Vol. 81, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 14, 1978 Pages 1000-1010

Regulation of the in vitro synthesis of the α-peptide of β-galactosidase directed by a restriction fragment of the lactose operon

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Received February 27, 1978

SUMMARY

The regulation of the in vitro synthesis of the N-terminal portion of the β-galactosidase molecule (α-peptide) has been investigated using DNA fragments of the lactose operon as template. DNA fragments of about 789 base pairs were isolated after endonuclease (Hin II) digestion of either λplac5, λh80dlacp^{s} or λh80dlacUV5 phage DNA or DNA from the recombinant plasmid PMC3. The regulation of the expression of these fragments is similar to that observed for the synthesis of β-galactosidase using total phage or plasmid DNA as template, indicating that the regulatory regions on the fragments are intact and functional. Thus, the synthesis of the α-peptide required an inducer due to the presence of lac repressor in the E. coli S-30 extract used. In addition a dependency on adenosine 3',5'-cyclic monophosphate (cAMP)[1] for α-peptide synthesis was obtained with the fragments isolated from λplac5 and λh80dlacp^{s} DNAs, whereas little effect of cAMP was seen with the fragment isolated from λh80dlacUV5 phage DNA or PMC3 plasmid DNA containing a UV5 promotor region. However, a significant difference

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[1]The abbreviations used are: IPTG, isopropylthiogalactoside; ONPG, orthonitrophenylgalactoside; ppGpp, guanosine-5'-diphosphate-3'-diphosphate; cAMP, cyclic adenosine 3',5'-monophosphate; CRP, cAMP receptor protein, PEG 6000, polyethylene glycol 6000.

0006-291X/78/0813-1000$01.00/0

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Aphid mycetocytes were obtained by puncturing the abdomen with a needle in physiological saline. Special care was taken not to injure the gut of the host insect. The mycetocytes were isolated by shaking the insect lightly in the chilled saline. Contaminating tissue debris were removed by passing the cells through the meshes and precipitating them several times under the natural gravity. To label the RNA species the isolated mycetocytes were incubated in Grace's T. C. medium (without insect plasma, Gibco)[4] containing radioactive uridine at 30°C. [5-^{3}H] Uridine, 25 Ci/mmole (Radiochemical Centre) was used at 0.1 mCi/ml. When necessary, the cells were incubated in the medium containing antibiotics for 30 min. before adding [^{3}H] uridine. *In vivo* labeling of aphid RNA was performed by injecting the insects with 1 µl of [U-^{14}C] uridine (Radiochemical Centre, 513 mCi/mmole) at 50 µCi/ml. Aphids were anesthetized in CO_2 and injected by inserting an ultra-thin capillary tube of glass into the bottom of the first left leg. The capillary tube was connected to a vacuum pump (Millipore Co.) by a length of polyethylene tubing and a semi-quantitative injection performed by applying the air pressure on the solution in the capillary tube for a short, fixed length of time.

Extraction of RNA from the whole tissue of host insects and the isolated mycetocytes were done by published procedures [5].

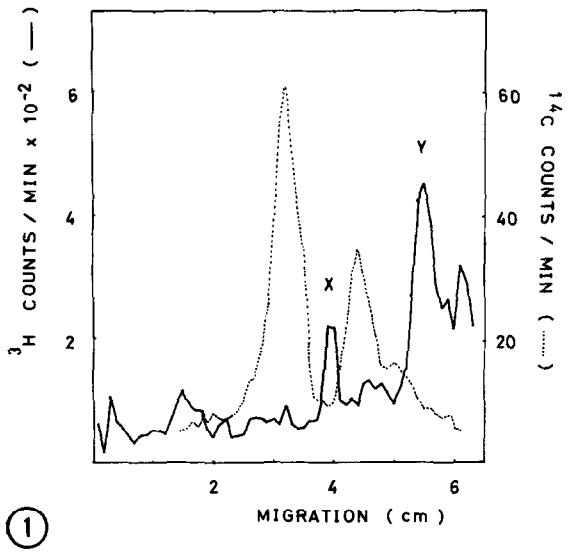
In polyacrylamide gel electrophoresis the method of Bishop *et al.* was followed with slight modifications [5, 6]. Gels of 2.5 % polyacrylamide (ethylene diacrylate cross-linked) were used. Electrophoresis was performed at room temperature at 5 mA per column for 300 min. The trough buffer was renewed once during the run. After electrophoresis the gels were frozen on powdered dry ice and sliced on a Mickle gel slicer (Mickle Lab.) into 1 mm slices. The slices were hydrolyzed overnight in a small volume of concentrated NH_4OH, and counted for radioactivity in PCS (Radiochemical Centre).

