UC Irvine

UC Irvine Previously Published Works

Title

Expression of a set of head-specific genes during Drosophila development.

Permalink

<https://escholarship.org/uc/item/2th0z08p>

Journal

Developmental biology, 94(2)

ISSN

0012-1606

Authors

Levy, LS
Manning, JE

Publication Date

1982-12-01

DOI

10.1016/0012-1606(82)90363-3

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Expression of a Set of Head-Specific Genes during *Drosophila* Development

LAURA SILVEN LEVY AND JERRY E. MANNING

Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717

Received March 18, 1982; accepted in revised form July 15, 1982

The expression of 20 cloned DNA sequences, selected on the basis of the prevalence of their transcripts in *Drosophila* adult head, was examined during *Drosophila* development. The amount of polyadenylated RNA complementary to these clones was measured in the cytoplasm of 0- to 20-hr embryos and on the polysomes of third instar larvae and 5- to 7-hr pupae. Four patterns of developmental expression were observed among the 20 clones, including 6 clones whose transcripts are prevalent only on polysomes of the adult head. Multiple genes closely clustered within the DNA of individual clones were shown to be expressed coordinately during development in some cases and differentially in others.

INTRODUCTION

In the accompanying paper, we reported the selection of 20 cloned DNA sequences whose mRNA complements appear from 3 to 177 times more frequently on the polysomes of the adult head than on those of the adult body. The transcripts comprise from 0.05–0.66% of the mass of polyadenylated mRNA of the head, and thus belong to the moderately abundant class of mRNA defined by Levy and McCarthy (1975). Although we did not define precisely the tissues within the head from which the mRNAs derive, the most probable candidates are the massive compound eyes, the other cephalic sensory organs, and the brain. Thus, we believe the sequences represent genes expressed in the terminally differentiated neural or sensory tissues of the head.

In this report, we examine the expression of the head-specific sequences during *Drosophila melanogaster* development. We performed these experiments to determine if the expression of genes controlled spatially in the adult is also regulated temporally during development, and to investigate various aspects of the regulation. The rapid and well-characterized development of *Drosophila* affords an excellent opportunity to conduct such a study. Embryogenesis at 25°C requires approximately 20 hr from oviposition to complete differentiation before hatching, including blastoderm formation at Hour 1.5, cellularization at Hour 2.5, gastrulation at Hour 3.5, and organogenesis from Hour 7 (Bownes, 1975; Zalokar, 1976). A large amount of maternally endowed RNA is found in the cytoplasm of the *Drosophila* egg, and protein synthesis is active in the embryo from oviposition (Goldstein and Snyder, 1973). Preformed maternal mRNA is recruited rapidly onto the polysomes of the embryo (Lovett and Goldstein, 1977; Mermod *et*

al., 1980), but no new mRNA is transcribed from zygotic nuclei until blastoderm formation (McKnight and Miller, 1976; Zalokar, 1976). Indeed, the embryonic histone genes are not active for the first 90 min, although DNA synthesis and mitosis are rapid from oviposition (Anderson and Lengyel, 1980). The rate of nuclear RNA synthesis increases rapidly from blastoderm (Anderson and Lengyel, 1979) and 72% of newly made RNA is associated with polysomes at gastrulation (Anderson and Lengyel, 1981). By Hour 9, more than 50% of the polyadenylated mRNA on polysomes has been transcribed since fertilization (Anderson and Lengyel, 1981), and the rate of total RNA synthesis increases sixfold in the first 15 hr of embryogenesis (Anderson and Lengyel, 1979). Population studies of mRNA sequence diversity have shown that only small qualitative changes occur during embryogenesis, and that most of the diversity is retained, at least during the first 17 hr of development (Arthur *et al.*, 1979). Further, we have recently reported (Levy and Manning, 1981) that only small qualitative changes in mRNA sequence diversity accompany the later development of *D. melanogaster*, from third larval instar to adult. However, a recent study of the expression of individual cloned gene species during *Drosophila* development illustrated that quantitative modulation of mRNA concentration is a frequent event. Of the 20 randomly selected clones examined in that study, the steady-state mRNA concentrations of six showed dramatic changes during development (Biessmann, 1981). Similarly, Mitchell and Peterson (1981) demonstrated in developing wing a rapidly changing population of mRNAs sufficiently abundant to be detected by *in vitro* translation.

In this report, we measure the steady-state concentrations throughout development of the mRNAs com-

plementary to 20 cloned DNA species. In particular, it is possible to determine whether the set of cloned sequences, related by virtue of their expression in the adult head, comprise a set or group of subsets with common developmental regulation. Further, we reported in the accompanying paper that four of the head-specific clones were observed to harbor multiple gene species. In two cases, clones 549 and 551, the complementary RNA species were found simultaneously on the polyosomes of adult head. In contrast, the genes located on clones 516 and 547 were apparently subject to distinct developmental influences. In the study reported here, we examine the expression of the four tightly clustered gene sets during development to determine what relationship, if any, exists between their spatial organization and expression. To investigate these and other aspects of the temporal control during development over a set of cloned gene sequences expressed in adult head, we have employed a quantitative dot hybridization assay to measure their expression throughout embryogenesis as well as in third instar larvae and pupae.

MATERIALS AND METHODS

Collection of Timed Developmental Stages

Drosophila melanogaster adults, strain Oregon R, were maintained at room temperature in population cages. Eggs were collected overnight on cornmeal agar trays spread with yeast paste and were discarded. To ensure clearing of eggs retained in the females, eggs were then collected on fresh trays for 1 hr after the light change and discarded. Eggs for experimental use were collected on fresh trays for the specified length of time and were allowed to develop at 25°C to the desired age. They were collected, washed, and stored at -70°C. Third instar larvae, 5- to 7-hr pupae, and adult heads were collected as previously described (Levy and Manning, 1981).

