The Evolution of Duplicate Glyceraldehyde-3-Phosphate Dehydrogenase Genes in Drosophila

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ABSTRACT

In Drosophila melanogaster there are two genes which encode the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Gapdh-43E and Gapdh-13F. We have shown that Gapdh-43E codes for the GAPDH subunit with an apparently larger molecular weight while Gapdh-13F encodes the GAPDH subunit having an apparently smaller molecular weight. Immunoblots of sodium dodecyl sulfate gels were used to survey species from throughout the genus and results indicated that two classes of GAPDH subunits are present only in Drosophila species of the melanogaster and takahashi subgroups of the melanogaster group. Only the smaller subunit is found in species of the obscura group while all other species have only a large subunit. Drosophila hydei was analyzed at the DNA level as a representative species of the subgenus Drosophila. The genome of this species has a single Gapdh gene which is localized at a cytogenetic position likely to be homologous to Gapdh-43E of D. melanogaster. Comparison of its sequence with the sequence of the D. melanogaster Gapdh genes indicates that the two genes of D. melanogaster are more similar to one another than either is to the gene from D. hydei. The Gapdh gene from D. hydei contains an intron following codon 26. Neither Gapdh gene of D. melanogaster has an intron within the coding region. Southern blots of genomic DNA were used to determine which species have duplicate Gapdh genomic sequences. Gene amplification was used to determine which species have a Gapdh gene that is interrupted by an intron. Species of the subgenus Drosophila have a single Gapdh gene with an intron. Species of the willistoni and saltans groups have a single Gapdh gene that does not contain an intron. Species of the obscura and melanogaster groups have two Gapdh genes neither of which have an intron. In Drosophila pseudoobscura these are located at cytogenetic positions homologous to those of D. melanogaster. Therefore, the simplest model for the evolution of the Gapdh genes proposes that the intron in the Gapdh gene was lost early in the Sophophoran lineage. Later in the Sophophoran lineage, at a point leading to the obscura and melanogaster groups, a duplication of the Gapdh gene occurred.

The genome of Drosophila melanogaster has been shown to contain two genes which encode iso-enzymes of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). In contrast to other well characterized duplications in Drosophila, the two Gapdh genes are unlinked. They have been localized to cytogenetic positions 13F and 43E, respectively (Sullivan et al. 1985). A comparison of the sequence divergence of the two genes is consistent with the duplication having occurred about 60 million years ago (T'so, Sun and Wu 1985). This would place the time of duplication at about the time that has been estimated for the origin of the genus Drosophila by Throckmorton (1975). This suggests that the Gapdh duplication could be a useful marker for studying the divergence of species groups which occurred early during the evolution of the genus.

We have shown previously that two subunit forms of GAPDH from D. melanogaster can be detected on immunoblots of extracts prepared from larvae or adults. These forms copurify and appear to differ by about 1–2 kD in molecular mass. However, the basis of this size difference is unclear, since translation of the nucleotide sequence does not predict a significant size difference between the gene products. In addition, in vitro translation of D. melanogaster mRNA in rabbit reticulocyte lysates yields two GAPDH polypeptides having the same sizes as the forms immunologically detected on western blots of whole animal extracts, thereby indicating that post-translational modification is not a likely basis for the size isoforms (Sullivan et al. 1985). Consequently, it is likely that the apparent size differences of the GAPDH subunits derive from some structural property that results in anomalous mobility in denaturing gels. The first goal of the work reported here has been to associate each subunit with a specific Gapdh gene.

Our second goal has been to describe the evolutionary history and events of significance during the evolution of the Gapdh loci. We report here the analysis of representative species from the major groups of the
genus Drosophila at the protein and DNA level. Immunoblots of extracts from a number of species from throughout the genus suggest that the Gapdh duplication occurred early in the Sophophoran radiation of the genus Drosophila. Drosophila hydei was selected as a representative species of the subgenus Drosophila for detailed study. A single Gapdh gene was found in D. hydei and this gene was discovered to have an intron which interrupts its coding region. Neither Gapdh gene of D. melanogaster has any introns in the coding region. Consequently, we have focused our studies on the possibility of simultaneous gene duplication and intron loss during evolution.

