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Genome and transcriptome analyses of *Leishmania* spp.: opening Pandora's box

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In the last 30 years, significant advances in genetic manipulation tools along with complete genome and transcriptome sequencing have advanced our understanding of the biology of *Leishmania* parasites and their interplay with the sand fly and mammalian hosts. High-throughput sequencing in association with CRISPR/Cas9 have prepared the ground for significant advances. Given the richness of the progress made over the last decade, in this article, we focused on the most recent contributions of genome-wide and transcriptome analyses of *Leishmania* spp., which permit the comparison of life cycle stages, the evaluation of different strains and species in their natural niches and in the field and the simultaneously comparison of the gene expression profiles of parasites and hosts.

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Introduction

The protozoan parasite *Leishmania* is the causative agent of leishmaniasis, a disease with a wide spectrum of clinical forms varying from asymptomatic to mild, morbid and fatal infections and involving tegumentary and visceral disease. The outcome of this disease depends on factors residing in the insect vector species, the parasite species and strain and the host immune system and genetics. Endemic areas are distributed over more than 80 countries, and over 1 billion individuals live in areas with a high risk of infection. More than 1 300 000 cases of leishmaniasis are reported, and 20 thousand deaths per year are caused by visceral leishmaniasis [1]. Despite these efforts, we still lack safe and effective chemotherapy and have no vaccines. *Leishmania* parasites are quite versatile as they alternate between two quite distinct but equally hostile environments: the gut of the sand fly and the phagolysosome of a professional phagocyte in the mammalian host. Understanding the molecular mechanisms underlying the success of this parasite is an urgent matter for the development of tools to combat leishmaniasis and also a fascinating subject of study because of the peculiarities of this early branching eukaryote. All available and upcoming high-throughput analyses and resources have positively impacted the ability to genetically manipulate the parasite. The difficulties in large-scale and comprehensive proteomics studies have directed the investigation of gene expression profiling to large-scale transcriptomics, despite the moderate correlation between transcript abundance and cellular protein levels in *Leishmania* spp. [2–4]. Given the richness of the progress over the last decade, this article focuses on the most recent contributions of the genome-wide and transcriptome analyses of *Leishmania* spp. (Figure 1).

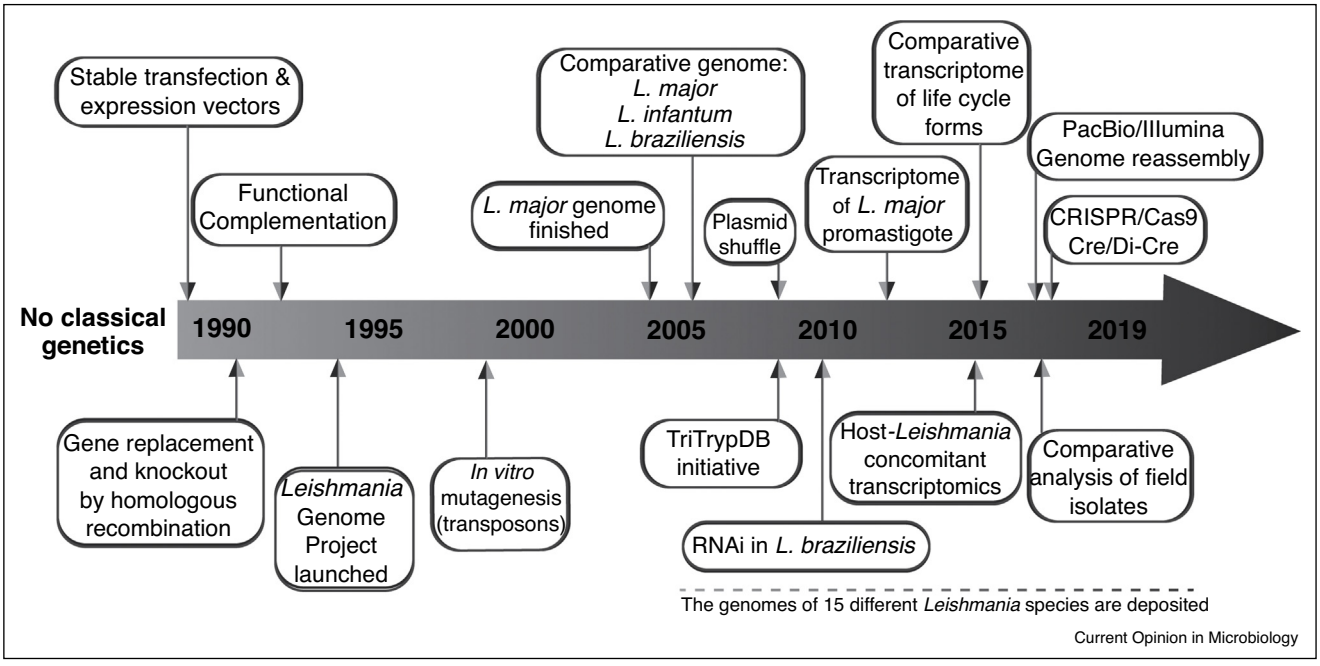
A brief view of genetic manipulation tools

