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Quantitative and qualitative changes in histone gene expression during early mouse embryo development

(histone mRNA/S1 nuclease)

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ABSTRACT **There are large amounts of histone mRNA present in mouse eggs. These RNAs are rapidly degraded, as are other mRNAs, after fertilization and prior to the second cleavage. During cleavage, the histone mRNA accumulates as the embryo divides. The same sets of histone genes are expressed in eggs and embryos, although there are large qualitative differences in the amounts of particular histone mRNAs. The function of the egg histone mRNA is unknown. The amount of histone mRNA in cleaving and blastocyst embryos is probably sufficient to code for the blastocyst histone proteins.**

It is now clear that the transition from maternally derived transcripts to zygote genome-derived transcripts in early development of the mouse embryo takes place during the two-cell stage. This conclusion has emerged as a result of a number of genetic and biochemical studies over the past two decades but has been focused recently by several key pieces of information. Paternally derived gene products that are the result of transcriptional activity in the embryo are detectable by the late two-cell stage (1). There is a loss of total poly(A)$^+$ mRNA sequences from the egg to the two-cell stage, followed by progressive accumulation of mRNA due to new synthesis (2–4). The changes in mRNA abundance are directly related to major changes in patterns of polypeptide synthesis during the transition period from the egg to the late two-cell embryo (5, 6). The observations on changes in abundance of mRNA populations during the preimplantation developmental period of the mouse embryo have included histone H3 mRNA as an example of nonpolyadenylylated mRNA (7).

Studies of histone protein synthesis in early mouse embryos have revealed that at least some of all classes of histone proteins are synthesized during embryogenesis (8). We have described (9) the isolation of a number of histone genes from the major mouse histone gene family. The expression of individual genes can be measured quantitatively by using an S1 nuclease mapping technique. In this communication we extend the studies on histone H3 mRNA to include the mRNAs of two other nucleosomal proteins, histone H2A and H2B. The results confirm previous observations that there is a marked degradation of maternal mRNA stores during the two-cell stage and indicate that all three histone mRNAs are regulated coordinately during this period of transition from maternal to embryonic control. While histone mRNA levels in early blastocysts have increased to levels similar to that in unfertilized eggs, there are qualitative differences in the amounts of particular histone H2A and H3 mRNAs between eggs and blastula embryos.

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MATERIALS AND METHODS

Animals and Embryo Recovery. Female random-bred Swiss albino CD1 mice (Charles River Breeding Laboratories) were used throughout the study. Superovulation and embryo recovery were conducted as described (7). After injection of human chorionic gonadotrophin (HCG) (time zero), unfertilized eggs were recovered at 14–16 hr; mid-two-cell embryos from the reproductive tract of mated females, at 42–44 hr; late two-cell embryos in transition to the four-cell stage, at 48 to 50 hr; eight-cell embryos, at 66 to 68 hr; and early blastocysts of ≈32 cells, at 92 to 96 hr. Total nucleic acid was extracted from mouse eggs and embryos exactly as described by Giebelhaus *et al.* (7).

Histone DNA Probes. The genomic DNA fragments used in blot-hybridization transfer and dot-blot experiments were as follows: for histone H3, plasmid pMH3.2, which contains a 900-base-pair (bp) segment from the H3.2 histone gene; for histone H2B, plasmid pMH2B.1, which contains a 1-kilobase (kb) *Hin*cII–*Ava* I DNA fragment from the H2B.1 gene in MM221 (8); for histone H2A, plasmid pMH2A.2 which contains a 1-kb *Eco*RI–*Pst* I fragment from the H2A.2 gene from phage MM614 (9). The cloned fragments were labeled by nick-translation to a specific activity of 2×10^8 cpm/μg.

RNA·DNA Hybridization. Total nucleic acid extracted from pools of eggs or embryos was subjected to electrophoresis on a 1.5% agarose gel containing 10 mM methylmercury(II) hydroxide (10), transferred to DBM (diazobenzyloxymethyl) paper (11), and hybridized with the radiolabeled probes as described (7). Standard curves for quantitation of mRNA levels were constructed by using a purified rabbit β-globin mRNA (Bethesda Research Laboratories) and a full-length rabbit globin cDNA clone (12).

S1 Nuclease Mapping. The amount of specific histone mRNAs was determined by using an S1 nuclease assay essentially as described (8, 9). The histone genes assayed have been described by Graves *et al.* (9).

