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# Autoregulation of YB-1 Synthesis in Cells

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**Abstract**—The Y-box binding protein 1 (YB-1) plays a crucial role in regulating essential cell functions, including transcription, translation, and DNA repair, through its interactions with nucleic acids and multiple protein partners. The multifunctionality of YB-1 makes the control of its levels critical for cellular homeostasis and adaptation to stress. The synthesis of YB-1 is regulated by gene transcription, protein stability (mediated by long non-coding RNAs), and translation of its mRNA. Autoregulation of *YB-1* mRNA translation remains the topic of ongoing debate. Some earlier *in vitro* studies suggested a role of the 5′ untranslated region (UTR) in inhibiting protein synthesis, while others demonstrated the importance of YB-1 binding to the 3′ UTR for reducing translation. This disagreement has been further complicated by the absence of evidence for these mechanisms in living cells. Here, we provide the first direct evidence that YB-1 represses its synthesis in cultured human cells. Using metabolic protein labeling and immunoprecipitation, we confirmed the effect of YB-1 on the translation of its mRNA. Experiments with reporter constructs showed that both UTRs of the *YB-1* mRNA are involved in autoregulation, thus resolving the contradiction in the literature. These results highlight a sophisticated mechanism for controlling YB-1 levels, which requires both 5′ and 3′ UTRs of the *YB-1* mRNA, and confirm their role in fine-tuning YB-1 synthesis.

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## INTRODUCTION

The Y-box binding protein 1 (YB-1) performs numerous functions in the cell via interaction with nucleic acids and binding to multiple partner proteins [1, 2]. It is involved in the regulation of transcription of a large set of genes and the control of both global translation and specific regulation of translation of a fairly large number of mRNAs. Additionally, it participates in DNA repair, metabolism of small non-coding RNAs, alternative splicing, and some other cellular events (see reviews [1-3] and references therein). This functional diversity ultimately determines the important role of YB-1 in cell proliferation, differentiation, apoptosis, etc. [1, 3, 4]. Notably, YB-1 plays a specific role in oncogenesis, as cancer cells use its diverse functions for their survival and adaptation to challenging conditions [5, 6].

Strict control over the YB-1 amount in the cell is essential for the normal cell life and survival under stress. The regulation of the *YB-1* gene transcription is one of the options to control the YB-1 levels. It typically happens during cell differentiation, when one set of transcription factors is replaced by another, thus reducing the *YB-1* mRNA synthesis [7]. Additionally, the stability of YB-1 protein can be regulated by long non-coding RNAs (for example, see [7]). However, for precise adjustment of the YB-1 level, controlling the translation of *YB-1* mRNA is likely the most crucial factor.

Though it has long been known that YB-1 regulates the translation of its mRNA [8-10], the knowledge of the regulatory mechanism remains sparse, and the available data are somewhat contradictory.

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For example, according to Fukuda et al. [8], the 5′ untranslated region (UTR) of *YB-1* mRNA is important for autoregulation because preincubation of the YB-1 protein with luciferase reporter mRNA containing the 5′ UTR of *YB-1* mRNA leads to the inhibition of luciferase synthesis in a cell-free translation system. On the other hand, in a series of our studies [9-11], we showed that YB-1 synthesis is inhibited by its specific binding to the 3′ UTR of *YB-1* mRNA.

A controversial point in the work of Fukuda et al. is that the site found within the 5′ UTR of *YB-1* mRNA seems to be absent from natural *YB-1* mRNAs in most cell lines (for details, see [7]). Furthermore, both these studies were conducted in cell-free translation systems, and the results have not been confirmed *ex vivo*. The evidence for the autoregulation of YB-1 synthesis in cells could be provided by an experiment with expressed exogenous tag-labeled YB-1 whose presence should suppress the synthesis of endogenous YB-1, thus reducing its amount. However, numerous experiments of this type reported no decrease in the amount of endogenous protein (see Fig. 4 in [12]; Fig. 1 in [13], and Fig. 1 in [14]), which might have been due to the following reasons. Firstly, it is quite difficult to ensure the long-term high expression of YB-1 from a plasmid. As reported, the most frequently achieved increase in the YB-1 amount is twofold, i.e., 1 : 1 relative to the endogenous protein, which may be insufficient to strongly inhibit the translation of the *YB-1* mRNA. Secondly, the high stability of YB-1 (according to our data, its half-life is ~60 h, see Fig. S1 in the Online Resource 1) may cause only a slight change in the amount of endogenous protein during the experiment (which is typically 48 h long), even if synthesis is decreased. Thirdly, such experiments show changes not in the level of YB-1 synthesis, but only in the amount of protein, which, along with the *YB-1* mRNA translation, depends on the *YB-1* mRNA synthesis and stability of the protein itself.

In this work, we used metabolic labeling of cellular proteins followed by immunoprecipitation with anti-YB-1 antibodies to provide the first direct evidence for the effect of YB-1 on its synthesis in cultured human cells. Also, using reporter constructs, we demonstrated that both 5′ and 3′ UTRs of the *YB-1* mRNA are required for the autoregulation of YB-1 synthesis in cultured cells.

## MATERIALS AND METHODS

**Cell cultures.** HEK293TΔYB-1 and HEK-293TΔYB-1+YB-1 cells were described previously [15]. HeLa cells were kindly provided by Elena Nadezhdina (Institute of Protein Research, Russian Academy of Sciences). HEK293T cells were cultured in plastic dishes (Corning, USA) in Dulbecco Modified Eagle Medium (DMEM) (Capricorn, Germany) supplemented with 10% fetal calf serum (Hyclone, USA; Capricorn), 100 U/ml penicillin, and 100 μg/ml streptomycin (PanEco, Russia; ServiceBio, China). HeLa cells were cultured similarly, except that the medium was DMEM/F12 (Capricorn). The cells were incubated in a $CO_2$ incubator at 37°C in a humidified atmosphere containing 5% $CO_2$. As the cells grew, they were periodically reseeded after pre-treatment with trypsin-EDTA (PanEco) to detach them from the plastic.