Preparation and Radiolabeling of RNA and DNA

Embryos (Hours 0-20) stored at -70°C were crushed with a cold mortar and pestle, and homogenized in 0.02 M Tris, pH 7.6, 0.025 M EGTA, 0.05 M MgCl₂, 0.025 M NaCl, 0.003 M dithiothreitol. Nuclei were removed by pelleting at 16,000g for 20 min at 4°C in a Sorvall HB-4 rotor. The supernatant was then brought to 0.1 M NaAc, pH 5.1, 0.1 M NaCl, 2% SDS, and extracted four times with saturated phenol and CHCl₃ (1:1). After two additional extractions with CHCl₃ alone, cytoplasmic RNA was precipitated from the aqueous phase with 2 vol of cold 95% EtOH. RNA was prepared from the polyosomes of third instar larvae, pupae, adults, and adult head as previously described (Levy and Manning, 1981). Polyadenylated RNA was purified by oligo-

(dT)-cellulose chromatography as described (Levy and Manning, 1981). The preparation of cloned DNAs and radiolabeling of RNA and DNA were performed as described in the accompanying paper (Levy *et al.*, 1982).

Restriction Enzymes, Electrophoresis, Southern Transfer, Northern Transfer, and Dot Blots

All restriction enzymes were purchased from Bethesda Research Labs (BRL) and were used as recommended. Electrophoresis of DNA and RNA, Southern and Northern transfers, and preparation of dot blots were conducted as described in the accompanying paper.

Hybridizations

Hybridization of Southern blots, Northern blots, and dot blots were conducted as described in the accompanying paper.

RESULTS

Expression of Cloned DNA Sequences during Development

The experiments reported here measure the concentration of RNA transcripts complementary to 20 cloned DNA sequences during Hours 0-20 of embryogenesis, in third larval instar, pupae, and adult. To accomplish this, we employed a method of dot hybridization shown in the accompanying paper to be quantitative and reproducible. For this assay, DNA (1 µg) from each lambda clone was denatured, neutralized, and spotted directly onto nitrocellulose (Thomas, 1980) into a 1-cm square demarcated on the filter. As a measure of background, wild-type lambda DNA (1 µg) was spotted into each of three squares on the filter. The dot blots were then hybridized to radiolabeled RNA from a defined developmental stage, and the squares were removed and counted by liquid scintillation. Internal standards described in the accompanying paper allow the correlation between the number of counts bound to each square and the proportion of RNA mass occupied by each sequence. Essential to the interpretation of the experiments reported here is the comparison between hybridizations using RNA from different periods in development. To allow a meaningful quantitative comparison, all hybridizations were conducted under identical conditions of salt, time, temperature, and DNA excess, with the same amount of RNA (1 µg) of constant specific activity (3×10^7 cpm/µg). Further, comparison between hybridizations to clones whose transcripts exhibit a wide range of concentration requires that the hybridization be at termination when the reaction is stopped. To determine the length of time required to reach termination under

FIG. 1. Kinetics of hybridization of ^{32}P -RNA to dot blots of four cloned DNAs. Dot blots of clones 536 (○), 555 (●), 525 (△), and 559 (▲) were hybridized for varying lengths of time to 6- to 9-hr embryo polyadenylated cytoplasmic RNA (1 μg) radiolabeled with polynucleotide kinase (sp act = 3×10^7 cpm/ μg). Intensity of the hybridization signal was determined by liquid scintillation counting of the hybridized dot blot. The proportion of RNA mass occupied by the hybridizing sequence was determined from the intensity of hybridization as described in the accompanying paper.

our conditions, we hybridized four cloned DNAs to radiolabeled RNA from 6- to 9-hr embryos for 5, 13, 22, 32, and 48 hr. The kinetics of hybridization to dot blots are such that termination is reached after approximately 20 hr over a wide range of RNA concentrations (Fig. 1). Dot blot hybridizations reported here and in the accompanying paper were conducted for 30 hr. The efficiency of hybridization is surprisingly low (<5%) compared to the efficiency reported using colony hybridization in a similar way (10–15%) (Lasky *et al.*, 1980). However, the number of counts bound to dot blots is directly proportional to the percentage of RNA mass occupied by a sequence as determined by an independent method (data in accompanying paper). Therefore, we have compared directly the number of counts bound to cloned DNA sequences when hybridized with RNA populations prepared from progressively older embryos, larvae, pupae, adults, and the adult head.

To accomplish this, the first 20 hr of embryogenesis were divided into eight periods (0–2, 2–4, 4–6, 6–9, 9–11, 11–13, 13–16, and 16–20 hr) and polyadenylated cytoplasmic RNA was prepared from each. Scarcity of material prohibited the preparation of polysomal mRNA from these stages. In addition, polyadenylated mRNA was prepared from the polysomes of third instar larvae, 5- to 7-hr pupae, and adults. These RNAs were then radiolabeled with polynucleotide kinase and hybridized three or more times to dot blots of the 20 cloned DNAs. The lower limit of detection in these experiments is considered to be the standard deviation of background measurements, 20 cpm, a number which represents approximately 0.007% of RNA mass (see accompanying

paper). We considered a peak containing approximately 100 cpm above background to represent a peak of gene activity or expression.