RESULTS

Total Histone mRNA Levels During Embryogenesis. Total nucleic acid was extracted from eggs and embryos, resolved by agarose gel electrophoresis, and transferred to DBM-paper. The filters were hybridized with labeled DNA probes coding for histone H3, H2B, and H2A mRNAs (Fig. 1). In each case, the levels of these mRNAs were observed to be high in the egg and markedly reduced in the two-cell embryos. The amount of histone mRNA per embryo then increased progressively to the early blastocyst stage. Reaccumulation

Abbreviations: HCG, human chorionic gonadotrophin; bp, base pair(s); kb, kilobase(s); DBM, diazobenzyloxymethyl.

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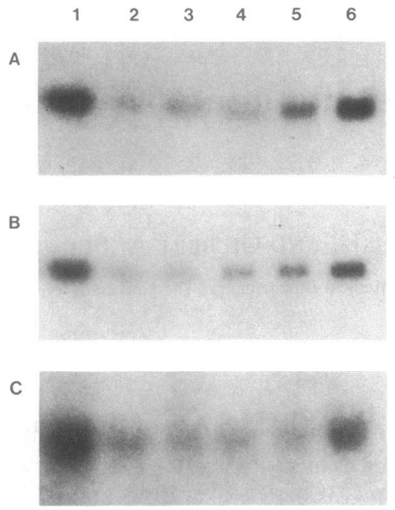

FIG. 1. Hybridization of subcloned histone DNA probes to RNA from mouse eggs and early embryos. Total RNA from pools of eggs and early embryos was resolved on 1.5% agarose gels containing 10 mM methylmercury(II) hydroxide (10) and transferred to DBM paper (11). The blots were hybridized with ^{32}P-labeled DNA probes homologous to histone H3 mRNA (*A*), histone H2A mRNA (*B*), and histone H2B mRNA (*C*). Lanes: 1, RNA from 1000 unfertilized eggs; 2, RNA from 1000–1200 mid-two-cell-stage embryos 42 hr after HCG injection; 3, RNA from 1000–1200 embryos in the transition from two-cell to four-cell 48 hr after HCG injection; 4, RNA from 500 eight-cell embryos 66 hr after HCG injection; 5, RNA from 300 early (32-cell) blastocysts 96 hr after HCG injection; and 6, RNA from 600 early blastocysts.

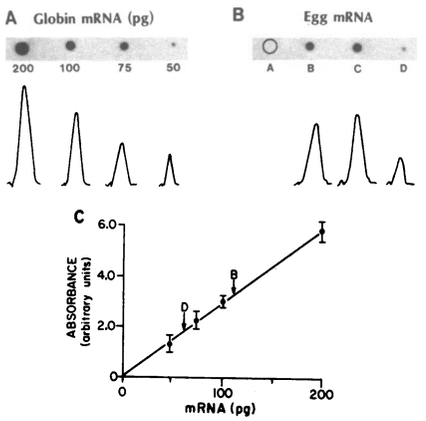

FIG. 2. Quantitation of histone H3 mRNA in unfertilized mouse eggs. DBM paper was dotted with nucleic acids as follows. (*A*) A series of purified β-globin mRNA standards ranging from 50 to 200 pg. (*B*) Total RNA from 2000 eggs treated with ribonuclease A (dot A), total RNA from 2000 eggs (dots B and C), and total RNA from 1000 eggs (dot D). Experimental procedures for the handling and hybridizing of the dot blots were the same as in Fig. 1. Absorbance measurements on the egg RNA dots were made and then converted to absolute amounts by using the standard curve (*C*).

of histone mRNA is such that the early blastocyst (32-cell embryo) has roughly the same histone content as in unfertilized eggs (in Fig. 1, compare lane 1, RNA from 1000 oocytes, with lane 6, RNA from 600 blastocysts). The differences in mRNA content observed are not due to variable losses during extraction procedures because ethidium bromide staining patterns indicated that recovery of total RNA did not vary significantly from one preparation to another (data not presented here; but see ref. 7).

