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The Selection, Expression, and Organization of a Set of Head-Specific Genes in *Drosophila*

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Comparative screening of a library of cloned *Drosophila* DNA with polyadenylated mRNA from *Drosophila* adult head and adult body identified 20 cloned sequences expressed more abundantly in the tissues of the head than in other tissues. Quantitation using a "dot blot" hybridization assay demonstrated that the DNA sequences are expressed on adult head polysomes from 3 to 177 times more abundantly than on body polysomes, and their transcripts represent from 0.05 to 0.66% of the polyadenylated mRNA mass of the head. The steady-state nuclear RNA concentrations of four species were determined to be from 13 to 48 times greater in head nuclei than in body nuclei, an indication that their expression is controlled at least in part at the transcriptional level. The chromosomal locations of all 20 head-specific clones were identified by *in situ* mapping, and no distinct clustering was observed. However, four of the clones were shown by Southern and Northern blot analysis to contain multiple RNA coding sequences. The genes in these tightly clustered sets were observed to be expressed simultaneously in some cases and differently in others.

INTRODUCTION

It is now evident that the processes of development and differentiation in eukaryotic organisms are accompanied by substantial changes in gene expression. Evidence for modulation of gene expression during development has come primarily from studies which show that individual messenger RNA species that accumulate in certain differentiated tissues may be rare or absent in other tissues. The molecular mechanisms responsible for this accumulation appear to be varied, and are currently a subject of intense study. There are clearly two levels at which control of mRNA concentration can be exerted: the transcriptional level and the broadly defined post-transcriptional level. As discussed below, regulatory action at both levels has been observed for several individual gene species.

Control of specific mRNA concentration at the level of transcription, the hallmark of bacterial gene regulation, is at least in part the action of steroid hormones in eukaryotes (Roop *et al.*, 1978; Chan *et al.*, 1978; Ganguly *et al.*, 1979, 1980; Derman, 1981; Hager and Palmiter, 1981). Individual genes other than those controlled by steroid hormones have been shown to be under transcriptional control, including eleven tissue-specific sequences from mammalian liver (Derman *et al.*, 1981), adenovirus transcripts from infected cells (Darnell, 1979; Wilson *et al.*, 1979a; 1979b), and certain gene sequences expressed differentially during development of both plants and animals (Daneholt, 1975; Williams *et al.*, 1979; Maekawa and Suzuki, 1980; Goldberg *et al.*, 1981).

Further evidence for transcriptional regulation is provided by population studies of steady-state RNA, particularly those which examine the appearance and frequency distribution of mRNA transcripts present in the nucleus. In several recent studies, no RNA transcripts of tissue-specific or developmentally regulated mRNAs were observed in nuclei from tissues in which the mRNA is not present in the cytoplasm (Lasky and Tobin, 1979; Affara *et al.*, 1980; Blumberg *et al.*, 1981). In *Drosophila* (Levy and McCarthy, 1976; Biessman, 1980), rat liver (Sippel *et al.*, 1977), and induced Friend cells (Mauron and Spohr, 1978), studies of the RNA frequency distribution in nuclei have shown that abundant cytoplasmic mRNAs are also abundant in nuclear RNA, a situation suggestive of transcriptional level control.

In contrast, the cytoplasmic accumulation of several specific mRNA species is apparently accomplished by regulation at the post-transcriptional level. Decreased half-life or stability of individual mRNA transcripts represents one type of post-transcriptional event which can influence cytoplasmic mRNA concentration. Examples of this include globin mRNA in erythroid cells (Volloch and Housman, 1981), casein mRNA in lactating mammary gland (Guyette *et al.*, 1979), and three adenovirus mRNAs expressed during infection (Wilson and Darnell, 1981). Other mRNA species whose cytoplasmic concentrations are apparently regulated by post-transcriptional event(s) include two sequences examined during sea urchin development (Lev *et al.*, 1980) and several mRNA species abundant in CHO cells (Harpold *et al.*, 1979).

Further evidence is again provided by population

studies of the steady-state relationship between nuclear and cytoplasmic mRNA transcripts. Several studies of this kind in sea urchin (Kleene and Humphreys, 1977; Wold *et al.*, 1978; Shepherd and Nemer, 1980) and tobacco (Kamalay and Goldberg, 1980) have confirmed that transcripts of both rare-class mRNAs and abundant mRNAs appear in nuclei at developmental stages when they are not found on polysomes. These and other results have prompted Davidson and Britten (1979) to suggest that structural genes encoding complex and moderately prevalent mRNA species are transcribed continuously at a basic rate, and post-transcriptional events determine the quantitative structure of cytoplasmic mRNA populations.

Additional regulation of gene expression may be modulated by a structural feature of gene organization, gene clustering. Recent studies have demonstrated that several sets of genes which exhibit tissue-specific or developmentally regulated expression exist in close proximity or in tight clusters on the chromosomes. This phenomenon has been reported in *Aspergillus* (Zimmerman *et al.*, 1980), *Dictyostelium* (Mangiarotti *et al.*, 1981) and *Drosophila*. In *Drosophila*, reports have shown tight clustering of the genes encoding two yolk proteins (Barnett *et al.*, 1980; Postlethwait and Jowett, 1980; Riddell *et al.*, 1981), four cuticle proteins (Snyder *et al.*, 1981), a discrete set of chorion proteins (Griffin-Shea *et al.*, 1980; Spradling *et al.*, 1980) several histone proteins (Lifton *et al.*, 1978), and four heat shock proteins (Corces *et al.*, 1980; Wadsworth *et al.*, 1980; Voellmy *et al.*, 1981). In addition, three of the four α -tubulin genes are located between regions 84B and 85E on the third chromosome of *Drosophila* (Kalfayan and Wensink, 1981). However, the genes encoding three subunits of a larval serum protein (Smith *et al.*, 1981) and those encoding six actin proteins (Fryberg *et al.*, 1980; Tobin *et al.*, 1980) are dispersed on *Drosophila* chromosomes. Further, extensive genetic studies have shown that genes affecting the same phenotype in *Drosophila* are not necessarily linked; for example, genes affecting eye color and those affecting body color occur on the first, second, and third chromosomes (Lindsley and Grell, 1968).

We have chosen to address these problems of gene regulation by first isolating a large set of genes expressed abundantly in the head of adult *Drosophila melanogaster*. The adult head is useful for our purpose because it represents a limited set of organs highly enriched in neural tissues and depleted in tissues from other physiological systems. In addition, pure preparations of adult head and body can be obtained with relative ease (Levy and Manning, 1981). *Drosophila* is an extraordinarily useful organism for this study because the expression of these genes may be conveniently studied throughout

the entire process of embryogenesis, as well as other selected periods during morphogenesis, and the chromosomal locations of the entire set of genes under consideration can be determined by *in situ* hybridization. In this and the accompanying paper, we discuss the selection and various aspects of the expression and organization of a set of 20 cloned DNA sequences expressed at a low frequency in the body, but abundantly in the head of adult *Drosophila*.

MATERIALS AND METHODS

Preparation of RNA and DNA

Polyadenylated mRNA from the polysomes of adult head and body of *Drosophila melanogaster* was prepared as described (Levy and Manning, 1981). Approximately 0.5–0.8% of polysomal RNA from adult tissues represents polyadenylated mRNA. Polyadenylated nuclear RNA was prepared from adult head and body using the method described by Zimmerman *et al.* (manuscript in preparation).

The DNA clones described here were selected from a library of *D. melanogaster* genomic DNA cloned in the lambda phage Charon 4 (Maniatis *et al.*, 1978), the gift of Dr. T. Maniatis, Harvard University. Selection was accomplished using the plaque hybridization technique of Benton and Davis (1977). The isolation of DNA fragments from agarose gels and their subcloning into the bacterial plasmids pACYC 184 (Chang and Cohen, 1978) and pBR 322 (Bolivar *et al.*, 1977) were performed as described by Fouts *et al.* (1981).

Radiolabeling of RNA and DNA

RNA was 5' end-labeled to a specific activity of 3–5 $\times 10^7$ cpm/ μ g using T₄ polynucleotide kinase (Boehringer-Mannheim). RNA (1–2 μ g) at 1 mg/ml in 50 mM Tris, pH 9.5, was hydrolyzed by incubation at 90°C for 20 min. The RNA was then incubated at 37°C for 45 min in 50 μ l of 70 mM Tris, pH 7.5, 10 mM MgCl₂, 5 mM dithiothreitol, with 100 μ Ci of [γ -³²P]ATP (2000–3000 Ci/mmol, New England Nuclear) and 3 units of polynucleotide kinase. The reaction was terminated by extraction with an equal volume of saturated phenol. Radiolabeled RNA was collected by chromatography over Sephadex G-50 (Pharmacia). Radioactivity in the excluded volume was pooled and used for hybridization.

