dumas C, Dube M, Sereno D, Breton M, et al. A common mechanism of stage-regulated gene expression in Leishmania mediated by a conserved 3'-untranslated region element. J Biol Chem 2002;277:19511–20. https://doi.org/10.1074/jbc.M200500200.
[38] Jensen BC, Ramasamy G, Vasconcelos EJ, Ingolia NT, Myler PJ, Parsons M. Extensive stage-regulation of translation revealed by ribosome profiling of Trypanosoma brucei. BMC Genomics 2014;15:911. https://doi.org/10.1186/1471-2164-15-911.
[39] Vasquez JJ, Hon CC, Vanselow JT, Schlosser A, Siegel TN. Comparative ribosome profiling reveals extensive translational complexity in different Trypanosoma brucei life cycle stages. Nucleic Acids Res 2014;42:3623–37. https://doi.org/10.1093/nar/gkt1386.
[40] Smircich P, Eastman G, Bispo S, Duhagon MA, Guerra-Slompo EP, Garat B, et al. Ribosome profiling reveals translation control as a key mechanism generating differential gene expression in Trypanosoma cruzi. BMC Genomics 2015;16:443. https://doi.org/10.1186/s12864-015-1563-8.
[41] Bente M, Harder S, Wiesgigl M, Heukeshoven J, Gelhaus C, Krause E, et al. Developmentally induced changes of the proteome in the protozoan parasite Leishmania donovani. Proteomics 2003;3:1811–29.
[42] Ingolia NT, Ghaemmaghami S, Newman JR, Weissman JS. Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. Science 2009;324:218–23. https://doi.org/10.1126/science.1168978.
[43] Kalesh K, Denny PW. A BONCAT-iTRAQ method enables temporally resolved quantitative profiling of newly synthesised proteins in Leishmania mexicana parasites during starvation. PLoS Negl Trop Dis 2019;13:. https://doi.org/10.1371/journal.pntd.0007651e0007651.
[44] Wiesgigl M, Clos J. Heat shock protein 90 homeostasis controls stage differentiation in *Leishmania donovani*. Mol Biol Cell 2001;12:3307–16.
[45] Cloutier S, Laverdiere M, Chou MN, Boilard N, Chow C, Papadopoulou B. Translational control through eIF2alpha phosphorylation during the Leishmania differentiation process. PLoS ONE 2012;7:. https://doi.org/10.1371/journal.pone.0035085e35085.
[46] Jara M, Maes I, Imamura H, Domagalska MA, Dujardin JC, Arevalo J. Tracking of quiescence in Leishmania by quantifying the expression of GFP in the ribosomal DNA locus. Sci Rep 2019;9:18951. https://doi.org/10.1038/s41598-019-55486-z.
[47] Dhalia R, Reis CR, Freire ER, Rocha PO, Katz R, Muniz JR, et al. Translation initiation in Leishmania major: characterisation of multiple eIF4F subunit homologues. Mol Biochem Parasitol 2005;140:23–41. https://doi.org/10.1016/j.molbiopara.2004.12.001.

