Molecular analysis of the yellow gene (*y*) region of *Drosophila melanogaster*

(gene regulation/development/mutation/transposable elements)

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ABSTRACT The yellow gene (*y*) is involved in pattern-specific melanin pigmentation of the cuticle of the adult fly and of larval mouth parts of *Drosophila melanogaster*. I have isolated some 70 kilobases (kb) of contiguous DNA from the *y* region. Chromosomal aberrations of *y*¹-type alleles (null alleles) and *y*²-type alleles that give rise to characteristic pattern mosaicism of pigmentation were mapped by Southern blot analysis. The *y*² allele is associated with the insertion of a “gypsy” transposable element 0.9 kb distal to the putative *y* coding region. A 3.1-kb region to which breakpoints of all *y*¹-type alleles could be mapped is homologous to a 2.0-kb polyadenylated mRNA, the expression of which is specifically regulated in development. This putative *y* gene transcript is present at high levels in pupae when melanization of the adult cuticle occurs, but its steady-state levels change dramatically during development, being highest in late embryos prior to hatching. This suggests that, in addition to melanin synthesis and/or deposition, the *y* gene product may have a role in other possibly neural functions.

The genetically well-characterized yellow gene (*y*) of *Drosophila melanogaster* is located at the tip of the X chromosome at 1B1 on the cytological map (1). The visible phenotypic effect of a *y* mutation is an altered (yellow) pigmentation of the adult cuticle and derivative structures as well as of the larval mouth parts (2, 3). Many mutant alleles change the pigmentation in only some regions, making the *y* gene ideal for studying effects of DNA sequence rearrangements on gene expression.

The process of cuticle formation in arthropods is under strict developmental control and is regulated by the titer of ecdysteroid hormone (for recent reviews, see refs. 4–6). Two processes are involved: sclerotization (hardening) and melanization (deposition of pigment), both of which use the same substrate (dopa). The characteristic adult melanization pattern is laid down at day 4 of puparium (7). Fifty hours after puparium formation, the epithelial cells in the hypoderm start secreting the imaginal cuticle, which subsequently is sclerotized. Deposition of melanin is first visible in the dorsal bristles of the head and thorax in 70-hr-old pupae, and it continues for the next 20 hr in a defined sequence (7, 8). The *y* function may be required during this developmental period to deposit black pigment in the correct part of the cuticle at the right time. Nothing is known about the *y* product itself, but it does not represent a structural gene for any of the known enzymes of tyrosine metabolism that generate products required for cuticle formation at every molt (9, 10).

The most intriguing feature of the *y* locus is the existence of many alleles that produce patterns of cuticle coloration that are normal in some areas and mutant in others (*y*²-type alleles; refs. 3 and 11). Each *y*² allele exhibits a distinguish-

able pattern of cuticle staining that is typical for the allele. In contrast, *y*¹-type mutations as well as *y* deficiencies exhibit the mutant phenotype throughout the entire adult cuticle and in all pigmented structures of the larvae.

In order to investigate the principles of tissue- and pattern-specific gene expression, I isolated DNA from the *y* gene region by molecular cloning (unpublished data). In this paper I report the localization of the *y* structural gene and its possible regulatory region by mapping breakpoints of various *y* alleles and, in addition, the nature and time of expression of a *y* region mRNA transcript.

While this work was in progress, the cloning of the proximally adjacent and overlapping region of the genome containing the *y*, *acheate* (*ac*), and *scute* (*sc*) loci was published (12). These loci are believed to form a cluster of functionally related genes that control the differentiation of cuticular structures (chaetes) and the pigmentation in a position-specific manner (13, 14).

MATERIALS AND METHODS

The following *D. melanogaster* strains were obtained from the California Institute of Technology Stock Center: *y*¹, *y*^{62d}*ac*², *y*⁴, *y*², *y*^{1d}, *In(1)y*^{3P}, *y*^{34c}, *y*^{v2}, and *y*^{CTS}. From the Bowling Green Stock Center were: *C(1)DX*, *y f/In(1)sc*⁸, *y*^{ox} *sc*⁸ *w sn*^{5.1}, *C(1)DX*, *y f/y*^{62a} *sc cv*; *C(1)RM*, *br ec/y*^{3d}; and *y*^{bg} *ct*⁶ *car*. *y*^{bl} was obtained from L. Sandler. The null alleles *y*^{80h16} and *y*^{80e14(6)} were obtained from M. M. Green. They have been induced on the *y*^{+13z} *gt*^{13z} X chromosome (15) and were analyzed at the molecular level recently (unpublished data). *y*^{m2v}, obtained from W. G. Nash, is a spontaneous derivative of *y*^m (8) and is fully mutant except for variegation in the sex combs and some bristles. The P/M-hybrid dysgenesis-induced alleles *y*^{CAA1}, *y*^{CAF2}, *y*^{CAG1}, *y*^{CAM3}, *y*^{CAP1}, *y*^{CU2}, and *y*^{BC16} are *y*¹-type alleles and express the fully mutant phenotype. They were generated by P. Adler by crossing Oregon R females to males of the Harwich P strain.

