who encodes a KH RNA binding protein that functions in muscle development

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SUMMARY

The Drosophila who (wings held-out) gene functions during the late stages of somatic muscle development when myotubes migrate and attach to specific epidermal sites. Animals lacking who function are capable of forming multinucleate myotubes, but these cells are restricted in migration. who mutants die at the end of embryogenesis with the posterior end of their cuticles arrested over the dorsal surface. Animals that possess weak who mutations either die as pupae, or survive as adults with defects in wing position. These phenotypes indicate that who also functions during metamorphosis, when muscles are reorganized to support adult structures and behavior. These embryonic and metamorphosis defects are similar to the phenotypes produced by previously identified genes that function in either muscle development or steroid signaling pathways. who transcription occurs in muscle and muscle attachment site cells during both embryogenesis and metamorphosis, and is inducible by the steroid ecdysone at the onset of metamorphosis. who encodes a protein that contains a KH RNA binding domain. Animals that possess a mutation in a conserved loop that links predicted α and β structures of this RNA binding motif lack who function. These results indicate that who plays an essential role in steroid regulation of muscle development.

Key words: Drosophila, muscle development, metamorphosis, steroid, ecdysone

INTRODUCTION

Somatic muscle development in Drosophila involves communication between cells of mesodermal and ectodermal origin, resulting in highly organized patterns of fibers. Within each reiterated segment of the larval cuticle there are specific repeated arrays of approximately 30 muscle fibers that form the larval body wall muscles. Following the specification of muscle cell precursors (reviewed by Bate, 1993; Abmayr et al., 1995) and the fusion of these cells to form multinucleate myotubes (Bate, 1990), muscle fiber precursors migrate across the epidermis to specific locations for attachment. The first recognized cellular change in myotube migration involves the extension of growth-cone-like processes that appear to be involved in navigation along the epidermis to appropriate attachment sites. Both the myotube and the epidermis seem to be required in migration, as mutations in segment-polarity genes that result in the absence or ectopic presence of epidermal border cells cause disorganized muscle fiber patterns (Volk and VijayRaghaven, 1994). The final aspect of muscle fiber development requires proper attachment to the ectoderm, after which this connection must be maintained.

Several genes have been implicated in the orientation and attachment of muscle cells to their ectodermal attachment sites. The transmembrane receptor gene Toll and the receptor tyrosine kinase gene derailed function in the interaction of a variety of cell types, and mutants of both genes exhibit defects in muscle cell migration and attachment (Halfon et al., 1995; Callahan et al., 1996). stripe mutants possess multinucleate muscle cells that express myosin heavy chain, but these cells form disorganized fiber patterns that are detached from attachment sites (Frommer et al., 1996). stripe encodes a zinc finger protein, which is thought to function in myotube orientation for attachment (Lee et al., 1995; Frommer et al., 1996). Studies of position-specific integrins indicate that these proteins are expressed in complementary patterns in both epidermal and muscles cell membranes at the site of muscle cell attachment (Bogaert et al., 1987; Leptin et al., 1989). Animals that lack myospheroid function (possessing mutations in β-s-integrin) are able to form multinucleate myotubes, but these muscles detach from the epidermis (Brown, 1994). myospheroid mutants are also defective in embryonic dorsal epidermal adhesion, adult wing position, and they develop blisters on their wing epithelia (Leptin et al., 1989; Zusman et al., 1990; Brown, 1994). The specific mechanism(s) that integrate the different genes that mediate proper cell-cell recognition, migration and attachment with appropriate temporal and spatial context during development of muscle are not clearly understood.