[48] Zinoviev A, Leger M, Wagner G, Shapira M. A novel 4E-interacting protein in Leishmania is involved in stage-specific translation pathways. Nucleic Acids Res 2011;39:8404–15. https://doi.org/10.1093/nar/gkr555.
[49] Morales MA, Watanabe R, Laurent C, Lenormand P, Rousselle JC, Namane A, et al. Phosphoproteomic analysis of*Leishmania donovani*pro- and amastigote stages. Proteomics 2008;8:350–63. https://doi.org/10.1002/pmic.200700697.
[50] Rosenzweig D, Smith D, Myler PJ, Olafson RW, Zilberstein D. Post-translational modification of cellular proteins during Leishmania donovani differentiation. Proteomics 2008;8:1843–50. https://doi.org/10.1002/pmic.200701043.
[51] Morales, M.; Watanabe, R.; Dacher, M.; Chafey, P.; Osorio y Fortéa, J.; Beverley, S.; Ommen, G.; Clos, J.; Hem, S.; Lenormand, P., et al. Phosphoproteome dynamics reveals heat shock protein complexes specific to theLeishmaniainfectious stage. Proc Natl Acad Sci U S A 2010, 107, 8381-8386.
[52] Wiese M. A mitogen-activated protein (MAP) kinase homologue of Leishmania mexicana is essential for parasite survival in the infected host. Embo J 1998;17:2619–28.
[53] Wiese M, Kuhn D, Grunfelder CG. Protein kinase involved in flagellar-length control. Eukaryot Cell 2003;2:769–77.
[54] Bengs F, Scholz A, Kuhn D, Wiese M. LmxMPK9, a mitogen-activated protein kinase homologue affects flagellar length in Leishmania mexicana. Mol Microbiol 2005;55:1606–15.
[55] Kuhn D, Wiese M. LmxPK4, a mitogen-activated protein kinase kinase homologue of Leishmania mexicana with a potential role in parasite differentiation. Mol Microbiol 2005;56:1169–82.
[56] Wang Q, Melzer IM, Kruse M, Sander-Juelch C, Wiese M. LmxMPK4, a mitogen-activated protein (MAP) kinase homologue essential for promastigotes and amastigotes of*Leishmania mexicana*. Kinetoplastid Biol Dis 2005;4:6.
[57] Erdmann M, Scholz A, Melzer IM, Schmetz C, Wiese M. Interacting protein kinases involved in the regulation of flagellar length. Mol Biol Cell 2006;17:2035–45. https://doi.org/10.1091/mbc.E05-10-0976.
[58] von Freyend, S.J.; Rosenqvist, H.; Fink, A.; Melzer, I.M.; Clos, J.; Jensen, O.N.; Wiese, M. LmxMPK4, an essential mitogen-activated protein kinase of Leishmania mexicana is phosphorylated and activated by the STE7-like protein kinase LmxMKK5. Int J Parasitol 2010, 40, 969-978, doi:S0020-7519(10)00062-7 [pii] 10.1016/j.ijpara.2010.02.004.
[59] Cayla M, Rachidi N, Leclercq O, Schmidt-Arras D, Rosenqvist H, Wiese M, et al. Transgenic analysis of the Leishmania MAP kinase MPK10 reveals an auto-inhibitory mechanism crucial for stage-regulated activity and parasite viability. PLoS Pathog 2014;10:. https://doi.org/10.1371/journal.ppat.1004347e1004347.
[60] Kaur P, Garg M, Hombach-Barrigah A, Clos J, Goyal N. MAPK1 of Leishmania donovani interacts and phosphorylates HSP70 and HSP90 subunits of foldosome complex. Sci Rep 2017;7:10202. https://doi.org/10.1038/s41598-017-09725-w.
[61] Silverman, J.M.; Clos, J.; de'Oliveira, C.C.; Shirvani, O.; Fang, Y.; Wang, C.; Foster, L.J.; Reiner, N.E. An exosome-based secretion pathway is responsible for protein export from Leishmania and communication with macrophages. J Cell Sci 2010, 123, 842-852, doi:jcs.056465 [pii] 10.1242/jcs.056465.
[62] Rachidi N, Taly JF, Durieu E, Leclercq O, Aulner N, Prina E, et al. Pharmacological assessment defines Leishmania donovani casein kinase 1 as a drug target and reveals important functions in parasite viability and intracellular infection. Antimicrob Agents Chemother 2014;58:1501–15. https://doi.org/10.1128/AAC.02022-13.