Genomic DNA was isolated from adults of both sexes or from males of the balanced stocks as described by Bingham *et al.* (16). Hybridization to Southern blots was done with ³²P-nick-translated probes, and *Bam*HI fragments from genomic DNA of the *y*² strain were cloned into the EMBL 4 λ phage vector (both procedures to be described elsewhere). Phage libraries were screened with nick-translated fragments subcloned in pBR329.

For heteroduplex analysis, linearized recombinant pBR322 DNA (2 μg/ml) containing the 6.8-kilobase (kb) *Xho* I fragment from the 7.3-kb “gypsy” transposable element represented in the recombinant phage bx^{34c}-6a2 (17) was mixed with 2 μg of *Bam*HI-digested recombinant EMBL 4 λ phage vector containing the 16.5-kb *Bam*HI fragment from *y*² DNA (Canton S strain, coordinates 36.5–45.9). After denaturation with 0.1 M NaOH, DNA was rehybridized by

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Abbreviation: kb, kilobase(s).

dilution (1:1) into 0.1 M Tris-HCl, pH 8.5/45% formamide and incubation for 30 min at room temperature and was prepared for electron microscopy (18).

RNA was isolated from different developmental stages by the guanidinium-HCl method and pelleted through 5.7 M CsCl (19). Poly(A)⁺ RNA (10 µg) selected by two passes over oligo(dT)-cellulose was loaded on each lane of an agarose-glyoxal gel (20) and, after electrophoresis, transferred onto APT (aminophenylthioether)-cellulose. The blot was probed by hybridization with the ³²P-nick-translated 4.5-kb *Eco*RI fragment (coordinates 33.5–38.0) that had been subcloned in pBR329.

RESULTS

Isolation of DNA from the Yellow Gene Region. A detailed description of the cloning of DNA from the *y* and the adjacent *ac* gene region will be given elsewhere. Briefly, DNA fragments from the tip of the X chromosome were obtained by microdissection and microcloning into λ phages (21). One of these fragments could be located near the proximal breakpoint of the homozygous viable deficiency for *y* and *ac*, *In(1)y^{3PL}sc^{8R}*, and was used to start a "chromosome walk" through the *y-ac* region. Thus, 70 kb of contiguous single-copy DNA, some clones from a Canton S genomic DNA library (22), and some (phages TG-1 and TG-2) from an Oregon R library generated by W. Bender were isolated and characterized by restriction mapping (see Fig. 2).

In comparison to many other regions of the *Drosophila* genome, the sequences surrounding *y* show few polymorphisms. The degree of polymorphism between Canton S and Oregon R wild types was assessed by hybridization of recombinant phages spanning the 70-kb region to Southern blots of genomic DNA cleaved with *Eco*RI, *Hind*III, *Bam*HI, and *Pst* I. The only polymorphism was detected at the most distal end of the "walk" between coordinates 65 and 68.5 (data not shown). Together with previously published results on the adjacent *ac* and *sc* regions (12, 23), this is the only detected restriction site polymorphism in a total of some 140 kb.

Physical Mapping of *y* Mutations. Southern blots of restricted genomic DNA from various *y* mutations were hybridized to nick-translated recombinant phages from the *y-ac* region (Fig. 1). Four different restriction enzymes used for these analyses (*Eco*RI, *Bam*HI, *Hind*III, and *Pst* I) gave a consistent localization for the altered sequence arrangements on the physical map. Since most of the mutations were isolated a long time ago, the parental chromosomes are no longer available for comparison. Therefore, the mutant chromosomes might carry sequence polymorphisms that could be mistaken for a DNA alteration causing the mutant phenotype. However, in the few cases where the parental chromosomes are known (hybrid dysgenesis-induced *y^l* alleles and *y* alleles induced on the *gt^{13z}* X chromosome), polymorphism can be ruled out. Moreover, other findings argue that the detected aberrations are unlikely to be polymorphisms: the *y-ac-sc* region contains few polymorphisms, and the alterations detected in the mutants are sequence rearrangements or insertions that can always be detected with more than one enzyme. Finally, all sequence rearrangements in *y^l*-type mutations mapped as a closely linked cluster (3.1 kb) within a larger region known to contain the *y* gene.