Transient transfection of cells was performed using Lipofectamine 3000 (Invitrogen, USA) according to the manufacturer's recommendations.

**Analysis of YB-1 synthesis levels in cultured cells using metabolic labeling.** For [$^{35}$S]-methionine labeling, the cells were cultured in 35-mm dishes in L-methionine-free DMEM supplemented with 0.1 mCi/ml L-[$^{35}$S]-methionine (Perkin Elmer, USA, 1000 Ci/mmol) for 1-2 h. The cells were then washed with PBS and lysed with 400 μl of buffer containing 20 mM Hepes-KOH, pH 7.6, 100 mM KCl, 5 mM $MgCl_2$, 2 mM DTT, 0.25% Nonidet P-40, 0.2% SDS, and protease inhibitor cocktail (Roche, Switzerland). Cell extracts were cleared by centrifugation at 10,000*g* for 15 min and incubated with anti-YB-1 antibodies (rat polyclonal antibodies against the 14-a.a. C-terminal peptide of YB-1; IMTEK, Russia) and 20 μl of Protein G-sepharose (GE Healthcare, USA) equilibrated with the lysis buffer for 2 h at 4°C. After extensive washing with PBS (6 times with 500 μl each), proteins were eluted with the acid-urea sample solution (8 M urea, 5% acetic acid, 0.025% methylene blue) and analyzed by acid-urea 10% polyacrylamide gel electrophoresis. To detect radiolabeled proteins, the dried gel was exposed to an intensifying screen followed by detection using a Cyclone®Storage PhosphorSystem (Packard Instrument Company Inc.). The relative amount of radioactivity was determined using the OptiQuant (ver. 03.00) software.

**Immunoblotting.** Proteins were transferred onto a nitrocellulose membrane (Cytiva, USA) using an electrophoretic transfer chamber and transfer buffer (25 mM Tris-HCl, pH 8.7, 90 mM glycine, 10% isopropanol, 0.1% SDS). To prevent non-specific adsorption, the membrane was incubated in TBS (10 mM Tris-HCl, pH 7.6, 150 mM NaCl) containing 5% dry fat-free milk for 1 h at room temperature. The membrane was then incubated in TBS-T (10 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween-20) containing 5% BSA and primary antibodies against YB-1 (Y0396; Sigma-Aldrich, USA; dilution, 1 : 10000) or rps6 (2217S; Cell Signaling Technology, USA; dilution, 1 : 10000) for 16-20 h at 4°C. The membrane was washed three times with TBS-T and incubated in TBS-T containing 5% dry fat-fee milk and secondary anti-rabbit IgG antibodies conjugated

**Table 1.** Sequences of primers used in the work

| Primer | Sequence (5′→3′) |
|---|---|
| F1 | TGGTGGCGCGTCGCGCCG |
| R1 | ATGGTCTTCACACTCGAAGATTTCGTTGG |
| F2 | TGGCGGGACAGGCGGGATAAG |
| R2 | ATGGTCTTCACACTCGAAGATTTCGTTGG |
| F3 | CGGCGCGACGCGCCACCATTCTCGCTAGTTCGATCGGTAGCGG |
| R3 | ATCTTCGAGTGTGAAGACCATGGTTGCGGTGATGGTGACTGGGG |
| F4 | CTTATCCCGCCTGTCCCGCCACCCTTTAGCTGCCATCTTGCGTC |
| R4 | ATCTTCGAGTGTGAAGACCATCGCCTTCCTCTCCTCCTCTGC |
| F5 | GCTGTTCCGAGTAACCATCAAC |
| R5 | GGTCCATACCGCTTTCTTGTG |
| F6 | GGATTACCAGGGATTTCAGTCGATG |
| R6 | GTTTTGTCACGATCAAAGGACTCTGGTAC |
| F7 | ACACTCTTTCCCTACACGACGCTCTTCCGATCTAAGCAGTG GTATCAACGCAGAGT |
| R7 | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGT CCATACCGCTTTCTTGTG |

with horseradish peroxidase (A9169; Sigma-Aldrich; dilution, 1 : 10000) for 1 h at room temperature. Next, the membrane was washed 3 times in TBS-T for 5 min each. Proteins were detected using an Amersham ECL Plus Western Blotting Detection System kit (Cytiva) according to the manufacturer's instructions.

**Plasmid constructs.** The pNL2.2 YB-1pr_YB-1_NlucP_YB-1, pNL2.2 ACTBpr_BTF3_NlucP_BTF3, and pNL2.2 ACTBpr_BTF3_Fluc_BTF3 plasmids were obtained previously [15, 16]. The digestion of these plasmids with the restriction endonucleases *Eco*RI and *Bam*HI produced the fragments containing 3′ UTRs of the *YB-1* and *BTF3* (basic transcription factor 3) mRNAs and vectors containing the *YB-1* or *ACTB* (beta-actin) promoter, the 5′ UTR of the *YB-1* or *BTF3* mRNAs, and *NlucP* cDNA. Ligation of the obtained fragments into the vectors yielded the pNL2.2 YB-1pr_YB-1_NlucP_BTF3 and pNL2.2 ACTBpr_BTF3_NlucP_YB-1 plasmids.

