NUCLEIC ACID SYNTHESIS IN *PSEUDOMONAS* P (2) THE FATE OF MESSENGER RNA SYNTHESIZED IN "CONC. CULTURE"

MASAMICHI TAKAGI, SHIGEKI MIZUNO and BUNJI MARUO

Institute of Applied Microbiology, University of Tokyo, Tokyo

Received February 24, 1965

It is widely accepted that protein synthesis in bacteria is mediated by an unstable, rapidly turning over RNA called messenger RNA (*m*-RNA). Several communications have been reported concerning the fate of *m*-RNA in intact cells. KITAZUME *et al.* (1) concluded in their report that *d*-RNA (a kind of RNA whose base composition is similar to that of the cellular DNA) is an obligatory precursor of *r*-RNA. MCCARTHY *et al.* (2-7) concluded after laborious studies that some of the "pulse labelled" RNA is a precursor of the stable *r*-RNA and that some is labile.

LEVINTHAL *et al.* (8) showed that in the cells of *B. subtilis*, in which RNA synthesis was inhibited by actinomycin, *m*-RNA breaks down into acid soluble, low molecular weight materials after being utilized in protein synthesis.

We reported in the previous paper (9) that *r*-RNA synthesis can be controlled by modification of the cell concentration. It seemed advantageous to use the system of "conc. culture-dil. culture" to learn the fate of *m*-RNA because *m*-RNA can be labelled in "conc. culture" without labelling *r*-RNA and its fate observed in "dil. culture".

EXPERIMENTAL

The incubation conditions and the compositions of the media were as reported in the previous paper (9), but instead of $^{32}\text{P}_i$, 1 μmole of ^{14}C -uracil was used as the labelling compound in "conc. culture".

In the experiments described below, "conc. culture 25 min.-dil. culture 15 min." was used. As shown in the previous paper, in "conc. culture 25 min.", most of the labelled RNA detected in the *r*-RNA fraction from MAK chromatography was *m*-RNA and when these cells were incubated in "dil. culture 15 min.", most of the labelled RNA in the *r*-RNA fraction became the *r*-RNA type. The specific activity of ^{14}C -uracil in the *r*-RNA fraction from MAK chromatography was determined after digestion of the RNA into four bases as follows: ^{14}C -uracil-RNA was digested with 85% formic acid in a sealed glass tube at 170-180°C for 30 min. Separation into four bases was carried out by paper chromatography using a solvent system of isopropanol-conc. HCl-distilled water=325/83/92. (ascending). The uracil spot was eluted with 0.01 N HCl. U.V. absorption at 260 m μ was measured

and the radioactivity counted by scintillation counter.

RESULTS

Distributions of ^{14}C -uracil in 5% cold trichloroacetic acid (TCA) soluble fraction and in 5% hot TCA soluble fraction after "conc. culture 25 min." are shown in Table 1. It should be noticed that the amount of ^{14}C -uracil in the cold TCA soluble fraction is not so large compared with that in the hot TCA soluble fraction. This indicates that after "conc. culture 25 min.", most of the ^{14}C -uracil incorporated into the cells was found in RNA (*m*-RNA, in this case).

Table 1. Incorporation of ^{14}C -uracil into cold-TCA soluble and hot-TCA soluble fractions in "conc. culture".

Incubation time (minutes)	Cold-TCA sol. counts (cpm)	Hot-TCA sol. counts (cpm)
5	50	174
10	95	256
25	75	365
100	35	860

The cells were incubated in "conc. culture" with ^{14}C -uracil. Aliquots were removed at intervals and washed with cold 5% TCA three times. The supernatant was used as a cold-TCA soluble fraction. The precipitate was extracted with 5% TCA in boiling water for 15 min. twice. The extract was used as a hot-TCA soluble fraction. The radioactivity of each fraction was counted.

To know whether RNA synthesized in "conc. culture" is the direct precursor of *r*-RNA synthesized in "dil. culture", or utilized after being degraded into low molecular weight compounds, three parallel experiments were carried out under the following conditions:

- "conc. culture 25 min." in medium containing $1\ \mu\text{C}$ -uracil ($0.05\ \mu\text{moles/ml}$).
- "conc. culture 25 min." same as (a) and after one washing, "dil. culture 15 min." in NB-P medium (containing no ^{14}C -uracil).
- "conc. culture 25 min." same as (a) and after one washing, "dil. culture 15 min." in NB-P medium containing $5\ \mu\text{moles/ml}$ of ^{12}C -uracil.

From these three cultures, *r*-RNA fractions were obtained by MAK chromatography as described in the previous paper (9). Hydrolysis of RNA and separation into four bases were carried out as shown in EXPERIMENTAL. The uracil spot was eluted and the specific activity determined. If *m*-RNA is the direct precursor of *r*-RNA, all ^{14}C -uracil of *m*-RNA in "conc. culture 25 min." should be retained in the *r*-RNA in "dil. culture 15 min.". And if *m*-RNA is once degraded into the acid soluble pool, ^{14}C -uracil should be diluted by ^{12}C -uracil added in the medium. The results shown in Table II indicate that neither of these possibilities is the case.

