HISTONE mRNA IN EGGS AND EMBRYOS OF STRONGYLOCENTROTUS PURPURATUS

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SUMMARY

Histone messenger RNA is detectable in both the maternal RNA which is stored in the unfertilized sea urchin egg and in the RNA species which are synthesized de novo after fertilization. Hybridization competition experiments show that sequences similar to pulse-labeled 9-12S RNA from morulae are present in total RNA from unfertilized eggs as well as that from later stages. The proportion of histone mRNA in cellular RNA increases after fertilization, reaching a maximum at the morula stage. Although these messengers are still present in hatched blastulae and gastrulae, they represent a smaller proportion of total RNA compared with earlier stages.

INTRODUCTION

Identification of histone mRNA as a component of pulse labeled 9-12S RNA in cleaving sea urchin embryos has established the fact that this message is synthesized after fertilization (1,2). However, de novo synthesis does not rule out the possibility that histone mRNA is also represented in the population of messages stored in the unfertilized egg. Mature sea urchin ova contain both the mRNA (3,4) and the auxiliary RNA and proteins necessary for their translation (5). The use of such stored components can apparently support normal development up to blastulation (6). The cellular events that occur during pre-blastula development imply that a large fraction of these maternal messages must be concerned with the synthesis of proteins necessary for cell division. On the other hand, analyses of chromosomal protein synthesis in the presence of actinomycin has suggested that not all of the histone-like peptides are synthesized on embryonic templates (7). However, direct identification of specific messages in the maternal mRNA has not been reported, although protein synthesis in the presence of actinomycin D and other data imply that the messages for microtubule

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protein (8) and ribonucleotide reductase (9) are components of the RNA stored in unfertilized eggs.

The small polysomes which are predominant in cytoplasmic extracts from morulae have received the most attention in studies of histone mRNA synthesis (1,10,11). However, cell division and histone synthesis continues through blastula and gastrula stages and small polysomes (12) and 9S mRNA (1) are both present, although not predominant, at later stages. For these reasons experiments were designed to compare the RNA present in sea urchins at various stages of development.

MATERIALS AND METHODS

Sea Urchin Embryo Culture. Strongylocentrotus purpuratus embryos were cultured at 9°C in Millipore-filtered sea water containing 250 µg/ml streptomycin and 100 µg/ml penicillin (13). One percent embryo suspensions, aerated by magnetic stirring produced morulae in 12 hr, hatched blastulae in 40 hr and gastrulae in 72 hr.

Isolation of Total Cellular RNA. De-membranized or hatched sea urchin embryos were collected by centrifugation, washed once with Millipore-filtered sea water and suspended in five volumes SD buffer (0.2 M LiCl, 0.02 M sodium acetate, pH 5.2, 0.5% SDS). Cells were broken in a Dounce B homogenizer and the RNA was phenol extracted as described elsewhere (14). All RNA preparations were treated with DNase (10 µg/ml) in 0.01 M $MgCl_2$, 0.01 M Tris, pH 7.5.

Isolation of DNA. Invertebrate DNA's were prepared from sperm or excised gonadal tissue by treatment with sodium dodecyl sulfate (SDS) and pronase (15). Liver or whole embryos were the source of vertebrate DNA's prepared by standard methods. DNA samples were treated with pancreatic RNase (Worthington) which had been heated at 80°C for 10 min to inactivate any trace amounts of DNase. RNase incubations were carried out at 37°C for 15 min in 1XSSC (0.15 M NaCl, 0.05 M sodium citrate) with 10 µg/ml RNase and approximately 1 mg/ml DNA.

Isolation of Histone mRNA. Strongylocentrotus purpuratus morulae or newly

hatched blastulae were pulse labeled with ^{3}H-uridine as previously described (16). Polyribosomes were prepared by the method of Iverson and Cohen (17). Purified RNA from isolated small polyribosomes was fractionated on sucrose gradients (16), and the pulse-labeled 9-12S RNA containing histone mRNA was used for hybridization.

