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Regulation of LacZ mRNA translatability in a cell-free system at heat shock by the last four sense codons

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Under heat shock conditions translation of *Xenopus laevis* normal mRNAs in a rabbit reticulocyte cell-free system is blocked whereas hsp70 mRNA is translated. mRNA for *E. coli* β-galactosidase containing the last four sense codons of *Drosophila* hsp70 at its 3'-end was constructed. This mRNA is efficiently translated in a rabbit reticulocyte cell-free system at 43°C.

Protein synthesis regulation; Heat shock; Cell-free translation

1. INTRODUCTION

The synthesis of normal proteins is repressed when *Drosophila* cells or frog oocytes are heat shocked but the synthesis of heat shock proteins (hsps) is induced [1,2]. Non-heat shock messages are not degraded but are reactivated after cells are returned to normal temperature [3,4]. It is suggested that translation under heat shock is regulated at the level of initiation [5,6] and at the level of elongation [7].

Recently this translational discrimination has been reproduced in a cell-free system [8]. It was found that at normal temperature all *Drosophila* mRNAs were translated in a rabbit reticulocyte cell-free system. When this system was heated to 43°C (heat shock conditions for rabbit reticulocytes) only mRNAs for hsps were translated. Most of the *Drosophila* normal proteins synthesized in vitro at 43°C were found exclusively in the ribosome fraction. It has been suggested that the translation of normal mRNAs under heat shock conditions was inhibited at the stage of termination.

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We have found that the rate of mRNA translation in a cell-free system at heat shock is controlled by the last four sense codons. mRNA for *E. coli* β-galactosidase containing the last four sense codons of *Drosophila* hsp70 at its 3'-end is translated with high efficiency.

2. MATERIALS AND METHODS

Four *X. laevis* oocytes were incubated in 20 μl of Barth solution containing 10 μCi of [^{35}S]methionine (500 Ci/mmol, 10 mCi/ml, V/O 'Isotop' USSR) for 30 min at either 20°C or 36°C. The oocytes were dissolved in SDS-sample buffer and electrophoresed [11]. RNAs from liver of *X. laevis* were purified by phenol-detergent deproteinisation [12]. Prior to this procedure the frog was heated at 35°C for 1 h. Composition of the cell-free translation system was: 15 μl of rabbit reticulocyte lysate [13] treated with micrococcal nuclease [14], 25 mM Hepes, pH 7.6, 10 mM creatine phosphate, 1 mM dithiothreitol, 19 amino acids (without methionine) 40 μM each, 0.6 mM spermidine, 1 mM ATP, 0.2 mM GTP, 1 mM Mg-acetate, 10 μCi [^{35}S]methionine and 25 mg of frog RNA. Final volume was 25 ml. K-acetate concentration was 50 mM or 200 mM. The system was incubated at 34°C or 43°C for 45 min, stopped by SDS-sample buffer addition and electrophoresed [11]. Gels were dried and exposed using PM-B X-ray film (Tasma, USSR).

Insertion of the LacZ gene under SP6 promoter has been done by digestion of pLZ4 [20] with *Eco*RI + *Sal*GI endonucleases and ligation of the LacZ fragment with digested plasmid pSP65. For insertion of restriction endonuclease sites at the 3'-end of LacZ gene, the *Eco*RI + *Hin*dIII fragment of

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plasmid pUR291 [21] was inserted instead of such a fragment in plasmid pLacR13. Oligonucleotides have been inserted in the final plasmid digested with *Sal*GI + *Hin*dIII. These recombinant plasmids were verified by sequencing. After digestion with *Hin*dIII plasmids pJCS and pIPC were transcribed by SP6 RNA-polymerase [22]. All manipulations were done by standard methods [23].

3. RESULTS AND DISCUSSION

The effect of temperature on translation of normal and heat shock mRNAs in a rabbit reticulocyte system is shown in fig.1. Preparation of total cytoplasmic RNAs from liver of heat-shocked frog was used. Heating of the system to 43°C predominantly represses the translation of normal messages. It should be noted that this discrimination can only be seen at physiological potassium concentration (200 mM K-acetate). At lower ionic strength (50 mM K-acetate) all mRNAs are equally translated at either 34°C or 43°C (fig.1b).

