FIDELITY OF PROTEIN SYNTHESIS AFFECTS THE READTHROUGH TRANSLATION OF TOBACCO MOSAIC VIRUS RNA

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1. Introduction

In many viral systems [1–5] and in the case of β-globin [6], translation of mRNA can continue past the termination codon by a process known as readthrough. In the abscence of suppressor tRNAs, such natural termination suppression is likely to involve misreading of the termination codon. One example of this is readthrough of the coat protein of the Qβ bacteriophage where the UGA-termination codon is misread at low frequency as tryptophan (UGG) [1]. The function of readthrough proteins is poorly known. However, the readthrough protein of the Qβ-bacteriophage, present in the virion coat, is essential for the infectivity of phage [7].

Readthrough translation is readily enhanced by excess of Mg^{2+} [2,8] and by polyamines [8,9]. It has been suggested that these agents act through specific effects on a normal tRNA, leading to increased misreading of the termination codons alone [8,9]. However, I present data here suggesting that readthrough is affected more generally by the fidelity of protein synthesis.

2. Experimental

The standard cell free system (25 μl) contained 20 μl nuclease-treated rabbit reticulocyte lysate [10], 10–30 μCi [^{35}S]methionine (800–1030 Ci/mmol, Amersham), 3.2 μg TMV RNA/ml, and 50 μM of all the 19 amino acids except methionine (British Drug House), 37.5 μg calf liver tRNA/ml (Boehringer Mannheim), 37.5 μg creatine kinase/ml, 10 mM creatine phosphate, 0.5 mM dithiothreitol (Sigma), 100 mM KCl and 0.5 mM $MgCl_2$ exogenously added. To study the effect of cycloheximide, lysates were supplemented with 0, 0.1, 0.2, 0.4 or 0.8 μM cycloheximide (Sigma) and incubated at 28°C. To study the effect of temperature, lysates were incubated at 19, 22, 25 or 28°C. In each case, lysates were sampled after 1, 2 or 3 h translation into 4 vol. 4% SDS, containing 65 mM Tris–HCl (pH 6.8), 10% glycerol, 5% 2-mercaptoethanol and bromophenol blue, boiled for 3 min, and analyzed by SDS–polyacrylamide gel electrophoresis [11] in 7.5% slab gels. After electrophoresis gels were fluorographed [12] using Kodak RP X-Omat films. The M_r-values were approximated with the following ^{14}C-labelled proteins (Amersham); heavy chain of myosin (212 000), phosphorylase B (92 000), bovine serum albumin (68 000), ovalbumin (43 000), carbonic anhydrase (30 000) and lysozyme (14 000).

To quantitate the synthesis of the 110 000 protein and its readthrough product (160 000), the respective polypeptide bands, located by fluorography, were excised from the gels and treated overnight with 0.5 ml 30% H_2O_2 at 37°C. After adding 6 ml scintillation solution (8 g Ultrablend, 750 ml NP-40 and 2000 ml xylene) radioactivity of the samples was measured. For each 110 000/160 000 pair the background radioactivity, measured from a slice cut out from the gel lane above the 160 000 region, was subtracted.

The effects of cycloheximide and temperature on the fidelity of poly(U)-translation were assayed in the standard cell-free system lacking TMV RNA, [^{35}S]-methionine and the amino acids, and containing 100 μg poly(U)/ml (Boehringer Mannheim), 4 mM $MgCl_2$, and either 20 μCi [^{3}H]phenylalanine (80 Ci/mmol, Amersham) and 13 μM leucine or 20 μCi [^{3}H]leucine

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(61 Ci/mmol, Amersham) and 10 μM phenylalanine. Lysates were incubated for 60 min and 2 μl was sampled in triplicates into 0.5 ml water. After adding 0.5 ml 1 M NaOH, 5% H_2O_2 and 1 mg/ml of either phenylalanine or leucine, samples were incubated at 37°C for 15 min, trichloroacetic acid-precipitated, filtered onto glass fibre filters (Whatman), and counted for incorporated radioactivity. Background incorporation in the absence of poly(U) was assayed in each case and subtracted from all experimental values to obtain poly(U)-directed incorporation. Incorporation of [^{3}H]Phe and [^{3}H]Leu were in the order of 10^5 cpm and 10^3 cpm, respectively.

