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Biphasic pattern of histone gene expression during *Drosophila* oogenesis

(oocyte/development/mRNA translation)

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ABSTRACT **The expression of histone genes during *Drosophila* oogenesis was compared to periods of DNA synthesis as well as to the pattern of actin gene expression. Accumulation of histone mRNAs was measured by RNA blot hybridization. Relatively low levels of histone mRNAs are present in egg chambers prior to stage 10, during the period of nurse and follicle cell polyploidization. Surprisingly, histone mRNAs accumulate rapidly and selectively after stage 10, coinciding with the onset of nurse cell degeneration and well after DNA synthesis and actin mRNA accumulation have ceased. A large proportion of the histone mRNAs is associated with polysomes at all times, indicating that expression of histone genes is not strictly coupled to DNA synthesis. The burst of histone mRNA accumulation near the end of oogenesis may provide a store of maternal histone mRNA to support the rapid cleavages that occur during early embryogenesis. These and previous results suggest that genes are independently regulated during differentiation of the *Drosophila* egg chamber.**

The *Drosophila* egg chamber consists of three cell types. A single oocyte is connected through cytoplasmic bridges to 15 sister nurse cells. Follicle cells surround this 16-cell complex. Oogenesis is extremely rapid: a stage 1 egg chamber grows about 5000-fold in a little over 3 days to become a mature stage 14 oocyte (1, 2). Growth is quasi-exponential; the bulk of the egg chamber components accumulate during the last day. Related to this intense gene activity, nurse and follicle cell nuclei become highly polyploid (3, 4) and extremely active in transcription, while the oocyte remains quiescent. Dramatic morphological changes occur during the last 5 hr of oogenesis, between stages 11 and 14: the nurse cells degenerate and transfer their contents to the oocyte, while the follicle cells secrete the egg shell, flatten, and are sloughed off during ovideposition. Although protein accumulation ceases abruptly at around stage 11, the polysome content remains high. This may be due to a drop in translational efficiency by a factor of ≈20 that occurs at this time (5). The oocyte stores about 6000 different RNA sequences (6), most of which are accumulated from the beginning of oogenesis (7).

It is not known if this overall pattern of gene expression during oogenesis is a composite of widely different profiles or if genes are regulated coordinately. We have recently shown that actin genes are preferentially expressed during oogenesis (8). Here we examine the expression of the histone genes and relate it to the expression of actin genes and to the periods of DNA synthesis during oogenesis and embryogenesis. We find that histone genes are uniquely regulated. Surprisingly, the major period of histone mRNA accumulation occurs after stage 10 of oogenesis. This is well after the main period of ovarian DNA synthesis or actin mRNA accumulation. The majority of the histone mRNAs is associated with polysomes at all times, even after cessation of DNA synthesis.

MATERIALS AND METHODS

Egg Chamber Purification and Embryo Collection. Wild-type *Drosophila melanogaster* of the P2 Oregon R strain were maintained in population cages. Egg chambers were purified by a mass fractionation procedure and embryos were collected as described (5).

Nucleic Acid Purification and RNA Blot Hybridization. Plasmid DNAs were purified by the procedures of Clewell (9) or Johnson and Ilan (10). Total RNA was purified from egg chambers and embryos by phenol/cresol/chloroform extraction (6). For RNA blot hybridization, nucleic acids were fractionated by electrophoresis on 1.5% agarose gels in the presence of 6% formaldehyde (11), blotted onto nitrocellulose, and hybridized with nick-translated cloned DNA probes (12). The histone probe was cDm500, a plasmid containing an 8.6-kilobase insert coding for the five *Drosophila* histone genes (13). In order to correct for RNA recovery, the blots were dehybridized and rehybridized with the ribosomal plasmid pKB7, containing the *Drosophila* 18S and 28S rRNA genes (14). The resulting autoradiograms were scanned at 540 nm with a Zeiss PMGII spectrophotometer connected to a chart recorder. The autoradiograms were within the linear range of densitometric quantitation. Peak areas were measured with an Apple computer graphics tablet. Histone mRNA peak area was divided by the rRNA peak area of each gel lane. Histone mRNA abundance was expressed relative to that of nucleic acids from 3-hr embryos by dividing the corrected peak area of the experimental sample by the corrected average peak area from 3-hr embryos.

