DEGRADATION OF ACCUMULATED DIFFERENT CHLORAMPHENICOL-RNA OF BACILLUS MEGATERIUM DURING TREATMENT WITH ACTINOMYCIN D

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Actinomycin D which blocks the DNA-dependent synthesis of RNA and is hence suitable to visualize the degradation of RNA components that are subject to turnover, was used to determine the life time of m-RNA in bacteria (Levinthal et al. 1963; Fan et al. 1964; Meselson et al. 1964; Leive 1965).

Acs et al. (1963) were the first to show that ribosomal precursor RNA accumulated in bacteria in the presence of Chloramphenicol (ClA) is partly degraded to acid-soluble material on subsequent exposure to actinomycin D. In our laboratory we found also that only part of the accumulated ribosomal precursor-RNA was degraded. Therefore we were interested in the problem of whether this incomplete degradation process might be due to a heterogenicity of the ClA particles.

Experimental

The bacteria used were Bac.meg. KM (uracil) No.239.[+] The nutrient medium consisted of inorganic salts, 25 ug Mg^{++}/ml,

[+]The bacterial strain was kindly donated by Prof. Wachsman, Urbana, Illinois.

200 ug P/ml, 0.5 % glucose, 0.5 % casein hydrolysate, 0.05 % yeast extract (Difco), 0.5 % Tris-buffer, pH 7.4 . Cultures and cell extracts were those described by Lindigkeit, Handschack, (1965). ^{3}H-adenine (210 uc/mg) and ^{14}C-uracil (47 uc/mg) were used as radioactive labels.

First, experiments were conducted to investigate whether the RNA synthesized in the bacteria during the initial period after ClA addition differed from RNA synthesized in a later period in its behaviour towards actinomycin D. In order to distinguish the early synthesized ClA-RNA from ClA-RNA synthesized during a later period successive labeling with ^{3}H-adenine and ^{14}C-uracil was carried out during ClA exposure. Subsequently, the bacteria thus labeled were exposed to actinomycin D in the presence of ClA. The results of one of these experiments are presented in Figure 1. It can be seen that the ^{14}C-labeled RNA was more sensitive to actinomycin D than the ^{3}H-labeled RNA.

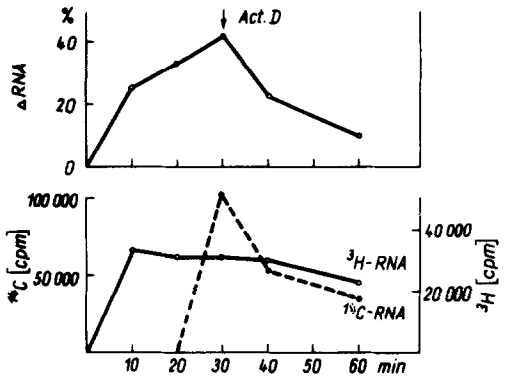

Figure 1
Successive labeling of bacterial RNA with ^{3}H-adenine and ^{14}C-uracil in the presence of ClA and subsequent degradation of RNA after addition of actinomycin D.

2 minutes after the addition of ClA (200 ug/ml) ^{3}H-adenine (0.4 uc/ml) was added. After 10 min cultivation the ^{3}H-adenine was excessively diluted by adding non-radioactiv adenine (400 ug/ml). After further 10 min cultivation ^{14}C-uracil was added. After another 10 minutes excess radioactivity was removed by centrifugation (for 1 min at 14 000 x g). The bacteria were then cultured for another 30 minutes in a freshly prepared ClA-

containing nutrient medium supplemented with 2 ug/ml of actinomycin D. The RNA was determined according to the method of Schmidt-Thannhauser. Determination of ^{3}H- and ^{14}C-radioactivity was carried out in a Tricarb-Scintillation counter (Nuclear Chicago Corp.).

Hitherto unpublished experiments from our laboratory had shown that in contrast to early synthesized ClA-particles, those ClA-particles synthesized in a later period preferably appear in rapidly sedimenting fractions. Together with the results of the above experiment this could mean that ClA-particles of the rapidly sedimenting fractions are degraded by actinomycin D to a greater extent than ClA-particles contained in the more slowly sedimenting fractions.

In order to check this assumption a study was made of the distribution of ^{14}C-labeled ClA-RNA in fractions obtained by fractional centrifugation of bacterial extracts before and after exposure of these bacteria to actinomycin D. Determinations of RNA concentration had shown that the content of RNA during exposure to ClA had increased by 30 %. Following the exposure to actinomycin D 21 % of the total RNA were degraded. During 30 min exposure to actinomycin D 61 % of the radioactively labeled RNA were lost. It is evident from Table 1 that the proportion of ^{14}C-labeled RNA found in the different fractions had decreased differently, the loss being greatest in the two heavy fractions.

