A CORRELATION BETWEEN RIBONUCLEASE II AND THE IN VIVO INACTIVATION OF MESSENGER RNA IN E. COLI

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Several attempts have been made to identify the enzyme or enzymes which inactivate and degrade mRNA* in E. coli. It is known that mRNA is degraded from the 3'-OH end (Baker and Yanofsky, 1968), and both RNase II and PNPase are suitable exonucleases which act in this direction (Thang, Guschlbauer, Zachau and Grunberg-Manago, 1967; Nossal and Singer, 1968; Klee and Singer, 1968). Studies of the degradation of pulse-labelled RNA in extracts of broken cells or subfractions derived from them appear to implicate RNase II (Spahr, 1964, Artman, Silman and Engelberg, 1967), PNPase (Sekiguchi and Cohen, 1963), or both enzymes (Wade and Lovett, 1961; Futai et al., 1966). These studies did not deal with the inactivation of mRNA, which may conceivably be a process distinct from the subsequent degradation. Moreover, it is not certain that such in vitro experiments are valid for the intact cell, where compartmentalization may exist, and it would seem preferable to employ in vivo systems where possible.

Investigations of this type, conducted with RNase I-deficient mutants and spheroplasts, have shown that RNAse I is probably not responsible for either the inactivation or degradation of mRNA in E. coli (Artman and Engelberg, 1965; Gesteland, 1966; Kivity-Vogel and Elson, 1967).

In our earlier work we tentatively suggested that the same might also be true of PNPase, since one of the mutants examined, Q-13, appeared to be deficient also in this enzyme; yet its mRNA was inactivated at a normal rate (Kivity-Vogel and Elson, 1967). It has since been shown, however, that Q-13 possesses an active, though mutated, PNPase (Natori and Mizuno, 1967; Thang, Thang and Grunberg-Manago, 1967), and we have also

*Abbreviations: mRNA, messenger ribonucleic acid; RNase I, ribonuclease I, endonuclease which does not require Mg; RNase II, K- or NH_4-activated phosphodiesterase; PNPase, polynucleotide phosphorylase; poly A, poly U, polyriboadenylic and uridylic acids.

observed such activity in cell extracts prepared differently and with a modified assay. Consequently, the possibility that PNPase and/or RNase II act on mRNA remains to be examined in in vivo systems.

The experiments reported here represent a first approach to this problem and apply to the inactivation of mRNA. We have examined three RNase I-deficient strains of E. coli. Two are the closely related strains Q-13 and 1113, both isolated in the laboratory of W. Gilbert, and both having a mutated but active PNPase; this mutation is presumably the same in both strains. They differ in that 1113 carries an additional mutation that renders its RNase II heat-labile. In the third strain, MRE-600 (Cammack and Wade, 1965), both enzymes are normal.

We have measured the rate at which mRNA is inactivated in cell cultures growing at different temperatures. This rate is known to increase with rising temperature. Upon further raising the temperature above the physiological optimum, we have observed, as was not unexpected, that a temperature is reached, above which the rate of mRNA inactivation now decreases. This temperature is lower for strain 1113, which carries a temperature-sensitive RNase II, than for the other two strains. In all three strains, however, this "critical temperature" corresponds to the temperature at which RNase II undergoes heat inactivation in vitro. Further experiments have shown that when cells are incubated for extended periods at a high temperature, there is a correlation between the amount of active RNase II within the cell and the rate of mRNA inactivation at the same time. We did not find a similar correlation with PNPase. These results indicate that RNase II is involved in the inactivation of mRNA. They provide no evidence that PNPase may also be involved, but do not eliminate such possibility.

METHODS

Cells were grown with vigorous shaking at the temperatures specified below in a glycerol-lactate-salts medium (Hershey and Chase, 1953) supplemented with 0.2% or 0.4% casamino acids (Difco). The rate of inactivation of the mRNA of induced β-galactosidase was measured with the inducer-withdrawal technique of Kepes (1963) as described previously (Kivity-Vogel and Elson, 1967). Protein was assayed according to Lowry et al. (1951).

