MOLECULAR CLONING OF α-AMYLASE GENES FROM *DROSOPHILA MELANOGASTER*. I. CLONE ISOLATION BY USE OF A MOUSE PROBE

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ABSTRACT

A cloned α-amylase cDNA sequence from the mouse is homologous to a small set of DNA sequences from *Drosophila melanogaster* under appropriate conditions of hybridization. A number of recombinant lambda phage that carry homologous Drosophila genomic DNA sequences were isolated using the mouse clone as a hybridization probe. Putative amylase clones hybridized *in situ* to one or the other of two distinct sites in polytene chromosome *2R* and were assigned to one of two classes, A and B. Clone λDm32, representing class A, hybridizes within chromosome section 53CD. Clone λDm65 of class B hybridizes within section 54A1-B1. Clone λDm65 is homologous to a 1450- to 1500-nucleotide RNA species, which is sufficiently long to code for α-amylase. No RNA homologous to λDm32 was detected. We suggest that the class B clone, λDm65, contains the functional *Amy* structural gene(s) and that class A clones contain an amylase pseudogene.

UNDERSTANDING cellular differentiation is in large measure a problem of understanding the mechanisms behind differential gene expression. The structural and regulatory genes involved in the expression of α-amylase in *Drosophila melanogaster* are being analyzed in this laboratory as a model system for understanding differential gene expression.

In Drosophila, α-amylase (EC 3.2.1.1) is a monomeric enzyme with a molecular weight of 54,500 (DOANE *et al.* 1975) that hydrolyzes α-1,4 glycosidic bonds in starch. It is found in a variety of tissues, but the midgut typically contains more amylase activity than other organs (KIKKAWA and ABE 1960; DOANE 1969a). Eight electrophoretic variants of amylase have been found in *D. melanogaster* (see review, DOANE *et al.* 1983), and allozymes have been used to genetically locate the amylase structural gene, *Amy*, on the linkage map of chromosome arm *2R* (KIKKAWA 1964; BAHN 1967; DOANE 1969b). On the basis of cytogenetic translocation analysis, *Amy* is reported to be situated between sections 54B and 55 in the salivary chromosome map of *2R* (BAHN 1971a). Furthermore, position effect variegation in amylase activity was reported for a translocation that inserts the segment from 54A-60, containing the *Amy* region, into the heterochromatic portion of the *X* chromosome (BAHN 1971b). Many strains of *D. melanogaster*, although isogenic for chromosome

2R, produce two amylase isozymes. This and other biochemical and genetic evidence (reviewed in DOANE *et al.* 1983) suggest that in these strains the structural gene for amylase is duplicated.

Amylase genes have been isolated from the mouse, *Mus musculus*, and analysis of this gene system at the molecular level has provided important new insights into eukaryotic genetic regulatory mechanisms (HAGENBÜCHLE, BOVEY and YOUNG 1980; SCHIBLER *et al.* 1982; SCHIBLER *et al.* 1983). These authors have shown that in the mouse there is more than one functional α-amylase structural gene, and that different transcripts are produced from a single structural gene in a tissue-specific manner. In *D. melanogaster*, the tissue-specific and temporal expression of amylase in the posterior midgut of the adult is, in part, under genetic control by a separate, *trans*-acting locus, termed *map* (midgut activity pattern; ABRAHAM and DOANE 1978; DOANE *et al.* 1983). It appears that the *map* gene regulates the level of translatable amylase mRNA, as shown by microinjection of appropriate fly RNA preparations into Xenopus oocytes and electrophoretic analysis of the Drosophila amylase isozymes subsequently produced by the oocytes (BUCHBERG 1983; DOANE *et al.* 1983). Although *map* regulates the level of amylase expressed in the adult posterior midgut, it seems to have little or no effect on amylase expression in the anterior midgut. There appears to be at least one other controlling element located in chromosome *2R* that is genetically separable from both *map* and *Amy* and that regulates amylase levels in the anterior midgut (DOANE 1980; DOANE *et al.* 1983). Isolation of a cloned DNA segment carrying all or part of the *Amy* gene from this species is the first step required for a molecular analysis of the complex genetic properties of the amylase system.

In this paper we report that a cDNA clone containing salivary α-amylase sequences from the mouse shares homology with a set of unique DNA sequences from *D. melanogaster*. This homology was exploited to isolate Drosophila DNA sequences that are in turn homologous to the *Amy* region in polytene chromosomes from *D. melanogaster*. One recombinant phage, λDm65, has many of the properties expected of an *Amy*-bearing clone. Final verification that λDm65 indeed contains *Amy* sequences is in our companion paper (LEVY, GEMMILL and DOANE 1985). A second group of isolated clones may represent an amylase pseudogene.

