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 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 w1118, 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 w1118 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 w1118, 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 w1118, 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 w1118 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 a-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, a-catenin, in vertebrates (Kemler, 1993). Recently the Vinculin and a-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 a-catenin (935 amino acids) sequences revealed that internal repeats and proline-rich domain are unique to the Drosophila vinculin. However, vinculin and a-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 a-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 a-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 a-catenin genes.

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Figure 1 (next page). Alignment of amino acid sequences of the Drosophila vinculin (Dmvincp) and a-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: 'i' - 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.