Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 2021–2025, March 1995
Genetics

Molecular basis of intracistronic complementation in the Passover locus of *Drosophila*

(neurogenetics/mutation/OPUS/synapse formation/connectivity)

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Communicated by Alan Garen, Yale University, New Haven, CT, November 28, 1994 (received for review October 1, 1994)

ABSTRACT **The only demonstrated mechanism for intracistronic genetic complementation requires physical interaction of protein subunits to create a functional molecule. We demonstrate another and perhaps quite general mechanism utilizing proteins with unique and shared domains. The *Drosophila* neural mutant Passover (*Pas*) disrupts specific synaptic connections. Alleles of a lethal complementation group exhibit a complex pattern of complementation with *Pas* alleles. Whereas all heterozygotes between these lethal alleles and *Pas* are viable, only some alleles complement the neural defect of *Pas*. Lethal and neural functions are separately encoded by two proteins that have distinct N-terminal domains and a common C-terminal portion. Neural-specific and lethal-specific mutations map to unique exons, while neural-lethal mutations map to shared exons. Combinations of lethal and neural alleles result in production of both proteins and demonstrate intracistronic complementation.**

Two recessive mutations are said to complement each other if wild-type function is restored in the heterozygote. If the mutations are in two different functional units, called cistrons (1), the mutations will complement each other because a wild-type copy of each cistron is present in the heterozygote. However, if the two mutations are in the same cistron, they will not usually be able to complement each other. Sometimes, however, particular pairs of mutations in the same cistron can complement each other. This is called intracistronic complementation. This paper is concerned with the mechanism of such intracistronic complementation.

A mechanism for this phenomenon was elucidated through the work of Garen and Garen (2) and Schlesinger and Levinthal (3). They studied alkaline phosphatase, an enzyme that is composed of two identical subunits (4). In studies of the *phoA* cistron of *Escherichia coli*, they showed that complementation could occur, *in vivo* and *in vitro*, when heterodimers formed between two different mutation polypeptide chains. In specific pairs of polypeptides, the heterodimer functioned at wild-type levels even though homodimers of each polypeptide were individually mutant. The mechanism for this is that different mutant subunits of a homomultimer compensate for each other and thereby give rise to a functional complex (5, 6). Subsequently, all examples of intracistronic complementation have been shown to involve such a mechanism. While the mutations studied in alkaline phosphatase were missense mutations, deletion mutations are also capable of such complementation, as in the case of the enzyme β-galactosidase (7).

In this report, we present evidence for a different mechanism of intracistronic complementation in the Passover (*Pas*) locus of *Drosophila melanogaster*. *Pas* disrupts specific synaptic connections in the neural circuit underlying the escape response of *Drosophila* (8). A light-off visual startle stimulus (9) or a shock to the brain (10) initiates the escape response. This reflex is mediated by the giant fiber system (GFS; see Fig. 1*A*), eight neurons that relay excitation from the eyes to the muscles of the thorax (10). The GF axons pass from the brain to the mesothorax, where they synapse with the peripherally synapsing interneuron (PSI) and the motoneuron of the jump muscle (TTM; tergotrochanteral motoneuron). The PSI synapses with the five motoneurons of the wing depressor muscles (DLMs; dorsal longitudinal motoneurons). A single shock activation of the GF elicits a single spike in the TTM and spikes in each of the DLM fibers, resulting in a jump and initial activation of the wings.

Pas flies fail to jump in response to a light-off stimulus. All the neurons are present but brain stimulation elicits no response from the DLMs and only a delayed and intermittent response from the TTM. The defect does not lie in the motor axons, neuromuscular junctions, or muscles; in *Pas* flies the muscles respond normally to direct stimulation of the motoneurons. Therefore, the abnormalities are in the synapses between the GF and the motoneurons it activates.

Molecular cloning of the *Pas* locus (11) showed that *Pas* is expressed specifically in the GFs and its postsynaptic targets in pupae and adults. The protein product is similar to the products of the *Drosophila l(1)* optic ganglion reduced (*ogre*) gene (12) and the *Caenorhabditis elegans unc-7* gene (13). Mutations of *unc-7* cause kinking during locomotion. Reconstruction of electron microscope serial sections shows that the connectivity of a premotor interneuron is altered. This is remarkably similar to *Pas*, where the connectivity of the premotor GF interneuron is disrupted. *ogre* is necessary for the development of postembryonic neuroblasts, but the mechanism of its action is not yet known. Thus, it appears that *Pas* is a member of a family of genes involved in neural development. Several other *C. elegans* cDNAs have sequence similarity and the name OPUS (ogre-passover-unc-shaking B) has been given to the gene family (14).