Since it has been suggested that the translation of heat shock mRNAs in vitro is regulated at the termination stage [8] we searched sequences of heat shock genes to find any possible regulatory elements. This proved to be the case. The last four sense codons of all known eukaryotic hsp68, 70 and 90 invariantly specify the tetrapeptide Glu-Glu-Val-Asp (this was first noted by Lowe and Moran [9]). Some of the sequences are shown in fig.2. We have not found this tetrapeptide at C-terminals of all other eukaryotic proteins sequenced to date (5205 sequences, Swiss-Prot Data Bank, Sept. 1987). We suggest that this sequence is a signal for proper termination under heat shock conditions.

In order to verify this hypothesis, we constructed an mRNA containing a coding region for a normal protein and terminating with the last four sense codons of *Drosophila* hsp70. Two plasmids were constructed: pJCS and pIPC (fig.3). These plasmids were used for in vitro transcription with SP6 RNA-polymerase to obtain mRNA for *E. coli* β-galactosidase either terminating with the last four sense codons of *Drosophila* hsp70: GAG GAG GTC GAC TAA (mRNALacZ.HSP70), or terminating with the slightly modified last four sense codons of rabbit myosin MLC: GGT ACC TCC ATC TAA (mRNALacZ.MLC). These mRNAs were translated in rabbit reticulocyte cell-free systems. Results are shown in fig.4. At 34°C

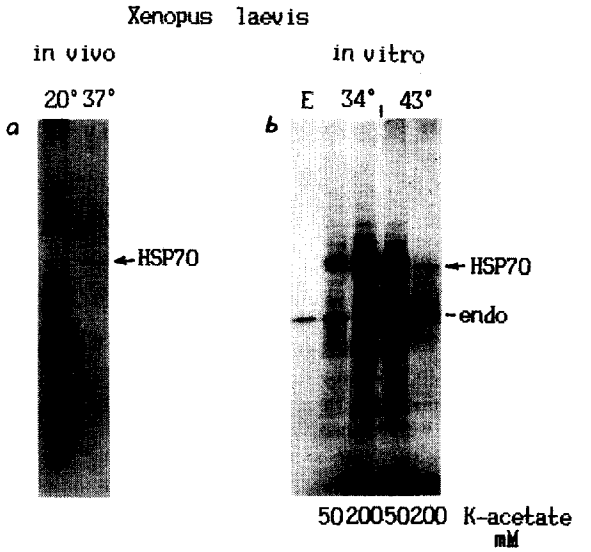

Fig.1. Effect of temperature on in vivo (a) and in vitro (b) protein synthesis. *X. laevis* oocytes (a) were incubated with [^{35}S]methionine at temperatures indicated. Equal amounts of proteins were loaded on each lane. Preparation of total cytoplasmic RNAs from liver of heat-shocked frog (b) were translated in rabbit reticulocyte systems under indicated conditions. Equal volumes of systems were loaded on each lane. E, no RNA in the system; endo, translation-independent labeling of a reticulocyte protein (depending on [^{35}S]methionine quality).

both mRNAs are translated equally well at 50 mM K-acetate, and equally poorly at 200 mM. At 43°C both mRNAs are again translated equally well at 50 mM K-acetate. But at 200 mM K-acetate the translation rate of mRNALacZ.HSP70 in contrast to the control mRNALacZ.MLC is increased and reaches the level observed at 50 mM K-acetate. Thus, translation of mRNA for β-galactosidase with the last four codons corresponding to Glu-Glu-Val-Asp is selectively stimulated by heat shock. This discrimination is observed only at physiological ionic strength, i.e. under the same conditions as for natural mRNAs.

yeast	HSP90 [12]	....	ATG	GAA	GAG	GTA	GAT	TAG ....
drosophila	HSP83 [13]	....	ATG	GAG	GAG	GTC	GAT	TAA ....
human	HSP70 [14]	....	ATT	GAG	GAG	GTA	GAT	TAG ....
drosophila	HSP70 [15]	....	GTC	GAG	GAG	GTC	GAC	TAA ....
xenopus	HSP70 [16]	....	ATA	GAA	GAA	GTT	GAC	TAA ....
chicken	HSP68 [6]	....	ATC	GAG	GAG	GTG	GAT	TAG ....
			Glu	Glu	Val	Asp	stop	

Fig.2. Last codons of some eukaryotic heat shock genes.

