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Genetics

Genetic regulation of tissue-specific expression of *Amylase* structural genes in *Drosophila melanogaster*

(temporal gene/*trans* action/midgut differentiation)

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ABSTRACT **Laboratory strains of *Drosophila melanogaster* were screened for spatial variations in adult midgut α-amylase (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1) expression. No strain-specific differences were found anteriorly, but three patterns of activity were discerned in the posterior midgut: A, activity throughout most of the region; B, activity in the anterior part of the region; and C, little or no activity. Alleles of a control gene, *map*, are responsible for this tissue-specific regulation of activity: e.g., map^A homozygotes produce the A pattern and map^C homozygotes the C pattern. The *map* locus was placed at 2-80± on the genetic map of chromosome 2R, about two crossover units distal to the *Amy* structural gene region for α-amylase. Electrophoretic studies showed that map^A is *trans* acting in map^A/map^C flies, allowing expression of amylase isozymes coded for by genes on the opposite chromosome. The *map* gene behaves as a temporal gene that is clearly separable from the tightly linked, duplicated *Amy* structural genes.**

Differential expression of genes in time and space plays an essential role in eukaryotic development. Two general classes of genes are involved: structural genes and genes with regulatory function (1–4). Among higher eukaryotes, genes with regulatory function may be subdivided into those that map very close to, or are part of, the structural gene (e.g., refs. 5–11) and those that map some distance from the structural gene (e.g., refs. 11–13). We describe here a control gene of the latter type which is *trans* acting in its effect on the expression of the structural genes for two discrete α-amylases (1,4-α-D-glucan glucanohydrolase, EC 3.2.1.1). This gene affects amylase activity in the posterior midgut of *Drosophila melanogaster* in a tissue-specific manner. It fulfills the definition of a temporal gene, i.e., one that encodes genetic information for the developmental program (2, 3, 10).

We assumed that regulatory variants for enzyme activities not critical to the life of the organism exist among different populations of *Drosophila*. Furthermore, a search for such variants would be greatly facilitated by the demonstration of changes in activity or distribution of a given enzyme in a particular tissue, rather than in the organism as a whole. Changes of this sort are especially relevant to the genetic analysis of development (14). The amylase system in *D. melanogaster* is well characterized at the genetic and biochemical levels and is well suited for this type of analysis (15–22).

In *D. melanogaster*, α-amylase activity is found mainly in the midgut and hemolymph, with smaller amounts in other tissues (17). We studied the genetic control of that activity in the midguts of adult females. During metamorphosis, the adult midgut arises *de novo* from stem cells located along the basement membrane of the larval midgut, which is histolyzed (23). The adult midgut has three morphologically distinguishable regions: anterior midgut, middle midgut, and posterior midgut. The anterior and posterior midguts are digestive in function. A technique described here permitted the visualization of amylase-active areas within these regions.

Many, perhaps all, of the strains in this study possess two closely linked structural genes for α-amylase (*Amy*). Evidence for the duplicated nature of these loci and for the monomeric nature of the enzyme in *Drosophila* has been reviewed (17, 18). The two structural loci are distinguishable when they code for enzymes with different electrophoretic mobilities (20). Superscripts designate which amylase isozymes characterize a given strain, e.g., isozymes 2 and 6 are produced by $Amy^{2,6}$ (= Amy^2 Amy^6). The duplicated loci are separable by rare recombinational events (16, 21) and are reported to show a linkage intensity of 0.008 map units (21). The duplication status of strains that produce only one isozyme species, e.g., Amy^{1-a}, remains unclear (17). Pooled data place the *Amy* region at 2-77 on chromosome 2R (16, 20, 21).