Powerful reverse and forward genetic tools in association with genome and transcriptome sequencing, proteomics and computational resources have been successfully developed and applied to the study of *Leishmania* biology and pathogenesis. Essential advances in genetic manipulation instruments started in 1990 when stable transfection was first effectively achieved in *Leishmania* [5,6]. Over the past three decades, a large repertoire of effective instruments for parasite genetic manipulation has been developed. Gene knockout was made possible by homologous recombination, the overexpression of genes was made achievable through ectopic or genome-integrated vectors, and episomal functional complementation is used *in vitro* and *in vivo* to examine essentiality. Functional complementation gained power with the use of a negative selectable marker in the vector, as in the plasmid shuffle methodology. In more recent years, *Leishmania* genome engineering by the Cre recombinase to mediate the excision of sequences flanked by locus loxP sites has been achieved using the inducible dimerization of split Cre enzyme fragments to form active DiCre. However, the most profound change and the revolution in genome editing came to *Leishmania* with the adaption of the CRISPR/Cas9 system for use in the parasite [7••,8]. There have been excellent reviews on the genetic manipulation approaches and achievements in *Leishmania* [9•,10].

What do genome comparisons tell us?

In 1994, the *Leishmania* Genome Initiative was launched and gathered a large number of laboratories to sequence a

Figure 1



A timeline depicting the main achievements on genetic manipulation and large-scale genomics and transcriptomics analyses of *Leishmania* spp.

reference strain of *Leishmania (Leishmania) major* [11]. Given the available resources, the status of sequencing technology and the computational power and tools, the first complete genome of the parasite was published in 2005. Since then, huge improvements in these technologies have permitted the sequencing of the genomes of 36 strains from 15 different species, according to TriTrypDB records (https://tritrypdb.org/tritrypdb/, March 2019). Deep sequencing using a combination of second-generation (Illumina) and third-generation (PacBio) sequencing technologies allowed the reassembly of *Leishmania* genomes, improving the available information [12,13]. The first comparative analysis of *Leishmania* genomes was conducted between *Leishmania (Leishmania) infantum* and *Leishmania (Viannia) braziliensis* with the available *L. major* genome published in 2007 [14]. These species are associated with different disease outcomes; typically, *L. major* leads to uncomplicated cutaneous disease (LCL), *L. infantum* leads to visceral disease (VL) and *L. braziliensis* is responsible for the morbid mucocutaneous forms (MCL and LCL) [15]. The study revealed the genomes to be highly conserved at the content and synteny levels, an expected result based on the conservation of the *L. major* genome with two other trypanosomatids [11,14], which was confirmed by PacBio reassembly. This comparative analysis contributed to unraveling similarities and differences due to the restricted number of species-specific genes detected; the highest number of *L. braziliensis*-specific genes was 49 [16]. Remarkably, this study revealed that in contrast to the two *Leishmania* from the subgenus *Leishmania*, *L. braziliensis*, from the *Viannia* subgenus, contains components of the RNA-mediated interference pathway, transposable elements and retrotransposons. The RNAi pathway was subsequently shown to be functional; tools have been developed for its technological application and utilized to study gene function [17,18].