Two other mutations (*shakB*^{2} and *njP181*) are known that fail to complement *Pas*^{1} and whose phenotypes show different intensities of the same disruption of the GFS. All allele combinations are viable and have similar phenotypes. These three will henceforth be called neural-only alleles. *Pas*^{1} and *shakB*^{2} were mapped cytologically to polytene band 19E3 near the base of the X chromosome (15) and *njP181* was shown molecularly to be in the same location (11).

The 19E3 band is also the location of the *R-9-29* complementation group, with nine known alleles. Homozygotes of these alleles are all lethal, and all allele combinations are also lethal. The lethal period is late embryonic or early larval. The *R-9-29* alleles, however, exhibited a complex pattern of complementation with the neural-only alleles (15). Heterozygotes combining any neural-only allele with any of the lethal *R-9-29* alleles are all viable. Most of these heterozygotes

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Abbreviations: GF, giant fiber; GFS, GF system; TTM, tergotrochanteral motoneuron; DLM, dorsal longitudinal motoneuron.

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exhibit the same neuronal defects as the neural-only homozygotes. Two *R-9-29* alleles, however, complement these phenotypes and the heterozygotes are viable wild-type individuals. These two alleles are called lethal-only alleles. The remaining *R-9-29* alleles, which complement neither the neural-only nor the lethal-only alleles, are termed neural-lethal alleles.

In this report, we show that the *Pas* locus encodes two proteins, one necessary for the GFS (pas^N; the neural protein) and one necessary for viability (pas^V; the vital protein).† The two proteins are coded by similar but distinct 5′ exons joined to a common set of 3′ exons. Neural-only mutations map to pas^N-specific exons and affect only one of the proteins. Lethal-only mutations map to a pas^V-specific exon and inactivate the other protein. Finally, neural-lethal mutations were mapped to the common set of 3′ exons and therefore inactivate both proteins. Thus, heterozygotes of lethal-only and neural-only alleles can result in production of both proteins and thereby manifest intracistronic complementation.

MATERIALS AND METHODS

***Drosophila* Stocks.** Descriptions of the deficiency-bearing chromosomes and the mutant alleles of the *Pas* locus can be found in Baird *et al.* (15) and Schalet and Lefevre (16).

Electrical Stimulation of the Neurons of the GFS and Recording of Muscle Potentials. The motor outputs of the GFS were recorded by using a modified version of the methods of Tanouye and Wyman (10).

PCR. All PCR mixtures contained 50 mM KCl, 10 mM Tris·HCl (pH 8.3), 1.0–2.5 mM $MgCl_2$, gelatin at 0.01 mg/ml, each deoxynucleoside triphosphate at 200 mM, each primer at 250 nM, and 0.5 unit of *Taq* DNA polymerase. Four microliters of genomic DNA from wild-type and mutant strains was used as a template. Exon-specific primers were derived from the intron sequences around the splice junction sequence. Amplification was performed with a Perkin–Elmer DNA thermal cycler as follows: 94°C for 2 min; 30 cycles of 94°C for 10 s, 55°C for 1 min, and 72°C for 2 min; and 72°C for 5 min.

Cloning and Sequencing of PCR Products. Amplified products were size fractionated by electrophoresis and subcloned into vector pPCR II from Invitrogen as supplied in the TA cloning kit. Computer programs used to analyze sequences have been described (11).

RESULTS

Genetic Mapping of the Mutations. A variety of mutations and deficiencies are known to affect the *Pas* locus. The known neural-only mutations of the locus are Pas^1, $shakB^2$, *njP181*; the lethal-only mutations are *L41* and *EF535*; the neural-lethal mutations are *R-9-29, HM437, E81, EC201, LB21, 17-189*, and *17-360* (15, 16).

Several deficiencies were used for the initial genetic and cytological mapping of the *Pas* alleles. *Df(1)16-3-35* eliminates *Pas* and several more distal genes but does not eliminate any more proximal genes. All *Pas* alleles are uncovered by *Df(1)16-3-35*. Molecular analysis shows that the proximal breakpoint of *Df(1)16-3-35* is in *Pas* near its 5′ end (11).