The F1/R1 and F2/R2 primer pairs (Table 1) and the above plasmids were used to generate the pNL2.2 YB-1pr_NlucP_BTF3, ACTBpr_NlucP_BTF3, pNL2.2 YB-1pr_NlucP_YB-1, and ACTBpr_NlucP_YB-1 vectors that were assembled by SLIC (sequence and ligation independent cloning) using the sequences containing the 5′ UTRs of the *YB-1* or *BTF3* mRNAs. These sequences were obtained by PCR with the F3/R3 (*YB-1* 5′ UTR) and F4/R4 (*BTF3* 5′ UTR) primers (Table 1) on the pNL2.2 YB-1pr_YB-1_NlucP_YB-1 and pNL2.2 ACTBpr_BTF3_NlucP_BTF3 plasmids, respectively. The resultant plasmids were pNL2.2 YB-1pr_BTF3_NlucP_BTF3, pNL2.2 YB-1pr_BTF3_NlucP_YB-1, pNL2.2 ACTBpr_YB-1_NlucP_YB-1, and pNL2.2 ACTBpr_YB-1_NlucP_BTF3.

**Measurement of luciferase activity in cells.** The next day after transfection with the plasmids encoding luciferases (NlucP/Fluc ratio, 10 : 1), the cells were reseeded into smaller dishes (to obtain 2 wells in 3 replicates for each cell line). After incubation for 24 h, half of the wells were used for the luciferase activity assay, and the rest – for the analysis of luciferase mRNA levels by RT-qPCR.

The activities of NlucP (nanoluciferase with the PEST sequence) and Fluc (firefly luciferase) were measured using the Nano-Dual-Glo Luciferase Assay System (Promega, USA). The cultured cells were lysed in passive lysis buffer (PLB, Promega) for 10 min at room temperature, and the enzymatic activity of luciferases was determined using a GloMax 20/20 luminometer (Promega).

The translation efficiency of the reporter mRNA was calculated as the ratio between the NlucP luciferase activity normalized to the activity of Fluc and the relative amount of the *NlucP* mRNA normalized to the amount of the control *Fluc* mRNA. When calculating the relative translation efficiency, the translation efficiency in HEK293TΔYB-1 cells was taken as 100%.

**Reverse transcription-quantitative PCR (RT-qPCR).** Total RNA was isolated using a Direct-Zol RNA Microprep kit (Zymo Research, USA) according to the manufacturer's recommendations. One μg of total RNA was reverse transcribed with Maxima H Minus reverse transcriptase (Thermo Fisher Scientific, USA) according to the manufacturer's recommendations. qPCR was performed in a QuantStudio 5 system (Thermo Fisher Scientific) using the qPCRmix-HS SYBR+ LowROX reaction mixture (Evrogen, Russia). The reaction mixture (25 μl) contained 0.5 μl of the reverse transcription reaction mixture and 0.2 μM of each primer pair (F5/R5 for NlucP and F6/R6 for Fluc; Table 1). The following amplification conditions were used: 5 min at 95°C followed by 35 cycles of 95°C for 10 s, 57°C for 20 s, and 72°C for 10 s. In the experiments measuring the relative mRNA amounts, the content of the transcripts was calculated using the QuantStudio™ Design & Analysis Software (Thermo Fisher Scientific).

**5′ RACE (rapid amplification of cDNA ends).** cDNAs for the 5′ RACE analysis were synthesized using a Mint RACE cDNA amplification kit (Evrogen) according to the manufacturer's recommendations using the PlugOligo adapter and oligod$T_{18}$. The first round of PCR was performed with the NlucP and PlugOligo-specific primers F7 and R7 (Table 1) carrying additional Illumina adapter sequences. PCR products were purified with SPRIselect beads (NEB, USA) according to the manufacturer's recommendations. The second round of PCR was performed using the primers from the NEBNext Dual Index Primers Set 1 for Illumina (NEB, USA). PCR products were purified with SPRIselect beads and sequenced on the NovaSeq6000 platform (Illumina) at the Skoltech Research Facilities Center (Genomics Facilities), Skolkovo Institute of Science and Technology, Russia. The resulting reads were processed with cutadapt v. 4.8 [17] to remove adapter sequences and 5′ poly-G tracks added by Mint reverse transcriptase. The mapping of the reads to pNL2.2 sequences was performed with bwa mem v. 0.7.17 [18]. The cumulative 5′ end coverage of the reads was calculated with bedtools v. 2.30.0 [19].

**Statistical analysis** of the experimental data was carried out using the one- or two-tailed Student's *t*-tests for independent samples in the R software environment.

## RESULTS AND DISCUSSION

**Autoregulation of YB-1 synthesis in HeLa cells.** To understand how YB-1 controls its synthesis at the translation level, we designed an experiment aimed at elucidating whether increasing the amount of YB-1 in a cell by introducing additional YB-1 synthesized from a plasmid would reduce the endogenous expression of YB-1 (Fig. 1). Note that we used HeLa cells because a significant portion of the *YB-1* mRNA in these cells is polysome-associated [20], providing conditions in which a clear decrease in the protein synthesis level can be expected in the case of autoregulation. HeLa cells were transfected with the pcDNA3-HA-YB-1 plasmid encoding hemagglutinin-tagged YB-1 (HA-YB-1) to distinguish between the exogenous and endogenous YB-1 and cultured in the presence of [$^{35}$S]-methionine after 24 or 48 h.