Type *y^l* Alleles. I analyzed the following *y^l*-type mutations (null alleles) that give rise to a uniformly yellow cuticle and larval mouth parts and are probably due to lesions in the structural gene: *y^l* (spontaneous), *y^{62d}* (origin unknown), *y⁴* (x-ray), *y^{ox}* (spontaneous), and the P/M hybrid dysgenesis-induced alleles *y^{CAAI}*, *y^{CAF2}*, *y^{CAG1}*, *y^{CAM3}*, *y^{CAP1}*, *y^{CU2}*, and *y^{BC16}*. All of the hybrid dysgenesis-induced *y^l*-type mutations, except *y^{CAP}*, and three of the spontaneous or x-ray

FIG. 1. Determination of chromosomal aberrations of *y^l*-type and *y²*-type alleles. Autoradiographs of genomic Southern blots of DNA from various *y* alleles, hybridized to ³²P-nick-translated probes from the cloned *y-ac* region. (A) DNA from hybrid dysgenesis-induced *y* null alleles digested with *Pst* I were hybridized to the *Eco*RI fragment py14b15-A (coordinates 33.5–49.0) subcloned in pBR329. (B) DNA from several *y* alleles digested with *Hind*III and hybridized to py14b15-A. (C) DNA from several *y* alleles digested with *Eco*RI and hybridized to phage 14-1 (coordinates 10.0–24.0). (D and E) *Hind*III-digested (D) or *Pst* I-digested (E) DNA from *y^{bl}* and *y^{m2v}* was hybridized to py14b15-A. The sizes of the wild-type DNA fragments hybridizing to the respective probes are indicated in kb.

induced *y^l*-type alleles (*y^{62d}*, *y⁴*, *y^{ox}*) had altered restriction fragments in the region between coordinates 33.5 kb and 36.5 kb (Fig. 1 A–C; see also Fig. 2). Another *y^l*-type mutation (*y^{80h16}*) that was induced on the *gt^{13z}* X chromosome has recently been shown to contain a "hobo" transposable element at position 36.4 kb, and another *y^l*-type mutation induced in this stock (*y^{80e14(6)}*) is due to the insertion of a different transposable element at coordinate 33.4 (unpublished data). In these cases the parental chromosome was available for comparison. For *y^l* no chromosomal breakpoint could be detected. These results suggest that the major part of the *y* gene coding region is located in the 3.1-kb region between coordinates 33.4 and 36.5 kb. No *y^l*-type mutations tested contained detectable DNA changes that mapped outside this region.

Type *y²*-Alleles. Twelve *y²*-type alleles were analyzed for detectable DNA abnormalities in the *y* gene region (Fig. 1 B–E). The characteristic phenotypes of these alleles result from the fact that they express different color states in different tissues, as summarized in Table 1. A more detailed classification of some of these *y²*-type alleles has been given by Nash and Yarkin (11).

The results of the mapping of *y²*-type alleles are summarized in Fig. 2 along with the mapping of data on the *y^l*-type alleles. Ten of the 12 *y²*-type alleles analyzed could be mapped. Of these, 6 exhibited altered restriction patterns compatible with a single discontinuity as would result from insertion of foreign sequence or chromosomal rearrange-

Table 1. Pigmentation phenotype of y^2 -alleles used in molecular mapping experiments

Allele	Origin	Body	Pigmentation phenotype			Larval mouth-parts
			Bristles	Sex combs	Wings	
2	Spont.	y	wt	wt	y	wt
<i>td</i>	Spont.	Tan	wt	wt	±wt	y
<i>3P</i>	X-rays	Tan; tip, wt	wt	wt	y	wt
<i>3d</i>	Spont.	Tan, y	y	wt	wt	y
<i>34c</i>	Spont.	Tan	wt	wt	±wt	wt
<i>v2</i>	Spont.	Tan; tho, y	wt	wt	wt	wt
<i>2S</i>	Spont.	y	±wt	±wt	y	wt
<i>bg</i>	Spont.	Tho, y; abd, wt	wt	wt	wt	wt
<i>62a</i>	Spont.	Tho, y; abd, wt	Tho:y abd:wt	wt	y	wt
<i>bl</i>	Spont.	wt	y	y	wt	wt
<i>m2v</i>	Spont.	y	±y	±y	y	y

Spont., spontaneous; tho, thorax; abd, abdomen; wt, wild type; y, yellow.

ment, and 4 showed detectable rearrangements at multiple sites (y^{bl} , y^{3d} , y^{2S} , y^{62a}) that are more difficult to interpret.

