MOLECULAR CLONING OF α-AMYLASE GENES FROM *DROSOPHILA MELANOGASTER*. II. CLONE ORGANIZATION AND VERIFICATION

JACK N. LEVY, ROBERT M. GEMMILL AND WINIFRED W. DOANE

Department of Zoology, Arizona State University, Tempe, Arizona 85287

Manuscript received August 14, 1984
Revised copy accepted February 19, 1985

ABSTRACT

Restriction maps of an α-amylase structural gene clone, λDm65, and of four putative α-amylase pseudogene clones are presented. Two α-amylase structural genes, inverted with respect to each other, are contained in λDm65. Subregions of internal DNA sequence homology within λDm65 and of cross-homology between the presumptive pseudogene clones and λDm65 were determined. Subregions of cross-homology between the Drosophila clones and the mouse α-amylase cDNA clone, pMSa104, were also determined. The presence of functional α-amylase structural genes in λDm65 was verified by injection of appropriate subclones into the germinal vesicle of Xenopus oocytes, followed by incubation of the oocytes under conditions that allowed coupled transcription and translation of injected genes to occur. Subclones of the 3.8- and 5.6-kb *Eco*RI fragments of λDm65 were shown to code for α-amylase isozymes 1 and 3, respectively, of *Drosophila melanogaster* Canton-S. Both sublcones are homologous to RNA of a size sufficient to accommodate the α-amylase-coding information. No RNA species homologous to other subcloned *Eco*RI fragments of λDm65 was detected.

IT is clear from the classical and molecular genetics of eukaryotes that many structural genes are members of gene families. Molecular studies have shown that such families may include elements (pseudogenes) which are related to the functional genes of a given family by DNA sequence homology, but which are not expressed.

In our companion paper (GEMMILL, LEVY and DOANE 1985) we demonstrated that under suitable conditions a mouse α-amylase cDNA sequence will hybridize to a small set of DNA sequences from the Canton-S strain of *Drosophila melanogaster* and that the mouse sequences can be used as a probe to isolate putative amylase clones from a genomic library of *D. melanogaster*. These clones were divided into classes based on their molecular characteristics and on the sites in polytene chromosomes to which they hybridize *in situ*. Indirect evidence suggested that the single class B clone, λDm65, contains the α-amylase (*Amy*) structural gene(s) and that class A clones may contain a pseudogene for α-amylase. It appears, then, that the amylase system in *D. melanogaster* may represent another example of a eukaryotic gene family.

In this paper we provide restriction maps of the class A ("pseudogene")

clones and of the single class B clone presumed to contain *Amy*-coding sequences. Regions of DNA sequence homology between a representative class A clone, λDm32, and the class B clone, λDm65, are identified, as are regions within λDm65 that show homology to larval RNA isolated from *D. melanogaster*. Finally, we show that two specific subclones of λDm65 are able to code for appropriate Drosophila α-amylases after their separate injection into the germinal vesicles (GV) of Xenopus oocytes. The α-amylases so produced are electrophoretically identical with those that characterize the Canton-S strain of *D. melanogaster* from which λDm65 is derived. This last experiment thus provides conclusive proof that λDm65 contains two functionally intact α-amylase structural genes from the Canton-S strain.

MATERIALS AND METHODS

Stocks and strains: A *c* $Amy^{2,3}$ strain of *D. melanogaster* used as a source of extracts for standards on electrophoretic gels is described by ABRAHAM and DOANE (1978). Sources of other Drosophila stocks and of bacteriophage and plasmids used are given by GEMMILL, LEVY and DOANE (1985). Females of *Xenopus laevis* were obtained from Carolina Biological Supply Company, Burlington, North Carolina, and were maintained in the Arizona State University Animal Resource Facility (NATIONAL ACADEMY OF SCIENCES 1974).

Xenopus oocyte preparation, injection and storage: Ovarian lobes, each containing many oocytes, were removed surgically through an abdominal incision. Oocytes were then dissected from the lobes with watchmaker's forceps and placed individually, with the animal pole up, in 1.1- or 1.2-mm diameter wells of specially made circular plexiglass planchettes which fit into the buckets of a Sorval HB-4 rotor. The planchettes were supported by hard plastic centrifuge tube adaptors which had been shortened on a lathe. Oocytes were spun 10 min at 3500–4000 rpm, forcing the GV of each to the surface at the animal pole. Oocytes were injected with DNA using glass micropipettes with bevel-ground tips connected to a 50-ml syringe via an air-filled system. Micropipettes were 15–25 μm in internal diameter at the tip. Injected DNA samples were 0.5 g/liter in Barth's solution; 5- to 100-nl aliquants were injected into the GV of each oocyte. Two injected oocytes were incubated together in 40 μl of Barth's solution in a microtiter plate well. Incubation was generally for 4 days at 18°. Barth's solution consisted of 88 mM NaCl, 0.96 mM KCl, 6.6 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.8 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid. For oocyte incubation this was supplemented with 10 μg/ml each of penicillin and streptomycin.