Adult and larval muscles are derived from a completely different set of cells, but the same genes appear to be utilized during both larval and adult muscle development. Near the end of Drosophila embryogenesis, cells are set aside that will form somatic muscles during metamorphosis for use in adult life (Bate et al., 1991). The development of adult muscle cells is mediated by genes that also function during embryogenesis including myospheroid and stripe (Fernandes et al., 1996). Late during Drosophila larval life, adult muscle cell lineages proliferate and differentiate to form myotubes with similar properties as larval muscles, but with distinct identities enabling...
them to support adult behaviors including the use of legs and wings (Robertson, 1936; Bate, 1993). While these muscles are forming, larval muscles are degenerating. The process of metamorphosis is initiated by the steroid hormone 20-hydroxycycodone (ecdysone) (Riddiford, 1993), and both the destruction of larval muscle and differentiation of adult muscle appear to be regulated by ecdysone. Consistent with steroid regulation of this process, flies with mutations in the ecdysonase-regulated early puff genes E74 and BR-C have defects in muscle development during metamorphosis. Mutations in E74B result in defects in head eversion (Fletcher et al., 1995): the sudden evagination of the presumptive adult head during metamorphosis that is caused by muscle contraction (Robertson, 1936). Furthermore, mutations in BR-C cause defects in muscle cell migration and attachment (Restifo and White, 1992). These defects appear similar to those observed in stripe mutants (Costello and Wyman, 1986; Frommer et al., 1996), indicating that ecdysone plays a pivotal role in muscle development during metamorphosis.

This report presents the isolation and characterization of the wings held-out gene, who. Animals that lack who function have defects indicating that this gene functions in myotube migration and/or attachment during both embryogenesis and metamorphosis. who transcription is inducible by ecdysone in culture and is observed in muscle and muscle attachment site cells during both of these stages, consistent with the defects observed in mutant animals. who encodes a member of the KH RNA binding protein family with greatest similarity to the mouse quaking and C. elegans gla-1 genes. These results indicate that who is a steroid-regulated component of the muscle development genetic pathway, a process that has been conserved in diverse animal groups.

MATERIALS AND METHODS

Drosophila stocks and genetics

Stocks were maintained on standard cornmeal/molasses/yeast medium at 18°C or 25°C. The following strains and chromosomes were used: Canton S wild-type, TM3 Sb e (Lindsley and Zimm, 1992), TM3 Sb e hblacZ (Drierer et al., 1989), TM6B Hu Tb e (Lindsley and Zimm, 1992), ru h st cu sr e ca (Lindsley and Zimm, 1992), Df(3R)E74B (Lindsley and Zimm, 1992), P-element insertion lines B1 3rd 12 and E7 3rd 4 (Bier et al., 1989), IA122 (Perrimon et al., 1991), and 2612 (provided by the Berkeley Drosophila Genome Project), and the imprecise-excision lines rev4 (who44) and rev17 (who17) that were generated from the B1 3rd 12 P-element line (kindly provided by Daniel Doherty and Yuh Nung Jan).

New mutations were created in who by conducting two genetic screens. In one screen, males that were homozygous for the B1 3rd 12 P-element that possesses the marker gene were irradiated with 4,000 rad from a 137Cs source. These flies were mated to w; TM3 Sb e/TM6B Hu Tb e virgin females. Approximately 75,000 progeny of this cross were screened for deletions of the w gene. Two lines were recovered that are useful for the analysis of who (Df(3R)93F2 and who25). Df(3R)93F2 is homozygous lethal and is a deletion that spans from 93F5 to 94A8 as determined by analysis of polytene chromosome squashes. who25 is homozygous viable and is a small lesion compared to Df(3R)93F2 based on the lack of a visible aberration of the polytene chromosomes. In another screen, isozenized ru h th st cu sr e ca males were mutagenized with 25 mM ethane methyl sulfonate following the method of Lewis and Bacher (1968). These flies were mated to TM3 Sb e/TM6B Hu Tb e virgin females and the male progeny of this cross were pair-mated to Df(3R)93F2/TM6B Hu Tb e virgin females. The progeny of the latter cross were screened for lethality in combination with Df(3R)93F2 and the resulting lethal lines were further screened for lethality in combination with the who44 and who17 mutant lines. After screening 7,914 viable lines, a single mutation was isolated, who44. Markers adjacent to who were removed by recombination with wild-type chromosomes following mutagenesis. The extent of who function was determined by comparing the phenotypes of homozygous mutants with the phenotypes of animals that were transheterozygous for mutant and deletion chromosomes, or homozygous for deletions in this locus.

