

NON-CODING RNAs FROM *Leishmania major*: LOOKING FOR TARGETS

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On the last decade novel classes of untranslated RNA molecules have been described. These noncoding RNAs (ncRNA) affect a large variety of processes including transcriptional regulation, chromosome replication, RNA processing/modification, mRNA stability and translation. We are currently investigating three putative *Leishmania* ncRNAs genes: *ODD1*, *ODD2* and *ODD3*, whose secondary structure presents hairpin-like motifs resembling those found in microRNAs. Based on the knowledge that ncRNAs can cause degradation of its target mRNA or block its translation by sequence complementarity, we designed sense oligonucleotides corresponding to the hairpin region found in the *ODD* genes. To identify putative mRNA targets, these oligonucleotides were used as probes in Northern blot and primers in RT-PCR experiments. In Northern experiments we detected transcripts of 1.7 to 2.2 kb with sense oligos *ODD1_2s*, *ODD2_3s*, *ODD3_1s* and *ODD3_2s*. The same oligonucleotides were used in RT-PCR experiments in conjunction with a primer designed to anneal to the splice leader region of any trans-spliced RNA. The template used in these experiments was total RNA extracted from promastigotes in mid-log phase. The products were cloned, sequenced and analysed *in silico* using the *L. major* Friedlin databank. One of the amplified RNA molecules, a 141bp fragment and potential *ODD2* target, is present twice within a 44kb region of chromosome 33 for which no gene was annotated. Curiously, another amplified RNA, potential *ODD1* target, is also present as a duplicated sequence within the same region of chromosome 33. The Northern blot and RT-PCR results suggest that this region might be transcribing for other ncRNAs possibly interacting with *ODD1* and *ODD2* final products. Other amplified RNAs were found dispersed in the genome. We are conducting some functional studies to understand the possible role of *ODD* genes and their correlation with the described RNA targets. We generated constructs of each of the *ODD* genes, which were transfected into *Leishmania* to obtain *ODD* overexpressing parasites. Transfectants were recovered and we found that *ODD3* overexpressor has severe growth impairment in axenic culture and morphological alterations. We are using this clone to further investigate altered genes and *ODD3* function. Our studies suggest that *ODD* genes may transcribe for functional ncRNAs and could act as riboregulators. **Support: FAPESP, CAPES and CNPq.**