Amylase assays: After incubation, pairs of oocytes were ground in the incubation medium with glass pestles. Each homogenate was transferred to a microcentrifuge tube and spun briefly, and the supernatant was saved as an extract for amylase assays. Polyacrylamide gels containing 1.5% Connaught starch were prepared between pairs of 3.25 × 4-inch lantern slide plates separated by microscope cover slip chips according to the procedure of DOANE (1967). Spot tests for amylolytic activity in oocyte extracts were performed by spotting 2–5 μl of the supernatant onto a starch-containing gel and incubating for 6 hr at room temperature. Gels were then stained for starch in IKI reagent (DOANE 1967).

Samples that gave positive spot tests were tested by discontinuous slab gel electrophoresis for analysis of the amylase isozyme(s) present. The method was essentially a modification of the procedure of DOANE (1967) in which the gel was poured as a 1.5-mm thick slab rather than in individual tubes, and correspondingly larger volumes of small-pore and large-pore gels were used. The loading gel was omitted. As standards for isozyme identification, aliquants of diluted crude aqueous extracts of adult flies of the $Amy^{1,6}$ and $Amy^{2,3}$ strains were run in parallel lanes. These usually were diluted 200-fold ($Amy^{2,3}$ extract) to 1000-fold ($Amy^{1,6}$ extract) before use. A starch-polyacrylamide test plate was laid over the slab gel after the run was completed. The test plate was incubated for 6–18 hr at room temperature prior to iodine staining.

Analysis of restriction endonuclease digestion products: Restriction endonucleases were obtained from New England Biolabs or Bethesda Research Laboratories, and digestions were carried out in buffers as directed by the suppliers. In some cases mercaptoethanol was omitted from the endonuclease buffer or replaced with 4 mM spermidine. The size of DNA fragments generated by restriction endonuclease digestion was estimated after separation of fragments on agarose gels (GEMMILL, LEVY and DOANE 1985).

Subcloning of fragments from λDm65: All plasmid subclones were constructed using the vector pBR325. Fragments produced by complete digestion of λDm65 with *Eco*RI, *Bam*HI or *Hin*dIII were either (1) ligated as mixed digests without prior size separation or (2) extracted from appropriate regions of agarose gels after electrophoretic separation, prior to ligation. In either case, the ligated vector-insert products were used to transform *E. coli* HB101. "Minipreps" of resulting plasmids were made from colony-purified transformed isolates having appropriate antibiotic resistance phenotypes, according to MANIATIS, FRITSCH and SAMBROOK (1982). Inserts were then identified by agarose gel electrophoresis after digestion of the miniprep with restriction endonucleases. Subclones derived from digests with each of the three enzymes were used in establishing the restriction map of λDm65 (Figure 1). Subclones of *Eco*RI fragments were also used for other experiments reported below.

Southern and Northern blot analyses: Preparation of DNA probes for Southern and Northern analyses, conditions of hybridization, wash and autoradiographic exposure are described by GEMMILL, LEVY and DOANE (1985).

RESULTS

Restriction maps of λDm65 and class A clones: Figure 1 presents the results of restriction enzyme-mapping analysis of λDm65 and of the four class A clones (λDm31, λDm32, λDm76 and λDm89). Class A clones were mapped with restriction endonucleases *Eco*RI, *Bam*HI and *Hin*dIII; λDm65 was mapped with these three enzymes and with *Xho*I and *Sal*I.

The four clones that make up class A appear, on the basis of their restriction maps (Figure 1), to be derived from the same genomic region and apparently represent an overlapping set. The restriction maps of these clones are clearly different from the restriction map of the class B clone, λDm65. The region spanned by the class A clones is approximately 20 kb in length.

Attention was focused on clone λDm65 since this clone appeared to contain the *Amy* sequences (GEMMILL, LEVY and DOANE 1985). The most significant feature of the restriction map of λDm65 is the presence of an apparently duplicated pattern of restriction endonuclease cleavage sites. A trio of *Bam*HI-*Sal*I-*Bam*HI cutting sites (referred to as a BSB triad) occurs in each of two locations within the map of λDm65. The two BSB triads occur within the 5.6- and 3.8-kb *Eco*RI fragments, respectively, and are separated by approximately 6.5 kb. The BSB triads appear to be inverted with respect to each other as judged by restriction fragment size measurements. In contrast, the restriction map of the class A clones shows no evidence for a duplicated restriction endonuclease cleavage pattern.