We estimated the absolute amount of histone H3, H2A, and H2B mRNAs at various stages of early mouse development over the period of loss of stored maternal (oocyte) mRNA and reaccumulation from zygote genome-derived transcription. Total mouse embryo nucleic acid samples were dotted onto DBM paper and hybridized with cloned ^{32}P-labeled histone DNA probes. An example of dots of RNA from mouse eggs hybridized with a histone H3 probe are shown in Fig. 2*B*. A series of purified β-globin mRNA samples ranging from 50 to 200 pg also were dotted in each experiment as a standard (Fig. 2*A*). The calculated amounts of histone H3, H2A, and H2B mRNA at selected stages of early mouse development are summarized in Table 1. Notable is the relatively high content of mRNAs for all three histones in the egg and the marked loss of 80–90% of these mRNAs as the embryo proceeds through the two-cell stage. The lowest histone mRNA content (about 15–20 fg per embryo) was observed at the late two-cell to early four-cell (48 to 50 hr after HCG injection) stage. After the four-cell stage, the embryo has a constant amount of mRNA (about 20,000 molecules) per cell for each histone protein. Thus, the amount of histone mRNA per embryo increases as the embryo divides.

Changes in Levels of Specific Histone mRNAs During Embryogenesis. The contribution of any specific histone gene to the total histone mRNA population can be measured by S1 nuclease mapping (9). Nine histone genes (four H3, three H2B, and two H2A genes) from three different genomic fragments were used as probes. The expression of all these genes is tightly coupled with DNA synthesis. These genes are members of the family (10–20 different genes for each histone protein) that comprises the major histone genes expressed in cultured mouse cells and fetal mice (9). All of the genes in this family coding for a particular histone have highly homologous (95%) coding regions and divergent untranslated regions. Two of these gene clusters, MM221 and MM291, are located on chromosome 13, and the other, MM614, is located on chromosome 3 (D. Cox, personal communication; unpublished data). The two genes, an H3 and an H2A gene, located on chromosome 3 are expressed in large amounts (30–40% of the total histone mRNA), while the seven genes on chromosome 13 are expressed in small amounts (<10% of the total) in cultured mouse cells (9).

Fig. 3*A* shows the levels of the four H3 genes in mouse eggs and blastocysts. Because all of the H3 genes share similar coding regions, all of the H3 DNAs protect a fragment of about 180 nucleotides extending to the initiation codon, labeled H3(5′). In addition, each gene protects a larger fragment that extends to the first nucleotide of the RNA derived from this gene. Two of the mRNAs, H3.291 and H3.1.221, are expressed in low amounts in both the egg and the blastocyst. This is similar to the level of expression of these genes in cultured mouse cells. Both of these genes code for H3.1 histone subtypes. The H3.614 and H3.2.221 mRNAs differ in expression between egg and blastocysts. Both of these mRNAs are expressed in high levels in the egg and represent a much lower proportion of the H3 mRNA in the blastocyst. In cultured mouse cells, the H3.614 mRNA is an abundant mRNA with levels similar to that found in the mouse egg (see Table 2). The low level of H3.614 mRNA in the blastocyst is still higher than that of the other H3 mRNAs studied. In contrast, the high levels of H3.2.221 mRNA in the egg have not been observed in other cell types. The low level of this mRNA in the blastocyst sample is similar to the level of this mRNA in cultured cells.

These results were confirmed by S1 nuclease mapping of the 3′ ends of the H3.614 and H3.2.221 mRNA (Fig. 3*B*). The 3′ ends of the histone mRNAs in the egg are the same as the 3′ ends in somatic cells.

Fig. 3*C* shows the results for the H2A genes. One of the H2A genes shows a striking variation between the egg and the blastula. The H2A.614 gene is by far the most prominent H2A

Table 1. Amounts of histone mRNA during early mouse development

Stage of development*	mRNA per egg or embryo,† fg			Molecules × 10^{-3} (per cell)
	H3	H2a	H2b	
Egg (14–16)	167 ± 9 (8)	146 ± 6 (4)	152 ± 8 (4)	610 (610)
Cells in zygote				
1 (30–32)	155 ± 6 (3)	ND	ND	570 (570)
2 (36–38)	59 ± 7 (3)	ND	ND	220 (110)
2 (42–44)	23 ± 7 (6)	23 ± 6 (4)	27 ± 4 (4)	82 (41)
2 (44–48)	16 ± 4 (3)	19 ± 4 (4)	20 ± 6 (4)	60 (30)
4 (54–56)	20 ± 6 (3)	ND	ND	75 (19)
8 (66–68)	41 ± 6 (7)	45 ± 4 (4)	60 ± 6 (4)	150 (19)
32 (92–96)	149 ± 14 (7)	146 ± 15 (4)	142 ± 12 (4)	550 (17)

Densitometric scans were made through the center of each dot (Fig. 2), and the amount of histone mRNA was determined by using the hybridization of globin mRNA to globin cDNA to construct a standard curve (Fig. 2). The values are corrected for recovery (78%) and for the fact that the histone probes contained only a portion of the histone mRNA. The molecules of mRNA per embryo and per cell were calculated from the amounts of histone H3 mRNA.

