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A SUBCELLULAR LOCALIZATION OF THE GENE PRODUCT OF THE DNASE-1 LOCUS IN *DROSOPHILA MELANOGASTER**

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Abstract—A study was undertaken to determine the subcellular location of DNase-1, a major acid deoxyribonuclease in *Drosophila melanogaster*. Embryonic tissue used in these experiments was derived from a wild type strain and from a strain homozygous at the *DNase-1* locus (3–61.8) for the null activity allele, DNase-1^{n324}. The majority of total acid DNase and DNase-1 activity is found in the small particulate fraction of tissue homogenates fractionated by differential centrifugation. The activity exhibits latency in these extracts indicating that it is membrane delimited. DNase-1 activity also co-equilibrates in sucrose density gradients with acid phosphatase activity which, in *D. melanogaster*, is known to be lysosomal. These results suggest that DNase-1 is localized largely within the lysosomes of embryonic tissue. Functional implications of a lysosomal location for DNase-1 are discussed.

Key Word Index: Drosophila melanogaster, DNase-1, lysosomes, acid hydrolase.

INTRODUCTION

ONE FREQUENTLY used approach to understanding the biological function of cellular constitutents has been the technique of subcellular tissue fractionation. Differential and isopycnic centrifugation analyses have been used for the subcellular localization of enzyme activities, following the classic work of DEDUVE *et al.* (1955). This general method for analyzing the fractions of tissue homogenates led to the use of 'marker enzymes' which are diagnostic of a particular subcellular fraction. Early in these studies, acid phosphatase became established as one of the few acid hydrolases which were widely characteristic of the lysosomal or 'light mitochondrial' fraction of various tissue homogenates (DEDUVE *et al.*, 1955; NOVIKOFF, 1961). Gradually many hydrolases (BARRET, 1972) came to be assigned a lysosomal location, often on the basis of their association in subcellular fractions with acid phosphatase. There has been, however, a growing appreciation of the heterogeneity with regard to the enzyme content of lysosomes, even within the same tissue (BEAUFAY, 1972). This has made histochemical and cytological criteria necessary complements to the biochemical criterion when a given enzyme is assigned to a particular subcellular location.

The applications of analytical tissue fractionation have been limited during the study of insects, possibly because of the difficulty of reproducibly fractionating tissue that is bound within a rigid chitinous exoskeleton (but see HENRIKSON and CLEVER, 1972). Even more rarely have these techniques been applied to eukaryotes in which the genetic locus responsible for the enzyme of interest is known.

To understand the processing of DNA during insect development better, we have been studying the enzyme DNase-1 in the tissues of *Drosophila melanogaster*. DNase-1 has a pH optimum of 4.8 and shows an apparent activation in the presence of EDTA. A structural locus for the enzyme has been mapped genetically to 61.8 on the third chromosome and cytogenetically localized on the salivary gland chromosome map to a position within the interval 90C2-D1 to 90E. In addition, a locus modifying the quantitative expression of the enzyme has been localized to the interval 67C to 70D on the left arm of the third chromosome (DETWILER and MACINTYRE, 1978). The developmental profile for the enzyme has also been established and eight null activity mutants have been produced at the structural locus (DETWILER, 1979). DNase-1 is maximally expressed during pupation-a period of extensive tissue histolysis.

The present study was undertaken to assign a subcellular location to the DNase-1 gene product. While no histochemical data are presented here, the distribution of acid DNase activity is compared with that of acid phosphatase which has been localized within the lysosomes of *D. melanogaster* by means of ultrastructural histochemical analysis of ovarian, embryonic and salivary gland tissue preparations (ANASTASIA-SAWICKI and MACINTYRE, 1975; SAWICKI, 1976).

MATERIALS AND METHODS

Stocks

Three different stocks were used for these studies. A wild

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type strain, BV1 (from Blacksburg, Virginia) was kindly provided by Dr. Bruce Wallace. The DNase-1 (3-61.8) null strain, DNase-1^{n324} was obtained by EMS mutagenesis following BELL et al. (1972). Homozygotes for the null allele, DNase-1^{n324} show no DNase-1 activity in an acrylamide gel assay (see DETWILER, 1979 for details). The Acph-1^{n13} strain, homozygous for a null allele of the acid phosphatase-1 locus (3-101.1) was isolated by BELL *et al.* (1972).