MATERIALS AND METHODS

Stocks of D. melanogaster: Fly stocks included a Canton-S strain which carries $Amy^{1,3}$ and an $Amy^{1,6}$ strain (DOANE 1969b). These strains were originally made isogenic for chromosomes *1*, *2* and *3* and have been maintained in this laboratory for many years. There is a ten-fold difference in total amylase activity between larval extracts of the Canton-S and $Amy^{1,6}$ strains, *i.e.*, 0.176 and 1.808 nmol of maltose equivalents per microgram of protein per minute released at 25°, pH 7.4, respectively (LYNDA G. TREAT-CLEMONS, unpublished data). Strains used for *in situ* hybridization experiments included gt^1 w^a, *FM6/y sc* gt^{X11} and *SM5/In(2R)G, Sp Bl N-2G*. The last strain was obtained from L. CRAYMER, California Institute of Technology, Pasadena; a derivative of this strain also contained *FM6/y sc* gt^{X11}. LINDSLEY and GRELL (1968) provide explanations of other genetic symbols for fly stocks used in this study.

DNA sources and manipulations: The cDNA clone for mouse salivary α-amylase, pMSa104 (HAG-

ENBÜCHLE, BOVEY and YOUNG 1980), was obtained from U. SCHIBLER, Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland. A library of DNA sequences from *D. melanogaster* Canton-S, inserted into the lambda vector Charon 4 (MANIATIS *et al.* 1978), was obtained from J. L. MARSH, University of California, Irvine. Plasmid DNA was purified using either a CsCl density gradient method (KUPERSZTOCH-PORTNOY, LOVETT and HELINSKY 1974; HERTZBERG *et al.* 1980) or an alkaline sodium dodecyl sulfate (SDS) method (BIRNBOIM and DOLY 1979). Restriction fragments were isolated (ZAIN and ROBERTS 1978) from preparative agarose gels (WU, JAY and ROYCHOUDHURY 1976). DNA preparations (plasmids, restriction fragments or lambda phage) were labeled to high specific activity with ^{32}P (α-^{32}P dCTP or α-^{32}P dATP, New England Nuclear) by nick translation (RIGBY *et al.* 1977).

Drosophila chromosomal DNA was isolated from adult flies by gentle homogenization in extraction buffer containing 100 mM tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 7.5, 200 mM sucrose, 50 mM ethylenediaminetetraacetic acid (EDTA) and 0.5% SDS. The homogenate was extracted three times with an equal volume of phenol equilibrated with buffer and DNA was recovered from the final supernatant by ethanol precipitation. DNA was digested with restriction endonucleases according to suppliers' specifications. Samples of digested DNA were resolved electrophoretically on gels containing 0.7–1.0% agarose and 90 mM Tris-borate, pH 8.3, 1 mM EDTA. Southern transfers (SOUTHERN 1975) were prepared according to the Gene Screen manual (New England Nuclear). The Drosophila Charon 4 library (MANIATIS *et al.* 1978) was screened by the methods of BENTON and DAVIS (1977). Recombinant phage were isolated preparatively using polyethylene glycol precipitation followed by centrifugation on CsCl step gradients (YAMAMOTO *et al.* 1970). Phage DNA was prepared by lysis of phage particles in 0.5% SDS and 5 mM EDTA at 60°. The solution was placed on ice and KCl was added to 0.5 M to precipitate the SDS. Following centrifugation, the supernatant was ethanol precipitated to recover the phage DNA.

Preparation of mouse amylase probe: Fifty micrograms of pMSa104 DNA were digested with *Pst*I and resolved on a 1.0% agarose gel. The inserted fragment that was recovered consisted of mouse α-amylase cDNA along with poly (dG-dC) tails at each end. Subsequent cleavage with *Hin*fI separated the amylase-coding sequences from the dG-dC tails. The three resulting internal *Hin*fI fragments constituted nearly the entire coding sequence for mouse salivary α-amylase (HAGENBÜCHLE, BOVEY and YOUNG 1980). These fragments were purified on an agarose gel, recovered and nick translated to generate the probe referred to as the *mouse amylase probe.*

Hybridization to filter bound DNA: Hybridizations were carried out in the following solution: 50% formamide, 0.2% polyvinyl pyrrolidone (PVP), 0.2% Ficoll, 0.2% bovine serum albumin (BSA), 50 mM Tris-HCl, pH 7.5, 1 M NaCl, 0.1% SDS, 250 μg/ml of denatured salmon testis DNA and 10% dextran sulfate. The mouse amylase probe was hybridized to filter-bound fly DNA under conditions of reduced stringency to optimize signal and reduce background. These conditions were determined empirically by systematically altering the temperature of hybridization and the temperature and salt concentration of subsequent washes. Optimal conditions included hybridization for 16 hr at 37° followed by two washes in 2 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M Na citrate), 0.1% SDS at room temperature for 15 min each. Two 15-min final washes were in 1 × SSC, 0.1% SDS at 50°. Autoradiograms were prepared by exposing Kodak X-Omat AR-5 film to filters at −70° for 12 hr to 3 days with DuPont Cronex Lightning-Plus intensifying screens. These conditions were used both for Southern analysis of Drosophila genomic DNA and for screening the Drosophila library with the mouse amylase probe.