*Numbers in parentheses are the hours after HCG administration.

†Values are means ± SD. Numbers in parentheses indicate the number of independent assays on pools of 500–1000 eggs or embryos; ND indicates not determined.

mRNA (>90% of the total) in eggs. This is shown in Fig. 3*C*, lanes 1 and 4, with S1 nuclease mapping from the *Xho* II and *Pst* I site, respectively. In contrast, in the blastocyst sample this RNA, although still abundant, is a much lower fraction of the total H2A mRNA and is found in similar amounts as in cultured cells. This RNA codes for an H2A.2 protein that is a minor (30–40%) component of the H2A histone in cultured cells. The H2A (var.) fragment (Fig. 3*C*, lanes 5 and 6) is due to cleavage of the mRNA·DNA hybrid at codon 16, the amino acid change between histones H2A.1 and H2A.2 (9). The H2A.614 gene is located within 1 kb of the H3.614 gene. This RNA shows the same pattern of expression as the H3.614 RNA (high in the egg and lower in the blastocyst), although quantitatively the H2A.614 RNA is expressed more than the H3.614 RNA at both stages. The other H2A mRNA assayed, H2A.291A, is expressed at low levels in both eggs and blastocysts. There is an H2A pseudogene closely linked to the H2B.291B gene, and band X is due to protection of this pseudogene by H2A mRNA to the point of divergence of the pseudogene and the real gene (9).

The three H2B genes analyzed are all expressed in low amounts in both egg and blastocyst. These three genes are also expressed in low amounts in cultured cells. One of these genes, H2B.221, is within 1 kb of the H3.2.221 gene, and these genes are transcribed from opposite strands of the DNA with their 5′ ends juxtaposed. This allows us to simultaneously assay both mRNAs by using an *Ava* I–*Sal* I fragment extending from codon 92 of the H2B to codon 58 of the H3 gene. The result is shown in Fig. 3*B*, lanes 14–16, and confirms the high level of H3.2.221 mRNA in the egg shown in Fig. 3*A*. The tightly linked H2B.221 gene is expressed in low amounts in both eggs and blastocysts. The changes in specific histone mRNA levels are summarized in Table 2.

DISCUSSION

The metabolism of histones and histone mRNA has been studied in the early development of several organisms: *Drosophila*, sea urchins, and *Xenopus*. The early development of these organisms differs from that of mammals in that the early DNA replications occur very rapidly without concomitant growth of the embryo. These three organisms solve the problem of providing large amounts of histones for the early cleavage stages by storing histones (*Xenopus*; refs. 15 and 16) and/or histone mRNA during oogenesis. No new mRNA is synthesized in the *Xenopus* embryo until the midblastula transition. In *Drosophila* (17) and sea urchin (18), there is a large store of maternal mRNA, which serves to provide the templates for histones during the early rapid DNA replication cycles. The histone genes are also very active during rapid cleavage, and both maternal and embryonic mRNAs contribute to the histone mRNA pool of the embryo during the period of most rapid DNA synthesis (17, 19). There is a well-documented change in the histone gene sets during early sea urchin development (20, 21). In contrast, in *Xenopus* (22) and in *Drosophila* (17), there is no change in the histone gene sets active in development.

Recent studies on mRNA and protein synthesis in early mouse embryos have shed some light on the changes that take place during early mouse embryogenesis. The mouse egg contains 350 pg of total RNA of which 6.5% (23 pg) has been estimated to contain poly(A) sequences (4). In the early blastocyst (32-cell stage) the amount of RNA has increased to 1420 pg of total RNA of which about 3% (42 pg) is poly(A)$^+$ mRNA. We estimate there is 160 fg of histone mRNA (which is not polyadenylylated) for each histone protein in the egg, or a total of 640 fg of total nucleosomal histone mRNA, which is equivalent to 3.0% of the putative [poly(A)$^+$] mRNA pool. In the blastocyst, the total histone mRNA content is about 1.6% of the total poly(A)$^+$ pool.

