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Mutations in the *DHR39* orphan receptor gene have no effect on viability.

The *DHR39* gene (also known as *FTZ-F1ß*) encodes an orphan member of the nuclear receptor superfamily (Ayer *et al.*, 1993; Ohno and Petkovich, 1992). A 3.5 kb *DHR39* mRNA is present in early embryos as a maternal component while a 5 kb mRNA is expressed at later stages of development. The 5 kb mRNA is significantly induced in late third instar larvae and prepupae as a direct response to the steroid hormone ecdysone (Horner *et al.*, 1995). DHR39 is highly related to the *Drosophila* FTZ-F1 orphan receptor, with 63% identity in the DNA binding domain. Consistent with this sequence similarity, both proteins can bind to the same regulatory sequences in the *ftz* zebra element and the *Adh* adult distal enhancer (Ayer *et al.*, 1993; Ohno and Petkovich, 1992; Ohno *et al.*, 1994).

The ecdysone-induced expression of *DHR39* during the onset of metamorphosis suggested that this gene may perform a critical function during this stage in development. To test this hypothesis, we set out to identify mutations in *DHR39*. Two lethal P element insertion stocks that mapped to the 39B4 region were obtained from the Berkeley Drosophila Genome Project, designated *P[11226]* and *P[13215]* (Spradling *et al.*, 1995). Inverse PCR was used to amplify genomic DNA flanking each P element insertion and these fragments were used as probes for Southern blot

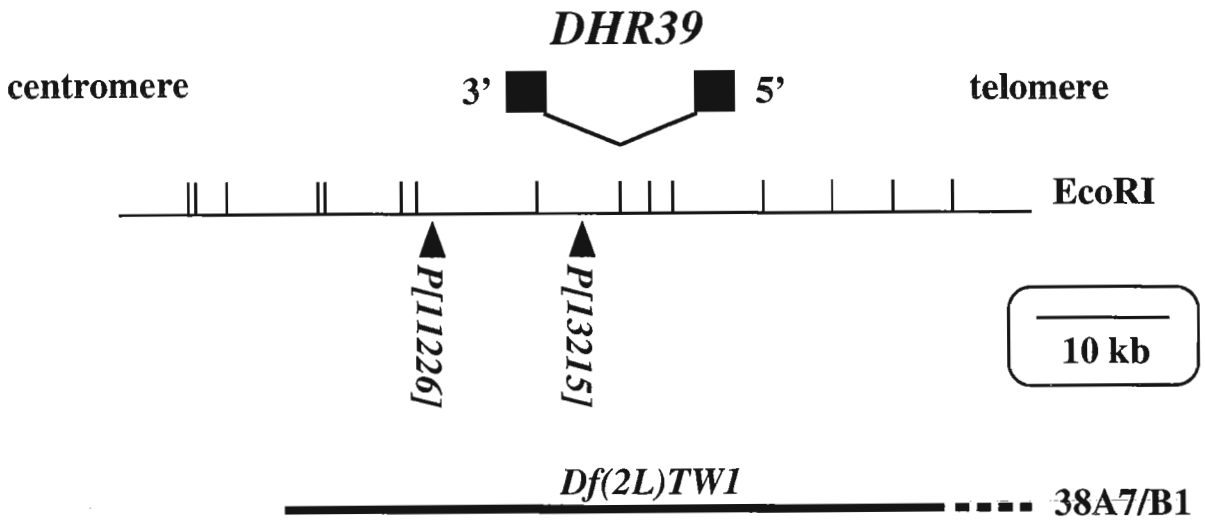

Figure 1. A map of the *DHR39* locus. The *DHR39* gene is depicted at the top with the large first intron represented by a line (intron A in Ohno and Petkovich, 1992). The two P element insertions discussed in the text are marked below an EcoRI restriction map of genomic DNA. Shown at the bottom is a deficiency that removes the *DHR39* locus. One endpoint of *Df(2L)TW1* appears to lie approximately 10-20 kb downstream from *DHR39* by genetic criteria.

hybridization to fragments of genomic DNA surrounding the *DHR39* region. In this manner, *P[11226]* was localized downstream from the 3' end of *DHR39* while *P[13215]* was mapped to the first intron of the *DHR39* gene (Figure 1). *P[13215]* thus lies upstream from the second exon, which contains the beginning of the *DHR39* protein coding region (Ohno and Petkovich, 1992). Curiously, however, only the lethality associated with *P[11226]* failed to complement the *TW1* deficiency that removes the *DHR39* locus (Figure 1). The lethality associated with *P[13215]* mapped outside of the region defined by this deficiency. The lethal mutation associated with the *P[13215]* chromosome was easily dissociated from the P element insertion by recombination. Henceforth, we will use the name *P[13215]* to refer to the stock from which the lethal mutation was removed by recombination.