Internal homologies of the λDm65 clone: The duplicated pattern of restriction endonuclease cleavage sites suggested the presence in λDm65 of a duplicated nucleotide sequence. A series of hyridization experiments were performed in order to determine regions of DNA sequence homology within λDm65. Five subcloned *Eco*RI fragments derived from this clone (pDm5.6, pDm3.8,

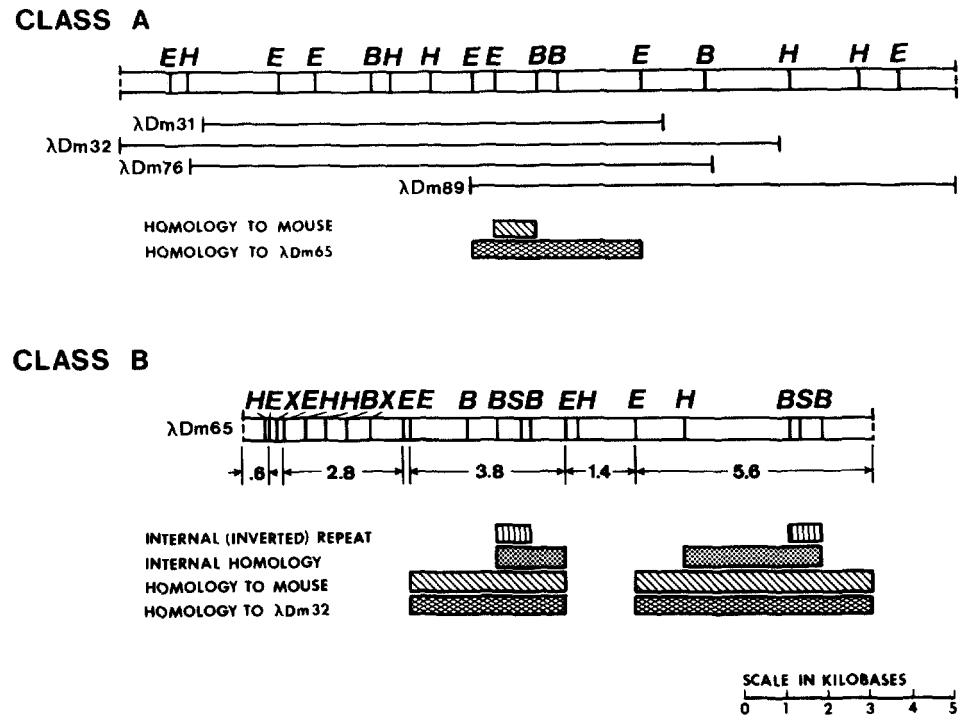

FIGURE 1.—Restriction endonuclease cleavage maps of the class A and class B clones. Sites for restriction endonucleases *Eco*RI (E), *Bam*HI (B), *Hin*dIII (H), *Sal*I (S) and *Xho*I (×) are shown. Homology of a 1-kb *Eco*RI/*Bam*HI fragment of λDm32 to the mouse amylase cDNA probe is indicated by ▧. Class A clone homologies to sequences of λDm65 are indicated by ▩. Clone λDm65 contains a repeated pattern of restriction endonuclease cutting sites (▥) referred to in the text as BSB triads. The region of internal homology determined by hybridization experiments is indicated by □. The last two sets of boxes for the class B clone indicate the *Eco*RI fragments of λDm65 that are homologous to both the mouse amylase probe (▧) and to the 3.8 kb *Eco*RI fragment of λDm32 (▩). In all cases the shaded boxes indicate the maximum extent of detected homology, as defined by the ends of homologous fragments. Actual homology may be confined to a portion of such regions. The locations of the 0.6-, 1.4-, 2.8-, 3.8- and 5.6-kb *Eco*RI fragments of λDm65 are indicated directly below the map of that clone.

pDm2.8, pDm1.4 and pDm0.6) were labeled by nick translation and used to probe Southern transfers of restriction endonuclease-digested λDm65. Figure 2 shows that on Southern transfers of *Eco*RI-digested λDm65, subclones pDm5.6 and pDm3.8 each show strong homology to both the 5.6- and 3.8-kb *Eco*RI fragments. This confirms the presence of duplicated sequences within λDm65. In contrast, pDm2.8, pDm1.4 and pDm0.6 each show homology to only a single *Eco*RI digestion product, that of the same size as the probe insert. Similar analyses were made of Southern transfers of double digests of λDm65 using endonucleases *Eco*RI, *Bam*HI and *Hin*dIII in various combinations. Such analyses indicated that pDm5.6 is homologous to the region of the 3.8-kb *Eco*RI fragment of λDm65 delimited by the BSB triad within that fragment and to a 0.85-kb *Bam*HI-*Eco*RI fragment immediately to the right of that BSB triad. Subclone pDm3.8 shows homology to the region of the 5.6-kb *Eco*RI