DNA was nick-translated to a specific activity of 8–10 $\times 10^7$ cpm/ μ g using a nick-translation kit (BRL) with 50 μ Ci each of [α -³²P]dATP and [α -³²P]dCTP (2000–3000 Ci/mmol, Amersham)/ μ g of DNA. The reaction was terminated by the addition of EDTA to 20 mM followed by digestion with proteinase K (0.5 mg/ml) for 30 min at 37°C. Protein was removed by repeated ex-

traction with phenol:CHCl₃ (1:1), and radiolabeled DNA was collected by chromatography over Sephadex G-100 (Pharmacia). The ³²P-DNA was then passed over hydroxyapatite (Bio-Rad) at 60°C in 0.12 M neutral phosphate with 0.2% SDS, and the bound DNA was collected after heating to 95°C for 5 min. Radiolabeled DNA released from hydroxyapatite was pelleted by centrifugation, resuspended, and used for hybridization.

Solution Hybridization

Nick-translated DNA (approximately 3×10^{-4} µg of isolated coding fragment) was hybridized in solution to a large sequence excess of head polyadenylated polysomal RNA (5 µg) under conditions which allow RNA-DNA hybridization, but do not allow DNA-DNA reannealing (Casey and Davidson, 1977). Hybridizations were carried out at 46°C in 80% formamide, 0.1 M Hepes, pH 7.8, 0.4 M NaCl, 0.005 M EDTA for as long as 24 hr, and samples were removed at appropriate times during the reactions. The R_0t value achieved was calculated based on the concentration of *Drosophila* RNA in each sample. Samples were analyzed for hybrid content using S₁ nuclease (Boehringer-Mannheim) and TCA precipitation as previously described (Zimmerman *et al.*, 1980). Control reactions containing nick-translated *Drosophila* DNA and heterologous (yeast) RNA demonstrated that no reannealing of the tracer occurred during the course of the reaction. For use as a standard, globin [³H]cDNA was prepared (Levy and Dixon, 1977) and hybridized to globin mRNA under the same conditions. The $R_0t_{1/2}$ of each reaction was determined by a least-squares computer solution of the data.

Restriction Enzymes, Electrophoresis, Southern Transfer, and Northern Transfer

All restriction enzymes were purchased from Bethesda Research Laboratories, Inc. (BRL) and were used as recommended. Restricted DNAs were electrophoresed and transferred to nitrocellulose (Southern transfer) as described (Fouts *et al.*, 1981). RNA for Northern transfer was denatured in the presence of DMSO and glyoxal (McMaster and Carmichael, 1977), fractionated by agarose gel electrophoresis, and transferred to nitrocellulose as described by Thomas (1980).

Preparation of Blots for Dot Hybridization

For dot blot analysis, a nitrocellulose sheet was demarcated into 1 cm. squares. DNA (1 µg) from each clone was denatured, neutralized and spotted within a square directly onto the filter as described by Thomas (1980). As a measure of background, lambda DNA (1 µg) was spotted into each of three squares on the filter.

Filter Hybridizations

Filters for library screening and Southern blots were hybridized, washed, and exposed to X-ray film as described by Fouts *et al.* (1981). Dot blots were hybridized as described (Fouts *et al.*, 1981) with the addition of dextran sulfate to 10%. In addition, the time (30 hr), and buffer volume (4 ml) of dot blot hybridizations were held constant to allow a quantitative comparison between experiments.

Northern blots were prehybridized and hybridized as described by Thomas (1980). After hybridization, the filters were washed for 4×10 min at room temperature in $2 \times$ SSC, 0.1% pyrophosphate, 0.1% SDS with vigorous agitation. They then were washed for 4×30 min at 50°C in $0.1 \times$ SSC, 0.1% pyrophosphate, 0.1% SDS. Autoradiography was conducted at -70°C as for Southern blots.

In Situ Hybridization to Polytene Chromosomes

Cloned *Drosophila* DNA was sheared by repeated passage through a 27-gauge needle, ethanol precipitated, and radiolabeled with ³H nucleotides as described above. The specific activity of the DNAs was $\sim 23 \times 10^6$ cpm/µg.

Procedures for salivary gland preparation and *in situ* hybridization were the same as described (Hayashi *et al.*, 1978; Strobel *et al.*, 1979). Hybridization was done under 22-mm² coverslips at 37°C for 16 hr in 15 µl buffer containing 50% deionized formamide, 0.4 M NaCl, 0.01 M Pipes, pH 6.4, and a heat-denatured nick-translated DNA probe ($\sim 3 \times 10^5$ cpm/slide). Following hybridization the slides were washed in hybridization buffer at 30°C for 15 min, rinsed with $2 \times$ SSC, and dehydrated. Autoradiography was done using Kodak NTB-2 nuclear track emulsion. The chromosomal localization of each cloned DNA was determined from the cytological map described by Lefevre (1976).

RESULTS

Identification of Cloned DNA Sequences Abundantly Expressed in Adult Head

To initiate this study, we wished to select from a genomic library cloned DNA sequences whose messenger RNA complements appear more frequently on the polyosomes of *Drosophila* adult head than in the body. We employed a strategy of comparison hybridization to accomplish this. Polyadenylated mRNA was prepared from the polyosomes of head and body, and these preparations as well as ribosomal RNA were radiolabeled with polynucleotide kinase to a high specific activity (3×10^7 cpm/µg). The three radiolabeled RNAs were then used to screen in parallel (Benton and Davis, 1977)

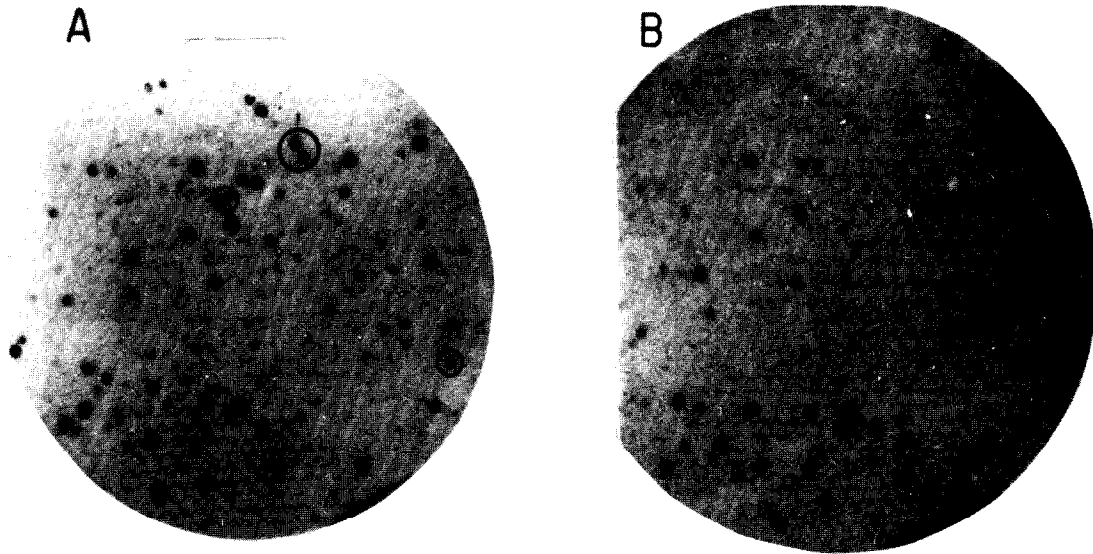


FIG. 1. Selection of head-specific clones by differential plaque hybridization. Replicas of petri plates containing approximately 5000 plaques from a library of *Drosophila* genomic DNA (Maniatis *et al.*, 1978) were prepared on nitrocellulose and hybridized to radiolabeled mRNA from the polysomes of adult head (A) or adult body (B) or to *Drosophila* ribosomal RNA (not shown). Plaques selected for further study, some of which are indicated (O), gave a strong signal only with RNA from adult head.

six genomic equivalents of a library of *D. melanogaster* nuclear DNA (a gift of T. Maniatis). Autoradiography of the filters was conducted at -70°C for 4 days, and the resulting films were examined for plaques which exhibited positive signals with head mRNA, but gave a weak or no signal with body mRNA or ribosomal RNA. Sixty-five such plaques were identified by the original library screening (Fig. 1). When these plaques were plated individually and screened again with head and body mRNA, 25 of them proved to contain DNA sequences whose mRNA complements are enriched on head polysomes. Of these, 20 were distinct species based on a restriction enzyme pattern and Southern blot analysis (data not shown). We next wished to quantitate the relative concentration of mRNA complementary to the 20 clones in head and body.

