Commentary

DISCRIMINATION OF MESSENGER RNA

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Received 28 November 1977

1. Introduction

Although in the past few years a large number of attempts to transfer and express eukaryotic genes in bacteria have been reported [1–5], confidence seems generally lacking that a eukaryotic messenger can at all be translated efficiently in bacteria. However, only recently has this problem been investigated with sufficient care:

There is no efficient heterologous translation of mRNA.

We will review here our data on a site specificity in the initiation region of mRNA preventing efficient heterologous translation and discuss briefly the consequences for genetic engineering experiments. We will then summarize recent evidence for mRNA discrimination in general.

2. Results and discussion

2.1. *Differences in recognition sites on mRNA of eukaryotic and prokaryotic origin*

The only possible direct test for the question whether eukaryotic gene copies are translated efficiently in a bacterial cell, is the translation of isolated mRNA in vitro in appropriate systems.

A set of messenger RNAs from various organisms was prepared and their translation examined in in vitro protein synthesizing systems from reticulocytes, wheat germ, Krebs ascites cells and *E. coli*. The outcome of these experiments was that, except for TMV RNA, all messenger RNAs were translated with drastically different efficiencies (table 1). Messenger RNA from eukaryotic cells were translated 200–500-times more efficiently in extracts from eukaryotic cells than in a cell-free system from *E. coli*. The reverse was observed for mRNA from prokaryotic sources. These RNAs were translated well in the bacterial extract but by a factor of 50–100 less efficiently in the cell-free systems from nucleated cells.

We realize that the protein synthesizing machineries differ in many respects between bacteria and eukaryotic cells [6–9]. The fact, however, that in the same extract two mRNAs can behave so differently, shows clearly that the two messenger RNA species are structurally different. Because the division of the RNAs into two groups followed the cross classification into pro- and eukaryotes, mRNAs from eukaryotes must possess a common structural property which prokaryotic RNA lacks, and vice versa. The tremendous reduction of total protein synthesis, in whatever heterologous combination, suggests that there is a deficiency at the initiation stage.

The heterologous recognition sites on mRNA are not rejected completely. The minute quantity of protein made, was still specific and the chains were completed (table 1) [10]. This may explain the long list of publications [11–19] describing successful

Table 1
Eukaryote and prokaryote specific initiation of translation

Protein synthesizing extract from	Relative rates of protein synthesis						
	MS-2 RNA	R17 RNA	T3 early RNA	T3 late RNA	Globin RNA	Oviduct RNA	TMV RNA
Krebs ascites cells	< 1.3		< 2.5	< 5.1	100	75.5	
Rabbit reticulocytes	< 2.6		< 0.6	< 2.6		100	
Wheat germ (a)	< 4.2	< 4.2	< 4.2		61.1	49.5	100
(b)			< 1.2			100	
Escherichia coli	31.4		92	100	< 0.2	< 0.3	98.5
						< 0.6	
						3.2	

Various messenger RNAs were used as templates for the synthesis in vitro of specific proteins. The rates of synthesis of individual proteins were determined by measuring the incorporation of radioactive amino acid into defined products. The proteins were resolved by SDS–slab-gel electrophoresis and their radioactivity measured by autoradiography and subsequent densitometry. The mRNAs and the products for which they code, were as follows: MS-2 RNA was isolated from phage, R17 RNA was obtained from Boehringer. Both RNAs directed the synthesis of replicase and of coat protein in the *E. coli* extract. T3 early and late RNAs were isolated from T3-infected cells [43] at 4.5 min and 10 min past infection (30°C). The RNAs code for the whole series of T3 proteins: T3 early RNA codes predominantly for early proteins such as *S*-adenosylmethionine hydrolase (SAMase), DNA ligase, protein kinase and RNA polymerase, T3 late RNA codes for the products of late T3 genes such as genes 6, 8, 9, 10, 12, 15, and for T3 lysozyme and unwinding protein. Globin and oviduct RNA were poly(A)-containing polysomal RNAs from hen reticulocytes and hen oviduct [10,44,45]. Major products are the α and β chains of globin, and ovalbumin, ovomucoid and lysozyme, respectively. Oviduct proteins determined individually in samples from the *E. coli* system, are listed in that order. TMV RNA was a gift of Dr K. Kloppstech. The products synthesized in vitro are discussed [10,46]. The protein synthesizing systems were prepared as in [41,47–49]. We thank Drs P. B. Hackett, D. Gallwitz and A. Sippel for samples of ascites cell extract, reticulocyte lysate and wheat germ system respectively. In this table, all radioactivity in products above 5000 daltons has been added up. Within the same protein synthesizing system, the highest rate of synthesis was set to be 100% corresponding to the following actual figures (in nmol leucine incorporated/ml incubation mixture): Ascites cell extract 6.1; reticulocyte lysate 0.22; wheat germ system (a) 0.24, (b) 0.11; *E. coli* system 8.1. The efficiency of translation of one RNA species in different systems can also be compared if ribosome concentrations are taken into account. The incubation mixtures contained: 1.1 mg/ml (ascites); 1.75 mg/ml (wheat germ); 3.1 mg/ml (*E. coli*). A comparison for instance between the efficiency of translation in the wheat germ and *E. coli* systems (per μg ribosome) reveals an at least 650-fold difference in the rate of ovalbumin synthesis, and a 50-fold difference in the rate of early T3 proteins for one given concentration of mRNA. For the small enzyme SAMase (measured by enzyme activity [43]) the difference was at least 22-fold [10]. Numbers in the table designated < (smaller than), are below the level detectable by the gel technique. The differences in lower limit of detection reflect differences in the length of film exposure