RNA-DNA Filter Hybridization. DNA filters were prepared by modification of standard procedures (18). Hybrid formation was carried out in 0.2 ml 2XSSC for 16 to 18 hr at 60°C or 67°C as indicated in the figure legend.

RESULTS

Hybridization of Pulse-Labeled RNA from Morulae and Hatched Blastulae. Pulse-labeled RNA was extracted from small polyribosomes of hatched blastulae to determine if histone mRNA synthesis could be detected at a stage later than morula. The specific radioactivity of hatched blastula polyribosomal RNA was about eight-fold higher than that from morula. Hybridization competition experiments with purified ribosomal RNA (rRNA) indicated that no detectable levels of rRNA were present in the pulse-labeled RNA isolated from either morula or blastula polyribosomes (19).

Hybridization of pulse-labeled RNA from small polyribosomes of hatched blastulae with DNA from divergent organisms was used as an assay for histone mRNA. Histones IV show the lowest rate of evolutionary divergence of all proteins for which comparative amino acid sequence data is available. This extremely slow rate of change is also reflected in partially purified histone mRNA preparations, which contain RNA species that are also highly conserved in evolution (11,16,20). Hybrids formed with hatched blastula polyribosomal RNA were compared with those formed with partially purified histone mRNA from morulae to determine if polyribosomal RNA from the later stage contained RNA molecules which were similarly conserved in evolution. The thermal stabilities of these hybrids are shown in Table 1. Hybridization reactions were carried out at 60°C in 2XSSC to maximize the reaction of

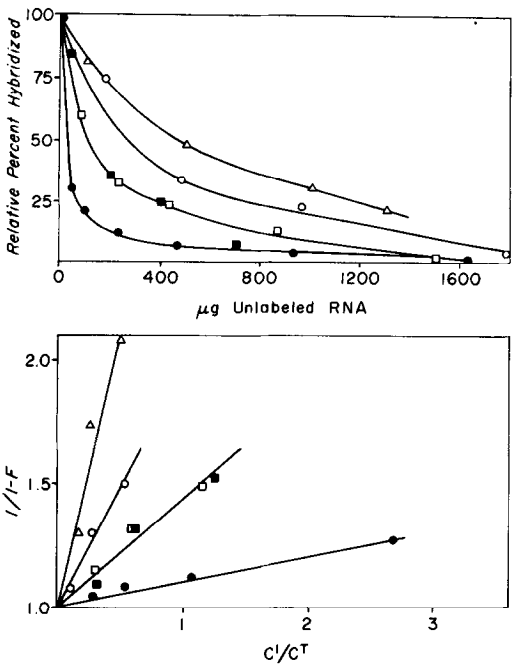

FIGURE 1. Competition hybridization of histone mRNA with total RNA from embryos at various developmental stages. Histone mRNA (0.5 μg, 700 cpm/μg) was incubated with S. purpuratus sperm DNA filters (14 μg/filter) in 0.2 ml 2XSSC at 67°C for 16 hr in the presence of the indicated amounts of unlabeled competitor RNA. Competition by (●) morula RNA, (□) unfertilized egg RNA, (■) 2 cell RNA, (○) hatched blastula RNA and (Δ) gastrula RNA are shown in the upper figure. The data are replotted in reciprocal form as described by Hansen et al (21) in the lower figure.

divergent (mismatched) sequences. The T_m values of the hybrids formed with hatched blastulae RNA and histone mRNA are analogous suggesting that similar RNA species react in each case.