Df(1)A118 and *Df(1)HC279* are both entirely proximal to *Df(1)16-3-35*. The former pair of deficiencies uncover the neural-only alleles but complement the lethality of both the lethal-only and neural-lethal alleles. This ordering places the neural-only mutations proximal to the lethal-only and neural-lethal mutations. In accordance with this hypothesis, crosses were made that facilitated the detection of recombinants between neural-only and lethal-only or neural-lethal alleles. A chromosome was constructed bearing Pas^1 and two closely linked flanking markers, a distal viable mutation, *mel*, and a proximal lethal mutation, $flil^{10}$. (The distance between *Pas* and $flil^{10}$ is ≈0.4 map unit.) In females heterozygous for this chromosome and a lethal-only or neural-lethal allele, the hypothesis suggests the following order:

$$\text{distal}\ \frac{mel \quad + \quad \text{neural} \quad flil^{10}}{+ \quad \text{lethal} \quad + \quad +}\ \text{proximal.}$$

If this female were crossed to a male marked with *Bar*, then only crossovers between the lethal and $flil^{10}$ would yield viable males marked with *mel*, some of which might be wild type for both functions of the *Pas* locus. If the order were the opposite of that which we expected,

$$\text{distal}\ \frac{mel \ \text{neural} \quad + \quad flil^{10}}{+ \quad + \quad \text{lethal} \quad +}\ \text{proximal,}$$

then all viable males marked with *mel* would bear the neural mutation.

As hypothesized, viable males, wild-type for *Pas*, were indeed recovered. Lines were established from each crossover male and tested electrophysiologically for *Pas* function. From a cross between *R-9-29* and *Pas*, there were 47 viable male crossovers (among 31,700 females) in the interval between *R-9-29* and $flil^{10}$; one of these proved to be Pas^+. From a cross between *L41* and *Pas* there were 38 viable male crossovers (among 18,400 females) in the interval between *L41* and $flil^{10}$; two of these proved to be Pas^+. These results suggest that *L41* and *R-9-29* are distal to *Pas*. Sequencing of these mutations (as reported below) confirms and extends this map order.

Electrophysiological Analysis of Complex Complementation. In wild-type flies, brain shocks elicit spikes in the jump and wing depressor muscles at very short latencies, even at very high frequencies of stimulation. The TTMs respond with a latency of only 0.9 ms and the DLMs respond with the slightly longer latency of 1.3 ms. Each stimulus elicits a response unless the stimuli are delivered with a frequency > 100 Hz. Fig. 1*B* shows the response of wild-type (Canton S) flies. Ten stimuli are delivered at a rate of 5 per s. Four muscles are recorded from—the right and left DLMs and the right and left TTMs. Each muscle responds to each shock at the expected latency. By contrast, in $shakB^2$ flies the DLMs do not respond at all to brain shocks and the TTMs respond only occasionally and then with a longer than normal latency. In Fig. 1*C* the DLMs responded to 0 of 10 stimuli. The right TTM responded to 2 of 10 and the left TTM responded to 3 of 10. The latency of responses was 1.7 ms instead of the wild-type value of 0.9 ms.

The neural-lethal allele *R-9-29* fails to complement this phenotype. Fig. 1*D* shows the electrophysiological response of the heterozygote between $shakB^2$ and *R-9-29*. The DLMs do not respond at all; neither does the right TTM, while the left TTM responds to 4 of 10 shocks. When the TTM does respond, its latency is abnormally long at 1.7 ms.

Fig. 1 *E* and *F* shows the responses in flies heterozygous for $shakB^2$ and either *EF535* (Fig. 1*E*) or *L41* (Fig. 1*F*). The responses are normal. All four muscles respond at the wild-type latencies with no failures. In both heterozygotes, the muscles respond to each shock up to stimulus frequencies of 100 Hz (data not shown). The two lethal alleles *EF535* and *L41* complement the electrophysiological phenotype of $shakB^2$.

These results are quite general. As well as *R-9-29*, the lethal alleles *17-189, 17-360, E81, EC201*, and *HM437* do not complement Pas^1 or $shakB^2$ (15). *EF535* and *L41* complement Pas^1 and *njP181* as well as $shakB^2$ (15).

The *Pas* Locus Encodes Two Transcripts. cDNAs mutated by the neural-only alleles have previously been isolated and sequenced (11). The transcript spans 30 kb and is composed of six coding exons and at least two noncoding exons. This sequence is denoted as pas^N. We have now found cDNA clones

†The sequence reported in this paper has been deposited in the GenBank data base (accession no. U17330).

that initiate within the third intron, which is 15 kb long. The 5′ end of the cDNAs is composed of a single exon that is encoded within the *pas*^{N} intron.

