EVIDENCE THAT THE s-POLYSOMES OF EARLY SEA URCHIN EMBRYOS MAY BE RESPONSIBLE FOR THE SYNTHESIS OF CHROMOSOMAL HISTONES[1]

Martin Nemer and David T. Lindsay

The Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania 19111, and Department of Zoology, University of Georgia, Athens, Georgia 30601

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SUMMARY

A class of slowly sedimenting "s-polysomes" derived from early sea urchin embryos supports incorporation of relatively high amounts of arginine and low amounts of tryptophan into nascent protein. These "s-polysomes" may be predominantly the sites of chromosomal histone synthesis.

INTRODUCTION

During early development of the sea urchin embryo, a class of slowly sedimenting "s-polysomes" accumulates as a result of the synthesis of new mRNA (Infante & Nemer, 1967). Correlated with the rise in the amount of these polysomes is a parallel rise in the synthesis of chromosomal histones through the course of development up to the early blastula (Lindsay, 1968, 1969). Both s-polysomal accumulation and chromosomal histone synthesis are inhibited by actinomycin, although the rest of the polysomal population and the bulk of protein synthesis are not immediately affected by the action of this antibiotic in blocking RNA synthesis. This correlation and the further observation in

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tissue culture cells that a class of slowly sedimenting polysomes are implicated in the synthesis of histones (Robbins & Borun, 1967; Borun, Scharff & Robbins, 1967) have led us to postulate that the polysomes responsible for chromosomal histone synthesis in the early sea urchin may belong to the class of "s-polysomes". To test this proposal, we have studied the simultaneous incorporation into the polysomes of sea urchin embryos of two amino acids: arginine, which is relatively abundant in histones, and tryptophan, which is usually not detectable in histones (Mirsky & Pollister, 1946; Hnilica, 1967).

METHODS

Gametes were obtained from Strongylocentrotus purpuratus (Pacific BioMarine Supply Co., Venice, Calif.), fertilized and developed at 18°C as described previously (Nemer & Infante, 1967). The morula stage, examined here, was reached in 8 hours. Embryos were incubated for 5 min in suspensions of 0.1 ml per 1.0 ml synthetic sea water (Nemer & Infante, 1967) with ^{3}H-tryptophan at 20 µC/ml (197 µC/µmole; New England Nuclear Corp., Boston, Mass.) and ^{14}C-arginine at 1 µC/ml (312 µC/µmole; New England Nuclear Corp.). The suspensions were diluted to 3 ml in the last minute of incubation. Embryos were centrifuged through 1 M dextrose in preparation for lysis by either homogenization by a single stroke of a tight Dounce homogenizer (Infante & Nemer, 1967) or by a modification of the method of Hinegardner (1962) to be reported separately (Nemer, 1969). The medium used for cellular lysis contained 240 mM KCl, 5 mM $MgCl_2$, and 50 mM triethanolamine HCl at pH 7.8. Cell lysates were centrifuged at 15,000 x g for 5 min, to yield cytoplasmic extracts free of nuclei and mitochondria. The cytoplasmic extracts were layered on 15 to 30% (w/w) sucrose gradients prepared in the above medium. Gradients were centrifuged for 27 min at 50,000 rev/min in the Spinco SW50 rotor. Fractions were collected after passage through a recording spectrophotometer, to measure the absorbance at 260 mµ (A_{260}). Fractions were plated on membrane filters (Infante & Nemer, 1968), dried, then assayed for radioactivity in toluene scintillation fluid.