Embryo collection

Strains used for embryo collection were reared in half pint bottles on standard cornmeal-molasses medium topped with 2 ml of a live yeast suspension, and a layer of dry granular yeast. During the period of peak emergence, bottles were cleared and adults collected 4 days later. Adults from 24 bottles were added to 6 egg laying chambers similar in design to those of HILDRETH and BRUNT (1962) except that vented petri dishes (100 × 15 mm) covered with gauze were used instead of plastic tubes. Egg laying medium was that of KRIEGSTEIN and HOGNESS (1974). It was supplemented with Norit A (Pfanstiehl Laboratories) to darken the color and poured into petri dish lids (100 × 15 mm). After hardening, a 0.5 cm wide strip was trimmed from the outer edge of the media, and yeast paste (40% yeast, 60% H_2O) was brushed onto the centre of the surface of the medium, covering an area of 3 cm in diameter. Two days before embryos were desired, egg laying chambers were placed on the prepared medium under conditions of high humidity at 25°C. After 24 hr the medium was replaced with a medium containing fresh yeast paste. Twenty hours later embryos were harvested into 0.12 M NaCl using a rubber spatula. Chambers were used over a period of 2–3 days for embryo collection.

Subcellular fractionation

Subcellular fractionation of embryos (Fig. 1) was carried out according to a modification of the method of HENRIKSON and CLEVER (1972). Embryos were washed free of faeces and yeast in 0.12 M NaCl, folded into a Nitex (48 μM) bag, dechorionated in 2–3% (w/v) NaOCl for 90 sec at 0°C, and rinsed in several changes of 0.12 M NaCl. Embryos were then weighed, suspended in H buffer (0.165 M Tricine, pH 7.5, 0.25 M sucrose, 10 mM KCl, 1 mM $MgCl_2$) (15% w/v), and gently ruptured in a Dounce homogenizer using 10–12 passages with a loose fitting pestle. This and all subsequent steps were carried out at 0–4°C. The extent of cell breakage was checked with phase microscopy; at least 70% of nuclei were released into solution. The homogenate was then filtered through Nitex (48 μM) and centrifuged at 4,300 ***g*** for 10 min in an SS-34 head of a Sorval RC-2B centrifuge to pellet nuclei and larger cell debris. The supernatant was recentrifuged at 25,000 ***g*** for 20 min. The pellet from this spin consisted of a small particulate (*SP*) fraction (by phase microscopy) and the supernatant was taken to represent the soluble (*S*) fraction of the homogenate. The nuclear or large particulate (*LP*) fraction described above was cleared of large cellular debris and undisrupted material by resuspension in a Dounce homogenizer in H buffer, centrifugation at 210 ***g*** for 10 min, resuspension of the resulting pellet in H buffer and recentrifugation at 121 ***g*** for 10 min. The final pellet was discarded and the combined supernatants (the LP fraction) were collected by centrifugation at 4300 ***g*** for 10 min. This fraction was largely nuclei and α-yolk spheres as judged by phase microscopy. All fractions were brought to the original volume of the homogenate with H buffer containing 0.1% Triton X-100 and frozen at −20°C prior to assays for enzyme activities.

Latency determinations

Two separate experiments were conducted to demonstrate latency of acid DNase activity. In the first study, latency was determined by assay of activities before and after sonication and freeze thawing of isotonic (0.24 M sucrose) embryo homogenates which had previously been cleared of cellular debris and large particulate material by centrifugation in an IEC clinical centrifuge (Model MB). Sonication consisted of five, 2-sec bursts at a setting of (2) on a Branson Sonifier. In the second study, subcellular fractionation was carried out as described in Fig. 1, except that isotonic homogenization buffer was either supplemented to 0.1% with Triton X-100 detergent or replaced by distilled water.