3. Results

Translation of tobacco mosaic virus (TMV) RNA yields 2 major polypeptides of 110 000 M_r and 160 000 M_r. The 160 000 M_r protein represents the readthrough product of the 110 000 M_r protein [2]. Using this system, I first examined the effect of cycloheximide on the relative synthesis of the two proteins. As shown in fig.1, the synthesis of the readthrough protein

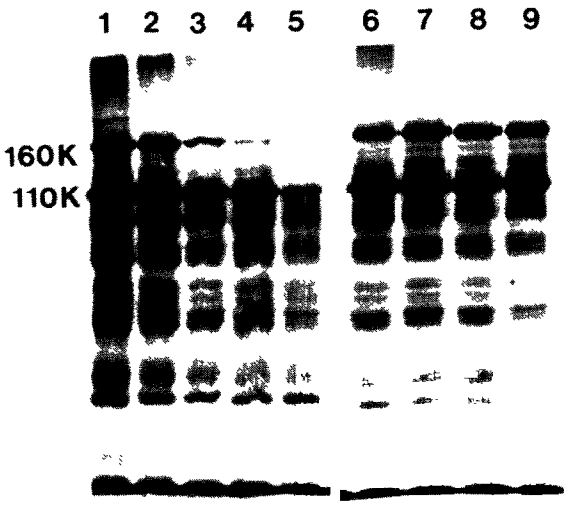

Fig.1. Effect of cycloheximide and temperature on the readthrough translation of TMV RNA. SDS gel analysis of the lysates translated for 2 h at 28°C in the presence of 0 (1), 0.1 (2), 0.2 (3), 0.4 (4) and 0.8 μM (5) cycloheximide; of the lysates translated for 3 h at 28°C (6), 25°C (7), 22°C (8) and 19°C (9). The 110 000 M_r protein is the major translation product of TMV RNA, and the 160 000 M_r protein represents its readthrough product [2]. The film is deliberately overexposed for photography, and gives an impression of higher readthrough than that shown in tables 1,2.

Table 1
Effect of cycloheximide on readthrough translation of TMV RNA

Translation time (h)	Cycloheximide (μM)	Incorp.[a] (cpm × 10^{-3})	Readthrough[b] (%)
1		103	12
2	–	124	13
3		93.7	10
1		74.6	(7.9)
2	0.1	72.2	12
3		71.4	9.0
1		10.2	(0.47)
2	0.2	22.9	6.1
3		19.3	5.3
1		1.06	(0.00)
2	0.4	19.2	2.6
3		24.6	4.4
1		0.585	(0.00)
2	0.8	2.78	0.00
3		9.47	0.22

[a] Summed radioactivity present in the 110 000 and 160 000 M_r proteins

[b] 100% × radioactivity of the 160 000 M_r protein/summed radioactivity of both proteins

Radioactivity incorporated into the 110 000 and 160 000 M_r (readthrough) proteins was quantitated as in section 2. Values in parentheses are considered as erroneous (see text)

(160 000 M_r), when compared to that of the 110 000 M_r protein, seems to be preferentially inhibited by cycloheximide. This effect of cycloheximide was quantitated and is given in table 1. It is clear that the readthrough translation of TMV RNA consistently decreases with the concentration of cycloheximide, so that apparently no readthrough protein is synthesized in the presence of 0.8 μM cycloheximide. The faint polypeptide bands seen between the 110 000 and 160 000 M_r bands in fig.1 probably represent prematurely terminated readthrough proteins. It is to be noted that they are not taken into account when calculating readthrough. However, this does not significantly change the results given in tables 1,2.

