Vol. 84, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 16, 1978 Pages 769-776

MODULATION OF THE LACTOSE OPERON mRNA TURNOVER BY INHIBITORS OF DIHYDROFOLATE REDUCTASE

Evelyne JOSEPH, Antoine DANCHIN (*) and Agnes ULLMANN

Département de Biochimie et Génétique Microbienne, Institut Pasteur
75015 PARIS (France) and

(*) Institut de Biologie Physico-Chimique 75005 PARIS (France)

Received August 31, 1978

SUMMARY. The decay rate of lactose messenger RNA is strongly decreased when inhibitors of dihydrofolate reductase are added to the growth medium. The effect is not immediate : it appears after one generation of growth in the presence of the inhibitor. Additional genetic evidence suggests that the level of one carbon-tetrahydrofolate might be involved in the processing of mRNA.

In a recent study, we have shown that the coordinate expression of the lactose operon of *E.coli* is dependent upon the availability of formylated initiator tRNA. If the level of formyl groups is lowered by the antifolic agent trimethoprim a strong polarity appears : the expression of the Lactose operon becomes uncoordinated (1).

In order to further investigate the role of folic acid metabolism in the expression of polycistronic messengers we studied the relationship between translation and messenger RNA decay in the lactose operon. In the present paper we show that the addition of trimethoprim or aminopterin to bacteria results in an increase in the differential rate of enzyme synthesis which can be accounted for by a slower decay of biologically active mRNA. This and further evidence suggest that the alteration in the available pools of one carbon tetrahydrofolate substrates might be responsible for this phenomenon.

MATERIALS AND METHODS

Strains and Media. The following *E.coli* strains were used : CP78 *(thr leu his arg)* ; CP78-T_1 *(thr leu his arg fol)* spontaneous mutant resistant to 2 µg/ml trimethoprim ; and the isogenic pairs : G16L *glyA*, G16L$^+$ *(glyA$^+$* ; P_1 transductant of G16L) and FA221 *hisC*, FA261 *(hisC$^+$* ; P_1 transductant of FA221) constructed for this work.

0006-291X/78/0843-0769$01.00/0

Bacteria were grown at 37°C (except G16 pairs grown at 33°C) in 63 minimal medium supplemented with the required amino acids (50 µg/ml), thiamine (5 µg/ml) and glycerol, glucose (0.4 %) or succinate as a carbon source.

Enzymatic assays. β-galactosidase was assayed according to Pardee *et al.* (2) and thiogalactoside transacetylase according to Leive and Kollin (3), with slight modifications, in bacterial suspensions permeabilized with toluene and deoxycholate. Enzyme units are expressed in nmoles of substrate converted per min at 28°C.

Lac mRNA decay measurements were performed according to Kepes (4). Exponential cultures were induced with isopropyl-β-D-thiogalactoside, 60 µM. After 90 seconds of induction rifampicin was added (150 µg/ml). The time course of the expression of mRNA initiated during the pulse induction was followed by sampling on chloramphenicol (50 µg/ml) at short intervals and measuring β-galactosidase and thiogalactoside transacetylase activities.

RESULTS

The addition of low concentrations of trimethoprim (0.5-1.5 µg/ml) to bacterial cultures lowers the extent of formylation of the aminoacylated initiator tRNA (5, 6). The kinetics of induction of the lactose operon enzymes of *E.coli* under these conditions were investigated.

It is well established that upon addition of inducer the differential rate of enzyme synthesis is constant and maximal from the start (7). If trimethoprim is added concomitantly with the inducer, the increase of enzyme synthesis as a function of bacterial mass increase exhibits a biphasic pattern. This is illustrated in Figure 1 : after one generation of growth in the presence of trimethoprim, the rates of both β-galactosidase and transacetylase synthesis are markedly increased. Initially, and for about one generation, the differential rate of enzyme synthesis is somewhat inhibited. The extent of inhibition depends upon the nature of the carbon source : in the presence of glucose or succinate the inhibition is more pronounced than in the presence of glycerol or lactate. Figure 2 shows the effect of trimethoprim on the rate of enzyme synthesis of a culture grown in the presence of glucose.

Aminopterin, another inhibitor of dihydrofolate reductase, shows similar effects to that observed with trimethoprim, whereas in the presence of other inhibitors of protein synthesis affecting the initiation (kasugamycin) or elongation (chloramphenicol) of polypeptide synthesis, the differential rate of enzyme synthesis is permanently inhibited (Fig. 3).