Importantly, the pcDNA3-HA-YB-1 plasmid did not encode the UTRs of the *YB-1* mRNA; therefore, the translation of the plasmid-synthesized *HA-YB-1* mRNA should not have been regulated by either endogenous YB-1 or exogenous HA-YB-1. The expression of the exogenous HA-YB-1 reached its maximum 24 h after the transfection (Fig. 1a). The amounts of HA-YB-1 and YB-1 were approximately equal, with no notable changes in the amount of the latter. However, the synthesis of endogenous YB-1 24 h after the transfection (Fig. 1, b and c) decreased in the HA-YB-1-expressing cells, while the level of total protein synthesis in these cells remained unchanged. Interestingly, 48 h after transfection, the synthesis of exogenous HA-YB-1 dropped down to the level of YB-1 synthesis, which returned to the values observed in the cells that did not express HA-YB-1. This means that 48 h after transfection, the amount of exogenous HA-YB-1 decreased (Fig. 1a) and was no longer sufficient to inhibit the synthesis of endogenous YB-1. This decrease might have been caused by the inability of HeLa cells to efficiently replicate the pcDNA3 plasmid. Accordingly, an approximately twofold decrease in the amount of exogenous HA-YB-1 could be expected after roughly one cell division cycle, as shown in Fig. 1a.

**The effect of YB-1 on the translation of reporter mRNAs containing the 5′ and 3′ UTRs of the *YB-1* mRNA in HEK293T cells.** A drawback of the above-described experiment is the inability to reveal the role of the *YB-1* mRNA UTRs in the regulation of translation of this mRNA by YB-1. This problem can be solved by using plasmid constructs encoding a reporter gene (*NlucP* in our case) flanked by the UTRs of the *YB-1* mRNA or some control mRNA (here, *BTF3* mRNA) (Fig. 2a). It is important to use the plasmids, because in the case of cell transfection with mRNAs, a significant fraction of these mRNAs remains inactive within the liposomal particles [21]. Also, we used a modified nanoluciferase that contained an instability element (PEST domain) that reduced its half-life to 15 min [16]. This allows measuring the amount of protein synthesized within a certain time interval, which, together with the known amount of *NlucP* mRNA, indicates the level of mRNA from which this protein was synthesized. Therefore, we were able