Phenotypic analysis

For analyses of embryonic phenotypes, mutant chromosomes were maintained over the marked balancer chromosome TM3 Sb e hblacZ to distinguish homozygous mutants from heterozygous and wild-type siblings. First larval instar cuticles were prepared and examined as previously described (Lamka et al., 1992). For examination of muscle cell morphology, embryos were dechorionated, fixed, and X-gal stained as described below. Muscle cells were visualized using a rabbit antiserum against myosin heavy chain (Kiehart and Feghali, 1986) using the Vectastain ABC Elite horseradish peroxidase system as described (Macdonald et al., 1986). Homozygous mutants were distinguished by the lack of X-gal staining. For analysis of pupal and adult phenotypes, mutant chromosomes were maintained over the TM6B Hu Tb e balancer chromosome to distinguish homozygous mutants using dominant pupal and adult markers. Pupae were analyzed by boiling pupal cases in water for 5 minutes, which enables clean dissection of the pharate adult from the pupal cuticle enclosure.

Molecular characterization of who

The B1 3rd 12, E7 3rd 4, IA122, and 2612 P-element insertion sites were mapped to the 93F13 locus of polytene chromosomes following the method of Chen et al. (1992). Genomic DNA adjacent to all four of the P-element insertions was isolated by plasmid rescue (Wilson et al., 1989). The site of transposable element insertions and the position of deletion breakpoints in this region were mapped onto genomic cosmid clones that had been isolated in previous studies of this locus (Baehrecke and Thummel, 1995) by Southern blot hybridization.

Genomic DNA fragments extending 40 kb on each side of the P-element insertion sites were hybridized to northern blots of RNA isolated from both embryos and prepupae (the two developmental stages when the lacZ gene is transcribed in the P-element enhancer trap lines) as previously described (Karim and Thummel, 1992). Genomic probes that detected transcripts were then used to isolate ten cDNAs from embryonic and prepupal libraries (provided by Patrick Hurban). Candidate who RNA transcripts were identified by in situ hybridization of random-labeled cDNA probes to Drosophila embryos as previously described (Tautz and Pfeifle, 1989). cDNAs that hybridized to embryos in a pattern that was similar to the pattern of lacZ expression in who enhancer trap lines were restriction mapped and positioned on genomic DNA by Southern blot hybridization.

Genomic DNA subclones and cDNAs were sequenced using an ABI 373 stretch sequencer (Applied Biosystems Inc.) as previously described (Baehrecke and Thummel, 1995). cDNAs were sequenced on both strands and the corresponding genomic DNA was sequenced on one strand. The amorphic who44 mutant chromosome was maintained over the marked balancer chromosome TM3 Sb e hblacZ. The entire open reading frame was sequenced from homozygous who44 mutant and the ru h th st cu sr e ca parental chromosome where this mutant was induced. This was accomplished by collecting embryos from a who44/TM3 Sb e hblacZ stock, and processing them for X-gal staining as described in the next section, except that paraformaldehyde was replaced with phosphate-buffered saline. Homozygous mutant embryos were collected, boiled for 10 minutes to facilitate disruption of embryonic cells, and the who open reading frame was PCR-amplified. The same procedure was repeated with ru h th st cu sr e...
Animals lacking who function possess defects in skeletal orientation and muscle development. (A) Wild-type and (B) whoe44 mutant embryonic cuticle preparations. Animals lacking who function die with the most posterior end of the cuticle arrested above the dorsal surface. (C) Wild-type and (D,E) two different whoe44 mutant embryos that were stained with myosin heavy chain antibodies. C illustrates the normal stretched repetitive pattern of differentiated muscle cells. (D,E) Animals lacking who function have differentiated multinucleate myotubes as indicated by anti-myosin staining, but possess defects in myotube migration and attachment as indicated by the disorganized and often round-shaped cells.