In addition to the typical extraction of information allowing the total or partial assembly and annotation of genomes, more recently, genome sequencing has been used to detect single nucleotide polymorphisms and gene copy number variation (CNVs) in comparative analysis of strains with phenotypic differences to illuminate possible phenotype/genotype correlation [19]. In particular, genome-wide association studies have been applied successfully to understand drug resistance versus responsive phenotypes. In an illustrative example, the authors generated the genome sequences of 26 *L. infantum* isolates from Brazilian patients with different miltefosine treatment outcomes. Wide-ranging information on genomic variation was obtained, such as SNPs, INDELs, and gene and chromosome copy number variations that were unique to the Brazilian parasites. Importantly, the study revealed a molecular marker of miltefosine treatment failure, the Miltefosine Sensitivity Locus (MSL) that contains four genes. Remarkably, the MSL has not been identified in reports of miltefosine resistance in *Leishmania* in the laboratory. Moreover, in the publicly available genomes of *Leishmania donovani*, a species also

responsible for VL and responsive to miltefosine, the MSL was universally present [20••]. MSL has not yet been confirmed as a biomarker for the prediction of patient response to the drug, but this study may help establish better therapeutic strategies for VL treatment after similar investigations have been conducted in different endemic zones. The take-home message from analysis of the few genome-wide studies on drug resistance/sensitivity in a large number of clinical isolates is that the results of studies on drug resistance acquired through artificial means do not necessarily translate into real-world drug resistance mechanisms. For a detailed retrospect and the prospect of genetic research on drug resistance and drug development, there are enlightening reviews [21••,22].

Combining genomes and transcriptomes: a path for epidemiological studies

The association of genome-wide (DNA-seq) and transcriptome (RNA-seq) high-throughput sequencing and analyses has allowed the comparison of field strains (and species) from different endemic areas and countries or long-maintained laboratory strains in a number of studies. Aneuploidy, chromosome-variable 'somy', restricted regions of amplification that may be either circular or linear, or expansion/deletions in gene arrays contribute in a variable way (depending on species and endemic areas) to gene copy number variations (CNVs). These issues have been revisited since the 1980s and are now central characteristics of the *Leishmania* genome, which leads to marked gene expression plasticity. The complexity of the genomic adaptation of the parasite to the field environment is high, and different reports have pointed so [23–27,28••,29]. One paradigmatic example of such complexity is a high-resolution study that have been conducted to analyze intraspecies differences in genetic diversity and gene expression by pairing genome-wide DNA-seq with RNA-seq. A reference genome and the genomes of 14 geographically dispersed field isolates of *Leishmania tropica* were used in the study, and the authors demonstrated a high degree of polymorphisms, more polymorphisms represented by SNPs in *L. tropica* strains than in *L. donovani* and *L. infantum* [28••]. It is remarkable, however, how variable the number of SNPs reported in different studies for the same species is [19,30,31]. In fact, Downing *et al.* [19] showed that fewer than half of the biallelic SNPs found in *L. tropica* field strains would distinguish *L. donovani* from *L. infantum* species, which pertain to the same complex [19]. In an *L. tropica* genetic diversity study, the authors highlighted that the gene dosage at the level of individual genes or chromosomal 'somy' accounted for greater than 85% of the total gene expression variation among genes presenting a twofold or greater change [28••]. It is worth mentioning, however, that several studies ([3,32,33•,34••], among others) indicated mostly modest differential expression levels ranging from ~1.5-fold to ~3.0-fold changes, and it was not clear in the study on *L. tropica* whether the differentially expressed (DE) genes with lower FCs could be explained by similar mechanisms. Relevant studies addressing the factors that govern *Leishmania* genome adaptation in the field or laboratory have recently been conducted, all of which point to the genomic regulation of gene expression levels [29,35–37]. One of these studies demonstrated the strain-based and species-based specificity of the observed karyotypic changes [29]. In summary, the genomic plasticity of *Leishmania* spp., is at least partially responsible for phenotypic variation and drives parasite fitness gains in response to different environmental constraints to different degrees.

What do *Leishmania* transcriptome comparisons tell us?

Transcriptome analysis via high-throughput sequencing (RNA-seq) captures significant changes in gene expression and has several advantages over other transcriptional profiling approaches, such as DNA microarrays or the serial analysis of gene expression [38]. A number of relevant communications using the later techniques are available, but they will not be discussed in this review [39] and have been reviewed previously [40].

Global RNA sequencing (RNA-seq) and analyses have been used in diverse transcriptomic profiling studies carried out to improve understanding of the interaction between the parasite with the mammalian and insect hosts, to capture changes in gene expression during life cycle progression and to compare axenic and natural environments at each stage.