Analysis of Histone mRNA Association with Polysomes. Polysomes were fractionated on sucrose gradients (5). Either individual gradient fractions or pooled polysomes (material sedimenting faster than the midpoint of the 80S monosome peak) and postpolysomal messenger ribonucleoprotein particles (mRNPs) (material sedimenting slower than the midpoint of the 80S monosome peak) were phenol extracted and ethanol precipitated. Equal aliquots of each sample were analyzed for histone mRNA content by blot hybridization with a histone probe, followed by densitometry of the autoradiograms. The proportion of histone mRNA associated with polysomes was calculated by dividing the polysome peak area (obtained by densitometry of appropriate lanes of the autoradiograms) by the combined peak areas of the polysome and postpolysomal mRNP lanes. Release of mRNA

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Abbreviations: mRNP, messenger ribonucleoprotein particle; histones H2–4, histones H2a, H2b, H3, and H4.

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from polysomes by puromycin was essentially as described (15).

RESULTS

Delayed Accumulation of Histone mRNAs During Oogenesis. Previous experiments (8) have demonstrated that actin genes are selectively regulated at the pretranslational level during oogenesis. Up to stage 10, actin mRNAs accumulate preferentially, resulting in a 2-fold enrichment over other mRNAs. Like actins, the histones are abundant proteins, but they have a totally different function. Thus, it is conceivable that histone genes are also independently regulated, to satisfy different requirements during oocyte differentiation. Changes in histone mRNA abundance as a function of oogenesis were measured by RNA blot hybridization, using plasmid cDm500 as a probe. This plasmid contains sequences coding for the five major histones. Fig. 1*A* shows an autoradiogram of a representative blot. Histone H1 mRNA migrates as a sharp upper band, whereas mRNAs encoding histones H2a, H2b, H3, and H4 (hereafter referred to as histones H2–4) are not resolved and migrate as a broad lower band. To verify that equal amounts of RNA were analyzed, each blot was rehybridized with the ribosomal RNA probe pKB7 (Fig. 1*B*). Histone mRNA abundance at different stages of differentiation was quantitated by scanning of the autoradiograms and correcting for differences in RNA recovery (see hybridization with pKB7 above and *Materials and Methods*). Fig. 2 depicts the changes in histone mRNA abundance (relative to that of 3-hr embryos) as a function of stage of oogenesis or embryogenesis. Histone mRNAs constitute a relative constant proportion of the total RNA during most of oogenesis; a small drop in abundance was observed between stages 8 and 10, despite this being the time of active polyploidization of nurse and follicle cell DNA. Surprisingly, a marked and abrupt increase in abundance of H1 and H2–4 mRNAs occurs late in oogenesis (stages 11–14), at a time when nurse cells begin to degenerate. Fig. 2 also indicates that H1 and the nucleosomal histone (H2–4) mRNAs are coordinately expressed during this developmental period.

Changes in Histone mRNA Content Are Temporally Unrelated to Actin mRNA Accumulation and to DNA Synthesis.

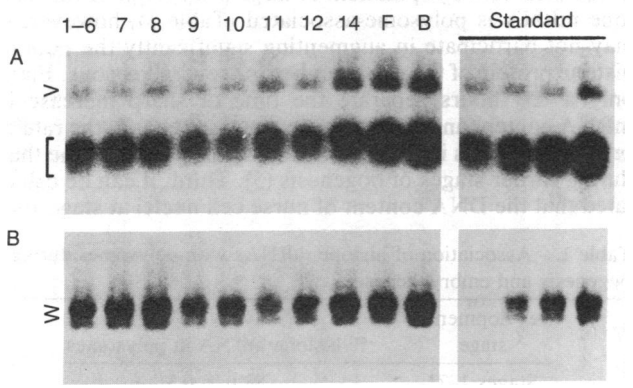

FIG. 1. Preferential accumulation of histone mRNAs late in oogenesis. (*A*) Four micrograms of egg chamber or embryo RNA was electrophoresed, blotted, and hybridized with the histone probe cDm500. Each lane is identified by the developmental stage from which the nucleic acids were obtained. Numbers refer to stages of oogenesis; F, 15-min embryos; B, 2-hr blastoderm embryos; Standard, standard curve constructed with RNA (0.8, 2.5, 3.8, and 5.0 μg) from 3-hr embryos. The arrowhead points to the position of migration of histone H1 mRNA; the bracket indicates the position of migration of histone H2–4 mRNAs (not resolved in this gel system). (*B*) The blot shown in *A* was dehybridized and rehybridized with the ribosomal probe pKB7. The arrowheads point to the 1.75- and 1.95-kilobase denatured rRNA species.

