μc/mg) (*6*) was treated with concentrated sulfuric acid to give $H^{14}CN$ which was collected by a vacuum line and trapped at 77°K. Ammonium cyanide solutions were prepared by adding NH_4OH solutions directly to the labeled HCN. Chromatography of the unirradiated solutions indicated the absence of any detectable cyanamide or dicyandiamide.

For the ultraviolet irradiations the solutions (Table 1) were placed in quartz tubes and irradiated for 20 hours with a high-pressure mercury arc (*7*) at a distance of 7.5 cm. During the irradiations the samples were kept at 25° to 35°C by an air stream. After irradiation, the reaction mixtures were evaporated to dryness at room temperature at reduced pressure, and the total (nonvolatile) radioactivity was determined. Portions were subjected to paper chromatography on Whatman No. 4 paper (washed with oxalic acid) or on "Ederol" chromatography paper (*8*). The initial solvent systems used were *n*-butanol, propionic acid, and water (75:36:49 by volume) and propanol, 16*N* NH_4OH, and water (6:3:1). Radioactive spots (shown by autoradiographs) that had the same R_F values as those for cyanamide and dicyandiamide were cut out, eluted, and co-chromatographed with the authentic compounds in (i) *n*-butanol, ethanol, and water (4:1:1) and (ii) isopropanol, methanol, and water (18:1:1). The cyanamide and dicyandiamide were made visible by spraying the paper with a solution of 5 percent potassium nitroprusside, 10 percent NaOH, 3 percent H_2O_2, and water (2:1:5:15).

The electron irradiations of the $^{14}CH_4$-NH_3-H_2O mixture were carried out as described (*4*), except that no hydrogen was used in the present experiment. After the irradiation, the chromatographic search for cyanamide and dicyandiamide was done in the same way as in the case of the cyanide solution that had been irradiated (Table 1).

The foregoing experiments lend support to the idea that the cyanamide dimer (dicyandiamide) was formed on prebiotic Earth—before the era of life as we know it—and that this compound could have played a role in chemical evolution.

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Ribonucleic Acids of the Ilyanassa Embryo

Abstract. *From the* Ilyanassa *embryo an RNA component having a base composition similar to that of DNA has been separated by elution from a methylated albumin column. This material is judged to be a messenger RNA.*

The descriptive, experimental, and chemical embryology of the *Ilyanassa* egg has been reviewed (*1*). I now report the separation, and characterization by base composition, of the RNA of the embryo of the marine mud snail *I. obsoleta*. The results support the conclusion that a messenger RNA (mRNA) is synthesized by the embryo before and during formation of organ primordia.

Forty thousand 5-day *Ilyanassa* embryos reared at 19°C were incubated in sea water containing phosphoric acid–P^{32} (1 μc/ml) for 4 hours at 20°C. The embryos were washed and homogenized, and the RNA was separated according to Scherrer and Darnell's modification (*2*) of the phenol procedure. The RNA was precipitated with a mixture of sodium chloride and ethanol, dissolved in tris buffer and 0.01*M* $MgCl_2$, *p*H 6.7, and treated with crystalline deoxyribonuclease (10 μg/ml) for 30 minutes at room temperature. After dialysis against 0.01*M* tris buffer containing 0.001*M* $MgCl_2$, the dialyzate was made 0.05*M* with NaCl and sorbed onto a methylated albumin column (*3*). The RNA was eluted from the column with a linear gradient, 0.05 to 1.0*M*, of NaCl in 0.05*M* phosphate buffer, *p*H 6.7; 3-ml fractions were collected. That the eluted RNA was protein-free was shown by its minimal absorption at 230 mμ, which reflects the absence of peptide bonds; the ratio of optical density at 260 mμ was 2.27.

The RNA eluted from the methylated albumin column was hydrolyzed, after the addition of carrier RNA, with 0.3*N* KOH for 20 hours at 37°C. The hydrolyzate was brought to *p*H 7.0, sorbed onto charcoal, and eluted with 50 percent ethanol containing 1 percent NH_4OH; the eluate was lyophilized to remove the eluant, and the residue was dissolved in tris buffer and applied to a Dowex-1 formate column. The nucleotides were separated by elution with formic acid and ammonium formate, and the base composition of the labeled sample was calculated from the specific activity of each nucleotide (*4*).

The base composition of bulk RNA was determined from RNA obtained from embryos that had been reared throughout in the presence of phosphoric acid–P^{32} (1 μc/ml). Thus, all RNA synthesized during embryogenesis was uniformly labeled with phosphorus-32. The base composition was determined by isotopic dilution as described.

The DNA was obtained from the digestive gland of the adult snail (*5*) and hydrolyzed with 72 percent perchloric acid for 1 hour at 100°C; the bases were separated by paper chromatography (*6*) and quantitatively determined by spectrophotometry.

The base composition, elution sequence, and specific activity of the RNA recovered from the column (Fig. 1) establish the presence of four distinct RNA's. The radioactivity profile describes newly formed RNA, whereas the optical-density profile depicts pre-existing RNA.