To date, the molecular nature of DNA lesions of most of the analyzed y alleles is unknown, and further work will be necessary to establish the cause of all of the observed chromosomal aberrations. The spontaneous y^2 mutation causes yellow body and wings but wild-type pigmentation of bristles, sex combs, and larval mouth parts. From *in situ* hybridization with *gypsy* sequences to the y^2 chromosome and from suppressibility of the y^2 mutation by the *gypsy*-specific suppressor *su(Hw)*, it has been concluded that the y^2 mutant is associated with a *gypsy* element insertion (17). When DNA Southern blots were hybridized to the nick-translated plasmid *py14b15-A* (coordinates 33.5–49.0) (Fig. 3), *Bam*HI fragments were detected that were 13 kb (23.4–36.5) and 9.4 kb (36.5–45.9) in wild-type DNA but were 16.5 kb and 13 kb in y^2 -DNA. The probe hybridized to a 12.5-kb *Eco*RI fragment in wild-type DNA but to two *Eco*RI fragments of about 10 kb each in y^2 DNA. These results are

FIG. 3. Autoradiograph of a Southern blot of y^2 and wild-type DNA digested with various restriction enzymes after hybridization to *py14b15-A* (coordinates 33.5–49.9). Molecular weight markers are indicated in kb.

consistent with the insertion of a *gypsy* element in the 1.2-kb *Hind*III fragment between coordinates 37.1 kb and 38.3 kb in the y^2 stock. I then isolated the 16.5-kb *Bam*HI fragment that should harbor the *gypsy* element from a *Bam*HI genomic DNA library of y^2 DNA in the λ phage vector EMBL 4. The presence and exact location at coordinate 37.4 kb of the *gypsy* insertion element in the 16.5-kb DNA fragment from the y^2 mutant was demonstrated by cross-hybridization on Southern blots, nuclease S1 mapping (data not shown), and heteroduplex analysis (Fig. 4).

Since y^2 -type alleles have a different phenotype, I expect their position to differ from the y^1 -type null mutations (mapping to coordinates 33.5–36.5). The DNA abnormalities of three alleles (y^{m2v} , y^{td} , and y^{v2}) were not resolved from the position of the y^1 alleles. These y^2 -type alleles were localized to a region that overlaps the y^1 -type alleles but includes a region some 1.7 kb distal. Four alleles (y^2 , y^{3P} , and the distal rearrangements of y^{2S} and y^{3d}) mapped clearly distally to the y^1 -region at coordinates 36.5–38.3. y^{bl} exhibited three detectable breakpoints at coordinates located distally at 40.8–41.5, 53.2–55.0, and 63.3–65.5. y^{62a} showed multiple alterations, all mapping proximally between coordinates 27.9–33.5. The more proximal DNA aberrations in y^{62a} may cause the *sc* phenotype that is associated with this chromosome. No breakpoint could be detected for y^{CTS} and y^{34c} . The only chromosomal breakpoint for y^{bg} maps between coordinates 11.8 and 12.8 kb, some 20 kb proximal to the presumed y gene coding region. Since this observed single breakpoint in y^{bg} is separated from the presumed y coding region by another transcribed region possibly coding for the *ac* transcript (12), it might be due to polymorphism and be unrelated to the y^{bg} phenotype. Alternatively, long range effects of the insertion

FIG. 2. Restriction map of 70 kb of contiguous DNA from the yellow-achaete region isolated from recombinant phage libraries containing wild-type *D. melanogaster* DNA from Oregon R (TG-1 phage) or Canton S (other phages). Below the kilobase scale, the size and designation of the isolated recombinant phages spanning this region are given. The locations on this physical map of DNA alterations of y alleles are indicated by bars above (y^1 -type alleles) or below (y^2 -type alleles) the line containing the restriction map. The lengths of these bars reflect the size of the smallest wild-type DNA fragment to which the abnormality could be mapped by genomic Southern blots using at least two restriction enzymes. The insertion sites of transposable elements from the alleles y^2 (*gypsy*), y^{80h16} (*hobo*), and $y^{80e14(16)}$ (unknown) are depicted as triangles. For easier reference to the text, coordinates of this map are given in kb beginning at the proximal end.

