Control of Embryonic Development: Effect of an Embryonic Inducer RNA on in Vitro Translation of mRNA†

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ABSTRACT: The transformation of undifferentiated early chick embryonic cells of the postnodal piece of stage 4 chick embryo into heartlike muscle tissues induced by a specific low-molecular-weight RNA fraction (7S CEH RNA) was recently documented in our laboratory [Deshpande, A. K., and Siddiqui, M. A. Q. (1977), *Dev. Biol. 58*, 230–247; Deshpande, A. K., Jakowlew, S. B., Arnold H. H., Crawford, P. A., and Siddiqui, M. A. Q. (1977), *J. Biol. Chem. 252*, 6521–6527]. The inducer 7S CEH RNA, which is rich in adenylate residues but does not translate under the experimental conditions used, is also capable of inhibiting both homologous poly[A(+)]RNA of chick embryonic heart and heterologous globin mRNA dependent cell-free translations in rabbit reticulocyte lysate with no apparent specificity. The inhibition appears to be due to the polyadenylate tract(s) of the RNA, since the poly(A) fragments generated by pancreatic RNase digestion of 7S CEH RNA or synthetic poly(A) can also produce similar inhibitory effects. Neither synthetic poly(A) nor the RNase digest of 7S CEH RNA is active in inducing the biochemical transition in the chick embryonic cells. These results would, therefore, suggest that a control at the level of translation is possibly not one of the mechanisms of action of the inducer 7S CEH RNA in causing a specific mode of differentiation in the undifferentiated cells of early chick embryo.

There are several examples where involvement of RNA has been postulated in the control of cellular functions, such as in specifications of antibody production (Pilch et al., 1975), interferon induction (Colby et al., 1971), in causing disease symptoms in plant tissues (Diener et al., 1972), and in others where RNA is implicated in the control of cell development and differentiations (see Bhargava and Shanmugum, 1971; Davidson and Britten, 1973; Dickson and Robertson, 1976; Siddiqui et al., 1977 for reviews). RNA is also known to be involved in the modulation of protein synthesis in cell-free systems (Bester et al., 1975; Lee-Huang et al., 1977; Sleger et al., 1977; Rao et al., 1977). Two classes of such low-molecular-weight RNA, translational control RNA (tcRNA), have been described in chick embryonic leg muscle and in *Artemia salina* embryos. The tcRNA elicit either a stimulatory or an inhibitory effect on cell-free protein synthesis. The RNA isolated from the mPNP particles of chick embryonic leg muscle, which is rich in uridylate residues, is known to inhibit myosin mRNA translation, presumably by way of a specific interaction with myosin mRNA (Heywood and Kennedy, 1976). Ochoa and co-workers (Lee-Huang et al., 1977) proposed that the RNA species of *A. salina* which exhibit similar properties might play a regulatory role in control of protein synthesis in the developing embryos.

In a recent communication from this laboratory (Deshpande et al., 1977), we described the isolation and partial characterization of a low-molecular-weight (7S) RNA fraction from the chick embryonic heart (CEH RNA). The RNA fraction when added to undifferentiated cells of the postnodal piece (PNP) of an early stage (stage 4) chick embryo induces a specific mode of morphological and biochemical transition similar to that of embryonic cardiogenic processes (Deshpande and Siddiqui, 1977). RNA from several other sources isolated under identical conditions and synthetic polynucleotides are not effective. We also observed that the RNA inhibits effectively the translational activity of globin mRNA and of rat liver poly[A(+)]RNA in vitro. Thus, the dependence of the onset of a specific mode of biochemical changes in the PNP upon a distinct RNA moiety and its activity in translational assays in vitro led us to postulate that the 7S CEH RNA might be involved, in some way, in the apparently selective control of synthesis of muscle-specific proteins at the level of translation in the host PNP cells.