At present the nomenclature with respect to the Drosophila Gapdh genes is in a confused state. The genes have been investigated by a group at Syracuse (Sullivan et al. 1985) and another at Cornell (T’so, Sun and Wu 1985; Sun, Lis and Wu 1988; Sun et al. 1988). Each group independently named these genes according to their own clone isolation history. In consultation with the Cornell laboratory and with their agreement, we propose to name each gene according to its cytogenetic localization. Hence the names Gapdh-43E and Gapdh-13F will be used for the genes located on the second and X chromosomes, respectively.

**MATERIALS AND METHODS**

**Animals:** D. melanogaster used in these studies were from our standard laboratory strain which was originally inbred from strain Oregon-R. D. hydei were from our standard laboratory strain which was inbred from a population originally collected in Mexico City by M. Wasserman. Drosophila pseudoobscura was an inbred line, Apple Hill 69. All other species were obtained from the species stock center at Bowling Green, Ohio.

**Blotting procedures:** Immunoblots were performed as previously described (Sullivan et al. 1985). Southern blots were conducted as described (Sullivan et al. 1985). For D. melanogaster blots a probe from Gapdh-43E, an approximately 3000-bp fragment from recombinant phage, λ G3, which extends from position 207 bp 5’ of the Gapdh translation start to 1600 3’ of the translation stop, was used. The probe used for interspecific analysis was a mixture of fragments from Gapdh-13F and Gapdh-43E. An 1100-bp fragment from the recombinant phage, λ G2, which extends from a Xhol site at position 218 bp 5’ of the translation start to a Ral site 930 bp 3’ to the translation start was mixed with an 1100-bp fragment of λ phage G3 including 207 bp 5’ to the translation start and 34 bp 3’ to the translation stop signal.

**Cytogenetic localization:** *In situ* hybridization to salivary chromosomes was conducted using a procedure based on that of Langer-Safer, Levine, and Ward (1982) with the following modifications. Biotinylated dUTP (15.5 nm) was used as a substrate for DNA polymerase (Klenow fragment) using random primers. Since we planned to conduct *in situ* hybridization to several species using a D. melanogaster probe, we adjusted hybridization and washing conditions such that a probe from Gapdh-13F gave signals of equal intensity at positions 13F and 43E of D. melanogaster salivary chromosomes. These were: hybridization at 37° in 2 x SSC and 50% formamide for 12–16 hr and washing at 55° for 10 min in 2 x SSC.

**DNA sequencing:** A 1.5-kb HindIII fragment from a D. hydei genomic clone obtained from an EMBL-4 genomic library was subcloned into M13mp18. Nucleotide sequencing was performed by the chain termination method (Hong 1982) using [32P]dATP. Buffer gradient gels of BgIII, Gibson and Hong (1983) were used. Gels were read and sequences ordered using a digitizer and computer programs from DNASTAR, Madison Wisconsin. Sequences were determined on one strand by synthesizing oligonucleotide primers spaced at about 350 to 400 bp. Each nucleotide was sequenced at least three times using both the Klenow fragment of Escherichia coli DNA polymerase and T7 DNA polymerase (Sequenase; U.S. Biochemical Corp.) in parallel lanes. This proved useful in resolving ambiguities since the two enzymes generate different sequence reading difficulties.

**Gene amplification:** The nucleotide sequences of the two Gapdh genes of D. melanogaster and the single Gapdh gene of D. hydei were used to design primers that were likely to form hybrids with a Gapdh gene of any Drosophila species. The 5’ primer is 23 nucleotides long and begins at the first nucleotide of codon 1. The 3’ primer is 20 nucleotides long and ends at the second nucleotide of codon 85. Amplification was conducted using 20 ng of genomic DNA of each species, and a gene amplification kit from U.S. Biochemical Corp. used according to the instructions supplied by the manufacturer.