Because *P[13215]* mapped within the *DHR39* gene, it seemed likely that it would effect *DHR39* transcription. To test this possibility, we isolated RNA from two control stocks, Canton S and w^{1118}, as well as *P[13215]* homozygotes. Equal amounts of RNA were analyzed by northern blot hybridization using three different radioactive probes derived from either the 5' or 3' ends of *DHR39*, or from the *white* gene that is carried by the *P[13215]* insertion. As expected, the 5 kb *DHR39* mRNA can be detected using both the 5' and 3' *DHR39* probes, in both Canton S and w^{1118} animals (Figure 2). In contrast, almost undetectable levels of *DHR39* mRNA are present in *P[13215]* homozygotes. A truncated *DHR39* mRNA could, however, be detected in these animals which showed strong cross-hybridization to the *white* probe.

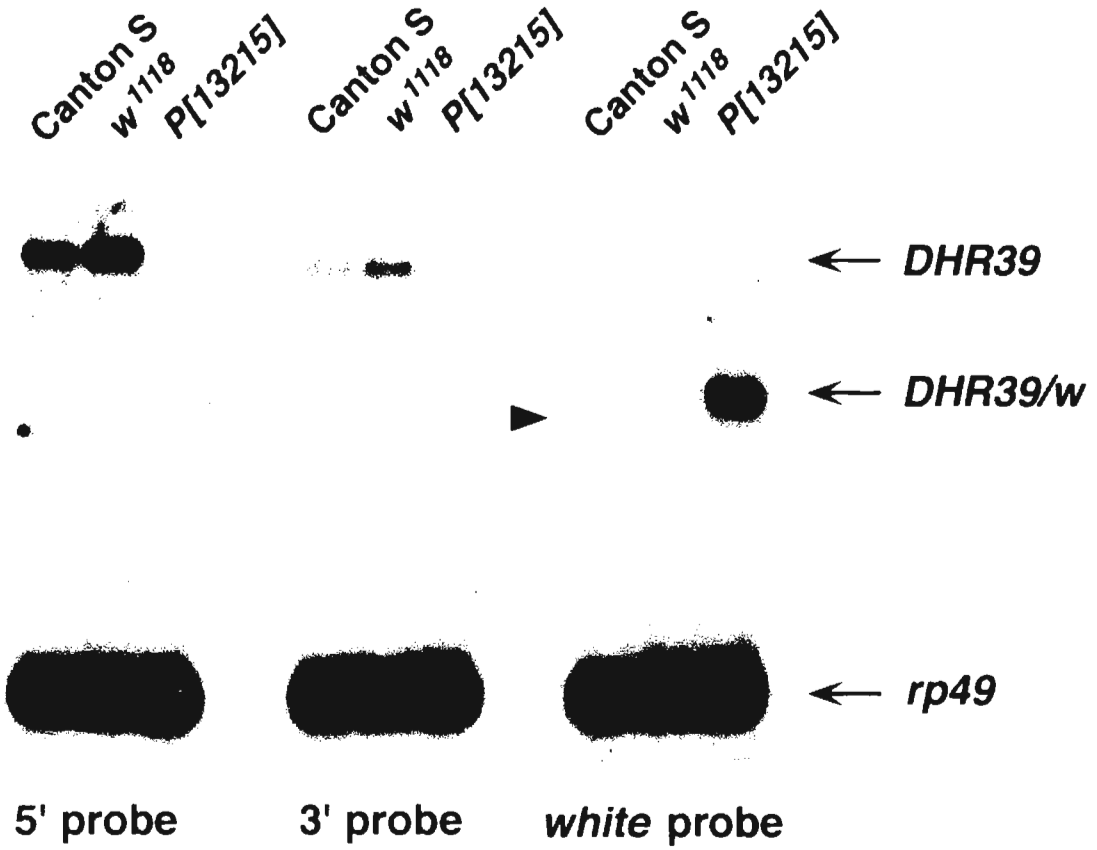

Figure 2. Northern blot hybridization analysis of RNA isolated from control and *DHR39* mutant prepupae. RNA was isolated from newly-formed prepupae of two control strains, either Canton S or w^{1118}, and *P[13215]* homozygotes. Equal amounts of RNA were loaded on a gel, fractionated by formaldehyde agarose gel electrophoresis, and hybridized with one of three radioactive probes. The 5' probe was derived from an EcoRI fragment that spanned the 5' end of the c10 cDNA clone (Ayer *et al.*, 1993). The 3' probe was derived from a HindIII fragment that spanned the 3' end of c10, and the *white* probe was derived from a SalI fragment isolated from pCaSpeR. The *DHR39* transcript and *DHR39/white* fusion mRNA are marked by arrows, as is *rp49* that was used as an internal control for loading and transfer. The arrowhead marks the wild-type *white* mRNA in Canton S animals.