MATERIALS AND METHODS

Genetic Stocks. *Amy* strains used for genetic analyses had been reared in this laboratory many years on standard food (24). Those listed in Table 1 were made isogenic for the three major chromosomes and co-isogenic for chromosomes 1 and 3 (16, 17); they were maintained by single-pair matings. Mass cultures were derived from them several generations before the survey and used in the experiments. "Isogenic" strains indicated in Tables 2, 3, and 4 came from the pair-mated lines or were newly synthesized by crosses to the H-40 stock. Major chromosomes in the H-40 stock are: *Basc; SM1; In(2LR)*bw^{V1}, *dp b* ds^{33k}; *C Sb; TM2* (see ref. 25). They contain dominant markers *B* (*Bar*), *Pm* (*Plum*) or *Cy* (*Curly*), and *Sb* (*Stubble*) or *Ubx* (*Ultrabithorax*) on the X, second, and third chromosomes, respectively. Using the *SM1* and *In(2LR)*bw^{V1}, *dp b* ds^{33k} chromosomes of the H-40 stock, an "iso-*al b c* sp^2" strain was established from one of 20 sublines made isogenic for chromosome 2 and used for the crosses in Table 3. For all analyses flies were reared at 25° without crowding during development and adult aging.

Midgut Amylase Activity Pattern Preparations. Midguts were dissected from adults 2–7 days old in Shen's solution (24). After three rinses in fresh saline, guts were stretched on an albumin-coated slide and air dried five min at room temperature. A starch/agar suspension of 0.25% Connaught hydrolyzed starch and 1.5% Bacto agar in 0.05 M Tris·HCl buffer (pH 7.4) containing 0.003 M $CaCl_2$, at 55°, was layered over each gut. Preparations with congealed starch-agar film were incubated 10 min at room temperature (22°±), fixed in Carnoy's solution,

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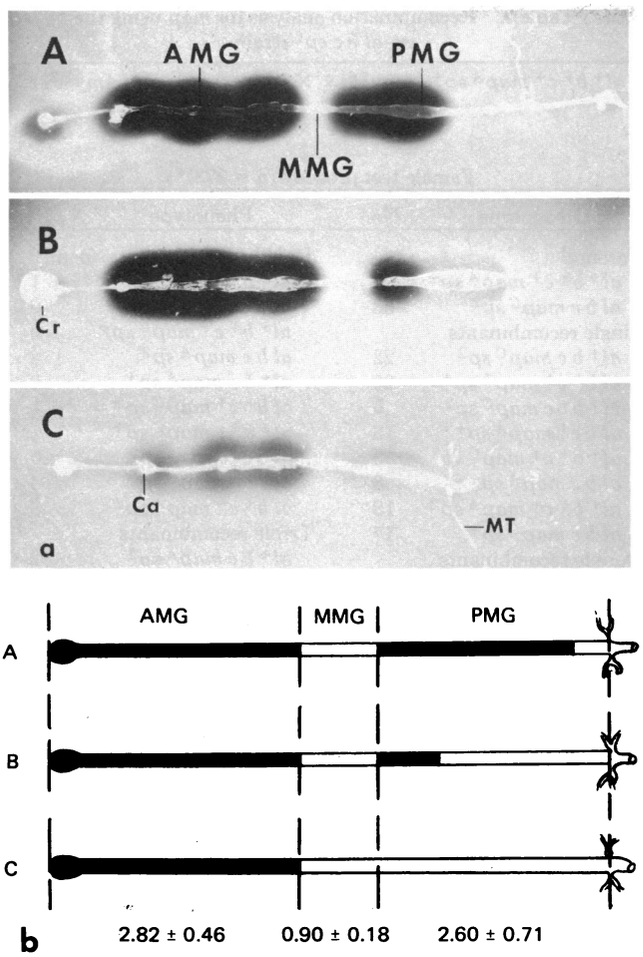


FIG. 1. (*a*) Reverse copy print of adult female midgut preparations showing amylase activity patterns. The starch/iodine film overlay appears as a light grey background; dark areas show where starch was hydrolyzed by amylase released from midgut cells. Three posterior midgut activity patterns are shown, A, B, and C. AMG, anterior midgut; MMG, middle midgut; PMG, posterior midgut; MT, Malpighian tubules; Cr, crop; Ca, cardia. (*b*) Diagram of midgut amylase activity patterns for adults aged 2–6 days from the *Amy* strains in Table 1. Blackened areas represent regions of activity as interpreted from *a*. Numbers indicate the average extended lengths (mm) and standard deviations for the midgut regions (n = 19).

stained in I/KI reagent (26), rinsed in water, and air dried. Areas where starch remained in the agar film stained dark blue; areas where starch was hydrolyzed by amylase activity appeared colorless, forming a pattern surrounding the midgut. Whole mount preparations served as photographic negatives for reverse copy prints. They were coded and scored blind for genetic analyses.

