A CHARACTERISTIC MESSENGER RNA IN THE SYSTEM OF INDUCED FORMATION OF CATECHOL OXIDIZING ENZYMES IN PSEUDOMONAS EFFUSA

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Attempts to find specific messenger RNA(m-RNA) corresponding to the genetic information for specific protein molecules are significant for confirmation of the hypothesis of induced enzyme forming mechanisms (Jacob and Monod, 1961). This communication describes some results on this problem.

In Pseudomonas bacteria sequentially induced enzymes which oxidize aromatic compounds are formed in the presence of these compounds (Stanier, 1947; Suda et al., 1950). Resting cells can still form induced catechol oxidizing enzymes in deficient nutritive conditions. Under these conditions, the m-RNA synthesized is easy to detect since the overall rate of RNA synthesis is low (Hayashi and Spiegelman, 1961). Thus this system is suitable for studies of m-RNA formation. When Pseudomonas effusa (M-6) is grown in nutrient broth supplemented with citrate at 30° for 16 hours and then suspended in 0.01 M Tris buffer, pH 7.3, containing 0.005 M $MgCl_2$, the rate of RNA synthesis is very low in the absence of a carbon source. When catechol was added to this suspension, oxidizing enzymes began to be synthesized after a constant lag period of about 15 minutes. It was found that the rate of RNA synthesis was much higher during induced enzyme formation than that in the presence of carbon sources not causing induction, such as citrate, succinate or glucose. In such experiments it was found that the ratio of ^{32}P incorporated into RNA to oxygen consumed during a 3 minute incubation period in the presence of catechol was 8040 (cpm/μatom O_2/ml), while the ratios with citrate, succinate and glucose were 3120, 1276 and 2410 respectively. A significant difference was observed in the sedimentation patterns of

RNA labeled under these conditions. The isotopic curve in the sedimentation diagram of RNA labeled during induction by catechol has a peak in the region sedimenting somewhat faster than 16 S ribosomal subunits. However, RNA labeled in the presence of substances not causing induction shows a peak in the region sedimenting slower than 16 S RNA. Diagrams from experiments with catechol and citrate are shown in Figure 1. The bulk of the labeled RNA is explained to be m-RNA, because of its similarity to DNA in base composition (Table 1) and to m-RNA in sedimentation behaviour in pulse and "chase" experiments.

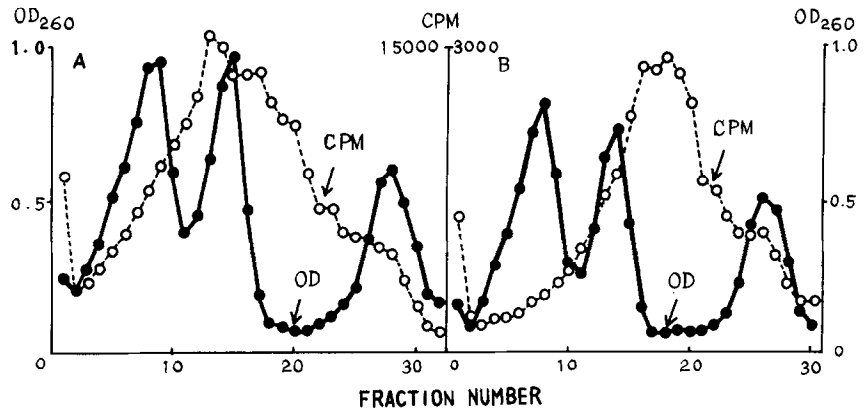

Figure 1. Sedimentation of pulse labeled RNA; (A) represents RNA labeled by a 3 minute pulse during catechol induction and (B) the same during citrate oxidation. The system contained 2.5 x 10^{-3}M catechol or 1 x 10^{-2}M citrate, 1 x 10^{-2}M Tris buffer (pH 7.3), 5 x 10^{-3}M $MgCl_2$ and a cell suspension (OD_{550}=0.670, in Coleman 12 x 75 mm round cuvettes). The total volume was 2 ml. Incubations were made aerobically at 30° in Warburg vessels. About 150 µc of carrier free ^{32}P was added after 40 minute incubation. Phenol RNA was prepared essentially according to the method of Okamoto et al. (1962). Swinging bucket centrifugation in a 5-20 % linear sucrose density gradient was carried out for 390 minutes at 36,000 rpm in the RPS 40 rotor of the Hitachi preparative centrifuge at 0°.

To demonstrate a specific relation between the synthesis of this heavier m-RNA and induction by catechol, a study was made of the variation in the rate of synthesis of the heavier m-RNA as induction ceased. It was found that when catechol had been removed by pyrocatechase there was a simultaneous decrease in

the rate of RNA synthesis (Figure 2, A). A transition was observed from synthesis of m-RNA to that of another form (Figure 2, B). Pattern I is characteristic for m-RNA labeled during induction by catechol and pattern II is characteristic for m-RNA labeled when the catechol has been used up. The disappearance of the characteristic peak in the isotopic curve of diagram I indicates that m-RNA labeled during the induction period is not synthesized during the subsequent 3 minute incubation period after cessation of the induction. Synthesis of the heavier m-RNA was never seen during the lag period, but its rate of synthesis increased as induced enzyme formation increased. Therefore, it is concluded that the heavier m-RNA is synthesized rapidly with specific relation to induced formation of catechol oxidizing enzymes. Similar results on m-RNA synthesis were observed during enzyme induction by benzoate as was expected, because catechol was one of metabolic intermediates in benzoate oxidation pathway. Although more direct evidence for the specificity of the heavier m-RNA is necessary, the results of these experiments strongly support our conclusion that the heavier m-RNA is the intermediate carrier of specific genetic information for the protein structure of catechol oxidizing enzymes.