Isolation of who mutations

who was first identified in the P-transposable element enhancer trap screens conducted in the Jan, Perrimon, and Scott laboratories (Bier et al., 1989; Perrimon et al., 1991). All of these P-element lines (B1 3rd 12, E7 3rd 4, 1A122, and 2612) are viable when combined with a deficiency that deletes the site of P-element insertion, although 2612 displays a weak adult wings held-out phenotype under these conditions. Several who mutants were isolated by perturbation of the B1 3rd 12 P-element, either by providing a source of transposase or irradiating flies with a 137Cs source. These mutants vary in their severity of who aberration, from complete loss-of-function (whoe44) to partial loss-of-function (whoe17, whoe23). The whoe44 deletion removes at least one other adjacent lethal complementation group. Finally, one ethane methyl sulfonate-induced mutation was isolated in a genetic screen for lethality in combination with a deficiency for this locus. This line (whoe44) completely lacks who function – the homozygous whoe44 phenotype is the same as the whoe44/Df(3R)93F2-transheterozygotes and homozygous deletion phenotypes.

Phenotypic analysis of who

who mutants fall into distinct classes based on their lethal phases during development and form an allelic series. whoe44 homozygotes appear to completely lack function. whoe44 have the same lethal phenotype as homozygotes and in combination with deficiencies of this region. Animals lacking who function die late during embryogenesis possessing defects that seem to occur sometime between myoblast fusion and muscle cell attachment. whoe44 mutants die with the most posterior region of the cuticle arrested above the dorsal surface (Fig. 1A,B), presumably caused by failure to complete germ band retraction. Further analyses revealed that whoe44 mutants have differenti-
Molecular characterization of the who locus

Genomic DNA that flanked the site of all four of the transposable P-element insertions was isolated by plasmid rescue as described by Wilson et al. (1989). This DNA was then mapped onto genomic cosmid clones extending approximately 40 kb on each side of the insertion site (Fig. 3). Three deficiencies that lack who function, as well as the site of the transposable element insertions, have been oriented on the physical map of the who locus. The Df(3R)eBS2 deficiency deletes from 93B to 93F14, the Df(3R)who4 deficiency spans approximately 18 kb, while the Df(3R)93F2 deficiency spans from 93F5 to 94A8, deleting the entire chromosomal walk (Fig. 3). All of the transposable P-elements used in these studies are inserted in the same genomic fragment, and no differences could be detected in the location of these insertions by restriction mapping (data not presented).

EcoRI restriction fragments from the entire genomic region were hybridized to northern blots of RNA isolated from both embryos and prepupae (the two developmental stages when the lacZ gene is transcribed in the P-element enhancer trap lines). Three transcripts were detected with genomic probes from this locus. These genomic probes were then used to isolate ten cDNAs. Several pieces of evidence led to the identification of the who RNA transcript. First, this transcript has the same embryonic expression pattern as detected in the transposable element enhancer trap lines (data not presented) as determined by in situ hybridization (Fig. 4). Second, the transposable element insertion site is adjacent to the 5'-end of this transcription unit – a feature common to many enhancer traps in Drosophila (Spradling et al., 1995). Furthermore, who17 possesses an aberration in the size of this RNA transcript (data not presented), and who17 fails to complement the who44 null allele. Most significantly, who44 possesses a missense mutation in a conserved structure in the product of this gene (see details below).

who is transcribed in muscle and muscle attachment site cells and is inducible by ecdysone

who transcription increases during embryonic and metamorphic stages of development, the latter being coincident with an increase in ecdysone titer. The temporal and spatial patterns of who transcription are consistent with the defects observed in mutant animals. who RNA is first detected in mesodermal precursors on the ventral side of the embryo at the onset of gastrulation (Fig. 4A). who transcript continues to be detected in presumptive mesoderm cells at the germ band extension stage (Fig. 4B). As germ band retraction occurs, who transcription is detected in the cells destined to form somatic and visceral muscle cells (Fig. 4C). Expression is also detected in the pharyngeal muscles at the anterior end of the embryo. who transcription is detected in cardiac precursors and muscle attachment cells of the epidermis as dorsal closure occurs (Fig. 4D). Late during embryogenesis, who is restricted to the heart and muscle attachment sites of the epidermis (Fig. 4E).
gene. We were interested in determining if who is expressed in a similar pattern during embryogenesis and metamorphism, since mutant phenotypes indicate that this gene has a similar function during these two stages. Similar to the pattern of expression during embryogenesis, who transcription is detected in muscle and muscle attachment cells during the onset of metamorphosis. who is detected in adult muscle cell precursors that are attached to the wing imaginal disc (Fig. 5A) and in parallel rows of epidermal muscle attachment site cells (Fig. 5B). In order to test if who is transcriptionally regulated by ecdysone, third larval instar tissues were isolated before the increase in ecdysone titer, cultured for 8 hours, and a physiologically high titer of ecdysone was added to cultures for varying periods of time. RNA was extracted from the tissues, electrophoresed, transferred to a membrane, and hybridized with a radiolabelled who probe. who is induced by ecdysone in cultured organs (Fig. 6).