The clones then continue with a sequence that is identical to that of the 3′ *pas*^{N} exons. Hybridization back to the genomic sequences indicates two separate 5′ sequences and a single 3′ sequence (Fig. 2). Thus, there are two alternative transcripts that have distinct promoters and 5′ ends but share a common 3′ end (Fig. 3). We denote the second sequence as *pas*^{V}.

The sequence of the pas^V-specific 5′ exon is highly similar to the first two coding exons of the pas^N transcript (Fig. 4); 69% of the amino acids coded for by these sequences are identical. Another 11% of the amino acids represent conservative substitutions. Thus, there has apparently been a duplication of the 5′ sequences with some divergence in the sequence and the introduction (or loss) of two introns in one of the sequences. The pas^V and pas^N sequences are also highly similar to the corresponding region of the ogre protein. pas^V and ogre are 50% identical and 64% similar in this region. pas^N and ogre are 49% identical and 61% similar. Both pas^V and ogre extend somewhat further in the N-terminal direction than pas^N; the 5′ exon encodes 120 aa in pas^V, whereas the corresponding sizes in ogre and pas^N are 119 and 109 aa, respectively.

Furthermore, all three of these *Drosophila* proteins are similar (Fig. 5) to the product of the *C. elegans* gene *unc-7*. These proteins and a variety of other sequence-similar but

