Individual Regulation of the Accumulation of H1 mRNA and Core Histone mRNAs in Sea Urchin Embryos

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The relative cytoplasmic accumulation of the individual histone mRNAs in sea urchins was determined by gel analysis of ^3H -labeled cytoplasmic RNA isolated from embryos of the early cleavage through the mesenchyme blastula stages. A number of separate determinations showed that H1 mRNA accumulates at a molar ratio of 0.5 or less compared with each of the H2 or H3 core histone mRNAs through approximately the first 12 h of embryonic development. After this time, the accumulation of H1 mRNA increases relative to the core histone mRNAs, and approximately equimolar amounts of the histone mRNAs are produced by about the 14-h stage. The equimolar synthesis of H1 mRNA appears to be transient, returning to 0.5-molar levels several hours later. The increase in H1 mRNA accumulation, relative to the core histone RNAs, is coincident with the transition from expression of the early (α) sea urchin histone gene set to the late histone genes. Since all five of the early histone genes occur in a 1:1 ratio within repeating units, the data suggest that the genes within a single repeat, or their immediate products, are individually regulated. Gel analysis of the proteins synthesized *in vivo* by embryos demonstrates that the pattern of synthesis of the histone proteins reflects the changing ratios of the histone mRNAs.

In early sea urchin embryos, the operative histone genes are organized in highly reiterated, tandem repeats, with each repeat unit containing one copy of each of the five histone genes (18, 28, 30). However, this 1:1 relationship in gene number is not reflected in the histone composition of chromatin. H1 histone is found in less than equimolar amounts, relative to the core histones, in all eucaryotic chromatin examined. Evidence indicates that there is one molecule of H1 for each two of the core histones, although deviation from this ratio may occur (for reviews, see references 16 and 17). It is of interest to determine at what regulatory level(s) the reduction in ratio occurs.

The organization of the histone genes immediately suggests the possibility of a polycistronic transcript as a means to ensure coordinated synthesis of the core histones, although a post-transcriptional down-regulation of H1 synthesis would be required. The question of whether a short-lived polycistronic histone gene transcript occurs in sea urchins is not clearly resolved, but it appears unlikely (9, 21, 29). Thus, possible mechanisms for ensuring a 0.5-molar ratio of H1 relative to the core histones range from differential transcriptional rates of the individual histone genes to synthesis of equimolar amounts of H1 protein and subsequent degradation of an unutilized half.

The analysis of histone RNA in sea urchins is complicated by the contribution of a second, less well-characterized histone gene set, the late histone genes (4, 10, 18), whose products predominate by 17 to 20 h of development. By the nomenclature of Newrock et al. (24), the early histone genes code for the α -histone variants of H1, H2A, and H2B, and the late histone genes code for the β , γ , and δ histone subtypes. No differences in early and late H3 and H4 histones have been identified, although the mRNAs are clearly different (8, 18). The late mRNAs produced from this gene set differ in electrophoretic mobility from their early counterparts, and thus, the contributions of the early and late histone gene sets can be at least partially resolved on this basis.

In this report, we ask whether the histone mRNAs for all five histones are being produced in equimolar amounts. We find that the ratio of newly synthesized H1 mRNA relative to the core histone RNAs is not constant during early development.

MATERIALS AND METHODS

Growth of embryos and preparation of RNA. Eggs from the sea urchin *Strongylocentrotus purpuratus* were fertilized and grown at 17°C as described previously (14). Embryos were labeled for 1 h with a mixture of [^3H]uridine and [^3H]adenosine (10 to 20

$\mu\text{Ci/ml}$ each) before homogenization. Embryos were washed and homogenized in 20 volumes of TNM buffer (50 mM Tris [pH 7.8] at 4°C , 240 mM NH_4Cl , 5 mM magnesium acetate, and 250 mM sucrose), containing 1.5 mg of bentonite per ml, with a Dounce homogenizer. A post-mitochondrial cytoplasmic fraction was prepared from the homogenate (5), and ethanol-precipitated material was suspended in 0.01 M sodium acetate (pH 6.0), 0.1 M NaCl, 0.001 M MgCl_2 , 0.005 M disodium EDTA, and 0.5% sodium dodecyl sulfate (SDS) and extracted twice with phenol-chloroform-isoamyl alcohol (50:50:1) and once with chloroform-isoamyl alcohol (100:1). RNA was ethanol precipitated at least twice and stored in water at -80°C .