Density gradient equilibrium centrifugation

For density gradient analysis of the SP fraction, SP pellets

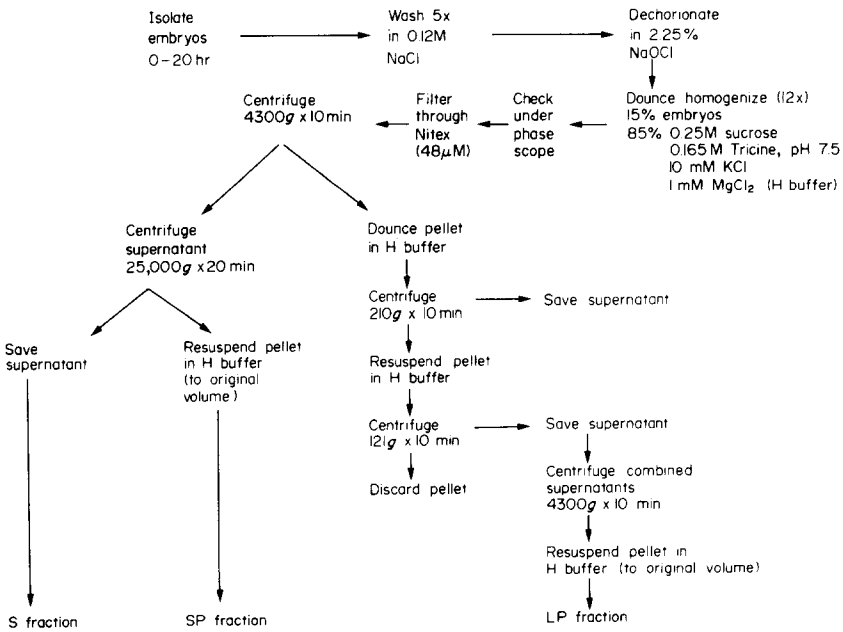

Fig. 1. Scheme for the subcellular fractionation of *D. melanogaster* embryos. Further details are given in the text.

were suspended in 0.1 M Na_2SO_4, a reversible inhibitor of acid DNase (DETWILER, unpublished observation; BERNARDI, 1971), in H buffer (0.83 ml/g embryos) and layered onto a 23 to 50% (w/v) sucrose gradient made up with H buffer. The gradients were centrifuged at 51,000 *g* for 7 hr at 4°C in a Beckman Model L5-50 ultracentrifuge, SW 50L rotor. Thirty-seven, 10-drop fractions were collected and frozen. Sucrose concentrations in the gradient were derived from refractive indices determined on a Bausch and Lomb Abbe 3-L Refractometer at 22°C.

Assays

Test tube assays for acid DNase activity (DETWILER and MACINTYRE, 1978) and acid phosphatase activity (MACINTYRE, 1971) have been reported elsewhere. α-Glycerophosphate dehydrogenase activity was assayed by following the reduction of NAD at 340 nm (O'BRIEN and MACINTYRE, 1972).

The DNA-acrylamide gel electrophoretic assay is that of BOYD and MITCHELL (1965) as modified by DETWILER and MACINTYRE (1978). After centrifugation for 10 min on an IEC clinical centrifuge (Model LB), at 13,000 *g*, 10 and 25 μl samples of supernatants of *LP*, *SP* and *S* fractions were subjected to electrophoresis in DNA acrylamide gels. For acid phosphatase determinations, 15 μl samples were subjected to electrophoresis and stained as described by MACINTYRE (1971). For gel assays of gradients, ten fractions across the peak (a total of 1.5 ml) were concentrated to 125 μl in a MicroProDiCon concentrator (Bio-Molecular Dynamics). Aliquots of this sample (25 μl for DNase-1 and 15 μl acid phosphatase activity measurements) were used for electrophoresis. Densitometry of the gel samples was carried out on a Canalco Model G Densitometer.

Table 1. Mean relative activities of acid DNase, acid phosphatase (Acph), and α-glycerophosphate dehydrogenase (αGPDH) in three subcellular fractions of embryonic cells of *Drosophila melanogaster**.

Enzyme	Fraction	Homogenization isotonic	hypotonic	detergent
Acph	*LP*	50.3±6.5	12.0±3.0	1.3±0.5
	SP	32.0±7.0	27.7±7.0	16.0±1.5
	S	17.7±0.6	60.3±4.0	82.7 ±2.0
	Total relative Activity	1.00	0.90	0.84
DNase	*LP*	29.0±7.0	8.0±2.0	0.0±0
	SP	38.7±6.5	30.7±6.0	14.7±8.0
	S	32.3±3.5	61.3±5.0	85.3±8.0
	Total relative Activity	1.00	1.14	1.4
α-GPDH	*LP*	2.6±0.5	2.0±1.0	0.3±0.5
	SP	10.0±4.5	12.0±4.0	1.3±1.0
	S	87.4±4.9	86.0±5.0	98.4±1.0
	Total relative Activity	1.00	0.83	1.00

* Each value represents an average of six determinations: two assays of each of three separate preparations. Results are given as mean ± range.

