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Formylation of Initiator tRNA Methionine in Procaryotic Protein Synthesis: In Vivo Polarity in Lactose Operon Expression

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Eucaryotic and procaryotic organisms differ in two aspects of their translation machinery: polycistronic messengers are expressed as a sequence of individual proteins only in procaryotes, and the initiation of protein synthesis proceeds with an initiator tRNA which is found to be modified (formylated) in procaryotes and not in eucaryotes. In the present study, we show that formylation is required in vivo for the coordinate expression of the *Escherichia coli* lactose operon. Our experiments are consistent with a translation mechanism using dissociated ribosomes at the 5′ end of the mRNA in a reaction that is only weakly dependent on formylation at this initiation step; the ribosomes then travel along the messenger and can reinitiate after the intracistronic barrier without dissociation. This latter initiation step is strongly dependent on the level of formylation: a low level of the formyl group, obtained by the antifolic agent trimethoprim, induces a strong polarity in the expression of the lactose operon. There exist mutant strains in which this polarity is much less apparent than in the wild type. We show here that such is the case of *rpsL* mutants. Ribosomes mutated in the S12 protein (*rpsL*) are found to be much more easily dissociated than the wild type. This might explain why the expression of the lactose operon on *rpsL* strains remains coordinated when the intracellular level of formylation is decreased.

Initiation of protein synthesis in most procaryotic organisms is coupled to the general cellular metabolism by the chemical modification (formylation) of the methionine carried by initiator $tRNA_f^{Met}$. However, as we have previously reported, formylated and unformylated Met-$tRNA_f^{Met}$ bind equally well to 30S ribosomal subunits in the presence of an mRNA (18). Furthermore, both species appear to be recognized by initiation factors and, upon addition of 50S subunits, are able to form the first peptide bond. Conversely, 70S ribosomes discriminate very efficiently between Met-$tRNA_f^{Met}$ and formyl-Met-$tRNA_f^{Met}$. Taken together, these two observations appear paradoxical since it is generally admitted that initiation of protein synthesis proceeds from ribosomal subunits and that formylation of intiator tRNA is universally required for polypeptide synthesis in procaryotes (8).

From a biochemical study of the 70S couples (19), we have proposed that two different mechanisms may coexist for initiation of translation of polycistronic mRNA: The first cistron of the messenger is translated using dissociated ribosomes for initiation (this would be rather insensitive to formylation), whereas the following cistrons initiate translation with undissociated 70S ribosomes (this would stringently require formylation). In this hypothesis the formyl group would act as a positive effector on a preexisting equilibrium between two conformations of 70S ribosomes: a major one, inactive and unable to bind fMet-$tRNA_f^{Met}$, and a minor one, active in initiation and binding fMet-$tRNA_f^{Met}$. Both forms are presumed to bind equally well the unformylated tRNA, whereas only the active form binds the formylated species, shifting the equilibrium toward activity.

This hypothesis suggests a general function for the regulatory action of the formyl group at the level of translation: since it would act as an antidissociating factor in polycistronic messenger translation, formylation would protect against premature termination of transcription and thus act as an antipolar effector. We have tested this in vivo by measuring different enzyme activities encoded by the same polycistronic messenger, namely the first and the last enzyme of the lactose operon of *Escherichia*

coli. In this article we present evidence showing that the expression of the lactose operon becomes uncoordinated when bacteria are grown in the presence of a low level of the antifolic agent trimethoprim.

In addition, it has been observed (3) that certain *rpsL* (formerly *strA*) alleles (mutants with altered S12 protein of the 30S ribosomal subunit) can overcome the inhibition of growth by antifolic agents in the presence of the one-carbon metabolites. This suggests that these mutants are much less sensitive to a defect in formylation compared to the wild type. We have therefore compared the expression of the lactose operon in the wild type and in such *rpsL* mutants.