**RESULTS**

Analysis of D. melanogaster extracts for GAPDH using immunoblots from sodium dodecyl sulfate gels reveals two subunit forms which differ in size. Since the two forms are also found in the immunoprecipitated products from in vitro translation of Drosophila mRNA (Sullivan et al. 1985) it is likely that each protein subunit is the product of one gene. However, no formal connection between a specific gene and protein has yet been established. Gapdh-43E is closely linked to the cinnabar (cn) locus. A. Howells and W. Warren of Australian National University (personal communication) have shown that Gapdh-43E and cn are located within a few thousand base pairs of each other, using the recombinant λ phage, G3, (Sullivan et al. 1985). Howells and Warren have used the series of small deletions, described by Alexandrov (1984) to localize the cn DNA region within the genome. Their success suggested to us that these deletions might be useful for associating the Gapdh-43E gene with a specific isoform.

The strains 79b9, 79bl3 and 74d2 each have deletions in the 43E region. Each is homozygous lethal but heterozygotes between them are viable while deficient for a small region of overlap in 43E (Alexandrov 1984). Figure 1A shows Southern blots of DNA from wild type flies and deletion strains. These blots were probed with a fragment from the Gapdh-43E gene. Two Gapdh bands are found in wild-type DNA (Figure 1A, lane 1). The stronger hybridizing 5.1-kb fragment is known to be derived from Gapdh-43E.
The weaker hybridizing fragment at 6.3 kb is known to be from Gapdh-13F (Sullivan et al. 1985). The relative intensity of the Gapdh-43E band in Figure 1A, lanes 2 through 5, is proportional to the dosage of wild-type alleles. Flies heterozygous for a single deletion show approximately one half the level of hybridization as compared to wild-type flies. This fragment is absent in DNA from flies heterozygous for overlapping deletions (Figure 1A, lanes 4 and 5). No additional hybridizing fragments are present in lanes 4 and 5 indicating that all or most of the Gapdh-43E gene is absent in these strains. Immunoblots of protein extracts of these strains reveal that the larger GAPDH is reduced in amount in deletion heterozygotes (Figure 1B, lanes 3, 4 and 5) and absent in deletion homozygotes (Figure 1B, lanes 6 through 10). The amount of the small isoform of GAPDH is similar in each of the flies. Therefore, we conclude that Gapdh-43E encodes the larger GAPDH isoform and Gapdh-13F encodes the smaller GAPDH isoform.

We surveyed a number of species from throughout the genus Drosophila using immunoblots as a preliminary strategy to determine the evolutionary history of the Gapdh duplication. A summary of all species analyzed is presented in Table 1. Species having two resolvable protein isoforms are found in the melanogaster and takahashi subgroups. Species of other subgroups from the melanogaster group have only a single GAPDH band. Species from the obscura group have one predominant band with mobility equivalent to the small GAPDH isoforms. All species of the subgenus Drosophila have a single large GAPDH isoform.

The number of protein bands revealed by Western blots is not a definitive guide to evolutionary history since it is conceivable that two protein isoforms might occupy the same position following electrophoresis. In addition, the molecular basis for the separation of the two isoforms of D. melanogaster is unknown because their conceptual translation does not predict isoforms of different subunit molecular weight. In all likelihood the separation of the GAPDH subunits on denaturing gels is due to retention of some secondary structure. We have reported earlier that two forms of GAPDH are immunoprecipitable from in vitro translation of D. melanogaster mRNA (Sullivan et al. 1985). Since the isoform pattern is phylogenetically coherent, we have used these observations as a guide for further study of the evolutionary history of the Gapdh duplication at the DNA level.