Gel electrophoresis of RNA. RNA samples were electrophoresed in 7 M urea on 11-cm, 4 to 8% or 6% polyacrylamide gels in TBE buffer (90 mM Tris base, 90 mM boric acid, 4 mM disodium EDTA, and 0.1% SDS (25)). All RNA samples, dissolved in 25 to 50 μl of RNA sample buffer (TBE buffer containing 7 M urea, 15% sucrose, and 0.01% bromophenol blue), were heated at 65°C for 3 to 5 min and quickly cooled in an ice-water bath before loading onto the gel. Electrophoresis was for 16 and 24 h for the 4 to 8% and 6% gels, respectively. Gels were fluorographed (3), and fluorographs within the linear range of exposure were quantitated by densitometry with an Ortec 4310 densitometer (20).

Method of calculation of histone RNA molar ratios. Molar ratios of histone RNAs were calculated by using the known sizes of the early and late histone messages, published by Childs et al. (4). The molar ratio of early H1 mRNA was determined relative to the band containing the early H2A, H2B, and H3 mRNAs. Because we know that the early H2A, H2B, and H3 mRNAs are made in approximately a 1:1:1 ratio from analysis of gels which resolve these three species (not shown), the density of the H2/H3 band is assigned the value 3. Therefore, the molar ratio of H1 is determined proportionally with the following formula:

$$\frac{\text{moles H1}}{\text{moles H2} + \text{H3}} = \frac{3(\text{H1})}{\text{H2} + \text{H3}} \times \frac{493}{660}$$

where H1 and H2 + H3 represent the incorporation into H1 and H2/H3 mRNAs (densities of the H1 and H2/H3 bands), respectively, as determined by scans of the fluorographic images (see, for example, Fig. 2), and 660 and 493 are the length of the H1 mRNA and the average length of the H2A, H2B, and H3 mRNAs (in nucleotides), respectively. The assumption here is that the adenosine plus uridine contents of the histone RNAs are approximately equivalent and that the labeling density is equivalent for each RNA.

The molar ratio of total H1 mRNA (early plus late species) to core histone mRNAs is calculated similarly, except that the assumption is made that all four core histone mRNAs, early and late, are synthesized in equimolar ratios. This assumption is approximately correct, but it has not been rigorously verified due to the comigration of early H4 mRNA with late histone mRNAs. H4 mRNA shows some variability in our analyses and is sometimes found in greater than equimolar amounts relative to the other core histone mRNAs. The lengths of the early H4 and late H2A, H2B, and H3 RNAs must be averaged because they overlap in the gels; however, the molecular weights of

these species are sufficiently close together that only minor error is introduced. The average length is taken to be 438 nucleotides. The lengths of late H1 RNA and the averaged late H4 RNAs are taken to be 695 and 363 nucleotides, respectively.