Since it has been shown (9) that the ribosomes found in *E. coli* wild-type cells growing in the presence of trimethoprim are mostly in the dissociated form, we have compared the in vitro dissociation pattern of ribosomes from wild-type bacteria with the pattern of the isogenic streptomycin-resistant ribosomes by using a light-scattering technique. Our experiments show that ribosomes from the *rpsL* strain dissociate more easily than those from the wild type; this might relate the increase in resistance to trimethoprim (in the presence of excess one-carbon metabolites) of the *rpsL* mutants to the easier dissociation of their ribosomes. This seems to be consistent with the mechanism that we propose for the translation of polycistronic mRNA in *E. coli*.

MATERIALS AND METHODS

Ribosomes: preparation, biochemical, and physical assays. Ribosomes were prepared as described by Dondon et al. (5) and washed once at high (1.5 M) and twice at low (60 mM) ammonium chloride concentrations and further purified by sucrose gradient centrifugation in the presence of 5 mM $MgCl_2$. Their association was measured by the light-scattering technique, as described by Godefroy-Colburn et al. (7). The measuring cuvette contained (in 2 ml) 2.5 to 7 absorbance at 260 nm (A_{260}) units of 70S ribosomal particles 10 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.4); 50, 150, or 450 mM K^+, and various magnesium concentrations (from 1 to 60 mM, as indicated). The assay solutions were made up individually at all measured points since sequential addition of magnesium chloride does not lead to full association. These measurements showed that more than 95% of the wild-type ribosomes were associated at 4 mM Mg^{2+} at low ionic strength (50 mM potassium chloride, 10 mM Tris-hydrochloride [pH 7.4]) (see below and Table 2 for other ionic conditions). This corresponds to the type A couples described by Debey et al. (4).

The assays for binding formylated and unformylated tRNA's, as well as the formation of the first peptidyl link, were performed as described by Petersen et al. (18). Assay tubes contained in 50 μl: 15 pmol of 70S ribosomes; 0.11 A_{260} unit of poly(A, U, G); 1 mM GTP; 50 mM Tris-hydrochloride (pH 7.4); 5 mM magnesium acetate; 50 mM ammonium chloride; 4 pmol fMet- or Met-$tRNA_f^{Met}$ (4,100 cpm/pmol); and, where indicated, 52 μg of crude initiation factors. Samples were passed through a membrane filter (Millipore Corp., Bedford, Mass.) after 30 min of incubation at 37°C, and for the puromycin-treated reactions, they were extracted with ethylacetate after 5 min of additional incubation with puromycin (0.5 mg/ml).

Bacterial strains and culture media. The ribosomes which were used for most of the in vitro experiments were extracted from strain MRE 600, which is a ribonuclease I^- mutant of *E. coli* C; to have a comparison below with this well-known bacterium, we isolated a spontaneous mutant MRE 600 which is resistant to 200 μg of dihydrostreptomycin per ml. This mutant can also grow on a synthetic medium supplemented with the one-carbon metabolites (serine, methionine, glycine, thymine, adenine, and pantothenate) in the presence of 100 μg of the antifolic agent trimethoprim (3) per ml. Ribosomes of this mutant were compared with those of the wild type.

The K-12 strains used throughout this work were CP 78 F^- *thr leu his arg thi rpsL*$^+$ and CP 781aF^- *thr leu his arg thy rpsL* isolated by M. Springer as a spontaneous streptomycin-resistant mutant. In some experiments we used strains 2001c F^- *thr leu rpsL* (Institut Pasteur collection). Strain 2177 (*lacZ2177 trp rpL*/F′ *lacZ2177*), carrying an operator proximal amber mutation in the *Z* gene and exhibiting about 50% polarity (25), was kindly provided by D. Zipser. The episome carrying the 2177 mutation was transferred into strain 2000 X 74 (Institut Pasteur collection), which carries a deletion of the entire *lac* region. As a control, an episome with a wild-type *lac* operon was transferred in the same F^- strain.