The results of these hybridizations are presented graphically below (Figs. 2–5), and demonstrate the numbers of counts bound to each clone and the proportion of the RNA mass occupied by each sequence during development. The data indicate that 14 of the 20 sequences, known to be expressed in the differentiated tissues of the adult head, also exhibit peaks of expression at other times in development. We refer throughout this report to the abrupt changes in transcript concentration observed during development as “peaks of expression” or “peaks of activity,” although it should be noted that we have no information on what combination of transcriptional or post-transcriptional mechanisms cause the observed peaks. Thus these terms are descriptive and refer to an increase in the level of detectable cytoplasmic mRNA concentration. The patterns of expression we observed fall roughly into four classes. The first class includes six clones, 512, 521, 525, 538, 551, and 557, none of which is expressed significantly at any time other than in the adult head (Figs. 2A–E). These comprise from 0.05 to 0.63% of the polyadenylated mRNA mass of the head, but represent <0.01% of the polyadenylated cytoplasmic RNA mass at other stages of development. Clones 538 and 551 have been localized by *in situ* mapping to the same chromosomal region, 28, and clone 551 has been shown to encode two mRNA species (see accompanying paper). Thus, all three mRNA species encoded at this chromosomal region are prevalent on head polysomes, but

FIG. 2. Developmental expression of six cloned DNA sequences which represent prevalent mRNAs in the head of adult *Drosophila*. Dot blots of clones 512 (A), 521 (B), 525 (C), 538 (D), 551 (E), and 557 (F) were hybridized to polyadenylated cytoplasmic RNA from embryos collected throughout Hours 0–20 of embryogenesis, and to polysomal polyadenylated mRNA from third instar larvae, 5- to 7-hr pupae, adult flies, and the adult head. RNA samples were radiolabeled with polynucleotide kinase (sp act = 3×10^7 cpm/ μg), and 3×10^7 cpm were included in each hybridization. Intensity of the hybridization signal was determined by liquid scintillation counting of the hybridized dot blot. The proportion of RNA mass occupied by the hybridizing sequence was determined from the intensity of hybridization as described in the accompanying paper.

are not expressed abundantly at other times. In addition, a small amount of RNA complementary to clones 521 and 525 was found in the cytoplasm of 0- to 2-hr eggs, presumably contributed maternally. In both cases, this RNA was no longer detectable after a few hours (Figs. 2B, C).

A second pattern of expression is that of clones 516 and 549 (Fig. 3). Both of these clones are actively expressed during a short period early in embryogenesis, predominantly between Hours 6 and 11. The short-lived

peak of activity of clone 516 was observed only between Hours 6 and 9 (Fig. 3A). At this time, the transcripts involved occupy approximately 0.04% of the RNA mass. Less transcription is apparent from Hours 2 to 6. At other times, including the newly laid egg, the transcript is rare. In the adult head, however, 516 is again expressed to a relatively high level. In contrast, the transcripts complementary to clone 549 are moderately prevalent throughout development (Fig. 3B). Sequence 549 occupies approximately 0.06% of the maternal RNA

mass. Transcription of this clone begins at Hour 2 and peaks between Hours 9 and 11 at a level substantially higher even than the expression in the adult head. The transcript level then drops throughout development until it rises slightly in the pupa.

Six clones demonstrate a third pattern of expression in which a peak of activity is seen later in development. Clones 528, 547, and 559 are actively expressed late in embryogenesis, Hours 13–20 (Figs. 4A–C). None of these is expressed significantly before Hour 13, even in the maternal RNA. The peak of activity of clone 559 is observed only between Hours 16 and 20. In contrast, 528 and 547 are expressed throughout late embryogenesis, and the level of sequence 547 drops only slightly after hatching (Figs. 4A, B). Clones 507, 514, and 527 are not expressed significantly throughout embryogenesis, but they exhibit a peak of activity at pupation (Figs. 4D–F). Transcripts of these clones appear on polysomes of pupa more frequently than in the whole adult, and in one case, more frequently than in the adult head (Fig. 4E).

The final and most complex pattern of expression during development is exhibited by six clones, 503, 506, 536, 543, 548, and 555 (Figs. 5A–F). Each of these is active during two short periods, predominantly Hours 4–9 of embryogenesis and again in the pupa. The six clones in this class comprise from 0.02 to 0.07% of the mass of maternal mRNA, but in most cases this level drops in the first 2 hr. The transcript concentrations rise thereafter, to a remarkably high level in clones 536, 543, and 548, where they occupy from 0.12 to 0.20% of the RNA mass of the embryo (Figs. 5C–E). The peak of activity is short-lived, particularly with clone 543, for which the transcript concentration rises and drops >fivefold in 4 hr (Fig. 5D). Each of the clones then exhibits a slight rise in activity late in organogenesis (Hours 13–16), and five of them show a large increase in the pupa. The sixth, clone 503, has a second peak of activity in the larva. All six of these clones are expressed more abundantly in the pupa than in the whole adult, and three are expressed at a higher level in pupa (or larva) than even in the adult head (Fig. 5).

Clones Containing Multiple Genes

In the experiments described above, cloned DNAs containing approximately 15 kb of the *Drosophila* genome (Maniatis *et al.*, 1978) were hybridized to 12 different RNA populations. Correct interpretation of the results depends on the knowledge that the same gene species in each clone is responsible for the hybridization observed throughout development. To test the possibility that multiple genes in any clone are expressed differentially during development, we digested the DNA

FIG. 3. Developmental expression of two cloned DNA sequences which represent prevalent mRNAs in the head of adult *Drosophila*. Dot blots of clones 516 (A) and 549 (B) were hybridized to polyadenylated cytoplasmic RNA from embryos collected throughout Hours 0–20 of embryogenesis, and to polysomal polyadenylated mRNA from third instar larvae, 5- to 7-hr pupae, adult flies, and the adult head. RNA samples were radiolabeled with polynucleotide kinase (sp act = 3×10^7 cpm/ μ g), and 3×10^7 cpm were included in each hybridization. Intensity of the hybridization signal was determined by liquid scintillation counting of the hybridized dot blot. The proportion of RNA mass occupied by the hybridizing sequence was determined from the intensity of hybridization as described in the accompanying paper.