Preparation of in vivo-labeled proteins. In vivo-labeled proteins were prepared by a number of methods to eliminate the possibility of artifactual results due to preferential extractions of different histones by different techniques. For all preparations, embryos were concentrated to 2.5% and labeled with $[4,5\text{-}^3\text{H}]\text{lysine}$ at 15 $\mu\text{Ci/ml}$ for 1-h periods preceding each time point. Whole-cell lysates were prepared according to Arceci and Gross (1). Briefly, embryos were washed in calcium- and magnesium-free sea water containing 2 mM disodium EDTA and then dissolved in lysis buffer (4% SDS and 2 mM disodium EDTA). (i) For one preparation, the entire lysate was mixed with an equal volume of 2 $\times$ Laemmli sample buffer (19). (ii) For another preparation, calf thymus histones (Sigma Chemical Co.) were added to the lysate at a concentration of 1.3 mg/ml and vortexed extensively, and an equal volume of 2 $\times$ sample buffer was added. (iii) For the preparation shown in Fig. 6, the lysate was acid extracted in the presence of 65 mg of calf thymus histones per ml. A total of 20 volumes of 0.4 N H_2SO_4 was added to the lysate, and the mixture was kept on ice with intermittent vortexing for 5 h. The acid-insoluble material was removed by centrifugation in an Eppendorf microfuge for 15 min, and the acid-soluble macromolecules in the supernatant were precipitated in four volumes of 95% ethanol. The ethanol precipitate was washed twice in 80% ethanol and once in acetone and then dissolved in Laemmli sample buffer. (iv) Another preparation was incubated on ice for 15 min with 1 mg of protamine sulfate (salmon sperm; Sigma Chemical Co.) per ml with extensive vortexing and then acid extracted as described above. (v) In the final preparation, the lysate was ethanol precipitated and dissolved in 5 mM sodium phosphate buffer (pH 6.8) with 1 mM CaCl_2 and staphylococcal nuclease (Boehringer-Mannheim Corp.) was added to a concentration of 40 mg/ml. The mixture was incubated for 1 h at 37°C , and an equal volume of 2 $\times$ sample buffer was added.

Gel electrophoresis of acid-extracted proteins. Protein samples were electrophoresed on 11-cm, 0.75-mm-thick slab gels of 15% acrylamide with a 5% stacking gel made according to Laemmli (19). The electrode buffer, which deviates from the Laemmli procedure, was 0.383 M glycine, 0.05 M Tris base, and 0.1% SDS. Samples were electrophoresed at 175 V for about 3.5 h. Gels were fluorographed and quantitated as described for RNA gels.

RESULTS

Analysis of changes in histone RNA synthesis in early development. Striking changes in histone gene expression during early sea urchin development are evident when newly synthesized histone mRNAs are displayed electrophoretically. Figure 1 shows a developmental series of histone mRNAs labeled for 1 h with $[^3\text{H}]\text{adenosine}$ before isolation at 1-h intervals between 12 and 17 h of development (early blastula). Most striking

FIG. 1. Newly synthesized histone mRNAs from 12 to 17 h of development. Embryos were labeled with [3 H]adenosine for 1 h before each time point, and cytoplasmic RNA was isolated and subjected to electrophoresis on a 4 to 8% acrylamide gel in the presence of 7 M urea as described in the text. The early histone mRNAs are indicated by the subscript E, whereas the late histone mRNAs are designated by the subscript L.

ing is the increase in accumulation of the late histone mRNAs, of different electrophoretic mobility than the mRNAs coding for the early histones during cleavage and morula stages. The accumulation and eventual predominance of the late histone mRNA species has been well documented (4, 10). Another change in histone gene expression is the large (about 10-fold) increase in histone mRNA synthesis per embryo between early cleavage and the early blastula stage, peaking at about 9 to 10 h of development (below, see Fig. 3; see also reference 22), concomitant with the peak of the cell division rate in the embryos. Therefore, in the stages shown here (12 to 17 h), the overall rate of histone RNA synthesis is decreasing. A third type of change in expression which is less obvious is a change in the ratios of mature histone mRNAs being synthesized. This change is the main subject of this report.

Gels as in Fig. 1 may be used to describe the relative expression of individual genes within the early histone gene set by quantitating relative levels of early H1 and H2/H3 mRNAs, since none of the late messages comigrates with these in this gel system. Figure 2 shows densitometric scans of the histone RNA regions of 12- and 17-h RNAs from Fig. 1. It can be seen that the late H1 mRNA was reasonably resolved from the early form, and no late histone mRNAs migrated near the early H2/H3 mRNAs. However, changes in the ratio of synthesis of early H4 mRNAs cannot

be assessed, because some of the late histone mRNAs did overlap with the early H4 mRNA in mobility. The optical densities of the mRNAs obtained in scans as illustrated in Fig. 2 can be converted into molar ratios by using the known lengths of each histone mRNA (4) as described above.