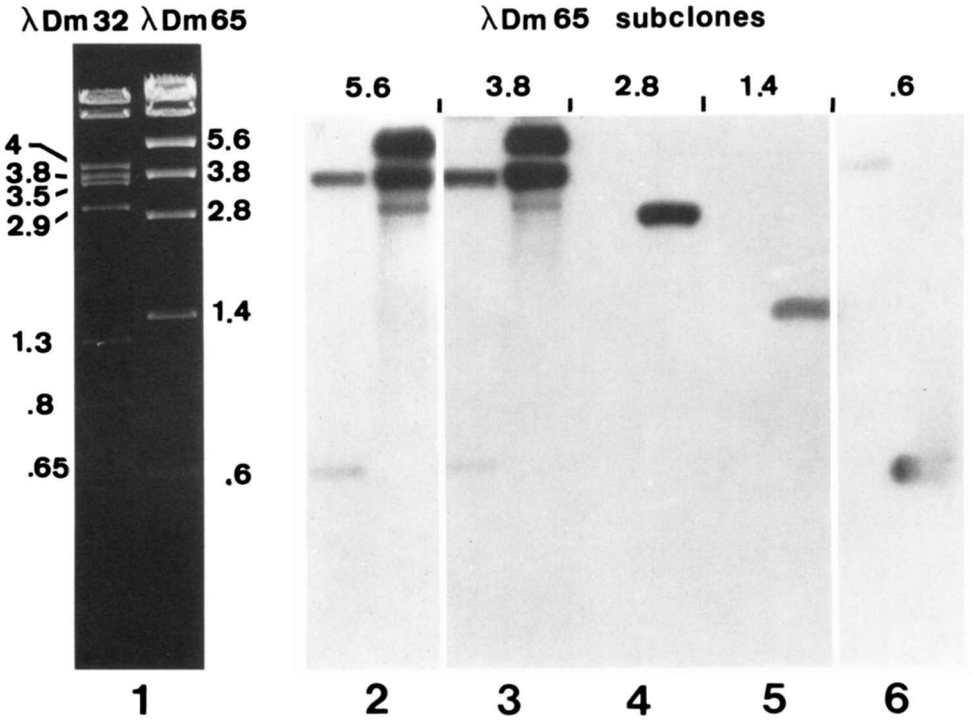

FIGURE 2.—Homologies between λDm32 and λDm65. One to 1.5 μg of DNA from λDm32 and λDm65 were digested to completion with *Eco*RI, resolved on adjacent lanes of a 1% agarose gel and stained with ethidium bromide (panel 1). The DNA was transferred to a filter, the filter cut into strips, and each strip hybridized with the indicated nick-translated subclone of λDm65 (panels 2–6). Each panel of the figure contains two lanes, the left lane containing *Eco*RI-cut λDm32 and the right lane containing *Eco*RI-cut λDm65. Sizes of fragments are in kilobases.

fragment of λDm65 delimited by the BSB triad of that fragment and to a 2.6-kb *Hin*dIII-*Bam*HI fragment just to the left of that BSB triad. The observation that the regions of internal homology extend in opposite directions from the two BSB triads confirms the earlier conclusion, based on restriction-mapping data, that the duplicated regions are inverted with respect to each other. The homologies described here and those discussed in the next section are summarized in Figure 1.

Homology between λDm65 and the class A clone λDm32: The five nick-translated, subcloned *Eco*RI fragments of λDm65 described above were tested in parallel for homology to the class A clone, λDm32. Figure 2 shows that pDm5.6 and pDm3.8 both have weak homology to the 3.8-kb *Eco*RI fragment of λDm32. All three fragments also show homology to the mouse α-amylase cDNA clone, as previously shown (GEMMILL, LEVY and DOANE 1985). The other three λDm65 subclones, pDm2.8, pDm1.4 and pDm0.6, show no detectable homology to any λDm32 fragments.

To summarize, the 3.8-kb *Eco*RI fragment of λDm32, the 3.8- and 5.6-kb *Eco*RI fragments of λDm65 and the mouse α-amylase cDNA insert of pMSa104