Electrophoresis. α-Amylase banding patterns characteristic of each strain were determined by polyacrylamide gel disc electrophoresis and the starch/iodine staining techniques of Doane (15–17).

Amylase Activity Assays. Amylase activities of whole-fly homogenates from different genetic strains were determined by starch/iodine assay (16).

RESULTS

Amylase Activity Patterns of Adult Midguts. Midgut amylase activity patterns were prepared for a series of different genetic strains. Preparations showed similar patterns for the anterior midgut in all cases, with activity extending from the anterior (cardia) to the posterior end of this region (Fig. 1). There was complete absence of activity in the highly acidic middle midgut region. The posterior midgut was characterized by strain-specific pattern differences. Three basic patterns emerged: an A pattern with activity along most of the posterior midgut, a B pattern with activity limited to the anterior end of this region, and a C pattern with little or no activity here. This is shown diagrammatically in Fig. 1*b*, which gives the average extended lengths of the anterior, middle, and posterior midgut regions based on morphology. Activity patterns typically showed further subdivisions as seen in Fig. 1*a*, where the A pattern of the posterior midgut appears made up of two smaller active regions while the B pattern shows only a single one at the anterior end. Anterior midgut patterns showed three subdivisions.

Table 1. α-Amylase activity in adult females with different midgut patterns

Amy strain*	Midgut pattern†	Total activity‡
$Amy^{1\text{-}a}$	A	78
$Amy^{1\text{-}b}$	A	66
$Amy^{1,6}$	A	251
$Amy^{2,6}$	A	267
$Amy^{3,6}$	A	237
$Amy^{1,4}$	B	92
$cn\ c\ Amy^{1,3}\ adp^{60}$	B	113
$Amy^{1\text{-}c}$	C	39
$c\ Amy^{2,3}$	C	173

* The origin of *Amy* strains has been published (17). Isozymes characteristic of each strain are denoted by superscripts, with a comma to indicate the *Amy* duplication; *a*, *b*, or *c* in superscripts denotes strain differences in total amylase specific activity.

† Amylase activity patterns are shown in Fig. 1; flies were aged 2–6 days.

‡ Activity is expressed as starch units (SU) per fly, in which one unit is equivalent to 0.1 μg of starch hydrolyzed per min at 25°, pH 7.4. With one exception, these data are from Doane (16).

Table 1 lists stocks surveyed for adult midgut patterns and the total amylase activity characteristic of females of each strain. The patterns were similar in males of the same strain but, because females are easier to dissect, only they were used. Five of the nine stocks displayed the A pattern. The pattern was invariant in four of these, although strain-specific variation in the intensity of the pattern was noted. The $Amy^{1\text{-}b}$ strain was included in the A group even though some of its flies (7 out of 20) produced B or C patterns. Both of the strains listed in the B group contained some flies with the A pattern. The C pattern strains showed this pattern exclusively. Data in Table 1 show that the midgut pattern does not appear to be a function of the particular isozyme present in the fly, nor is there a correlation between total activity and midgut pattern.

Linkage Analysis for Pattern Controlling Factor(s). Males from a cloned H-40 line that produced nothing but C patterns were crossed to females of the isogenic $Amy^{1,6}$ strain (Table 2). F_1 heterozygotes produced a pattern similar to the A pattern of $Amy^{1,6}$ homozygotes, but with slightly reduced amylase activity in the posterior midgut. F_1 females were backcrossed to cloned H-40 males and their female progeny were scored for midgut patterns.