**who encodes a putative RNA binding protein**

The who transcript has been characterized by sequencing cDNA clones on both strands, and sequencing genomic DNA for the entire transcript on one strand. who spans greater than 35 kb of genomic DNA, contains seven introns with conserved splice-site consensus sequences (Mount et al., 1992), and is spliced to form a 4.0 kb mRNA transcript (Figs 3, 7). The largest conceptual translation product encodes a 405 amino acid open reading frame (Fig. 7).

Blast database searches reveal strong similarity with a rapidly growing family of K homology (KH domain) RNA binding proteins (Fig. 8). The KH domain encoded by who is extremely similar to and distinguishes a group of proteins in this gene family. The most similar members of the KH RNA binding protein family include mouse quaking (QKI; Ebersole et al., 1996), *C. elegans* ovarian tumor suppressor *gld-1* (GLD-1; Jones and Schedl, 1995), mouse p62 RasGAP (Richard et al., 1995), and human Sam68 (Wong et al., 1992; Fig. 8). These proteins possess three features that distinguish them from other members of the KH RNA binding protein family – they have a single KH domain, they have extended similarity of unknown functional significance called the GSG domain (Jones and Schedl, 1995), and they contain a conserved loop structure in the middle of the RNA binding motif (Musco et al., 1996; Fig. 8).

To determine the site and type of lesion in the chemically induced who44 null allele, the exons containing coding sequence of homzygous mutant and the parental chromosome that was used for this mutagenesis were sequenced. who44 contains a missense mutation in a codon that results in an R to C change (Fig. 7). This mutation occurs in an amino acid conserved in all members of this unique group of KH domain proteins (Fig. 8), in the conserved loop that may function in protein-protein or protein-nucleic acid interaction (Musco et al., 1996).

**DISCUSSION**

Proper who function is required for muscle formation during two stages of *Drosophila* development. who mutant defects are similar to previously characterized genes that function in muscle cell migration and attachment, but who is unique for several reasons. First, who is steroid regulated, and this is reflected in mutant lethality associated with the ecdysone regulated process of metamorphosis. Second, who encodes a KH RNA binding protein family member. Members of this gene family include the *C. elegans* ovarian tumor suppressor gene *gld-1* (Jones and Schedl, 1995), the mouse myelination gene *quaking* (Ebersole et al., 1996), and the human fragile X-linked mental retardation gene *FMR1* (Sioini et al., 1993). The fact that who encodes an RNA binding protein indicates that this gene plays a regulatory role, and thus must interact with genes in either the steroid signaling or muscle development genetic regulatory pathways.

**who mutants are defective in muscle development**

Genetic characterization of who has revealed important features of this gene. At the cellular level, who mutants display defects following the formation of multinucleate myotubes. The myotubes of who mutant animals express myosin heavy chain indicating that they are differentiated muscles, but they fail to migrate and attach to the epidermal attachment sites. who loss-of-function mutant cuticles exhibit a defect with the most posterior region being arrested over the dorsal surface. Weak who mutants die as pupae with their head stuck in the thorax, while the weakest mutants survive as adult with defects in wing position. Both head eversion and wing position depend on proper muscle function that appears to be defective in who mutants. Furthermore, who is required during both embryogenesis and metamorphosis, the stages when larval and adult muscles develop.