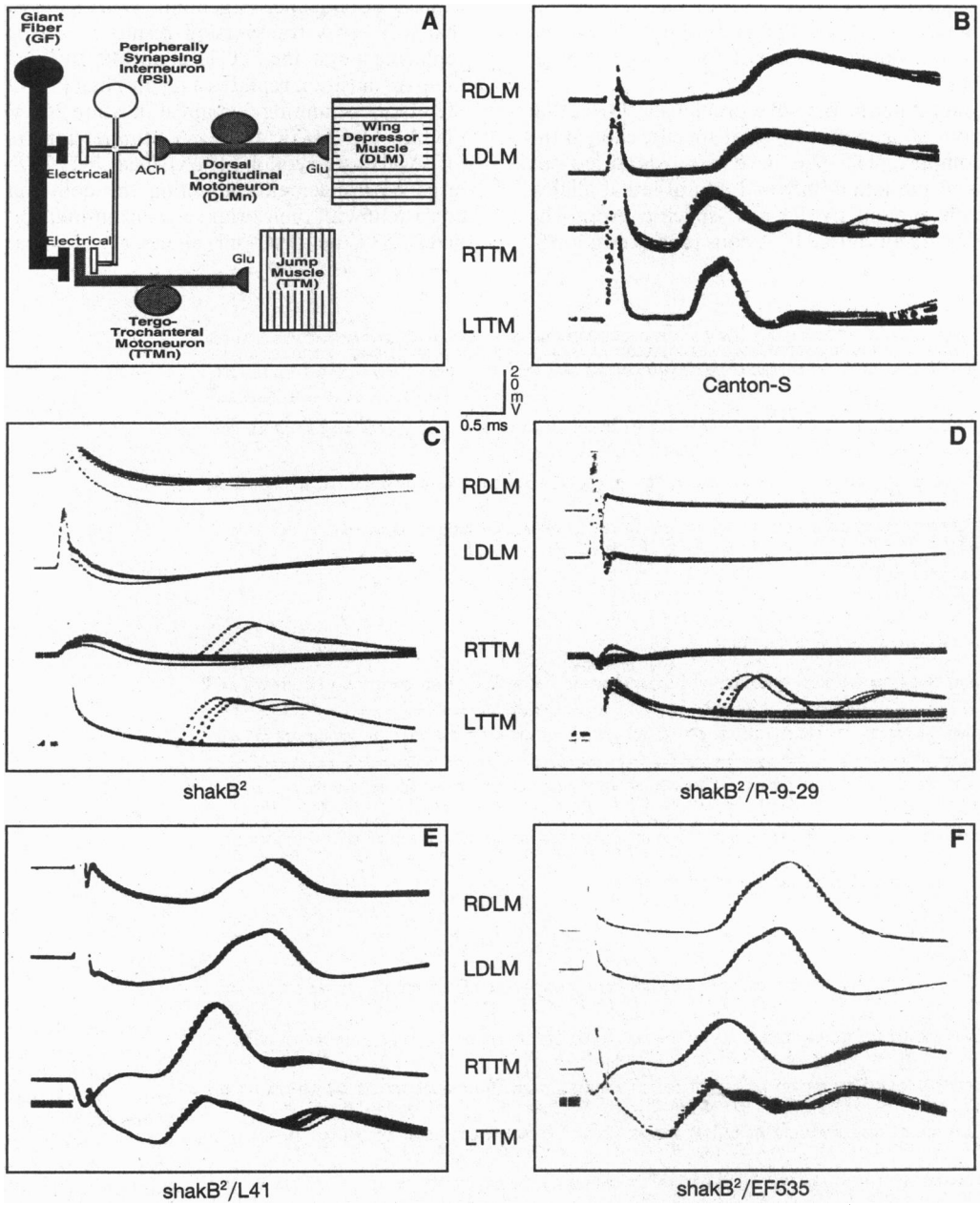

FIG. 1. GFS: connections, electrophysiology, and complementation test. (*A*) Schematic representation of the GFS. (*B–E*) Electrophysiological phenotypes. (*B*) Wild type (Canton S). All four muscles responded to each of the 10 shocks. Wild types can respond to stimuli at rates up to 100 per s without failures. (*C*) *shakB*^{2}. Neither DLM responded to any of the shocks. In traces where they fail to respond, the baseline is flat. The right (R) TTM responded to 2 of 10 shocks, the left (L) TTM responded to 3 of 10 shocks; the latencies of the responses, however, were quite long. (*D–F*) Complementation tests. (*D*) *shakB*^{2}*/R-9-29. R-9-29* fails to complement. As in the homozygote *shakB*^{2}, the DLMs do not respond at all and the TTMs respond weakly: 0 of 10 for the right TTM and 4 of 10 for the left TTM. The response latencies are longer than that of wild type. (*E* and *F*) *shakB*^{2}*/L41* and *shakB*^{2}*/EF535. L41* and *EF535* complement the phenotype. As for Canton S, all four muscles responded to each of the 10 shocks; the latencies are normal.

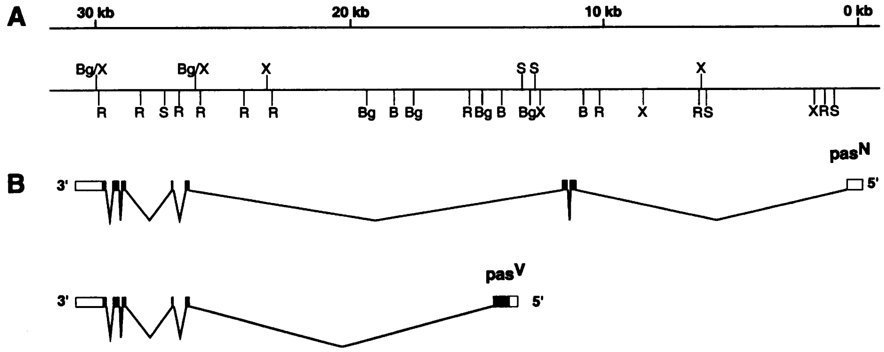

FIG. 2. Physical organization of the *Pas* locus. (*A*) Map of genomic DNA with restriction sites shown. Scale shows distances in kb; 0 kb indicates insertion site of the P element of *njP181.* Centromere on the DNA map is to the right; telomere is to the left. B, *Bam*HI; Bg, *Bgl* II; R, *Eco*RI; S, *Sal* I; X, *Xba* I. (*B*) Organization of the pas^V and pas^N transcription units. Exons are represented by boxes below the physical map. Open boxes indicate noncoding sequences. Solid boxes indicate coding sequences.

otherwise uncharacterized cDNAs from *C. elegans* share a pentapeptide sequence, YYQWV, that is absolutely conserved in all members of the family but is not found in any other known sequence (14).