Fraction *LP* is the pellet from the centrifugation of the initial homogenate at 4300 *g* for 10 min.

Fraction *SP* is the pellet from the centrifugation of the LP supernatant at 25,000 *g* for 20 min.

Fraction *S* is the supernatant from the centrifugation providing the SP fraction. See text for details.

RESULTS AND DISCUSSION

Acid DNase activity in subcellular fractions of Drosophila *embryos*

An analysis of the subcellular distribution of acid DNase activity was undertaken using embryonic tissues. Embryonic tissue was chosen for two reasons: (1) compared to other stages of the life cycle, embryos are less heterogeneous with respect to cell type (in this regard, a cultured cell line, DM2 of Schneider, exhibited no detectable DNase-1 activity) and (2) embryos, since they lack chitinous exoskeleton, can be gently disrupted with minimum damage to intracellular structures. Homogenates of embryos were subdivided into three fractions: a large particulate fraction (*LP*), a small particulate fraction (*SP*), and a soluble fraction (*S*), by the series of differential contrifugations outlined in Materials and Methods. After subjecting the particulate fractions to Triton X-100, followed by freezing and thawing of all fractions, acid DNase, acid phosphatase and α-glycerophosphate dehydrogenase (α-GPDH) activities were determined. Three separate fractionation experiments were performed, and replicate assays were done on each fraction for each enzyme. Results are shown in Table 1. Activity values are reported as percentages of the total activity in the homogenate. The range of values is reported after each mean value.

For isotonic homogenates (0.25 M sucrose, no detergent), specific enzyme activities were determined based on measufements of total protein (LOWRY *et al.*, 1951) in each fraction. From these values, relative specific enzyme activities, based on total activity in the homogenate, were calculated for each fraction. The relative specific activity for each fraction is then plotted against percentage of total protein present in the fraction ('DEDUVE plots') as shown in Fig. 2. In each plot the fractions represented in order from the left are: *LP*, *SP* and *S*. The rectangular area for each fraction is a measure of the relative distribution of enzyme activity in each subcellular fraction. The mean relative activities and the relative specific activities of acid DNase indicate that it is significantly more abundant in the small particulate fraction of embryonic cells following fractionation under isotonic conditions. The result is consistent with a lysosomal location for acid DNase, assuming that the majority of the mitochondria and lysosomes are present in the small particulate fraction. Considerable acid DNase activity is also present in the soluble (*S*) fraction. Given the fragility of lysosomal membranes (BEAUFAY, 1972), however, some of this activity is probably due to lysosome breakage during homogenization and some acid DNase, freed in solution due to lysosome rupture, may be nonselectively adsorbed to a variety of cellular constitutents (BEAUFAY, 1972) found in either the (*LP*) or (*S*) fractions. On the other hand, some acid DNase activity may originate in the (*S*) fraction, which probably contains some lysosomes as well as the peroxisomal and microsomal fractions of these homogenates.

The existence of acid DNase in the large particulate fraction may mean that acid DNase is found in or functions in embryonic nuclei which may also be the case in calf thymus nuclei (SLOR and LEV, 1971). It is more probable however that lysosomes are present in

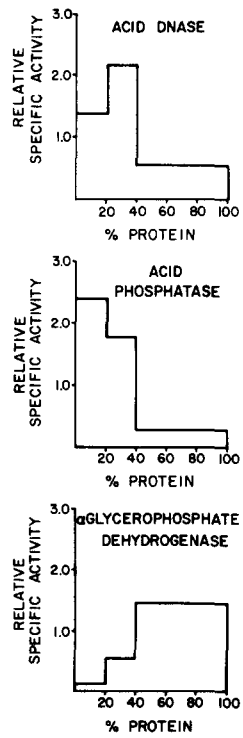

Fig. 2. DEDUVE plots of acid DNase, acid phosphatase, and α-glycerophosphate dehydrogenase activities in *D. melanogaster* embryonic tissues. Fractions are plotted in the order of their isolation (from left to right), large particulate (*LP*), small particulate (*SP*) and soluble (*S*). Each fraction is represented in the ordinate scale by it relative specific activity (percentage of total activity/percentage of total protein). In the abscissa scale, each fraction is represented (cummulatively from left) by its percentage of total protein; *LP* represents the first 20% of the protein, SP the protein from 20–40% and S from 40–100%.

the (*LP*) fraction in the form of lysosomal aggregates, some of which in embryos appear to be as large as nuclei (SAWICKI and MACINTYRE, 1977).

