Translational control of the human hemojuvelin via upstream open reading frames

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Iron is an essential element for many biological reactions carried out by living systems. A tight regulation of systemic iron homeostasis is crucial to avoid the pathological conditions of iron deficiency or overload. Juvenile hemochromatosis, is an early-onset inherited disorder associated to iron overload caused by mutations on the hepcidin gene or in the gene encoding hemojuvelin (HJV). HJV is a glycosylphosphatidylinositol (GPI)-linked membrane protein shown to be a co-receptor for class of ligands called bone morphogenetic proteins (BMPs). Thus, HJV is involved on iron homeostasis through regulation of hepcidin transcription levels.

A better knowledge of the mechanisms implicated in HJV gene expression is crucial to understand its role in the iron homeostasis. The 5' leader sequence of the human HJV mRNA has two upstream AUGs (uAUGs) that share the same codon stop forming two upstream open reading frames (uORF) with 28 and 19 codons. To evaluate the effect of these uORFs in the translational regulation of HJV, reporter constructs containing several HJV 5'-leader sequences fused to the Firefly luciferase cistron were tested in HeLa and HepG2 cells. Luciferase activity was measured by luminometry and normalized to the corresponding mRNA levels, quantified by real-time RT-PCR, to obtain translation efficiencies.

The results revealed that the HJV uORFs decrease the translational efficiency of the main ORF in about 6-fold. Furthermore, we have observed that the HJV mRNA has a low leaky scanning ability that contributes to the translational repression of the main ORF. Thus, reinitiation is the mechanism mainly involved in the production of HJV protein. Aiming to characterize the mechanism through which the HJV uORFs affect downstream translation, we have observed that the amino acid sequences of the uORFs encoded peptides seem to cause ribosomal stalling, which also impede translation of the downstream main ORF.