Quantitation of mRNA Prevalence in Head and Body

Having identified 20 head-specific clones by visual examination of autoradiographic signals, we wished to evaluate quantitatively the increased abundance of mRNA transcripts on head polysomes as compared to body polysomes. To determine the concentration of RNA complementary to a large number of cloned DNA species, we used "dot hybridization," a method similar to that employed by others to quantitate the relative abundance of a species in a population (Biessman *et al.*, 1979; Kafatos *et al.*, 1979; Sim *et al.*, 1979; Lasky *et al.*, 1980). This method requires that the precise concentration of a few RNAs be determined by R_0t analysis

and that their mRNA size be determined in order that these species may be used as internal standards. We chose four clones, 507, 512, 527, and 557, for this purpose. Initially, it was necessary to subclone a coding fragment from the original phage recombinant DNA clones of each of the four (Fig. 2). *EcoRI* restriction fragments generated from clones 507 (2.3 kb) and 527 (2.6 kb) were subcloned in the plasmid pACYC 184 (Chang and Cohen, 1978). *BamHI* restriction fragments generated from clones 512 (3.8 kb) and 557 (1.4 kb) were subcloned in the plasmid pBR 322 (Bolivar *et al.*, 1977). We then hybridized in solution a large excess of polyadenylated mRNA from head to the isolated, nick-translated coding fragment from subclones 507, 512, 527, and 557. We employed hybridization conditions which permit RNA-DNA hybridization but do not permit reannealing of the DNA tracer (Casey and Davidson, 1977). A least-squares computer solution of the data provided an estimate of the $R_0t_{1/2}$ of each hybridization (Fig. 3; Table 1). To determine the complexity of the RNA driving each solution hybridization, the messenger RNA size was determined by Northern blot analysis. The nick-translated coding fragments from clones 507, 512, 527, and 557 hybridized to unique species of head mRNA of sizes 1700, 1500, 1500, and 1150 nucleotides, respectively (Figs. 5a, c, e, g). The observed $R_0t_{1/2}$ values could then be compared to the $R_0t_{1/2}$ of the hybridization of globin mRNA to its cDNA under the same conditions ($R_0t_{1/2} = 4 \times 10^{-3}$; data not shown) after correction for differences in tracer length and driver complexity. The results of the experiments demonstrate that clones 507,

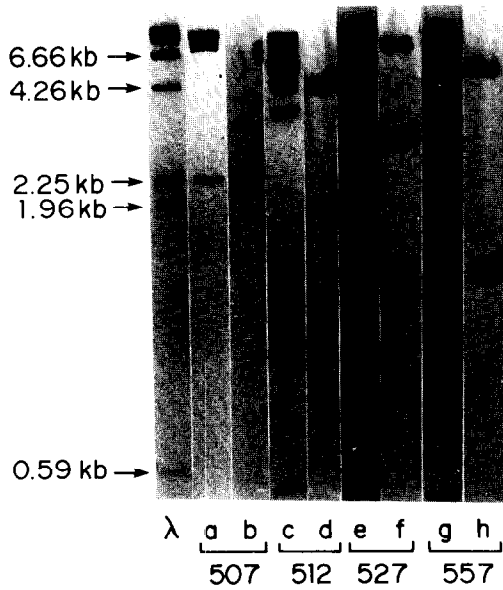


FIG. 2. Southern blot analysis of four recombinant DNA clones. DNA was isolated from clones 507, 512, 527, and 557, digested with the restriction enzyme *EcoRI* (507 and 527) or *BamHI* (512 and 557), and fractionated by gel electrophoresis (a, c, e, g visualized by EtBr staining). Fractionated DNA was transferred to nitrocellulose (Fouts *et al.*, 1981) and hybridized to polyadenylated mRNA from *Drosophila* adult head (b, d, f, h). The hybridizing restriction fragments indicated (*) were subcloned into the bacterial plasmid pACYC 184 (Chang and Cohen, 1978) (507 and 527) or pBR 322 (Bolivar *et al.*, 1977) (512 and 557). DNA from wild-type bacteriophage λ digested with the restriction enzyme *HindIII* is included as a size marker.

512, 527, and 557 comprise from 0.11% to 0.66% of the poly(A)⁺ messenger RNA mass of the head (Table 1).

The RNA concentrations determined above served as internal standards in the dot hybridization assay. For this assay, a nitrocellulose filter was demarcated into 1-cm squares, and 1 μ g of each recombinant phage DNA was spotted in a square as described (Thomas, 1980). As a measure of background, 1 μ g of wild-type lambda DNA was also spotted onto the filter. The filter was then hybridized to 1 μ g of polyadenylated mRNA from head or body radiolabeled with polynucleotide kinase to a specific activity of 3×10^7 cpm/ μ g. Thus, the filter-bound DNA coding sequence contained in 1 μ g of cloned phage DNA, assuming an average gene size for *Drosophila* of 1250 nucleotides (Zimmerman *et al.*, 1980), was in excess over its mRNA complement within the range of mRNA concentrations examined here. The conditions for hybridization were chosen to represent a plateau for reactions over a wide range of relative RNA concentration (data in accompanying paper). After hybridization and washing, the squares containing radioactivity were excised from the filter and counted individually by liquid scintillation. The number of counts bound to the DNAs from the four clones representing internal standards proved to be related to the concentration of their RNA transcripts as determined by solution hybridization (Fig. 4). Thus, the number of counts bound to all of the cloned DNAs could be cor-

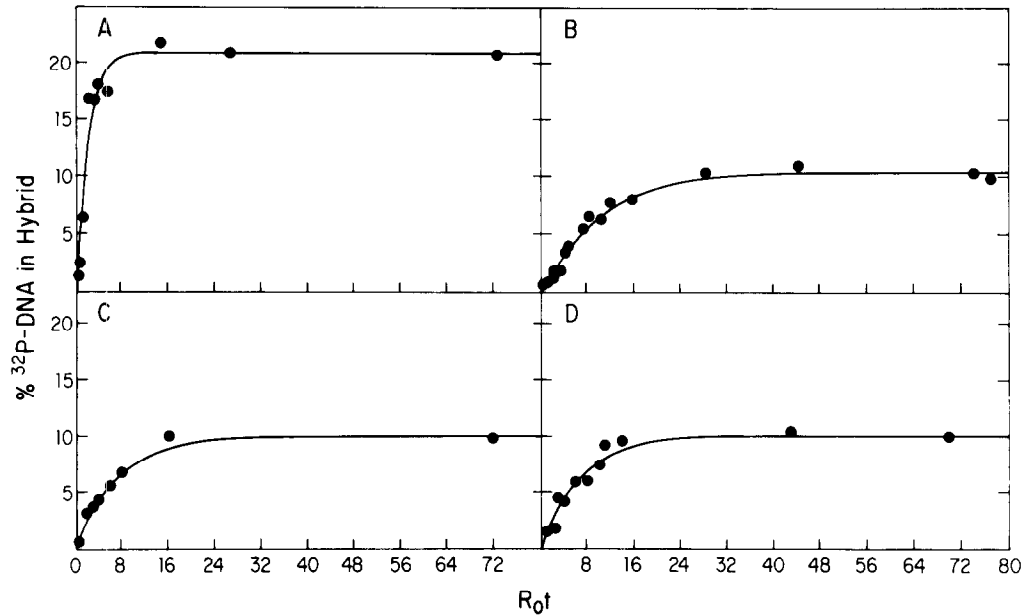


FIG. 3. Saturation hybridization of cloned *Drosophila* DNA to polyadenylated mRNA from *Drosophila* adult head. Trace quantities ($\sim 3 \times 10^{-4}$ μ g) of the nick-translated DNA coding fragments of clones 507 (A), 512 (B), 527 (C), and 557 (D) were hybridized in solution to a large sequence excess of polyadenylated mRNA (5 μ g) from the polysomes of *Drosophila* adult head. Hybridization was monitored by resistance to S1 nuclease digestion.

TABLE 1

| Clone | $R_{0t_{1/2}}$ obs ^a | Driver complexity ^b (NT) | $R_{0t_{1/2}}$ cor ^c | Percentage mRNA ^d |
|-------|---------------------------------|-------------------------------------|---------------------------------|------------------------------|
| 507 | 1.3 | 1700 | 0.6 | 0.66 |
| 512 | 7.0 | 1500 | 3.7 | 0.11 |
| 527 | 5.0 | 1500 | 2.6 | 0.15 |
| 557 | 4.6 | 1150 | 3.2 | 0.13 |

^a A least-squares computer solution of the data presented in Fig. 3.