syntheses in heterologous systems, although other explanations, such as organelle contamination, seem possible. There were exceptions to the rule in that globin RNA translation in an *E. coli* extract was initiated at an unnatural site [20] and ovalbumin message carries an efficient internal initiation site for *E. coli* ribosomes [10]. *E. coli* ribosomes should, therefore, protect fragments in binding assays of these RNAs against nuclease digestion. But this may well depend on kinetic properties of the reaction studied [22a]. Plant virus RNA seems well accepted in the *E. coli* extract [21,22]. However, TMV RNA yielded different products in eukaryotic extracts and in an *E. coli* extract [10].

In a large series of similar experiments, the borderline between organisms containing mRNA with the 'eukaryotic' recognition site, and organisms with mRNA carrying the 'prokaryotic' recognition site, was defined [23]. Although in these experiments, gross leucine incorporation was used and, for obvious reasons, no check on the specificity of the products was possible, the 'all or none' character of the data permits the conclusion:

The eukaryote-type recognition site is carried by

mRNA from nucleated cells including yeast and several algae such as Euglena and Chlorella and by mRNA from blue–green algae.

Mitochondria and chloroplasts are apparently of the prokaryotic type [24–26]. They share this property with bacteria and bacteriophages. Already at the time of the early experiments, the block of the heterologous combinations appeared to be in the step of initiation [23].

The difference in translational efficiency between heterologous and homologous systems may become an obstacle to the cloning experiments planned in many laboratories. The advantage of an increased copy number of eukaryotic genes attached to bacterial plasmid vectors, may be abolished by the low translational yield in bacteria. And the substitution of bacterial genes for human gene deficiencies may, in the future, suffer from the same problem. A prerequisite of all these experiments is the transfer of appropriate recognition sites to genes selected for cloning. In two experiments demonstrating expression of yeast genes in *E. coli* [27,28], the appropriate recognition sites may either have been present by chance, or been selected for and obtained by insertion or recombination, or the auxotroph *E. coli* strain may be able to live on low levels of yeast enzyme produced at say 1% efficiency. Low translational yield may also be compensated for by a tremendous plasmid copy increase through selective pressure. On the basis of our data we would predict that translation would be low if, in such experiments, the selective pressure were omitted.

For the sake of the discussion here, our data demonstrate the existence of recognition sites on mRNA for which the corresponding protein synthesizing apparatus has been adapted.

The data cannot decide whether the initiation signals are used as regulatory elements within the same cell.

2.2. *Recognition sites on mRNA used for translational control*

Cells infected with viruses contain two types of mRNA which may carry different recognition sites. The colivirus T7 is one very favorable example. In vivo, T7 shuts off host DNA, RNA and protein synthesis [29–31]. A specific discrimination of mRNA molecules is observed in cells infected with T7 wild type if the fate of RNA phage translation is followed. f2 coat protein synthesis was completely blocked while T7 specific translation occurred at optimal rate (fig.1). The inhibition of f2 protein synthesis required T7 gene expression since no inhibition was observed upon rifampicin addition prior to T7 infection.