These findings were in contrast to results obtained by Yudkin and Davis (1965) who found that pulse-labeled RNA of the membrane fraction (30 000 x g sediment) is very stable towards actinomycin D whereas the cytoplasmatic fraction is degraded in the presence of actinomycin D. In order to check if there is a fundamental difference in the behaviour of accumulated

ClA-RNA and pulse-labeled RNA towards actinomycin D we investigated the degradation of pulse labeled RNA. The bacteria were first labeled during the log phase with ^{14}C-uracil

Table 1

Distribution of total RNA synthesized in the presence of ClA over the different fractions of cell extracts obtained by fractional centrifugation, before and after exposure to actinomycin D.

Fractions	Controls		After 30 min exposure to actinomycin D		
	RNA (mg)	^{14}C-radio-activity (cpm)	RNA (mg)	^{14}C-radio-activity (cpm)	loss of ^{14}C-RNA (%)
9 000 x g	2.28	14 200	2.09	2 900	79.4
30 000 x g	2.38	18 000	0.94	7 000	61.0
100 000 x g	3.46	15 000	3.14	7 600	49.0
180 000 x g	0.705	8 700	0.71	5 600	37.5

2 minutes after the addition of ClA (200 ug/mg) ^{14}C-uracil was added for 15 minutes. 200 ug/ml of non-radioactive uracil and 2 ug/ml of actinomycin D were added to one half of the culture and cultivated for another 30 minutes. Bacterial extracts were fractionated by centrifugation for 15 min at 11 000 x g, for 30 min at 30 000 x g, for 1 hr at 100 000 x g, and for 3 hrs at 180 000 x g. The RNA was determined as described in Figure 1.

(0.06 uc/ml) and then exposed for 10 minutes to actinomycin D (2 ug/ml). After exposure for 10 minutes to actinomycin D determinations of RNA concentration indicated that 7 % of the RNA were lost by degradation and measurements of radioactivity showed that 78 % of the pulse-labeled RNA were degraded. Data on the degradation of RNA in the different fractions are listed in Table 2.

It can be seen in this table that both in the present case of pulse-labeling and in the case of ClA-exposure the largest proportions of ^{14}C-labeled RNA degraded are found in the two

Table 2

Distribution of total RNA and RNA synthesized in the log phase after 1 min pulse-labeling, before and after exposure of the different fractions of cell extracts to actinomycin D.

Fractions	Controls		After 10 min exposure to actinomycin D		
	RNA (mg)	^{14}C-radio-activity (cmp)	RNA (mg)	^{14}C-radio-activity (cmp)	loss of ^{14}C-RNA (%)
9 000 x g	0.37	5 600	0.087	307	94
30 000 x g	1.21	20 700	0.79	1 730	92
100 000 x g	2.36	15 900	3.70	6 580	58
180 000 x g	1.62	9 100	0.64	2 580	72

After pulse-labeling 200 ug/ml of non-radioactive uracil and 2 ug of actinomycin D were added to one half of the bacterial culture. The bacteria were then cultivated for another 10 minutes. Preparation of the different fractions and determination of RNA were carried out as indicated in Table 1.

heavy fractions. Hence, there is no essential difference in the behaviour of accumulated ClA-RNA and pulse-labeled RNA towards actinomycin D. The present results do not confirm the findings of Yudkin and Davis (1965).

Discussion

It is evident from the above experiments that particularly those portions of pulse-labeled RNA and RNA accumulated in the presence of ClA that appear in the two rapidly sedimenting fractions are degraded on subsequent exposure to actinomycin D. Although no particular distinction has been made in these experiments between

DNA-like RNA and ribosomal precursor-RNA the degradation of a large proportion of ClA-RNA nevertheless suggests that during ClA inhibition predominantly ribosomal precursor-RNA is lost by degradation. In view of the fact that after 1 min pulse-labeling during log growth the larger part of the newly synthesized bacterial RNA consists of ribosomal precursor-RNA (Midgley and McCarthy, 1962) it may be assumed that also in the absence of ClA both DNA-like RNA and ribosomal precursor-RNA are converted to acid-soluble material in the presence of actinomycin D. It is likely that the RNA of the slowly sedimenting fraction which is relatively stable to actinomycin D is largely composed of the RNA which had been synthesized during the initial period after ClA addition to these bacteria. This can be concluded from the experiment using successive labeling with ^{3}H-adenine and ^{14}C-uracil. Other hitherto unpublished studies in our laboratory had shown that in cell extracts prepared in 10^{-2}m $MgCl_2$ the "older" ClA-particles, contrary to the "younger" ClA-particles are found to occur preferably in a free form. Hence, we assume that the "younger" ClA-particles occurring in cell extracts in a combined form are more susceptible to actinomycin D than the "older" ClA particles which are found to be present in a free form.

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