Analysis of the 5' untranslated region of human *UPF1* mRNA indicates both cryptic promoter and internal ribosome entry site activity

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Apart from its role in nonsense-mediated mRNA decay, a mechanism that promotes rapid degradation of transcripts carrying premature translation termination codons, 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. Due to its relevance for several physiological roles, and to the fact that it is expressed during G2/M phase, in which overall protein synthesis is reduced, we hypothesized that its translation may occur *via* an internal ribosome entry site (IRES). IRESs can occur at the 5' untranslated region (UTR) of transcripts and allow the direct recruitment of the ribosome to the vicinity of the main AUG, therefore bypassing the need of scanning the entire UTR.

To test this hypothesis, we cloned the human *UPF1* 5'UTR in the dicistronic vector p_Renilla_Firefly and transfected HeLa cells 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, which suggests the presence of an IRES. However, these levels of luciferase activity could be due to the presence of a cryptic promoter. Hence, we transfected cells with promoterless plasmids and observed a 20-fold increase in relative luciferase activity levels. These data demonstrate that *UPF1* 5'UTR contains a cryptic promoter, whose activity may be masking IRES activity. To check the IRES activity alone, we have transfected cells with *in vitro* transcribed, capped and polyadenylated mRNAs and observed a 2-fold increase in protein levels. This is also observed in two other cell lines. Besides, *UPF1* IRES activity is maintained under conditions of global protein synthesis inhibition. Deletional analysis of *UPF1* 5'UTR revealed that the first 50 nucleotides at the 5' end of this region are essential for both cryptic promoter and IRES activity. These results evidence, for the first time, the existence of both a cryptic promoter and an IRES element within *UPF1* 5'UTR and provide new insights on the regulation of UPF1 expression in human cells.