The histone mRNA content in late two-cell embryos is only about 10% of the amount in unfertilized eggs. The mRNA for three histone mRNA classes is degraded and resynthesized with similar kinetics. The decay (loss) of maternal histone mRNA parallels that of total poly(A)$^+$ mRNA (4) and actin mRNA (23). From *in vitro* translation experiments with RNA derived from two-cell embryos cultured in the presence or absence of the transcriptional inhibitor α-amanitin, it now seems clear that the transition from maternal to embryonic control of development occurs during the two-cell stage (5, 6). The late two-cell to early four-cell histone mRNA populations (and those of later stages) are probably derived from the zygote genome, while levels at the early- to mid-two-cell stage are a mixture of both maternal and zygote-genome-derived transcripts.

A mouse embryo blastula cell contains the same amount of histone mRNA as does a rapidly growing cultured cell. Thus, the cells in the embryo contain sufficient histone mRNA to code for their own histone protein. In contrast, a mouse egg contains at least 20 times the amount of histone mRNA (and total mRNA) as in a growing cell. About 2% of the protein made by mouse eggs is histone (13), consistent with the fact that 3% of the mRNA in mouse eggs is histone mRNA. The egg has a low efficiency of translation relative to the embryo (24, 25). The limited translation capacity has been confirmed by mRNA microinjection experiments (25). Since the early