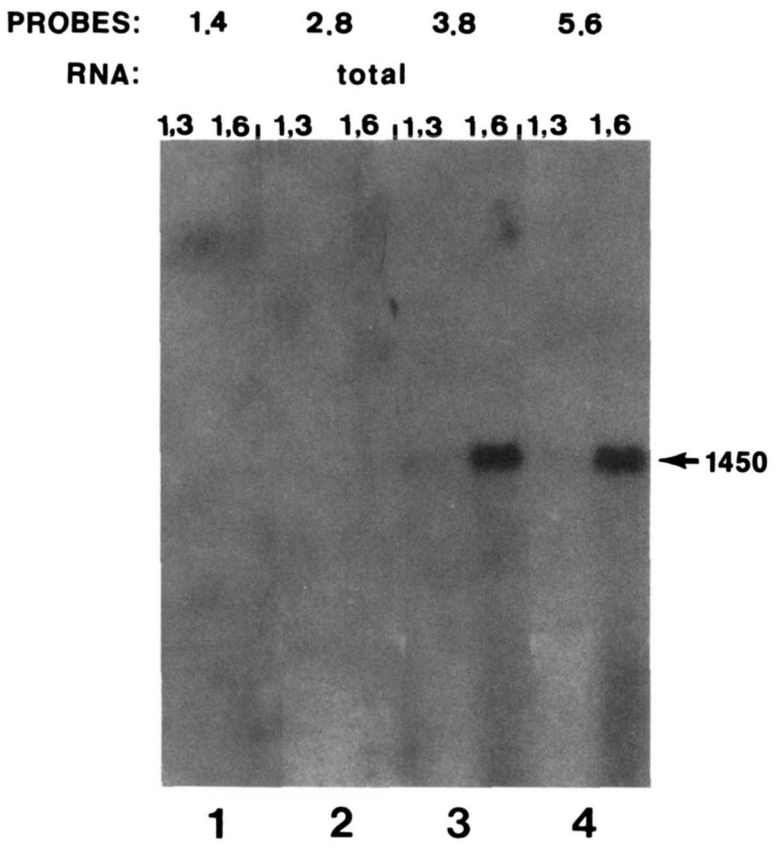

FIGURE 3.—Hybridization of λDm65 subclones to RNA on Northern transfers. Total RNA was isolated from $Amy^{1,6}$ (1,6) and $Amy^{1,3}$ (1,3) larvae, and Northern transfers were prepared as described by GEMMILL, LEVY and DOANE (1985). For each of the eight lanes, 20 μg of RNA were resolved on a denaturing agarose gel and transferred to a filter. Filter strips, each carrying transferred RNA from an $Amy^{1,3}$ preparation and from an $Amy^{1,6}$ preparation, were hybridized with the four nick-translated subclones. Panel 1 shows the result using pDm1.4 as probe, and panels 2, 3 and 4 show comparable results using pDm2.8, pDm3.8 and pDm5.6, respectively. In each panel, the lane with 1,3 RNA is on the left and that with 1,6 RNA is on the right. The size of the RNA homologous to pDm3.8 and pDm5.6 (panels 3 and 4) was estimated at 1450 nucleotides as previously described (GEMMILL, LEVY and DOANE 1985).

all show DNA sequence complementarity, in varying degree, when compared *inter se*.

Identification of transcribed regions of λDm65: Nick-translated subclones of the four largest *Eco*RI fragments derived from the insert of λDm65 were used as hybridization probes of a Northern transfer of total RNA prepared from third instar larvae of the $Amy^{1,3}$ and $Amy^{1,6}$ strains of *D. melanogaster*. The results of this experiment are presented in Figure 3. No RNA homologous to the 1.4- or the 2.8-kb *Eco*RI fragments of λDm65 could be detected in preparations made from either *Amy* strain. In contrast, RNA homologous to both the 3.8- and the 5.6-kb *Eco*RI fragments was detected in preparations made from both

TABLE 1

Induction of α-amylase activity in Xenopus oocytes injected with subcloned D. melanogaster *DNA sequences*

Experimental treatment of oocytes[a]	No. of oocytes treated	No. of positive tests[b]
Uninjected	36	0
Barth's injected	64	0
pDm1.4 injected	75	0
pDm3.8 injected	87	30
pDm5.6 injected	162	6

[a] Four of 12 batches of injected oocytes yielded some positive results. Results from these four batches are tabulated.

[b] Starch-iodine spot tests were performed as described in MATERIALS AND METHODS.

Amy^{1,3} and *Amy*^{1,6} larvae. The level of RNA homologous to these probes was much higher in the *Amy*^{1,6} strain than in the *Amy*^{1,3} strain, in agreement with the results using intact λDm65 presented previously (GEMMILL, LEVY and DOANE 1985).

Together, the four subclones used as probes in the above experiment contain more than 90% of the sequences from *D. melanogaster* present in the "parent" clone, λDm65. As shown in the restriction map of this clone (Figure 1), the 1.4-kb *Eco*RI fragment, for which no transcript was detected, maps between the 5.6- and 3.8-kb fragments. The latter two fragments are homologous to RNA(s) produced by the *Amy*^{1,3} and *Amy*^{1,6} larvae, suggesting that one complete *Amy* gene might be contained in each fragment.

Verification that λDm65 contains coding information for D. melanogaster *Canton-S α-amylase*: We tested the ability of the subcloned 3.8- and 5.6-kb fragments of λDm65 to direct synthesis of functional α-amylase after injection into the GV of stage V and VI Xenopus oocytes (DUMONT 1972). Since Xenopus oocytes have no endogenous amylase activity (W. W. DOANE unpublished observation), positive starch iodine spot tests for amylase activity in extracts prepared after incubation of injected oocytes suggested *de novo* amylase synthesis. Confirmation came from subsequent electrophoretic analyses (see below). The 1.4-kb *Eco*RI fragment, which maps between the two larger fragments, was similarly tested; this subclone provided a negative control for the injection experiments.