[63] Martel, D.; Beneke, T.; Gluenz, E.; Spath, G.F.; Rachidi, N. Characterisation of Casein Kinase 1.1 in Leishmania donovani Using the CRISPR Cas9 Toolkit. Biomed Res Int 2017, 2017, 4635605, doi:10.1155/2017/4635605.
[64] Hombach-Barrigah A, Bartsch K, Smirlis D, Rosenqvist H, MacDonald A, Dingli F, et al. Leishmania donovani 90 kD Heat Shock Protein - Impact of Phosphosites on Parasite Fitness, Infectivity and Casein Kinase Affinity. Sci Rep 2019;9:5074. https://doi.org/10.1038/s41598-019-41640-0.
[65] Reinbothe S, Parthier B. Translational regulation of plastid gene expression in Euglena gracilis. FEBS Lett 1990;265:7–11.
[66] Keller M, Chan RL, Tessier LH, Weil JH, Imbault P. Post-transcriptional regulation by light of the biosynthesis of Euglena ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit. Plant Mol Biol 1991;17:73–82.
[67] Myler PJ, Beverley SM, Cruz AK, Dobson DE, Ivens AC, McDonagh PD, et al. The Leishmania genome project: new insights into gene organization and function. Med Microbiol Immunol (Berl) 2001;190:9–12.
[68] Lee, M.G.; Atkinson, B.L.; Giannini, S.H.; Van der Ploeg, L.H. Structure and expression of the hsp 70 gene family of Leishmania major [published erratum appears in Nucleic Acids Res 1988 Dec 9;16(23):11400- 1]. Nucleic Acids Res 1988, 16, 9567-9585.
[69] Bock JH, Langer PJ. Sequence and genomic organization of the hsp70 genes of Leishmania amazonensis. Mol Biochem Parasitol 1993;62:187–97.
[70] Hübel A, Clos J. The genomic organization of the HSP83 gene locus is conserved in three Leishmania species. Exp Parasitol 1996;82:225–8.
[71] Shapira M, Zilka A, Garlapati S, Dahan E, Dahan I, Yavesky V. Post transcriptional control of gene expression in Leishmania. Med Microbiol Immunol (Berl) 2001;190:23–6.
[72] Folgueira C, Requena JM. A postgenomic view of the heat shock proteins in kinetoplastids. FEMS Microbiol Rev 2007;31:359–77. https://doi.org/10.1111/j.1574-6976.2007.00069.x.
[73] Cruz A, Coburn CM, Beverley SM. Double targeted gene replacement for creating null mutants. Proc Natl Acad Sci USA 1991;88:7170–4.
[74] Cruz AK, Titus R, Beverley SM. Plasticity in chromosome number and testing of essential genes in Leishmania by targeting. Proc Natl Acad Sci USA 1993;90:1599–603. https://doi.org/10.1073/pnas.90.4.1599.
[75] Krobitsch S, Clos J. Cross-species homologous recombination in *Leishmania donovani* reveals the sites of integration. Mol Biochem Parasitol 2000;107:123–8.
[76] Imamura H, Dujardin JC. A guide to next generation sequence analysis of leishmania genomes. Methods Mol Biol 2019;1971:69–94. https://doi.org/10.1007/978-1-4939-9210-2_3.
[77] Mannaert A, Downing T, Imamura H, Dujardin JC. Adaptive mechanisms in pathogens: universal aneuploidy in*Leishmania*. Trends in parasitology 2012;28:370–6. https://doi.org/10.1016/j.pt.2012.06.003.
[78] Lachaud L, Bourgeois N, Kuk N, Morelle C, Crobu L, Merlin G, et al. Constitutive mosaic aneuploidy is a unique genetic feature widespread in the Leishmania genus. Microbes Infect 2014;16:61–6. https://doi.org/10.1016/j.micinf.2013.09.005.
[79] Sterkers Y, Crobu L, Lachaud L, Pages M, Bastien P. Parasexuality and mosaic aneuploidy in Leishmania: alternative genetics. Trends Parasitol 2014;30:429–35. https://doi.org/10.1016/j.pt.2014.07.002.
[80] Sundar S. Drug resistance in Indian visceral leishmaniasis. Trop Med Int Health 2001;6:849–54.
[81] Dube A, Singh N, Sundar S. Refractoriness to the treatment of sodium stibogluconate in Indian kala-azar field isolates persist in in vitro and in vivo experimental models. Parasitol Res 2005.
[82] Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clin Microbiol Rev 2006;19:111–26.