We selected D. hydei as a representative species of the subgenus Drosophila for detailed study. Southern blots of D. hydei genomic DNA using a D. melanogaster Gapdh-43E probe yield a series of hybridizing fragments which are best interpreted as a single gene because none of eight enzymes generated more than a single hybridizing fragment. Five of these are shown in Figure 2. The others, EcoRI, BamHI, and SalI all generated large single Gapdh fragments (data not shown). Therefore, in D. hydei all Gapdh genes probably reside on a single fragment and one of these fragments, that generated by Hind III digestion is only 1.5 kb which is too small to contain more than one Gapdh gene. A genomic library of D. hydei DNA was prepared as a partial MboI digest in the lambda vector EMBL-4 (Mennotti-Raymond, Starmer and Sullivan 1991). Nine recombinant lambda clones were isolated using the Gapdh-43E probe. All clones were judged by their restriction maps to overlap and come from the same chromosomal region (data not shown). A single HindIII fragment of 1.5 kb was found to hybridize to a Gapdh probe. This fragment was isolated and its nucleotide sequence determined. The sequence and its translation is shown in Figure 3. This sequence starts 346 bp 5' to the translation start codon and ends 165 bp 3' to the termination signal. A striking aspect of the D. hydei gene is the presence of a 69-bp intron (nucleotides 434–502) which interrupts the coding region following amino acid 29. Neither D. melanogaster gene has any introns within the coding region. Comparison of the D. hydei and D. melanogaster Gapdh genes reveals that the two D. melanogaster Gapdh genes are more similar to each other than either is to the D. hydei gene (Table 2). This is the case when comparisons of the nucleotide sequences or the encoded amino acid sequences are performed.

In situ hybridization of a Gapdh probe to D. hydei salivary chromosomes results in a single site of hybridization on chromosome 5 (Figure 4). This is equivalent
TABLE 1
GAPDH isozyme distribution in the genus Drosophila

<table>
<thead>
<tr>
<th>Subgenus</th>
<th>Group</th>
<th>Subgroup</th>
<th>Species</th>
<th>GAPDH forms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drosophila</td>
<td>Virilis</td>
<td>Virilis</td>
<td>virilis</td>
<td>1 large</td>
</tr>
<tr>
<td></td>
<td>Picture Winged</td>
<td>Hawaiensis</td>
<td>mirmshawi</td>
<td>1 large</td>
</tr>
<tr>
<td></td>
<td>Repleta</td>
<td>Hydei</td>
<td>hydei</td>
<td>1 large</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mulleri</td>
<td>mjavensis</td>
<td>mjavensis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mulleri</td>
<td>1 large</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>arizona</td>
<td>1 large</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>bushii</td>
<td>1 large</td>
</tr>
<tr>
<td></td>
<td>Dorsilopha</td>
<td>Willistoni</td>
<td>willistoni</td>
<td>2 large</td>
</tr>
<tr>
<td></td>
<td>Sophophora</td>
<td>Saltans</td>
<td>saltans</td>
<td>1 large</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Obscura</td>
<td>persimilis</td>
<td>1 small</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>subobscura</td>
<td>1 small</td>
</tr>
<tr>
<td></td>
<td>Melanogaster</td>
<td>Affinis</td>
<td>affinis</td>
<td>1 small</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scaptodrosophila</td>
<td>Victoria</td>
<td>Victoria</td>
<td>lebanonensis</td>
</tr>
</tbody>
</table>

**D. hydei**

![Figure 2.—Restriction map of D. hydei genomic DNA determined by probing with a D. melanogaster Gapdh gene.](image)

The Gapdh site in D. hydei is clearly within the first third of chromosome 5.