RESULTS

(a) *In vitro* labeling of mycetocyte RNA

It was advantageous for the present purpose that in a matured aphid the mycetocytes do not form the mycetome but are scattered as free cells around the mid- and hindgut in the body cavity [7]. The mycetocytes are characterized by their gigantic size and very high density [8]. These facts also made it easy to isolate the mycetocytes. A small amount of isolated mycetocytes was suspended in 1 ml of Grace's medium containing 0.1 mCi of [^{3}H] uridine and incubated aseptically at 30°C for up to 120 min. RNA species were extracted from these cells and resolved on polyacrylamide gel electrophoresis. In these experiments, the aphid RNAs which had been labeled *in vivo* with [^{14}C] uridine for 18 hr were run on the same gel as internal standards.

It is evident that a short pulse *in vitro* up to 120 min. did not label any of the mature rRNAs of eukaryotic hosts. After 30 min, the three highly-labeled species were conspicuous (Fig. 1). In comparison with the mobility of the host rRNAs with molecular weight of 1.6 x 10^{6} and 0.9 x 10^{6}, two heavier ones of the above three were with apparent molecular weight of 1.2 x 10^{6} and 0.6 x 10^{6}, and designated X and Y, respectively. It will be safely said that these



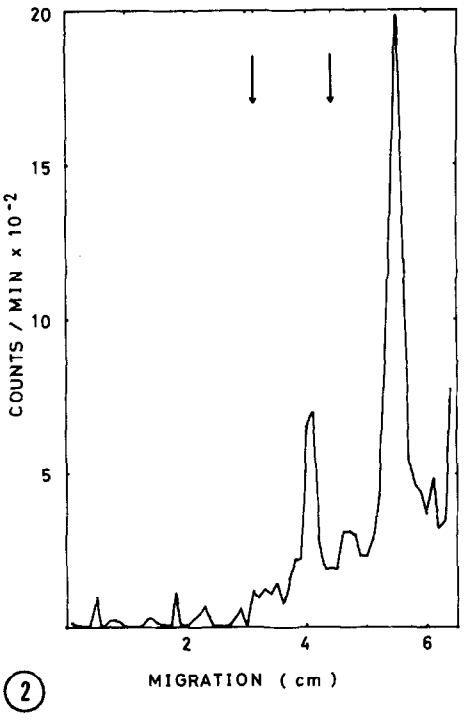


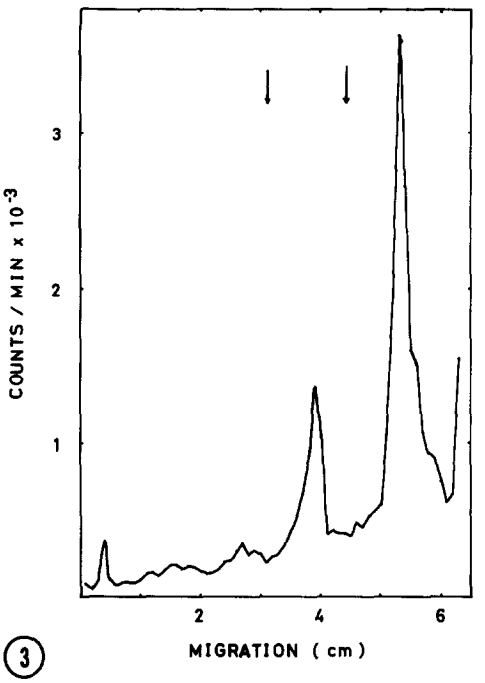
Fig. 1. Polyacrylamide gel electrophoresis of the mycetocyte RNAs labeled in vitro for 30 min. The isolated mycetocytes were incubated aseptically in Grace's medium containing 0.1 mCi/ml of [^{3}H] uridine at 30 °C and the RNA extracted 30 min. later. The RNA sample (about 50 μg in 50 μl) was subjected to polyacrylamide gel (2.5 %, ethylene diacrylate-cross linked) electrophoresis. Electrophoresis was performed at 5 mA/gel for 300 min. The aphid total RNAs were labeled with [^{14}C] uridine in vivo for 18 hr, and employed as internal standards. The gel was sliced into 1 mm slices and counted for radioactivity.