Table 2. ^{14}C -Uracil in RNA purified by MAK-column chromatography.

Treatment	Specific activity (cpm/O.D. 260 $\text{m}\mu$)	Ratio
(a)	545	1.00
(b)	299	0.54
(c)	253	0.47

^{14}C -uracil-RNA was extracted from the cells which had been treated by the following method:

- (a) The cells were incubated with ^{14}C -uracil in "conc. culture 25 min."
- (b) After "conc. culture", the cells were washed once and then incubated in "dil. culture 15 min."
- (c) Same as "(b)", but with the addition of 5 $\mu\text{moles/ml}$ of ^{12}C -uracil.

Nucleic acids were extracted from each culture and separated by MAK-column chromatography. *r*-RNA fractions were pooled and hydrolyzed with 85% of formic acid in a sealed glass tube at 170–180°C for 30 min. The bases in the resulting hydrolysate were separated by paper chromatography. The radio-activity and O.D. 260 $\text{m}\mu$ were determined and the specific activity calculated.

DISCUSSION

In the previous paper (9), we reported that in "conc. culture 25 min.", most of the newly synthesized RNA found in the *r*-RNA fraction from MAK chromatography was *m*-RNA and that RNA in the *r*-RNA fraction obtained from the cells of "dil. culture 15 min." after "conc. culture 25 min." was the true *r*-RNA, judging from the patterns of MAK chromatography and base composition analysis. Using this system, the possibility of precursor-product relationship between *m*-RNA and *r*-RNA was investigated. As any detectable increase was not observed in the net quantity of RNA obtained by SDS-phenol method during "conc. culture 25 min.-dil. culture 15 min.", the specific activity of ^{14}C -uracil was expected to be proportional to the total amount of ^{14}C -uracil in the RNA.

From Table 2, the following conclusions were obtained:

1. All the *m*-RNA was not the direct precursor of *r*-RNA, because the specific activity of ^{14}C -uracil in "dil. culture 15 min." decreased to about half of the specific activity in "conc. culture".
2. Some of the ^{14}C -uracil in *m*-RNA was incorporated into *r*-RNA without mixing with acid soluble compounds, because the specific activity of ^{14}C -uracil was scarcely lowered by addition of a large amount of ^{12}C -uracil to the medium of "dil. culture".

The latter conclusion does not necessarily mean that *m*-RNA is the direct precursor of *r*-RNA because an alternative possibility is still conceivable. That is, *m*-RNA is degraded into the specific pool which is not exchangeable with the compounds added to the medium, and re-utilized for *r*-RNA synthesis effectively. But it is very probable that some of the *m*-RNA is

incorporated into *r*-RNA without being degraded into low molecular weight compounds, because the preliminary experiment in which ^{14}C -uracil was added in "dil. culture 15 min." showed that uracil was incorporated very effectively into *r*-RNA.

SUMMARY

1. To learn the fate of *m*-RNA, we used the system of "conc. culture 25 min.-dil. culture 15 min.", characterization of which was shown in the previous paper.

2. About half of the ^{14}C -uracil incorporated into *m*-RNA during "conc. culture 25 min." was found in *r*-RNA in "dil. culture 15 min.", which was scarcely diluted by extracellularly added ^{12}C -uracil.

3. These results indicate that some *m*-RNA was incorporated into *r*-RNA without mixing with acid soluble compounds, while some degraded into the acid soluble pool.

REFERENCES

- (1) Y. KITAZUME, M. YCAS, W. S. WINCENT: *Proc. Natl. Acad. Sci. U.S.*, **48**, 265 (1962).
- (2) B. J. MCCARTHY: *Biochem. Biophys. Acta*, **39**, 563 (1960).
- (3) B. J. MCCARTHY: *Biochem. Biophys. Acta*, **55**, 880 (1962).
- (4) B. J. MCCARTHY, J. E. MIDGLEY: *Biochem. Biophys. Acta*, **61**, 696 (1962).
- (5) B. J. MCCARTHY, A. I. ARONSON: *Biophys. J.*, **1**, 227 (1961).
- (6) B. J. MCCARTHY, R. J. BRITTEN: *Biophys. J.*, **2**, 35 (1962).
- (7) R. B. ROBERTS, R. J. BRITTEN, B. J. MCCARTHY: "Molecular Genetics", ed. by J. H. TAYLOR: Academic Press Inc., New York, Vol. **1**, p. 291 (1963).
- (8) C. LEVINTHAL, A. KEYNAN, A. HIGA: *Proc. Natl. Acad. Sci. U.S.*, **48**, 1631 (1962).
- (9) M. TAKAGI, S. MIZUNO, B. MARUO: *J. Gen. Appl. Microbiol.* **11**, 61 (1965).