All enzyme assays were performed with crude cell extracts, at 30° in order to avoid heat inactivation during the assay. Enzyme activities were calculated as activity units per mg protein; only relative values are presented here. At non-inactivating temperatures, the 3 strains showed very similar values for both enzymes. RNase II was assayed according

to Singer and Tolbert (1964), with C^{14}-poly A as substrate. When K was replaced by Na in the assay medium, activity fell by 95% or more. PNPase was determined with the phosphorolysis assay of Kimhi and Littauer (1967), but with poly U (Miles) as substrate. With poly A (Miles), assays of the PNPase of Q-13 and 1113 gave low and erratic values. With poly U, tested because of information reported by Natori and Mizuno (1967), the PNPase assays were reproducible and gave values as high as those obtained with other strains.

Further details are given in the legends of the figures.

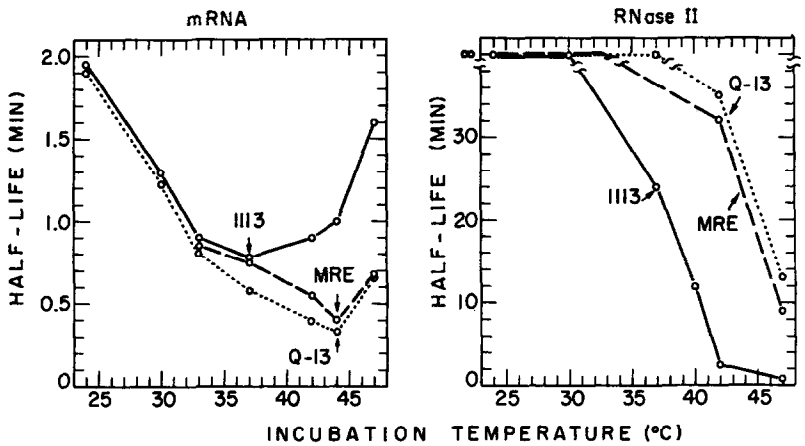

Fig. 1. The rates of inactivation of mRNA and RNase II as a function of temperature. mRNA: At 37° or lower, cells were cultured and induced and mRNA half-life was determined at the indicated temperature. For higher temperatures, cells were first cultured at 37° and then incubated at the higher temperature for 30 min, after which β-galactosidase induction and the determination of mRNA half-life were carried out at the higher temperature. Arrows indicate the temperature at which mRNA was inactivated most rapidly. RNase II: Exponentially growing cells were broken in a French pressure cell and centrifuged for 20 min at 30,000 g. Supernatant samples were incubated at the indicated temperatures, and aliquots taken at intervals were rapidly chilled in ice and subsequently assayed. When heat-labile, activity decayed exponentially, and a semi-logarithmic plot of activity versus incubation time gave a straight line from which the half-life could be taken directly.

RESULTS AND DISCUSSION

Fig. 1 shows the effect of temperature on the rate of inactivation of mRNA *in vivo* and on the stability of RNase II in crude cell extracts *in vitro*. With rising temperature, the mRNA was inactivated more and more rapidly until a certain temperature range was reached. Parallel assays of RNase II *in vitro* have shown its activity to increase correspond-

The temperature range at which this reversal occurred was the same for strains MRE-600 and Q-13 but was lower for strain 1113. In each case, however, it corresponded to the temperature range at which RNase II was sharply inactivated.

The experiment illustrated in Fig. 2 was performed in order to obtain a closer approximation of the amount of active enzyme in the same cells in which the rate of mRNA inactivation was determined. A small culture was rapidly brought to 47° and kept at that temperature. At intervals, samples were taken for the determination of the rate of mRNA inactivation. In a separate but identical experiment the samples were quickly chilled and subsequently assayed for RNase II and PNPase at 30°, a temperature at which all active enzyme retains full activity.