Fig. 2. Polyacrylamide gel electrophoresis of the mycetocyte RNAs labeled in vitro for 60 min. The arrows indicate the position of the 28S and 18S rRNAs of aphids that was located with the internal standards as in Fig. 1.

two RNA species are those which arose the most rapidly when the total RNA of aphids labeled in vivo [3]. At 60 min, there is a continued increase in radioactivity in the X and X species (Fig. 2). By 120 min, the above two were labeled increasingly heavily and no other peak of radioactivity appeared (Fig. 3).

(b) Effects of antibiotics

Isolated mycetocytes were incubated in Grace's medium containing a concentration of actinomycin D for 30 min. previously before labeled with [^{3}H] uridine for 60 min. in the continued presence of the antibiotic. The RNAs were extracted and resolved on polyacrylamide gel as above. At the three concentrations



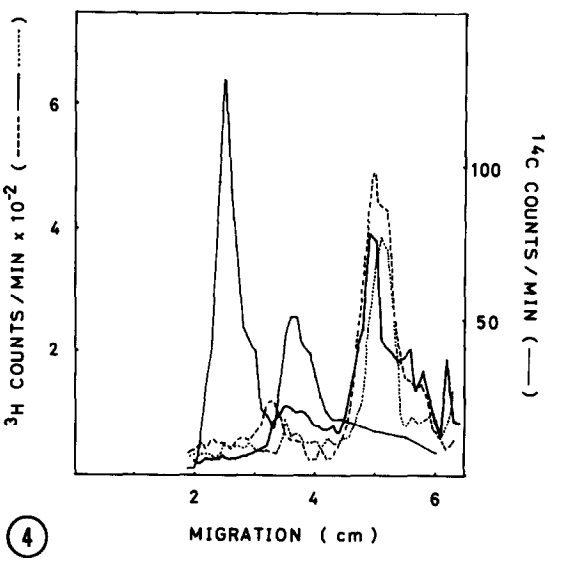


Fig. 3. Polyacrylamide gel electrophoresis of the mycetocyte RNAs labeled in vitro for 120 min.

Fig. 4. Effect of actinomycin D on the RNA synthesis in the mycetocyte. The mycetocytes were incubated at 30°C in Grace's medium containing actinomycin D at 1 μg/ml (---), 5 μg/ml (——) and 20 μg/ml (-----) for 30 min. Immediately after that the cells were labeled in vitro with [^{3}H] uridine for 60 min in the presence of the antibiotic, and the RNA resolved as before. ^{14}C-labeled rRNAs of aphids (——) were run together with the ^{3}H-RNA sample from the cells exposed to 1 μg/ml of actinomycin D. For convenience, three separate electropherograms were redrawn on a single figure.

examined, the effect of actinomycin D was to inhibit the overall synthesis of RNA in the mycetocyte (Fig. 4). Also noted is a broadening of the radioactive peaks of X and Y. This will indicate that the antibiotic incurs a partial degradation of these RNA molecules and/or a crippled synthesis of them. By increasing the concentration up to 20 μg/ml the effect of actinomycin D was gradually strengthened. And yet, a considerable amount of RNA was synthesized at the highest concentration tested.

In contrast with this, the effect of rifampicin on the RNA synthesis was highly selective at a lower concentration and nearly complete at a higher concentration (Fig. 5). Under the influence of rifampicin at 50 μg/ml virtually no RNA species of high molecular weight were synthesized in the mycetocyte for 60 min. In particular, rifampicin exerted strong effect on the