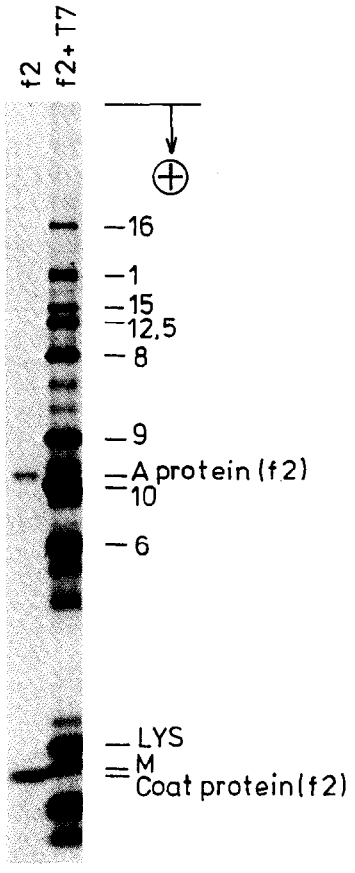

Fig.1. T7-induced translation control in vivo. *E. coli* W 1655 carrying the T7 permissive mutant sex factor F 3343 [50], were grown to A_{600} = 0.2 in M9 minimal medium supplemented with 0.4% glucose, 20 μg/ml each of methionine and thiamine, and 10^{-4} M $MgSO_4$. The cells were irradiated with ultraviolet light for 60 s (standard conditions [51]) and infected immediately with f2 at a multiplicity of infection of 10. The culture was divided. All cultures received a pulse of amino [^{14}C]acids (25 μCi/ml) from 41–43 min past f2 infection. One part of the culture was superinfected with T7 wild type (multiplicity of infection: 10) at 30 min after f2 infection. One part of the culture was superinfected but rifampicin at 100 μg/ml added at the same time. After harvest, total protein of each sample was resolved by SDS–acrylamide electrophoresis (10–20% acrylamide gradient). An autoradiogram is shown.

f2 RNA can be considered as host-type mRNA. Thus, in vivo, host-type and T7 virus-type messenger RNA species are distinguished, which permits again the conclusion that these two types of messenger RNA must carry group-specific recognition sites. It seems that in cells infected with other coliviruses, mRNAs with such group-specific recognition sites also exist [32–39].

In the T7 system, the control protein operating on these group-specific recognition signals, was identified (translational repressor [40]), and partially purified. In vitro, the translational repressor did what had been observed in vivo: it strongly inhibited the translation of MS-2 or f2 RNA but did even stimulate the expression of late T7 genes (fig.2). The control protein inhibited also the synthesis of host enzymes such as β-galactosidase [40]. The translational repressor thus blocks the initiation of translation and this apparently discriminates between host and late T7 viral messenger RNA species.

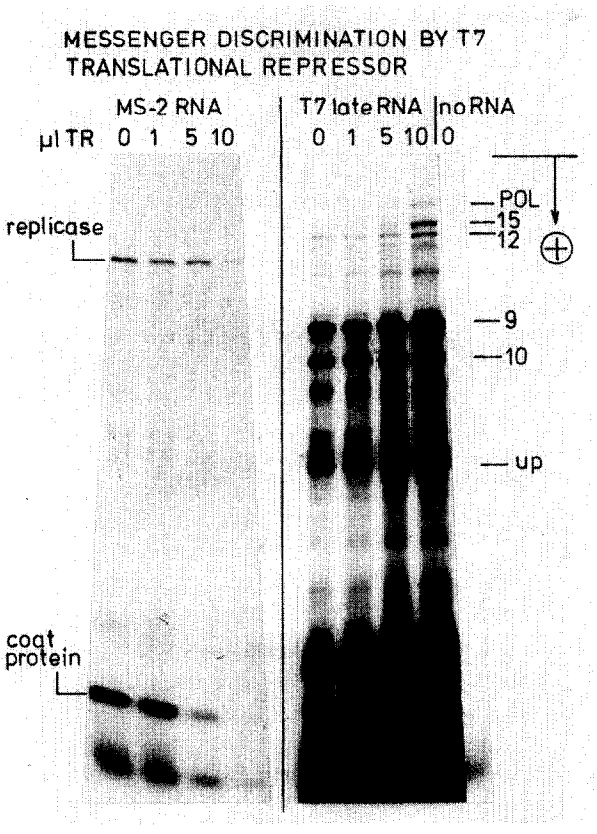

Fig.2. Messenger discrimination by the T7 translational repressor in vitro. The translational control protein was isolated from the ribosomal wash and partially purified by ammonium sulfate precipitation and DEAE cellulose chromatography (unpublished). Its action was tested by protein synthesis in vitro using MS-2 RNA and late T7 RNA as messenger. The protein synthesizing system has been described [10,41]. Protein was labeled with amino [^{14}C]-acids during in vitro synthesis, and resolved by SDS–acrylamide gel electrophoresis (10–25% acrylamide gradient). The gel was treated with 50% trichloroacetic acid for 2 h, transferred to H_2O for 1 h and activated with PPO [52]. The gel was dried. An autoradiogram is shown.