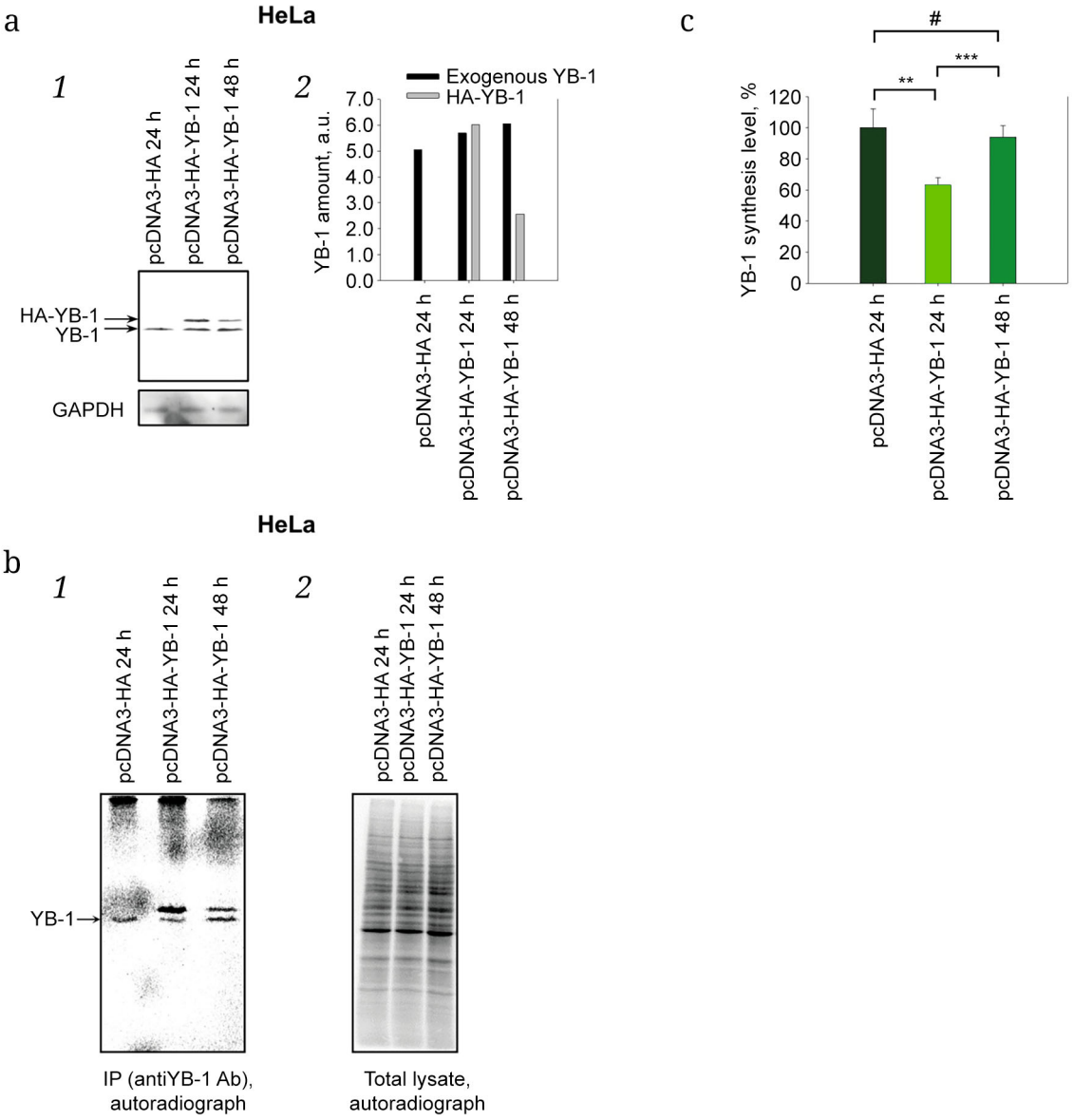


**Fig. 1.** The autoregulation of YB-1 synthesis in HeLa cells. a) The content of YB-1 (endogenous) and HA-YB-1 (exogenous) proteins in HeLa cells transfected with pcDNA3-HA-YB-1 or pcDNA3-HA 24 and 48 h after transfection was analyzed by immunoblotting with anti-YB-1 antibodies (*1*). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. Band intensities for endogenous (YB-1) and exogenous (HA-YB-1) proteins (*2*). b) The levels of synthesis of endogenous (YB-1) and exogenous (HA-YB-1) proteins were determined by metabolic protein labeling with [$^{35}$S]-methionine followed by YB-1 immunoprecipitation (*1*). All samples contained the same amount of total protein (*2*). c) Relative levels of YB-1 protein synthesis as determined from the radioactivity of the YB-1-corresponding bands in the autoradiograph shown in panel b (*2*). The level of YB-1 synthesis in the cells transfected with pcDNA3-HA was taken as 100%. The values are shown as means ±2 standard deviations (SD) from three independent experiments. The two-tailed Student's *t*-test was used to assess the statistical significance of differences; ** $p < 0.01$; *** $p < 0.005$; # statistically insignificant.

to determine the translation efficiency of the reporter mRNA. The results were normalized against the translation of the control RNA. Note that the *YB-1* and *ACTB* promoters were used as the reporter gene promoters in the pNL2.2 plasmids (Fig. 2a).

To demonstrate the effect of YB-1 on the translation of reporter mRNAs, two types of cells should be used – with low or no YB-1 and with the normal YB-1 level. Accordingly, we used HEK293TΔYB-1 cells in which the synthesis of endogenous YB-1 was blocked using the CRISPR/Cas9 genome editing system, and HEK293TΔYB-1+YB-1 cells stably expressing YB-1 at a level comparable to that of endogenous protein in normal HEK293T cells (Fig. 2b). These cells were transfected with pNL2.2 plasmids encoding the *NlucP* mRNAs with the 5′ and 3′ UTRs from the *YB-1* and/or *BTF3* mRNAs under control of the *YB-1* or *ACTB* promoters. As an internal control, we introduced

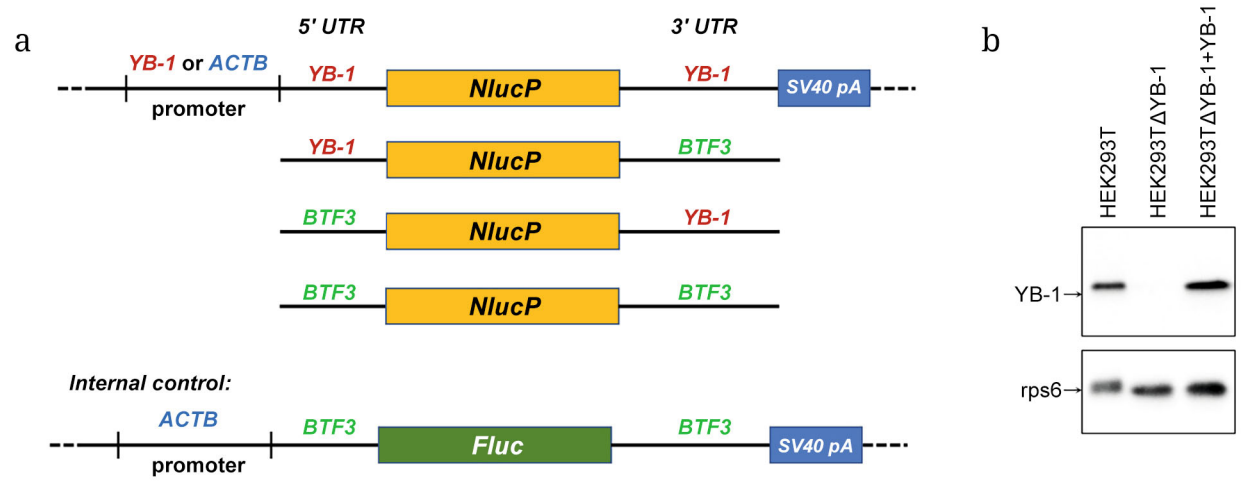


**Fig. 2.** Genetic constructs and cell lines used in the study. a) pNL2.2 plasmids encoding the reporter *NlucP* mRNAs containing a combination of UTRs from the *YB-3* and *BTF3* mRNAs and pNL2.2BTF3_Fluc_BTF3 plasmid encoding the *Fluc* mRNA (internal control) that were used for the transfection of HEK293TΔYB-1 and HEK293TΔYB-1+YB-1 cells. b) Analysis of YB-1 content in HEK293T, HEK293TΔYB-1, and HEK293TΔYB-1+YB-1 cells. Rps6 (ribosomal protein S6) was used as a loading control.