The first global RNA profiling study of *L. major* was conducted with axenic promastigotes [41]. Following this article, a series of comparative analyses on the transcriptomes at the major life cycle stages were reported. Three of these studies were conducted in *L. major*, *Leishmania mexicana* and *L. braziliensis* species [32,33•,34••]. In general, these reports illustrate the improvement of genomic annotation, gene structure, and existing expression datasets for each species and stage. Comparison of the reported results indicates that alternative processing sites are a common finding, but no correlation between stage-related alternative processing and expression levels was found [33•]. In each of these studies, the profiles of the DE genes were thoroughly analyzed, and lists of genes preferentially expressed at different stages were compiled. These results are relevant and allow the identification of specific coexpression networks that may be useful for interspecies and intraspecies comparative analysis. After a groundwork study towards the discovery of noncoding RNAs (ncRNAs) in *Leishmania* spp. transcriptomes [42], a comparative transcriptomics study performed with *L. braziliensis* was the first to systematically search for ncRNAs in the transcriptomes; a large number of novel putative ncRNAs were detected. The analysis of

DE ncRNAs indicated that the most prominent differences were observed between the transcriptomes of the proliferative insect and mammalian forms [34••], which was similar to the results found for protein-coding genes [32,34••,43••].

A Gene Ontology (GO) functional enrichment analysis of the DE protein-coding genes described in the studies on *L. major*, *L. mexicana* and *L. braziliensis* revealed that the functional GO terms of the clustered DE genes were upregulated in promastigotes, metacyclics or amastigotes and fell in similar GO classes across species, which was not surprising. This analysis also revealed that the approximate number of genes preferentially expressed at each stage (by pairwise analysis) was similar among species. Nonetheless, using orthology as a parameter to compare DE gene content, the percent of orthologous genes was low, ranging between 12 and 35%. These results suggest that in spite of synteny, coding sequence conservation, and the conservation of DE genes, according to the functional categories (GO), orthology-based differences may contribute to species diversity. A possible explanation for the lack of orthologs in the subpopulations of upregulated genes at any of the compared stages from different species might be that paralogous genes carry out similar functions; these paralogous genes are a consequence of gene duplication events, the expansion of tandem arrays or 'somy' changes [34••].

Simultaneous interrogation of the parasite and host transcriptomes

The best scenario to interpret the host–parasite interaction is to evaluate the parasite and host gene expression profiles concomitantly. In this context, a thorough examination of the transcriptomes of the main sand fly stages (procyclic, nectomonad, and metacyclic promastigotes) of *L. major* within the insect gut infected with tissue-derived amastigotes was carried out [43••]. The authors described the modulation of gene expression throughout differentiation, highlighting the main differences between the transcriptomes of each sand fly stage. Some of the most marked differences and relevant findings to help understand the parasite in its natural niche were thoroughly discussed. A remarkable and positive surprise from this study was the similarity between the sand fly and culture-derived metacyclic transcriptomes, which had only 26 mRNAs that differed in abundance.

Switching to the mammalian host/parasite interaction, there have been some remarkable contributions. In 2015, Dillon *et al.* performed transcriptome profiling using RNA-seq to simultaneously identify global changes in murine macrophage and *L. major* gene expression, following a time-course to cover the progress of infection from 4 to 72 hours. Differential gene expression and gene ontology analysis led to the identification of host and parasite responses during infection. The most substantial and dynamic gene expression responses by both the macrophages and parasites were observed during early infection [44•]. Fernandes, in 2016, reported an elegant study characterizing the gene expression signatures of *L. major* and *Leishmania amazonensis* and the coordinated response of *in vitro*-infected human macrophages using a similar time course to cover the progression of infection [45••]. The authors reported a parasite-specific response in the human macrophages early in infection that was reduced at later time points in association with a similar expression pattern observed in the parasites. These analyses provided specific insights into the interaction between human macrophages and *Leishmania* parasites. Moreover, the abovementioned previous and similar study, performed in murine macrophages, allowed the authors to establish a parallel between the similarities and differences of the responses of macrophages from diverse origins to parasite infection. These reports constitute preeminent resources for the study of parasite evasion from host defense and the subversion of host cell physiology to allow intracellular survival.