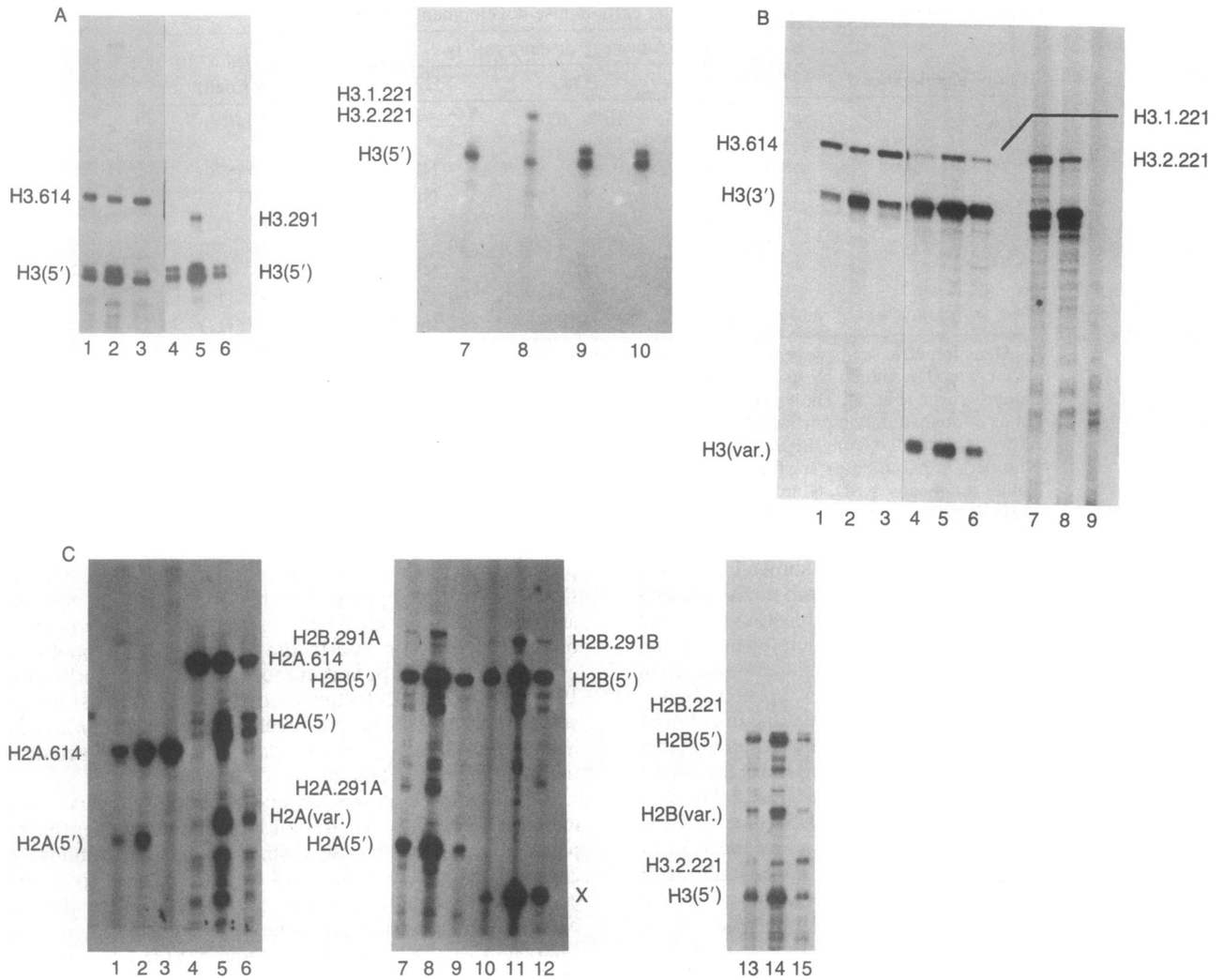

FIG. 3. Levels of specific histone mRNAs in egg and blastocysts. The amount of specific histone mRNAs was determined by using an S1 nuclease assay essentially as described (9, 13, 14). The H3 genes were digested with *Sal* I, which cleaves the genes at codon 58. The H2A.614 gene was digested with either *Xho* II (codon 61) or *Pst* I (codon 85). The H2A and H2B genes on MM291 are tightly linked and transcribed from opposite strands of the DNA with their 5′ ends juxtaposed. The genes were digested with *Xho* II (which released a 600-base fragment extending from codon 94 of the H2B gene to codon 61 of the H2A gene). This fragment was end-labeled, and both H2A and H2B mRNAs were measured simultaneously. The H2B and H3.2 genes on MM221 were assayed simultaneously by using an *Ava* I–*Sal* I fragment extending from codon 92 of the H2B.221 gene to codon 58 of the H3.2.221 gene (8). For S1 nuclease mapping of the 3′ end of the H3.2.221 gene and the H3.614 gene, the DNA was labeled at the *Sal* I site (8). RNA from 50 or 100 eggs or blastocyst embryos was hybridized with 20 fmol of end-labeled DNA for 16 hr (8). The hybrids were digested with S1 nuclease, and the resistant DNA fragments were resolved by polyacrylamide gel electrophoresis in 7 M urea. The fragments denoted H3(5′), H2A(5′), and H2B(5′) result from protection to the AUG codon by a family of histone mRNAs that share common coding regions; fragments denoted H3.614, etc., result from protection due to the specific mRNA derived from a particular gene. The H3(3′) fragment extends to the TAA codon at the end of the H3 coding regions. The "614," "221," or "291" refers to the particular recombinant phage from which the gene was derived (9). The variant H3(var.), H2A(var.) and H2B(var.) fragments result from cleavages within the coding regions at known amino acid changes in histone proteins (8, 9). (*A*) H3 genes 5′ end-labeled at the *Sal* I site: H3.614 (lanes 1–3), H3.291 (lanes 4–6), H3.2.221 (lanes 7 and 8), and H3.1.221 (lanes 9 and 10). Lanes: 1 and 6, myeloma RNA (70 ng, 3500 cells); 2 and 5, blastocyst RNA (186 ng, 120 embryos); 3 and 4, egg RNA (46 ng, 120 eggs); 7 and 9, egg RNA (27 ng, 77 eggs); 8 and 10, blastocyst RNA (110 ng, 77 embryos). The samples in lanes 7–10 are from different pools of blastocysts and eggs than the samples used for the other assays in this figure. These blastocysts had probably undergone one less division than the other samples, accounting for the smaller amounts of histone mRNA in these blastocysts compared with the egg RNA. (*B*) H3 genes 3′ end-labeled at the *Sal* I site: H3.614 (lanes 1–3), H3.1.221 (lanes 4–6), and H3.2.221 (lanes 7–9). Lanes: 1, 6, and 8, myeloma RNA (92 ng, 4600 cells); 3, 4, and 7, egg RNA (60 ng, 160 eggs); 2 and 5, blastocyst RNA (176 ng, 115 embryos); 9, yeast tRNA (5 μgms). (*C*) H2A and H2B genes: H2A.614, labeled at *Xho* II site (lanes 1–3); H2A.614, labeled at *Pst* I site (lanes 4–6); H2A.291 and H2B.291, labeled *Xho* II fragments extending from H2B codon 94 to H2A codon 61 for the two H2A–H2B pairs [A (lanes 7–9) and B (lanes 10–12) from recombinant phage MM291; fragment X is due to protection of part of the H2A.291B pseudogene (9)]; and H2B.221 and H3.2.221, a labeled *Ava* I–*Sal* I fragment extending from H2B codon 92 to H3 codon 58 (8) (lanes 13–15). Lanes: 1 and 6, myeloma RNA (46 ng, 2300 cells); 2, 5, 8, 11, and 14, blastocyst RNA (185 ng, 120 embryos); 3, 4, 9, 10, and 15, egg RNA (46 ng, 120 eggs); 7, 12, and 13, myeloma RNA (70 ng, 3500 cells).