TABLE I

Base Composition of Pulse Labeled RNA

Fraction	Moles per cent				% GC	Pu/Py
	C	A	U(T)	G		
No.12, 13 of Fig.1,(A)	28.0	21.8	22.4	27.8	55.8	0.99
No.17, 18 of Fig.1,(B)	30.7	20.5	20.3	28.5	59.2	0.96
Bulk RNA	21.9	24.1	23.7	30.3	52.2	1.19
Ribosomal RNA	21.2	25.0	23.0	30.8	52.0	1.26
Soluble RNA*	29.2	24.4	20.5	25.9	56.1	0.97
DNA*	32	18	18	32	64	1.00

Analysis of mononucleotides in alkaline digests of labeled RNA was carried out according to the method of Bautz and Hall (1962). * Hayashi and Spiegelman (1961).

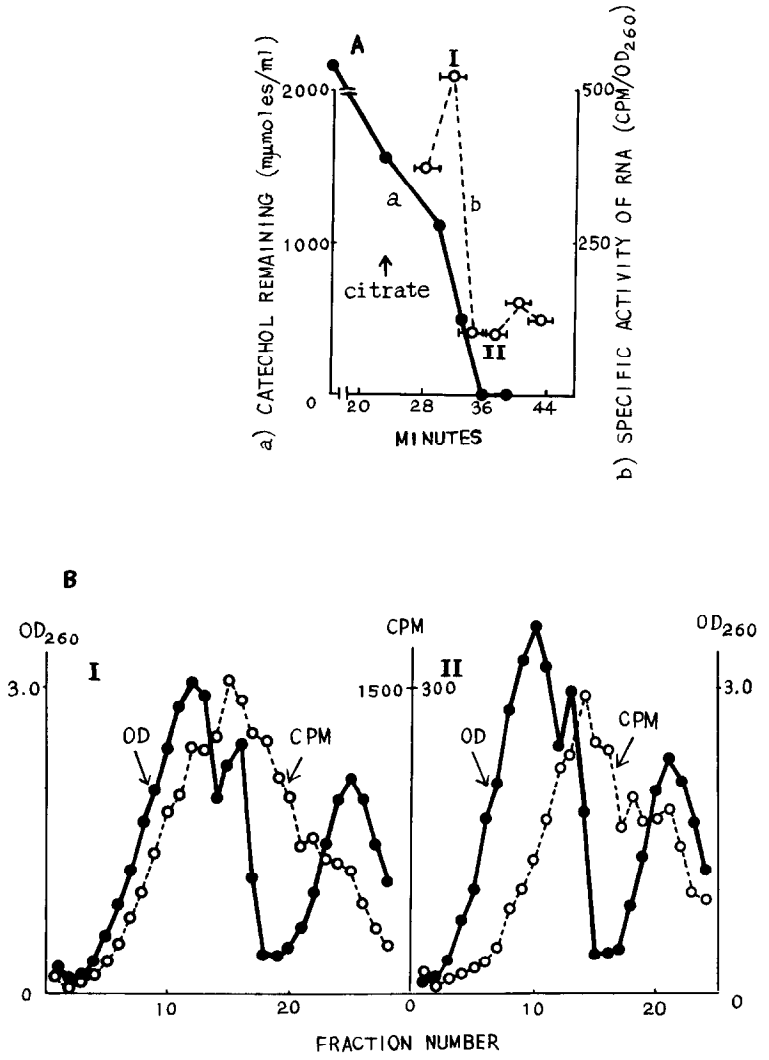

Figure 2, (A). Rate of RNA synthesis during the disappearance of catechol: The system is the same as in Fig. 1. 1 x 10^{-2}M citrate was added after 23 minute incubation as an energy source after exhaustion of the catechol. The pulse period is represented by the symbol (⊢O⊣). (B). Variation in sedimentation behaviour of labeled RNA during the disappearance of catechol: I and II correspond to I and II in (A).

In this system, pyrocatechase formation proceeded for several minutes after the heavier m-RNA synthesis had ceased because of exhaustion of catechol. Its formation was inhibited by chloramphenicol. However, this is compatible with the

specific template function of this m-RNA. From "chase" experiments, the heavier m-RNA seems to be stable during aerobic incubation of several minutes duration at least. Probably, pyrocatechase is formed by the template action of remaining m-RNA which was synthesized before cessation of induction. By analogy with the current concept for the lactose system of E. coli (Jacob and Monod, 1961), the mechanism of catechol induction system might be explained on the basis of the specific m-RNA in the following way. Catechol acts as a direct inducer at the level of m-RNA synthesis and the template function of m-RNA is independent of the inducer.

Complete details of this work will be reported elsewhere (Imamoto et al., in preparation).

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