The examination of parasite–host interplay in real-world infection/disease moved a step forward with the meta-transcriptome profiling of human-*L. braziliensis* cutaneous lesions [46••]. The authors faced the challenge of pauciparasitary *L. braziliensis* lesions and succeeded in simultaneously analyzing the patterns of parasite and host gene expression. The stratification of patients into two groups, with or without detectable parasite transcripts, allowed the authors to examine host responses as a function of parasite persistence. Very briefly, they confirmed a previous transcriptomic analysis of early and late *L. braziliensis* lesions [47] but also encountered unexpected novelties, such as B-cell transcripts associated with persistent parasite lesions. Unanticipated findings such as the uniformity of parasite gene expression across all patients irrespective of lesion characteristics were also revealed in the parasite transcriptome profile. Overall, these reports are major resources to understand *L. braziliensis* and host interplay and should be carefully examined.

Final remarks

The most recent contributions on genome-wide and transcriptome analyses of *Leishmania* spp. bestow considerable knowledge of *Leishmania* genetics, from their structural to their functional characteristics. The life cycle stages of different species and strains have been compared, and genetic strategies of the parasite to survive in mammalian and insect environments have been evaluated. Moreover, the comparative analysis of large numbers of clinical isolates from different corners of the world and from different species is unravelling how substantial the genetic plasticity of these parasites is and how diverse the different species are. In addition, the simultaneous analysis of the gene expression profiles of parasites and hosts is a milestone in the field.

There exist a colossal amount of data, including similarities and differences, open to being explored with rigor and acuity. We must not forget that genetic plasticity, species and strain specificities and different experimental conditions and computational settings may lead to quite different results. The field has reached its maturity, and it is time to innovate and design strategies to understand parasite biology and pathogenesis by inspecting publicly available information using novel and powerful genetic manipulation tools.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. WHO: *Leishmaniasis*. WHO; 2017.

2. McNicoll F, Drummelsmith J, Müller M, Madore E, Boilard N, Ouellette M, Papadopoulou B: **A combined proteomic and transcriptomic approach to the study of stage differentiation in *Leishmania infantum***. *Proteomics* 2006, **6**:3567-3581.

3. Lahav T, Sivam D, Volpin H, Ronen M, Tsigankov P, Green A, Holland N, Kuzyk M, Borchers C, Zilberstein D *et al.*: **Multiple levels of gene regulation mediate differentiation of the intracellular pathogen *Leishmania***. *FASEB J* 2011, **25**:515-525.

4. de Almeida-Bizzo JH, Alves LR, Castro FF, Garcia JB, Goldenberg S, Cruz AK: **Characterization of the pattern of ribosomal protein L19 production during the lifecycle of *Leishmania* spp**. *Exp Parasitol* 2014, **147**:60-66.

5. Laban A, Tobin JF, Curotto de Lafaille MA, Wirth DF: **Stable expression of the bacterial neor gene in *Leishmania enriettii***. *Nature* 1990, **343**:572-574.

6. Kapler GM, Coburn CM, Beverley SM: **Stable transfection of the human parasite *Leishmania major* delineates a 30-kilobase region sufficient for extrachromosomal replication and expression**. *Mol Cell Biol* 1990, **10**:1084-1094.

7. •• Beneke T, Madden R, Makin L, Valli J, Sunter J, Gluenz E: **A CRISPR Cas9 high-throughput genome editing toolkit for kinetoplastids**. *R Soc Open Sci* 2017, **4**:170095.
The best CRISPR/Cas9 toolkit adapted to *Leishmania*; it permits rapid tagging and gene knockout without requiring the user to perform any DNA cloning.

8. Beneke T, Demay F, Hookway E, Ashman N, Jeffery H, Smith J, Valli J, Becvar T, Myskova J, Lestinova T *et al.*: **Genetic dissection of a *Leishmania* flagellar proteome demonstrates requirement for directional motility in sand fly infections**. *bioRxiv* 2018. 476994.

9. • Duncan SM, Jones NG, Mottram JC: **Recent advances in *Leishmania* reverse genetics: manipulating a manipulative parasite**. *Mol Biochem Parasitol* 2017, **216**:30-38.
Excellent review that describes the expanding repertoire of molecular tools for genetic manipulation in *Leishmania*.