In the present study, we examined the effect of 7S CEH RNA on translations of mRNA preparations obtained from both homologous and heterologous sources. The CEH RNA, which is rich in adenylate residues but is not translatable under the experimental conditions used (Deshpande et al., 1977), exhibits a strong inhibition of globin mRNA and the polysomal chick heart poly[A(+)]RNA-dependent translations in vitro with no apparent specificity. The effect, however, appears to be indistinguishable from that of synthetic poly(A) or poly(A) fragment(s) generated by enzymatic digestion of 7S CEH RNA. In view of these results, the significance of a control at the level of translation in the regulation of cell development by low-molecular-weight RNAs is discussed.

Materials and Methods

Preparations of 7S CEH RNA. The 7S CEH RNA was isolated from the total poly(A)-containing RNA from the 16-day-old chick embryonic heart as described earlier (Deshpande et al., 1977). The electrophoretic run on 7.2% polyacrylamide gel in 98% formamide was, however, prolonged to about 11 h, which apparently resolved the fraction 1′a′ (Deshpande et al., 1977) into a minor slow- and a major fast-moving spot. Of these, only the fast-moving spot possessed the activity to induce the development in PNP similar to embryonic heart formation, which was characterized according to criteria described earlier (Deshpande and Siddiqui, 1977). The RNA which was similar to fraction 1′a′ RNA in base composition and migrated on gel with a mobility of 7S will be referred to from hereon as 7S CEH RNA.

Preparation of Poly[A(+)]RNA Fraction from Chick Embryonic Heart. The 16-day-old chick embryonic hearts, stored frozen in liquid N_2, were thawed, washed quickly, and suspended in ice-cold buffer containing 50 mM Hepes (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.4),

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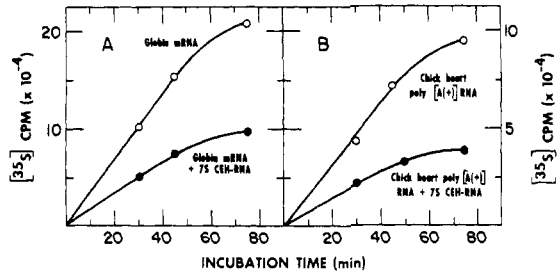

FIGURE 1: Effects of 7S CEH RNA on translations of globin mRNA and chick embryonic heart poly[A(+)]RNA. Translation of globin mRNA (A), 2.2 μg/mL, and chick heart poly[A(+)]RNA (B), 20 μg/mL, was done in the standard incubation mixture in the presence of 0.9 μg of 7S CEH RNA added to each assay tube at the start of the incubation. Aliquots, 5 μL each, were removed at the times indicated. Note that the concentration of 7S CEH RNA used was effective for about 50% inhibition of translation only (see also Figure 2): (○) control; (●) mRNA plus 7S CEH RNA.

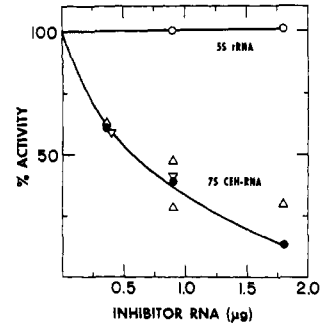

FIGURE 2: Inhibition of translation by 7S CEH RNA at varying concentrations. Translation of globin mRNA, 2.2 μg/mL, and chick heart poly[A(+)]RNA, 20 μg/mL, was done in the standard incubation mixture in the presence of varying concentrations of 7S CEH RNA or 5S rRNA as before. Aliquots were taken after 30 min of incubation at 30 °C. The effect of 5S rRNA of the posterior silk gland of *B. mori* (Chen and Siddiqui, 1975) was tested for globin mRNA translation alone: (●) 7S CEH RNA plus globin mRNA; (○) 5S rRNA of *B. mori* plus globin mRNA; (△ or ▽) 7S CEH RNA plus chick heart poly[A(+)]RNA (△ and ▽ represent two separate experiments).

50 mM NaCl, 5.0 mM $MgCl_2$, 0.14 M sucrose, and 500 μg/mL heparin. The heart tissues were homogenized in a motor-driven glass–Teflon homogenizer, and intact polysomes were prepared essentially as described by Schimke et al. (1974). Membrane-bound polysomes were freed by the addition of 2% Triton X-100 and 1% sodium deoxycholate to the first homogenate. Polysomes were then treated with 1% sodium dodecyl sulfate ($NaDodSO_4$) and the RNA was precipitated overnight in 2 M LiCl and 10 mM Na_2EDTA (pH 7.2) at 4 °C. The RNA recovered by centrifugation was reprecipitated in 2.5 volumes of ethanol containing 0.2 M sodium acetate, pH 4.5. The total poly[A(+)]RNA was then obtained by oligo(dT)-cellulose chromatography according to Aviv and Leder (1972) and as described earlier (Deshpande and Siddiqui, 1977).