The base compositions of all four RNA's (Fig. 1) and of the bulk RNA (Table 1) show that adenylic and uridylic acids are the predominant nucleotides in RNA of the *Ilyanassa* embryo. Ribonucleic acids having high adenylate and uridylate contents have also been reported for the bulk RNA of *Drosophila melanogaster* eggs (*7*) and for the nucleolar and cytoplasmic RNA's of the salivary gland of *Chironomus* larvae (*8*). Investigation of invertebrates other than echinoderms may show that RNA's rich in adenylate and uridylate occur throughout the invertebrate phyla. How-

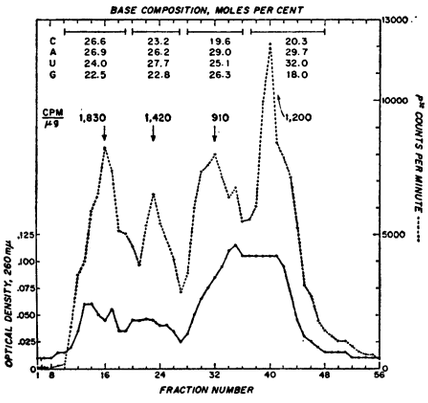

Fig. 1. Fractionation of pulse-labeled RNA. *CPM*, count/min; other abbreviations as for Table 1.

ever, the base composition of RNA from the *Ilyanassa* embryo should be confirmed by methods not dependent on P^{32}-labeling.

The order of elution of polynucleotides from a methylated albumin column is determined primarily by the molecular weight and secondarily by base composition. By these criteria the RNA of fractions 10 to 21 is transfer RNA; that of fractions 28 to 48 is probably ribosomal RNA that is unresolved or poorly resolved by the optical-density profile. The two peaks depicted by the radioactivity profile indicate that fractions 28 to 48 probably correspond in part to the characteristic RNA's of animal ribosomes. That the last component to emerge from the column, fractions 37 to 48, does not correspond entirely to ribosomal RNA is shown by its higher specific activity and its unique base composition; it is judged to be an informational or mRNA because of its singular base composition, similar to that of DNA (Table 1). Definitive interpretation of the nature of this RNA component must await demonstration of its capacity to program protein synthesis.

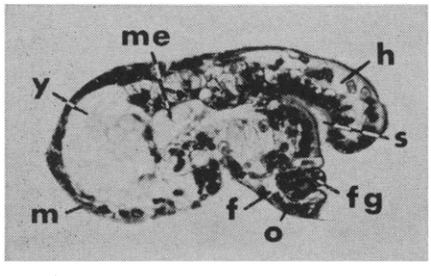

Fig. 2. Sagittal section of a 5-day *Ilyanassa* embryo, the stage that yielded mRNA. Abbreviations: *f*, foot; *fg*, foot gland; *h*, head vesicle; *m*, mantle; *me*, mesenteron anlage; *o*, operculum; *s*, stomodeum; *y*, yolk.

The nature of the RNA of fractions 21 to 28 is not clear. That this component is not DNA, although it is eluted from the column in a position corresponding to that of DNA, is shown by its resistance to treatment with deoxyribonuclease, its alkaline lability, and by the isolation from it of uridylate. Pending further evidence, the possibility should be entertained that this RNA is a degradation product of ribosomal RNA.

The specific activity for incorporation of P^{32} by the peak fraction of each of the four RNA's is of the same order of magnitude for each component. The higher specific activity of fractions 10 to 21 may reflect terminal addition of nucleotides, a characteristic of transfer RNA.

The association of the DNA-like RNA of the *Ilyanassa* embryo with ribosomal RNA probably resulted from the relatively high salt concentration used in fractionation on a methylated albumin column. Similar elution profiles were obtained for the separation of mRNA of *Escherichia coli* on a methylated albumin column (*9*). Thus it is not possible to judge the size of the DNA-like RNA of the *Ilyanassa* embryo from the experiments reported.

Brown and Littna (*10*) recently described a DNA-like 18*S* RNA in the embryo of *Xenopus laevis*, and Davidson *et al.* (*11*) report the occurrence of a 5 to 25*S* RNA with high contents of adenylate and uridylate. The oocyte RNA of *Xenopus* (*11*) is exceedingly rich in uridylate, and the RNA of fractions 37 to 48 of the *Ilyanassa* embryo differs chiefly from bulk RNA in its high uridylate content, although this RNA is also richer in adenylate. It cannot be decided at this point whether or not there is a special significance to the high uridylic acid content of these RNA's.

The rates of incorporation of P^{32} into the messenger and ribosomal RNA, as judged by specific activity, are of the same order of magnitude, indicating that these nucleic acids are synthesized at approximately the same rate. Whether this is characteristic of mRNA synthesis in this embryo or arises from the fact that ribosomal RNA also is rapidly synthesized at the stage studied was not determined.

Presence of an mRNA in the 5-day embryo correlates with the differentiation of organ primordia (Fig. 2). Synthesis of mRNA in the 3-day embryo has been observed in other experiments. These findings show that gene transcription, as evidenced by mRNA synthesis, is initiated from 24 to 36 hours before and is concurrent with organogenesis in the *Ilyanassa* embryo.

Table 1. Nucleic acid base composition (in moles percent) of the *Ilyanassa* embryo.

Base*	DNA	Bulk RNA
C	16.9	21.5
A	32.5	25.5
U (T)	33.1	27.0
G	17.1	25.8
A + U	66.0	52.5
C + G	34.0	47.3

* C, cytidylate; A, adenylate; U (T), uridylate (or thymidylate); G, guanylate.

The isolation and characterization of an mRNA in an embryo presents the possibility of isolating different mRNA's associated with the differentiation of various embryonic structures, and promises an approach to the question of whether mRNA, and therefore gene transcription, is associated with determination as well as actual differentiation. If embryonic determination, which occurs during early cleavage in the molluscan embryo, is achieved by transcription of genes either at the time a cell is determined or by the localization of preformed materials derived from a previous reading of the genome, then the question arises of whether the same genes are read at different times, once for determination and later for differentiation, or whether different groups of genes are related to these two processes.

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