with restriction endonucleases *Hind*III and *Eco*RI, fractionated the products by gel electrophoresis, and transferred the pattern to nitrocellulose paper (Southern, 1975). The resulting Southern blots were then hybridized to radiolabeled RNA from each developmental stage. We examined the pattern of hybridization to restriction fragments with each RNA in order to observe any changes which might indicate expression of an additional gene. For 18 of the 20 clones, the pattern of hybridization was identical with RNAs from embryos of increasing age, larvae, pupae, adults, and adult head (data not shown). In most cases, the hybridizing fragment or fragments in each clone contained <4 kb of DNA. However, clone 549, shown in the accompanying paper to encode three mRNA species in head, is composed of several large DNA restriction fragments whose pattern of hybridization is identical throughout development and in head (data not shown). To test directly the suggestion that the three mRNA species observed in head are expressed simultaneously throughout de-

FIG. 4. Developmental expression of six cloned DNA sequences which represent prevalent mRNAs in the head of adult *Drosophila*. Dot blots of clones 528 (A), 547 (B), 559 (C), 507 (D), 514 (E), and 527 (F) were hybridized to polyadenylated cytoplasmic RNA from embryos collected throughout Hours 0–20 of embryogenesis, and to polysomal polyadenylated mRNA from third instar larvae, 5- to 7-hr pupae, adult flies, and the adult head. RNA samples were radiolabeled with polynucleotide kinase (sp act = 3×10^7 cpm/ μ g), and 3×10^7 cpm were included in each hybridization. Intensity of the hybridization signal was determined by liquid scintillation counting of the hybridized dot blot. The proportion of RNA mass occupied by the hybridizing sequence was determined from the intensity of hybridization as described in the accompanying paper.

velopment, cytoplasmic or polysomal polyadenylated RNA from embryos, larvae, pupae, adult head, and adult body were fractionated by gel electrophoresis and transferred to nitrocellulose paper (Thomas, 1980). The resulting Northern blots were hybridized to radiolabeled 549 DNA. The results of these hybridizations, several of which are shown in Fig. 6, demonstrate that the three mRNA species encoded by 549 DNA are expressed as a set throughout development. Further, visual inspection suggests that the species are expressed in the same relative proportions throughout development.

In contrast, the pattern of hybridization to Southern blots of 2 of the 20 clones revealed that additional genes other than those expressed in the adult head were expressed during development. For clone 516, it is apparent from the patterns with 6- to 9-hr embryo RNA (Fig. 7b) and with head RNA (Fig. 7c) that distinct DNA sequences are responsible for the hybridization observed at these developmental periods. The developmental expression of clone 516 is of the second class described above, with activity that begins at Hour 2 and peaks at Hours 6–9 (Fig. 3A). To determine directly the RNA

FIG. 5. Developmental expression of six cloned DNA sequences which represent prevalent mRNAs in the head of adult *Drosophila*. Dot blots of clones 503 (A), 506 (B), 536 (C), 543 (D), 548 (E), and 555 (F) were hybridized to polyadenylated cytoplasmic RNA from embryos collected throughout Hours 0–20 of embryogenesis, and to polysomal polyadenylated mRNA from third instar larvae, 5- to 7-hr pupae, adult flies, and the adult head. RNA samples were radiolabeled with polynucleotide kinase (sp act = 3×10^7 cpm/ μ g), and 3×10^7 cpm were included in each hybridization. Intensity of the hybridization signal was determined by liquid scintillation counting of the hybridized dot blot. The proportion of RNA mass occupied by the hybridizing sequence was determined from the intensity of hybridization as described in the accompanying paper.

sequences expressed during the active periods, cytoplasmic polyadenylated RNA from 2- to 4-, 4- to 6-, and 6- to 9-hr embryos and polyadenylated mRNA from head were fractionated by gel electrophoresis and transferred to nitrocellulose (Thomas, 1980). Hybridization of radiolabeled 516 DNA to these revealed that a single large RNA species (4100 nt) occurs on head polysomes which is not found in the cytoplasm of early embryos. Two other RNA species appear in early embryos (2200 nt and 1180 nt), one of which is not prevalent until Hour 4 (Figs. 7 d–g).

Similarly, the hybridization of radiolabeled RNAs from late embryo and adult head to Southern blots of restricted clone 547 DNA revealed that distinct DNA sequences encode the RNAs expressed at those times (Figs. 8b, c). The pattern observed with RNA from larva and pupa is identical to that with 13- to 20-hr RNA (data not shown). Interestingly, hybridization with radiolabeled adult body RNA demonstrated activity of all the DNA sequences expressed during development (data not shown). Hybridization of nick-translated 547 DNA to a Northern blot of RNA from 13- to 20-hr embryo,

FIG. 6. Northern blot analysis of the transcripts complementary to clone 549. Polyadenylated cytoplasmic RNA (1 μ g) from 6- to 11-hr embryos (a), and polyadenylated polysomal mRNA (1 μ g) from pupae (b), and adult head (c) were denatured in the presence of glyoxal (McMaster and Carmichael, 1977), fractionated by agarose gel electrophoresis, and transferred to nitrocellulose (Thomas, 1980). The Northern blots were hybridized to 0.2 μ g of nick-translated DNA (sp act = 1×10^8 cpm/ μ g) of clone 549. The sizes indicated by the arrows are those of bacteriophage λ -DNA digested with the restriction enzyme *Hind*III, radiolabeled with polynucleotide kinase using [32 P]ATP, and electrophoresed in parallel with RNA samples to provide size markers.

pupa, adult head, and adult body illustrated that two RNA species are encoded by clone 547. The larger (1700 nt) appears on head polysomes (Fig. 8f), and the smaller (1200 nt) is expressed earlier in development (Figs. 8d, e). However, both appear on the polysomes of adult body (Fig. 8f) as predicted by the Southern blot analysis.