Developmental changes in the relative synthesis of early H1 and H2/H3 mRNAs. A number of developmental series of 1-h pulse-labeled RNAs, such as in Fig. 1, were subjected to electrophoresis, and the densities of the early H1 and H2/H3 bands of fluorographs were converted to molar ratios (Fig. 3A). The ordinate in Fig. 3A is the molar ratio of H1 mRNA relative to the H2A, H2B, and H3 mRNAs, which have been assigned the value 3 (i.e., a 1:1:1 ratio, as explained above). The data demonstrate that H1 mRNA was being produced at 0.5 mol or less for each mole of the H2 and H3 mRNAs produced during early cleavage stages. At about 12 h of development a relative change in expression occurred, and by 17 h about two molecules of early H1 mRNA accumulated for each one of the early H2 and H3 mRNAs during the 1-h labeling period.

Figure 3B shows a steady and directly parallel increase in the synthesis of H1 and core histone mRNAs during the cleavage stages, with the peak of synthetic activity in this developmental series occurring at 10 h. Thereafter, the labeling of both the H1 mRNA and the core histone

FIG. 2. Densitometric scans of 12- and 17-h histone RNAs from the fluorograph shown in Fig. 1, showing the relative synthesis of the early and late histone mRNAs.

FIG. 3. Synthesis of early H1 mRNA relative to early H2/H3 mRNAs. (A) ^3H -labeled, 1-h cytoplasmic RNA from a number of developmental stages was subjected to electrophoresis. The relative incorporation into early H1 mRNA and early H2/H3 mRNAs was determined for each developmental stage by densitometric scanning of appropriately exposed fluorographs, and relative optical densities were converted into molar ratios as described in the text. Each symbol represents an independent determination with a different batch of embryos. The ordinate represents the molar ratio of early H1 mRNA relative to each one of the early H2 and H3 mRNAs. (B) Embryos were labeled for 1-h periods at several times during the first 15 h of development. Equivalent amounts of total cytoplasmic RNA from embryos at each time point were electrophoresed, and the relative incorporation into the early H1 and H2/H3 mRNAs was determined from densitometric scans of fluorographs as for Fig. 2. The maximum degree of labeling of the histone mRNAs occurred at 10 h, and these values were set equal to 100%. The dashed line gives the relative radioactivity in the H2/H3 mRNAs at the different times of labeling. The solid line shows the labeling of H1 mRNA.

mRNAs decreased, but H1 mRNA decreased less rapidly; it is this difference which results in the increased molar ratio of H1 mRNA depicted in Fig. 3A. Although the curve shown in Fig. 3B

cannot be taken to be precisely accurate, because no corrections were made for any possible changes in isotope uptake and pool specific activities which may occur during development, the overall shape of the curve is very similar to one published by Maxson and Wilt (22) showing pool-corrected rates of synthesis of total histone mRNA. An interpretation of these data is that the early H1 genes are remaining relatively more active than the early H2 and H3 genes.

Analysis of ratios of mRNAs labeled for 1-h periods, as in the experiment in Fig. 3, actually yields data on relative rates of accumulation rather than synthesis. However, the shorter the labeling period, the more closely the incorporation ratios will approximate the synthetic ratios. The histone RNAs synthesized during the labeling times ranging from 10 to 60 min were electrophoretically separated, and the fluorograph is displayed in Fig. 4B. The ratio of incorporation into H1 and core histone mRNAs remained approximately constant during the labeling period, and for the morula stage examined here, the molar ratio of H1 to core histone RNA was about 0.5 (Fig. 4A). The invariant ratio is consistent with the possibility that the molar ratios of the 1-h labeled mRNAs observed during development reflect relative rates of synthesis. This does not, however, eliminate the possible equimolar synthesis of all the histone mRNAs with a portion of H1 mRNA degrading very rapidly, thereby producing a 0.5 molar ratio.