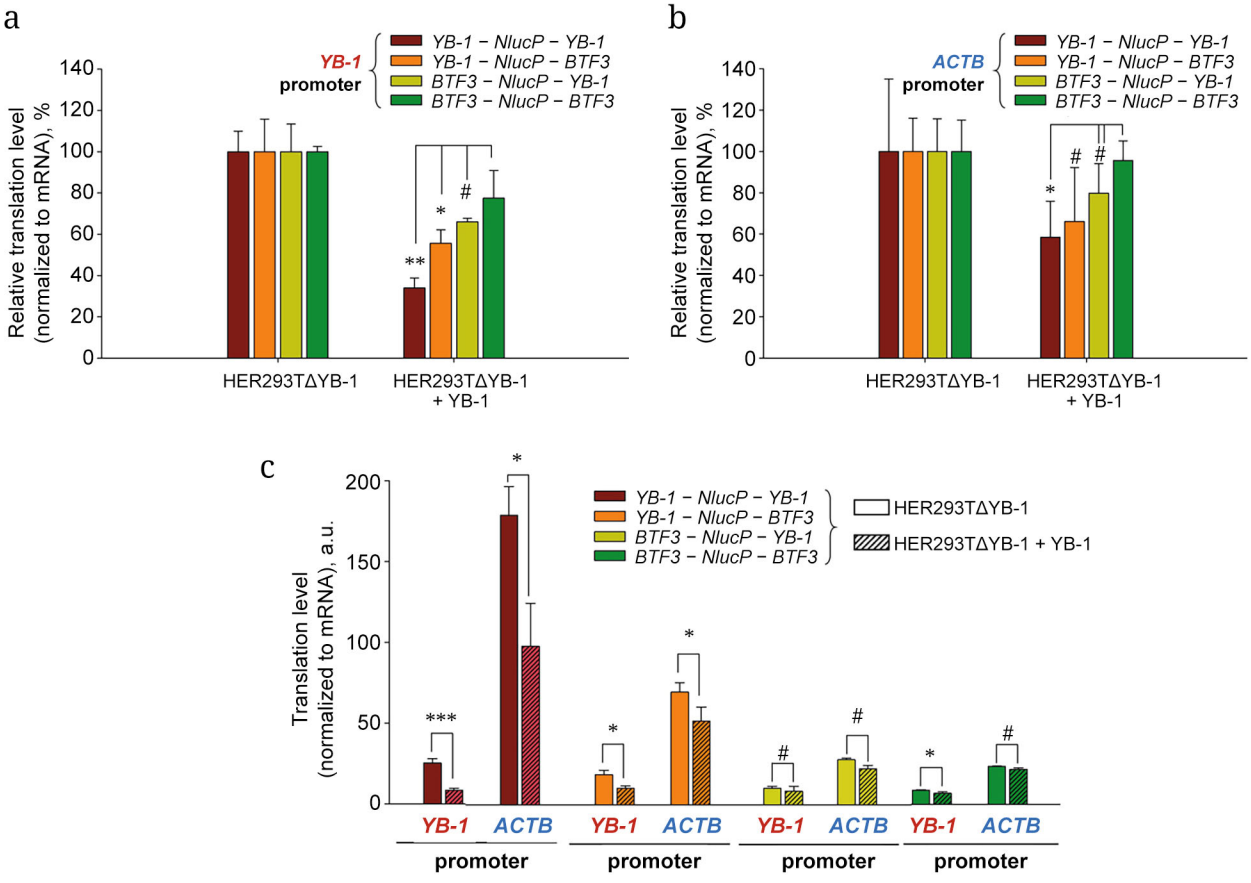


**Fig. 3.** Translation of the *NlucP* mRNA containing the 5′ and 3′ UTRs from the *YB-1* and/or *BTF3* mRNA under control of the *YB-1* (a) or *ACTB* (b) promoters in HEK293TΔYB-1 and HEK293TΔYB-1+YB-1 cells. Twenty-four hours after transfection with the pNL2.2 plasmids (Fig. 2a), the cells were harvested and divided into two fractions. Total RNA isolated from one fraction were used for measuring the amounts of *NlucP* and *Fluc* mRNAs by RT-qPCR. Cells from the other fraction were used for determining the activities of NlucP and Fluc. The relative level of NlucP translation was calculated as the NlucP activity normalized to the amount of mRNA. The level of translation in HEK293TΔYB-1 cells is taken as 100%. c) Same as (a) and (b), except that the graph shows the absolute values of the translation level. The values shown are as means ±SD from three independent experiments. The one-tailed Student's *t*-test was used to assess statistical significance; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; # statistically insignificant.