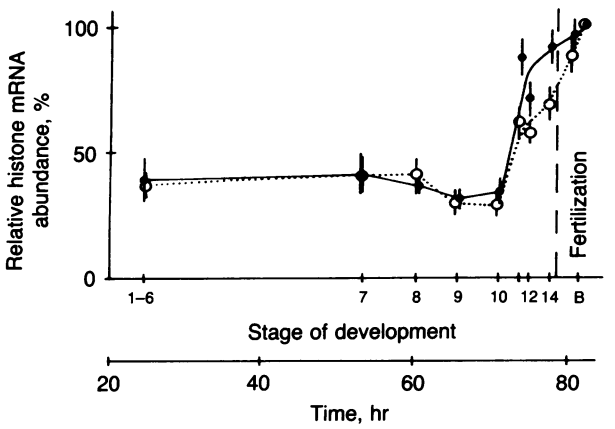

FIG. 2. Relative histone mRNA abundance as a function of development. The relative abundance of histone mRNAs in the total RNA population at each stage of development was measured by RNA blot hybridization with the histone probe cDm500 and then with the ribosomal probe pKB7 (see Fig. 1), followed by densitometry of the autoradiograms. Each point represents the mean ± SEM of 7–15 determinations. The abundance of histone mRNAs at each stage of development is expressed relative to that of 3-hr embryos, taken as 100%. ●, Histone H1 mRNA; ○, histone H2–4 mRNAs. The stages of oogenesis (1) as well as the temporal midpoint of each stage of development (16) are indicated on the abscissa. The time axis uses the formation of the stage 1 egg chamber as "zero time." B, 2-hr blastoderm embryos.

The relative histone and actin mRNA content of egg chambers and embryos shown in Fig. 3*A* was derived from the measured histone (Fig. 2) and actin (8) mRNA abundance, taking into consideration the RNA content of egg chambers and embryos (5). These values were related to the changes in DNA synthesis during the same developmental time (Fig. 3*B*). Two main conclusions can be drawn from these data. First, the accumulation of histone mRNAs follows a distinctly different temporal profile from that of actin mRNA accumulation. Actin mRNA content per egg chamber increases about 20-fold between stages 7 and 11 of oogenesis, whereas a similar increase in histone mRNA is delayed by several hours. It should be noted that many measurements of actin and histone mRNA content were performed on the same RNA blots to preclude artifactual variations due to differences in staging of the egg chambers. Second, the accumulation of histone mRNAs appears to be uncoupled from DNA synthesis, at least during late oogenesis. Prior to stage 10, a time of active polyploidization of nurse and follicle cells, the increase in histone mRNA content is relatively small. Following stage 10, the histone mRNA content abruptly increases by about 4-fold, at a time when nurse cell degeneration has already begun (19).

A Large Proportion of the Histone mRNAs Is Associated with Polysomes of Constant Size Throughout Oogenesis and Early Embryogenesis. The experiments described in this section examine the functional state of the histone mRNAs during different stages of development and address the following questions. (*i*) Is there a relationship between DNA synthesis and histone mRNA translation during the course of egg chamber differentiation? (*ii*) Are the histone mRNAs that accumulate during the late burst translated or stored for translation after fertilization?

Postmitochondrial supernatants from egg chambers or embryos were fractionated by sucrose gradient centrifugation, and fractions were analyzed by RNA blot hybridization. Fig. 4 displays representative profiles of egg chambers stages 10 and 14 and of 1-hr embryos. Polysome size remains approximately constant at two to six ribosomes per histone mRNA. Additional experiments (Table 1) quantitated mRNA distri-