Cell-Free Translation in Rabbit Reticulocyte Lysates. Analysis of translational activity was none essentially as described by Pelham and Jackson (1976). Rabbit reticulocyte lysates were prepared according to Hunt et al. (1974). The standard incubation assay, in a final volume of 50 μL, contained 25 μL of lysate, 20 mM Hepes (pH 7.4), 1 mM ATP, 0.2 mM GTP, 1 mM $MgCl_2$, 10 mM creatine phosphate, 100 mM KCl, 20 μM hemin, 2.5 μg of creatinine phosphokinase, 5–20 μCi of [^{35}S]methionine (sp act. 880 Ci/mM), a mixture of nonlabeled amino acids (except methionine), 50 μM each, 1–2 μg of poly[A(+)]RNA from the chick heart, and 0.1–0.2 μg of globin mRNA. Incubation was done at 30 °C and the Millipore membrane-bound radioactivity in CCl_3COOH-insoluble proteins was measured in Bray's solution in a Beckman LS350 spectrometer at 94% and 40% efficiency for ^{35}S and ^{3}H, respectively. The optimum Mg^{2+} concentration for translation of both mRNAs was 1.0 mM. Concentrations of 80 and 100 mM K^+ were used for globin and chick heart poly[A(+)]RNA, respectively. The rate of incorporation was linear for at least 60 min of incubation, and the lysate responded linearly for both mRNA at concentrations up to 2.2 μg/mL for globin mRNA and 12 μg/mL for chick heart poly[A(+)]RNA.

$NaDodSO_4$–Polyacrylamide Gel Electrophoresis. Analysis of the labeled translational products was carried out in 10% polyacrylamide gels containing 1% $NaDodSO_4$. Electrophoresis was done according to Laemmli (1970). Aliquots containing 1000 to 20 000 cpm were first centrifuged in a Beckman airfuge (Beckman Instruments) at 150 000*g* for 10 min to remove the polysomes and other insoluble material, and the supernatant was applied directly to gel slots along with appropriate markers. Radioactivity was detected by fluorography of the dried gels (Bonner and Laskey, 1974). Gels with nonlabeled marker proteins were stained with Coomassie blue and destained in 10% acetic acid.

Enzymatic Digestion of RNA. 7S CEH RNA or poly(A)-RNA, 1–2 μg, was digested with 16–20 μg of prewashed insoluble pancreatic RNase (Sigma) in suspension (134 Kunitz units/g) in 60 μL of 0.05 M Tris-Cl[1] (pH 7.2) at 37 °C for 2 h. The RNase was removed by centrifugation and the supernatant was tested for RNase activity by incubation with 5S [^{32}P]rRNA from the posterior silkgland of *Bombyx mori* (Chen and Siddiqui, 1975). After 4 h of incubation at 37 °C, only 4.8% of radioactivity became CCl_3COOH soluble. Under the same conditions of incubation with the insoluble RNase (pellet), 97% of labeled 5S rRNA was hydrolyzed by the enzyme. For digestion with micrococcal nuclease (Worthington), 0.3–0.6 μg of RNA was incubated at 37 °C for 60 min with 8 units of the enzyme in 10 mM Tris–borate, (pH 8.5), 1 mM $CaCl_2$. At the end of the incubation, the enzyme was inactivated by the addition of 3 mM EGTA. 5S [^{32}P]rRNA of *Bombyx mori* was also incubated under identical conditions to monitor the hydrolysis. The RNA samples were heated to 85 °C for 3 min and rapidly chilled in ice-cold H_2O prior to digestions.