DISCUSSION

In this report, we have measured the changing concentration of RNA complementary to 20 cloned DNA sequences as a function of developmental time. The clones, originally selected on the basis of their abundant expression in the differentiated tissues of the adult head,

were examined throughout embryogenesis as well as in third instar larva, pupa, and the whole adult. We have focused intently on activity during the first 20 hr after oviposition because complete differentiation of the larval form requires approximately this length of time (Bownes, 1975). Further, the morphological changes during embryogenesis have been well characterized (Demerec, 1950; Bownes, 1975), as has the metabolism of DNA and RNA (Zalokar, 1976; Lovett and Goldstein, 1977; Anderson and Lengyel, 1981). Of particular relevance here, development of the nervous system and sensory organs during embryogenesis has been described in some detail (Demerec, 1950).

Embryogenesis is initiated with a large endowment

FIG. 7. Southern and Northern blot analyses of the transcripts complementary to clone 516. DNA was isolated from clone 516, digested with the restriction enzymes *Eco*RI and *Hind*III, and fractionated by agarose gel electrophoresis (a) visualized by EtBr staining; the sizes indicated by arrows were determined by comparison to DNA size markers electrophoresed in parallel. Fractionated DNA was transferred to nitrocellulose (Southern, 1975), and hybridized to polyadenylated cytoplasmic RNA (1 μ g) from 6- to 9-hr embryos (b) or to polyadenylated polysomal mRNA (1 μ g) from adult head (c), which had been radiolabeled with polynucleotide kinase. Polyadenylated cytoplasmic RNA (1 μ g) from 2- to 4-hr (d), 4- to 6-hr (e), and 6- to 9-hr (f) embryos, and polyadenylated polysomal mRNA (1 μ g) from adult head (g) were denatured in the presence of glyoxal (McMaster and Carmichael, 1977), fractionated by gel electrophoresis, and transferred to nitrocellulose (Thomas, 1980). Northern blots were hybridized to 0.2 μ g of 516 DNA nick-translated to a specific activity of $\sim 1 \times 10^8$ cpm/ μ g. The arrows adjacent to lane d indicate sizes of bacteriophage λ -DNA digested with *Hind*III, radiolabeled with polynucleotide kinase using [γ - 32 P]ATP, and electrophoresed in parallel with RNA samples to provide size markers.

of maternal RNA (0.19 $\mu\text{g}/\text{egg}$; Hough-Evans *et al.*, 1980). Protein synthesis is active at ovulation, increasing at the time of fertilization (Goldstein and Snyder, 1973), although no transcriptional activity can be detected prior to blastulation (90–120 min) (McKnight and Miller, 1976; Zalokar, 1976). Nine of the twenty clones examined here were observed to be expressed at a significant level in maternal RNA (0- to 2-hr eggs), where they represented from 0.02 to 0.08% of the cytoplasmic polyadenylated RNA mass. The concentration of four of these declined sharply during the first 2 hr (Figs. 2B, C, 4D, 5F). Recently, comparative screening of a *Drosophila* library with 0- to 2-hr RNA and 2- to 5-hr RNA revealed that a proportion of the sequences present in maternal RNA disappeared rapidly in the first 2 hr (Scherer *et al.*, 1981). Further, a significant loss of the maternal RNA complementary to two cloned sea urchin genes has been reported to occur in the first hours of embryogenesis (Lev *et al.*, 1980).

Transcription of the *Drosophila* embryonic genome begins at approximately 90 min, as documented in the case of the histone genes (Anderson and Lengyel, 1980). Scherer *et al.* (1981) employed a comparative library screening procedure to report that 50–70 new sequences whose abundance is $>0.05\%$ appear at Hours 2–5. Indeed, they have selected the cloned DNAs of several of these sequences, and report that most of them peak in activity at 6–9 hr, declining thereafter (Scherer *et al.*, 1981). Our results demonstrate abrupt transcriptional activity of 6 of the 20 clones at Hours 2–4 (Figs. 3B, 5B–F). The patterns of expression thereafter can be divided into four categories. Six of the clones are not expressed significantly at any time other than in the adult head (Fig. 2), where their transcripts comprise from 0.05 to 0.63% of the polyadenylated mRNA mass. These may derive from terminally differentiated tissues of the head, for example, the compound eyes, which do not differentiate fully until late in metamorphosis (Demerec, 1950). They are indeed good candidates to be among the messages responsible for abundant proteins characteristic of the differentiated state. The other 14 clones encode genes which apparently have a role in embryogenesis. They are expressed in three different patterns which can be correlated with general developmental events and particularly those of the structures contained in the adult head.