10. Beverley SM, Akopyants NS, Goyard S, Matlib RS, Gordon JL, Brownstein BH, Stormo GD, Bukanova EN, Hott CT, Li F *et al.*: **Putting the *Leishmania* genome to work: functional genomics by transposon trapping and expression profiling**. *Philos Trans R Soc Lond B Biol Sci* 2002, **357**:47-53.

11. Ivens AC, Peacock CS, Worthey EA, Murphy L, Aggarwal G, Berriman M, Sisk E, Rajandream MA, Adlem E, Aert R *et al.*: **The genome of the kinetoplastid parasite, *Leishmania major***. *Science* 2005, **309**:436-442.

12. Lypaczewski P, Hoshizaki J, Zhang WW, McCall LI, Torcivia-Rodriguez J, Simonyan V, Kaur A, Dewar K, Matlashewski G: **A complete *Leishmania donovani* reference genome identifies novel genetic variations associated with virulence**. *Sci Rep* 2018, **8**:16549.

13. González-de la Fuente S, Peiró-Pastor R, Rastrojo A, Moreno J, Carrasco-Ramiro F, Requena JM, Aguado B: **Resequencing of the *Leishmania infantum* (strain JPCM5) genome and de novo assembly into 36 contigs**. *Sci Rep* 2017, **7**:18050.

14. Peacock CS, Seeger K, Harris D, Murphy L, Ruiz JC, Quail MA, 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.

15. Reithinger R, Dujardin JC, Louzir H, Pirmez C, Alexander B, Brooker S: **Cutaneous *Leishmaniasis***. *Lancet Infect Dis* 2007, **7**:581-596.

16. Smith DF, Peacock CS, Cruz AK: **Comparative genomics: from genotype to disease phenotype in the *Leishmaniases***. *Int J Parasitol* 2007, **37**:1173-1186.

17. de Paiva RM, Grazielle-Silva V, Cardoso MS, Nakagaki BN, Mendonça-Neto RP, Canavaci AM, Souza Melo N, Martinelli PM, Fernandes AP, daRocha WD *et al.*: **Amastin knockdown in *Leishmania braziliensis* affects parasite-macrophage interaction and results in impaired viability of intracellular amastigote**. *PLoS Pathog* 2015, **11**:e1005296.

18. Lye LF, Owens K, Shi H, Murta SM, Vieira AC, Turco SJ, Tschudi C, Ullu E, Beverley SM: **Retention and loss of RNA interference pathways in trypanosomatid protozoans**. *PLoS Pathog* 2010, **6**:e1001161.

19. Downing T, Imamura H, Decuypere S, Clark TG, Coombs GH, Cotton JA, Hilley JD, de Doncker S, Maes I, Mottram JC *et al.*: **Whole genome sequencing of multiple *Leishmania donovani* clinical isolates provides insights into population structure and mechanisms of drug resistance**. *Genome Res* 2011, **21**:2143-2156.

20. •• Carnielli JBT, Crouch K, Forrester S, Silva VC, Carvalho SFG, Damasceno JD, Brown E, Dickens NJ, Costa DL, Costa CHN *et al.*: **A *Leishmania infantum* genetic marker associated with miltefosine treatment failure for visceral leishmaniasis**. *EBioMedicine* 2018, **36**:83-91.
A sound genome-wide study from a large number of *L. infantum* clinical isolates from Brazilian patients; it reveals a genetic marker associated with miltefosine treatment failure.

21. •• Jones NG, Catta-Preta CMC, Lima APCA, Mottram JC: **Genetically validated drug targets in *Leishmania*: current knowledge and future prospects**. *ACS Infect Dis* 2018, **4**:467-477.
A complete and informative revision on drug development approaches, current knowledge and prospects in the field.

22. Leprohon P, Fernandez-Prada C, Gazanion É, Monte-Neto R, Ouellette M: **Drug resistance analysis by next generation sequencing in Leishmania**. *Int J Parasitol Drugs Drug Resist* 2015, **5**:26-35.

23. Leprohon P, Légaré D, Raymond F, Madore E, Hardiman G, Corbeil J, Ouellette M: **Gene expression modulation is associated with gene amplification, supernumerary chromosomes and chromosome loss in antimony-resistant *Leishmania infantum***. *Nucleic Acids Res* 2009, **37**:1387-1399.