^b RNA size (nucleotides) determined from data shown in Fig. 5.

^c The observed $R_{0t_{1/2}}$ has been corrected to allow comparison with the $R_{0t_{1/2}}$ of globin mRNA hybridized under identical conditions to globin cDNA. Corrections have been made for driver complexity and tracer length according to the following relationship (Smith *et al.*, 1975): $R_{0t_{1/2}}$ corrected = $R_{0t_{1/2}}$ observed \times (300/500) - (L/1320), where 300 and 500 are the tracer lengths of nick-translated cloned DNA and globin cDNA, respectively, and L and 1320 represent the complexity of the RNA driver and of rabbit globin mRNA (α and β), respectively.

^d The percentage of the polyadenylated mRNA mass occupied by each species determined by comparison of the $R_{0t_{1/2}}$ cor with the $R_{0t_{1/2}}$ of globin mRNA hybridized to its cDNA under our conditions ($R_{0t_{1/2}} = 4 \times 10^{-3}$).

related with the concentration of each species in the hybridizing population (Table 2). The lower limit of detection by this method, the standard deviation of background counts, is approximately 20 cpm. By comparison with internal standards, this number represents 0.007% of the mRNA mass. Therefore, RNA concentrations lower than 0.007% cannot be measured accu-

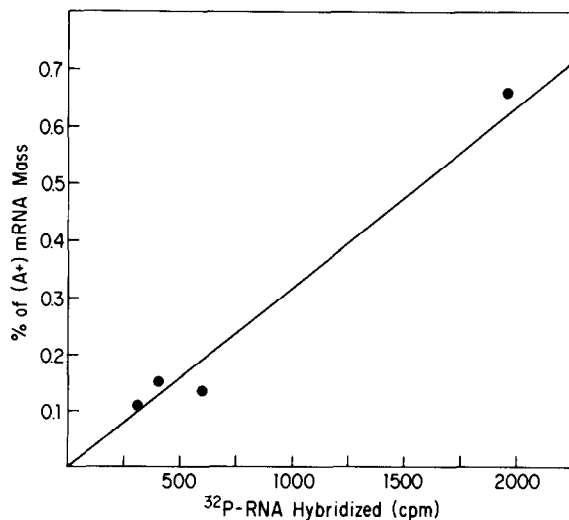


FIG. 4. Relationship between the intensity of hybridization to dot blots of clones 507, 512, 527, and 557, determined by liquid scintillation counting of the hybridized blot, and the amount of hybridizable sequence present in polyadenylated mRNA from polysomes of adult head. The proportions of RNA mass occupied by the transcripts of clones 507, 512, 527, and 557 were determined precisely using RNA excess saturation hybridization.

TABLE 2

| Clone | Percentage head mRNA ^a | Percentage body mRNA ^a | Ratio (Head/Body) |
|-------|-----------------------------------|-----------------------------------|-------------------|
| 503 | 0.12 | 0.008 | 15 |
| 506 | 0.09 | <0.007 | >13 |
| 507 | 0.66 | <0.007 | >95 |
| 512 | 0.11 | <0.007 | >16 |
| 514 | 0.05 | 0.018 | 3 |
| 516 | 0.12 | 0.008 | 15 |
| 521 | 0.05 | 0.010 | 5 |
| 525 | 0.10 | <0.007 | >14 |
| 527 | 0.15 | <0.007 | >21 |
| 528 | 0.05 | <0.007 | >7 |
| 536 | 0.07 | <0.007 | >10 |
| 538 | 0.64 | 0.060 | 11 |
| 543 | 0.26 | 0.011 | 24 |
| 547 | 0.30 | 0.011 | 27 |
| 548 | 0.16 | <0.007 | >23 |
| 549 | 0.12 | 0.036 | 3 |
| 551 | 0.24 | <0.007 | >34 |
| 555 | 0.05 | 0.014 | 4 |
| 557 | 0.13 | <0.007 | >19 |
| 559 | 0.18 | <0.007 | >26 |

^a Based on comparison to internal standards (see text). No correction has been made for differences in mRNA length. The percentage mRNA represents the average of at least three independent measurements whose values varied from 0.5 to 1.7 times the mean. Background was 154 cpm \pm 20 cpm.

rately by this technique. The results presented in Table 2 demonstrate that the mRNA transcripts of the cloned sequences examined accumulate on head polysomes to a level 3 to 95 times greater than on body polysomes, and represent from 0.05% to 0.66% of the polyadenylated mRNA mass of the adult head.

Quantitation of Transcript Prevalence in Steady-State Nuclear RNA

Examination of the steady-state nuclear RNA of sea urchin blastula, gastrula, and adult tissues has shown that transcripts complementary to blastula mRNA appear in all heterogeneous nuclear RNAs at approximately the same concentration, although the qualitative and quantitative structure of cytoplasmic mRNA differs among the samples (Wold *et al.*, 1978; Lev *et al.*, 1980). These results suggest that single-copy structural genes are transcribed continuously at a similar rate, and that the frequency distribution of cytoplasmic mRNA is controlled post-transcriptionally (Davidson and Britten, 1979). The cloned DNA species described above are appropriate subjects for further examination of this possibility because their transcripts are abundant on polysomes from the limited set of tissues comprising the adult head of *Drosophila*, and are rare on polysomes from the body. We wished to determine whether the

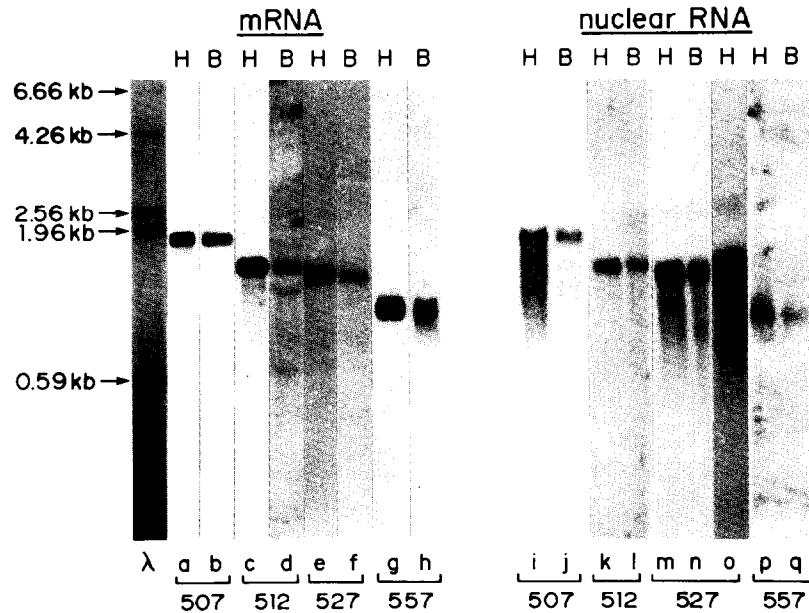


FIG. 5. Northern blot analysis of the nuclear and messenger RNA transcripts of clones 507, 512, 527, and 557. Polyadenylated polysomal mRNA and polyadenylated nuclear RNA were prepared from adult head (H) and adult body (B) of *D. melanogaster*. Messenger and nuclear RNAs from head (1 μ g) and body (10 μ g) were denatured in the presence of glyoxal (McMaster and Carmichael, 1977), fractionated by agarose gel electrophoresis, and transferred to nitrocellulose paper (Thomas, 1980). Northern blots were hybridized to 0.2 μ g of nick-translated recombinant plasmid DNA (sp act $\sim 1 \times 10^8$ cpm/ μ g). DNA from bacteriophage λ was digested with the restriction enzyme *Hind*III, radiolabeled with polynucleotide kinase using [γ - 32 P]ATP, and electrophoresed in parallel with RNA samples to provide size markers. Lane o represents an overexposure of lane m, included to demonstrate the presence of a large minor RNA species.

abundance of transcripts on head polysomes reflects a similar abundance of the corresponding sequences in head nuclei, or whether the sequences are present in head and body nuclei at similar concentrations.