The growth media were either the rich broth LB or synthetic medium M63 (14) supplemented with the required amino acids (50 μg/ml), thiamine (5 μg/ml), and either glucose or glycerol (0.4%) as a carbon source.

In vivo induction. To exponential cultures, different concentrations of protein synthesis inhibitors and an inducer of the *lac* operon were added concomitantly. After 1.5 generations of growth at 37°C (representing a mass increase of about 100 to 150 μg [dry weight] of bacteria per ml), the amounts of β-galactosidase and thiogalactoside transacetylase were determined. The results are expressed in units of enzyme per milligram (dry weight) of increase of bacteria.

Enzymatic assays. Enzymes were assayed in toluene-treated bacterial suspensions (to 2 ml of bacterial suspension, 50 μl of toluene and 50 μl of 1% sodium deoxycholate were added, and the mixtures were agitated for 30 min at 37°C). β-Galactosidase was assayed by the method of Pardee et al. (17). One unit is the amount of enzyme that hydrolyzes 1 μmol of *O*-nitrophenyl-β-D-galactoside per min at 28°C. Thiogalactoside transacetylase was assayed as described by Leive and Kollin (12) with slight modifications. One unit is the amount of enzyme which produces 1 μmol of acetyl-isopropyl-β-D-thiogalactoside per min at 28°C.

Chemicals. The protein synthesis inhibitors were chloramphenicol (0.1 to 5 μg/ml) or kasugamycin (5 to

150 μg/ml; gift from Bristol Laboratories, Syracuse, N.Y.). Trimethoprim was from Calbiochem, La Jolla, Calif. L-[*methyl*-^{3}H]methionine (8.2 Ci/mmol) was purchased from Amersham Little Chalfont, Buckinghamshire, England and was used without further purification.

Isopropyl-β-D-thiogalactoside (IPTG), *O*-nitrophenyl-β-D-galactoside, vitamins, and amino acids (all in the L-form) were purchased from Sigma Chemical Co., St. Louis, Mo.

RESULTS

70S ribosomes from wild type and *rpsL* mutants behave similarly in vitro. To assay for the influence of mutational modifications of the S12 protein (yielding streptomycin-resistant ribosomes), we measured the binding of initiator tRNA and formation of the first peptidyl link with streptomycin-resistant 70S couples (isolated from an MRE 600 Str^r strain). No significant difference was found either at the binding step or during the formation of the first peptidyl link (Table 1). Using ribosomes from wild-type MRE 600 and a streptomycin-resistant mutant, we found, in agreement with previous results (18), that fMet-tRNA$_f^{Met}$ binding is stimulated whereas unformylated Met-tRNA$_f^{Met}$ binding is inhibited by initiation factors. Since predissociated ribosomes are able to initiate peptide synthesis in the absence of formylation (18; data not shown), care was taken to be sure that our ribosomes were fully associated; this was obtained by using a low amount of monovalent cations (50 mM) (Table 2).

In addition, we checked that trimethoprim in high concentrations (100-fold higher than that known to inhibit bacterial growth) does not interfere with either binding of charged tRNA or formation of the first peptidyl link (Table 1).

Light scattering by wild-type and streptomycin-resistant ribosomes. To obtain further information concerning a possible difference between wild-type and streptomycin-resistant ribosomes, we compared their dissociation pattern under different conditions. We chose the light-scattering method, which does not interfere with the experimental system and can easily be monitored at various temperatures and ionic strengths.

Table 2 shows the magnesium concentration at a constant temperature and ionic strength at which half of the ribosomes from different *E. coli* strains are in associated form. The numbers were computed from association curves, such as shown in Fig. 1, where the whole range of Mg^{2+} concentrations was explored. A much higher magnesium concentration was needed to obtain half association of the streptomycin-resistant ribosomes than for the wild-type ones. (Table 2). The most extreme case seems to appear in strain 2001c where, at 450 mM KCl, no significant association can be obtained at magnesium concentration as high as 60 mM.