Results of this linkage analysis (Table 2) show that control of the posterior midgut pattern is associated with chromosome 2. A single control gene was postulated and named *map* for midgut activity pattern. Presumably, there existed three alleles

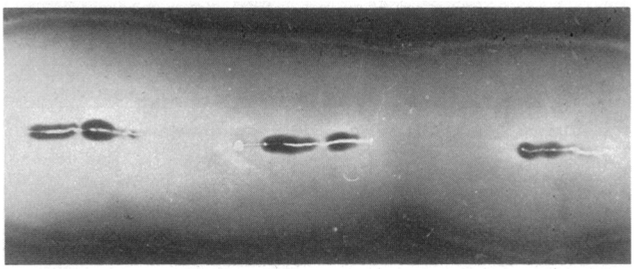


FIG. 2. Midgut activity patterns produced by females, aged 4–6 days, of the genotypes c^+ $Amy^{1,6}$ map^A (A pattern), c^+ $Amy^{1,6}$ map^A/c $Amy^{2,3}$ map^C (reduced A pattern), and c $Amy^{2,3}$ map^C (C pattern), left to right.

among the stocks examined, map^A, map^B, and map^C, and these determined the A, B, and C patterns, respectively, in homozygotes.

Location of *map* on Chromosome 2. The iso-*al b c sp*^{2} strain indicated in Table 3 consistently produced C midgut patterns and was assumed to contain the map^C allele. This multiply marked second chromosome strain and the $Amy^{1,6}$ (map^A) strain were used in a recombination analysis to roughly locate the hypothetical *map* factor. F_1 heterozygotes produced midgut patterns that resembled the A type and that could be readily distinguished from the C pattern of the iso-*al b c sp*^{2} strain.

The raw data in Table 3 place *map* about 8 crossover units right of *c*, or 5.8 units right of *Amy*. However, viability was not equal in all reciprocal classes of the test cross progeny and neither parental strain was fully viable (~80% for $Amy^{1,6}$ and ~50% for *al b c sp*^{2}). Removal of the bias imposed by lowered viabilities tends to shorten the distance between *Amy* and *map* to a range compatible with data (below) from the use of *Amy* as a genetic marker.

Recombination Analysis for *map* with *Amy* as Marker. A recombination analysis was performed to determine if *map* was separable from *Amy*. Two "isogenic" strains, $Amy^{1,6}$ map^A and c $Amy^{2,3}$ map^C, were chosen from Table 1. Females of these strains consistently produced A and C patterns, respectively, up to the age of 12 days. Heterozygotes produced A patterns similar to $Amy^{1,6}$ map^A homozygotes, but not as pronounced (Fig. 2). Results of the three-point analysis involving *c*, *Amy*, and *map* are given in Table 4. The tightly linked, duplicated *Amy* loci behaved as a single genetic unit whose alleles were detected by electrophoretic analysis of fly remains after midgut removal.

Table 2. Linkage analysis for adult posterior midgut pattern control factor

+/+; +/+; +/+ ♀ × *B/Y; Cy/Pm; Sb/Ubx** ♂
($Amy^{1,6}$, A pattern) ↓ (Amy^1, C pattern)
F_1 (A pattern) ♀ × *B/Y; Cy/Pm; Sb/Ubx* ♂
↓
Female test progeny†

Midgut pattern	*Cy* or *Pm*/+				*Cy/Pm*			
	Sb or *Ubx*/+		*Sb/Ubx*		*Sb* or *Ubx*/+		*Sb/Ubx*	
	B/B	*B/+*	*B/B*	*B/+*	*B/B*	*B/+*	*B/B*	*B/+*
A	7	13	3	8	—	—	—	—
B	—	—	—	—	—	—	—	—
C	—	—	—	—	4	7	1	4

* Each of the major chromosomes in this "cloned H-40" strain derives from a single chromosome in the original H-40 stock.

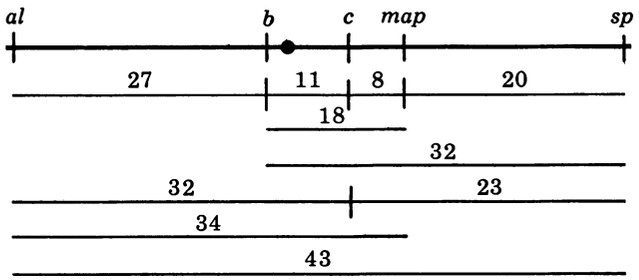
† *Basc* carries *B* and w^a, so the eye color phenotype of the *Pm* chromosome, *In(2LR)bw*^{V1}, *dp b ds*^{33k}, is masked in *B/B* progeny. To distinguish *B/B; Cy/Pm* and *B/B; Cy/+* females, each was individually crossed to a *b/b* male prior to dissection and her progeny were examined for the *b* body color. All test cross progeny were aged 3–6 days on fresh food.