This transcript migrates slightly slower than the *white* mRNA, which is expressed in Canton S but not w^{1118}, as detected with the *white* probe (arrowhead in Figure 2). These observations are consistent with a *DHR39/white* fusion transcript that contains a short length of the *DHR39* 5' untranslated region joined to the *white* coding region. Densitometric scans of the full-length *DHR39* mRNA expressed in *P[13215]* homozygotes revealed that this level is approximately 0.1% of wild-type levels. Similar results were obtained by northern blot hybridization using RNA samples isolated from *P[13215]* over a deficiency, or from 0-3 hour *P[13215]* homozygous mutant embryos (data not shown). This mutation thus appears to severely reduce both maternal and zygotic *DHR39* activity.

Surprisingly, the *P[13215]* insertion could be easily maintained as a homozygous viable stock. This suggests that there is no essential function for either the maternal or zygotic functions of *DHR39*. Furthermore, when both parents carried *P[13215]* over a balancer, the *P[13215]* homozygous offspring comprised the expected one-third of the population (30.9%; n = 313). In order to test for embryonic *DHR39* function, embryos were collected from *P[13215]* homozygotes and followed to hatching. Of these, 91% hatched on time (n = 625), as compared to 95.2% from a w^{1118} control stock (n = 666). Furthermore, no effects on *ftz* expression could be seen in *P[13215]* embryos by *in situ* hybridization, and no defects were evident in the cuticle (data not shown).

These results indicate that *DHR39* does not play an essential role during *Drosophila* development. Similar results have been obtained with mutations in the *E78* orphan receptor gene, although these mutations did lead to subtle changes in the puffing patterns of the polytene chromosomes (Russell *et al.*, 1996). Further insights into *DHR39* function will require a screen for second-site interacting mutations in *P[13215]* homozygotes.

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Alatortsev, V.E. Institute of Molecular Genetics, Russian Academy of Sciences, Kurchatov Sq. 46, Moscow, 123182, Russia. An indication on overlapping functions of the *Vinculin* and *α-catenin* loci in *Drosophila melanogaster*.

Vinculin, a conservative protein of the cellular cytoskeletal and anchorage system, was localized in adherent contacts of cells (Geiger *et al.*, 1980; Burridge *et al.*, 1988; Geiger *et al.*, 1990). Vinculin is homologous to the other peripheral cytoplasmic protein, α-catenin, in vertebrates (Kemler, 1993). Recently the *Vinculin* and *α-catenin* genes were described in *Drosophila melanogaster* (Alatortsev *et al.*, 1997; Oda, *et al.*, 1993). Structures of the corresponding *Drosophila* proteins are compared in this note.

Alignment of the vinculin (962 amino acids) and α-catenin (935 amino acids) sequences revealed that internal repeats and proline-rich domain are unique to the *Drosophila* vinculin. However, vinculin and α-catenin contain three extended regions of homology which occupy greater parts of their sequences (Figure 1). These regions lie within the highly conservative N- and C-domains of vinculin, as well as in the central part of the vinculin sequence. Given this multiple homology, it is possible to suggest that vinculin and α-catenin have some functions in common.

Interestingly, sequence of the central part of vinculin is variable in different vinculins (Weller *et al.*, 1990). High level of similarity between vinculin and α-catenin found for central region (71.5%) reflects co-evolution of two proteins in *Drosophila* and represents a special indication on overlapping functions of the *Vinculin* and *α-catenin* genes.

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Figure 1 (next page). Alignment of amino acid sequences of the *Drosophila* vinculin (Dmvincp) and α-catenin (Dmcatp) produced with the help of the GENEBEE program (Brodsky *et al.*, 1995). Standard parameters were used. Only regions with reliable homology are shown. The meaning of signs at the top of the alignment is following: ' ' - the average weight of column pair exchanges is less than weight matrix mean value; '.' - is less than mean value plus one SD; '+' - is less than mean value plus two SD; '*' - is more than mean value plus two SD.