CONTROL OF GENE EXPRESSION AND GENETIC PLASTICITY IN *Leishmania*

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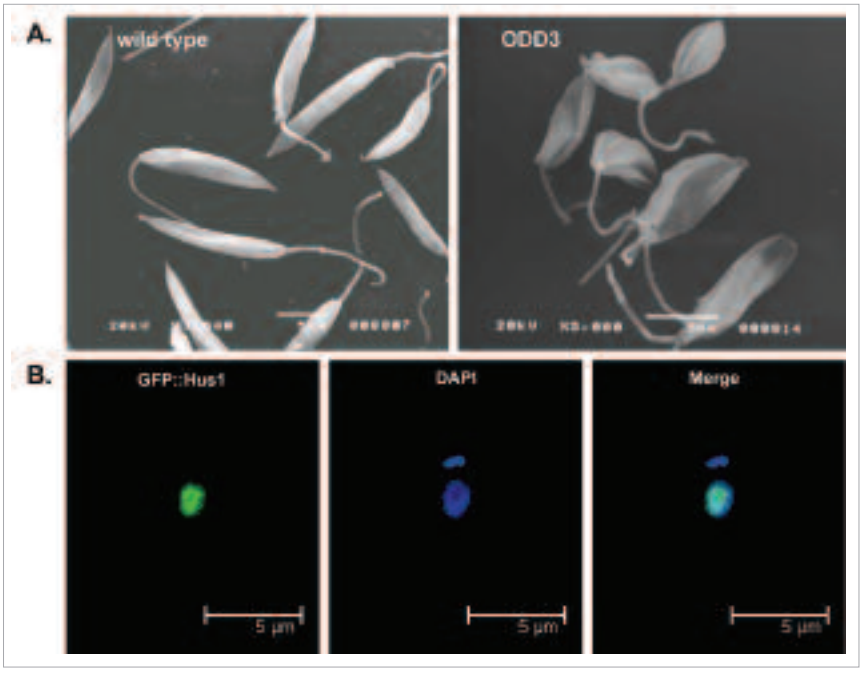

Different approaches are used to understand the control of gene expression in Leishmania

The proposal includes the studies of control of gene expression, genetic recombination and plasticity associated with comparative genomics of *Leishmania*. Different approaches and study objects are used to understand the control of gene expression in the parasite we are investigating: (i) noncoding RNAs as regulatory elements; (ii) a putative extraribosomal role of a ribosomal protein; (iii) the role of 5' and 3'UTR GKC to control its level of expression. Comparative genomics approaches. Parasite's genes possibly involved in DNA repair and recombination leading to genetic plasticity are also under investigation. Studies on aspects of genome organization affecting gene expression is being conducted by using bioinformatic tools followed by *in vivo* validation.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We worked on the comparative analysis of three *Leishmania* genomes: *L. major*, *L. infantum* and *L. braziliensis*. This comparison revealed the presence of a few species-specific genes and a higher divergence of *L. braziliensis* genome and the presence of intact transposable elements and RNAi machinery only in this species1,2,3. We developed a pipeline to identify *in silico* conserved elements present in noncoding regions of the three *Leishmania* species. More than 70 conserved elements were found and we are currently investigating proteins which interact with two of these elements and their role.

Different routes used by the parasite to control gene expression are also under investigation; one of them is focused on noncoding SEQ CHAPTER \h \r 1RNAs (ncRNA). The overexpression of a putative ncRNA, the ODD3 gene, has been shown to impair growth rate of promastigotes in culture, to drastically alter the cell morphology and to diminish the level of a putative target transcript previously identified *in silico*. The co-transfection of this target transcript into *L. major* ODD3 overexpressors led to partial reversion of the original phenotype.

The investigation of mechanisms of genetic recombination and their role in the extensive genome plasticity of *Leishmania* is another theme of interest. In mammalian cells the protein Hus1 is involved in chromosome stability and DNA repair mechanisms. We found that the *L.major* LMHUS1 protein localizes to the nucleus and protects DNA from genotoxic stress. Our results also suggest the participation of LMHUS1 in cell cycle progression. We therefore hypothesize that LMHUS1 participates in chromosome stability and DNA rearrangements leading to DNA amplification. Another gene involved in DNA repair, control of replication, transcription and cell cycle progression is the Histone chaperone Anti-Silencing factor-1(ASF-1). We investigated its putative homologue from *L. major*, LmASF-1, and our results indicate functional conservation of the gene. Finally, the putative homologues of *Rad*51 and *BrcA*2, which are possibly involved in DNA repair mechanisms, are also being investigated.

While attempting to disrupt a telomere located essential gene of the parasite4 we have generated a heterozygous cell line bearing the selectable marker SAT (streptothricin acetyl transferase) integrated adjacent to the telomere of chromosome 20. The expression of SAT in this cell line was notably different when compared to a cell line in which the same selectable marker was integrated into an internal locus of chromosome 23. Notably, the pattern of SAT expression from this telomeric locus was affected by the increased expression of the LmASF-1 protein mentioned above. Altogether, our results suggested the possible existence of a telomere position effect that may participate in the control of *Leishmania* gene expression across telomeric loci.

MAIN PUBLICATIONS

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