Change in the combined expression of the early and late histone genes. The synthetic ratio of total H1 mRNA (early plus late) to total nucleosomal histone mRNAs was evaluated during the period of development (12 to 23 h) representing the transition from predominantly early histone gene expression to predominantly late. In this case, the molar ratio of H1 mRNAs was determined relative to core histone mRNAs by assuming a 1:1:1:1 ratio of synthesis of the four core histone mRNAs, as described above. Figure 5 shows the ratio of production of total H1 mRNA relative to total nucleosomal histone mRNAs. During this period of development, H1 mRNA increased from less than 0.5 to 1 mol per each mole of the averaged nucleosomal histone mRNAs. It appears that this increase in ratio is transient, as it returned to approximately 0.5 by the time the late mRNAs became predominant.

Histone synthesis during the transition period. Since we and others have previously demonstrated that more than 90% of the newly synthesized histone mRNAs are on polysomes in early blastula embryos (2, 7), these data suggested that the ratio of histones being synthesized during early development might change in concert with the changing synthetic ratio of H1 mRNA

FIG. 4. Kinetics of accumulation of cytoplasmic histone mRNAs. (A) Embryos at 9.5 h of development were incubated in [3 H]adenosine and [3 H]uridine as described in the text for the lengths of time indicated. RNA was extracted from a cytoplasmic fraction, and the specific activity of the labeled RNA was determined (●). The open symbols (○, Δ) give the molar ratios of H1 mRNA (relative to H2/H3 histone mRNAs as described in the text) for each time of incubation. (B) The fluorograph shows the newly made histone mRNAs present in the cytoplasmic extracts of embryos labeled for various lengths of time. This series of gels was used to obtain one set of the H1 mRNA molar ratios given in (A). The labeling times were 10, 20, 30, 40, 50, and 60 min and are given consecutively in lanes a through f.

to nucleosomal histone mRNAs. Histone protein synthesis *in vivo* was examined to determine whether this was the case. Embryos were labeled for 1-h periods with [3 H]lysine, and acid-soluble or total protein from the whole-cell lysate was prepared by a number of procedures. A representative gel of acid-soluble proteins is shown in Fig. 6. The proportion of H1 being synthesized relative to H2A and H2B increased during the period from early cleavage to the early blastula stage. Data from a number of separate experiments have been summarized in Fig. 7. To include data from a variety of prepara-

FIG. 5. The synthesis of total (early plus late) H1 mRNA relative to total (early plus late) nucleosomal histone RNA. Data points were generated as described in the legend to Fig. 3, except that all histone mRNAs were included in the calculation. The ordinate represents the molar ratio of H1 mRNA to each of the core histone mRNAs. Each symbol represents a different batch of embryos.

tions, the H1 to H2A/B histone ratio is plotted as the percent difference from the ratio observed at the earliest cleavage time point of each series. It is clear that the ratio of H1 synthesized relative to H2A/B does not change significantly in the period from 5 to 10 h of development. By about 16 h, however, this ratio had increased, roughly parallel with the increase observed in the synthesis of H1 mRNA relative to the core histone mRNAs.

We attribute the scatter in the data points in Fig. 7 to the fact that different protein preparation procedures invariably yield somewhat different histone ratios. The difficulty in obtaining

FIG. 6. Acid-soluble proteins synthesized *in vivo* by embryos from 8 to 16 h of development. Embryos were labeled with [3 H]lysine for 1 h, and acid-soluble protein was prepared from whole embryo lysates as described in the text (method iii). Proteins were subjected to electrophoresis on a 15% SDS-acrylamide gel.