Lesions in Mutant Alleles. We have previously shown that the neural mutations all map in the neural-specific exon at the 5′ end of the complex (11). We have now identified the molecular lesions in the lethal-only and neural-lethal alleles. The lethal-only alleles map to the pas^V-specific exon. The neural-lethal alleles are all caused by lesions in the common 3′ exons. The sequence of genomic DNA from *E81* flies revealed that a T → A transversion results in a stop codon (TGA) replacing a cysteine (TGT) at aa 140. In *R-9-29* and *EC201*, a G → A transition replaces a tryptophan (TGG) in the putative membrane-spanning domain at position 262 with a stop codon (TGA). In *HM437*, a T → A transversion converts a leucine (TTA) to a stop codon (TAA) at aa 299. *17-189* and *17-360* are large rearrangements affecting the common exons. This is consistent with their origin as neuron-induced mutations. *L41* and *EF535*, the lethal-only alleles, are identical 17-bp deletions

A

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         njP181
1    ACAGGCAACTCGTGAAAAGGTGGCCTTAGCTTGTGCGACTGAATAACCAAACCAAATCAATTCATCGATTAATGATAACCGATAAGCAATAAGCTGATTAACGAATCAGCGACTAATCGA
121  CTTAAGAGCGATATCCCGAAATCGAAATCACGAACGATCTCAACGCAAGAGCCGGCGGCGGCGATAACAAATATCGATAGCCGATAGACTGGCTCTGAACCACTGCTGGCGGTGTGTGTG
241  TGTGTGCCGCGTGTGTGTGCGGTGCCGCCCACGCCCATCCATCCCCACCCCCGCCAAAAGCAAAGTGATAGAAA ATG GTG TCT CAT GTC AAA ATC GAT TCA CCT GTT T
1                                                                                M   V   S   H   V   K   I   D   S   P   V   F

                                                   shakB2                                      Pas1
350  CGC CTG CAC ACC AAC GCA ACG GTT ATA TTA CTG ATC ACA TTC TCG ATT GCT GTG ACG ACG CGG CAG TAC GTA GGA AAT CCC ATA GAC TGT
13   R   L   H   T   N   A   T   V   I   L   L   I   T   F   S   I   A   V   T   T   R   Q   Y   V   G   N   P   I   D   C

440  GTA CAT ACA AG A GAC ATT CCC GAA GAT GTT CTC AAC ACA TAT TGT TGG ATA CAC TCA ACG TAT ACA GTG GTC GAT GCG TTT ATG AAG AAG
43   V   H   T   R    D   I   P   E   D   V   L   N   T   Y   C   W   I   H   S   T   Y   T   V   V   D   A   F   M   K   K

530  CAA GGT TCC GAG GTG CCT TTT CCC GGA GTT CAC AAT TCA CAG GGC CGT GGG CCT CTT ACA ATA AAG CAC ACA AAA TAT TAT CAA TGG GTA
73   Q   G   S   E   V   P   F   P   G   V   H   N   S   Q   G   R   G   P   L   T   I   K   H   T   K   Y   Y   Q   W   V

620  GCG TTT ACA CTA TTT TTT CAG
103  A   F   T   L   F   F   Q
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B

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                                                                   L41, EF535
1    GATAAAAGCAAGAAGGGAGGCTAGAGAGAATCATCGCCGATCTTGCCAGCTTTCGCTGACGACAAACC ATG TTA GAT ATA TTT CGT GGA TTG AAA AAC CTT GTA AAG
1                                                                         M   L   D   I   F   R   G   L   K   N   L   V   K

108  GTC AGT CAC GTT AAA ACA GAT TCG ATA GTA TTC CGC CTG CAT TAC TCA ATA ACT GTG ATG ATT CTG ATG TCA TTC TCG CTA ATT ATA ACC
14   V   S   H   V   K   T   D   S   I   V   F   R   L   H   Y   S   I   T   V   M   I   L   M   S   F   S   L   I   I   T

198  ACG CGC CAG TAC GTT GGC AAT CCG ATC GAT TGT GTG CAC ACC AAA GAT ATT CCA GAG GAT GTG CTC AAC ACT TAC TGC TGG ATC CAG TCC
44   T   R   Q   Y   V   G   N   P   I   D   C   V   H   T   K   D   I   P   E   D   V   L   N   T   Y   C   W   I   Q   S

288  ACC TAC ACG CTC AAG AGC CTC TTC CTG AAG AAG CAG GGC GTG TCG GTT CCA TAT CCG GGC ATC GGC AAC TCC GAC GGT GAT CCG GCG GAC
74   T   Y   T   L   K   S   L   F   L   K   K   Q   G   V   S   V   P   Y   P   G   I   G   N   S   D   G   D   P   A   D