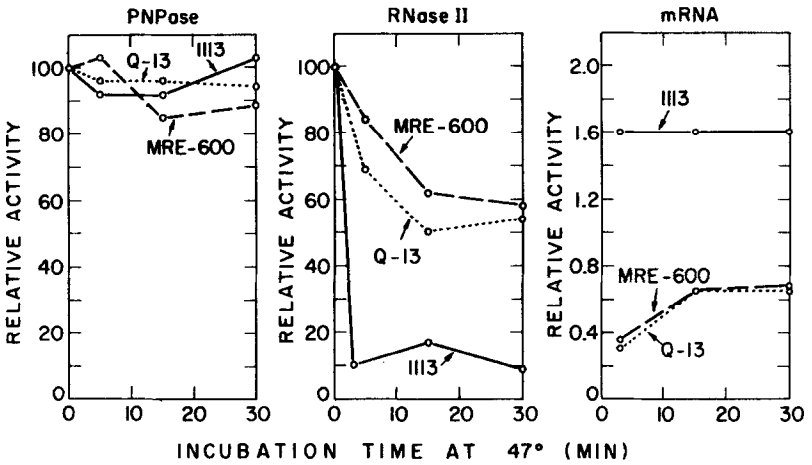

Fig. 2. The effect of incubation at 47° on the intracellular content of RNase II and PNPase and the rate of the inactivation of mRNA. Ten ml samples of an exponentially growing 37° culture were pipetted into 250 ml flasks maintained at 47° (The samples reached 47° within 0.5-1.0 min). After varying periods at 47°, the samples were assayed for β-galactosidase mRNA half-life (see methods). In a parallel experiment PNPase and RNase II activities were determined as follows: After incubation at 47°, as above, the samples were cooled immediately in liquid air, reaching 4-6° within5 sec. The bacteria were collected by centrifugation, washed with 10 mM Tris (pH 7.4)-1.5 mM Mg acetate and resuspended in 0.35 ml of this buffer. The bacterial suspensions were treated simultaneously in a sonic oscillator (Raytheon) at 10 Kc/sec for 60 min at 4°. This treatment released maximum enzyme activity; further 60 min treatment neither increased nor decreased the activity of the two enzymes examined. After a 20 min centrifugation at 30,000 g, the supernatant was removed and assayed as described in methods. The activity at 37° (zero time) was taken as hundred percent.

The results again show a correlation between RNase II activity and the rate of inactivation of mRNA. In strains MRE-600 and Q-13, the RNase II activity slowly fell to about half its initial value and then remained constant, and over the same period the rate of mRNA inactivation also fell to a new value and then remained constant. In the temperature-sensitive strain 1113, RNase II activity fell much more rapidly and to a much lower value, and a correspondingly more rapid and larger change occurred in the rate of mRNA inactivation. There was no such correlation with PNPase.

It may be noted that RNase II appears to be inactivated by heat more extensively in vitro (Fig. 1) than in vivo (Fig. 2). While it is possible that a portion of the enzyme is stable in the cell but not outside of it, it may well be that the enzyme is inactivated equally in both cases, but that in vivo there is a compensating synthesis of new enzyme. All three strains are clearly able to synthesize protein at temperatures as high as 47°, since they can be induced to form active β-galactosidase under these conditions. When cells are brought from 47° to a lower temperature, their content of active RNase II quickly rises to the level characteristic of that temperature, as does the rate of mRNA inactivation. It has not yet been proven, however, that this is due to the synthesis of new RNase II and not to the reactivation of preexisting enzyme; this is now under investigation.

Because of the method employed in this study, our data are directly relevant to the inactivation of the biological function of mRNA, but not to its degradation. Our results indicate strongly that RNase II is involved in the inactivation of mRNA in E. coli. They provide no evidence for the involvement of PNPase, but do not exclude this possibility.

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