FIG. 4. Heteroduplex between the 16.5-kb *Bam*HI fragment from y^2 DNA cloned into λ phage vector EMBL 4, and the 6.8-kb *Xho*I fragment of the *gypsy* element. The 6.8-kb *Xho*I fragment was cloned into the *Sal*I site of pBR322, and the recombinant plasmid was linearized with *Bam*HI that does not cleave within the *gypsy* fragment. The double-stranded region, indicating the extent of *gypsy* homology, is located between the arrows.

of transposable elements on distant genes, as demonstrated in the case of w^{DZL} (24, 25), are also possible.

Transcription at the Yellow Locus. Melanin synthesis and deposition in the adult cuticle requires a functional *y* gene product and occurs in a precise sequence from anterior to posterior during the second half of pupal development (7, 8). Therefore, the main transcriptional activity of the *y* gene is expected at this developmental stage. To determine the steady-state concentrations of the *y* mRNA transcript in different stages of development, poly(A) RNA was blot-hybridized and probed with the nick-translated 4.5-kb *Eco*RI fragment *py14a3-B* (coordinates 33.5–38.0) to which all of the y^1 -type breakpoints had been mapped (see Fig. 2). This probe hybridized to a single poly(A) mRNA of 2.0 kb (Fig. 5) that very likely represents the *y* gene transcript. Its size appeared not to change during development. The developmental profile of *y* gene expression was consistent with the timing of melanization in development, and as expected, high levels of mRNA could be found in pupae. Low levels of *y* mRNA could be detected in adults and in the larval instars, probably due to new pigmentation of mouth parts at larval molts. The *y* gene apparently is not transcribed in early embryos, but high levels of mRNA are present in late embryos (16–24 hr) just prior to hatching.

DISCUSSION

The molecular analysis of the *y* locus presented here has revealed several interesting features that are relevant for the elucidation of its function and the mechanisms that control the expression of this gene during development. The *y* gene plays an important role in cuticle pigmentation, and it is closely associated with the *achaete-scute* complex that governs the formation and differentiation of micro- and macrochaetes on the cuticle of the adult fly. This physical proximity is clearly established by the molecular mapping of mutant *y* alleles in this report, together with the independent

mapping of *ac*, *sc*, and *y* gene regions as published recently (12). Since all three genes are involved in modifying pattern on the adult cuticle in a position-specific way, it is tempting to speculate that the clustering of these three gene loci reflects some functional coordination between them.

Classification and Mapping of *y* Alleles. Two classes of mutant *y* alleles can be distinguished: y^1 -type alleles are amorphic and exhibit the mutant coloration phenotype throughout the adult cuticle and in the pigmented structures of the larvae. In contrast, y^2 -type alleles exhibit allele-specific pigmentation of the cuticle (resulting from functional expression of the gene in some structures of the cuticle that become partially or fully wild type) and mutant (yellow) expression in other parts of the cuticle. As a working hypothesis, I propose that the y^1 -type alleles are due to mutations in the coding region and y^2 -type alleles are caused by mutations that alter its proper temporal or spatial expression (8). In addition, position effects and variegation due to proximity of the *y* gene to β -heterochromatic regions in some inversions (e.g., *In(1)y^{3P}*) will also affect proper expression of the *y* gene and result in a y^2 -type phenotype (13). As was pointed out by Campuzano *et al.* (12), the position of the *In(1)y^{3P}* breakpoint defines the most distal limit of the *y* coding region. The proximal extension of the *y* gene region may be defined by the presumed *ac* transcription unit some 13 kb away. All of the y^1 -type alleles for which rearrangements were detected and, with the exception of y^{bg} , those y^2 -type alleles for which single sites of rearrangement were detected map to this region.

The chromosomal aberrations of 11 y^1 -type alleles were localized more precisely to a 3.1-kb DNA region between coordinates 33.4 and 36.5 kb. No y^1 -type mutations map outside this region. Therefore, I conclude that this region must contain a major portion of the *y* gene coding region. This is corroborated by the finding that a DNA fragment containing this region hybridizes to a single polyadenylated mRNA species of 2.0 kb, which is transcribed during the pupal stage when the adult cuticle is becoming pigmented.

According to our working hypothesis, y^2 -type mutations affect the regulation of the gene, thus altering its proper temporal and spatial expression. I have not yet mapped the transcription unit and the different mutations with sufficient accuracy to directly test this hypothesis. However, I note that the locations of the y^2 -type rearrangements differ from those of the y^1 -type null alleles. Of the y^2 -type alleles that have simple rearrangements, three of six map distal to the y^1 -type alleles, while two map indistinguishably from the y^1 region, and one maps proximally.