Acid phosphatase, which has been shown to be a lysosomal enzyme from electron microscope studies of *D. melanogaster* salivary gland tissue (ANASTASIA-SAWICKI and MACINTYRE, 1975), exhibits, in embryonic tissue, a higher relative specific activity in the (*LP*) fraction. A peculiarity of embryonic tissue which may explain this result is the presence in early embryos of α-yolk spheres which contain large amounts of acid phosphatase activity (SAWICKI, 1976). These yolk spheres, being similar in size to nuclei,

Table 2. Enzyme activities* of α-glycerophosphate dehydrogenase (αGPDH) and acid DNase in homogenates of *Drosophila melanogaster* embryos before and after freezing and sonication.

Enzyme	Before	After
Acid DNase	0.050 ± 0.005	0.097 ± 0.008
α-GPDH	0.091 ± 0.003	0.097 ± 0.012

* For acid DNase, one unit of activity corresponds to an increase in acid soluble salmon sperm DNA oligonucleotides corresponding to a change in OD_{260nm} of 1.00 at 37°C, for 60 min. See DETWILER and MACINTYRE (1978). For α-GPDH, the units are in terms of changes in OD_{340nm} per minute at 37°C in a reaction volume of 2.0 ml. See O'BRIEN and MACINTYRE (1972) for details.
Each value represents the mean and range of two determinations Latency = $activity_{after}$ — $activity_{before}$)/$activity_{after}$ × 100% = 48.5% (acid DNase) = 6.2% (α-GPDH)

would be in the (LP) fraction, increasing the relative amount of acid phosphatase activity found there.

The relative specific activity of α-GPDH is highest in the soluble fraction as would be expected for a cytoplasmic enzyme (SACKTOR and COCHRAN, 1957; O'BRIEN and MACINTYRE, 1972).

The latency of the acid hydrolase activities (DEDUVE *et al.*, 1955) was demonstrated in two separate experiments described in the Materials and Methods section. The latency values for acid DNase and α-GPDH in crude homogenates (Table 2) were derived from two separate assays using the formula indicated in Table 2. If all of the acid DNase activity is assumed to be associated with or contained within membranous structures, then the latency value suggests that approximately half of the total acid DNase activity in these homogenates is liberated by the homogenization procedure alone. By contrast, α-GPDH, a known cytoplasmic enzyme (SACKTOR and COCHRAN, 1957) exhibits little or no latency in these homogenates.

In the second experiment, as can be seen in Table 1, acid DNase and acid phosphatase activities both exhibit latency; there is a considerable shift of activity from the particulate fractions to the soluble fraction. This shift is common for enzymes which, under isotonic homogenization conditions, are isolated from exogenous substrates within membrane delimited particles. The α-GPDH activity distributions by contrast, were not greatly affected by membrane disruption.

A necessary control for these experiments was the

Table 3. Mean relative activities of DNase-1 and acid phosphatase-1 in subcellular fractions LP, SP and S* of embryonic cells from two strains of *Drosophila melanogaster*.

Strains	Enzyme activities†					
	DNase-1			Acph-1		
	LP	*SP*	*S*	*LP*	*SP*	*S*
BV 1	13.6 (± 8.4)	46.1 (± 3.1)	40.3 (± 5.3)	39.4 (± 4.1)	29.5 (± 7.9)	31.1 (± 3.8)
DNase-1^{n324}	0.0 (± 0.0)	0.0 (± 0.0)	0.0 (± 0.0)	46.7 (± 6.2)	25.7 (± 5.1)	27.6 (± 1.1)

* See Table 1 legend for a description of these fractions.
† Values in parentheses are the ranges of two determinations for each fraction.

