

Translational control of the human erythropoietin *via* an upstream open reading frame in cardiac tissue

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Cellular stress activates an integrated stress response, which includes rapid changes in global and gene-specific translation. Translational regulation of specific transcripts mostly occurs at translation initiation and is mediated *via* different *cis*-acting elements present in the mRNA 5' untranslated region (5'UTR); these elements include upstream open reading frames (uORFs). uORFs modulate translation of the main ORF by decreasing the number and/or efficiency of scanning ribosomes to reinitiate at the start codon of the main ORF.

In its classical hormonal role, human erythropoietin (EPO) is a glycoprotein synthesized and released mainly from the kidney, which has a key role in hematopoiesis. However, recent studies have revealed that EPO is a multifunctional molecule produced and utilized by many tissues that rapidly responds to different cell stress stimuli and tissue injuries. The 5' leader sequence of the human EPO mRNA has one uORF with 14 codons, which is conserved among different species, indicating its potential role in translational regulation. Indeed, we have recently shown that translation of human EPO mRNA is regulated by its uORF in response to hypoxia in HeLa cells (Barbosa & Romão, RNA 2014). **In the present work, we aimed to test whether EPO** is translationally regulated in response to amino acid starvation induced by exposure to the histidine analog Reporter constructs containing several EPO 5' leader sequences fused to the Firefly luciferase cistron were tested in H9C2 (rat heart/myocardium myoblast) and C2C12 (mouse muscle myoblast) cells to evaluate the effect of this uORF in the translational regulation of EPO in cardiac and muscular cells, respectively. Luciferase activity was measured by luminometry and the corresponding mRNA levels, quantified by real-time RT-PCR.

The results revealed that the EPO uORF represses the translation of the main ORF at about 60-70%, in H9C2 and C2C12 cell lines. Furthermore, we have observed that the translation reinitiation is the main mechanism involved in the production of

EPO protein. Thus, EPO mRNA has a low leaky scanning ability that contributes to the translational repression of the main ORF. Additionally, the 3'UTR is able to increase in about 2.5-fold the protein levels of luciferase in C2C12 cells probably by stabilizing the mRNA. On the contrary, in H9C2 cells the 3'UTR does not alter the protein levels showing that this structure has no influence in the translation regulation in the cardiac tissue.

A better knowledge of the mechanisms implicated in EPO gene expression may be valuable in the determination of therapies for several human disorders, including cardiac disorders.