To genetic element C of the virilis-repleta radiation and chromosome 5 of Drosophila virilis (WASSERMAN 1982). WHITING et al. (1989) have conducted an extensive series of in situ hybridizations to D. virilis salivary chromosomes using probes of D. melanogaster origin. Five of these probes are localized to chromosome 5 of D. virilis. Their site 555 hybridizes to 43A-B of D. melanogaster and at a position near the tip of the D. virilis chromosome. Another probe, 514, hybridizes to 51B of D. melanogaster and at a position about 1/3 the distance from the D. virilis centromere. Another probe DT83b which hybridizes to 56C of the D. melanogaster chromosome is distal to 514 on the D. virilis chromosome. Therefore, one might expect that a probe from 43E of D. melanogaster might hybridize either near 555 at the distal end of the chromosome or to a site proximal to the sites of 514 and DT83b or within the proximal third of chromosome 5 depending on how the cytogenetic events which have occurred during evolution separated positions 43A-B, 43E and 51B. The site of hybridization of the Gapdh probe to D. hydei chromosomes is clearly within the first third of chromosome 5. Therefore, within the limits of cytogenetic comparisons possible, we conclude that the D. melanogaster 43E site and D. hydei sites are homologous and that the 43E site in D. melanogaster is homologous to the ancestral position of the single Gapdh gene.
of the species from the major groups of the two major subgenera, Sopho-
phora (melanogaster, obscura, willistoni and saltans groups) and Drosophila (virilis, repleta and immigrans groups) and the subgenera Dorsilopha and Scaptodro-
phora (melanogaster, obscura, willistoni and saltans groups) and Drosophila (virilis, repleta and immigrans groups) and the subgenera Dorsilopha and Scaptodro-
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prime the hypothesis that the original Gapdh duplication occurred early in the evolution of the Sophophoran radiation and raises the possibility that the mechanism of duplication was through a retrotransposition event thereby accounting for the loss of the intron. We have proceeded to survey the genomes of other species with respect to the number of Gapdh genes and whether an intron at this location is present. Since it is likely that the duplication occurred early in the evolution of the genus we have selected for study species from the major groups of the two major subgenera, Sopho-

### Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Amino acid differences</th>
<th>Percent nucleotide similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gapdh-43E/Gapdh-Hy</td>
<td>13</td>
<td>95.8</td>
</tr>
<tr>
<td>Gapdh-13F/Gapdh-Hy</td>
<td>12</td>
<td>96.4</td>
</tr>
<tr>
<td>Gapdh-43K/Gapdh-Hy</td>
<td>8</td>
<td>97.6</td>
</tr>
</tbody>
</table>

*Percent nucleotide similarity is calculated for nucleotides within the coding region only.*

This comparison of the Gapdh genes of *D. melanogaster* and *D. hydei* supports the hypothesis that the original Gapdh duplication occurred early in the evolution of the Sophophoran radiation and raises the possibility that the mechanism of duplication was through a retrotransposition event thereby accounting for the loss of the intron. We have proceeded to survey the genomes of other species with respect to the number of Gapdh genes and whether an intron at or near codon 29 is present. Since it is likely that the duplication occurred early in the evolution of the genus we have selected for study species from the major groups of the two major subgenera, Sopho-

![FIGURE 3.—Nucleotide sequence of the Gapdh region of D. hydei.](image-url)
FIGURE 5.—Southern blots of DNA from various species probed with a mixture of Gapdh 13F and Gapdh 43E fragments. (A) Lanes 1, 2 and 3 are D. busckii DNA; lane 1, EcoRI; lane 2, EcoRI-PstI; lane 3, PstI. Lanes 4, 5 and 6 are D. immigrans DNA; lane 4, HindIII; lane 5, HindIII-PstI; lane 6, HindIII. Lanes 7, 8 and 9 are D. auraria DNA; lane 7, NdeI; lane 8, NdeI-HindIII; lane 9, HindIII. (B) D. saltans DNA; lane 1, BamHI; lane 2, EcoRI; lane 3, HindIII-EcoRI; lane 4, HindIII; lane 5, HindIII-BamHI. (C) D. willistoni DNA digested with CiaI.