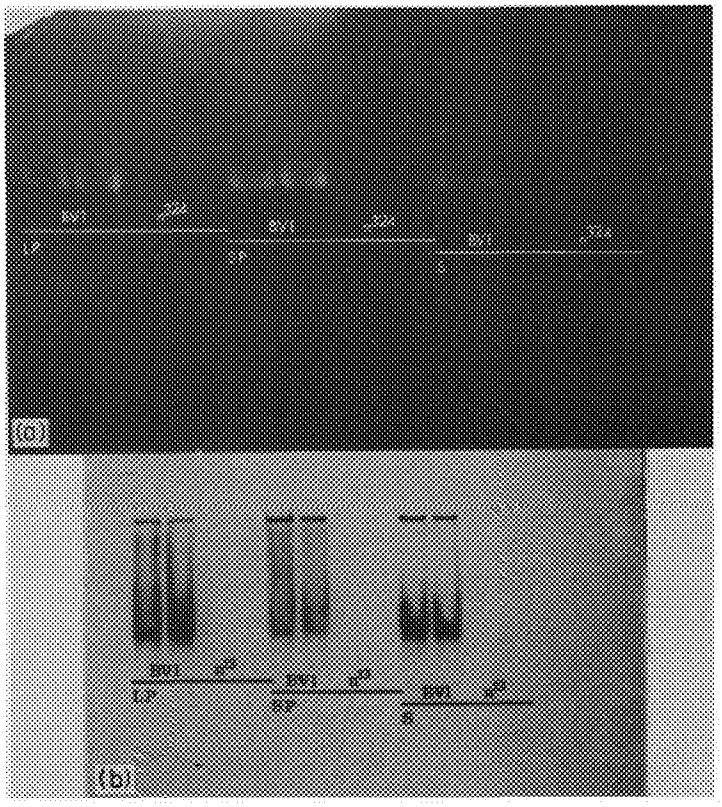

Fig. 3. DNase-1 (panel A), and acid phosphatase-1 (panel B) activities in the large particulate (*LP*), small particulate (*SP*), and soluble (*S*) fractions of embryonic cells from BV1, *DNase-1*^{n324}, and *Acph-1*^{n13} strains of *D. melanogaster*.

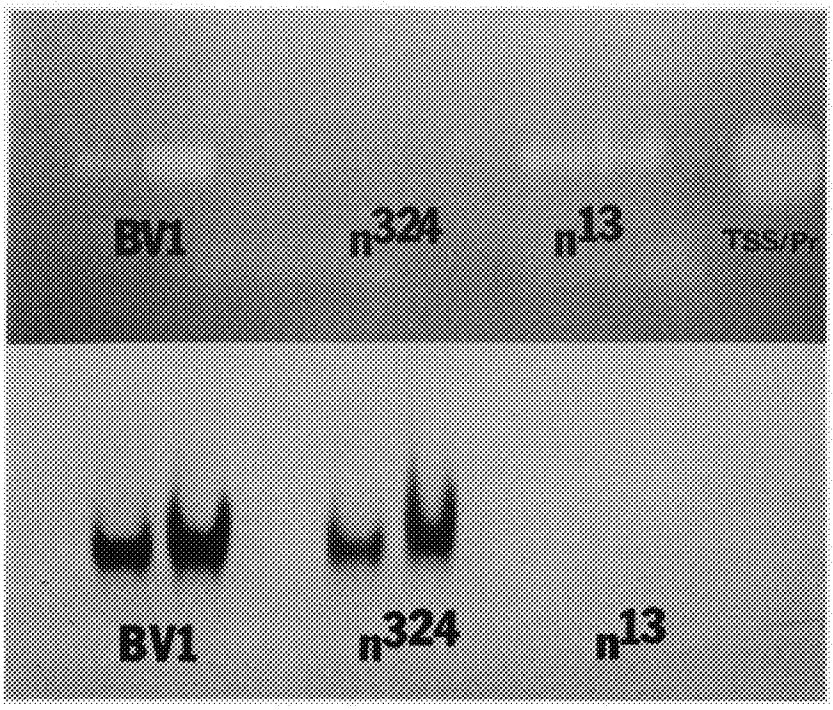

Fig. 6. Peak fractions of DNase-1 and Acph-1 activities from sucrose density gradients of the small particulate fraction of embryonic cells from the BV1, *DNase-1*^{n324}, and *Acph-1*^{n13} strains of *D. melanogaster*. TSS/Pr designates DNase-1 activity from extracts of adult individuals from a strain used in the genetic localization of the *DNase-1* structural gene. Ten peak fractions for each strain were concentrated to 125 μl, and samples of the concentrated material were subjected to electrophoresis as described in Materials and Methods.

determination of 'total relative activities' which are a measure of the total enzyme activity in all fractions of a homogenate relative to the total enzyme activity in all fractions of the corresponding isotonic honogenate. These values were generally close to unity with the possible exception of DNase activity in the Triton X-100 homogenates. This apparent increase in total DNase activity could be due to differences in the initial degree of polymerization of the DNA substrate used in the assays or possibly to increased release of DNase activity resulting from the exposure of the homogenate to the detergent.

