An upstream open reading frame regulates de translational efficiency of the human erythropoietin transcript

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Among the various cis-acting elements present in the 5' leader sequence of mRNAs there are upstream open reading frames (uORFs). Although their function is still poorly understood, they are known to downregulate the main ORF expression of several human transcripts that code for key regulatory genes.

The human erythropoietin (EPO) is a glycoprotein that was initially characterized has a hormone mainly synthesized and released from the kidney, with a key role in hematopoiesis. However, many recent reports have implicated EPO in several non-hematopoietic functions and have shown its production in several other organs. Consequently, it might be used as a therapeutic target for the treatment of several human disorders. We found a natural occurring 14-codon-uORF on the human EPO transcript. Our belief is that understanding the molecular mechanisms through which the EPO uORF controls translation may be valuable in the determination of these EPO-based therapies.

To explore the mechanisms by which EPO uORF controls translational efficiency, HepG2, HEK293 and REPC cells were transfected with several constructs carrying the luciferase reporter gene with the intact or disrupted EPO uORF, with or without the EPO 3' untranslated region (3'UTR). Luciferase activity was measured by luminometry and normalized to the corresponding mRNA levels to obtain translation efficiencies. The mRNA levels were quantified by real-time RT-PCR. Furthermore, we also analyzed its response to several cell stress stimuli. Results show that the EPO uORF can decrease the main ORF translation efficiency in about 3-fold. In addition, our data support the conclusion that reinitiation, and in less extent leaky scanning, are responsible for the main ORF translation. In addition, the 3'UTR does not affect the role of the uORF, but it increases the luciferase levels, probably by stabilizing the mRNA. Specifically in REPC

cells, translational inhibition mediated by the EPO uORF is overridden in response to chemical hypoxia, which is due to less recognition of the uAUG.