We conclude from this that:

mRNA with different recognition sites exists within the same cell, and that such signals are indeed used for regulation of gene expression on the level of translation.

2.3. *An inhibitor specific for recognition sites on mRNA*

An antibacterial agent (nitrofurantoin) helped to reveal mRNA discrimination also in non-infected bacterial cells. Whereas most antibacterial agents inhibit by a general mechanism such as inhibition of transcription of all bacterial genes, or inhibition of a ribosomal function again involving any translation, nitrofurantoin acts selectively on the expression of some genes, not of others. Nitrofurantoin interfered in *E. coli* preferentially with the expression of inducible genes such as β-galactosidase, tryptophanase or galactokinase, but did not inhibit the synthesis of most other proteins (fig.3) [41].

The preferential inhibition of gene expression by nitrofurantoin occurs at the level of translation: this is shown by experiments with preformed lac mRNA (fig.3). The translation of preformed RNA was measured as in [34]. *Lac* transcription was induced and mRNA allowed to accumulate in the presence of a tryptophan analogue. Then rifampicin was added and the synthesis of active enzyme initiated by the addition of excess tryptophan. Nitrofurantoin inhibited the translation of preformed RNA to the same extent as it inhibited the complete reaction without rifampicin (fig.3). The synthesis of a T7 enzyme, however, was distinctly less sensitive to nitrofurantoin. These results are in agreement with earlier in vitro observations [41] and experiments with fusion mutants [41].

Nitrofurantoin apparently interferes with a mechanism which discriminates between messenger RNA species within the same bacterial cell. With the ques-

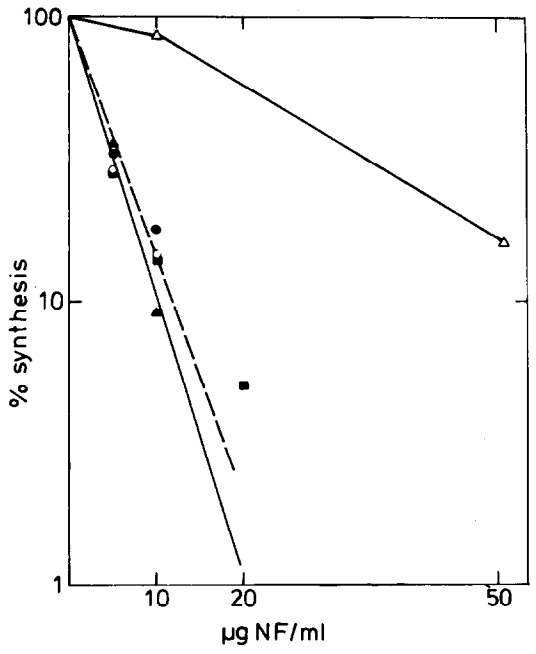

Fig.3. Specific inhibition by nitrofurantoin of translation of preformed *lac* mRNA in vivo. *E. coli* B_{S-1} were grown in M9-glucose medium until A_{600} = 0.5. Part of the culture was induced for β-galactosidase synthesis by the addition of 10^{-3} M isopropyl-thio-galactoside (IPTG) and the synthesis allowed to proceed in the absence or presence of nitrofurantoin (■). The other part of the culture was treated with 7-azatryptophan and 5-methyltryptophan at 4 min prior to IPTG [34,42]. The culture was divided into two; only one part received rifampicin at 100 µg/ml and at 5 min after IPTG (●,○), but both parts were treated with an excess of tryptophan plus increasing amounts of nitrofurantoin at 6.5 min past induction (▲). At various times thereafter, β-galactosidase activity was determined. Evaluation of plateau levels reached (●). Evaluation of initial rate of synthesis (○). The remainder of the culture was infected with T7 H280. At 1 min, amino [^{14}C]acids (25 µCi/ml) were added plus increasing doses of nitrofurantoin, and the cells collected at 15 min. Total protein was resolved by SDS–gel electrophoresis. The synthesis of the T7 M protein evaluated by densitometry is plotted here (△).

tion initially posed in mind the data demonstrate:

The existence of recognition sites on bacterial messenger RNA which are essential to the process of initiation of translation [41,42].