who defects are similar to the aberrations exhibited by previously identified mutants that function in the migration and attachment of somatic muscle cells. The myotubes of animals that lack the function of the βPS-integrin gene *myospheroid*, for example, migrate to appropriate attachment sites, but fail to maintain attachment (Brown, 1994). Like who mutants, *myospheroid* mutants exhibit a cuticle defect that is caused by incomplete germ band retraction (Leptin et al., 1989). who mutant muscle fibers are perturbed earlier than the muscles of *myospheroid* mutants, however, and appear to be defective in migration rather than attachment. This migration defect is more similar to the phenotype of *stripe* (Frommer et al., 1996) and *derailed* (Callahan et al., 1996) mutants, both of which form myotubes that exhibit defects in orientation during migration. Analysis of weak who mutants revealed further similarity to the genes known to function during adult muscle development. Specifically, they either die during metamorphosis or survive with defects in wing position and have wing blisters, similar to *myospheroid* mutant phenotypes (Leptin et al., 1989; Zusman et al., 1990; Brown, 1994).

The pattern of who expression has distinct similarities to and differences from the expression of previously characterized genes that function in somatic muscle migration and attachment. who is transcribed in muscles and in the epidermal locations where muscles attach. This is similar to the expression of *derailed*, which is expressed in the same population of cells (Callahan et al., 1996). These genes differ from the position-specific integrins, however, which are expressed in complementary patterns in either muscle or muscle attachment site cells (Bogaert et al., 1987; Leptin et al., 1989). who and *derailed* also differ from *stripe* expression, which is restricted to epidermal muscle attachment sites (Frommer et
al., 1996). Like who, both the integrins and stripe are expressed in a similar population of cells when adult muscle development is occurring during metamorphosis (Fernandes et al., 1996).

The characterization of the genes that function in the migration and attachment of somatic muscle cells has provided significant insights into this developmental process, yet gaps exist in the integration of the functions of these genes. The position-specific integrins function in the maintenance of cell junctions (Brown, 1994), indicating that they function late in this process. derailed, stripe, and who appear to function in the migration of the presumptive muscle fiber, but it is unclear how these genes may be involved in integration of this process. derailed is a receptor tyrosine kinase that also functions in neuronal migration (Callahan et al., 1995). It is easier to envision how cell surface molecules such as a receptor tyrosine kinase or integrins may biochemically function in cell migration and attachment. In contrast, it is more difficult to predict how genes that encode DNA or RNA binding proteins like stripe and who may function in muscle cell migration. Presumably, stripe and who act in the regulation of genes that are more directly involved in the cellular process of migration. RNA binding proteins encoded by genes like who could have various functions including RNA transport, stability and splicing. Future studies of who will determine how a gene that encodes an RNA binding protein functions in muscle development.

Steroid-regulation of who and muscle development

The metamorphosis from a larva to an adult is regulated by the steroid hormone ecdysone (Riddiford, 1993). Phenotypic
**Fig. 7.** Nucleotide and predicted amino acid sequences of *who*. The intron boundaries are indicated in lower case above the splice junctions. The location of the *who* C to T (nucleotide 854) missense mutation and corresponding R to C (amino acid 185) changes are indicated with boxes. The amino acids encoding the KH domain are overscored. The GenBank accession number is U85651.
analyses indicate that who functions in adult muscle development during metamorphosis. Furthermore, the defects in who mutants observed during metamorphosis are similar to those of previously identified genes that function in a steroid regulatory pathway. Partial-loss-of-who-function mutant animals die during metamorphosis and often possess defects in the muscle dependent process of head eversion, similar to animals that possess mutations in the ecdysone-regulated process of head eversion, similar to animals that undergo metamorphosis. Furthermore, the defects in head eversion, similar to animals that undergo metamorphosis. Moreover, the defects in head eversion, similar to animals that undergo metamorphosis.

Fig. 8. (A) is a member of a conserved family of RNA binding proteins. WHO is aligned with the most similar proteins that were identified in Blast database searches. (A) This family of proteins is typified by a conserved central region that includes the Maxi-KH RNA binding domain (black regions) as defined previously (Musco et al., 1996). A number of proteins in this gene family share regions of identity outside the KH region that are of uncertain functional importance including the GSG domain (cross-hatched regions). The percentage identity of WHO with these proteins following Clustal analysis is indicated to the right. (B) Alignment of the Maxi-KH RNA binding domains illustrates the degree of sequence conservation. The α and β structures, the highly conserved loop that distinguishes these proteins from other members of this gene family (Musco et al., 1996), and the location of the who e44 R to C mutation (asterisk) are indicated.