According to Demerec (1950), neuroblasts, from which the central nervous system derives are first distinguishable in the 4-hr embryo, where they exhibit distinctive morphology and mitotic activity. Their differentiation continues somewhat beyond Hour 7. Later, during the singularly important Hours 9–11, ganglionic masses which will give rise to the brain pull away from the surface and begin to compact, rudiments of the optic

FIG. 8. Southern and Northern blot analyses of the transcripts complementary to clone 547. DNA was isolated from clone 547, digested with the restriction enzymes *EcoRI* and *HindIII*, and fractionated by agarose gel electrophoresis ((a) visualized by EtBr staining; the sizes indicated by arrows were determined by comparison to DNA size markers electrophoresed in parallel). Fractionated DNA was transferred to nitrocellulose (Southern, 1975) and hybridized to polyadenylated cytoplasmic RNA (1 μg) from 13- to 20-hr embryos (b) or to polyadenylated polysomal mRNA (1 μg) from adult head (c) which had been radiolabeled with polynucleotide kinase (sp act = 3×10^7 cpm/ μg). Polyadenylated cytoplasmic RNA (1 μg) from 13- to 20-hr embryos (d), and polyadenylated polysomal mRNA from pupae (e), adult body (f), and adult head (g) were fractionated by gel electrophoresis, and transferred to nitrocellulose (Thomas, 1980). Northern blots were hybridized to 0.2 μg of 547 DNA nick-translated to a specific activity of $\sim 1 \times 10^8$ cpm/ μg . The arrows adjacent to lane d indicate the sizes of bacteriophage λ -DNA digested with *HindIII*, radiolabeled with polynucleotide kinase using [γ - ^{32}P]ATP, and electrophoresed in parallel with RNA samples to provide size markers.

lobes invaginate, and the first cholinesterase activity is observed. Development of the larval cephalic sense organs occurs late in embryogenesis, Hours 14–16. In the larva, the central nervous system consists of the two brain hemispheres and the ventral ganglionic mass which will appear in the adult but are located in the larval third thoracic segment. The imaginal eye and antennal discs, attached to the larval brain, reach their maximal size late in larval development. During metamorphosis, the central nervous system of the larva is not destroyed, but undergoes various changes in shape, size, and internal anatomy to become the cephalic and thoracic structures of the adult central nervous system, the brain and ventral ganglionic mass. The compound

eyes differentiate fully only late in metamorphosis. Of the 14 head-specific clones expressed during development, 2 peak in activity early in embryogenesis, Hours 6–11 (Fig. 3). Six others are active later in development, 3 between Hours 13 and 20 of embryogenesis (Figs. 4A–C), and 3 in the 5- to 7-hr pupa (Figs. 4D–F). Of the latter, the transcripts appear on polysomes of pupa more frequently than on those of the whole adult or even the adult head (Fig. 4E). Finally, a group of 6 clones is expressed both early in embryogenesis (Hours 4–9) and in pupa (or larva, in one case; Fig. 5). The transcripts complementary to all 6 of these clones are more abundant on polysomes of pupa (or larva) than on those of the whole adult or adult head. Thus, the activity which gives rise to the abundance of transcripts observed in adult head may actually begin in the pupa or earlier. Most importantly, the data illustrate that 14 of 20 cloned DNA sequences expressed prevalently in the tissues of the adult head are also active for brief periods at other times in development. Further, subsets of the 20 sequences are regulated temporally in one of four patterns. Studies of the changing prevalence of cloned mRNA species during development of sea urchin and *Xenopus* have shown a similar phenomenon to occur in a few cases. In particular, a gene expressed in the early sea urchin embryo was shown to disappear from the polysomes of gastrula, but to reappear on the polysomes of adult intestine (Lev *et al.*, 1980). In *Xenopus*, some of the >200 cloned species followed throughout development exhibited patterns of expression which suggest some common regulation (Dworkin and Dawid, 1980). However, the whole of embryogenesis in these organisms is too long to permit a detailed analysis; therefore, brief periods of activity would not have been detected.

In some cases reported here, peaks of expression during embryogenesis are indeed quite brief (Figs. 3A, B, 5B–E). For example, the transcript of clone 543 is observed to rise and fall fivefold in concentration between Hours 2 and 6 (Fig. 5D). The concentrations of sequences 536 and 549 rise and drop from 2.5- to 4-fold between Hours 2 and 11. Newly synthesized mRNA in sea urchin embryos turns over with a half-life of 5.7 hr (Galau *et al.*, 1977), a length of time comparable to the peaks of activity we observe during *Drosophila* embryogenesis. Lengyel and Penman (1977) reported that mRNA in a cultured *Drosophila* cell line is composed of two components with half-lives of 1 hr and 6–7 hr.

A further issue we address here is the relationship between the spatial organization of genes and their expression. Nineteen of the twenty cloned sequences were shown in the accompanying paper to occur at unique loci on all five polytene chromosome arms. No correlations are obvious between the 19 loci identified and the patterns of expression exhibited by the cloned

sequences. Two clones, 507 and 547, occur at locus 66D, and appear to have in common the gene sequence encoding the larger of the two mRNA species complementary to 547 DNA. It is not known whether the clones were derived from the identical genomic DNA or from a repeat element (see accompanying paper). However, we demonstrate in this report that the gene sequence held in common is expressed significantly only in the adult head. Two other clones, 538 and 551, map to closely adjacent loci (28A and 28C), and encode three distinct mRNA species in head (see accompanying paper). Interestingly, both clones 538 and 551 clearly demonstrate the pattern of expression characteristic of class I described above, in which no significant transcription is apparent in the cytoplasm of any developmental stage except in the adult head.

Of more significance than the relationship between cloned DNAs is the relationship between genes tightly clustered within a single clone. In several specific cases, sets of closely clustered genes are expressed coordinately during development. This is true of four genes encoding the larval cuticle proteins of *Drosophila* (Snyder *et al.*, 1981), four heat-shock genes at the locus 67B on *Drosophila* chromosomes (Corces *et al.*, 1980; Wadsworth *et al.*, 1980; Voellmy *et al.*, 1981), and several sets of developmentally regulated genes in *Dictyostelium* (Mangiarotti *et al.*, 1981). Further, the genes encoding *Drosophila* chorion proteins are clustered at a few chromosomal loci which may represent coordinately regulated gene sets (Griffin-Shea *et al.*, 1980; Spradling *et al.*, 1980). We report here that two clusters of genes, those on clones 551 and 549, are subject to common regulation during development. Clone 551 encodes two large mRNA species (see accompanying paper) which are prevalent only in the adult head where together they occupy 0.24% of the mRNA mass (Fig. 2E). The three RNA species encoded by clone 549 are expressed coordinately throughout development (Fig. 6). Although their total level of expression is temporally regulated (Fig. 3B), the relative amounts of each species appear not to change (Fig. 6).