In situ *hybridization to polytene chromosomes*: Chromosome squashes were prepared from salivary glands of "giant" (*y sc* gt^{X11}/gt^{1} w^{a}) larvae, derived from the cross *FM6/y sc* gt^{X11} ♀ × gt^{1} w^{a} ♂. The procedure of PARDUE and GALL (1975), as modified by HAYASHI *et al.* (1978), was used for *in situ* hybridization of labeled probe to salivary polytene chromosomes. For some experiments, squashes were prepared from larvae of the genotype *y sc* gt^{X11}/gt^{1} w^{a}; *In(2R)G/+*. Tritium-labeled cRNA was synthesized (WENSINK *et al.* 1974) using purified recombinant phage DNA as template. Hybridization of cRNA (10^{5} cpm/slide) to chromosome preparations was performed for 16 hr at 42° in a fluid containing 50% formamide, 2 × SSC, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, 200 μg/ml of denatured salmon testis DNA, 5 mM Na phosphate, pH 6.5, and 10% dextran sulfate. Slides were washed in four changes of 2 × SSC at room temperature followed by two washes in 0.5 × SSC at 65° for 15 min each. Treatment with RNase was omitted. Slides were coated with nuclear track emulsion (Kodak) and exposed for 4–7 days at 4°.

Isolation of total and poly(A)$^+$ RNA and analysis on Northern blots: Total RNA was isolated from third instar larvae of the *Amy*^{1,3} and *Amy*^{1,6} strains using the guanidinium thiocyanate method (CHIRGWIN *et al.* 1979). Larvae were reared on a starch-yeast diet (DOANE 1969a). Oligo-dT cellulose chromatography (AVIV and LEDER 1972) was used to prepare a poly(A)$^+$ RNA fraction from each RNA preparation. Three milligrams of total RNA typically yielded 50–65 μg of poly(A)$^+$ RNA.

RNA samples were denatured with 10 mM methyl mercuric hydroxide and resolved by electrophoresis in 1.1% agarose gels containing 2 mM methyl mercuric hydroxide (BAILEY and DAVIDSON 1976). Gels were stained in 0.5 M NH_4 acetate containing 0.5 μg of ethidium bromide/ml. The RNA was transferred to membrane filters and hybridized according to the method of THOMAS (1980).

RESULTS

Hybridization of a mouse α-amylase cDNA probe to specific sequences in Drosophila DNA: Plasmid pMSa104 contains a 1.66-kilobase (kb) cDNA fragment inserted into the *Pst*I site of pBR322 and carries nearly all of the coding sequence for the mouse salivary α-amylase gene (HAGENBÜCHLE, BOVEY and YOUNG 1980). We had conducted a number of preliminary experiments to determine whether the mouse amylase sequences present on pMSa104 could be used to detect homologous sequences in DNA from *D. melanogaster*. These experiments (DOANE *et al.* 1983) were performed using labeled pMSa104 DNA as a hybridization probe on Southern transfers of *Eco*RI-cut DNA isolated from our Oregon-R strain of *D. melanogaster*. We found that under appropriate conditions (MATERIALS AND METHODS), labeled pMSa104 does hybridize to the Drosophila DNA. However, the high level of background signal seen with this probe prevented its direct use. Experiments using pBR322 as probe demonstrated that much of the background could be attributed to hybridization by vector sequences. To lower the level of background hybridization we prepared a probe consisting of mouse amylase-coding sequences purified away from the vector sequences of pMSa104 (MATERIALS AND METHODS; DOANE *et al.* 1983). This probe hybridized to at least two discrete *Eco*RI fragments in *D. melanogaster* Oregon-R DNA (figure 8 in DOANE *et al.* 1983) with little or no background. Because our genomic library was derived from a Canton-S strain (MANIATIS *et al.* 1978), we have here repeated these experiments using Southern transfers of *Eco*RI-cut Canton-S DNA. Figure 1, lane 1, shows that the mouse amylase probe hybridized to three restriction fragments approximately 3.8, 5.8 and 20 kb in size in *Eco*RI-cut Canton-S DNA. These results taken together with previous findings (DOANE *et al.* 1983) indicate that some homology exists between the mouse salivary amylase-coding sequence and a set of DNA sequences in *D. melanogaster*.