Table 3. Recombination analysis for *map* using the iso-*al b c sp*^{2} strain

al^+ b^+ c^+ map^A sp^+ ($Amy^{1,6}$) ♀ × *al b c* map^C sp^2 ($Amy^{1,3}$) ♂
↓
F_1 ♀ × *al b c* map^C sp^2 ♂
↓
Female test progeny (n = 273)*

Phenotype	No.	Phenotype	No.
Parental		Double recombinants (cont.)	
al^+ b^+ c^+ map^A sp^+	76	al^+ b^+ c map^A sp^+	1
al b c map^C sp^2	53	*al b* c^+ map^C sp^2	0
Single recombinants		al^+ b^+ c^+ map^C sp^+	0
al^+ *b c* map^C sp^2	22	*al b c* map^A sp^2	2
al b^+ c^+ map^A sp^+	25	al^+ *b c* map^A sp^+	2
al^+ b^+ *c* map^C sp^2	5	*al* b^+ c^+ map^C sp^2	1
al b c^+ map^A sp^+	15	al^+ b^+ *c* map^C sp^+	1
al^+ b^+ c^+ map^C sp^2	3	*al b* c^+ map^A sp^2	0
al b c map^A sp^+	8	al^+ *b c* map^C sp^+	4
al^+ b^+ c^+ map^A sp^2	18	*al* b^+ c^+ map^A sp^2	8
al b c map^C sp^+	17	Triple recombinants	
Double recombinants		al^+ *b c* map^A sp^2	3
al^+ *b* c^+ map^A sp^+	6	*al* b^+ c^+ map^C sp^+	1
al b^+ *c* map^C sp^2	1	al^+ *b* c^+ map^A sp^2	1
		al b^+ *c* map^C sp^+	0

Crossover intervals based on raw data:



* Flies were aged 2–7 days on fresh food. Triple recombinant classes include only those found among the progeny.

Table 4. Recombination analysis for *map*, using *Amy* variants as markers

c^+ $Amy^{1,6}$ map^A ♀ × *c* $Amy^{2,3}$ map^C ♂
↓
F_1 ♀ × *c* $Amy^{2,3}$ map^C ♂
↓
Female test progeny*

Phenotype	No.
Parental	
c $Amy^{2,3}$ map^C	74
c^+ $Amy^{1,6}$ map^A	59
Recombinant	
c $Amy^{1,6}$ map^A	1
c^+ $Amy^{2,3}$ map^C	3
c $Amy^{2,3}$ map^A	1
c^+ $Amy^{1,6}$ map^C	2

Interval	% recombination	
	Uncorrected	Corrected†
c–Amy	2.9	2.6
c–map	5.0	4.5
Amy–map	2.1	1.9

* Test cross progeny were aged 4–6 days on fresh food.

† Corrected values assume an equal frequency of parental types.

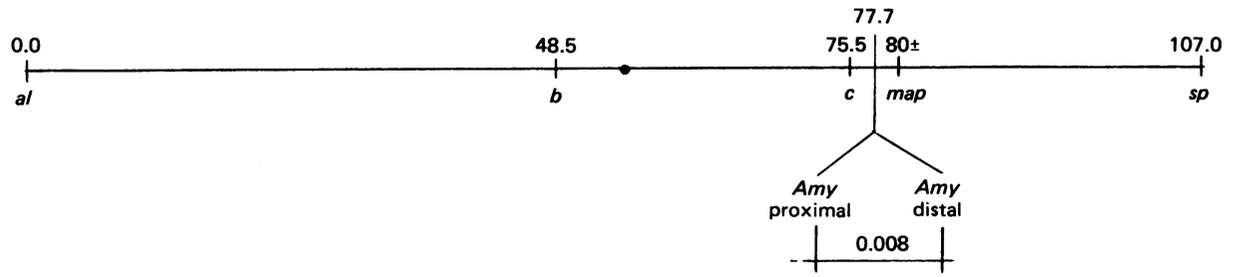


FIG. 3. Genetic map of chromosome 2 showing the location of *map* based on data in Table 4. Positions for other loci shown are from the literature (16, 21, 25).