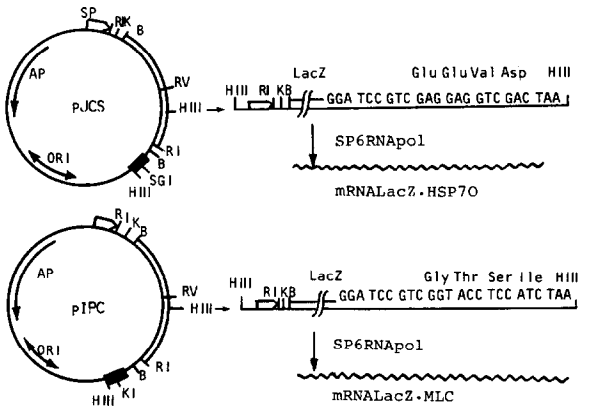

Fig.3. Construction of recombinant plasmids. Sequence of pBR322 is shown by thin line. LacZ. open box; SP6-promoter, open pentagon.

The effects of temperature and salt concentration on translation of natural and SP6-transcribed mRNAs are not exactly the same (cf. figs 1b and 4). Both mRNAs for β-galactosidase in contrast to

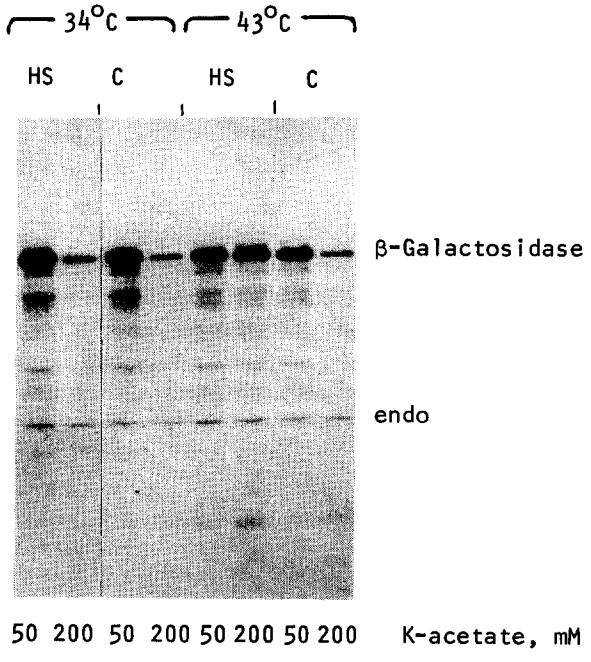

Fig.4. Effects of temperature and salt concentration on mRNALacZ.HSP70 and mRNALacZ.MLC translation in rabbit reticulocyte systems. Translation and analysis were as described in the legend to fig.1. 1.5–2 μg of each mRNA were added to the systems. HS, mRNALacZ.HSP70; C, mRNALacZ.MLC.

natural mRNAs are translated with low efficiency at 200 mM K-acetate at 34°C. These differences could be explained by the absence of a CAP-structure on in vitro synthesized mRNAs.

As low molecular mass *Drosophila* hsps do not possess Glu-Glu-Val-Asp at their C-terminals [13], we wonder if there are other sequences that similarly facilitate mRNA translation under heat shock.

The results presented in this paper demonstrate that the last four sense codons (or corresponding peptide) control mRNA translation efficiency in a cell-free system under heat shock conditions: they are the codons for Glu-Glu-Val-Asp, thus providing high mRNA translatability. Since it has been shown that the 5′-untranslated region is critical for hsp70 mRNA translation [5], the problem has become more interesting.

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