Results

Inhibition of Cell-Free mRNA Translations by 7S CEH RNA. We have recently reported our preliminary findings that the 7S CEH RNA inhibits globin mRNA and rat liver poly[A(+)]RNA-dependent translations in vitro. This suggested that the observed biological activity of 7S CEH RNA in inducing a specific mode of biochemical transition in the PNP might be related, in some way, to an effect on translation process in vivo. If this is so, then it must also affect the in vitro utilization of mRNA from the same source from which the inhibitor 7S RNA was originally isolated. Figure 1 illustrates that globin mRNA as well as the total chick heart poly[A(+)]RNA-dependent translations in rabbit reticulocyte lysates were inhibited effectively. The translational activity of globin mRNA was reduced by about 87% when 1.8 μg of 7S CEH RNA was present in the incubation mixture (Figure 2). This represents a 40-fold molar excess of 7S CEH RNA over globin mRNA, assuming an approximate molecular weight

[1] Abbreviations used are: Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

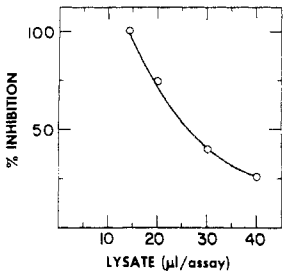

FIGURE 3: Effect of 7S CEH RNA on globin mRNA translation performed with varying amounts of rabbit reticulocyte lysate. Translation of globin mRNA (2.2 g/mL) was done in the standard 50-μL incubation mixture, except that the amount of rabbit reticulocyte lysate was varied as indicated. 7S CEH RNA, 0.9 μg, was added at the start of the incubation and 5-μL aliquots were removed after 30 min. In each case, duplicate controls (without 7S CEH RNA) were used and percent inhibition due to the inhibitor RNA was calculated from the average values of corresponding controls.

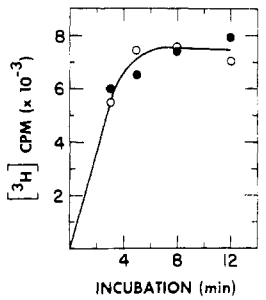

FIGURE 4: Effect of 7S CEH RNA on the endogenous activity of rabbit reticulocyte lysate in the presence of edeine. 7S CEH RNA, 1.6 μg, was added to the incubation mixture containing 20 μL of rabbit reticulocyte lysate without micrococcal nuclease and 15 μM of edeine. Ten microcuries of [^{3}H]leucine (sp act. 60 Ci/mM) was the labeled amino acid. Incubation was done in the presence of (○) and in absence of (●) of 7S CEH RNA, and 5-μL aliquots were removed at the times indicated and processed for radioactivity as before.

of 80 000 for 7S CEH RNA based on its electrophoretic mobility on 7.2% polyacrylamide in 98% formamide. Addition of 5S rRNA of *B. mori* at comparable concentrations, on the other hand, had no effect. The inhibition by 7S CEH RNA of globin mRNA translation could not be overcome by increasing the concentration of globin mRNA in the reaction mixture. The lack of stoichiometric relationship between the mRNA and the inhibitor RNA would suggest that the latter is not similar, in its mechanism of inhibition, to the myosin mRNP-tcRNA of the chick embryonic leg muscle described by Heywood and co-workers (1976). The 7S CEH RNA apparently competes with some component in the rabbit reticulocyte lysate, since increasing the amount of lysate in the reaction mixture diminished the inhibitory effect of 7S CEH RNA (Figure 3).

Mode of Action of 7S CEH RNA. Since we used the overall translation in rabbit reticulocyte lysate as the standard assay for the 7S CEH RNA activity, the site of action of the RNA and the steps involved in inhibition are not known. The inhibitor RNA from *A. salina* embryos blocks the elongation step preferentially (Lee-Huang et al., 1977), whereas the tcRNA of chick leg muscle appears to act by way of interaction with messenger RNA (Heywood and Kennedy, 1976). As a preliminary step toward investigating the mechanism of 7S CEH RNA inhibition of protein synthesis, the RNA was added to rabbit reticulocyte lysate (not treated with nuclease) in the presence or absence of the antibiotic, edeine, which is known to prevent a chain-initiation process (Pestka, 1977). As shown in Figure 4, the stimulation of [^{3}H]leucine incorporation,