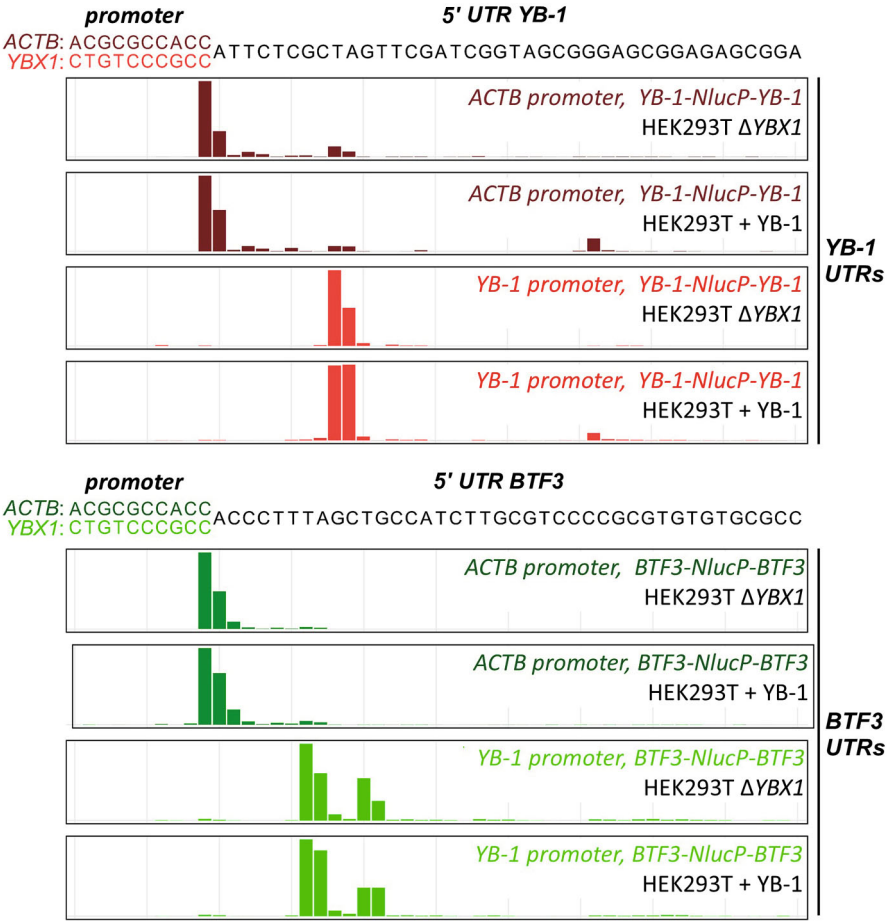


**Fig. 4.** Analysis of the transcription start sites from the pNL2.2 plasmids in HEK293TΔYB-1 and HEK293TΔYB-1+YB-1 cells. The cells were transfected with the pNL2.2-YB-1_YB-1-NlucP-YB-1, pNL2.2-YB-1_BTF3-NlucP-BTF3, pNL2.2-ACTB_YB-1-NlucP-YB-1, and pNL2.2-ACTB_BTF3-NlucP-BTF3 plasmids. After 24 h, total RNA was isolated from the cells, and the 5′ ends of the reporter mRNAs were analyzed by 5′ RACE.

a plasmid encoding the *Fluc* mRNA with the 5′ and 3′ UTRs from the *BTF3* mRNA along with the pNL2.2 plasmid.

After 24 h of culturing, RNA was isolated from a fraction of cells to measure the amount of synthesized *NlucP* and *Fluc* mRNAs, while the remaining cells were used to measure the activity of NlucP and Fluc luciferases. The activity of the former was normalized to the Fluc activity (internal control); the amount of the *NlucP* mRNA was normalized to the amount of the *Fluc* mRNA.

Figure 3a shows that upon the restoration of the YB-1 amount in the cells, the decrease in the translation efficiency of the *NlucP* mRNA containing both UTRs of the *YB-1* mRNA was much stronger (~3-fold) than that of the reporter mRNA with the UTRs from the control *BTF3* mRNA (~1.3-fold). The presence of either of the reporter mRNAs containing solely the 5′ or 3′ UTR of the *YB-1* mRNA was insufficient for the suppression of mRNA translation by YB-1 to the same extent as when both UTRs from the *YB-1* mRNA were used. Although the translation of the reporter mRNA with the *YB-1* mRNA 5′ UTR was more sensitive to YB-1, both UTRs of the *YB-1* mRNA were required for the efficient regulation of the *YB-1* mRNA translation by YB-1 (Fig. 3a).

Interestingly, the use of plasmid constructs with the reporter genes under control of the *ACTB* promoter produced the same results (Fig. 3b). The greatest sensitivity of translation to the presence of YB-1 was observed for the reporter mRNA with both UTRs of the *YB-1* mRNA, although the effect was less pronounced.

This observation may have different explanations. The start of the mRNA sequence containing the *YB-1* mRNA 5′ UTR and the *YB-1* promoter corresponds to the start of the main transcription site for HEK293T cells, according to the FANTOM project [22] and the dbTSS database [23]: the length of the *YB-1* mRNA 5′ UTR is 140 nucleotides. The use of the *ACTB* promoter increases the 5′ UTR sequence by 9 nucleotides at the 5′ end (Fig. 4, Fig. S2 in the Online Resource 1).

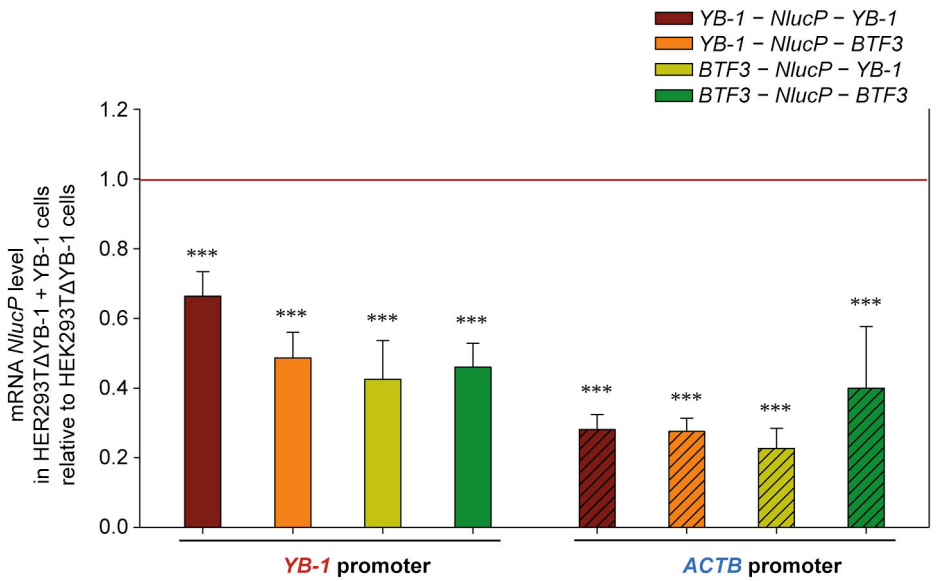


**Fig. 5.** The content of *NlucP* mRNA containing 5′ and 3′ UTRs from the *YB-1* and/or *BTF3* mRNAs synthesized from the *YB-1* or *ACTB* promoters in HEK293TΔYB-1 and HEK293TΔYB-1+YB-1 cells. The amounts of mRNAs are taken from Fig. 3. The level of *NlucP* mRNA in HEK293TΔYB-1 cells was considered as 1. The results shown are the means ±SD of three independent experiments. The one-tailed Student's *t*-test was used to assess statistical significance; *** $p < 0.001$.