Results of these experiments are summarized in Table 1. Injected oocytes were homogenized after 4 days of incubation and small samples (2–5 μl) of extracts prepared from them were spot tested for amylolytic activity. Activity was never detected in oocytes that had been sham injected with Barth's solution or injected with subclone pDm1.4. However, some positive tests were obtained among extracts of oocytes injected with pDm3.8 and among those injected with pDm5.6. Oocytes injected with pDm3.8 gave more frequent and stronger positive tests for amylase activity than did those injected with pDm5.6.

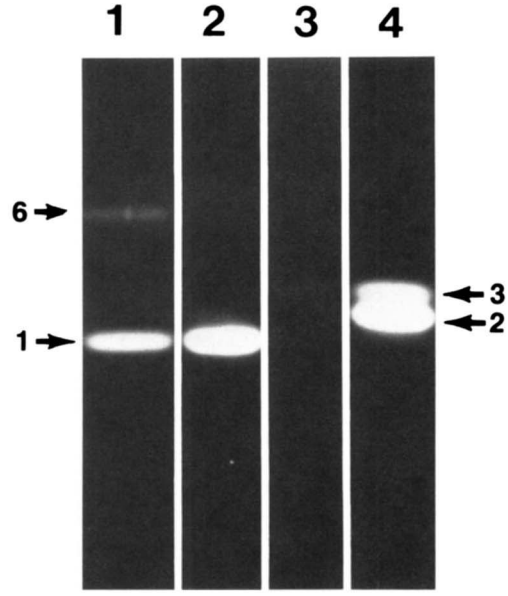

FIGURE 4.—Amylase isozymes produced after injection of pDm3.8 and pDm5.6 into Xenopus oocytes. Procedures used are in MATERIALS AND METHODS. The isozyme patterns of fly extracts of the $Amy^{1,6}$ (lane 1) and $Amy^{2,3}$ (lane 4) strains serve as standards for identifying isozymes produced by injected oocytes. Lane 2 shows the isozyme pattern obtained from an oocyte injected with the pDm3.8 clone, and lane 3 shows the pattern obtained from an oocyte injected with pDm5.6. Lanes are from the same gel and were aligned by their origins; superfluous intervening lanes were removed.

Oocyte extracts that tested positively for amylase activity were stored at −20° and aliquants were analyzed by discontinuous electrophoresis in polyacrylamide slab gels for the identity of the amylase isozyme(s) they contained. As standards, appropriate dilutions of whole fly extracts of adults from the $Amy^{1,6}$ and $Amy^{2,3}$ strains were run in separate, parallel lanes. The results of one such experiment are shown in Figure 4. Here, it can be seen that the pDm3.8 subclone codes for α-amylase isozyme 1, whereas the pDm5.6 subclone codes for α-amylase isozyme 3.

The recombinant phage library from which λDm65 was isolated is derived from the Canton-S strain of *D. melanogaster*. Canton-S individuals produce α-amylase isozymes 1 and 3; it has been assumed that these isozymes are encoded in duplicated structural genes designated Amy^1 and Amy^3 ($=Amy^{1,3}$), respectively (DOANE 1969a). The amylase isozymes produced when the pDm3.8 and pDm5.6 subclones of λDm65 were injected into Xenopus oocytes migrated to positions indistinguishable from those of amylase isozymes 1 and 3 from the Canton-S strain. This experiment confirms that the two isozymes of the Canton-S strain are indeed coded for by two complete and separate structural genes.

DISCUSSION

The electrophoretic analysis of amylase variants in *D. melanogaster* described by KIKKAWA (1964) led to the hypothesis that certain strains of *D. melanogaster* carry a duplication of the α-amylase structural gene in chromosome *2R*. His data suggested that the duplicated *Amy* genes are so tightly linked that they are usually inherited as a single unit. This hypothesis could explain the fact that certain strains that were known to be homozygous for the *Amy* region produced two amylase isozymes. Genetic, biochemical and molecular evidence indirectly supported this hypothesis (reviewed by DOANE 1969b; DOANE *et al.* 1983). Most pertinent was the demonstration by BAHN (1967) that, among progeny of testcrosses, about one in 13,000 flies produced a nonparental combination of amylase isozymes. BAHN (1967) interpreted such progeny as evidence of meiotic recombination between two separable *Amy* structural genes located 0.008 cM apart on the genetic map of chromosome *2R*. His results, however, did not rule out the possibility that such distinct isozymes might have resulted from differential processing of a single transcript or from multiple primary transcripts read from the same coding information. A mechanism of the latter type operates in the mouse salivary-liver α-amylase gene system, in which a single structural gene is associated with two promoters. One promoter is functional in both liver and salivary gland tissue and the other in liver only (SCHIBLER *et al.* 1983). In this example from the mouse, the two distinct primary transcripts differ in their 5′ untranslated regions but presumably result in identical polypeptides. In contrast, it has been proposed (MEISLER *et al.* 1983; TOSI *et al.* 1984) that the multiple pancreatic α-amylase isozymes of certain mouse strains are encoded in closely linked but independent structural genes. This model is similar to that proposed for the *Amy* system of *D. melanogaster* (KIKKAWA 1964; BAHN 1967).