The finding that the expression of the lactose operon is significantly increased in the presence of inhibitors of dihydrofolate reductase

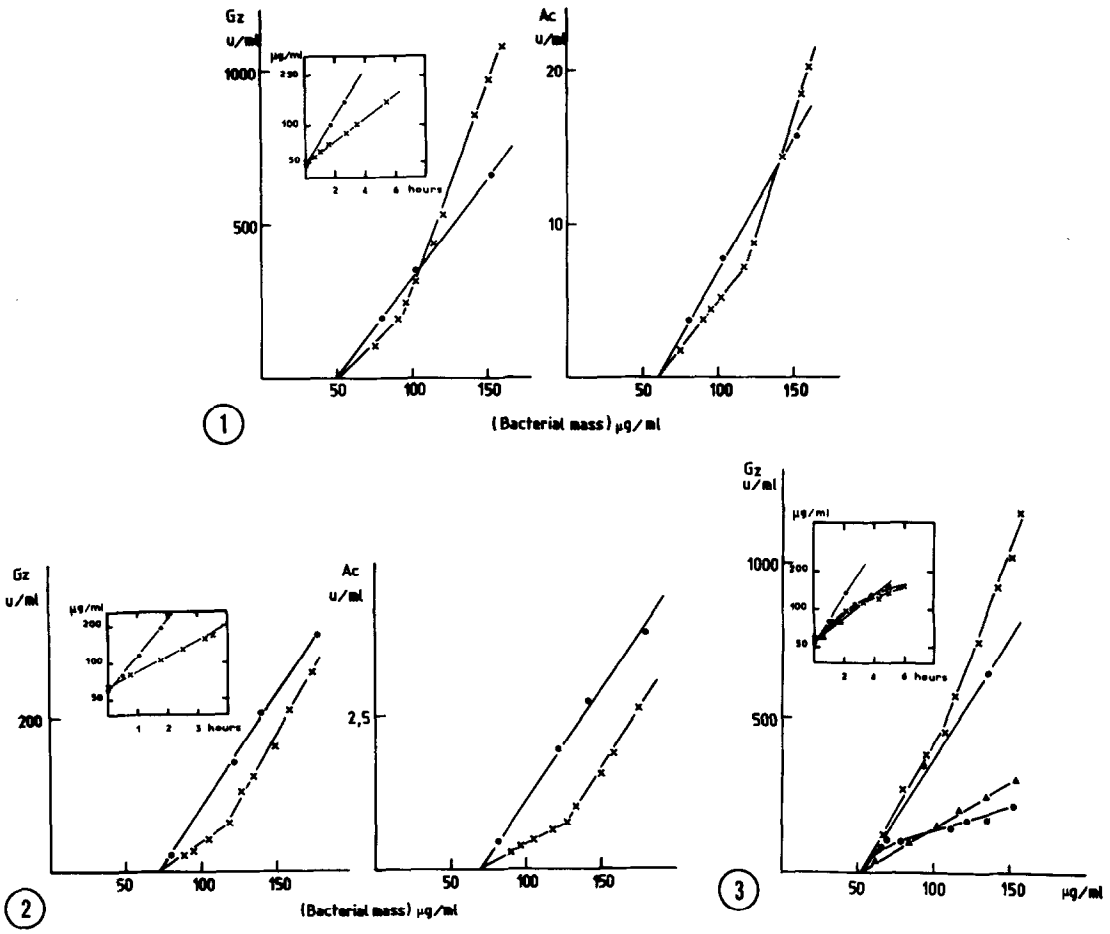

Fig. 1 *Effect of trimethoprim on the differential rate of enzyme synthesis : cultures grown in glycerol.*

To exponential cultures of strain CP78, trimethoprim (1 µg/ml) and IPTG (1 mM) were added concomitantly. At different time intervals the amounts of β-galactosidase (GZ) and transacetylase (Ac) were determined. Units of enzyme are plotted as a function of bacterial dry weight. ●——● without trimethoprim, x——x with trimethoprim. The growth curves of the cultures are shown in the insert.

Fig. 2 *Effect of trimethoprim on the differential rate of enzyme synthesis : cultures grown in glucose.*

Experimental conditions are similar as described in Fig. 1.

Fig. 3 *Effect of different inhibitors on the differential rate of β-galactosidase synthesis.*

Experimental conditions are as described in Fig. 1. To glycerol-grown cultures the following inhibitors were added concomitantly with inducer : aminopterin 20 µg/ml (x——x), chloramphenicol 2 µg/ml (▲——▲), kasugamycin 120 µg/ml (o——o). Without inhibitor : ●——●.

can be accounted for by several mechanisms :

a) enhancement of initiation of protein synthesis ;

b) increase in the frequency of transcription initiation of the lactose operon ;

c) slower degradation of the lac mRNA.

Since trimethoprim exerts a severe growth inhibition,it seems unlikely that the amount of lac mRNA or its rate of translation increase under these conditions. Moreover, a decrease in the level of formylation of aminoacylated initiator tRNA would slow down the initiation of protein synthesis rather than enhance it.