Similarly, the length of the *BTF3* mRNA 5′ UTR upon the use of the *ACTB* promoter increases from 221 to 228 nucleotides. This slight extension may affect the regulation of its translation by the YB-1 protein. An alternative and more probable explanation is that mRNAs synthesized from different promoters might be modified differently or recruit different sets of RNA-binding proteins, which ultimately affects the efficiency of its translation and regulation. For example, as reported recently, a promoter can affect not only the efficiency of transcription, but also the efficiency of translation [24].

The latter explanation is indirectly supported by the fact that the translation level (translation per RNA in absolute values) of mRNAs synthesized from the *ACTB* promoter was significantly higher than that of mRNAs synthesized from the *YB-1* promoter (Fig. 3c).

Another important fact about the effect of YB-1 on the synthesis or amount of its mRNA is that during the YB-1 synthesis, the amount of reporter mRNA decreased, which may indicate a negative effect of YB-1 on transcription not only from the *YB-1* promoter, but also from the *ACTB* promoter (Fig. 5). The effect of YB-1 on transcription has long been reported [1], but this might have been a non-specific effect on the overall transcription, probably, due to the YB-1 involvement in the regulation of many genes participating in transcription. Nevertheless, it cannot be ruled out that both the *YB-1* and *ACTB* promoters can be YB-1-regulated in certain situations, which requires additional studies.

## CONCLUSION

Taken together, the above facts provide evidence for the existence of the autoregulation of YB-1 synthesis in cultured mammalian cells. We can also state that both 5′ and 3′ UTRs of the *YB-1* mRNA mediate the inhibitory effect of YB-1 on its translation. Previously, *in vitro* experiments showed that removal of the regulatory element from the *YB-1* mRNA 3′ UTR is sufficient to eliminate the inhibitory effect of YB-1 [11]. Still, the situation may be more complicated in cultured eukaryotic cells, where the *YB-1* mRNA 5′ UTR has been shown to participate in the autoregulation of YB-1 synthesis [8].

Presumably, the spatial proximity of the 5′ and 3′ UTRs of the *YB-1* mRNA can allow YB-1, through its specific interaction with the regulatory element in the 3′ UTR, to influence the initiation of the *YB-1* mRNA translation. Presumably, such proximity can be provided by proteins interacting with both UTRs of this mRNA. Also, it cannot be ruled out that YB-1 itself interacts simultaneously with the 5′ and 3′ UTRs of the *YB-1* mRNA. Besides, in many mRNAs, the 5′ and 3′ ends are close to each other due to the secondary structure architecture [25]. This suggests that the 5′ UTR of the *YB-1* mRNA might be in proximity to the 3′ UTR, thereby contributing to the effect of the 3′ UTR-bound YB-1 on translation. In any case, the 5′ UTR of the *YB-1* mRNA is likely an element providing an increased sensitivity of the *YB-1* mRNA translation to YB-1, while the 3′ UTR serves as a YB-1 carrier.

The discussion on the inhibitory effect of YB-1 on its synthesis cannot ignore a surprising fact recently reported by Wang et al. [26] who found that the 5′ UTR of the *YB-1* mRNA participates in the regulation of its translation. Overexpression of Flag-YB-1 from a plasmid in glioblastoma cells stimulated the synthesis of both endogenous YB-1 and a reporter mRNA containing the *YB-1* mRNA 5′ UTR. This suggests that the effect of YB-1 on the translation of its mRNA can vary depending on the cellular context and, probably, other proteins interacting with the *YB-1* mRNA UTRs. However, it cannot be ruled out that the properties of Flag-YB-1 used by Wang et al. were altered due to a high negative charge of the Flag tag (more precisely, 3xFlag tag) that could interact with or block positively charged amino acid residues of the YB-1 C-terminal domain.

Some other proteins interacting with the *YB-1* mRNA UTRs may also be involved in the regulation of translation of the *YB-1* mRNA in the cells. For example, involvement of the poly(A)-binding protein (PABP) and heterogeneous nuclear ribonucleoprotein Q (hnRNP Q) in this process has been shown *in vitro* [11, 27]. Therefore, the mechanism of regulation of the *YB-1* mRNA translation by YB-1 requires further investigation.

**Abbreviations**

| | |
|---|---|
| ACTB | beta-actin; |
| BTF3 | basic transcription factor 3; |
| Fluc | firefly luciferase; |
| NlucP | nanoluciferase with the PEST sequence; |
| UTR | untranslated region; |
| YB-1 | Y-box binding protein 1. |

**Supplementary information**

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**Contributions**

D.N.L. developed the study concept and supervised the study; D.N.L., I.A.E., V.S.K., and A.I.B. conducted the experiments; D.N.L. and I.A.E. validated the data; D.N.L. wrote the text of the article; I.A.E. edited the manuscript.

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**Ethics approval and consent to participate**

This work does not contain any studies involving human or animal subjects.

**Conflict of interest**

The authors of this work declare that they have no conflicts of interest.

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