in each digest is larger than any known Gapdh gene, these data require that the genome contains more than one gene or alternatively the existence of a 2-kb or greater intron positioned so as to occupy most of the smallest restriction fragment seen in the digests. Furthermore, this would have to be a newly created intron since such an intron is not found in other species which have common ancestors with D. auraria. This combination of unlikely events makes it improbable that D. auraria has a single Gapdh gene. The genomes of Drosophila saltans (Figure 5B) and Drosophila willistoni (Figure 5C) generate only single Gapdh genomic restriction fragments and in the case of D. willistoni this band is less than 1.5 kb. Since it is unlikely that two nontandemly arranged genes would each have two CiaI sites identically positioned on such a small fragment, it is likely that the genomes of these related species contains a single Gapdh gene.

In situ hybridization to D. pseudoobscura salivary gland chromosomes reveals several sites of hybridization. Two of these, marked A and B in Figure 6, are on chromosomes XL and 3, respectively. These are likely to be homologous to the 13F and 43E positions of D. melanogaster. A third region of hybridization, C, which is in or near the heterochromatin of chromo-
some XR and sometimes appears as two separate regions can also be seen in Figure 6. The nature of these heterochromatic regions has not been further investigated. Southern blots of genomic DNA generate a pattern of fragments consistent with two Gapdh genes (not shown). This indicates that species in the obscura group contain duplicate Gapdh genes and that the presence of a single isoform in these species (Table 1) is best interpreted as resulting from two proteins occupying the same gel position or the translational silencing of one gene.

Since the single Gapdh gene of D. hydei has an intron, while neither of the two Gapdh genes of D. melanogaster has an intron, we have explored the possibility that duplication and intron loss happened coincidentally during evolution which would be the case if the Gapdh duplication occurred by means of a retrotransposition event. We have used a gene amplification technique to measure the distance from codon 1 to codon 85 of the Gapdh gene in genomic DNA of a number of species. The two primers were designed to complement conserved regions in Gapdh genes so as to hybridize equally well to both of the D. melanogaster genes and the D. hydei gene in order to be effective with any Gapdh from the genus. Since the amplified fragment spans the position of the intron (codon 29) found in the D. hydei gene, a Gapdh gene without the intron in its genomic DNA should result in a fragment of 254 bp, while a fragment larger than this would be found when the intron is present in genomic DNA. The exact size of the fragment might vary with variation of intron size but in the case of D. hydei the amplified fragment should be 325 bp. The results of the gene amplification analysis are shown in Figure 7. All species of the subgenus Sophophora, including D. melanogaster, D. saltans, D. pseudoobscura, D. willistoni and D. auraria, generate a 254-bp fragment indicating that there is no intron in the Gapdh gene(s) of these species. D. immigrans, (a member of the immigrans group of the quintaria section), D. hydei (a member of the repleta group) and Drosophila americana (a member of the virilis group), have an amplified fragment of about 320 bp. D. busckii of the subgenus Dorsilopha (lane 7) has an amplified fragment larger than 320 bp. Drosophila lebanonensis of the subgenus Scaptodrosophila has an amplified fragment a few nucleotides shorter, but close to the size of the amplified fragment of D. hydei, lanes 6 and 12. The initial amplification of D. lebanonensis DNA (lane 6) was only partially successful so a second DNA sample was prepared and amplified (lane 12) and compared to a second sample of D. hydei DNA (lane 11). Since GAPDH is an extremely conserved protein, flexibility in amino acid number internally in the protein is highly unlikely. Accordingly, species with
an amplified fragment larger than 254 bp must have an intron in their Gapdh gene. Since species from each of the four groups of the subgenus Sophophora have a Gapdh gene without an intron but only two of these species groups have a duplication, these results indicate that the loss of the intron in the Gapdh genes occurred very early in the evolution of the Sophophoran lineage and in all likelihood preceded the Gapdh duplication.