In contrast, clone 516 encodes three RNA species, only two of which are expressed during embryogenesis (Hours 2–9; Figs. 2A, 7D–F). The third is expressed only in the adult head, and is the only one of the three species found on head polysomes (Fig. 7g). Clone 547 encodes two developmentally controlled RNA species, also subject to distinct regulatory influences. The larger species represents 0.30% of the mass of polyadenylated mRNA of the head (Fig. 4B), and is the only one expressed on head polysomes (Fig. 8f). The smaller is found in the cytoplasm of late embryos (13–20 hr), and on the polysomes of pupa and adult body (Figs. 8d, e, g). Thus, it is clear that immediate physical proximity of gene se-

quences does not ensure coordinate regulation of expression. Similarly, Scherer *et al.* (1981) selected cloned DNAs expressed during *Drosophila* embryogenesis, some of which encode differentially regulated RNA species. Other clones of this type have been described from *Dicystostelium* (Mangiarotti *et al.*, 1981) and *Aspergillus* (Zimmermann *et al.*, 1980). The globin gene clusters of humans also represent adjacent genes which are expressed distinctly during development, although the genes of each cluster may have arisen from a common ancestor (Fritsch *et al.*, 1980; Lauer *et al.*, 1980). Each of the cloned DNA sequences in this study has been mapped to a single locus on the chromosomes of *D. melanogaster* under conditions of *in situ* hybridization in which a repeat element as small as 500 nucleotides can be identified. Thus, the genes appear to represent single-copy DNA (see accompanying paper). However, the closely clustered genes which reside on a single-cloned species may represent members of a small multigene family. Such a possibility has not been ruled out, and may indeed influence significantly the regulation of their expression.

To summarize, we have described in this and the accompanying paper many aspects of the expression and organization of a set of cloned DNA sequences selected because of the prevalence of their transcripts on polyosomes of the adult head. Our data indicate that increased transcriptional activity contributes to the abundance on head polyosomes, although some post-transcriptional mechanism may also exert an influence. We report here that the RNA transcripts are found as moderately abundant species not only in the adult head, but also for brief periods at other times in development. Further, we have shown that although the head-specific clones are not clustered on polytene chromosomes, many of them contain multiple gene sequences clustered on the approximately 15 kb of DNA. The expression of closely clustered genes is shown to be coordinately regulated in some cases and not in others.

The authors are sincerely grateful to Dr. David Fouts for his cooperation throughout this project and Dr. K. K. Tewari for helpful suggestions. This work was supported by Grant CA 00522 awarded by the National Cancer Institute, and Grant GM 22207 awarded by the National Institute of Health. L.S.L. was supported as a predoctoral trainee by Public Health Service Training grant. A portion of this work will be submitted by L.S.L. in partial fulfillment for the degree of Doctor of Philosophy.