In our experiments, subclones containing nonoverlapping, putative α-amylase structural genes from λDm65 are able to direct synthesis of functional amylase after injection into the GV of Xenopus oocytes. This demonstrates beyond a doubt that (1) λDm65 does, indeed, contain two transcriptionally active α-amylase structural genes and (2) the Canton-S strain from which λDm65 was derived carries a duplication of the *Amy* gene. These conclusions are further substantiated by the fact that the electrophoretic mobilities of Canton-S amylase isozymes 1 and 3 are indistinguishable from those of the amylases produced, respectively, by the subcloned 3.8- and 5.6-kb *Eco*RI fragments of λDm65. Thus, the machinery used to transcribe, process and translate α-amylase message and to modify the resulting polypeptide (if modifications occur) appears to operate in an identical or nearly identical manner in Drosophila and in Xenopus. Accordingly, we have assigned the Amy^1 gene of the Canton-S strain to the 3.8-kb fragment and the Amy^3 gene of this strain to the 5.6-kb fragment.

Covalently closed circular DNA (ccDNA) is the most effective physical form for functional activity of an injected transcriptional template in the Xenopus oocyte expression assay (HARLAND, WEINTRAUB and MCKNIGHT 1983). Therefore, we used ccDNA prepared from plasmid subclones of λDm65 in our assays

for α-amylase expression. Not all injected oocytes produced detectable levels of amylase (Table 1). In our experiments, greater levels of amylase activity were observed in oocytes injected with the subcloned 3.8-kb fragment than in those injected with the 5.6-kb subclone; the reason for this difference remains to be explored. We note that the cloned genes used in these experiments were derived from a strain (Canton-S) that produces a relatively low level of α-amylase. It may be possible to obtain more consistently positive results using cloned genes derived from "high activity" strains.

The genetic fine structure analysis of amylase structural genes in *D. melanogaster* (BAHN 1967) implies the presence of a proximal and a distal *Amy* locus in chromosome *2R*. Bahn's results were consistent with multiple alleles for amylase isozymes segregating at each locus and, based on the fly strains used, he deduced that his Amy^1 gene segregated at the proximal locus and his Amy^3 gene at the distal locus. If these are the same Amy^1 and Amy^3 genes (or alleles at their specific loci) that characterize the Canton-S strain, then the 3.8-kb fragment is to the left of the 5.6-kb fragment when λDm65 is in parallel orientation with chromosome *2R*, provided the centromere lies at the left end of the chromosomal arm (the customary orientation). Orientation of the map of λDm65 in Figure 3 reflects this consideration.

The restriction map and results of Southern analysis show that the two copies of the α-amylase structural gene are inverted with respect to each other in λDm65. The distance between the two copies must be greater than 1.4 kb (because a 1.4-kb *Eco*RI fragment maps between the two *Eco*RI fragments carrying the *Amy* genes) and is presumably less than 6.2 kb, the distance separating the interior *Bam*HI sites of the two BSB triads. This distance agrees well with estimates based on genetic mapping data (BAHN 1967).

The class A clones are at present an enigma. They possess demonstrable homology with both the mouse amylase-coding sequences of pMSa104 and the two amylase-coding regions of λDm65. Additional evidence (GEMMILL, LEVY and DOANE 1985) suggests that the amylase-like sequence present in these clones is not functional. Multiple, putative α-amylase pseudogenes have been demonstrated recently in the rat (CRERAR *et al.* 1983), and the α-amylase gene system of *D. melanogaster* may represent a similar gene family.

In summary, clone λDm65 contains two active α-amylase structural genes derived from the Canton-S strain of *D. melanogaster*. These *Amy* genes are inverted with respect to each other. We have determined which copy of the structural gene in this clone codes for each of the two α-amylase isozymes characteristic of the Canton-S strain, and we have tentatively assigned an orientation to the clone with respect to chromosome arm *2R*. Restriction maps for several clones containing a putative α-amylase pseudogene are also presented.