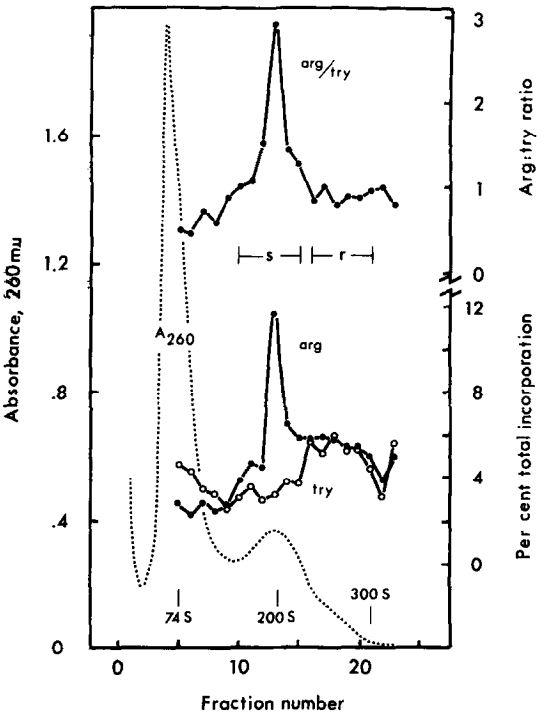

Figure 1. Incorporation of arginine and tryptophan in polysomes of sea urchin morulae. Procedures in text. Four such experiments have been performed with similar results.

RESULTS

Figure 1 is a sedimentation diagram of polysomes derived from sea urchin morulae of 100 to 200 cells. As previously shown (Infante & Nemer, 1967), two classes of polysomes are discernible, a prominent class of slowly sedimenting "s-polysomes" (mean S value of 200S) and a lesser class of rapidly sedimenting "r-polysomes" (mean S value of 300S). ^{3}H-Tryptophan and ^{14}C-arginine incorporations after 5 min pulse labeling were examined in these polysomal classes. The pattern of incorporation displayed by arginine is distinctly different from that of tryptophan. Whereas tryptophan incorporation is decidedly low in the s-polysome region, arginine incorporation is intense. On the other hand, the relative incorporations of these amino acids are essentially identical in the r-polysome region of the gradient. These

patterns are reflected by the curve in Figure 1 displaying arginine/tryptophan incorporation ratios. The s-polysomes display the highest ratio, which is, on the average of four experiments, 1.76 ± .20 times the value for the r-polysomes. The incorporations of these amino acids in polysomes after the 5-min incubation period employed represent steady state labeling of nascent protein (Nemer, unpublished) and hence reflect the amino acid composition. Therefore, the amino acid compositions of the nascent proteins of the two polysomal classes are strikingly different with respect to arginine and tryptophan.

A subclass of proteins of the s-polysomes entirely lacking in tryptophan may be histones (Phillips, 1961; Hnilica, 1967). If the area under the tryptophan curve is taken to represent the amount of protein of overall average amino acid composition, we can estimate from the additional arginine incorporation in the s-polysomes that the output of this subclass (presumed histones) may be as much as 25% of the production of proteins at this embryonic stage.

DISCUSSION

The rate of arginine incorporation into chromosomal histone rises sharply in the late cleavage stages and reaches a peak in the early blastula (Lindsay, 1968, 1969). Actinomycin inhibits this synthesis, in a manner parallel to its inhibition of the accumulation of s-polysomes during the same period. Chromosomal histone synthesis thus depends upon concomitant RNA transcription (Lindsay, 1968, 1969) and appears to be correlated with the accumulation of s-polysomes, which have been shown to contain newly synthesized mRNA (Spirin & Nemer, 1965). On the other hand, the bulk of embryo protein synthesis at this time is independent of new RNA synthesis and its kinetics correlates closely with the accumulation and early actinomycin insensitivity of "r-polysomes" (Infante & Nemer, 1967). Cleavage and early blastula are periods of intense DNA replication and cell division, possibly involving a close linkage between

DNA and histone synthesis (Prescott, 1966; Robbins & Borun, 1967; Hardin, Einem & Lindsay, 1967). It is therefore reasonable to find at these stages increasing synthesis of chromosomal histone and accumulation of s-polysomes supporting histone-like incorporation of arginine and tryptophan.

We have demonstrated that the prominent s-polysomes are responsible for the synthesis of a special class of proteins, whose amino acid composition differs markedly from that of proteins generally synthesized by the embryo. The arginine/tryptophan incorporation ratio of these nascent proteins is consistent with the formulation that they are largely histones (Phillips, 1961; Hnilica, 1967). Further investigation is now indicated to establish with certainty their histone character and their relationship to chromosomal histones.

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