Competition hybridization was used to assay for histone mRNA at other developmental stages. Pulse-labeled 9-12S RNA from morulae was incubated at relatively low RNA:DNA ratios with DNA filters in the presence of increasing amounts of total RNA from embryos at various stages of development. These conditions favor the formation of well-matched, high G+C hybrids typical of histone mRNA and minimize the reaction of other RNA sequences which are present in the 9-12S mRNA fraction (19). Hybrid formation of ^3H histone mRNA in the presence of increasing amounts of total unlabeled RNA from embryos at various stages of development is shown in Figure 1. In the upper figure

TABLE 1

Comparison of Hybrids Formed with Histone mRNA from Morulae and Polysomal RNA from Hatched Blastulae (HB)

DNA Source	T_m of Hybrid °C	
	9-12S mRNA	HB Polysomal RNA
Jellyfish	69	71
Oyster	69	70.5
Salmon	69	70
Toad	69	65.5
Chick	67.5	66
Mouse	67	66.5

^3H pulse labeled 9-12S mRNA (1.6 µg, 700 cpm/µg) or HB ^3H pulse labeled polysomal RNA (1 µg, 1500 cpm/µg) was incubated with 80-100 µg filter bound DNA in 2XSSC at 60°C for 16 hr. Thermal stabilities were measured in 1XSSC. The T_m is the temperature at which 50% of the radioactivity is eluted from the filter.

TABLE 2

Relative Amounts of Histone mRNA in Total RNA from Sea Urchin Embryos at Different Stages of Development

Stage	Relative Amount of Histone mRNA
Unfertilized egg	1.0
Two cell	1.0
Morula	4.4
Hatched blastula	0.4
Gastrula	0.2

Relative amounts of histone mRNA were calculated from ratios of the reciprocals of the slopes (21) of the hybridization competition data shown in Figure 1.

the data are plotted in standard form; complete competition was obtained with RNA from the early stage embryos and appears to be about 85% complete with RNA from gastrulae. The same data are replotted in reciprocal form as recommended by Hansen et al (21). Extrapolation of the data to infinite concentration of competitor RNA implies that total gastrula RNA does in fact contain all of the RNA sequences present in the pulse-labeled 9-12S fraction. Complete competition by RNA from unfertilized eggs with histone mRNA identifies these messengers as components of the maternal RNA stored during oogenesis.

The relative proportions of histone mRNA in various preparations can be determined by comparing the ratios of the slopes of the competition reactions when they are plotted in reciprocal form (Fig. 1). These ratios, based on an assigned value of 1.0 for the amount of histone mRNA present in unfertilized eggs, are shown in Table 2. The highest proportion of histone mRNA occurs in RNA from morulae, the stage at which the small polysomes predominate (1, 12). The relative amount of histone mRNA declines substantially during blastula and gastrula stages, probably reflecting an increase in synthesis of other types of RNA (3, 13) as well as an actual decrease in histone mRNA synthesis as the rate of cell division declines (1).

DISCUSSION

Gross and co-workers (1,22) have proposed the existence of two distinct classes of mRNA in developing sea urchins: 1) maternal mRNA, which is stored in the unfertilized egg and translated only fertilization and 2) mRNA synthesized post fertilization. Certainly both of these types of RNA exist, but it appears that many RNA molecules are found in both maternal and newly synthesized populations; i.e., synthesis of maternal type mRNA continues after fertilization (23). Clearly histone mRNA occurs in both classes. The requirement for large amounts of histone mRNA necessary during rapid cleavage is fulfilled both by storage of this RNA in the egg and through *de novo* synthesis. Little RNA synthesis occurs in developing sea urchins before the 16 cell stage (24); maternal histone mRNA is probably utilized up to this stage. However, increasing amounts of RNA synthesis

occur after the fourth cleavage and by the time the morula stage is reached, 9-12S RNA is the predominant pulse labeled species. Although histone mRNA is still synthesized in hatched blastulae, it is not the predominant fraction and is apparently representative of only a small proportion of the total RNA present in gastrulae.

The apparent short half-life of histone mRNA (25), compared to the 10-20 hrs during which large amounts of histones are synthesized, implies that the need for large amounts of mRNA is met by transcription of multiple genes. However, it is also possible that not all the histone genes are transcribed during development; perhaps the real need for all of the gene copies occurs during oogenesis while maternal messenger accumulates. A possible precedent for this notion has recently been described for 5S rRNA of Xenopus laevis: comparison of nucleotide sequences indicates that some 5S RNA genes are transcribed only during oogenesis and that such 5S RNA species are absent from somatic tissues (26).

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