Nitrofurantoin may either block the initiation site specific for the mRNA species from inducible genes, or it may react with a specific control protein involved in translation of this group of mRNA.

3. Conclusions

In conclusion, all three types of experiments revealed rate-determining recognition sites on messenger RNA:

(i) The protein synthesizing systems from pro- and eukaryotic cells distinguished naked protein-free messenger RNA from either pro- or eukaryotes.

(ii) Viral and host-bacterial messenger RNA carry recognition sites which a viral protein uses for discrimination.

(iii) The inhibitor studies with nitrofuran depicted signal differences on mRNA from the same cell.

The in vitro experiments are interpreted here in all caution; since only drastic effects were evaluated, there was a strict correlation between in vivo observation and in vitro data, at least in two of the experiments, and care was taken to assure high rate of in vitro protein synthesis. The rate-determining recognition sites on mRNA and their counterpart, the ribosomal machinery or control proteins, offer two possibilities with respect to the regulation of gene expression:

1. An inbuilt program of translational rate.

2. A versatile translational control.

References

[1] Morrow, J. F., Cohen, S. N., Chang, A. C. Y., Boyer, H. W., Goodman, H. M. and Helling, R. B. (1974) Proc. Natl. Acad. Sci. USA 71, 1743–1747.
[2] Chang, A. C. Y., Lansman, R. A., Clayton, D. A. and Cohen, S. N. (1975) Cell 6, 231–244.
[3] Meagher, R. B., Tait, R. C., Betlach, M. and Boyer, H. W. (1977) Cell 10, 521–536.
[4] Maniatis, T., Gek Kee, S., Efstratiadis, A. and Kafatos, F. C. (1976) Cell 8, 163–182.
[5] Gubbins, E. J., Newlon, C. S., Kann, M. D. and Donelson, J. E. (1977) Gene 1, 185–207.
[6] Lucas-Lenard, J. and Lipmann, F. (1971) Ann. Rev. Biochem. 40, 409–448.
[7] Pestka, S. (1971) Ann. Rev. Microbiol. 25, 487–562.
[8] Klein, H. A. and Ochoa, S. (1972) J. Biol. Chem. 247, 8122–8128.