Drosophila genomic DNA clones homologous to the mouse α-amylase cDNA probe: The mouse α-amylase cDNA fragments (mouse amylase probe) purified from pMSa104 were used as a hybridization probe to isolate homologous sequences from the Maniatis library (MATERIALS AND METHODS). The conditions of hybridization were identical with those used for the identification of homologous sequences on the Southern transfer shown in Figure 1, lane 1. A total of 12 clones was isolated in this screen from an estimated 1.6×10^5 phage, repre-

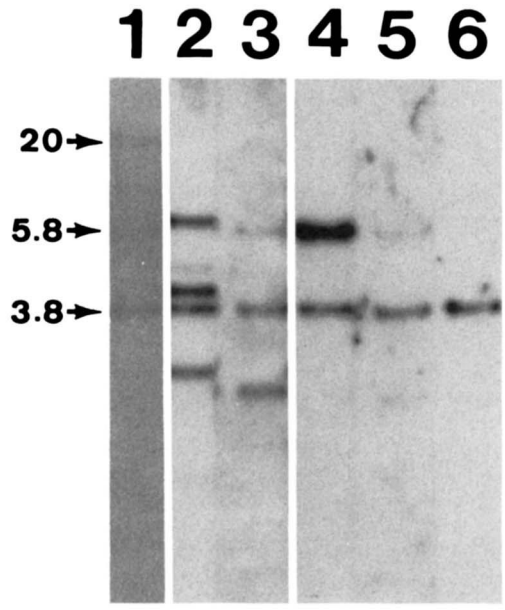

FIGURE 1.—Southern analysis of *D. melanogaster* Canton-S DNA. *Eco*RI-digested DNA from the Canton-S strain of *D. melanogaster* was resolved on parallel lanes (10 μg/lane) of a 1% agarose gel. A Southern transfer was prepared from the fractionated DNA, and individual lanes were hybridized with the following ^{32}P-labeled probes: purified mouse amylase probe (lane 1); λDm32 (lane 2); λDm65 (lane 3); 5.6-kb subclone of λDm65 (lane 4); 3.8-kb subclone of λDm65 (lane 5); 3.8-kb subclone of λDm32 (lane 6). Hybridizations in lanes 2–6 were carried out at high levels of stringency, whereas the hybridization in lane 1 was carried out under the conditions described in MATERIALS AND METHODS for use of the mouse amylase probe. The sizes of the three DNA fragments in lane 1 are given in kilobases.

senting 15 haploid genome equivalents of fly DNA. Preliminary characterization of these isolates by restriction endonuclease digestion indicated that a number of clones carried overlapping fragments derived from the same genomic region. The clones were divided into groups on this basis. Representative members of each group were tested for the sites to which they hybridized on polytene chromosomes. Clones from two classes, A and B, hybridized to chromosome arm *2R*, which contains the *Amy* region, whereas all other clones tested hybridized to other chromosomes. We chose to focus our efforts on the clones in class A and class B since *Amy* sequences would most likely be found in one of these two groups.

Class A contained five clones, λD31, λDm32, λDm76, λDm89 and λDm713; class B contained two clones, λDm65 and λDm41. From restriction endonuclease digestion patterns, clone λDm713 appeared to be identical with λDm31, and λDm41 was apparently identical with λDm65. Thus, we obtained four independent class A clones and a single class B clone.

Figure 2 shows the results of hybridization of the mouse amylase probe to a Southern blot of *Eco*RI-digested DNA isolated from these phage. Panel A shows the ethidium bromide-stained gel. In panel B, the resulting blot was