**DISCUSSION**

All available evidence is consistent with the Gapdh duplication having occurred at an early point during the evolution of the Sophophoran subgenus. There is no evidence of duplicate Gapdh genes in any species of the subgenus Drosophila. In D. hydei a number of independent lines of evidence clearly indicate a single gene. Therefore it appears that early in the evolution of the subgenus Sophophora two events occurred. One was the loss of the intron and the second was duplication of the intron-less Gapdh gene. We began these studies attracted to the hypothesis that the loss of the intron and the duplication might have occurred simultaneously by a single retrotransposition event. However, the simplest, most economical hypothesis based on the analysis of species presented here is that the intron was lost substantially earlier in the Sophophoran lineage than the time at which the duplication occurred. The species of the related groups, saltans and willistoni, clearly have a Gapdh gene without an intron. Yet Southern blots indicate that these species each have only a single Gapdh gene. More complicated hypotheses can be invoked that might connect the events of gene duplication and intron loss. Accordingly, the current data are consistent with a model in which an original duplication occurred through retrotransposition and the subsequent loss of the intron positioning predates the prokaryotic-eukaryotic divergence (QUIGLEY, MARTIN and CERF 1988; SHIH, HEINRICH and GOODMAN 1988). The manner through which introns are lost has not been ascertained but it seems reasonable that the precision of intron loss requires reverse transcription. FINN (1987) has argued that the scarcity of introns in the yeast genome is the result of reverse transcription of a large fraction of yeast genes over evolutionary time. Accordingly, if one accepts the position that introns are old and only subject to loss, it seems most likely that the presence of an intron in the Gapdh gene of D. hydei and its absence in the genes of D. melanogaster is due to loss of the intron in the D. melanogaster lineage. There is no direct demonstration that the history of Gapdh evolution in the genus Drosophila could not have involved the gain of a new intron at codon 29 early in the evolution of the subgenus Drosophila. However, D. lebanonensis, a member of the subgenus Scaptodrosophila, has an intron in its Gapdh gene.
The early evolution of subgenera in the genus Drosophila is not unambiguously determined but the subgenus Scaptodrosophila is thought to have diverged before the divergence of the Sophophora and Drosophila subgenera (Beverley and Wilson 1982). If this view is correct, then there would have been an intron in the Gapdh genes of the ancestors of the Sophophoran groups thereby making it highly likely that the absence of the intron in the Gapdh genes of species from these groups is due the loss of an old intron.

Since GAPDH has been the object of extensive physical structural characterization the position of introns in Gapdh genes from several sources has been studied in order to further investigate the relation between intron position and the evolution of protein structure. For the most part the position of introns in Gapdh genes correlate well with the boundaries of proposed domains in GAPDH in the Gapdh genes of: chickens (Stone, Rothblum and Schwartz 1985); nematodes (Yarbrough et al. 1987); humans (Ercolan et al. 1988); maize (Quigley, Martin and Cerf 1988); and nuclear and mitochondrial encoded GAPDH of arabidopsis (Shih, Heirich and Goodman 1988). However, the relation between protein domains and intron position is not well correlated in the Gapdh/GAPDH of Aspergillus (Punt et al. 1988). An intron at codon 29 as in D. hydei Gapdh has not been observed in other species. The detailed comparison of GAPDH domain organization and Gapdh intron position by Shih, Heirich and Goodman (1988) reveals that introns have not yet been found at all potential domain delineating positions. Interestingly, these authors predict that an intron might be found in the interval between codons 20 and 30. The intron in the D. hydei Gapdh gene at codon 29 apparently confirms this prediction. Therefore, this intron is at a position that is located near the borders of two protein domains.

This research was supported by National Institutes of Health grant 26830 to D.T.S. We thank Wyatt Anderson and Marvin Wasserma for their assistance in the cytogenetic localization in D. pseudoobscura and D. hydei, respectively. The nucleotide sequence of the Gapdh gene of D. hydei has been submitted to the EMBL data base and has been assigned Acc. No. Z14144.

LITERATURE CITED


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