378  AAG AAG CAC TAC AAG TAC TAC CAG TGG GTC TGC TTC TGC CTC TTC TTT CAG
104  K   K   H   Y   K   Y   Y   Q   W   V   C   F   C   L   F   F   Q
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C

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641                      GCA ATC TTA TTT TAT ACA CCA AGA TGG CTG TGG AAA TCT TGG GAG GGT GGC AAG ATT CAT GCG CTC ATC
110                      A   I   L   ·F  Y   T   P   R   W   L   W   K   S   W   E   G   G   K   I   H   A   L   I
                                 E81
710  ATG GAC TTA GAC ATA GGC ATT TGT TCC GAA GCC GAG AAA AAA CAA AAA AAG AAA TTA CTT TTA GAT TAT TTG TGG GAA AAT TTA AG A TAT
133  M   D   L   D   I   G   I   C   S   E   A   E   K   K   Q   K   K   K   L   L   L   D   Y   L   W   E   N   L   R    Y

800  CAC AAT TGG TGG GCG TAC AGA TAT TAC GTG TGT GAG CTG CTC GCC CTT ATA AAT GTG ATA G GT CAA ATG TTT CTT ATG AAT CGA TTT TTC
163  H   N   W   W   A   Y   R   Y   Y   V   C   E   L   L   A   L   I   N   V   I   G    Q   M   F   L   M   N   R   F   F

890  GAT GGC GAA TTT ATA ACA TTT GGC CTG AAA GTG ATA GAT TAT ATG GAG ACC GAT CAG GAA GAT CGC ATG GAT CCG ATG ATT TAC ATA TTT
193  D   G   E   F   I   T   F   G   L   K   V   I   D   Y   M   E   T   D   Q   E   D   R   M   D   P   M   I   Y   I   F

980  CCC AGA ATG ACC AAA TGT ACA TTT TTT AAA TAT GGT TCC GGG GAG GTG GTG GAG AAA CAC GAC GCC ATT TGC ATT TTA CCA TTA AAC GTT
223  P   R   M   T   K   C   T   F   F   K   Y   G   S   S   G   E   V   E   K   H   D   A   I   C   I   L   P   L   N   V
                                           EC201, R929
1070 GTT AAT GAG AAG ATT TAC ATT TTC CTT TGG TTT TGG TTT ATA TTA TTA ACG TTT CTC ACA TTA TTA ACG CTA ATA TAC AGG GTG GTT ATT
253  V   N   E   K   I   Y   I   F   L   W   F   W   F   I   L   L   T   F   L   T   L   L   T   L   I   Y   R   V   V   I
                                                                HM437
1160 ATA TTC TCT CCT CGA ATG AGG GTC TAC TTA TTT CGT ATG CGA TTT AG G TTA GTG CGT CGT GAC GCT ATT GAA ATA ATC GTT CGT CGT TCA
283  I   F   S   P   R   M   R   V   Y   L   F   R   M   R   F   R    L   V   R   R   D   A   I   E   I   I   V   R   R   S

1250 AAG ATG GGC GAT TGG TTT TTG TTG TAT TTA CTA GGT GAA AAC ATA GAT ACA GTT ATA TTT CGT GAT GTT GTA CAG GAC TTA GCG AAT CGT
313  K   M   G   D   W   F   L   L   Y   L   L   G   E   N   I   D   T   V   I   F   R   D   V   V   Q   D   L   A   N   R

1340 TTA GGA CAT AAC CAA CAC CAC AGG GTG CCT GGC TTA AAA GGT GAA ATA CAG GAT GCA TGA TATTGGGAGTATTAGAAACAATACAAAATGCAATTTGTC
343  L   G   H   N   Q   H   H   R   V   P   G   L   K   G   E   I   Q   D   A
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FIG. 3. Nucleotide and deduced amino acid sequences of pas^V and pas^N. (*A*) pas^N-specific exon. (*B*) pas^V-specific exon. (*C*) Common 3′ exons. Arrows above the sequence indicate lesions in the allele noted (see text for details). Positions of introns are indicated by arrowheads. Stop codon is indicated by a period. Possible signal sequences (*A* and *B*) and transmembrane domain (*C*) are shown by a double underline. Nucleotide sequence and numbering are shown in roman type; amino acid sequence and numbering are italicized.