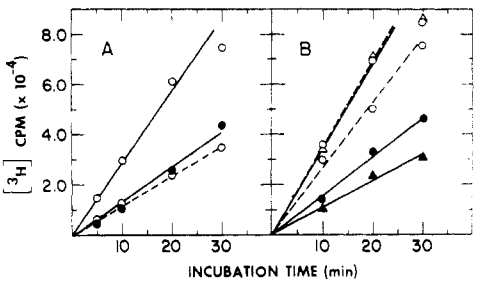

FIGURE 5: Effect of RNase treatment of inhibitor RNA on globin mRNA translations. Translation of globin mRNA was done under standard incubation conditions using 10 μCi of [^{3}H]leucine as before. 7S CEH RNA, 0.64 μg, or synthetic poly(A), 0.4 μg, alone or after digestion with nuclease as described under "Materials and Methods" was added at the start of incubation, and radioactivity in 5-μL aliquots was measured at the times indicated. (A) Effect of pancreatic RNase digestion of 7S CEH RNA on globin mRNA translation: (○) control; (●) plus 7S CEH RNA; (○- -○) plus 7S CEH RNA after pancreatic RNase digestion. (B) Effect of micrococcal nuclease digestion of 7S CEH RNA or of synthetic poly(A) on globin mRNA: (○) control; (●) plus 7S CEH RNA; (○- -○) plus 7S CEH RNA after micrococcal nuclease digestion; (△) plus poly(A); (△- -△) plus poly(A) after nuclease treatment.

which should reflect the elongation of preexisting polypeptides, was the same whether the 7S CEH RNA was present or not, suggesting that some step(s) in the initiation process of protein synthesis is the likely site of inhibition by 7S CEH RNA.

The previously described low-molecular-weight RNAs implicated in the control of translation are rich in one or the other nucleoside content. Heywood's tcRNA from chick leg muscle, for example, contains 48% U (Heywood and Kennedy, 1976), Bogdanovsky's rabbit reticulocyte RNA contains 46% A (Bogdanovsky et al., 1973; Lee-Huang et al., 1977), the inhibitor RNA from *A. salina* has 47% U, and the activator RNA from the same source contains 51% G (Lee-Huang et al., 1977). Since the 7S CEH RNA described in these studies is rich in A (53%) and contains poly(A) tract(s) (Deshpande et al., 1977), we deemed it necessary to ascertain whether the inhibitory effect of the RNA could possibly be due to the poly(A) tract(s) alone. Single-stranded poly(A), poly(U), poly(I) and double stranded RNA have previously been shown to cause inhibition of cell-free translations (Darnbrough et al., 1972; Hunter et al., 1972; Hardesty et al., 1963). For this purpose, the 7S CEH RNA was first digested with insoluble pancreatic RNase, and the supernatant obtained after centrifugation, which should contain the undigested poly(A) fragment(s), was tested for globin mRNA translation. As indicated in Figure 5A, the nuclease-digested material was as active in inhibiting translation as the control. Under the same conditions of digestion, 5S [^{32}P]rRNA of *B. mori* became 95% CCl_3COOH soluble. Digestion with micrococcal nuclease, on the other hand, resulted in a significant loss of inhibition due to RNA (Figure 5B). The inhibitory effect, in fact, was mimicked by synthetic poly(A)$_{11-19}$, which when added to the translation mixture at comparable concentrations caused an inhibition of amino acid incorporation, and treatment of poly(A) with micrococcal nuclease totally diminished the inhibition. These results, therefore, suggested that the 7S CEH RNA inhibition of translation, for at least globin mRNA, might be due to the poly(A) tract(s) present in the RNA. Poly(A) also inhibited the translation of total chick embryonic heart poly[A(+)]RNA as well as that of globin mRNA (see Figure 6). It is possible, however, that the inhibition of chick heart poly[A(+)]RNA due to 7S CEH RNA, at least at a specific concentration, may be selective for some fraction of translatable mRNA in the total chick embryonic heart poly[A(+)]RNA population, and the effect produced by