We selected four of the clones for this purpose, clones 507, 512, 527, and 557, because the concentration of mRNA complementary to these is known precisely from solution hybridization and because they are expressed on head polysomes 16 to 95 times more abundantly than on body polysomes (Table 2). Polyadenylated polysomal mRNA from head (1 μ g) and body (10 μ g) and polyadenylated nuclear RNA from head (1 μ g) and body (10 μ g) were fractionated by gel electrophoresis and transferred to nitrocellulose paper (Thomas, 1980). The resulting Northern blots were then hybridized to 2×10^7 cpm of nick-translated recombinant plasmid DNA (sp act = 1×10^8 approximately) containing the subcloned coding fragment (see Fig. 2). The results of the hybridizations clearly demonstrate the abundance of transcripts in head mRNA as compared to body mRNA, particularly considering the 10-fold greater amount of RNA per lane from body (Figs. 5a-h). Hybridizations to nuclear RNA reveal that each clone is complementary to only a single species which has the same size as the mRNA transcript in all cases (Figs. 5i-q). However, overexposure of the hybridization with clone 527 to nu-

clear RNA from both head and body shows that a minor species, larger than the mRNA transcript, is also homologous to the cloned coding fragment (Fig. 5o). In addition, it is generally apparent that the homologous nuclear transcripts are more abundant in head than in body, particularly considering the 10-fold greater amount of RNA per lane from body nuclei.

To quantitate these relationships, a small area of each blot containing only the intensely hybridizing band was removed and counted by liquid scintillation (Table 3). The smear of apparently degraded RNA below the main band, particularly obvious in lanes i, m, and n, was determined to contain less than 10% of the total radioactivity in each case, and was not included in the quantitation. Because the hybridizations were carried out with the same number of counts of DNAs of uniform specific activity, and under conditions which represent saturation (data not shown), hybridizations to different RNA populations can be directly compared. As seen in Table 3 (ratio A/B), the mRNA transcripts on head polysomes are from 21 to 177 times more abundant than on body polysomes for clones 507, 512, 527, and 557. In addition, their transcripts are more abundant in the nuclei of head by a factor of 13-48 (ratio C/D). Therefore, the transcripts complementary to the genes examined here do not appear at a uniform level in the

TABLE 3

| Clone | Counts per minute bound to RNA ^a | | | | Ratios | | | |
|-------|---|-------------------------|-------------------------------|--------------------------------|--------|-----|-----|-----|
| | A. Head mRNA (1 µg) | B. Body mRNA (10 µg) | C. Head nuclear RNA (1 µg) | D. Body nuclear RNA (10 µg) | A/B | C/D | A/C | B/D |
| 507 | 3832 | 217 | 261 | 128 | 177 | 20 | 15 | 1.7 |
| 512 | 534 | 34 | 69 | 51 | 110 | 14 | 8 | 0.7 |
| 527 | 295 | 138 | 402 | 314 | 21 | 13 | 0.7 | 0.4 |
| 557 | 1064 | 83 | 178 | 37 | 130 | 48 | 6 | 2.2 |

^a Radiolabeled cloned DNAs were hybridized to Northern blots of RNA samples. The area of each blot containing only the intensely hybridizing band was removed and counted by liquid scintillation (see text).

nuclei from all tissues, but are more abundant in nuclei from the tissues in which their cytoplasmic counterparts are more abundant.

The data in Table 3 further demonstrate that the mRNA sequences on head polysomes accumulate as much as 15 times their level in head nuclei (ratio A/C), although the same sequences on body polysomes accumulate only 1–2 times their level in body nuclei (ratio B/D). Clone 527 is unusual in this regard because more transcripts appear in the nuclei of head and body than on the polysomes of head and body.

Localization on Polytene Chromosomes

Several recent studies in *Drosophila* have shown that genes expressed tissue specifically or in a common developmental pattern may be organized in a clustered array on polytene chromosomes (see, for example, Griffin-Shea *et al.* (1980); Wadsworth *et al.*, 1980; Snyder *et al.*, 1981). We report here an investigation by *in situ* mapping of any such relationships which may exist among the 20 cloned DNA sequences we have shown to be expressed abundantly in the adult head. For this purpose, the cloned DNA of each species was radiolabeled by nick-translation and hybridized *in situ* to polytene chromosomes (Fig. 6). The chromosomal regions identified as homologous to the 20 species are shown in Table 4. All of the clones were observed to hybridize to single sites on the chromosomes. Although no tight clustering is evident, the loci identified are not randomly distributed along the chromosome arms (Fig. 7). In particular, five of the clones hybridize to chromosome 2R between loci 43A and 48F. One pair of cloned DNAs hybridized to the same locus. Both 507 and 547 map to 66D, a region already demonstrated to contain genes for chorion proteins (Spradling *et al.*, 1980). However, clones 507 and 547 are not identical on the basis of cross-hybridization, restriction enzyme digestion pattern, and the mRNAs they encode (data in accompanying paper).

Further evidence for gene clustering was provided by

Southern blot analysis of all 20 clones (data not shown). For most of the clones, the coding region could be localized by this analysis to restriction fragments of 3–4 kb or less, suggesting the presence of a single gene sequence. In some cases, however, the size of the restriction fragments which hybridized to radiolabeled mRNA or the varied developmental expression of distinct restriction fragments (see accompanying paper) indicated the presence of more than one sequence clustered within the DNA of a single clone. Based on this type of information, we chose five of the clones to examine by Northern blot analysis for the presence of multiple gene sequences. Three of the five, clones 516, 538, and 547, were observed to encode single mRNA species on head polysomes of sizes 4100, 950, and 1700 nucleotides, respectively (data not shown). However, clones 551 and 549 were shown to encode two and three species, respectively, all of which are found on head polysomes (Fig. 8). Clone 549 encodes RNA species of sizes 3000, 1800, and 1050 nucleotides (Fig. 8a). Clone 551 encodes two large mRNA species of sizes 4450 and 4000 nucleotides (Fig. 8b). Clones 538 and 551 map to loci 28C and 28A, respectively; thus, three gene sequences expressed abundantly in adult head are located at region 28, two of which are clustered within the approximately 15 kb of *Drosophila* DNA on clone 551. In addition, clones 516 and 547 were observed to contain other gene sequences not expressed in adult head, but which are expressed for brief periods during the development of *Drosophila* (data in accompanying paper). Thus, clones 516 and 547 represent tightly clustered sets of genes which are subject to distinct developmental regulation.

DISCUSSION

We describe here the selection of 20 cloned DNA sequences whose mRNA transcripts appear abundantly in the head but not the body of adult *Drosophila melanogaster*. These were selected by comparing the hybridization to a cloned library of *Drosophila* DNA with mRNA from head polysomes to that with mRNA from



FIG. 6. Chromosomal localization of clone 528, one of 20 adult head-specific cloned DNA sequences. *In situ* hybridization to polytene chromosomes from the salivary gland of *D. melanogaster* demonstrates that clone 528 DNA hybridizes to band 47E on the right arm of the second chromosome.

the body. We have not identified the tissues within the head from which these sequences derive; however, the most likely candidates are the large compound eyes, other cephalic sensory organs, and the brain. Indeed, a mass of neural tissue functionally analogous to the brain exists in the thorax of the adult (Demerec, 1950). Thus, neural tissue-specific sequences selected because

of their abundant expression in the head might be expected to be expressed to some degree in the body.

The mRNA species complementary to the 20 cloned sequences accumulate on the polysomes of adult head as much as 177 times their level on the polysomes of body (Table 4). They represent from 0.05 to 0.66% of the polyadenylated mRNA mass of the head (Table 2),

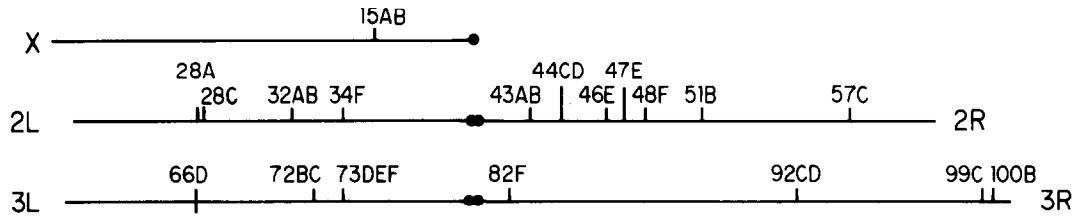


FIG. 7. Chromosomal locations of 20 head-specific cloned DNA sequences represented diagrammatically on the five arms of the salivary gland polytene chromosomes of *D. melanogaster*. Two cloned sequences are located at band 66D.

and thus belong to the moderately abundant mRNA class identified by Levy and McCarthy (1975). Assuming the average *Drosophila* cell contains 6.6 pg of RNA (Lamb and Laird, 1976) of which 90% is cytoplasmic and 68% of that is polysomal (Lovett and Goldstein, 1977), it is possible to estimate the number of copies of these species per average cell of the head. Since 0.5% of polysomal RNA of adult represents polyadenylated mRNA, the most abundantly expressed head-specific clone, 507, occurs at an average level of 140 copies per cell in the head, but only 0.8 copies per cell in the body.