Data confirm that the *map* gene is located distal to *c* and that its alleles recombine with those at the *Amy* loci. No double crossovers were recovered, which is compatible with the genetic map distances involved and the sample size. On the basis of the raw data, *map* is located about 2.1 crossover units right of the *Amy* region. This distance reduces to 1.9 when correction is made for the lower viability of the c^+ $Amy^{1,6}$ map^A parental type. The corrected distance between *c* and *Amy* (2.6 units) is close to the value of 2.2 found in the literature (25). Assuming an interval of about 2 map units between *Amy* and *map*, the *map* gene was placed at 2-80± on chromosome 2R (Fig. 3). This is considered a more accurate estimate of its location than that from the previous cross in Table 3 for two reasons. First, *Amy* variants furnished genetic markers closer to *map* than *c* and, second, the viability of test cross progeny was considerably better in the Table 4 analysis.

Effect of *map* on Midgut Isozyme Expression. Midgut amylase isozymes produced by females of the strains used for the experiment in Table 4 were analyzed electrophoretically. Zymograms produced by $Amy^{1,6}$ map^A and *c* $Amy^{2,3}$ map^C homozygotes are shown in Fig. 4. Differences in the intensities of amylase bands between gels of the same genotype and gut region reflect sample sizes.

Isozymes 1 and 6 were similarly expressed in the anterior and posterior midgut of $Amy^{1,6}$ map^A flies. Flies homozygous for *c* $Amy^{2,3}$ map^C produced isozymes 2 and 3 in the anterior midgut but neither of these were detected in gels of the posterior midgut shown in Fig. 4. This is consistent with *map* affecting *Amy* expression posteriorly but not anteriorly. Anterior midguts from heterozygotes ($Amy^{1,6}$ map^A/*c* $Amy^{2,3}$ map^C) showed isozymes 1, 2, 3, and 6, as expected for codominant expression of *Amy* alleles here. Isozymes expressed in the posterior midgut, on the other hand, were additive with respect to isozymes 1, 3, and 6, but not for 2, which was barely visible. The fact that only a trace of isozyme 2 was expressed may explain why these heterozygotes showed a less pronounced A midgut activity pattern than the homozygotes for map^A (Fig. 2.)

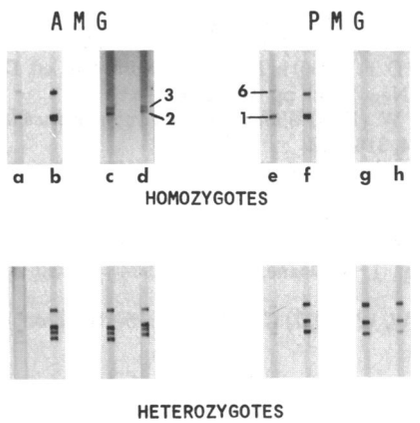


FIG. 4. Electrophoretic banding patterns made by amylase isozymes in the anterior midgut (AMG) and posterior midgut (PMG) regions of adult females aged 6–7 days. Genotypes were $Amy^{1,6}$ map^A and *c* $Amy^{2,3}$ map^C (*Upper*) and heterozygotes between them (*Lower*). AMG samples are on the left and PMG samples on the right. Midgut regions from one to three flies were included in sample gels. In the top row, samples a, b, e, and f were from $Amy^{1,6}$ map^A flies; c, d, g, and h represent *c* $Amy^{2,3}$ map^C flies. The starch/iodine reaction was used to show amylase activity.

It is of theoretical importance to know whether or not the *map* gene acts *cis* or *trans* in relation to the expression of the *Amy* structural genes in heterozygotes. The zymograms in Fig. 4 indicate that map^A exerts a *trans* effect on the expression of the $Amy^{2,3}$ loci (= Amy^2 Amy^3) in the posterior midgut of $Amy^{1,6}$ map^A/*c* $Amy^{2,3}$ map^C heterozygotes. However, the Amy^2 locus is barely expressed in terms of activity, while the Amy^3 locus is expressed at a level similar to Amy^1 and Amy^6.