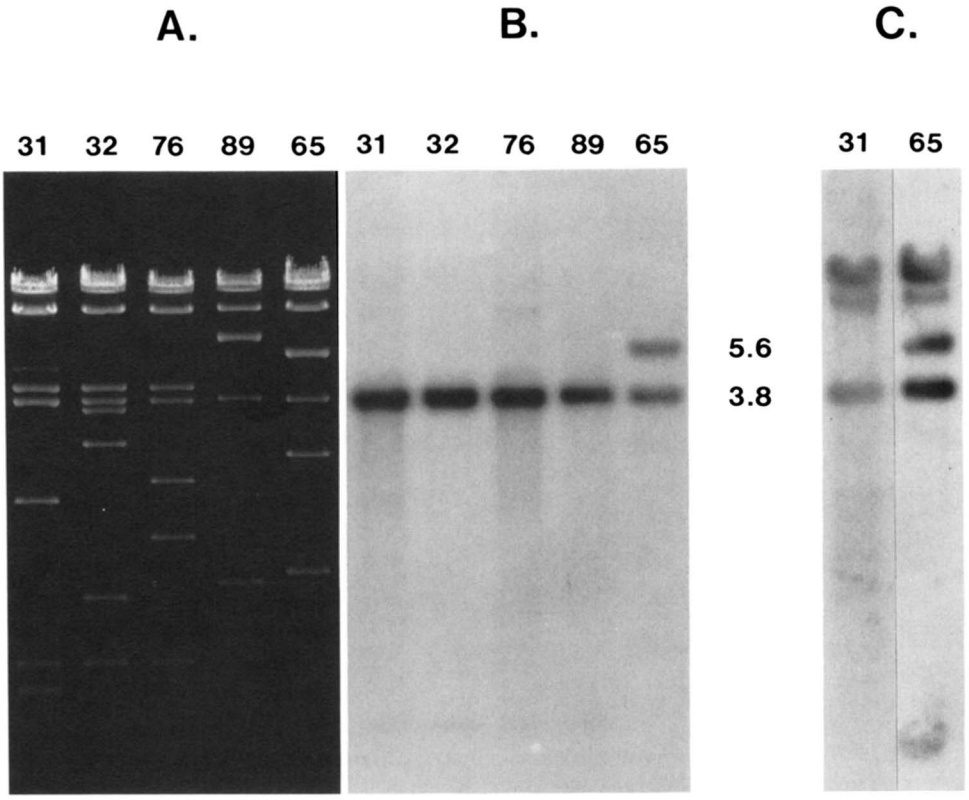

FIGURE 2.—Hybridization of mouse amylase cDNA sequences to the class A and class B clones. DNA (~1 μg) of λDm31, λDm32, λDm76, λDm89 (all class A) and λDm65 (class B) were digested with *Eco*RI and the products resolved on a 0.7% agarose gel. After transfer, the resulting filter was hybridized with 1.5×10^6 cpm of nick-translated mouse amylase probe (*i.e.*, cDNA insert fragments; panel B) or intact pMSa104 (panel C) using the conditions of lowered stringency described in MATERIALS AND METHODS. Panel A shows the ethidium bromide-stained pattern of DNA fragments. Panels B and C show the resulting autoradiograms from the two probes used. In panel C, the two lanes shown contain λDm31 (class A) and λDm65 (class B), respectively, and these filters were washed under conditions of increased stringency (1 hr in 0.5 × SSC, 0.1% SDS at 55°).

hybridized with the mouse amylase probe. All four class A clones contained a single *Eco*RI-generated fragment about 3.8 kb in size that hybridized with the mouse probe. The class B clone, λDm65, contained two *Eco*RI fragments, 5.6 and 3.8 kb in size, which were homologous to the probe. In panel C, the blot was probed with labeled pMSa104 and subsequent washes were performed at a higher stringency (0.5 × SSC and 55°). Under these conditions, clone λDm65 was much more effective in retaining the probe than was the representative class A clone, λDm31, probably indicating a higher degree of homology between λDm65 and mouse amylase sequences. We have observed this apparent difference in a number of hybridization experiments comparing class A and B clones, but not in all (*e.g.*, Figure 2B; see DISCUSSION).

Probes generated by nick translation of λDm32 (class A) and λDm65 (class

B) were hybridized to Southern blots of *Eco*RI-cut Canton-S DNA. Figure 1, lane 2, shows that λDm32 hybridized to a set of *Eco*RI fragments, including one fragment that coincides with the 3.8-kb fragment identified in lane 1 by the mouse amylase probe. Clone λDm65 (lane 3) hybridized to a different set of fragments on a similar blot, including two fragments, 3.8 and 5.8 kb in size, that coincide with two fragments identified with the mouse amylase probe in lane 1. Figure 2 demonstrates that the amylase-like sequences present in our isolates reside in a 3.8-kb *Eco*RI fragment in class A clones and in 3.8- and 5.6-kb *Eco*RI fragments in the class B clone. These three *Eco*RI fragments, subcloned into the *Eco*RI site of pBR325 (LEVY, GEMMILL and DOANE 1985), were used separately as probes against similar Southern blots. The 5.6-kb subclone of λDm65 hybridized to both the 5.8- and 3.8-kb genomic fragments homologous to the mouse amylase probe (Figure 1, lane 4). Likewise, the 3.8-kb subclone of λDm65 hybridized to the same two fragments (Figure 1, lane 5), although the relative hybridization intensity is now reversed. In contrast, the subclone from λDm32 (Figure 1, lane 6) hybridized only to the 3.8-kb genomic fragment.

Our results show that the clones we have isolated contain the sequences originally identified on Southern blots using the mouse amylase probe. The 5.6-kb *Eco*RI fragment present in λDm65 is 0.2 kb shorter than the corresponding genomic fragment. This difference is explained by the fact that this fragment is located at one end of the restriction endonuclease map of λDm65 (LEVY, GEMMILL and DOANE 1985) and it apparently contains an artificial *Eco*RI site introduced during library construction (MANIATIS *et al.* 1978). The class A and class B clones do not contain sequences corresponding to the 20-kb fragment identified in Figure 1, lane 1. If this sequence is present in the group of clones we isolated, it must be located in one of the clones that mapped to a chromosome arm other than *2R*. These clones have not yet been characterized in detail.

In situ *hybridization of cloned sequences to polytene chromosomes*: Clones λDm32 and λDm65 were hybridized *in situ* to polytene chromosomes from a number of strains to precisely locate their hybridization sites on the cytological map of *D. melanogaster*. Tritium-labeled cRNA synthesized from clone λDm32 (class A) hybridized to polytene chromosome arm *2R* within section 53CD (Figure 3A). A comparable probe synthesized from λDm65 (class B) hybridized within section 54A1-B1, probably proximal to 54B1 (Figure 3B). A mixture of these two probes hybridized to both sites on chromosome *2R*, thus confirming that the sites of hybridization are distinct (Figure 3C). Our conclusions concerning the position of hybridization of these two clones have also been confirmed by hybridization to chromosomes heterozygous for *In(2R)G* (not shown), which has breakpoints at 50E and 54D (LINDSLEY and GRELL 1968). Both clones hybridized within the inversion loop but at separate sites consistent with each clone's expected distance from the 54D breakpoint.