REFERENCES

- ANDERSON, K. V., and LENGUEL, J. A. (1979). Rates of synthesis of major classes of RNA in *Drosophila* embryos. *Dev. Biol.* **70**, 217-231.
- ANDERSON, K. V., and LENGUEL, J. A. (1980). Changing rates of histone mRNA synthesis and turnover in *Drosophila* embryos. *Cell* **21**, 717-727.
- ANDERSON, K. V., and LENGUEL, J. A. (1981). Changing rates of DNA and RNA synthesis in *Drosophila* embryos. *Dev. Biol.* **82**, 127-138.
- ARTHUR, C. G., WEIDE, C. M., VINCENT, W. S., III, and GOLDSTEIN, E. S. (1979). mRNA sequence diversity during early embryogenesis in *Drosophila melanogaster*. *Exp. Cell Res.* **121**, 87-94.
- BIESSMANN, H. (1981). Changes in the abundance of polyadenylated RNAs in development of *Drosophila melanogaster* analyzed with cloned DNA fragments. *Dev. Biol.* **83**, 62-68.
- BOWNES, M. (1975). A photographic study of development in the living embryo of *Drosophila melanogaster*. *J. Embryol. Exp. Morphol.* **33**, 789-801.
- CORCES, V., HOLMGREN, R., FREUND, R., MORIMOTO, R., and MELSELSON, M. (1980). Four heat shock proteins of *Drosophila melanogaster* coded within a 12-kilobase region in chromosome subdivision 67B. *Proc. Nat. Acad. Sci. USA* **77**, 5390-5393.
- DEMEREK, M. (ed.). (1950). "Biology of *Drosophila*." Wiley, New York.
- DWORKIN, M. R., and DAWID, I. B. (1980). Use of a cloned library for the study of abundant poly (A+) RNA during *Xenopus laevis* development. *Dev. Biol.* **76**, 449-464.
- FRITSCH, E. F., LAWN, R. M., and MANIATIS, T. (1980). Molecular cloning and characterization of the human B-like globin gene cluster. *Cell* **19**, 959-972.
- GALAU, G. A., LIPSON, E. D., BRITTEN, R. J., and DAVIDSON, E. H. (1977). Synthesis and turnover of polysomal mRNAs in sea urchin embryos. *Cell* **10**, 415-432.
- GOLDSTEIN, E. S., and SNYDER, L. A. (1973). Protein synthesis and distribution of ribosomal elements in ovarian oocytes and early developmental stages of *Drosophila melanogaster*. *Exp. Cell Res.* **81**, 47-56.
- GRIFFIN-SHEA, R., THIREOS, G., KAFATOS, F. C., PETRI, W. H., and VILLA-KOMAROFF, L. (1980). Chorion cDNA clones of *D. melanogaster* and their use in studies of sequence homology and chromosomal location of chorion genes. *Cell* **19**, 915-922.
- HOUGH-EVANS, B. R., JACOBS-LORENA, M., CUMMINGS, M. R., BRITTEN, R. J., and DAVIDSON, E. H. (1980). Complexity of RNA in eggs of *Drosophila melanogaster* and *Musca domestica*. *Genetics* **95**, 81-94.
- LASKY, L. A., LEV, Z., XIN, J.-H., BRITTEN, R. J., and DAVIDSON, E. H. (1980). Messenger RNA prevalence in sea urchin embryos measured with cloned cDNAs. *Proc. Nat. Acad. Sci. USA* **77**, 5317-5321.
- LAUER, J., SHEN, C.-K., and MANIATIS, T. (1980). The chromosomal arrangement of human α -like globin genes: Sequence homology and α -like globin gene deletions. *Cell* **20**, 119-130.
- LENGUEL, J. A., and PENMAN, S. (1977). Differential stability of cytoplasmic RNA in a *Drosophila* cell line. *Dev. Biol.* **57**, 243-253.
- LEV, Z., THOMAS, T. L., LEE, A. S., ANGERER, R. C., BRITTEN, R. J., and DAVIDSON, E. H. (1980). Developmental expression of two cloned sequences coding for rare sea urchin embryo messages. *Dev. Biol.* **76**, 322-340.
- LEVY, L. S., and MANNING, J. E. (1981). Messenger RNA sequence complexity and homology in developmental stages of *Drosophila*. *Dev. Biol.* **85**, 141-149.
- LEVY, W. B., and MCCARTHY, B. J. (1975). Messenger RNA complexity in *Drosophila melanogaster*. *Biochemistry* **14**, 2440-2446.
- LEVY, L. S., GANGULY, R., GANGULY, N., and MANNING, J. E. (1982). The selection, expression, and organization of a set of head-specific genes in *Drosophila*. *Dev. Biol.* **94**, 451-464.
- LOVETT, J. A., and GOLDSTEIN, E. S. (1977). The cytoplasmic distribution and characterization of poly (A)+ RNA in oocytes and embryos of *Drosophila*. *Dev. Biol.* **61**, 70-78.
- MANGIAROTTI, G., CHUNG, S., ZUKER, C., and LODISH, H. F. (1981). Selection and analysis of cloned developmentally-regulated *Dictyo-*

- stelium discoideum* genes by hybridization-competition. *Nucl. Acids Res.* **9**, 947-963.
- MANIATIS, T., HARDISON, R. C., LACY, E., LAUER, J., O'CONNELL, C., QUON, D., SIM, G. K., and EFSTRADIATIS, A. (1978). The isolation of structural genes from libraries of eucaryotic DNA. *Cell* **15**, 687-701.
- MCKNIGHT, S. L., and MILLER, O. L. (1976). Ultrastructural patterns of RNA synthesis during early embryogenesis of *Drosophila melanogaster*. *Cell* **8**, 305-319.
- MERMOD, J. J., SCHATZ, G., and CRIPPA, M. (1980). Specific control of messenger translation in *Drosophila* oocytes and embryos. *Dev. Biol.* **75**, 177-186.
- MITCHELL, H. K., and PETERSON, N. S. (1981). Rapid changes in gene expression in differentiating tissues of *Drosophila*. *Dev. Biol.* **85**, 233-242.
- SCHERER, G., TELFORD, J., BALDARI, C., and PIRROTA, V. (1981). Isolation of cloned genes differentially expressed at early and late stages of *Drosophila* embryonic development. *Dev. Biol.* **86**, 438-447.
- SNYDER, M., HIRSH, J., and DAVIDSON, N. (1981). The cuticle genes of *Drosophila*: A developmentally regulated gene cluster. *Cell* **25**, 165-177.
- SOUTHERN, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**, 503-517.
- SPRADLING, A. C., DIGAN, M. E., MAHOWALD, A. P., SCOTT, M., and CRAIG, E. A. (1980). Two clusters of genes for major chorion proteins of *Drosophila melanogaster*. *Cell* **19**, 905-914.
- THOMAS, P. (1980). Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Nat. Acad. Sci. USA* **77**, 5201-5205.
- VOELLMY, R., GOLDSCHMIDT-CLERMONT, M., SOUTHGATE, R., TISSIERES, R., LEVIS, R., and GEHRING, W. (1981). A DNA segment isolated from chromosomal site 67B in *D. melanogaster* contains four closely linked heat-shock genes. *Cell* **23**, 261-270.
- WADSWORTH, S. C., CRAIG, E. A., and MCCARTHY, B. J. (1980). Genes for three *Drosophila* heat-shock-induced proteins at a single locus. *Proc. Nat. Acad. Sci. USA* **77**, 2134-2137.
- ZALOKAR, M. (1976). Autoradiographic study of protein and RNA formation during early development of *Drosophila* eggs. *Dev. Biol.* **49**, 425-437.
- ZIMMERMANN, C. R., ORR, W. C., LECLERC, R. F., BARNARD, E. C., and TIMBERLAKE, W. E. (1980). Molecular cloning and selection of genes regulated in *Aspergillus* development. *Cell* **21**, 709-715.