FIG. 7. Increase in the ratio of H1 synthesis to H2A/B synthesis during early development. Proteins (labeled for 1 h *in vivo*) were prepared by five different methods from whole embryos and resolved on 15% acrylamide gels. Ratios of label incorporated into H1 to H2A/B were determined by densitometric scanning of fluorographs. Data are plotted as the percent increase in the H1 to H2A/B ratio from the ratio observed at the earliest time point (5 or 6 h) of each developmental series of proteins, i.e., the earliest data point for each series is taken to be 0 (base line). Most preparations had time points at 5, 8, and 10 h; these were all zero, and for clarity, only one symbol (x) is given on the base line at these time points. In two preparations (●, △) there was no apparent change until after 14 h.

quantitative histone yields has been discussed by several authors (6, 17, 26). Furthermore, the amount of calf thymus histone added as a carrier in any procedure has a direct effect on quantitation by fluorography and densitometry. We find that increasing amounts of calf thymus histones result in decreasing optical density of H3 and H4 histone fluorographic images. This effect is apparently due to quenching of the radioactivity of comigrating histone species in the gel (data not shown). Thus, we have chosen to present the data on histone synthesis by quantitation of the changes in the ratio of synthesis of H1 relative to the core histones, H2A and H2B, avoiding a questionable determination of the actual molar ratios of histones being synthesized. The trend during development is similar for the five preparations shown in Fig. 7. We cannot, however, eliminate the possibilities that the increase in H1 protein obtained at later developmental times might reflect a different chromatin organization, rather than increased synthesis, or that differential lysine compositions of early and late species cause the change in relative densities. Although

these data must be viewed with caution, they are supportive of the RNA data.

DISCUSSION

We have demonstrated that H1 histone mRNA does not accumulate in a fixed ratio relative to the core histone mRNAs. Calculation of the molar ratios of H1 mRNAs relative to core histone mRNAs at different developmental stages indicates that H1 mRNA is produced at less than 0.5 molar amounts during cleavage and morula stages, but during the transition to a free-swimming blastula (13 to 16 h), H1 mRNA is accumulated in amounts equimolar with core histone mRNAs.

The time of this synthetic ratio change coincides with the down-shift in the activity of the early histone gene set. The activity of these genes parallels the rate of cell division in the embryos, with both reaching a peak at about 10 h of development (11, 22, 23). The data presented in this report suggest the possibility that the down-shift in transcriptional activity is not coordinate for each of the early histone genes within a repeat unit, since H1 mRNA synthesis continued at a rate greater than that of the core histone RNAs (at least H2A, H2B, and H3 RNAs) for several hours. The changing ratios of histone mRNAs strongly suggest that the regulation of the H1 gene is independent of the core histone genes within a repeat unit. It seems unlikely that the typically reduced (less than equimolar) production of early H1 mRNA during the cleavage stages (before 12 h) is due simply to a weaker promoter mechanism, since H1 mRNA accumulation actually increases relative to that of the other histone mRNAs in the early blastula stage.

Differential rates of transcription of the individual histone genes may be responsible for regulating the ratio of newly made H1 mRNA to core histone mRNAs in the cytoplasm. This implies that during the first 10 to 12 h of development the early histone H1 gene is transcribed about one-half or less as frequently as the early histone H2 and H3 genes, whereas after 12 h, two times more early H1 mRNA is transcribed than early H2 and H3 mRNAs. Since all the genes of the early gene set are being turned off during this transition period (Fig. 3B), this change in relative expression of these genes may be due to a slower rate of repression of the H1 gene. This possibility may be tested through measurements of absolute rates of synthesis and turnover of the individual mRNAs. Although we have not rigorously demonstrated that we are observing relative rates of synthesis when we examine 1-h labeled histone mRNA ratios, the finding that the ratios of the RNAs are identical within limits of experimental measurements, whether labeled for 10 or 60 min, suggests that

the mature cytoplasmic histone messages have comparable turnover rates. Differential processing, e.g., very rapid degradation of 50 to 70% of the newly transcribed H1 RNA sequences within the nucleus during cleavage and morula stages to produce a 0.3 to 0.5 molar ratio, cannot be eliminated. Similarly, we cannot eliminate the possibility that the stability of the early H1 mRNA relative to the early H2 and H3 mRNAs increases during the period of 10 to 16 h to yield a molar ratio of about 2.0 as shown in Fig. 3A.