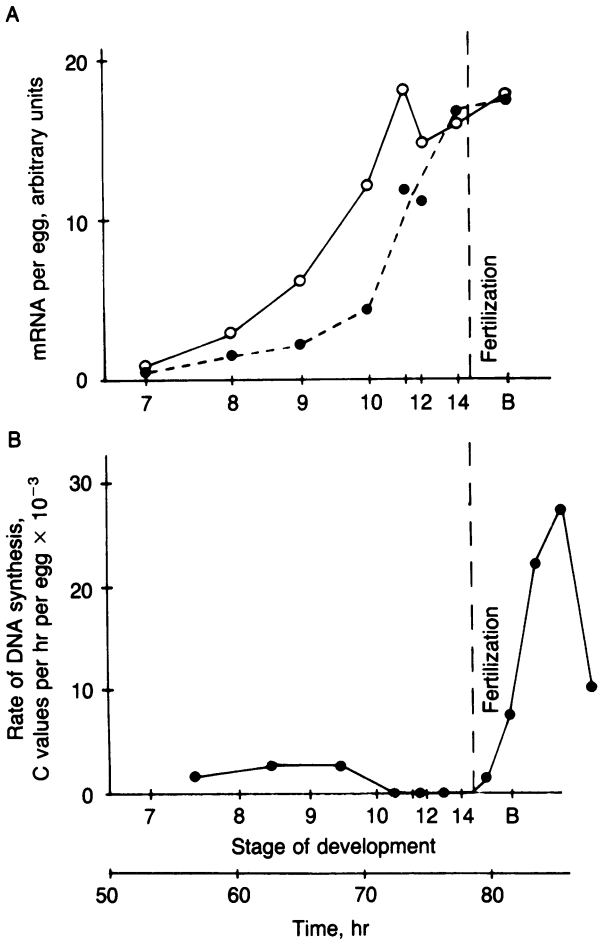

FIG. 3. Accumulation of histone mRNAs during oogenesis compared to actin mRNA accumulation and DNA synthesis. (*A*) The mRNA content of egg chambers was derived from the relative histone mRNA abundance (see Fig. 2) and from the relative actin mRNA abundance (8) by taking into consideration the RNA content per egg chamber. ●, Histone mRNA content; ○, actin mRNA content. (*B*) The rate of DNA synthesis at each interval was estimated by calculating the increase in nurse cell, follicle cell, and embryo DNA content (C = haploid content), taking into consideration the duration of each developmental stage (3, 4, 17, 18).

bution between polysomes and postpolysomal supernatants. Despite a tendency toward a decrease in polysome association during differentiation, it is apparent that at any stage a large proportion of the histone mRNAs is associated with polysomes. Association of histone mRNAs with polysomes most likely represents active translation complexes (rather than artifactual aggregation of histone mRNPs) because incubation of polysomes with puromycin causes >90% of the histone mRNAs to migrate as 80S or smaller particles (results not shown). We conclude that translation of histone mRNAs throughout oogenesis and early embryogenesis is temporally unrelated to DNA synthesis.

DISCUSSION

Histone genes are expressed in two distinct phases during oogenesis (Fig. 3). The first phase, which occupies most of oogenesis (up to stage 10), is characterized by relatively low levels of histone mRNAs, despite extensive polyploidization (up to 1000 times the haploid content per nucleus) of nurse and follicle cells. This relatively low histone mRNA content appears to be sufficient to provide enough histone proteins to support chromatinization of the newly synthesized DNA, for two possible reasons: (*i*) this interval is long enough (70

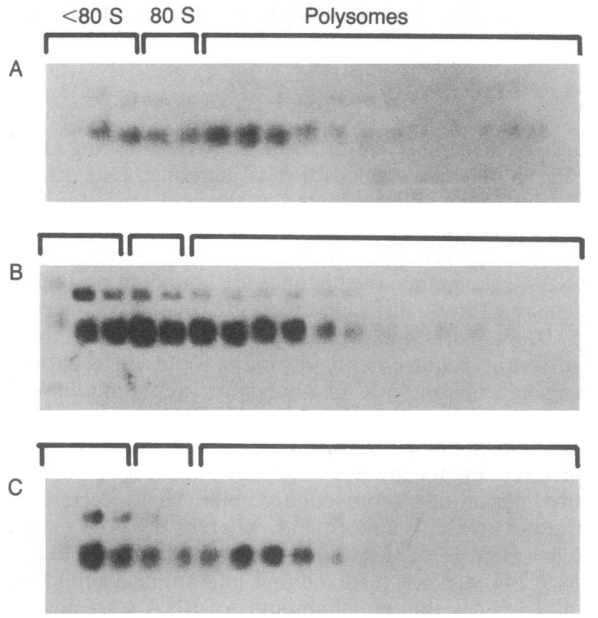

FIG. 4. Distribution of histone mRNAs between polysomes and postpolysomal mRNPs. Postmitochondrial supernatants from 400 egg chambers or embryos were resolved on 15–50% sucrose gradients, and the absorption profile at 254 nm was recorded (see ref. 3 for representative profiles). The histone mRNA content of each gradient fraction was analyzed by RNA blot hybridization with the histone probe cDm500. The position of migration of monosomes and polysomes in each gradient is indicated at the top of *A*. (*A*) Stage 10 egg chambers. (*B*) Stage 14 egg chambers. (*C*) One-hour embryos.

hr) to allow many rounds of histone mRNA translation and (*ii*) each mRNA is likely to be translated about 20-fold more efficiently during this period than at later developmental stages (5).

