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JKTBP1 Is Involved in Stabilization and IRES-Dependent Translation of NRF mRNAs by Binding to 5′ and 3′ Untranslated Regions

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Heterogeneous nuclear ribonucleoprotein D-like protein (JKTBP) 1 was implicated in cap-independent translation by binding to the internal ribosome entry site in the 5′ untranslated region (UTR) of NF-κB-repressing factor (NRF). Two different NRF mRNAs have been identified so far, both sharing the common 5′ internal ribosome entry site but having different length of 3′ UTRs. Here, we used a series of DNA and RNA luciferase reporter constructs comprising 5′, 3′ or both NRF UTRs to study the effect of JKTBP1 on translation of NRF mRNA variants. The results indicate that JKTBP1 regulates the level of NRF protein expression by binding to both NRF 5′ and 3′ UTRs. Using successive deletion and point mutations as well as RNA binding studies, we define two distinct JKTBP1 binding elements in NRF 5′ and 3′ UTRs. Furthermore, JKTBP1 requires two distinct RNA binding domains to interact with NRF UTRs and a short C-terminal region for its effect on NRF expression. Together, our study shows that JKTBP1 contributes to NRF protein expression via two disparate mechanisms: mRNA stabilization and cap-independent translation. By binding to 5′ UTR, JKTBP1 increases the internal translation initiation in both NRF mRNA variants, whereas its binding to 3′ UTR elevated primarily the stability of the major NRF mRNA. Thus, JKTBP1 is a key regulatory factor linking two pivotal control mechanisms of NRF gene expression: the cap-independent translation initiation and mRNA stabilization.

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Abbreviations used: UTR, untranslated region; IRES, internal ribosome entry site; NRF, NFκB-repressing factor; JKTBP, JKT-binding protein or heterogeneous nuclear ribonucleoprotein D-like protein; RBD, RNA binding domain; GST, glutathione *S*-transferase; EGFP, enhanced green fluorescent protein; EMSA, electrophoretic mobility shift assay; siRNA, short interfering RNA; ARE, AU-rich RNA element; TNF-α, tumor necrosis factor-α; hnRNP, heterogeneous nuclear ribonucleoprotein; TFR, transferrin receptor.

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Introduction

Heterogeneous nuclear ribonucleoprotein D-like proteins (JKTBPs) belong to the DNA- and RNA-binding heterogeneous nuclear ribonucleoprotein (hnRNP) family.[1] Three isoforms of JKTBP are known: JKTBP1, its splice variant JKTBP1Δ6 and JKTBP2. The major isoform of JKTBPs, JKTBP1, was shown to shuttle between cytoplasm and nucleus.[2] It consists of a short N-terminus, two contiguous RNA binding domains (RBDs), a short glutamine-rich motif (6xQ) and a glycine/tyrosine-rich C-terminal domain.[1,3] Although abundantly expressed in many cell lines and tissues, JKTBP1 expression level was found to be highly elevated in epithelial cells in response to pro-inflammatory cytokines, interferon-γ, interleukin-6 and interleukin-1β.[3,4]

We have previously shown that JKTBP1 binds to an AU-rich sequence in 5′ untranslated region (UTR) of the NF-κB-repressing factor (NRF) and enhances the internal translation initiation at a downstream open reading frame (ORF) in bicistronic constructs.[5] Additionally, JKTBP1 was shown to bind to an AU-rich RNA element (ARE) in 3′ UTR of the pro-inflammatory factor tumor necrosis factor-α (TNF-α).[6] However, sequence comparison of NRF 5′ UTR and TNF-α 3′ UTR revealed no homologous JKTBP1 binding element. In a search for a possible JKTBP1 binding consensus sequence, an ACUAGC motif was identified from pools of 20-nt random sequence RNAs *in vitro*.[7] However, this sequence shows no homology to NRF 5′ UTR or TNF-α 3′ UTR. Although no specific RNA binding sequence could be defined yet, JKTBP1 seems to have a high affinity to AU-rich RNA sequences.