To investigate this, measurements of lac mRNA turnover were undertaken. The decay rate of biologically active lac mRNA was determined at different times after the addition of trimethoprim to the growth medium. Such an experiment is shown in Figure 4. Forty minutes after the addition of trimethoprim, the amount of coding capacity of the preformed mRNA was similar to that of the control. However after 2.5 hours, whereas the amount of coding capacity was still practically unchanged, the half lives of both β-galactosidase and transacetylase messengers were significantly increased (by approximately a factor of 2). After several hours of growth in the presence of trimethoprim, the amount of coding capacity was lowered (interpreted as a consequence of the alteration of cellular metabolism) but its half life still remained increased. It is noteworthy (see Fig. 1) that the rate of enzyme synthesis starts to increase after one generation of growth (about 3 hours) in the presence of trimethoprim in exact parallel with the slow down of mRNA decay. We tentatively interpret the increase of enzyme synthesis as being a result of the decreased decay rate of the corresponding mRNA.

Table I shows that the turnover rate of mRNA remains stable under various conditions of protein synthesis inhibition, and it decreases ($t_{1/2}$ increases) only when cells are grown in the presence of an inhibitor of dihydrofolate reductase. Other alterations in the available pools of tetrahydrofolate derivatives were investigated. For this purpose we used strains carrying different mutations :

a) affecting enzymes leading to the production of tetrahydrofolate (*fol* mutants, with altered dihydrofolate reductase regulation and/or activity)

b) exhibiting low serine transhydroxymethylase activity (*glyA* mutants) thus affected in transferring one-carbon residues to tetrahydrofolate

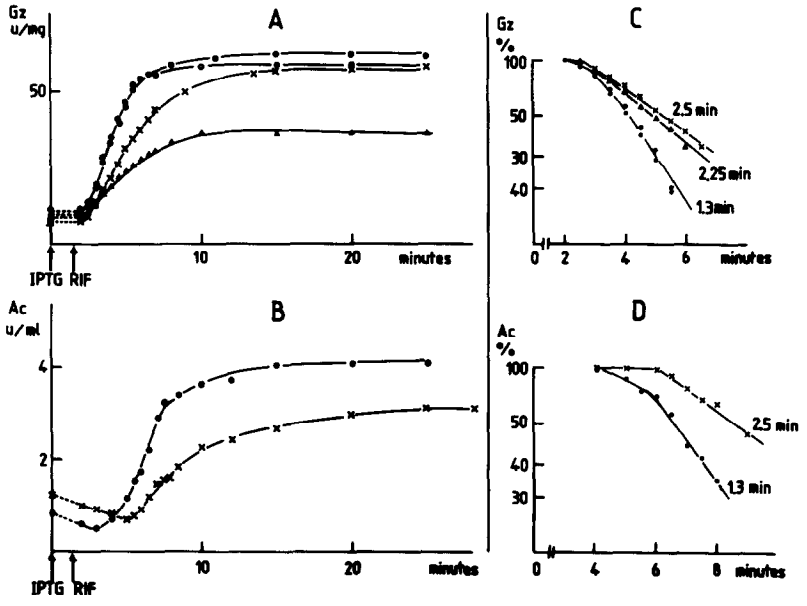

Fig. 4 *Effect of trimethoprim on enzyme synthesis and on loss of enzyme synthesizing capacity after a 90 sec induction.*

Trimethoprim (1 µg/ml) was added to exponential cultures of strain CP78. At different time intervals (40 min, 2.5 hours and 3.5 hours after addition of trimethoprim) samples were withdrawn for pulse induction. The procedure is given in Materials and Methods.

A and B : Time course of β-galactosidase (GZ) and transacetylase (Ac) synthesis. The decreasing rates of enzyme synthesis reflect the decay of specific messenger RNA molecules.

C and D : Decrease of enzyme synthesizing capacity determined from curves A and B. At any given time the residual synthesizing capacity is the difference between the actual amount of enzyme and the potential maximum amount given by the asymptotes of the curves. From the semi-logarithmic plot of these differences the half-lives of mRNA's (indicated by numbers) were determined.

●——● without trimethoprim ;
O——O 40 min after addition of trimethoprim ;
X——X 2.5 hours after addition of trimethoprim ;
▲——▲ 3.5 hours after addition of trimethoprim.

(See fig. 1 for bacterial growth and fully induced rates of enzyme synthesis).

c) with increased turnover of one-carbon tetrahydrofolate substrates (*hisC* mutants, overproducing the purine precursor, 5-aminoimidazole-4-carboxamide ribotide) which, combining with 10-formyl-tetrahydrofolate, limits the amount of the latter in the cell (8)

It can be seen in Table I that *fol*, *glyA* and *hisC* mutants exhibit a decreased decay rate of the lac mRNA as compared to the parental strains.