In summary, the high specific activity of acid DNase in the SP fraction and its latency prior to membrane disruption suggest that the majority of acid DNase in *D. melanogaster* embryonic tissue is found within small membrane bound particles. This observation is consistent with a lysosomal localization for acid DNase activity.

Distribution of DNase-1 in subcellular fractions

In *D. melanogaster*, acid DNase and acid phosphatase activities result mainly from the products of two genetically determined loci: *DNase-1* (DETWILER and MACINTYRE, 1978) and *Acph-1* (MACINTYRE, 1966). From studies of null activity mutants it has been determined that about 77% of acid DNase activity in embryos (DETWILER, 1979) and >90% of acid phosphatase activity in adult flies (BELL and MACINTYRE, 1973) are contributed by the gene products of these two loci (DETWILER, 1979). Acrylamide gel assays of a homozygous DNase-1 null strain and a wild type strain (Table 3 and Fig. 3) were made to examine the distributions of DNase-1 and Acph-1 activities in the subcellular fractions of embryonic tissues. Densitometric data shown in Table 3 indicate that DNase-1 and Acph-1 activity distributions are similar to the distributions of total acid DNase and acid phosphatase activities reported in Table 1. DNase-1 activity is the highest in the *SP* fraction. In the same electrophoretic system, however, subcellular fractions of embryos homozygous for the null activity allele, *DNase-1*^{n324} exhibit a complete absence of DNase-1 activity from all subcellular fractions. Similar results are obtained for acid phosphatase-1 activity when a strain of embryos homozygous for the null allele, *Acph-1*^{n13}, is used. Thus, it is probable that in the *SP* fraction with its relatively high specific acid DNase activity, most of the total acid DNase activity is contributed by the DNase-1 gene product.

Density gradient analysis of acid DNase and DNase-1 activity

The small particulate fractions of embryos of a wild type strain (BV1) were subjected to equilibrium density gradient centrifugation to determine the distribution of acid phosphatase and acid DNase activities within the gradients. Since acid phosphatase-1 has a lysosomal location in embryonic tissue (SAWICKI and MACINTYRE, 1977), acid DNase activity might be expected to equilibrate at a density similar to that of acid phosphatase activity in these gradients if the acid DNase activity is also lysosomal in embryonic tissue. Results of a typical gradient experiment are shown in Fig. 4. Although acid DNase activity is distributed throughout the gradient, its peak activity coincides with that of acid phosphatase. Both enzyme activity peaks lie near the peak protein fraction in the gradient. The presence of maximal succinic dehydrogenase activity in this region of the gradient

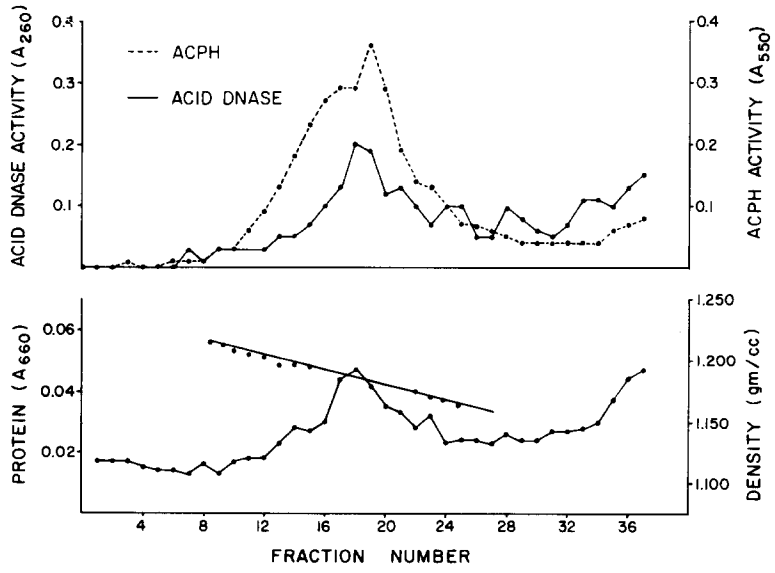

Fig. 4. Distributions of acid DNase activity, acid phosphatase activity and total protein in a sucrose density gradient of the small particulate (*SP*) fraction of a homogenate of embryonic tissue. Embryos were derived from the BV1 wild type strain of *D. melanogaster*. Protein was determined by the LOWRY *et al.* (1951) method. Sucrose density was determined as described in Materials and Methods. Two gradients were analyzed which were qualitatively identical. The data in this figure are from the gradient exhibiting the larger amounts of enzyme activities.