The experiments presented in this paper and in our companion article (GEMMILL, LEVY and DOANE 1985) provide the foundation for further studies on the regulation of the α-amylase gene system in *D. melanogaster*. The inverted orientation of the two *Amy* structural genes is consistent with a model in which they are associated with separate promoters, although at present there is no

direct evidence bearing on this point. We would like to know whether they are expressed coordinately under certain conditions and whether the two structural genes may function differentially at different times during development or in different tissues, as has been proposed (DOANE 1969b; HICKEY 1981; DOANE *et al.* 1983). We would particularly like to know whether portions of the genes and/or their flanking sequences are responsible for interaction with the *map* regulator of ABRAHAM and DOANE (1978). Experiments designed to provide insight into these questions are in progress.

We thank SUSAN RHODES, TIMOTHY COOK and GREGORY HENDRICKS for technical assistance, LARRY NIENABER for care of our experimental animals, AIMEE BAKKEU, University of Washington, Seattle, for advice about Xenopus experiments and DENNIS GLANZMAN, Department of Psychology, Arizona State University, for use of his facilities for preparation of glass needles used for DNA injections. Primary support for this work came from United States Public Health Service research grant GM-25255 to W.W.D. Additional support was received from National Institutes of Health Biomedical Research Support grant SO 7 RR07112 to Arizona State University and from National Science Foundation grant PCM-8302909 to J.N.L.

LITERATURE CITED

ABRAHAM, I. and W. W. DOANE, 1978 Genetic regulation of tissue-specific expression of amylase structural genes in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **75:** 4446–4450.

BAHN, E., 1967 Crossing over in the chromosomal region determining amylase isozymes in *Drosophila melanogaster*. Hereditas **58:** 1–12.

CRERAR, M. M., W. F. SWAIN, R. L. PICTET, W. NIKOVITS and W. J. RUTTER, 1983 Isolation and characterization of a rat amylase gene family. J. Biol. Chem. **258:** 1311–1317.

DOANE, W. W., 1967 Quantitation of amylases in *Drosophila* separated by acrylamide gel electrophoresis. J. Exp. Zool. **164:** 363–377.

DOANE, W. W., 1969a Amylase variants in *Drosophila melanogaster*: linkage studies and characterization of enzyme extracts. J. Exp. Zool. **171:** 321–342.

DOANE, W. W., 1969b *Drosophila* amylases and problems in cellular differentiation. pp. 73–109. In: *RNA in Development*, Edited by E. W. HANLY. University of Utah Press, Salt Lake City, Utah.

DOANE, W. W., L. G. TREAT-CLEMONS, R. M. GEMMILL, J. N. LEVY, S. A. HAWLEY, A. BUCHBERG and K. PAIGEN, 1983 Genetic mechanisms for tissue-specific control of alpha-amylase expression in *Drosophila melanogaster*. pp. 63–90. In: *Isozymes: Curent Topics in Biological and Medical Research*, Vol. 9. Alan R. Liss, New York.

DUMONT, J. N., 1972 Oogenesis in *Xenopus laevis* (Daudin): stages of oocyte development in laboratory maintained animals. J. Morphol. **136:** 153–180.

GEMMILL, R. M., J. N. LEVY and W. W. DOANE, 1985 Molecular cloning of α-amylase genes from *Drosophila melanogaster*. I. Clone isolation by use of a mouse probe. Genetics **110:** 299–312.

HARLAND, R. M., H. WEINTRAUB and S. L. MCKNIGHT, 1983 Transcription of DNA injected into *Xenopus* oocytes is influenced by template topology. Nature **302:** 38–43.

HICKEY, D. A., 1981 Regulation of amylase activity in *Drosophila melanogaster*: variation in the number of enzyme molecules produced by different amylase genotypes. Biochem. Genet. **19:** 783–796.

KIKKAWA, H., 1964 An electrophoretic study on amylase in *Drosophila melanogaster*. Jpn. J. Genet. **39:** 401–411.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratories, Cold Spring Harbor, New York.

MEISLER, M., J. STRAHLER, K. WIEBAUER and K. K. THOMSEN, 1983 Multiple genes encode mouse pancreatic amylases. pp. 39–57. In: *Isozymes: Current Topics in Biological and Medical Research*, Vol. 7. Alan R. Liss, New York.

NATIONAL ACADEMY OF SCIENCES, 1974 *Amphibians: Guidelines for the Breeding, Care, and Management of Laboratory Animals.* National Academy of Sciences of the United States of America, Washington, D. C.

SCHIBLER, U., O. HAGENBÜCHLE, P. K. WELLAUER and A. C. PITTET, 1983 Two promoters of different strengths control the transcription of the mouse alpha-amylase gene *Amy-1*^{a} in the parotid gland and liver. Cell **33:** 501–508.

TOSI, M., R. BOVEY, S. ASTOLFI, S. BODARI, M. MEISLER and P. K. WELLAUER, 1984 Multiple non-allelic genes encoding pancreatic alpha-amylase of mouse are expressed in a strain-specific fashion. EMBO J. **3:** 2809–2816.

Communicating editor: W. W. ANDERSON