Similar experiments were performed by using conditions considered to be very close to those usually encountered in the *E. coli* cytoplasm in vivo (37°C, 150 mM K^+). The results of those experiments are shown in Fig. 1, where we compared two isogenic strains CP78 (*rpsL*$^+$) and CP781a (*rpsL*). Here, too, we found that ribo-

TABLE 2. *Magnesium concentration required for 50% association of ribosomal subunits at 24°C*

Ribosomal source	Mg_{50}^{2+} (mM)[a]	
	50 mM KCl	450 mM KCl
MRE 600 (*rpsL*$^+$)	3.5	6
MRE 600 (*rpsL*)	5	12
2001c (*rpsL*)	10	>50
CP78 (*rpsL*$^+$)	3	10
CP781a (*rpsL*)	5.5	17.5

[a] Mg_{50}^{2+} value at high ionic strength is extremely sensitive to the type of ribosomes; the values given here are the lowest obtained and correspond to the type A ribosomes described by Debey et al. (4).

TABLE 1. *Binding of Met- and fMet-tRNA$_f^{Met}$ to ribosomes from streptomycin-sensitive and -resistant strains of MRE 600 and puromycin reactivity of the bound fMet-tRNA*

Ribosomal source	Met-tRNA$_f^{Met}$	fMet-tRNA$_f^{Met}$	IF[a]	Radioactivity bound (cpm) to:	
				Ribosomes	Puromycin
MRE 600 (*rpsL*$^+$)	+		−	6,600	
	+		+	730	
		+	−	1,170	1,080
		+	+	13,700	11,000
Plus trimethoprim (100 μg/ml)		+	+	12,700	11,600
MRE 600 (*rpsL*)	+		−	8,100	
	+		+	440	
		+	−	600	510
		+	+	8,400	9,700

[a] IF, Crude mixture of initiation factors.

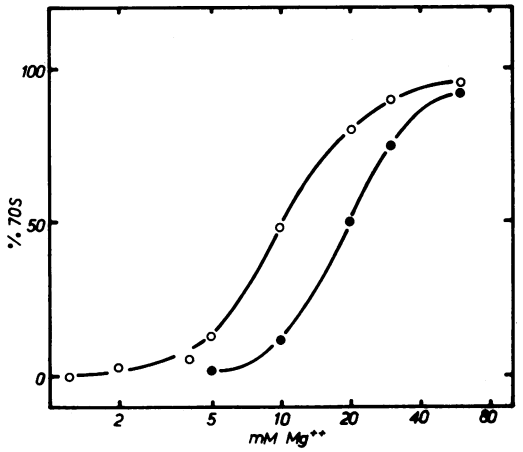

FIG. 1. *Light-scattering measurement of the association of ribosomal subunits as a function of magnesium concentration at 150 mM potassium chloride and at 37°C. Symbols:* ○, *Ribosomes from strain CP 78 (rpsL⁺);* ●, *ribosomes from strain CP 781a (rpsL).*

somes isolated from the streptomycin-resistant mutant are more dissociated than those from the wild type at magnesium concentrations varying from 5 to 50 mM. The concentration necessary for 50% association (Mg^{2+}_{50}) is 10 mM for the wild-type and 20 mM for the *rpsL* mutant ribosomes. Apart from this significant difference, both dissociation curves exhibit the same type A ribosome structure (4), which is somewhat similar to the cooperative behavior of "tight" ribosomes (15).

Thus, all the *rpsL* mutants that we have so far tested exhibit a dissociation pattern clearly distinct from that of the wild type. The association curves appear to be similar, but the optimum in magnesium concentration necessary to obtain half-associated particles is always higher for the streptomycin-resistant ribosomes than for those of the wild type. These results suggest that in vivo (150 to 250 mM K^+, 7 mM Mg^{2+}) (20) the proportion of dissociated ribosomes is much higher in the streptomycin-resistant mutants than in the wild type.

