Human UPF1: a cap-independent translation initiation mechanism and a cryptic promoter regulate its gene expression in cancer cells

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Cervical (CC) and colorectal (CRC) cancers are among the leading causes of death worldwide and their development and progression is dependent on regulation of gene expression. Eukaryotic cells possess a variety of post-transcriptional mechanisms by which they regulate gene expression, namely at translation initiation level. Although in most cases it occurs *via* the cap-dependent mechanism, several oncogenes, growth factors or proteins involved in the regulation of programmed cell death, among others, have a different translation initiation mechanism. It involves the direct recruitment of the ribosome to the vicinity of the initiation codon without the involvement of the cap structure. This allows the maintenance of protein synthesis under several cellular stresses and promotes tumourigenesis.

Apart from its role in nonsense-mediated decay, the human up-frameshift 1 (UPF1) DNA and RNA helicase protein plays a crucial role in telomere replication and homeostasis, and in cell cycle progression. In addition, its expression levels are maintained in every phase of the cell cycle, thus indicating that its translation may occur *via* a cap-independent mechanism. To test this hypothesis, we cloned the human *UPF1* 5'UTR in a dicistronic vector and transfected CC and CRC cell lines with either this construct or the control counterparts. We observed a 15- to 25-fold increase in relative luciferase activity of the *UPF1* 5'UTR-containing construct compared to the levels obtained from the empty counterpart in all tested cell lines, suggesting a cap-independent translation initiation. To control whether these levels of luciferase activity could be due to the presence of a cryptic promoter, we transfected cells with promoterless plasmids and observed the same result, demonstrating that *UPF1* 5'UTR contains a cryptic promoter. To check the cap-independent translation activity alone, we transfected cells with *in vitro* transcribed, capped and polyadenylated mRNAs and observed a 2-fold increase in protein levels, which shows that translation can occur in a cap-independent way. This is maintained under conditions of global protein synthesis inhibition. Deletional analysis of *UPF1* 5'UTR revealed that first 50 nucleotides are essential for both cryptic promoter and cap-independent activities. These results provide new insights on the regulation of UPF1 expression in human cancer cells.