Selection of these clones affords an opportunity to measure the steady-state level of sequences in nuclear RNA from tissues in which their abundance on polyosomes differs by as much as two orders of magnitude. We determined the steady-state concentration of four of the clones in nuclei from head and body by quantitating the hybridization of radiolabeled cloned DNA to Northern blots of nuclear RNA (Fig. 5; Table 3). Most importantly, we observed that the concentration of all sequences in head nuclei is substantially greater (13–48

times) than their concentration in body nuclei. Thus, an increase in cytoplasmic mRNA concentration is paralleled by an increase in the nuclear concentration of these species, a result which suggests that their expression is controlled at the level of transcription. Such a result is predicted by population studies of RNA from cultured *Drosophila* cells which demonstrate that frequently occurring sequences in nuclear RNA are also abundant in cytoplasmic RNA (Levy and McCarthy, 1976). A similar correlation has been reported for several liver-specific genes in mouse (Derman *et al.*, 1981) and for some of the mRNA sequences studied from

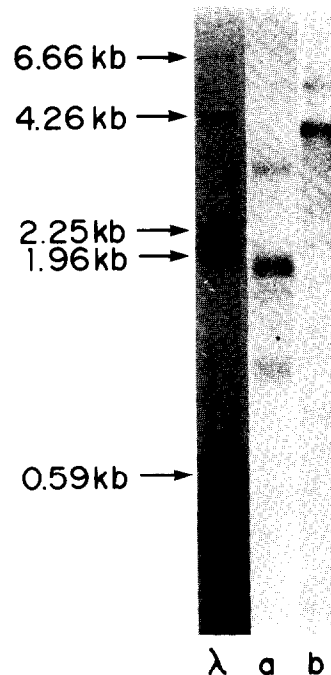


FIG. 8. Northern blot analysis of the transcripts of clones 549 (a) and 551 (b) in polyadenylated polysomal mRNA from *Drosophila* adult head. RNA (1 μ g) was denatured in the presence of glyoxal (McMaster and Carmichael, 1977), fractionated by agarose gel electrophoresis, and transferred to nitrocellulose paper (Thomas, 1980). Northern blots were hybridized to 0.2 μ g of nick-translated cloned DNA (sp act $\sim 1 \times 10^8$ cpm/ μ g). DNA from bacteriophage λ was digested with the restriction enzyme *Hind*III, radiolabeled with polynucleotide kinase using [γ - 32 P]-ATP, and electrophoresed in parallel with RNA samples to provide size markers.

TABLE 4

| Clone | Chromosome arm | Band |
|-------|----------------|-------|
| 503 | 2L | 32AB |
| 506 | 3R | 82F |
| 507 | 3L | 66D |
| 512 | 3R | 92CD |
| 514 | 2R | 51B |
| 516 | 3R | 100B |
| 521 | 3L | 73DEF |
| 525 | 2R | 57C |
| 527 | 2L | 34F |
| 528 | 2R | 47E |
| 536 | 2R | 44CD |
| 538 | 2L | 28C |
| 543 | 2R | 48F |
| 547 | 3L | 66D |
| 548 | X | 15AB |
| 549 | 2R | 46E |
| 551 | 2L | 28A |
| 555 | 2R | 43AB |
| 557 | 3L | 72BC |
| 559 | 3R | 99C |

Chinese hamster ovary (CHO) cells (Harpold *et al.*, 1979). In both of these studies, measurement of transcription in isolated nuclei clearly demonstrates that cytoplasmic abundance of these species is paralleled by increased transcription.

However, studies of sea urchin steady-state RNAs have shown that complex class structural gene sequences occur in nuclei from a variety of tissues and developmental stages at a constant level, although their frequency on polysomes differs (Wold *et al.*, 1978). Indeed, this phenomenon has been documented for two cloned sequences which encode specific developmentally regulated mRNAs in sea urchin (Lev *et al.*, 1980). Davidson and Britten (1979) have recently interpreted these and other data to suggest that structural genes which encode complex class and moderately prevalent mRNAs are transcribed at a basic, continuous rate characteristic of a particular cell type or organism. The data reported here contrast with those from sea urchin and suggest that increased transcriptional activity in the head contributes to the cytoplasmic abundance of the species examined.

Although the increased abundance of four mRNA species on head polysomes is paralleled by an increase in head nuclei, the relationship is not direct. That is, the mRNAs on polysomes are from 21 to 177 times more frequent in head than in body, but the transcripts are only 13 to 48 times more frequent in head nuclei than in body nuclei (Table 3). This comparison suggests that post-transcriptional events also contribute to the cytoplasmic mRNA abundance. An increased rate of processing or transport from head nuclei or an increased mRNA half-life in head are post-transcriptional events which may account for these data. Consistent with these possibilities are the observations of Lenk *et al.* (1978) which show that the decay of abundant polyadenylated mRNAs in cultured *Drosophila* cells is slower than the decay of low frequency species.

In each case examined here by Northern blot analysis, cloned DNAs hybridized to a single major nuclear RNA species of the same size as messenger RNA. Therefore, although somewhat larger molecules are found in the nuclei of *Drosophila* after a 15-min pulse label (Lengyel and Penman, 1975), only message size molecules were observed in steady-state nuclear RNA, perhaps as a result of rapid *in vivo* processing. Similarly, Levy *et al.* (1976) reported the mean size of pulse-labeled polyadenylated nuclear RNA to be 20 S, although steady-state polyadenylated nuclear RNA was substantially smaller (14 S). A minor species, larger than the major band, is apparent in the hybridization of clone 527 to nuclear RNA from head and body (Fig. 5). It is not known whether the minor species represents a precursor molecule, although its concentration is approximately

10-fold greater in head than in body. The transcription of clone 527 is unusual also in that the concentration of the major species of RNA is greater in the nucleus of head or body than in the cytoplasm of head or body. We have not determined the meaning of this relationship. In the case of clones 507, 512, and 557, the transcript concentration is from 6 to 15 times higher on head polysomes than in head nuclei (Table 3). Biessmann (1980) reported an average fourfold relative abundance over nuclear levels for eight moderately abundantly cytoplasmic mRNAs in cultured *Drosophila* cells. However, clones 507, 512, and 557 occur at a frequently typical of rare class messages (Levy and McCarthy, 1975) on body polysomes. In this case, the cytoplasmic concentration is only one to two times greater than the concentration in nuclei (Table 3). Thus, not only is the transcription of these sequences apparently less active in body nuclei than in head nuclei but also a post-transcriptional event has influenced the ratio of cytoplasmic to nuclear RNA concentration.

The chromosomal locations of all 20 cloned sequences have been determined by *in situ* hybridization (Table 4). All of the clones were observed to hybridize to only single loci, suggesting that they are single-copy genes. If the genes are repetitive, the repeats must occur within the same locus, a phenomenon which has been reported for ovalbumin and its homologous relatives (Heilig *et al.*, 1980). Further, it is possible that the cloned DNAs used for *in situ* mapping contain small repetitive elements located at multiple sites on the chromosomes, but which are too small to provide a positive hybridization signal. We consider this unlikely based on the interspersed pattern of repetitive DNA in *Drosophila*, in which long blocks of middle repetitive DNA (average 5.6 kb) lie next to large regions of single-copy DNA (average 13 kb) (Manning *et al.*, 1975). Furthermore, we can detect the 500 nucleotide internal EcoRI fragment of the *cop* gene when hybridized *in situ* under our conditions (Fouts *et al.*, 1981). Thus, the genes appear to represent single-copy DNA although they encode mRNAs in head which belong to the moderately abundant class. Craig *et al.*, (1981) identified the reiteration frequency in the genome of cultured *Drosophila* cells of 20 genes which encode rare-class and moderately abundant mRNAs. Of the 20, seventeen were determined to be single copy and three belonged to the middle repetitive DNA class.