As an important regulatory mode of gene expression, AREs in 3′ UTRs have been implicated in both degradation and stabilization of mRNAs.[8] AREs of human mRNAs have been divided into three classes regarding the number of copies of an AUUUA motif. Whereas class I AREs contain a single AUUUA pentamer, class II AREs enclose multiple copies of this motif and are subdivided into four clusters depending on the frequency of AUUUA motifs.[9,10]

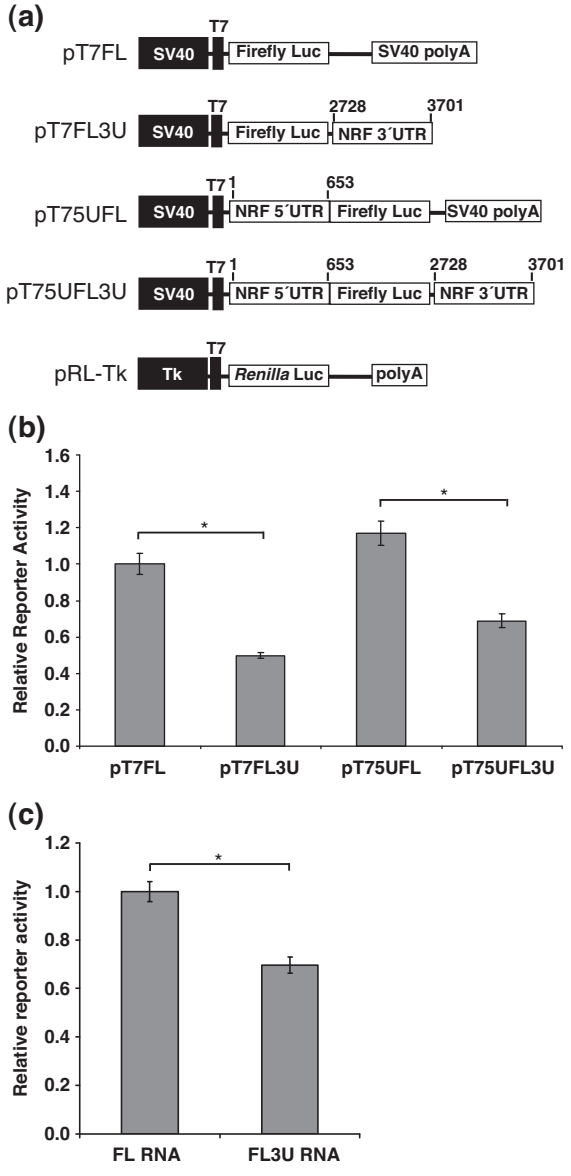

Fig. 1. The NRF UTRs differentially affect reporter gene expression. (a) A schematic diagram of the transfected reporter constructs is shown on the top. pT7FL contains an SV40 early promoter/enhancer (SV40), a minimal T7 promoter (T7), the firefly luciferase gene (Firefly Luc) and an SV40 polyadenylation signal. pT75UFL, pT7FL3U and pT75UFL3U additionally contain the NRF 5′ UTR (nucleotides 1–653), the NRF 3′ UTR (nucleotides 2728–3701) including a polyadenylation signal and both UTRs, respectively. pRL-Tk served as internal control containing a thymidine kinase promoter (Tk), the *Renilla* luciferase gene (*Renilla* Luc) and an SV40 polyadenylation signal. (b) HeLa cells were transfected with reporter construct pT7FL, pT75UFL, pT7FL3U or pT75UFL3U and internal control vector pRL-Tk. Two days after transfection, the cells were harvested, and luciferase activities were determined. Firefly luciferase activities were normalized to the corresponding *Renilla* luciferase activities to obtain the relative firefly luciferase activity shown here as the mean ± standard error of the mean (SEM) of three independent transfection experiments. Relative firefly luciferase activity of cells transfected with pT7FL was set to 1. A p-value <0.05 is indicated (∗). (c) The T7 promoter of pT7FL, pT7FL3U and pRL-Tk was used to express reporter RNAs *in vitro*. HeLa cells were transfected with 2.5 μg reporter RNAs FL, FL3U and RL-RNA. Six hours following transfection, the cells were harvested, and luciferase activities were determined. The relative firefly luciferase activity was obtained as described above. Relative firefly luciferase activity of cells transfected with FL-RNA was set to 1. A p-value <0.05 is indicated (∗).

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