24. Beverley SM, Coderre JA, Santi DV, Schimke RT: **Unstable DNA amplifications in methotrexate-resistant *Leishmania* consist of extrachromosomal circles which relocalize during stabilization**. *Cell* 1984, **38**:431-439.

25. Ouellette M, Hettema E, Wüst D, Fase-Fowler F, Borst P: **Direct and inverted DNA repeats associated with P-glycoprotein gene amplification in drug resistant *Leishmania***. *EMBO J* 1991, **10**:1009-1016.

26. Ubeda JM, Légaré D, Raymond F, Ouameur AA, Boisvert S, Rigault P, Corbeil J, Tremblay MJ, Olivier M, Papadopoulou B *et al.*: **Modulation of gene expression in drug resistant *Leishmania* is associated with gene amplification, gene deletion and chromosome aneuploidy**. *Genome Biol* 2008, **9**: R115.

27. Ubeda JM, Raymond F, Mukherjee A, Plourde M, Gingras H, Roy G, Lapointe A, Leprohon P, Papadopoulou B, Corbeil J *et al.*: **Genome-wide stochastic adaptive DNA amplification at direct and inverted DNA repeats in the parasite *Leishmania***. *PLoS Biol* 2014, **12**:e1001868.

28. •• Iantorno SA, Durrant C, Khan A, Sanders MJ, Beverley SM, Warren WC, Berriman M, Sacks DL, Cotton JA, Grigg ME: **Gene expression in *Leishmania* is regulated predominantly by gene dosage**. *mBio* 2017, **8**.

An important genome-wide study combining genomics and transcriptomics of field isolates of *L. tropica* from various endemic areas; it reveals the relevance of gene dosage at the level of individual genes or chromosomal somy to determine major transcript abundance differences.

29. Bussotti G, Gouzelou E, Côrtes Boité M, Kherachi I, Harrat Z, Eddaikra N, Mottram JC, Antoniou M, Christodoulou V, Bali A *et al.*: **Genome dynamics during environmental adaptation reveal strain-specific differences in gene copy number variation, karyotype instability, and telomeric amplification**. *mBio* 2018, **9**.

30. Rogers MB, Hilley JD, Dickens NJ, Wilkes J, Bates PA, Depledge DP, Harris D, Her Y, Herzyk P, Imamura H *et al.*: **Chromosome and gene copy number variation allow major structural change between species and strains of Leishmania**. *Genome Res* 2011, **21**:2129-2142.

31. Zhang WW, Ramasamy G, McCall LI, Haydock A, Ranasinghe S, Abeygunasekara P, Sirimanna G, Wickremasinghe R, Myler P, Matlashewski G: **Genetic analysis of *Leishmania donovani* tropism using a naturally attenuated cutaneous strain**. *PLoS Pathog* 2014, **10**:e1004244.

32. Fiebig M, Kelly S, Gluenz E: **Comparative life cycle transcriptomics revises *Leishmania mexicana* genome annotation and links a chromosome duplication with parasitism of vertebrates**. *PLoS Pathog* 2015, **11**:e1005186.

33. • illon LA, Okrah K, Hughitt VK, Suresh R, Li Y, Fernandes MC, Belew AT, Corrada Bravo H, Mosser DM, El-Sayed NM: **Transcriptomic profiling of gene expression and RNA processing during *Leishmania major* differentiation**. *Nucleic Acids Res* 2015, **43**:6799-6813.

This article is the first one to compare poly(A) transcriptome from axenic cultures of *L. major* procyclic and metacyclic promastigotes.

34. •• Ruy PC, Monteiro-Teles NM, Miserani Magalhães RD, Freitas-Castro F, Dias L, Aquino Defina TP, Rosas De Vasconcelos EJ, Myler PJ, Kaysel Cruz A: **Comparative transcriptomics in *Leishmania braziliensis*: disclosing differential gene expression of coding and putative noncoding RNAs across developmental stages**. *RNA Biol* 2019:1-22.

The first comparative transcriptome study using total RNA from the three *L. braziliensis* life stages grown axenically to perform a systematic computational analysis of the putative ncRNA content of the parasite.

35. Dumetz F, Imamura H, Sanders M, Seblova V, Myskova J, Pescher P, Vanaerschot M, Meehan CJ, 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**.