synthetic poly(A) (or by the digestion product of 7S CEH RNA) may not be the same. We, therefore, examined the translational products of the chick heart poly[A(+)]RNA at several concentrations of 7S CEH RNA and synthetic poly(A) on 10% polyacrylamide gels in $NaDodSO_4$ (Figure 6). The patterns of labeled polypeptides obtained after 30, 50, and 70% inhibition of translations due to the two RNA were identical. All bands appeared to be inhibited without an apparent difference in intensity of label or their migration properties. The presumptive heavy-chain myosin, that should migrate with a mobility of about 200 000 mol wt, was made in considerably less amounts than expected in both inhibited and uninhibited mixtures. In most translation assays, the heavy-chain myosin-like band was almost absent, although bands in the region of 100 000 to 150 000 mol wt were visible. We do not, at present, know whether this is due to the possible degradation of the myosin heavy chain or its incomplete synthesis. It is, therefore, difficult to evaluate the specificity of inhibition, if any, by 7S CEH RNA for the myosin heavy chain mRNA translation.

Thus, it appears that the effect of 7S CEH RNA on in vitro translations is due to the poly(A)-rich segment of the RNA, but it is certain that the biological activity of 7S CEH RNA on PNP is not due to the poly(A) segment of the RNA, since neither the poly(A)-containing fragment(s) generated by nuclease digestion of 7S CEH RNA alone nor synthetic poly(A) at various concentrations is able to cause any morphological and biochemical changes in the PNP (Deshpande et al., 1977; Deshpande and Siddiqui, 1977). Poly(A)-containing RNA from a variety of other sources is also not effective, suggesting a great degree of specificity for the phenomenon.

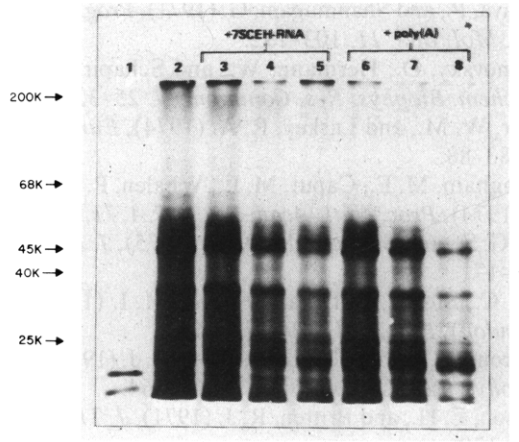

FIGURE 6: Polyacrylamide gel-separation pattern of labeled translational products of chick heart poly[A(+)]RNA in the presence or absence of inhibitor RNA. Translation of chick heart poly[A(+)]RNA, 20 μg/mL, was done under the standard incubation conditions with or without 7S CEH RNA or poly(A), and the aliquots were examined by electrophoresis as described under "Materials and Methods". Nonlabeled marker proteins were run simultaneously on the same gel and located by staining with Coomasie blue. Arrows indicate the position of the marker proteins. (1) Control nuclease treated lysate; (2), chick heart poly[A(+)]RNA; (3, 4, and 5) poly[A(+)]RNA plus 0.32, 0.54, and 1.1 μg of 7S CEH RNA, respectively; (6, 7, and 8) the same as 2 but contain 0.23, 0.46, and 0.92 μg of poly(A), respectively.