[83] Ostyn B, Hasker E, Dorlo TP, Rijal S, Sundar S, Dujardin JC, et al. Failure of miltefosine treatment for visceral leishmaniasis in children and men in South-East Asia. PLoS ONE 2014;9:. https://doi.org/10.1371/journal.pone.0100220e100220.
[84] Leprohon P, Legare D, Raymond F, Madore E, Hardiman G, Corbeil J, et al. Gene expression modulation is associated with gene amplification, supernumerary chromosomes and chromosome loss in antimony-resistant*Leishmania infantum*. Nucleic Acids Res 2009;37:1387–99. https://doi.org/10.1093/nar/gkn1069.
[85] Laffitte MN, Leprohon P, Papadopoulou B, Ouellette M. Plasticity of the Leishmania genome leading to gene copy number variations and drug resistance. F1000Res 2016;5:2350. https://doi.org/10.12688/f1000research.9218.1.
[86] Dumetz, F.; Imamura, H.; Sanders, M.; Seblova, V.; Myskova, J.; Pescher, P.; Vanaerschot, M.; Meehan, C.J.; Cuypers, B.; De Muylder, G., et al. Modulation of Aneuploidy in Leishmania donovani during Adaptation to Different In Vitro and In Vivo Environments and Its Impact on Gene Expression. MBio 2017, 8, doi:10.1128/mBio.00599-17.
[87] Dumetz, F.; Cuypers, B.; Imamura, H.; Zander, D.; D'Haenens, E.; Maes, I.; Domagalska, M.A.; Clos, J.; Dujardin, J.C.; De Muylder, G. Molecular Preadaptation to Antimony Resistance in Leishmania donovani on the Indian Subcontinent. mSphere 2018, 3, doi:10.1128/mSphere.00548-17.
[88] Sterkers Y, Lachaud L, Crobu L, Bastien P, Pages M. FISH analysis reveals aneuploidy and continual generation of chromosomal mosaicism in Leishmania major. Cell Microbiol 2011;13:274–83. https://doi.org/10.1111/j.1462-5822.2010.01534.x.
[89] Sterkers Y, Lachaud L, Bourgeois N, Crobu L, Bastien P, Pages M. Novel insights into genome plasticity in Eukaryotes: mosaic aneuploidy in Leishmania. Mol Microbiol 2012;86:15–23. https://doi.org/10.1111/j.1365-2958.2012.08185.x.
[90] Domagalska MA, Imamura H, Sanders M, Van den Broeck F, Bhattarai NR, Vanaerschot M, et al. Genomes of Leishmania parasites directly sequenced from patients with visceral leishmaniasis in the Indian subcontinent. PLoS Negl Trop Dis 2019;13:. https://doi.org/10.1371/journal.pntd.0007900e0007900.
[91] Pellman D. Cell biology: aneuploidy and cancer. Nature 2007;446:38–9. https://doi.org/10.1038/446038a.
[92] Torres EM, Williams BR, Amon A. Aneuploidy: cells losing their balance. Genetics 2008;179:737–46. https://doi.org/10.1534/genetics.108.090878.
[93] Rutledge SD, Cimini D. Consequences of aneuploidy in sickness and in health. Curr Opin Cell Biol 2016;40:41–6. https://doi.org/10.1016/j.ceb.2016.02.003.
[94] Kröber-Boncardo C, Lorenzen S, Brinker C, Clos J. Casein kinase 1.2 over expression restores stress resistance to Leishmania donovani HSP23 null mutants. Sci Rep 2020;10:15969. https://doi.org/10.1038/s41598-020-72724-x.
[95] Brochu C, Haimeur A, Ouellette M. The heat shock protein HSP70 and heat shock cognate protein HSC70 contribute to antimony tolerance in the protozoan parasite leishmania. Cell Stress Chaperones 2004;9:294–303.
[96] Vergnes B, Gourbal B, Girard I, Sundar S, Drummelsmith J, Ouellette M. A proteomics screen implicates HSP83 and a small kinetoplastid calpain-related protein in drug resistance in Leishmania donovani clinical field isolates by modulating drug-induced programmed cell death. Mol Cell Proteomics 2007;6:88–101. https://doi.org/10.1074/mcp.M600319-MCP200.
[97] Vacchina P, Norris-Mullins B, Carlson ES, Morales MA. A mitochondrial HSP70 (HSPA9B) is linked to miltefosine resistance and stress response in Leishmania donovani. Parasites Vectors 2016;9:621. https://doi.org/10.1186/s13071-016-1904-8.