Dissociation of ribosomes has usually been studied by using ultracentrifugation in sucrose gradients at 4°C. With this procedure, no significant difference was found between wild-type and streptomycin-resistant ribosomes (data not shown). Unfortunately, this simple experimental procedure has three drawbacks: it introduces a very high hydrodynamic pressure (which might interfere with dissociation); the chosen temperature is very far from the temperature generally used for growth of *E. coli* (20 to 45°C); and, finally, sucrose itself masks the normal dissociation pattern. Moreover, it has been shown (6) that, at 4°C, the initiation step of protein synthesis is stopped while elongation can proceed. It therefore seems necessary that the experimental conditions should be as close as possible to the in vivo ones. Indeed, using the light-scattering method at 5°C, we found no difference between the dissociation curves of ribosomes from strains CP78 and CP781a.

These results raise the question whether this in vitro-observed dissociation equilibrium has a meaningful function in vivo. In this respect, the study of translation of a polycistronic messenger seems a suitable system: assuming that 70S ribosomes travel along the messengers without dissociating at the intercistronic barrier, formylation would be stringently required for the translation of all cistrons except the first one. This requirement would, however, be overcome in strains having a significant amount of free ribosomal subunits, which might be the case of streptomycin-resistant mutants.

In vivo expression of the lactose operon in the presence of an inhibitor of formylation. It is well established that the expression of the three enzymes encoded by the lactose operon are coordinatedly expressed under various conditions (11). In a first set of experiments, we lowered the extent of formylation by adding low concentrations of trimethoprim (3, 9); these concentrations (0.05 to 1.5 μg/ml) moderately inhibit the growth of bacteria. The differential rates of β-galactosidase and thiogalactoside transacetylase synthesis (first and last enzymes of the lactose operon) were measured after induction during 1.5 generations of growth. Table 3 shows the results of such experiments where the two isogenic strains (except for *rpsL*) that we used for the in vitro ribosomal dissociation studies were compared. In both strains, in the presence of increasing concentrations of trimethoprim, the generation time of bacteria was increased and the differential rate of β-galactosid-

TABLE 3. *Effect of trimethoprim (TRM) on the differential rate of β-galactosidase (Gz) and thiogalactoside transacetylase (Ac) synthesis*

Strain	TRM[a] (μg/ml)	Generation time[b] (min)	Gz (U/mg)	Ac (U/mg)	Ratio (Gz/Ac)
CP78		70	4,460	108	1
	0.75	170	3,000	48	1.5
	1.5	225	2,700	27	2.4
CP781a		70	3,600	83	1
	0.75	150	2,770	56	1.15
	1.5	220	2,330	45	1.2

[a] Added concomitantly with inducer (IPTG 1 mM).
[b] Bacteria were grown in M63 glucose medium.

ase synthesis was reduced. The most significant feature of the experiment is that in the *rpsL*$^+$ strain (wild-type ribosomes) the expression of the operon becomes markedly uncoordinated, whereas in the *rpsL* mutants this uncoordination is only weak.

Trimethoprim is known to interfere with several biosynthetic pathways (e.g., 2), especially that of methionine synthesis. Thus, overall protein synthesis is reduced upon addition of this antimetabolite. Therefore, we used different inhibitors of protein synthesis as controls. Chloramphenicol was used to assay for restriction of overall protein synthesis and kasugamycin, a known inhibitor of initiation of polypeptide synthesis (16), was used as a control for a direct inhibition at this step. Finally, we added methionine to the growth medium in an experiment to check whether our observation was not due to a starvation effect (10). No loss of coordination was observed in the presence of chloramphenicol or kasugamycin under conditions where the growth rates are similar to those observed in the presence of trimethoprim (Table 4). Conversely, methionine addition does not reverse the effect of trimethoprim. Therefore, it appears that the effect of this antibiotic agent is specific for the uncoordination (polar effect) observed.