blastocyst embryo with the same amount of mRNA synthesizes 5 times as much total protein as well as histone protein (13), this suggests that the translation of all mRNAs (including histone mRNA) increases 4- to 5-fold between egg and blastula (13). If we assume that the 32-cell early-blastocyst embryo synthesizes all of its histone protein in an 8-hr S phase, then the mouse egg could synthesize a haploid complement of histone protein in 2–3 hr. It is probable that the mouse egg accumulates histone proteins, and these could possibly be used during early development, although the cleaving embryo also synthesizes substantial amounts of histone proteins. One definite function of the egg histones is to displace the sperm protamines after fertilization (14).

The S1 nuclease assay demonstrates that the representa-

Table 2. Relative abundance of specific histone gene transcripts during early mouse development

Histone gene	Egg	Blastocyst	Myeloma
H3.2.614	46	14	45
H3.2.221	42	7	7
H3.1.221	3	8	6
H3.1.291	<3	7	9
H2A.614	>90	30	33
H2A.291A	<3	11	10
H2B.221	<5	<5	6
H2B.291A	<3	5	8
H2B.291B	<3	12	11

The abundance of specific histone mRNAs relative to the total histone mRNA (of that class) detected was determined by densitometric scanning of the autoradiographs. The data is presented as a percentage of the total amount of histone mRNA of that class (e.g., H3 mRNA). Because of difficulties in comparing two bands of widely varying intensities, the values for the minor histone mRNAs represent the maximum possible value for these mRNAs. The values for the myeloma cells are taken from ref. 9. Each value represents the average of at least two independent determinations.

tion of different histone mRNAs changes during development. The mouse egg contains large amounts of the H3.614 mRNA and the H3.2.221 mRNA. The levels of the H3.614 mRNA are similar (proportion of total H3 mRNA) to the level in cultured mouse cells and 18-day fetal mice (9). The levels of H3.2 mRNA are greatly elevated and nearly all of the H2A mRNA detected is H2A.614 mRNA. In the blastocysts the proportion of all of these mRNAs is greatly reduced. In particular the H3.614 mRNA is a lower proportion of the total H3 mRNA than in any other cells examined. The proportion of H3.2.221 and H2A.614 mRNAs is similar to that in other mouse cells.

The amount of a particular mRNA is determined both by its rate of synthesis and degradation. The level of histone mRNAs in cultured mouse cells is controlled by altering both the rate of mRNA synthesis and mRNA degradation (26, 27). Thus, changes in the proportion of individual mRNAs could be due to variations in either synthesis or degradation rate. It is possible that the high levels of the H3.614, H2A.614, and H3.2.221 mRNAs in the egg are due to increased stability of these mRNAs compared to other mRNAs and not to differences in rate of synthesis. However, it is known that the relatively high amounts of H3.614 and H2A.614 mRNA in cultured cells are due to an increased rate of synthesis (28).

A consequence of the qualitative variation in histone mRNA levels is a change in the histone protein variants expressed in the egg and blastocyst. The H2A mRNAs in the egg code almost exclusively for the H2A.2 protein and the H3 mRNAs for the H3.2 protein. Since the genes we have analyzed together code for at most half of the H2A and H3 mRNA in the blastocyst, we cannot draw firm conclusions about the histone protein variants expressed in the blastocyst. It is possible that the altered pattern of histone protein variants, particularly the large increase in H2A.2, plays a role after fertilization or in early cleavage.

This study demonstrates that it is possible to measure levels of specific RNAs by S1 nuclease mapping in mouse eggs and embryos. The rare histone mRNAs we detect (with an exposure time of 24 hr and with RNA from 100 blastocysts) are present at about 600 copies per blastocyst cell. By increasing the specific activity of the probe and using more RNA, and longer exposure times, it should be possible to detect relatively rare mRNAs (25–50 molecules per cell). The study of the mRNAs from many specific genes in mouse embryos is attainable.

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