We have attributed the increase in H1 mRNA relative to the core histone RNAs to a prolonged production of the early H1 mRNA, because the greatest incorporation into H1 mRNA from 12 to 16 h of development is into a band of appropriate mobility. It is possible that part of that incorporation is due to a comigrating late H1 mRNA variant. We have observed a number of variants of both H1 and H4 mRNAs in different batches of embryos (unpublished data).

H1 histone appears to be produced in a fixed ratio, relative to the core histones, during the first 12 h of sea urchin development. If the relative ratios of the mRNAs are an indication of the relative rates of synthesis of their protein products, then the RNA data suggest that histone H1 may be synthesized in less than 0.5 molar amounts during early embryogenesis (see Fig. 3 and 4) and that a doubling of the relative amount of H1 synthesized may occur at a later stage. A prediction of this occurrence might be a detectable difference in chromatin structure in cleavage and morula embryos (9 to 10 h) relative to hatched blastulae (16 to 18 h). Arceci and Gross (1) have demonstrated a general decrease in nuclease susceptibility through *S. purpuratus* embryogenesis, but they do not specifically report a change in digestion properties between the morula and blastula stages which might be the consequence of increased H1 levels. Keichline and Wasserman (15) have reported that both the rate and extent of nuclease digestion decrease between morula-early blastula and late blastula stages in the sea urchin *Arbacia punctulata*. Recently, Savic et al. (27) reported an increase in nucleosome repeat lengths between 16-cell embryos and blastulae in *S. purpuratus* and *Lytechinus pictus*. Although such data are consistent with the possibility that an increased H1 content in sea urchin chromatin during blastula stages results in an observable structural change, correlations with the decrease in cell division rate or the appearance of new histone subtypes are equally valid. Clearly, a quantitative analysis of sea urchin chromatin before and after the transition period is required.

Analyses of histone synthesis in at least two early embryo types indicate that H1 is synthesized at particularly low levels during early

embryonic stages. Flynn and Woodland (6) have shown that H1 is synthesized in very low amounts relative to the core histones in *Xenopus laevis* eggs and early embryos, whereas it is synthesized and recovered from chromatin in normal amounts at the late blastula stage. A similar rise in H1, relative to the core histones, has been described in newt embryos (12, 13). Woodland (31) speculates that a possible depletion of H1 in early embryonic chromatin may be responsible for the large and diffuse character of cleavage-stage nuclei.

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LITERATURE CITED

1. Arceci, R., and P. Gross. 1980. Histone variants and chromatin structure during sea urchin development. *Dev. Biol.* 80:186-209.
2. Baker, E., and A. Infante. 1982. Non-random distribution of histone messenger RNAs into polysomes and nonpolysomal ribonucleoprotein particles in sea urchin embryos. *Proc. Natl. Acad. Sci. U.S.A.* 79:2455-2459.
3. Bonner, W., and R. Laskey. 1974. A film detection method for tritium labeled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* 46:83-88.
4. Childs, G., R. Maxson, and L. Kedes. 1979. Histone gene expression during sea urchin embryogenesis: isolation and characterization of early and late messenger RNAs of *Strongylocentrotus purpuratus* by gene-specific hybridization and template activity. *Dev. Biol.* 73:153-173.
5. Dworkin, M., and A. Infante. 1976. Relationship between the mRNA of polysomes and free ribonucleoprotein particles in the early sea urchin embryo. *Dev. Biol.* 53:73-90.
6. Flynn, J., and H. Woodland. 1980. The synthesis of histone H1 during early amphibian development. *Dev. Biol.* 75:222-230.
7. Goustin, A. S. 1981. Two temporal phases for the control of histone gene activity in cleaving sea urchin embryos (*S. purpuratus*). *Dev. Biol.* 87:163-175.
8. Grunstein, M. 1978. Hatching in the sea urchin *Lytechinus pictus* is accompanied by a shift in histone H4 gene activity. *Proc. Natl. Acad. Sci. U.S.A.* 75:4135-4139.
9. Hentschel, C., and M. Birnstiel. 1981. The organization and expression of the histone gene families. *Cell* 25:301-313.
10. Hieter, P., M. Hendricks, K. Hemminki, and E. Weinberg. 1979. Histone gene switch in the sea urchin embryo. Identification of late embryonic histone messenger ribonucleic acids and the control of their synthesis. *Biochemistry* 18:2707-2716.
11. Hinegardner, R. 1967. Echinoderms, p. 139-155. In F. Wilt and N. Wessels (ed.), *Methods in developmental biology*. Thomas Crowell Co., New York.
12. Imoh, H. 1977. Changes in H1 histone during development of newt embryos. *Exp. Cell Res.* 108:57-62.
13. Imoh, H. 1978. Re-examination of histone changes during development of newt embryos. *Exp. Cell Res.* 113:23-29.
14. Infante, A., and M. Nemer. 1968. Heterogeneous ribonucleoprotein particles in the cytoplasm of sea urchin embryos. *J. Mol. Biol.* 32:543-565.
15. Keichline, L., and P. Wasserman. 1977. Developmental study of the structure of sea urchin embryo and sperm chromatin using micrococcal nuclease. *Biochim. Biophys. Acta* 475:139-151.
16. Kornberg, R. 1974. Chromatin structure: a repeating unit