DISCUSSION

The data clearly show the existence of a gene in *D. melanogaster* that controls the level of amylase activity in the posterior midgut of adult females. This gene, *map*, behaves in a tissue-specific manner, having no effect on activity in the anterior midgut. It appears to be a temporal gene (2, 3). A number of examples supporting the concept of temporal genes have been cited in the literature (see ref. 10), including one for aldehyde oxidase in *Drosophila* (6). Such genes presumably comprise the set of DNA sequences that contain the genetic information for the developmental program. It is noteworthy that, with respect to the *map* gene, the cells from which the anterior and posterior portions of the midgut arise have different embryonic origins (ref. 27; cf. ref. 28), perhaps implicating cellular origin in the developmental response to this control locus. The effect of *map* on amylase expression in males and in tissues other than the midgut remains to be fully explored.

Three different posterior midgut amylase activity patterns were found among the eleven laboratory stocks screened. These were attributed to three alleles at the *map* locus. Two of them, map^A and map^C, were well defined by independent genetic analyses. Preliminary data (unpublished) indicate that map^B will be less easy to define because of overlapping phenotypes between homozygotes and heterozygotes for this allele. In any event, allelic variation at the *map* locus is apparently common among laboratory stocks with no serious detrimental effect on viability.

The *map* gene is on chromosome 2R about two crossover units right of the amylase structural gene region. The amount of DNA between *map* and the *Amy* region is sufficient to

contain information for many other genes, assuming one map unit represents approximately 18 chromomeres (cistrons) (cf. ref. 29). Such estimates are based on the total number of chromomeres seen in salivary gland chromosomes (30, 31) and the total map units (25) for the species. At least two unrelated morphological mutants are located within this region (25).

Consistent with the separation between *map* and *Amy* loci, the map^A gene is *trans* acting in its effect on the expression of *Amy* structural genes in heterozygotes. Thus, in the double heterozygote, $Amy^{1,6}$ map^A/c $Amy^{2,3}$ map^C, map^A brought about expression of amylase isozymes 2 and 3 in the posterior midgut, but these isozymes showed little or no expression in the posterior midgut of map^C double homozygotes (c $Amy^{2,3}$ map^C/c $Amy^{2,3}$ map^C). Significantly, this *trans* action of map^A extended to both structural genes in the *Amy* region, but the effect was not equivalent for the two tightly linked structural loci. Isozyme 2 activity appeared in trace amounts compared to 1, 3, and 6. By contrast, activities of all four isozymes were additive and roughly comparable in the anterior midgut. This suggests that somehow *Amy* genes, or their products, are differentially recognized by products of the *map* gene in addition to being differentially recognized in different parts of the midgut. Differential expression of duplicated *Amy* structural genes was previously observed among different tissues and in different developmental stages of homozygotes (17).

It is premature to speculate on the mechanism by which the *map* gene affects amylase activity in the posterior midgut. First to be determined is whether or not the level of amylase protein itself is altered. Changes in the rates of synthesis or degradation, and in the activation or inactivation of amylase molecules must be considered. The amount of amylase can be approached immunologically (ref. 32, unpublished data). If the number of amylase molecules is altered by the *map* gene, we would like to know if the effect is caused by a change at the transcriptional or post-transcriptional level.

There is preliminary evidence for the existence of another control gene besides *map* that influences midgut amylase activity patterns (33). It appears to be located close to *map* on chromosome 2R and affects amylase activity in the anterior midgut. A number of different amylase activity patterns for the anterior midgut were found among flies from various laboratory and natural populations; these patterns were produced by midguts which displayed the A, B, or C pattern posteriorly. This suggests that *map* is just one of a series of temporal genes that affect the expression of amylase activity in the midgut.

It is conceivable that *map* exercises its control at the transcriptional level and has a molecular role analogous to that of an integrator gene, as described by Britten and Davidson (4). According to this model, products of the *map* locus would interact with regulatory regions at the *Amy* region, which, in turn, would affect the structural portion of the gene. There is good evidence for a regulatory region very close to the structural gene for xanthine dehydrogenase in *Drosophila* (5). The differential effect of map^A on amylase isozyme activities in the midguts of heterozygotes might be explained in these terms. While other models cannot be excluded, it is clear that the amylase system provides the opportunity to test the various possibilities.

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