The hybridization of probes to salivary chromosome squashes was done under conditions of high stringency. Thus, although λDm32 and λDm65 showed homology to the mouse amylase probe and showed homology *inter se* under

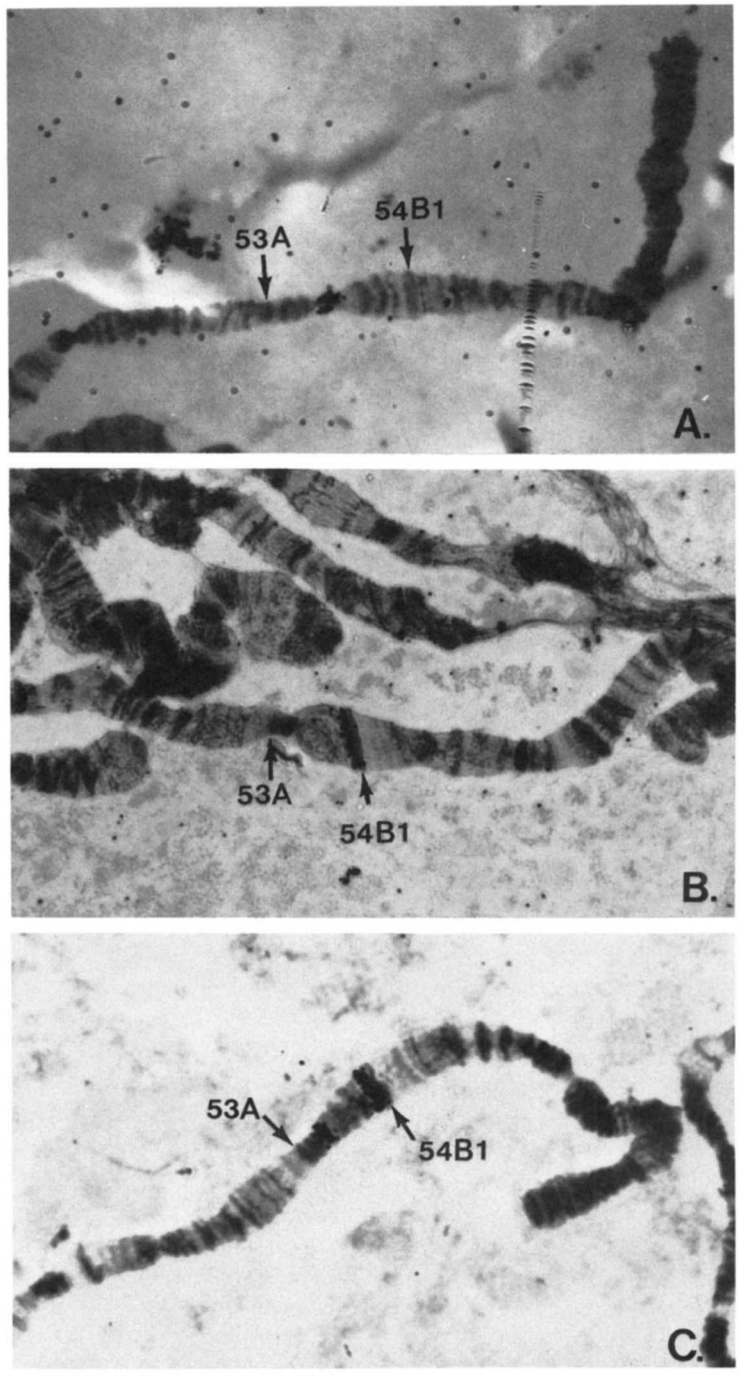

FIGURE 3.—*In situ* hybridization of λDm65 and λDm32 to polytene chromosomes of *D. melanogaster*. Salivary chromosome squashes prepared from giant larvae were hybridized with tritium-labeled cRNA prepared from λDm32 and λDm65 (MATERIALS AND METHODS). Each panel shows the distal portion of chromosome arm *2R*. The landmark bands at 53A and 54B1 are pointed out by arrows on each chromosome. Chromosome preparations in panels A through C were hybridized with the following probes: panel A, λDm32; panel B, λDm65; panel C, a mixture of λDm32 and λDm65.