The 20 genes examined here represent a unique set in that they are expressed abundantly only in the limited number of tissues, predominantly neural tissues, that comprise the adult head. Recent studies have demonstrated that gene sets which are tissue specific or which share common developmental regulation may exist as a tight gene cluster on the chromosomes. This phenom-

enon has been reported from the organisms *Aspergillus* (Zimmerman *et al.*, 1981), *Dictyostelium* (Mangiarotti *et al.*, 1981), and *Drosophila* (Spradling *et al.*, 1980; Snyder *et al.*, 1981; Voellmy *et al.*, 1981). The chromosomal loci identified by 20 head-specific clones are dispersed over the chromosome arms, although not randomly (Fig. 7). Twenty-five percent lie on chromosome 2R between regions 43 and 48. In addition, several tight clusters of gene sequences have been identified, two of which contain genes with common developmental regulation. Three genes were identified within the *Drosophila* DNA insert in clone 549, localized to bands 46EF (Fig. 8a). These are expressed together in the head and to some extent in the body of the adult (Table 2). Three genes were localized to region 28 (Table 4), two of which exist within approximately 15 kb of *Drosophila* DNA in clone 551 (Fig. 8b). These three genes are expressed abundantly only in the head of adult *Drosophila* (Table 2; additional data in accompanying paper).

Two other gene clusters were identified which contain sequences subject to distinct developmental regulation. Three genes exist at band 100B within the *Drosophila* DNA in clone 516. One of these is expressed abundantly only in adult head and the others are expressed during embryogenesis (data in accompanying paper). Region 66D, the location of clones 507 and 547, contains at least two gene sequences within the 15 kb of *Drosophila* DNA in clone 547. Only one of these is expressed in the adult head, and the other is expressed at other times during development (data in accompanying paper). Clones 507 and 547 partially cross-hybridize and contain a gene which encodes a 1700-nucleotide mRNA. It is not yet known if these two cloned genes derive from the identical genomic site or represent a repeat. The locus 66D also contains sequences which encode chorion proteins (Spradling *et al.*, 1980), genes subject to developmental regulation distinct from the sequences on clones 507 and 547.

Thus, in summary, we have described the selection and various aspects of the expression and organization of a set of 20 cloned DNA sequences which are expressed abundantly only in the head of adult *Drosophila melanogaster*. In the accompanying paper, we examine the expression of these sequences during *Drosophila* development.

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REFERENCES

- AFFARA, N. A., ROBERT, B., JACQUET, M., BUCKINGHAM, M. E., and GROS, F. (1980). Changes in gene expression during myogenic differentiation. *J. Mol. Biol.* **140**, 441-458.
- BARNETT, T., PACHL, C., GERGEN, J. P., and WENSINK, P. C. (1980). The isolation and characterization of *Drosophila* yolk protein genes. *Cell* **21**, 729-738.
- BENTON, W. D., and DAVIS, R. W. (1977). Screening λ gt recombinant clones by hybridization to single plaques in situ. *Science* **196**, 180-182.
- BIESSMANN, H. (1980). Concentrations of individual RNA sequences in polyadenylated nuclear and cytoplasmic RNA populations of *Drosophila* cells. *Nucl. Acids Res.* **8**, 6099-6111.
- BIESSMANN, H., CRAIG, E. A., and MCCARTHY, B. J. (1979). Rapid quantitation of individual RNA species in a complex population. *Nucl. Acids Res.* **7**, 981-996.
- BLUMBERG, D. D., and LODISH, H. F. (1981). Changes in the complexity of nuclear RNA during development of *Dictyostelium discoideum*. *Dev. Biol.* **81**, 74-80.
- BOLIVAR, F., RODRIGUEZ, R. L., GREENE, P. J., BETLACH, M. C., HEYNECKER, H. L., and BOYER, H. W. (1977). Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene* **2**, 95-113.
- CASEY, J., and DAVIDSON, N. (1977). Rates of formation and thermal stability of RNA: DNA and DNA:DNA duplexes at high concentrations of formamide. *Nucl. Acids Res.* **4**, 1539-1550.
- CHAN, K.-M., KURTZ, D. T., and FEIGELSON, P., (1978). Transcription of the α_2 -globulin gene in male rat liver nuclei *in vitro*. *Biochemistry* **17**, 3092-3096.
- CHANG, A. C. Y., and COHEN, S. N. (1978). Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the p15A cryptic miniplasmid. *J. Bacteriol.* **134**, 1141-1156.
- CORCES, V., HOLMGREN, R., FREUND, R., MORIMOTO, R., and MELSELSON, M. (1980). Four heat shock proteins of *Drosophila melanogaster* coded. *Proc. Nat. Acad. Sci. USA* **77**, 5390-5393.
- CRAIG, E. A., BIESSMANN, H., and MCCARTHY, B. J. (1981). Cloned cDNA of *Drosophila* cells: Relationship of genome copy number to messenger RNA abundance. *Biochemistry* **20**, 2651-2656.
- DANEHOLT, B. (1975). Transcription in polytene chromosomes. *Cell* **4**, 1-9.
- DARNELL, J. E., JR. (1979). Transcription units for mRNA production in eukaryotic cells and their DNA viruses. *Prog. Nucl. Acids Res.* **22**, 327-353.
- DAVIDSON, E. H., and BRITTEN, R. J. (1979). Regulation of gene expression: possible role of repetitive sequences. *Science* **204**, 1052-1059.
- DEMEREK, M. (ed.) (1950). "Biology of *Drosophila*." Wiley, New York.
- DERMAN, E. (1981). Isolation of a cDNA clone for mouse urinary proteins: Age- and sex-related expression of mouse urinary protein genes in transcriptionally controlled cells. *Proc. Nat. Acad. Sci. USA* **78**, 5425-5429.
- DERMAN, E., KRAUTER, K., WALLING, L., WEINBERGER, C., RAY, M., and DARNELL, J. E., JR. (1981). Transcriptional control in the production of liver-specific mRNAs. *Cell* **23**, 731-739.
- FOUTS, D. L., MANNING, J. E., FOX, G. M., and SCHMID, C. W. (1981). A complex repeated DNA sequence within the *Drosophila* transposable element *copia*. *Nucl. Acids Res.* **9**, 7053-7064.
- FRYBERG, E. A., KINDLE, K. L., and DAVIDSON, N. (1980). The actin genes of *Drosophila*: a dispersed multigene family. *Cell* **19**, 365-378.
- GANGULY, R., MEHTA, N. M., GANGULY, N., and BANERJEE, M. R. (1979). Glucocorticoid modulation of casein gene transcription in mouse mammary gland. *Proc. Nat. Acad. Sci. USA* **76**, 6466-6470.
- GANGULY, R., GANGULY, N., MEHTA, N. M., and BANERJEE, M. R. (1980). Absolute requirement of glucocorticoid for expression of the