KH RNA binding proteins are conserved molecules with diverse developmental functions

WHO is a member of the KH RNA binding protein family. Members of this gene family have been identified from diverse organisms and are involved in a variety of cellular functions. Prokaryotic members of the KH family include proteins that are as different as ribonuclease PNP (Regnier et al., 1987) and transcription elongation factor NusA (Liu and Hanna, 1995). Eukaryotic members of the KH family have been identified in organisms as different as yeast (Nandabalan et al., 1992) and humans (Siomi et al., 1993). Members of this family have been implicated in biochemical functions including RNA splicing and stability. WHO shares the most extensive homology with a unique group of proteins that has a single RNA binding motif and a conserved loop that could serve in interactions with other molecules (Fig. 8). WHO is most similar to the products of the mouse quaking gene (Ebersole et al., 1996), the C. elegans gld-1 gene (Jones and Schedl, 1995), the mouse p62 RasGAP gene (Richard et al., 1995), and the human Sam68 gene (Wong et al., 1992). These genes have diverse properties and functions including the regulation of growth and myelination, ovarian tumor suppression, and the association with signaling molecules during mitosis.

who is a member of the class of KH RNA binding protein genes that includes quaking and gld-1 and, thus, offers the strength of extensive genetic analyses. For example, more than 30 gld-1 mutants have been isolated (Francis et al., 1995). The location of each of these lesions has been determined and most of the missense mutations map to the KH RNA binding domain (Jones and Schedl, 1995). The location of the WHO mutation in the conserved loop of the KH domain is consistent with these results, and clearly points to the significance of this motif in the function of this class of genes. The codon that is mutated in who e44 was not mutated in the analyses of gld-1. However, missense mutations in close and conserved codons of this loop structure result in gld-1 gain-of-function (Francis et al., 1995).
Furthermore, genetic analyses indicated that the gain-of-function mutant defects associated with the conserved loop structure require RNA binding function. This is intriguing, as it has been suggested that the conserved loop structure may function as a protein-protein interaction domain (Musco et al., 1996). Thus, it is possible that members of this family bind RNA as part of a protein complex.

Considerable evidence indicates that KH domains bind single-stranded RNA (Leffers et al., 1995; Liu and Hanna, 1995; Arning et al., 1996). Assuming that who encodes a molecule that binds RNA, one can hypothesize biochemical functions that are as different as splicing, transport, and stability. The role of KH RNA binding proteins is no clearer if one considers the diversity of developmental functions that have been attributed to this gene family. Mutations in previously identified members of this gene family result in developmental aberrations including ovarian tumors in C. elegans (Jones and Schedl, 1995), defects in growth and neuron myelination in mice (Ebersole et al., 1996), and fragile X-linked mental retardation in humans (Siomi et al., 1993). WHO is very similar to QKI and GLD-1 (Fig. 8), but mutations affect such different tissues. The complexity of developmental processes that are regulated by KH RNA binding proteins combined with the evolutionary diversity of organisms that utilize these molecules, therefore, illustrates the importance of members of this gene family during development.

This work was initiated in the laboratory of Carl Thummel and I am grateful for his support. I also thank Daniel Wendel and Iam Madanat for technical assistance. Daniel Kiehart for antibodies. Patrick Hurban for cDNA libraries, Felix Karim for sharing northern blots, Daniel Doherty, Yuh Nung Jan, Todd Laverty, Andrea Brand, Kathy Matthews and the Drosophila Stock Center in Bloomington for fly stocks, the CAB sequencing facility, Michael Horner, Daniel Cimbora, Zhongchi Liu and Rolf Bodmer for numerous helpful discussions, and Stephen Mount and Zhongchi Liu for constructive comments on the manuscript. This research was initiated while E. H. B. was supported by a Howard Hughes Medical Institute fellowship from the Life Sciences Research Foundation.

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