TABLE I

Effect of inhibitors and specific mutations on the rate of decay of β-galactosidase-specific mRNA

Strains	*Carbon source*	*Inhibitor*	*Relative half-life*
CP78	Glycerol	-	1.0
CP78	"	Chloramphenicol	1.0
CP78	"	Kasugamycin	1.0
CP78	"	Aminopterin	1.7
CP78	Glucose	Trimethoprim	1.8
CP78	Succinate	"	1.8
CP78-T_1 *(fol)*	Glycerol	-	1.9
G16L$^+$ *(glyA$^+$)*	"	-	1.0
G16L *(glyA)*	"	-	1.5
FA261 *(hisC$^+$)*	"	-	1.0
FA221 *(hisC)*	"	-	1.6

Experimental conditions and inhibitor concentrations are as described in Figs 1-4. The half-life of the control was normalized to 1.0. The actual half-life is 1.1-1.8 min depending on the strain and growth conditions.

It is of interest to note that the differential rate of β-galactosidase synthesis is 25-40 % higher in the mutants than in the parental strains (data not shown).

DISCUSSION

It is well established that, in bacteria, messenger RNA is highly unstable. The turnover of various mRNA species appears to be somewhat different ; moreover decay rates can be modulated under specific conditions of protein synthesis inhibition (9, 10). Although a large number of studies have been undertaken, no specific mechanism leading to mRNA degradation has so far been found. It has been suspected for some time that RNase III might play a role in this degradation (11), but more recent results have shown that if this enzyme has a function related to mRNA

degradation, it can only be a minor one (12, 13). Imamoto and Schlessinger showed (14) that endonucleolytic cleavage appears to be irrelevant to the basic mechanism of decay, and proposed that the fate of mRNA is determined by *processes* that introduce the 5' to 3' sense of degradation. Our present results, showing that the availability of one-carbon tetrahydrofolate substrates might modulate the functional decay of lac mRNA, could have some bearings on such hypothetical processes. We would like to suggest, as a working hypothesis, that a one-carbon tetrahydrofolate substrate acts either as an effector of the degradative process or as a methylating substrate whose target could be either the 5' end of the mRNA or some of the initiating ribosomes themselves. This would act as a regulatory signal for the initiation of mRNA degradation. If methylation were involved in this process, one would expect S-adenosyl-methionine, rather than the unusual tetrahydrofolate substrate, to be the methyl donor. However, since the addition of 1 mM methionine to the growth medium did not alter the phenomenon observed with trimethoprim (data not shown) it seems unlikely that S-adenosyl-methionine plays a role in this degradation process. Folic acid metabolism would appear to be well suited to involvement in such an important process as mRNA degradation because of its compulsory coupling to many metabolic pathways of the cell.

ACKNOWLEDGMENTS

This work was supported by the following grants : Centre National de la Recherche Scientifique (Laboratoire Associé n° 270 and Groupe de Recherche n° 18) ; Délégation Générale à la Recherche Scientifique et Technique and Ligue Nationale Française contre le Cancer (Comité de la Seine).

REFERENCES

1. Petersen, H.U., Joseph, E., Ullmann, A. and Danchin, A. (1978) *J. Bact.* in press.
2. Pardee, A.B., Jacob, F. and Monod, J. (1959) *J. Mol. Biol.* 1 165-178.
3. Leive, L. and Kollin, V. (1967) *J. Mol. Biol.* 24 247-259.
4. Kepes, A. (1963) *Biochim. Biophys. Acta* 76, 293-309.
5. Harvey, R.J. (1973) *J. Bact.* 114 309-322.
6. Danchin, A. (1973) *FEBS-Letters* 34 327-332.
7. Monod, J.,Pappenheimer Jr., A.M. and Cohen-Bazire, G. (1952) *Biochim. Biophys. Acta* 9 648-660.
8. Berberich, M.A., Kovach, J.S. and Goldberger, R.F. (1967) *Proc. Natl. Acad. Sci. USA* 57 1857-1864.

9. Kennell, D. and Simmons, C. (1972) *J. Mol. Biol.* 70 451-464.
10. Walker, A.C., Walsh, M.L., Pennica, D., Cohen, P.S. and Ennis, H.L. *Proc. Natl. Acad. Sci. USA* 73 1126-1130.
11. Kindler, P., Keil, T.U. and Hofschneider, P.H. (1973) *Molec. gen. Genet.* 126 53-69.
12. Apirion, D. (1974) *Bookhaven Symp. in Biology* 26 286-306.
13. Apirion, D. and Watson, N. (1975) *Molec. gen. Genet.* 136 317-326.
14. Imamoto, F. and Schlessinger, D. (1974) *Molec. gen. Genet.* 135 29-38.