The second phase of histone gene expression begins during late stage 10, *after* the nurse cells and follicle cells achieve maximal ploidy. At this time the rate of histone mRNA accumulation increases abruptly, resulting in an ≈4-fold increase in content and a 2-fold enrichment with respect to the total RNA population. A large proportion of this histone mRNA is polysome-associated (Table 1); however, it may not participate in augmenting significantly the pool of histone protein of the egg chamber for several reasons. First, only a few hours separate the time of sharp increase in mRNA content and the end of oogenesis. Second, the rate of protein synthesis is likely to be much lower at this time than during earlier stages of oogenesis (5). Third, it can be calculated that the DNA content of nurse cell nuclei at stage 10 is

Table 1. Association of histone mRNAs with polysomes during oogenesis and embryogenesis

Developmental stage	% histone mRNA in polysomes
Stages 1–7*	83.6 ± 6.3
Stage 10	64.9 ± 3.0
Stage 14	63.4 ± 3.4
1-hr embryo	59.8 ± 3.4
5-hr embryo	51.8 ± 2.1

The proportion of histone mRNA (H1, H2–4) associated with polysomes was quantitated by blot hybridization of RNA extracted from the polysomal (>80S) and postpolysomal mRNP (<80S) regions of sucrose gradients, followed by densitometry of the resulting autoradiograms. The values obtained for H1 and H2–4 histone mRNAs were similar and the results were combined. The mean ± SEM of 3–4 determinations is shown.

*Ovaries from newly eclosed flies, containing stage 1–7 egg chambers.

enough for ≈5000–6000 diploid cells (20). The fate of the histone protein that presumably is associated with this DNA is not known. If all nurse cell histone protein is preserved and transferred to the oocyte, new histone synthesis at the end of oogenesis is not likely to add to this large pool by a significant amount. In contrast to the above considerations for histone proteins, the histone mRNA that is synthesized near the end of oogenesis certainly makes a major contribution to the pool of maternal mRNA. This mRNA may play a vital role in supporting the rapid nuclear divisions that occur during embryogenesis (17). Using *in situ* hybridization to tissue sections, Ambrosio and Schedl (30) have observed a similar biphasic pattern of histone mRNA accumulation during *Drosophila* oogenesis. Moreover, they have evidence that late histone mRNA synthesis occurs in nurse cells.

Histone mRNA transcription and translation in somatic cells is usually coupled to periods of DNA synthesis (21). However, uncoupled histone gene expression is often observed when a large store of histone mRNA and protein is required to support rapid embryonic cleavages (22–25). In *Drosophila*, histone gene expression appears to fall into both categories. Before stage 10 of oogenesis, histone mRNA content may be correlated with the extent of DNA polyploidization. After stage 10, when DNA begins to be degraded, a period of very rapid histone mRNA accumulation is observed. This mRNA appears to be recruited into polysomes without delay—in fact, slightly more histone mRNA is polysome-associated during this time than in early embryos (Table 1), when the rate of DNA synthesis is extremely high. Coupling of histone gene expression and DNA synthesis may again resume during embryonic development (17).

Different, as yet unidentified, regulatory mechanisms must operate during these rapidly succeeding transitions of histone gene expression. In mammalian tissue culture cells, inhibition of protein synthesis results in a rapid and several-fold increase of histone mRNA levels (26). This increase is not mediated by changes in transcription rates but rather is a consequence of histone mRNA stabilization. This stabilization is thought to be mediated by the loss of a rapidly turning-over "mRNA-destabilizing protein" (26). This general model would fit *Drosophila* quite well: toward the end of oogenesis the rate of protein synthesis declines sharply (5), which could result in the rapid decrease in the levels of the putative destabilizing protein. Thus, the histone mRNA level would increase, a result that we actually observe.

The overall pattern of gene expression during oogenesis that was suggested by our earlier results (7, 27) is that the majority of the genes whose products are found in the egg are expressed synchronously from very early stages. Results of this study suggest that the expression of two classes of genes coding for abundant products—actins and histones—is independently modulated. About 5 hr separate the sharp rise in the rate of accumulation of each mRNA at the end of oogenesis. Furthermore, another set of abundant mRNAs coding for ribosomal proteins were found to be primarily regulated at the translational level during early *Drosophila* development (unpublished data). While this manuscript was in preparation, evidence for the delayed accumulation of histone mRNAs during sea urchin oogenesis has also been reported (28). In contrast to *Drosophila*, however, these mRNAs appear to be contained within the oocyte nucleus in a nonfunctional state. Our observations also contrast with those made in *Xenopus*, in which most nonmitochondrial mRNAs are accumulated to their maximal levels synchronously and very early during oogenesis (29). What mechanisms operate during *Drosophila* oogenesis to independently regulate the level of individual gene products is a fundamental question that requires further investigation. Conceivably, similar mechanisms of differential gene expression may operate during the critical periods of cell determination and differentiation during embryogenesis.

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