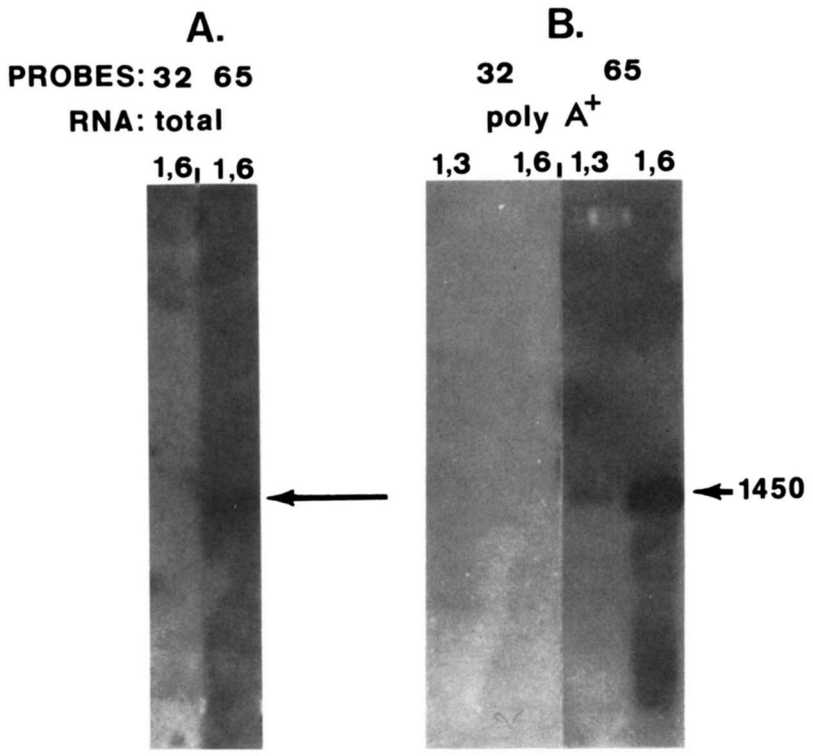

FIGURE 4.—Hybridization of λDm65 and λDm32 to RNA on Northern transfers. RNA was isolated from $Amy^{1,6}$ (1,6), and $Amy^{1,3}$ (1,3) larvae and Northern transfers were prepared and hybridized as described in MATERIALS AND METHODS. In panel A, 20 μg of total RNA from $Amy^{1,6}$ larvae were resolved on a denaturing agarose gel prior to transfer. In panel B, 5 μg of poly(A)$^+$ RNA from $Amy^{1,3}$ and $Amy^{1,6}$ larvae were resolved in adjacent lanes of a comparable gel prior to transfer to a filter. The filter strips were hybridized with either nick-translated λDm32 or λDm65 probes as indicated. Arrows mark the position of the homologous RNA which is approximately 1450 nucleotides in size.

conditions of lowered stringency (LEVY, GEMMILL and DOANE, 1985), each clone hybridized to only a single site in these experiments.

Hybridization of clones to RNA on Northern blots: RNA prepared from two strains of flies was tested for the presence of transcripts homologous to λDm32 and λDm65. RNA gel transfers were prepared from RNA isolated from larvae of $Amy^{1,6}$ and $Amy^{1,3}$ (Canton-S) strains. When λDm32 was used as a hybridization probe against RNA isolated from $Amy^{1,6}$ larvae, no homologous RNA was detectable from either 20 μg of total RNA (Figure 4A) or 5 μg of poly(A)$^+$ RNA (Figure 4B). However, λDm65 hybridized to a discrete RNA species, approximately 1450 nucleotides in size, that was detectable in both the total RNA and the poly(A)$^+$ fraction. This size was estimated by comparison to known sizes of rRNA and mitochondrial rRNA (LONG and DAWID 1979; ZIMMERMAN, FOUTS and MANNING 1980). The strong signal present in the poly(A)$^+$ sample (Figure 4B) indicates that at least a portion of the RNA homologous to λDm65 was polyadenylated and presumably functioned as a message. These

results suggest that λDm65 carries a gene(s) functional in late third instar larvae, whereas λDm32 does not.

Based on visual inspection, the amount of homologous RNA present in $Amy^{1,6}$ and $Amy^{1,3}$ larvae was quite different (*cf.* 1,3 and 1,6 tracks in Figure 4B). It appears that $Amy^{1,6}$ larvae contained considerably more of this RNA than did $Amy^{1,3}$ larvae. The difference in RNA levels thus correlates well with the different amounts of amylase activity in these two strains (see fly stocks in MATERIALS AND METHODS).

DISCUSSION

Homology between a mouse amylase cDNA clone and specific DNA sequences in *D. melanogaster* is weak but detectable. To detect this homology, it was essential to use intermediate levels of stringency for both the hybridization and subsequent filter washes. In addition, it was necessary to purify the amylase-coding sequences away from vector sequences in order to lower the level of background hybridization (DOANE *et al.* 1983).