The *gypsy* insertion at position 37.4 in y^2 is located distally to all mappable y^1 -type alleles, and there are strong arguments that the y^2 mutation is due to *gypsy* element insertion. However, there is some ambiguity with regard to the ordering of *y* alleles. Green (26) reported apparent recombination between y^1 and y^2 and between y^1 and y^{59b} . In each case, a single recombinant female was found. From these results it

FIG. 5. Autoradiograph of an RNA blot hybridized with the DNA fragment *py14a3-B* (coordinates 33.5–38.0) subcloned in pBR329. Poly(A)-selected RNA (10 μ g) from different developmental stages was electrophoresed in an agarose-glyoxal gel and transferred to APT-cellulose paper. Exposure time was 48 hr. The molecular weights of the linear DNA fragments used as size markers are indicated in kb.

was concluded that the y^1 -type allele is distal to the y^2 -type allele. However, these mapping experiments were done without using a distal marker to rule out the possibility that reversion of one of the alleles had occurred. Since y^2 is caused by the insertion of a *gypsy* transposable element, reversion of y^2 is not such an infrequent (2×10^{-5}) event (27). Padilla-Nash (28) found no recombination between y^1 and y^2 among 40,588 progeny scored, which may have been an insufficient number to detect crossing-over between these two alleles. Likewise, y^2 and y^{329} (an x-ray-induced y^1 -type allele) were inseparable (689,164 progeny scored), and only one apparent recombinant was found between y^{1d} and y^{329} (230,760 progeny scored). Thus, y^1 and y^2 types of alleles are extremely closely linked. At this point it is not possible to correlate the molecular and the genetic data, because I have not been able to detect a molecular breakpoint in y^1 . However, I do find that the *gypsy* insertion in y^2 is located at least 0.9 kb distal from any mappable y^1 -type allele. Therefore, I suggest that most y^2 -type alleles lie distal to y^1 -type alleles.

The Putative y Gene Transcript. A 2.0-kb transcript is detectable with a subcloned DNA fragment (coordinates 33.5–38.0) that includes the region of all y^1 -type breakpoints. This transcript is almost certainly identical to the 1.9-kb transcript T6, which is absent in the null allele *In(1)y⁴* (12). I have not attempted to define the limits of the transcription unit that code for this 2.0-kb putative y mRNA, but results published by Campuzano *et al.* (12) suggest that it hybridizes to fragments covering a region of about 13 kb. This mRNA is quite abundant in pupae, when melanin is deposited in the adult cuticle. It remains to be demonstrated whether y is expressed in a spatially restricted pattern and how this might be altered by y^2 -type mutations.

The putative y mRNA is present at much lower levels in the larval instars and in adults. While no y transcript is detectable in early embryos, unexpectedly high levels are present in late embryos a few hours prior to hatching. Since larvae repigment their mouth parts at every molt (2), it is unlikely that the extremely high y mRNA levels in late embryos solely reflect melanization of the developing larval mouth parts. Therefore, it is tempting to speculate that besides melanization, the y gene might have an as yet unknown function during embryonic development.

There is some circumstantial evidence that the y gene may also have a neural function. It is well established that males hemizygous for y are at a mating disadvantage when paired with wild-type females because of a reduced level of locomotion (29, 30). Furthermore, no correlation between the effect of y on secondary cuticle structures, like sex combs and genital apparatus, and the abnormal behavior of y^2 -type males was found (31). These studies suggest that the y gene may also have an effect on behavior, possibly through altered levels of neuroactive catecholamines (32) that are synthesized via dopa-like melanin. However, levels of dopamine and other dihydroxylated phenol compounds in the tyrosine pathway analyzed by HPLC are unchanged in total extracts from y^1 flies (Bruce Black, personal communication).

Supporting evidence for a link between melanization and neural development comes from studies on behavior and brain anatomy in pigmentless (albino) mutants of mammals. In several species, albinos exhibit a higher degree of misrouting of optical axons in the chiasm compared to wild type, resulting in a higher number of axons innervating the ipsilateral hemisphere (for example, see refs. 33–35). The degree of this abnormal axonal growth generally correlates with the degree of melanin pigmentation in retinal pigment

epithelium (33), and it was proposed that melanin or its breakdown products are directly associated with nerve outgrowth and routing (36).

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