(DETWILER, unpublished observation) and the density range across the protein peak (ρ = 1.200–1.175) suggests that these fractions represent the equilibrium position of the mitochondrial fraction. It is worth noting that the equilibrium position of mitochondria and lysosomes in sucrose gradients of rat liver tissue are within 0.02 g/ml of each other (BEAUFAY *et al.*, 1964). On this basis, overlap of the mitochondrial and lysosomal distributions in these gradients would be expected. The sucrose density in the vicinity of maximal enzyme activity for acid phosphatase and acid DNase is ρ = 1.185–1.187 g/ml). This range of density is very similar to the average density (ρ = 1.19 g/ml) reported for rat spleen lysosomes (Bowers and DEDUVE, 1967) and to the average density at which HENRIKSON and CLEVER (1972) found acid phosphatase and acid protease in sucrose gradients of extracts from the salivary glands of *Chironomus tentans*. Thus, the sucrose density at which acid DNase activity equilibrates and the co-equilibration of acid DNase and acid phosphatase activities are observations which strongly suggest that much of the acid DNase activity in *D. melanogaster* embryonic tissue is lysosomal. Increases in enzyme activity and total protein at the top of the gradients probably result from disruption of particulate material during resuspension of the *SP* pellet. In spite of some variability in the condition of the DNA substrate and in gradient materials which interfere with absorbency measurements, virtually all of the acid DNase activity present in the *SP* fraction which was layered onto the gradient could be accounted for either in the peak fractions or remaining on the top of the gradients.

When 40 μl aliquots from selected fractions of the gradient are subjected to acrylamide gel electrophoresis, acid phosphatase-1 and DNase-1 gene products are distributed as indicated in Fig. 5. In gels, *DNase-1* activity was observable in fractions 16, 19 and 22, but intervening fractions presumably contain *DNase-1* activity as well. These fractions correspond to the peak of acid phosphatase-1 activity as well as the peak of total acid DNase and acid phosphatase activities. This result is consistent with the earlier observation that most of the total acid DNase activity is contributed by DNase-1.

Finally, when *SP* fractions of embryos from the homozygous null strain *DNase-1*^{n324} are subjected to density gradient analysis, the lysosomal fraction of the gradient, as determined by the peak of acid phosphatase activity, contains no DNase-1 activity (Fig. 6). Conversely, when *SP* fractions from embryos of the *Acph-1*^{n13} null strain are used, the lysosomal fraction as determined by the peak of acid DNase activity, contains no acid phosphatase-1 activity (Fig. 6). These results show that a region of the density gradient, in which acid phosphatase containing particles are localized, contains no DNase-1 activity in the *DNase-1*^{n324} strain.

Taken together, the above results indicate that the *DNase-1* locus in *D. melanogaster* contributes a gene product that is localized within the lysosomes of developing embryos. This location within lysosomes suggest a degradative function for DNase-1. That DNase-1 is utilized in degradation of total cellular DNA is also suggested by its increased activity during pupation (DETWILER and MACINTYRE, 1978), a time of extensive tissue histolysis. Further studies of the substrate specificities of *DNase-1* will be useful for an understanding of the role of nucleases in insect development.

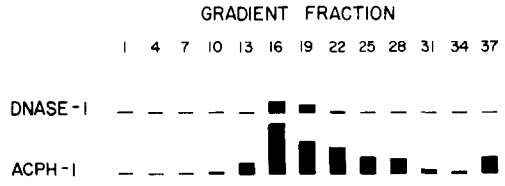

Fig. 5. Co-sedimentation of DNase-1 and acid phosphatase-1 in a sucrose density gradient. Small particulate fractions from the BV1 wild type strain were subjected to density gradient centrifugation as described in the Materials and Methods section. Gradients were fractionated and frozen; 40 μl aliquots (DNase-1) or 15 μl aliquots (Acph-1) were subjected to electrophoresis in acrylamide gels. DNase-1 and Acph-1 activity in gels was scanned on a densitometer; peaks were cut out and weighed. Heights of bars represent relative intensities of enzyme activity. Fractions from the gradient selected for electrophoresis are indicated above the bars. Data represent the means from two experiments.

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