[9] Wool, I. G. and Stöffler, G. (1974) in: Ribosomes (Nomura, M., Tissières, A. and Lengyel, P. eds) pp. 417–460, Cold Spring Harbor Lab. NY.
[10] Herrlich, P. and Groner, B., unpublished.
[11] Di Girolamo, A., Di Girolamo, M., Gaetani, S. and Spadoni, M. A. (1967) Eur. J. Biochem. 1, 164.
[12] Rekosh, D. M., Lodish, H. F. and Baltimore, D. (1970) J. Mol. Biol. 54, 327–340.
[13] Siegert, W., Konings, R. N. H., Bauer, H. and Hofschneider, P. H. (1972) Proc. Natl. Acad. Sci. USA 69, 888–891.
[14] Aviv, H., Boime, I., Loyd, B. and Leder, P. (1972) Science 178, 1293–1295.
[15] Crawford, L. V. and Gesteland, R. F. (1973) J. Mol. Biol. 74, 627–634.
[16] Morrison, T. G. and Lodish, H. F. (1973) Proc. Natl. Acad. Sci. USA 70, 315–319.
[17] Schreier, M. H., Staehelin, T., Gesteland, R. F. and Spahr, P. F. (1973) J. Mol. Biol. 75, 575–578.
[18] Davies, J. W. and Kaesberg, P. (1973) J. Virol. 12, 1434–1441.
[19] Anderson, C. W., Atkins, J. F. and Dunn, J. J. (1976) Proc. Natl. Acad. Sci. USA 73, 2752–2756.
[20] Noll, M., Noll, H. and Lingrel, J. B. (1972) Proc. Natl. Acad. Sci. USA 69, 1843–1847.
[21] Clark, J. M., jr, Chang, A. C. Y., Spiegelman, S. and Reichmann, M. E. (1965) Proc. Natl. Acad. Sci. USA 54, 1193.
[22] Schwartz, J. H. (1967) J. Mol. Biol. 30, 309–327.
[22a] Legon, S., Model, P. and Robertson, H. D. (1977) Proc. Natl. Acad. Sci. USA 74, 2692–2696.
[23] Schweiger, M., Herrlich, P. and Zillig, W. (1969) Hoppe-Seyler's Z. Physiol. Chem. 350, 775–783.
[24] Harthy, M. R., Wheeler, A. and Ellis, R. J. (1975) J. Mol. Biol. 91, 67–77.
[25] Herrlich, P., Ponta, H., Richter, D., Pfennig-Yeh, M., Hirsch-Kauffmann, M. and Schweiger, M. (1977) Molecular and Cellular Biochemistry 14, 143–148.
[26] Scragg, A. H. and Thomas, D. Y. (1975) Eur. J. Biochem. 56, 183–192.
[27] Struhl, K., Cameron, J. R. and Davis, R. W. (1976) Proc. Natl. Acad. Sci. USA 73, 1471–1475.
[28] Ratzkin, B. and Carbon, J. (1977) Proc. Natl. Acad. Sci. USA 74, 487–491.
[29] Brunovskis, I. and Summers, W. C. (1972) Virology 50, 322–327.
[30] Schweiger, M. and Herrlich, P. (1974) Curr. Top. Microbiol. Immunol. 65, 59–132.
[31] Ponta, H., Rahmsdorf, H. J., Pai, S. H., Hirsch-Kauffmann, M., Herrlich, P. and Schweiger, M. (1974) Mol. Gen. Genet. 134, 281–297.
[32] Kennell, D. (1970) J. Virol. 6, 208–217.
[33] Cohen, P. S., Zetter, B. R. and Walsh, M. L. (1972) Virology 49, 808–810.
[34] Svenson, S. B. and Karlström, D. H. (1976) J. Virol. 17, 326–334.
[35] Wagner, E., Ponta, H. and Schweiger, M. (1977) Mol. Gen. Genet. 150, 21–28.
[36] Hattman, S. and Hofschneider, P. H. (1967) J. Mol. Biol. 29, 173–190.
[37] Goldman, E. and Lodish, H. F. (1971) J. Virol. 8, 417–429.
[38] Cohen, P. S. (1972) Virology 47, 780.
[39] Swebilius Singer, B. and Gold, L. (1976) J. Mol. Biol. 103, 627–646.
[40] Herrlich, P., Rahmsdorf, H. J., Pai, S. H. and Schweiger, M. (1974) Proc. Natl. Acad. Sci. USA 71, 1088–1092.
[41] Herrlich, P. and Schweiger, M. (1976) Proc. Natl. Acad. Sci. USA 73, 3386–3390.
[42] Wagner, E., Schweiger, M., Ponta, H. and Herrlich, P. (1977) FEBS Lett. 83, 337–340.
[43] Herrlich, P. and Schweiger, M. (1974) Methods Enzymol. 30, 654–669.
[44] Knöchel, W., Hendrick, D., Schröter, S. and Tiedemann, H. (1973) Hoppe-Seyler's Z. Physiol. Chem. 354, 1389–1394.
[45] Hynes, N. E. Groner, B., Sippel, A. E., Nguyen-Huu, M. C. and Schütz, G. (1977) Cell 11, 923–932.
[46] Hunter, T. R., Hunt, T., Knowland, J. and Zimmern, D. (1976) Nature 260, 759–764.
[47] Hackett, P. B., Egberts, E. and Traub, P. (1978) J. Virol. in press.
[48] Shafritz, D. A., Prichard, P. M. and Anderson, W. F. (1972) in: Protein Biosynthesis in Nonbacterial Systems (Last, J. A. and Laskin, A. J. eds) pp. 265–294, Marcel Dekker, New York.
[49] Marcu, K. and Dudock, B. (1974) Nucleic Acids Res. 1, 1385–1397.
[50] Anthony, W. M., Deonier, R. C., Lee, H.-J., Hu, S., Ohtsubo, E., Davidson, N. and Broda, P. (1974) J. Mol. Biol. 89, 647–650.
[51] Hirsch-Kauffmann, M., Schweiger, M., Herrlich, P., Ponta, H., Rahmsdorf, H. J., Pai, S. H. and Wittmann, H. G. (1975) Eur. J. Biochem. 52, 469–474.
[52] Bonner, W. M. and Laskey, R. A. (1974) European J. Biochem. 46, 83–88.