TABLE 4. *Effect of different inhibitors on the coordinate synthesis of β-galactosidase (Gz) and thiogalactoside transacetylase (Ac)*

Strain	Addition[a]	(μg/ml)	Generation time (min)	Ratio (Gz/Ac)
CP78			80	1
	Chloramphenicol	1.5	150	1.06
	Chloramphenicol	2	200	1.14
	Kasugamycin	40	160	0.92
	Trimethoprim	1.5	200	2.02
	Trimethoprim	1.5		
	Methionine	50	200	2.1
CP781a			110	1
	Chloramphenicol	1.5	170	1.1
	Chloramphenicol	2	240	1.2
	Kasugamycin	20	220	0.96
	Trimethoprim	1.2	300	0.98

[a] Added concomitantly with inducer (IPTG 1 mM).
[b] Bacteria were grown in M63 glucose-medium.

The experiments that we have just described deal with protein synthesis occuring at the normal initiation site of a polycistronic operon. It is known that, in some cases, protein synthesis can be initiated at secondary sites located inside a coding cistron. Investigating the relationship between polarity and translational punctuation, Zipser (23) has been led to study the polar effects of termination codons introduced in the *Z* gene. As expected, the general trend is that polarity increases as the mutation is situated closer to the operator site. However, mutations occurring in two well-defined regions do not fit this general picture; they exhibit much less polarity than their position would suggest. To account for this finding, Zipser postulated that a potential initiation site was located soon after the termination codons introduced in these regions. In agreement with this proposition, our own hypothesis would suggest that the 70S ribosomes running along the *Z* gene could be triggered for a proper reinitiation, provided that soon after the terminator mutation an initiation codon appears and that formylated initiator tRNA be present. Therefore, one would expect that inhibition of the formylation potential of the cell should result in restoration of polarity.

Since there is no simple internal standard of the expression of the *lac* genes when β-galactosidase is not functional, we designed the following experiment, which is meant to measure the efficiency of the transacetylase expression. Iso-

TABLE 5. *Restoration of polarity in a weakly polar operator proximal lacZ mutant in the presence of trimethoprim*

Inhibitor[a]	(μg/ml)	200X74/F'*lac*$^+$				2000X74/F'*lacZ2177*			
		Generation time[b] (min)	GZ[c] (U/mg)	Ac[d] (U/mg)	Ratio GZ/Ac	Generation time[b] (min)	Ac (U/mg)	Ac (% wild type)	% Polarity
		45	7,340	180	1	45	58	32	68
Trimethoprim	1.0	110	3,750	78	1.2	100	7	9	91
	1.5	200	3,040	33	2.3	180	5.5	17	83
Chloramphenicol	1.75	90	1,570	44	0.9	95	15	34	66
	3	180	545	14	1	180	6	42	58
Kasugamycin	90	70	1,160	38	0.8	70	14	37	63
	140	120	800	18	1.1	100	7	39	61

[a] Added concomitantly with inducer (IPTG 1 mM).
[b] Bacteria were grown in M63 glucose medium.
[c] GZ, β-Galactosidase.
[d] Ac, Thiogalactoside transacetylase.

genic strains harboring either a wild-type F'*lac* episome or an F'*lacZ2177* episome (carrying an operator proximal amber mutation) were compared. The results (Table 5) show that: (i) the 2000X74/F'*lac*$^+$ control strain exhibits the same uncoordination effect in the presence of trimethoprim as strain CP78; (ii) trimethoprim induces a strong polar effect on the differential rate of transacetylase synthesis in the strain carrying the amber mutation. Indeed, by calculating the relative amounts of transacetylase produced by the mutant with respect to the control (Table 5, last column), it can be seen that in the presence of trimethoprim the polarity was greatly increased, whereas neither chloramphenicol nor kasugamycin had similar effects.