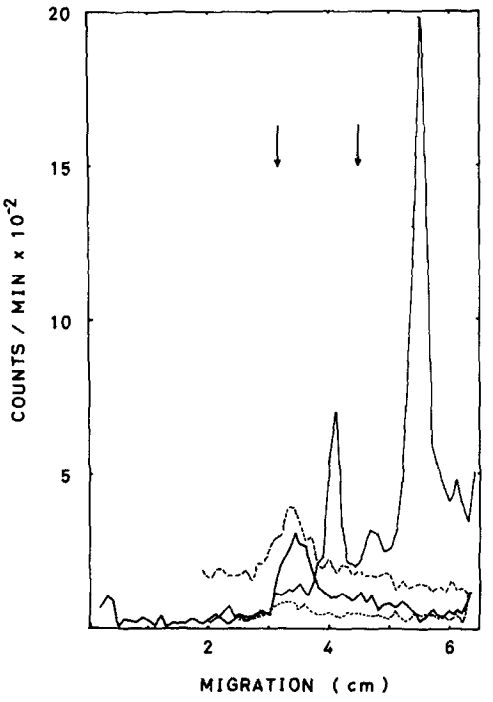


Fig. 5. Effect of rifampicin on the RNA synthesis in the mycetocyte. The cells were incubated in the presence of 0 (——), 1 (– – –), 10 (——) and 50 (·····) μg/ml of rifampicin, and labeled with [^{3}H] uridine for 60 min. Four separate electropherograms were redrawn. The arrows indicate the position of the 28S and 18S rRNAs of aphids.

synthesis of X and Y components. At the concentration as low as 1 μg/ml the antibiotic shut off the synthesis of these molecules dramatically. Apparently the synthesis of heterogeneous RNA species seen at the heavier shoulder of the peak X was more resistant to the antibiotic or even augmented at the lower concentrations. There differential effects of rifampicin may be plausibly explained by assuming two kinds of RNA polymerases functioning in the mycetocyte. Judging from its high sensitivity to rifampicin, the RNA polymerase that is involved in synthesizing the RNA species X and Y will be apparently of the prokaryotic type [9].

DISCUSSION

In the aphid mycetocyte the two different genomes, one eukaryotic and the other prokaryotic, together form a harmonious system. For this system to function, gene expression of these two must be regulated very accurately and coordinatedly. That this is true for the expression of rRNA genes has

been implied in the present experiments.

One of the most striking characteristics of the aphid mycetocyte was that it does not synthesize either the rRNAs of the eukaryotic type or their precursors of high molecular weight for 120 min. when incubated in vitro. No discrete components other than X and Y took up the RNA precursor added for the length of time (Fig. 3). It is also evident that the newly-synthesized molecules of X and Y were stable and that no indication of these two to be processed was observed. This taken together with their apparent molecular weights, the component X and Y likely are the rRNAs of the symbiotic microorganisms in the mycetocyte. The fact that a low concentration of rifampicin ruined the synthesis of these molecules thoroughly will lend further support for the above assumption (Fig. 5). The assumption also is consistent with Hinde's observation on electronmicrographs [10]. According to the micrographs the cytoplasm of the symbionts in the mycetocyte contains many ribosomes. Thus, it will be safely said that in the mycetocyte the ribosome genes of the symbionts are preferentially expressed. With this in mind, one of the temping possibilities is that the host cells and the host insect also live upon the rRNA molecules thus produced by their symbionts. As has been suspected, the host cytoplasm may digest, as occasion demands, the envelopes of several symbionts [1, 11, 12]. In so doing, the host cell will make use of the contents of them, whether directly or after modifications, for its eukaryotic life. By assuming these events to occur, not only the exceptional properties of aphid rRNAs [3, 13-15] but the extraordinary fecundity [16] pertained to the aphids could be plausibly interpreted. In this connection, it is significant that in forms and in their reaction to lead staining the ribosomes in the symbiont closely resemble those seen in the host cell cytoplasm [10]. Therefore, the symbiont's rRNA and ribosomes will be among the many candidates along with cholesterol [17], fatty acids [18] and amino acids [19-21] that are produced by the symbiont and are eventually utilized by the host cell.

ACKNOWLEDGEMENTS

I am grateful to Dr. M. Shiga of F.T.R.S. for supplying the chestnut aphids. My gratitude is also due to Dr. S. Nasu of National Institute of Agricultural Science for his technical advices. I gratefully acknowledge that the study on the mycetocyte symbiont was started with the advice of Dr. G. R. Wyatt, Queen's University.

This work was supported by grants from the Ministry of Education, Science and Culture (Nos. 264240 and 234041) and the Itoh Science Foundation.

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