[98] Grondin K, Haimeur A, Mukhopadhyay R, Rosen BP, Ouellette M. Co-amplification of the gamma-glutamylcysteine synthetase gene gsh1 and of the ABC transporter gene pgpA in arsenite-resistant Leishmania tarentolae. EMBO J 1997;16:3057–65. https://doi.org/10.1093/emboj/16.11.3057.

[99] Ubeda JM, Raymond F, Mukherjee A, Plourde M, Gingras H, Roy G, et al. Genome-wide stochastic adaptive DNA amplification at direct and inverted DNA repeats in the parasite*Leishmania*. PLoS Biol 2014;12:. https://doi.org/10.1371/journal.pbio.1001868e1001868.

[100] Iantorno, S.A.; Durrant, C.; Khan, A.; Sanders, M.J.; Beverley, S.M.; Warren, W. C.; Berriman, M.; Sacks, D.L.; Cotton, J.A.; Grigg, M.E. Gene Expression in Leishmania Is Regulated Predominantly by Gene Dosage. mBio 2017, 8, doi:10.1128/mBio.01393-17.

[101] Bartsch K, Hombach-Barrigah A, Clos J. Hsp90 inhibitors radicicol and geldanamycin have opposing effects on Leishmania Aha1-dependent proliferation. Cell Stress Chaperones 2017;22:729–42. https://doi.org/10.1007/s12192-017-0800-2.

[102] Perez-Victoria FJ, Gamarro F, Ouellette M, Castanys S. Functional cloning of the miltefosine transporter. A novel P-type phospholipid translocase from Leishmania involved in drug resistance. J Biol Chem 2003;278:49965–71.

[103] Laffitte MC, Leprohon P, Legare D, Ouellette M. Deep-sequencing revealing mutation dynamics in the miltefosine transporter gene in Leishmania infantum selected for miltefosine resistance. Parasitol Res 2016;115:3699–703. https://doi.org/10.1007/s00436-016-5195-y.

[104] Bifeld E, Chrobak M, Zander D, Schleicher U, Schonian G, Clos J. Geographical sequence variation in the Leishmania major virulence factor P46. Infect Genet Evol 2015;30:195–205. https://doi.org/10.1016/j.meegid.2014.12.029.

[105] Beverley SM, Turco SJ. Identification of genes mediating lipophosphoglycan biosynthesis by functional complementation of Leishmania donovani mutants. Ann Trop Med Parasitol 1995;89(Suppl 1):11–7.

[106] Cotrim PC, Garrity LK, Beverley SM. Isolation of genes mediating resistance to inhibitors of nucleoside and ergosterol metabolism in Leishmania by overexpression/selection. J Biol Chem 1999;274:37723–30.

[107] Hoyer C, Mellenthin K, Schilhabel M, Platzer M, Clos J. *Leishmania*and the Leishmaniases: Use of genetic complementation to identify gene(s) which specify species-specific organ tropism of*Leishmania*. Med Microbiol Immunol 2001;190:53–6.

[108] Clos J, Choudhury K. Functional cloning as a means to identify*Leishmania*genes involved in drug resistance. Mini Rev Med Chem 2006;6:123–9.

[109] Nuhs, A.; Schafer, C.; Zander, D.; Trube, L.; Tejera Nevado, P.; Schmidt, S.; Arevalo, J.; Adaui, V.; Maes, L.; Dujardin, J.C., et al. A novel marker, ARM58, confers antimony resistance to Leishmania spp. International journal for parasitology. Drugs and drug resistance 2014, 4, 37-47, doi:10.1016/j.ijpddr.2013.11.004.

[110] LeBowitz JH, Smith HQ, Rusche L, Beverley SM. Coupling of poly(A) site selection and trans-splicing in Leishmania. Genes Dev 1993;7:996–1007.

[111] Gazanion E, Fernandez-Prada C, Papadopoulou B, Leprohon P, Ouellette M. Cos-Seq for high-throughput identification of drug target and resistance mechanisms in the protozoan parasite Leishmania. Proc Natl Acad Sci U S A 2016;113:E3012–3021. https://doi.org/10.1073/pnas.1520693113.

[112] Fernandez-Prada, C.; Sharma, M.; Plourde, M.; Bresson, E.; Roy, G.; Leprohon, P.; Ouellette, M. High-throughput Cos-Seq screen with intracellular Leishmania infantum for the discovery of novel drug-resistance mechanisms. International journal for parasitology. Drugs and drug resistance 2018, 8, 165-173, doi:10.1016/j.ijpddr.2018.03.004.

[113] Potvin JE, Leprohon P, Gazanion E, Sharma M, Fernandez-Prada C, Ouellette M. Cos-Seq: a high-throughput gain-of-function screen for drug resistance studies in leishmania. Methods Mol Biol 2019;1971:141–67. https://doi.org/10.1007/978-1-4939-9210-2_7.

[114] Borsari, C.; Jimenez-Anton, M.D.; Eick, J.; Bifeld, E.; Torrado, J.J.; Olias-Molero, A.I.; Corral, M.J.; Santarem, N.; Baptista, C.; Severi, L., et al. Discovery of a benzothiophene-flavonol halting miltefosine and antimonial drug resistance in Leishmania parasites through the application of medicinal chemistry, screening and genomics. Eur J Med Chem 2019, 183, 111676, doi:10.1016/j.ejmech.2019.111676.