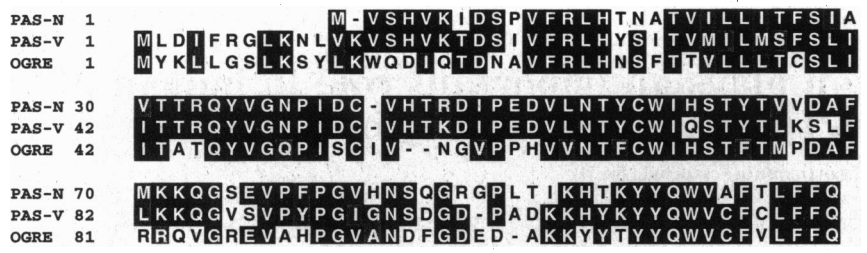

FIG. 4. Amino acid sequence of pas^V compared to pas^N and ogre. The ogre sequence is described by Watanabe and Kankel (12). Identities are highlighted with a black background and conservative substitutions are indicated with a shaded background.

at the start of the pas^V sequence that remove the initiation site and the first two codons in their entirety. Given that *L41* was x-ray induced and *EF535* was ethyl methanesulfonate induced, it is surprising that both stocks exhibit the same lesion; a mislabeling may have occurred. Lefevre and Watkins (17) report many changes between an early and a later characterization of the large set of X-chromosome mutants from which these were obtained.

DISCUSSION

Our data show that the *Pas* locus encodes two polypeptides, pas^V and pas^N. These proteins are encoded by transcripts whose 5′ exons are unique but are spliced onto common 3′ exons. The unique exons confer distinct N-terminal domains on each protein. Viable neural-only mutations map to the exons unique to pas^N. They inactivate the pas^N protein, while pas^V remains intact, resulting in a viable fly with the electrophysiological phenotype. Lethal-only mutations map to the exons unique to pas^V. They inactivate pas^V but not pas^N. These flies die as embryos. If such flies could be made to survive, as in a mosaic or in a temperature-sensitive variant, we expect that they would be wild type for GFS function even in those cells that do not produce pas^V. Neural-lethal mutations map to the common 3′ exons. They inactivate both pas^V and pas^N.

Our model for intracistronic complementation (Fig. 5) provides that in a neural-only/neural-lethal heterozygote, the neural-only chromosome codes for pas^V but not pas^N. The neural-lethal chromosome codes for neither. This heterozygote thus has a viable neural-only phenotype. By contrast, in a neural-only/lethal-only heterozygote, the lethal-only chromosome codes for pas^N and the neural-only chromosome codes for pas^V. While neither protein may be produced in wild-type amounts, the amount produced is sufficient to result in a wild-type fly. Therefore, intracistronic complementation could occur without a multimerization step.

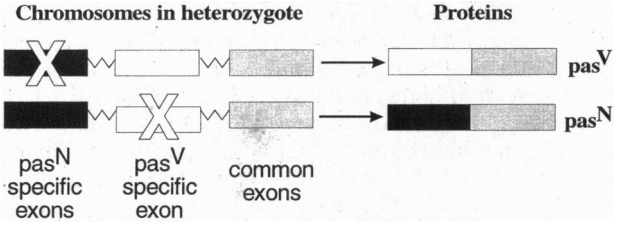

FIG. 5. Model for intracistronic complementation. A chromosome bearing a neural-only mutation can make only the pas^V protein. Similarly, a chromosome bearing a lethal-only mutation can make only the pas^N protein. A heterozygote carrying both these chromosomes can make both proteins and is phenotypically wild type.

We believe this mechanism for intracistronic complementation might be common. Molecular analysis shows that many loci encode two transcripts with unique 5′ exons spliced onto common 3′ exons. Recent examples in *Drosophila* include the Punch locus (18) and the pointed locus (19). In other cases, the 5′ exons are shared but spliced onto different 3′ exons (20). While mutations were not always mapped onto these exons in these papers, nor were the complementation tests performed, it is likely that a situation similar to that of *Pas* will be found. Indeed, it should be possible to demonstrate complex complementation in any multiply spliced gene. It is likely that the mechanism of intracistronic complementation discussed in this paper will be more common than the classical mechanism.

We thank Drs. Douglas Dimlich and Haig Keshishian for helpful discussion and for help in the preparation of the manuscript. E.F. thanks Dr. Walter Schaffner for generous support, the Stiftung Prof. Dr. Max Cloëtta for a fellowship, and Swiss National Science Foundation Grant 31-37768-93. This work was supported by National Institutes of Health Grant NS-07314 and (U.S.) National Science Foundation Grant IBN-9213387 to R.J.W.

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