



Differential Gene Expression in Different *Leishmania* Species

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ABOUT THE STUDY

Leishmania is a severe global threat in many countries, causing a wide range of clinical conditions known as leishmaniasis. The genome sequences of *Leishmania major* and *Leishmania infantum* were recently completed, allowing researchers to study global gene expression across these parasites' embryonic life stages. Global gene expression profiling with *Leishmania* microarrays revealed that 2%-9% of all genes examined were developmentally regulated, with genome coverage ranging from 22% to 97.5 percent. Using a DNA oligonucleotide microarray covering the complete genomes of *L. major* and *L. infantum*, the current study provides a thorough examination of whole-genome stage- and species-specific gene expression profiles within *L. major* and *L. infantum*. To date, studies have compared worldwide changes in mRNA abundance during the maturation of *Leishmania* species associated with various disease tropisms (e.g. cutaneous vs. visceral leishmaniasis).

Comparing *L. major* and *L. infantum*, quantitative analyses revealed significant changes in state-regulated gene expression patterns. Despite largely conserved genomes, these species-specific changes may explain some of the variances in clinical diseases. As a result, the *Leishmania* genome might be termed constitutively expressed, with only a few genes expressing at different stages. The bulk of differentially expressed genes are species specific, with only a few differentially expressed genes in common between these two *Leishmania* species, according to comparative genomic analysis of gene expression levels in *Leishmania*.

The expression of genes involved in cellular structure, biogenesis, and cell motility differed significantly between the promastigote and amastigote phases of both *Leishmania* species. In contrast to aflagellated amastigotes, motile flagellated promastigotes overexpress dyneins, which are large minus-end-directed microtubule motors that provide the force for flagellar movement, microtubules and a variety of microtubule-associated proteins, kinesins, and trypanosomatid-specific PFR genes.

Microtubule-associated protein genes were discovered to be increased in *L. major* but not in *L. infantum* promastigotes, which was surprising. In *L. major* and *L. infantum* promastigotes, genes coding for calpains, calcium-dependent cysteine proteinases involved in a number of cellular activities such as cytoskeletal/membrane attachments and signal transduction pathways, were increased. Intracellular amastigotes, on the other hand, increased the expression of lysosomal cathepsin-L, which is similar to cysteine proteinases or amino peptidases.

Leishmania's adaptive response includes morphological, physiological, and metabolic changes. The creation of *in vitro* settings that promote transformation in different *Leishmania* species has aided studies of alterations that occur during adaptation. The technique is ideal for examining gene expression regulation during adaptive differentiation. Trans-splicing and RNA editing are two mRNA processing methods that are unique to these protozoa. Several genes that are expressed differently in the two stages have previously been investigated. There are no clear regulatory motifs in the DNA. It is crucial to utilise the same normalisation and statistical tests when conducting comparative genomic research with DNA microarrays, as different approaches utilizing different algorithms may result in findings that are not directly comparable.

However, the mRNA expression profiles of *Leishmania* species were analyzed using *Leishmania major* DNA oligonucleotide microarrays, which had 1124-mer oligonucleotides per gene comprising 8160 genes. Evaluation of differential gene expression in *Leishmania major* Friedlin procyclics and metacyclics using DNA microarray analysis was recently completed. In this study, fluorescent probes generated from *L.*

Major Friedlin RNA were hybridized with DNA microarrays comprising PCR-amplified DNA from a randomly amplified genomic library of *L. major* Friedlin at five time periods during differentiation from procyclics to metacyclics. The relative

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abundance of RNA for each site was computed after the data was standardized for background and probe intensity. During the transition, over 15% of the DNAs (1387/9282) showed statistically significant ($P < 0.01$) changes in expression, with 1.16 percent exhibiting up-regulation at two or more time points and

0.14 percent showing down-regulation. The findings were validated by Northern blot analysis of selected genes. These analyses also validated the stage-specific expression of several known genes while also identifying a number of novel genes that are up-regulated in either procyclics or metacyclics.