Preliminary analysis of isolated clones by restriction endonuclease digestion resulted in the grouping of clones that seemed to be related into separate classes. Clones from two of these classes, A and B, hybridized to chromosome arm *2R*, known to contain the *Amy* region. Further analysis of these clones has shown that the two classes are quite distinct. Sequences represented by λDm32 (class A) are localized to polytene chromosome section 53CD, whereas sequences in λDm65 (class B) are present at 54A1-B1. The only clone that detects a larval poly-adenylated RNA on Northern blots is λDm65, indicating that this clone has strong homology to a transcribed gene. The class A clones all contain a single *Eco*RI fragment that retains homology with mouse amylase, whereas λDm65 (class B) contains two such fragments. The degree of homology between mouse amylase and these two groups of clones appears to differ as judged by Southern blot hybridization. We have repeatedly seen λDm65 retain the mouse amylase probe at higher levels of stringency than does λDm32 (Figure 2C). Although Figure 2B appears to be an exception to this, when this filter was rewashed at a higher stringency, the probe was again retained more effectively by λDm65 than by the class A clones.

The results presented here strongly suggest that clone λDm65 contains at least one *Amy* gene from *D. melanogaster*. First, this clone retains significant homology with mouse amylase-coding sequences. Second, it hybridizes within polytene chromosome section 54A1-B1, which very likely includes the *Amy* region (see below). Third, it contains a gene(s) that is expressed as a polyadenylated RNA. Fourth, this RNA is of sufficient size (1450 nucleotides) to code for a protein the size of Drosophila amylase (molecular weight = 54,500; 1350 nucleotides required). Fifth, the relative amounts of this homologous RNA present in $Amy^{1,6}$ and $Amy^{1,3}$ (Canton-S) larvae were quite different; $Amy^{1,6}$ larvae contained significantly more of this RNA than did $Amy^{1,3}$ larvae. Since extracts of $Amy^{1,6}$ larvae contain about ten-fold more total amylase activity per microgram of protein than do extracts of $Amy^{1,3}$ larvae when reared under the same conditions as those used for RNA isolation, the strain differences in levels

of RNA homologous to λDM65 appear to correlate with the strain differences in total amylase activity. Sixth, the presence in λDm65 of two *Eco*RI fragments which hybridize with the mouse amylase probe is consistent with the presence of a duplicated amylase-coding sequence. *Amy* is presumed to be duplicated in strains that produce two different major amylase isozymes and yet are homozygous for chromosome *2R* (reviewed by DOANE 1969a), including our $Amy^{1,3}$ strain which was derived from a Canton-S stock. The genomic library that we screened (MANIATIS *et al.* 1978) was also derived from a Canton-S strain.

Since we have, in fact, verified that λDm65 contains the *Amy* region (LEVY, GEMMILL and DOANE 1985), the *in situ* hybridization site of this clone raises a question about the previous cytogenetic localization of *Amy*. BAHN (1971a) showed that *Amy* is located within the translocation of strain *T(1;2)OR72*, which reportedly contains segment 54B-60 of chromosome *2R*. The breakpoint in chromosome *2R* of *T(1;2)OR72* is listed by LINDSLEY and GRELL (1968) as 54B. However, this breakpoint has not been published elsewhere or corroborated, and E. BAHN (personal communication) did not verify it cytologically. Although our results may be consistent with inclusion of *Amy* within 54B1, it appears more likely from Figure 3B that λDm65 hybridized in 54A. Thus, the original localization of the *2R* breakpoint of *T(1;2)OR72* in 54B may be in error and should be reexamined. We have been unable to locate any existing strain of *T(1;2)OR72* and, therefore, have not examined the cytology of the breakpoints ourselves.

Characterization of the class A clones, exemplified by λDm32, demonstrated that these clones probably do not contain functional *Amy* genes in spite of the homology to mouse amylase sequences. In most experiments, the degree of homology between the mouse amylase sequence and the class A clones appeared to be quantitatively less than between the mouse amylase sequence and the class B clone, λDm65 (Figure 2C). The polytene chromosome site at which these clones hybridized was within section 53CD, a significant distance proximal to the presumptive site of *Amy*. We were unable to detect any RNA from third instar larvae that was homologous to λDm32. The class A clones probably represent some form of *Amy* pseudogene, although we cannot yet exclude the possibility that they contain some functional gene(s). DNA sequence analysis may resolve this question.

Interspecific homology has been observed for a number of essential genes, *e.g.*, calmodulin (MUNJAAL *et al.* 1982), actin (SCHULER and KELLER 1981) and tubulin (CLEVELAND *et al.* 1980). These genes are highly conserved during evolution because their gene products form a basic and indispensable part of the cellular machinery. Amylase, however, is a dispensable activity in *D. melanogaster* (HAJ-AHMAD and HICKEY 1982) and is assumed to evolve relatively rapidly (KARN and MALACINSKY 1978). In spite of this, previous results (DOANE *et al.* 1983), as well as those shown in Figure 1, suggest that amylase structural gene sequences may be sufficiently conserved to retain observable homology between the amylase genes in mouse and Drosophila. In our companion paper we show conclusively that λDm65 contains the Canton-S *Amy* genes. Our finding that a mouse α-amylase probe can be used to identify an amylase gene in a library of genomic clones from *D. melanogaster* raises the hope that it will be

possible to isolate many such dispensable genes from a variety of organisms by taking advantage of partial homology.

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