Since cycloheximide, in the concentrations used here, is known to inhibit mainly the elongation step of protein synthesis [13] erroneously low readthrough will be observed simply by sampling the translation system too early. For example, the low readthrough observed after 1 h translation in the presence of cyclo-

Table 2
Effect of temperature on readthrough translation of TMV RNA

Translation time (h)	Temp. (°C)	Incorp.[a] (cpm × 10^{-3})	Readthrough[b] (%)
1		109	9.6
2	28	106	11
3		83.6	11
1		70.1	14
2	25	110	17
3		88.0	17
1		24.1	(7.4)
2	22	56.9	18
3		58.8	20
1		5.46	(2.0)
2	19	39.4	21
3		48.3	24

[a] Summed radioactivity present in the 110 000 and 160 000 M_r proteins

[b] 100% × radioactivity of the 160 000 M_r protein/summed radioactivity of both proteins

Radioactivity incorporated into the 110 000 and 160 000 M_r (readthrough) proteins was quantitated as in section 2. Values in parentheses are considered as erroneous (see text)

heximide is due in part to the decreased elongation rate which increases the time of completion of the 160 000 M_r protein close to 1 h. However, by measuring readthrough at longer time points this effect can be eliminated (see tables 1, 2).

Table 2 shows the data of an experiment where TMV RNA was translated at different temperatures. Decreasing the translation temperature appears to increase readthrough, that is, proportionally more of the 160 000 M_r protein in comparison to the 110 000 M_r protein is synthesized at low temperatures. Also in this experiment, the low readthrough measured at 19°C and 22°C after 1 h translation is erroneous and a consequence of the decreased rate of elongation.

To study whether the changes seen in the synthesis of the readthrough protein could be correlated with the accuracy of protein synthesis, I next examined the effects of cycloheximide and temperature on the fidelity of poly(U)-translation. Initial experiments carried out at the same ionic conditions as used in the translation of TMV RNA (100 mM KCl and 0.5 mM $MgCl_2$) were unsuccessful due to the very low level of misreading in the control poly(U)-translation. However,

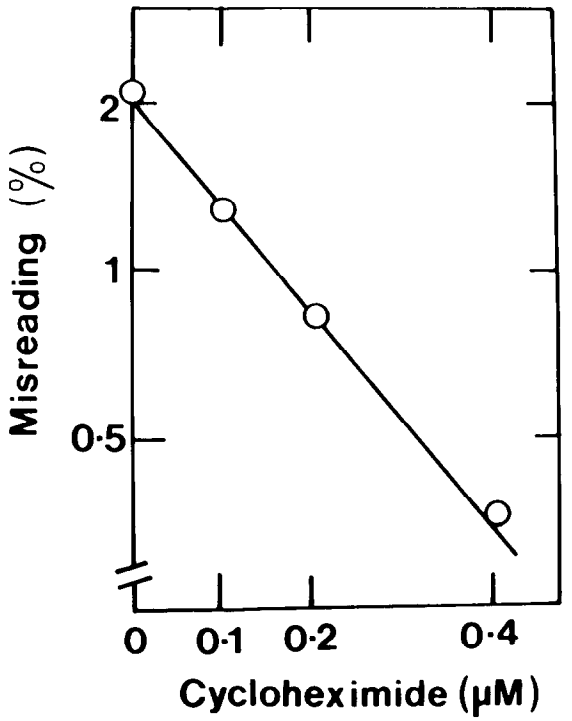

Fig.2. Effect of cycloheximide on the fidelity of poly(U)-translation. Misreading (%) is plotted on log-scale and was calculated as: 100% × poly(U)-directed trichloroacetic acid-insoluble pmol [^{3}H]leucine/pmol [^{3}H]phenylalanine.

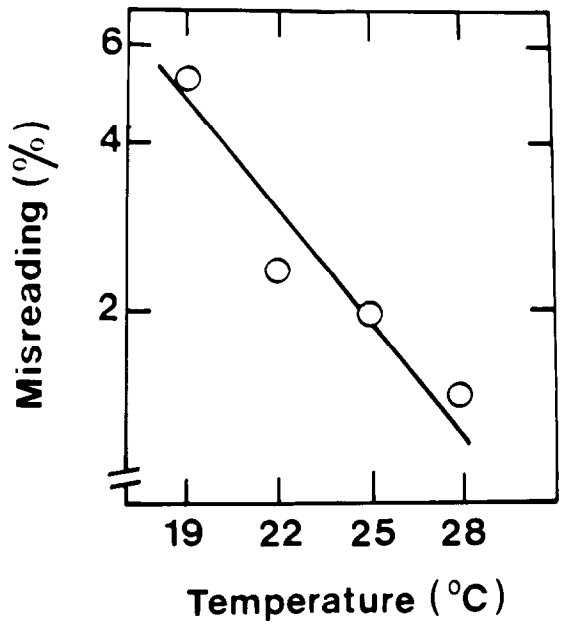

Fig.3. Effect of temperature on the fidelity of poly(U)-translation. Misreading (%), plotted on log-scale, was calculated as in fig.2.