- of histones and DNA. *Science* 184:868-871.
17. Kornberg, R. 1977. Structure of chromatin. *Annu. Rev. Biochem.* 46:931-954.
 18. Kunkel, N., and E. Weinberg. 1978. Histone gene transcripts in the cleavage and mesenchyme blastula embryo of the sea urchin, *S. purpuratus*. *Cell* 14:313-326.
 19. Laemmli, U. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
 20. Laskey, R., and A. Mills. 1975. Quantitative film detection of ^3H and ^{14}C in polyacrylamide gels by fluorography. *Eur. J. Biochem.* 56:335-341.
 21. Maun, A., S. Levy, G. Childs, and L. Kedes. 1981. Monocistronic transcription is the physiological mechanism of sea urchin embryonic histone gene expression. *Mol. Cell. Biol.* 1:661-671.
 22. Maxson, R., and F. Wilt. 1981. The rate of synthesis of histone mRNA during the development of sea urchin embryos (*Strongylocentrotus purpuratus*). *Dev. Biol.* 83:380-386.
 23. Moav, B., and M. Nemer. 1971. Histone synthesis. Assignment of a special class of polyribosomes in sea urchin embryos. *Biochemistry* 10:881-888.
 24. Newrock, K., C. Alfageme, R. Nardi, and L. Cohen. 1977. Histone changes during chromatin remodeling in embryogenesis. *Cold Spring Harbor Symp. Quant. Biol.* 42:421-431.
 25. Peacock, A., and C. Dingman. 1968. Resolution of multiple ribonucleic acid species by polyacrylamide gel electrophoresis. *Biochemistry* 18:3056-3063.
 26. Ruderman, J., H. Woodland, and E. Sturgess. 1979. Modulations of histone messenger RNA during the early development of *Xenopus laevis*. *Dev. Biol.* 71:71-82.
 27. Savic, A., P. Richman, P. Williamson, and D. Poccia. 1981. Alterations in chromatin structure during early sea urchin embryogenesis. *Proc. Natl. Acad. Sci. U.S.A.* 78:3706-3710.
 28. Schaffner, W., G. Kunz, H. Daetwyler, J. Telford, H. Smith, and M. Birnstiel. 1978. Genes and spacers of cloned sea urchin histone DNA analyzed by sequencing. *Cell* 14:605-617.
 29. Spinelli, G., M. Melli, E. Arnold, C. Casano, F. Gianguzza, and M. Ciaccio. 1980. High molecular weight RNA containing histone messenger in the sea urchin *Paracentrotus lividus*. *J. Mol. Biol.* 139:111-122.
 30. Sures, I., J. Lowry, and L. Kedes. 1978. The DNA sequence of sea urchin (*S. purpuratus*) H2A, H2B, and H3 histone coding and spacer regions. *Cell* 15:1033-1044.
 31. Woodland, H. 1980. Histone synthesis during the development of *Xenopus*. *FEBS Lett.* 121:1-7.