- casein gene in the presence of prolactin. *Proc. Nat. Acad. Sci. USA* **77**, 6003-6006.
- GOLDBERG, R. B., HOSCHEK, G., DITTA, G. S., and BREIDENBACH, R. W. (1981). Developmental regulation of cloned superabundant embryo mRNAs in soybean. *Dev. Biol.* **83**, 218-231.
- 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.
- GUYETTE, W. A., MATUSIK, R. J., and ROSEN, J. M. (1979). Prolactin-mediated transcriptional and post-transcriptional control of casein gene expression. *Cell* **17**, 1013-1023.
- HAGER, L. J., and PALMITER, R. D. (1981). Transcriptional regulation of mouse liver metallothionein I gene by glucocorticoids. *Nature (London)* **291**, 340-342.
- HARPOLD, M. M., EVANS, R. M., SALDITT-GEORGIEFF, M., and DARNELL, J. E., JR. (1979). Production of mRNA in Chinese hamster cells: Relationship of the rate of synthesis to the cytoplasmic concentration of nine specific mRNA sequences. *Cell* **17**, 1025-1035.
- HAYASHI, S., GILLAM, I. C., DELANEY, A. D., and TENER, G. M. (1978). Acetylation of chromosome squashes of *Drosophila melanogaster* decreases the background in autoradiographs from hybridization with [¹²⁵I]-labeled RNA. *J. Histochem. Cytochem.* **26**, 677-679.
- HEILIG, R., PERRIN, F., GANNON, F., MANDEL, J. L., and CHAMBON, P. (1980). The ovalbumin gene family: Structure of the X gene and evolution of duplicated split genes. *Cell* **20**, 625-637.
- KAFATOS, F. C., JONES, C. W., and EFSTRATIADIS, A. (1979). Determination of nucleic acid sequence homologies and relative concentrations by a dot hybridization procedure. *Nucl. Acids. Res.* **7**, 1541-1552.
- KALFAYAN, L., and WENSINK, P. C. (1981). α -Tubulin genes of *Drosophila*. *Cell* **24**, 97-106.
- KAMALAY, J., and GOLDBERG, R. B. (1980). Regulation of structural gene expression in tobacco. *Cell* **19**, 935-946.
- KLEENE, K. C., and HUMPHREYS, T. (1977). Similarity of hnRNA Sequences in blastula and pluteus stage sea urchin embryos. *Cell* **12**, 143-155.
- LAMB, M. M., and LAIRD, C. D. (1976). The size of poly (A)-containing RNAs in *Drosophila melanogaster* embryos. *Biochem. Genet.* **14**, 357-371.
- LASKY, L., and TOBIN, A. J. (1979). Transcriptional regulation in avian erythroid cells. *Biochemistry* **18**, 1594-1598.
- 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.
- LEFEVRE, G., JR. (1976). A photographic representation and interpretation of the polytene chromosomes of *Drosophila melanogaster* salivary glands. In "The Genetics and Biology of *Drosophila*" (M. Ashburner and E. Novitsk, eds.), Vol. 1a, pp. 31-66. Academic Press, New York.
- LENGYEL, J., and PENMAN, S. (1975). hnRNA size and processing as related to different DNA content in two dipterans: *Drosophila* and *Aedes*. *Cell* **5**, 281-290.
- LENK, R., HERMAN, R., and PENMAN, S. (1978). Messenger RNA abundance and life time: A correlation in *Drosophila* cells but not in HeLa. *Nucl. Acids Res.* **5**, 3057-3070.
- 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, W. B., JOHNSON, C. B., and MCCARTHY, B. J. (1976). Diversity of sequences in total and polyadenylated nuclear RNA from *Drosophila* cells. *Nucl. Acids Res.* **3**, 1777-1788.
- LEVY, W. B., and MCCARTHY, B. J. (1976). Relationship between nuclear and cytoplasmic RNA in *Drosophila* cells. *Biochemistry* **15**, 2415-2419.
- LEVY, W. B., and DIXON, G. H. (1977). Diversity of sequences of polyadenylated cytoplasmic RNA from rainbow trout (*Salmo gairdneri*) testis and liver. *Biochemistry* **16**, 958-964.
- LIFTON, R. P., GOLDBERG, M. L., KARP, R. W., and HOGNESS, D. S. (1978). The organization of the histone genes in *Drosophila melanogaster*: Functional and evolutionary implications. *Cold Spring Harbor Symp. Quant. Biol.* **42**, 1047-1051.
- LINDSLEY, D. L., and GRELL, E. H. (1968). "Genetic Variations of *Drosophila melanogaster*." *Carnegie Institution of Washington, publication No. 627*.
- 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.
- MAEKAWA, H., and SUZUKI, Y. (1980). Repeated turn-off and turn-on of fibroin gene transcription during silk gland development of *Bombyx mori*. *Dev. Biol.* **78**, 394-406.
- MANGIAROTTI, G., CHUNG, S., ZUKER, C., and LODISH, H. (1981). Selection and analysis of cloned developmentally-regulated *Dictyostelium discoideum* genes by hybridization-competition. *Nucl. Acids Res.* **9**, 947-963.
- MANIATIS, T., HARDISON, R. C., LACY, E., LAUER, J., O'CONNEL, C., QUON, D., SIM, G. K., and EFSTRATIADIS, A. (1978). The isolation of structural genes from libraries of eucaryotic DNA. *Cell* **15**, 687-701.
- MANNING, J. E., SCHMID, C. W., and DAVIDSON, N. (1975). Interspersion of repetitive and nonrepetitive DNA sequences in the *Drosophila melanogaster* genome. *Cell* **4**, 141-155.
- MAURON, A., and SPOHR, G. (1978). Frequency distribution of mRNA and pre-mRNA in growing and differentiated Friend cells. *Nucl. Acids Res.* **5**, 3013-3032.
- MCMASTER, G. K., and CARMICHAEL, G. G. (1977). Analysis of single- and double-stranded nucleic acids on polyacrylamide and agarose gels by using glyoxal and acridine orange. *Proc. Nat. Acad. Sci. USA* **74**, 4835-4838.
- POSTLETHWAIT, J. H., and JOWETT, T. (1980). Genetic analysis of the hormonally regulated yolk polypeptide genes in *D. melanogaster*. *Cell* **20**, 671-678.
- ROOP, D. R., NORDSTROM, J. L., TSAI, S. Y., TSAI, M.-J., and O'MALLEY, B. (1978). Transcription of structural and intervening sequences in the ovalbumin gene and identification of potential ovalbumin mRNA precursors. *Cell* **15**, 671-685.
- RIDDELL, D. C., HIGGINS, M. J., McMILLAN, B. J., and WHITE, B. N. (1981). Structural analysis of the three vitellogenin genes in *Drosophila melanogaster*. *Nucl. Acids Res.* **9**, 1323-1330.
- SHEPHERD, G. W., and NEMER, M. (1980). Developmental shifts in frequency distribution of polysomal mRNA and their posttranscriptional regulation in the sea urchin embryo. *Proc. Nat. Acad. Sci. USA* **77**, 4653-4656.
- SIM, G. K., KAFATOS, F. C., JONES, C. W., KOEHLER, M. D., EFSTRATIADIS, A., and MANIATIS, T. (1979). Use of a cDNA library for studies on evolution and developmental expression of the chorion multigene families. *Cell* **18**, 1303-1316.
- SIPPEL, A. E., HYNES, N., GRONER, B., and SCHUTZ, G. (1977). Frequency distribution of messenger sequences within polysomal mRNA and nuclear RNA from rat liver. *Eur. J. Biochem.* **77**, 141-151.
- SMITH, D. F., McCLELLAND, A., WHITE, B. N., ADDISON, C. F., and

- GLOVER, D. M. (1981). The molecular cloning of a dispersed set of developmentally regulated genes which encode the major larval serum protein of *D. melanogaster*. *Cell* **23**, 441-449.
- SMITH, M. J., BRITTEN, R. J., and DAVIDSON, E. H. (1975). Studies on nucleic acid reassociation kinetics: Reactivity of single stranded tails in DNA-DNA renaturation. *Proc. Nat. Acad. Sci. USA* **72**, 4805-4809.
- SNYDER, M., HIRSH, J., and DAVIDSON, N. (1981). The cuticle genes of *Drosophila*: A developmentally regulated gene cluster. *Cell* **25**, 165-177.
- 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.
- STROBEL, E., DUNSUMIR, P., and RUBIN, G. M. (1979). Polymorphisms in the chromosomal locations of elements of the 412, Copis and 297 dispersed repeated gene families in *Drosophila*. *Cell* **17**, 429-439.
- THOMAS, P. (1980). Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Nat. Acad. Sci. USA* **77**, 5201-5205.
- TOBIN, S. L., ZULAUF, R., SANCHEZ, F., CRAIG, E. A., and MCCARTHY, B. J. (1980). Multiple actin-related sequences in the *Drosophila melanogaster* genome. *Cell* **19**, 121-131.
- VOELLMY, R., GOLDSCHMIDT-CLERMONT, M., SOUTHGATE, R., TISSIERES, A., 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.
- VOLLOCH, V., and HOUSMAN, D. (1981). Stability of globin mRNA in terminally differentiating murine erythroleukemia cells. *Cell* **23**, 509-514.
- 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.
- WILLIAMS, J. G., LLOYD, M. M., and DEVINE, J. M. (1979). Characterization and transcription analysis of a cloned sequence derived from a major developmentally regulated mRNA of *D. discoideum*. *Cell* **17**, 903-913.
- WILSON, M., FRASER, N. W., and DARNELL, J. E., JR. (1979a). Mapping of RNA initiation sites by high doses of uv irradiation: evidence for three independent promoters within the left 11% of the Ad-2 genome. *Virology* **94**, 175-184.
- WILSON, M. C., NEVINS, J. R., BLANCHARD, J.-M., GINSBERG, H. S., and DARNELL, J. E., JR. (1979b). Metabolism of mRNA from the transforming region of adenovirus 2. *Cold Spring Harbor Symp. Quant. Biol.* **44**, 447-455.
- WILSON, M. C., and DARNELL, J. E., JR. (1981). Control of messenger RNA concentration by differential cytoplasmic half-life. *J. Mol. Biol.* **148**, 131-151.
- WOLD, B. J., KLEIN, W. H., HOUGH-EVANS, B. R., BRITTEN, R. J., and DAVIDSON, E. H. (1978). Sea urchin embryo mRNA sequences expressed in the nuclear RNA of adult tissues. *Cell* **14**, 941-950.
- ZIMMERMAN, J. L., FOUTS, D. L., and MANNING, J. E. (1980). Evidence for a complex class of nonadenylated mRNA in *Drosophila*. *Genetics* **95**, 673-691.
- ZIMMERMAN, 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.