after increasing Mg^{2+} up to 4 mM (which increased misreading to the level of 1.4–2.1%) the effects of cycloheximide and temperature could be reproducibly measured. Fig.2 shows that the fidelity of poly(U)-translation increases in the presence of cycloheximide, so that at 0.8 μM cycloheximide no misreading could be detected. Fig.3 shows the data of an experiment in which the fidelity of poly(U)-translation was studied at different temperatures. Increasing the temperature seems to enhance the fidelity of translation.

4. Discussion

Here, I show that in the presence of cycloheximide both misreading, as measured by the fidelity of poly(U) translation, and the readthrough translation of TMV RNA are decreased. However, low translation temperature was shown to increase misreading and to enhance readthrough. Based on these data it is suggested that fidelity of protein synthesis in general affects readthrough translation. The following observations are compatible with such a notion. Excess of Mg^{2+} increases the readthrough translation of TMV RNA (2) and Qβ RNA [8]. From other studies it is known that excess of Mg^{2+} enhances misreading [14,15]. Readthrough of the 0-protein of λ bacteriophage is inhibited in a cell-free system utilizing streptomycin-resistant ribosomes [3]. As noted below, these ribosomes display reduced misreading. Finally, one intriguing exception exists: polyamines have been reported to increase the fidelity of poly(U)-translation [16] and yet they are known to enhance readthrough in the TMV [9] and Qβ [8] systems.

The reduced misreading of poly(U) in the presence of cycloheximide, noted here, is possibly due to the decreased rate of elongation. According to [15], elongation rate, and specifically the translocation step can affect the fidelity of protein synthesis. Thus, more misreading of poly(U) was observed when the rate of elongation was increased by adding EF-G to a factor-free translation system. That increased rate of elongation induces more misreading is also supported by the theoretical considerations in [17]. Interestingly, the effect of mutations affecting the accuracy of protein synthesis by altering ribosomal proteins is consistent with the above notion. For example, streptomycin-resistant ribosomes, containing altered S12-protein, display less misreading [18] and show decreased rate of elongation [19,20].

Low temperature, by reducing the rate of elongation, would be expected to decrease misreading. However, the opposite result was actually observed here, suggesting that low temperature, in fact, increases misreading. Indirect evidence in support of this idea has been reported. It is known that misreading can reduce the specific activity of β-galactosidase synthesized in vivo [18], and that the specific activity of the enzyme synthesized at 25°C is ~60% of the specific activity of the enzyme synthesized at 37°C [21]. Taken together, these observations also suggest that more misreading occurs at low temperature.

Previously, low temperature (25°C) was shown to induce suppression of amber (UAG) mutants in vitro in extracts of *Escherichia coli* lacking suppressor tRNA activity, whereas no effect was detected in the case of ochre (UAA) or opal (UGA) mutants [21]. In addition, readthrough of the Qβ coat protein, which terminates in the UGA-codon, was not enhanced at low temperature (unpublished data cited in [21]). Readthrough translation of TMV RNA involves the suppression of the UAG-termination codon [2] and, as shown here, low temperature enhanced its suppression. Thus, if the low temperature suppression is due to increased misreading, as suggested above, it is difficult to see why it does not operate in the case of the UAA- and UGA-termination codons.

Finally, it is quite clear that the termination codon of TMV RNA (or any other respective system) as such is suppressed much too frequently to represent average codon misreading alone. Compared to the in vivo misreading frequency of 10^{-4}/amino acid incorporated [22,23], synthesis of readthrough proteins is always ≥100-fold more abundant. Obviously, the reading context [24] of the 'readthrough' termination codons must enhance their misreading.

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