36. Prieto Barja P, Pescher P, Bussotti G, Dumetz F, Imamura H, Kedra D, Domagalska M, Chaumeau V, Himmelbauer H, Pages M *et al.*: **Haplotype selection as an adaptive mechanism in the protozoan pathogen *Leishmania donovani***. *Nat Ecol Evol* 2017, **1**:1961-1969.

37. Imamura H, Downing T, Van den Broeck F, Sanders MJ, Rijal S, Sundar S, Mannaert A, Vanaerschot M, Berg M, De Muylder G *et al.*: **Evolutionary genomics of epidemic visceral Leishmaniasis in the Indian subcontinent**. *eLife* 2016, **5**.

38. Nowrousian M: **Next-generation sequencing techniques for eukaryotic microorganisms: sequencing-based solutions to biological problems**. *Eukaryot Cell* 2010, **9**:1300-1310.

39. Rochette A, Raymond F, Ubeda JM, Smith M, Messier N, Boisvert S, Rigault P, Corbeil J, Ouellette M, Papadopoulou B: **Genome-wide gene expression profiling analysis of *Leishmania major* and *Leishmania infantum* developmental stages reveals substantial differences between the two species**. *BMC Genomics* 2008, **9**:255.

40. Alonso A, Larraga V, Alcolea PJ: **The contribution of DNA microarray technology to gene expression profiling in *Leishmania* spp.: a retrospective view**. *Acta Trop* 2018, **187**:129-139.

41. Rastrojo A, Carrasco-Ramiro F, Martín D, Crespillo A, Reguera RM, Aguado B, Requena JM: **The transcriptome of *Leishmania major* in the axenic promastigote stage: transcript annotation and relative expression levels by RNA-seq**. *BMC Genomics* 2013, **14**:223.

42. Freitas Castro F, de Cássia Ruy P, Zeviani KN, de Freitas Santos R, Toledo JS, Cruz AK: **Evidence of putative non-coding RNAs from *Leishmania* untranslated regions**. *Mol Biochem Parasitol* 2017, **214**:69-74.

43. •• Inbar E, Hughitt VK, Dillon LA, Ghosh K, El-Sayed NM, Sacks DL: **The transcriptome of *Leishmania major* developmental stages in their natural sand fly vector**. *mBio* 2017, **8**.

This article is the first and thorough study to perform a multi-comparative gene expression analysis of the main *L. major* sand-fly stages, amastigotes from mice lesions, and axenic metacyclic forms.

44. • Dillon LA, Suresh R, Okrah K, Corrada Bravo H, Mosser DM, El-Sayed NM: **Simultaneous transcriptional profiling of *Leishmania major* and its murine macrophage host cell reveals insights into host-pathogen interactions**. *BMC Genomics* 2015, **16**:1108.

This article is the first comprehensive transcriptome analysis that simultaneously identifies, using murine macrophages and *L. major*, global changes in host and parasite gene expression during infection.

45. •• Fernandes MC, Dillon LA, Belew AT, Bravo HC, Mosser DM, El-Sayed NM: **Dual transcriptome profiling of Leishmania-infected human macrophages reveals distinct reprogramming signatures**. *mBio* 2016, **7**.

The authors performed a thorough multi-comparative transcriptome analysis that simultaneously identifies global gene expression changes in human macrophage infected with *L. major* and *L. amazonensis* over time (at early and late intervals post-infection).

46. •• Christensen SM, Dillon LA, Carvalho LP, Passos S, Novais FO, Hughitt VK, Beiting DP, Carvalho EM, Scott P, El-Sayed NM *et al.*: **Meta-transcriptome profiling of the human-*Leishmania braziliensis* cutaneous lesion**. *PLoS Negl Trop Dis* 2016, **10**: e0004992.

The authors simultaneously analyzed the human and the parasite transcriptomes in the pauciparasitary lesions of *L. braziliensis* obtained from infected patients. They observed substantial differences in host responses to infection depending on the absence or detection of active transcription of parasite genes.

47. Novais FO, Carvalho LP, Passos S, Roos DS, Carvalho EM, Scott P, Beiting DP: **Genomic profiling of human *Leishmania braziliensis* lesions identifies transcriptional modules associated with cutaneous immunopathology**. *J Invest Dermatol* 2015, **135**:94-101.