EXPRESSION AND CHARACTERIZATION OF THE HUMAN PROTEIN ISG95: A PUTATIVE RNA-METHYLTRANSFERASE AND RNA-GUANYLYLTRANSFERASE

Vaz, T. H., Silva, T. C. L. and Zanchin, N. I. T.

Center for Structural Molecular Biology (CEBIME)
Brazilian Synchrotron Light Laboratory

The main mechanism of single-cell resistance to viral infection involves several genes from the interferon signaling pathway, called ISGs (interferon stimulated genes). Some of these genes can also be induced by alternative pathways. ISG95 expression is enhanced in response to INF and CpG treatment, to HCV (hepatitis C virus) and VV (vaccinia virus) infection and in leukemic cells. Primary sequence analysis of ISG95 revealed four conserved domains that include: Gpath (RNA binding), FtsJ (RNA methylation), DNA ligase/mRNA capping family domain and WW (protein-protein interaction). ISG95 was expressed in insect cells and showed triphosphatase activity, but did not show guanylyltransferase or methyltransferase activity, which would be expected for an mRNA capping enzyme. Complementation assays in *Saccharomyces cerevisiae* were negative for the three mRNA cap biosynthesis steps. A yeast two-hybrid screen was performed using ISG95 as bait. This screen identified a group of proteins involved in RNA synthesis and processing, strongly indicating that ISG95 function is related to RNA splicing. Regulation of ISG95 expression is being investigated using a reporter gene to assay the response of the ISG95 promoter to interferon treatment. For this purpose, different regions of the ISG95 promoter were cloned into the pTAL-SEAP plasmid (Clontech), which will be transected into Vero Cells for interferon response assays.

Supported by CNPq/ FAPESP/ LNLS