Discussion

Embryonic development and differentiation are controlled possibly by a range of components, the nature of which remains obscure. Both RNA and proteins have been implicated as regulatory elements affecting the pattern of gene activity during development (Davidson and Britten, 1971; Dickson and Robertson, 1976), but the precise nature of these elements and the mechanisms by which they would control the normal cellular processes are not known. In a recent communication (Deshpande et al., 1977), we reported the presence of a low-molecular-weight RNA fraction (7S CEH RNA) in the embryonic chick heart. A unique property of the RNA is its ability to cause a specific mode of differentiative transition in the otherwise undifferentiated cells of an early stage chick embryo. The RNA also shares some characteristics previously described for the low-molecular-weight RNA species isolated from embryonic chick muscle (Bester et al., 1975; Heywood and Kennedy, 1976), *A. salina* embryos (Lee-Huang et al., 1977), rabbit reticulocytes (Bogdanovsky et al., 1973), and Novikoff hepatoma ascites cells (Rao et al., 1977). These RNAs have been shown to participate as modulators of protein synthesis in cell-free translational systems and, therefore, are implicated to play a role in the regulation of cell development and differentiation. In the present communication, we report that the 7S CEH RNA is capable of inhibiting the in vitro translation of both homologous and heterologous mRNAs effectively. The inhibition is noncompetitive with respect to mRNA and is overcome by some factor(s) present in the rabbit reticulocyte lysate, a feature similar to that of a U1 RNA inhibition of translation in the wheat germ extract (Rao et al., 1977). The inhibition, however, appears to be effected by the adenine-rich segment(s) of 7S CEH RNA. It would be of interest, therefore, to examine whether the translation inhibitory effects of previously reported tcRNA can be discriminated from those likely to be produced by the corresponding nucleotide-rich polynucleotides.

The relationship between the 7S CEH RNA induced biological transition in the PNP and the inhibition of in vitro translations due to the RNA is not totally clear. Since the product of pancreatic RNase digestion of 7S CEH RNA results in a loss of its inducing activity and the fact that synthetic poly(A) alone, at several concentrations, cannot reproduce the effect of 7S CEH RNA on PNP, it would be reasonable to assume that a control at the level of translation, based on the observations on in vitro translational assays, is not likely the mechanism of action of the RNA on PNP. However, a mechanism envisaging a selective discrimination of specific mRNA translation under distinct physiological conditions, which are not necessarily duplicated by rabbit reticulocyte lysates, cannot be excluded. A control at the translation level is reported to exist in the primary myoblast cell culture, the terminal differentiation of which, with respect to muscle-specific protein synthesis, is preceded by the stabilization of myosin mRNA and its subsequent translation (Buckingham et al., 1974). Such a stabilization of mRNA can be visualized by way of prevention of specific mRNA translations in vivo due to an inhibitor RNA molecule. It was suggested that the inhibition of protein synthesis due to RNA in *A. salina* is overcome by an activator RNA molecule, thus permitting the onset of protein synthesis in the developing embryo. Whether such a molecule exists in chick embryonic heart tissue remains to be seen.

Acknowledgments

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Selectivity of RNA Chain Initiation in Vitro. 1. Analysis of RNA Initiations by Two-Dimensional Thin-Layer Chromatography of 5′-Triphosphate-Labeled Oligonucleotides†

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ABSTRACT: A method for the rapid and quantitative analysis of 5′-terminal oligonucleotides of RNAs made in vitro is described. The method involves synthesis of RNA in the presence of [γ-^{32}P]ATP or GTP, isolation of the RNA, and digestion with T1 or pancreatic ribonucleases to release labeled 5′-triphosphate terminated oligonucleotides. The oligonucleotides are then subjected to chromatography on a polyethylenimine cellulose thin-layer system using 2 M LiCl, 0.01 M EDTA (pH 6.5) in the first dimension and 1.5 M LiCl, 1.8 M formic acid, 0.005 M EDTA (pH 2.0) in the second. RNAs made with *E. coli* RNA polymerase and λcb2, T7, T4, and adenovirus 2 DNA yield characteristic fingerprint patterns. The utility of this method in studying selectivity of in vitro RNA chain initiation is discussed.

Synthesis of specific functional RNAs is dependent on the correct initiation of transcription by RNA polymerase at a specific site or sites on the DNA template. The region of DNA which signals the start of a new RNA is called the promoter (Reznikoff, 1972). Each promoter gives rise to an RNA transcript beginning with a characteristic 5′-triphosphate terminal sequence. Purified *E. coli* RNA polymerase is capable of transcribing a DNA template selectively in vitro. It has been demonstrated by hybridization, electron microscopy, and direct sequence techniques that these transcripts are selectively initiated and are homologous to in vivo RNAs from the same DNA. The factors governing this selectivity of initiation of transcription are not completely understood (for review, see Chamberlin, 1974). In order to study the selectivity of initiation, it is necessary to be able to measure initiation of the RNA at different promoters. The number and quantity of 5′-terminal

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