DISCUSSION

One of the striking differences between procaryotic and eucaryotic protein synthesis is that, whereas procaryotes initiate translation at several sites on polycistronic messengers, eucaryotic mRNA's are either monocistronic or translated as a single polypeptide, subsequently cleaved into individual proteins, (e.g., viruses). Polarity in the expression of polycistronic operons is thus a salient feature of procaryotic gene expression. It can be controlled at several levels: early termination of transcription, endonucleolytic cleavage of the mRNA covered by polysomes, or differential initiation of translation.

The presently available experimental data do not allow us to distinguish between these three hypotheses. However, the observation that the expression of the first cistron is favored compared to the last one would rather favor the first hypothesis over the others.

In vitro assay of initiation of polypeptide synthesis shows that there is a marked difference between 70S associated and (30S + 50S) dissociated ribosomes in their requirement for formylation. On the other hand, we showed that the ribosomes isolated from streptomycin-resistant mutants are significantly dissociated, compared to those isolated from the wild type, under temperature and ionic strength conditions comparable to those found in vivo. A decrease in formylation results in a polar effect in strains (*rpsL*$^+$) in which ribosomes do not easily dissociate. This is consistent with the hypothesis of a preexisting equilibrium in the 70S ribosomal conformation, shifted towards activity (for initiation of protein synthesis) by fMet-tRNA at the intercistronic barrier. This involvement of formylation is further emphasized by the lack of polar influence of other inhibitors of protein synthesis acting either at the elongation or the initiation steps.

Our in vivo experiments appear to strongly suggest that the translation of polycistronic messengers proceeds with a significant proportion of 70S ribosomes running along the mRNA without dissociating at intercistronic barriers, provided that there is no limitation in the availability of formylated initiator tRNA. In addition, our experiments are consistent with the interpretation given by Zipser (23) of his results showing a lack of expected polarity when termination codons are introduced at several loci in the *lacZ* gene. One can indeed hypothesize that a start codon situated in the vicinity of these termination loci triggers a correct reinitiation without dissociation of the 70S ribosomes running along the message, at least when enough fMet-tRNA$_f^{Met}$ is provided. These results might appear to conflict with the results obtained by Webster and Zinder (21), who interpret their data as showing dissociation of 70S ribosomes at a termination signal. In fact, using purely in vitro experimental conditions, they have shown that at least 30% of intact 70S ribosomes remained attached to the mRNA after a termination codon. Since their experimental conditions imply a long delay and a large dilution factor, it is difficult to extrapolate their results to what actually happens in vivo with respect to dissociation of the ribosomes. Zalkin et al. (22) have also studied in vitro the expression of various enzymes of the biosynthetic *trp* operon. Using kasugamycin as a specific inhibitor of initiation of translation, they concluded that there is obligatory dissociation of the ribosomes at the intercistronic barriers. However, their interpretation is grounded on the assumption that kasugamycin specifically inhibits 30S-mediated initiation. In fact, although located on the 30S subunit, the site of action of kasugamycin results in inhibition of protein synthesis initiation both on dissociated ribosomes and 70S ribosomes (16). Therefore, the results of Zalkin et al., rather than being contradictory, are consistent with our results. It has been shown a long time ago (1) that the expression of different enzymes of the *his* operon was sensitive to one-carbon pool metabolism. The various enzyme activities were expressed in an uncoordinated (sequential) fashion upon limitation of 10-formyl-tetrahydrofolate, whereas they were found to be coordinated when an excess one-carbon supply was provided to the growing cells. At that time this result was difficult to explain in a convincing way. We think that our present experiments are strikingly consistent with these former studies. Moreover, the fact that both a catabolic operon and a biosynthetic one appear to respond in a similar way to a variation in the one-carbon pool metabolism

would suggest that our model might be quite general. Thus, the folic acid metabolism might exert an important regulatory control mechanism in the expression of polycistronic messengers.

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