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

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SUMMARY

The *Drosophila who* (<u>w</u>ings <u>h</u>eld-<u>o</u>ut) 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 mult-inucleate 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 βPS-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-hydroxyecdysone (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 ecdysone-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 w̲ings h̲eld-o̲ut 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 gld-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* (Driever et al., 1989), *TM6B Hu Tb e* (Lindsley and Zimm, 1992), *ru h th st cu sr e^s ca* (Lindsley and Zimm, 1992), *Df(3R)e^BS2* (Lindsley and Zimm, 1992), P-element insertion lines *B1 3rd 12* and *E7 3rd 4* (Bier et al., 1989), *1A122* (Perrimon et al., 1991), and *2612* (provided by the Berkeley *Drosophila* Genome Project), and the imprecise-excision lines *rev4* (*who^r4*) and *rev17* (*who^r17*) 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 *w* marker gene were irradiated with 4,000 rad from a ^{137}Cs 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)93F^x2* and *who^x3*). *Df(3R)93F^x2* is homozygous lethal and is a deletion that spans from 93F5 to 94A8 as determined by analysis of polytene chromosome squashes. *who^x3* is homozygous viable and is a small lesion compared to *Df(3R)93F^x2* based on the lack of a visible aberration of the polytene chromosomes. In another screen, isogenized *ru h th st cu sr e^s 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)93F^x2/TM6B Hu Tb e* virgin females. The progeny of the latter cross were screened for lethality in combination with *Df(3R)93F^x2* and the resulting lethal lines were further screened for lethality in combination with the *who^r4* and *who^r17* mutant lines. After screening 7,914 viable lines, a single mutation was isolated, *who^e44*. 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*, *1A122*, 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 *who^e44* mutant chromosome was maintained over the marked balancer chromosome *TM3 Sb e hblacZ*. The entire open reading frame was sequenced from homozygous *who^e44* mutant and the *ru h th st cu sr e^s ca* parental chromosome where this mutant was induced. This was accomplished by collecting embryos from a *who^e44/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^s*

Fig. 1. Animals lacking *who* function possess defects in skeletal orientation and muscle development. (A) Wild-type and (B) *who^e44* 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 *who^e44* 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.
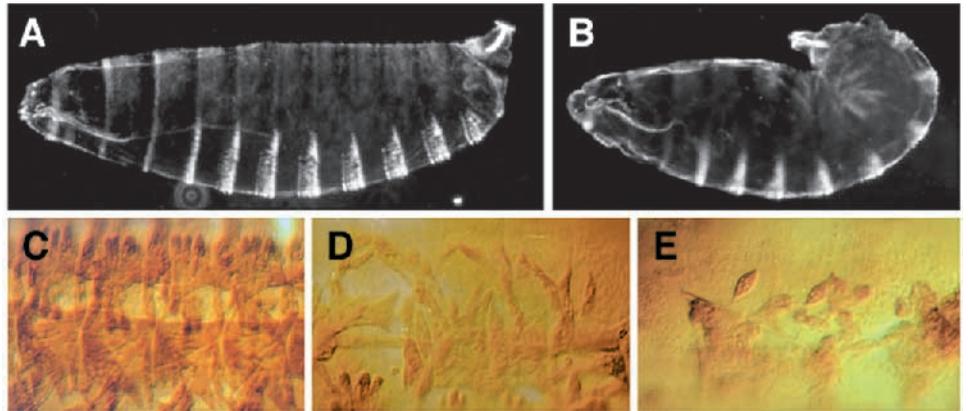
ca embryos. Each exon was amplified independently by using primers designed against intron sequences. PCR products were purified from agarose using the Wizard Purification System (Promega). DNA was then sequenced as described. Multiple templates that were generated from independent samples of each genotype were sequenced on both strands.

Analysis of expression patterns

lacZ activity was analyzed in *who* enhancer trap lines during two stages of development – embryogenesis and metamorphosis. For embryonic analyses, embryos were collected, dechorionated, fixed in equal amounts of 4% paraformaldehyde and heptane, and analyzed for X-gal activity as described by O'Kane and Gehring (1987). A similar technique was used for staged larvae and pupae, except that they were dissected by making an incision along the anterior to posterior axis prior to fixation.

In situ detection of transcripts was conducted using previously described techniques (Tautz and Pfeifle, 1989). *who* probes were synthesized by random priming a 800 bp *Bst*XI fragment from *who* cDNA 4.

To determine if *who* transcription is inducible by ecdysone, northern blots were hybridized with a random-primed 800 bp *Bst*XI fragment from *who* cDNA 4 as previously described (Karim and Thummel, 1992). These northern blots have been used to study ecdysone regulation of numerous steroid responsive genes.

RESULTS

Isolation of *who* mutations

who was first identified in the P-transposable element enhancer trap screens conducted in the Jan, Perrimon, and Scott labora-

tories (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 ^{137}Cs source. These mutants vary in their severity of *who* aberration, from complete loss-of-function (*who^r4*) to partial loss-of-function (*who^r17*, *who^x3*). The *who^r4* 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 (*who^e44*) completely lacks *who* function – the homozygous *who^e44* phenotype is the same as the *who^e44/Df(3R)93F^x2* 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. *who^e44* homozygotes appear to completely lack function. *who^e44* 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. *who^e44* 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 *who^e44* mutants have differenti-

Fig. 2. Animals with partial-loss-of-*who*-function either die during metamorphosis with defects in head eversion or survive to adulthood and possess an altered wing position phenotype. (A) Wild-type and (B) *who^r17* mutant pupae. Weak *who* mutants frequently die during pupal development with their head stuck in their thorax (note the partial exposure of the eye indicated with an arrow). (C) Wild-type adult flies with normal wing position. (D) Many of the flies that are homozygous for the *who^r17* allele die during metamorphosis, but escapers that survive to adulthood have a strong held-out wing phenotype and often have blisters on the wing (arrow).
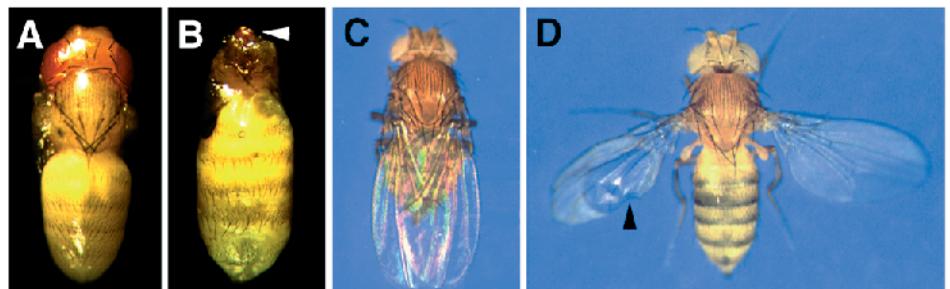
Fig. 3. Physical map of the *who* locus. The chromosomal walk (cosmid clones) spans from 93F9 to 93F14. Genetic aberrations (transposable element insertions and deficiencies) are indicated with the position of their insertion sites and breakpoints relative to the physical map. The *who* RNA transcript is mapped onto the genomic DNA. The *Df(3R)e^{BS2}* and *Df(3R)who^{r4}*

deficiencies remove part of the *who* transcript, while the *Df(3R)93F^{x2}* deficiency deletes the entire region. The *who* open reading frame starts in the first exon and stops in the seventh exon.
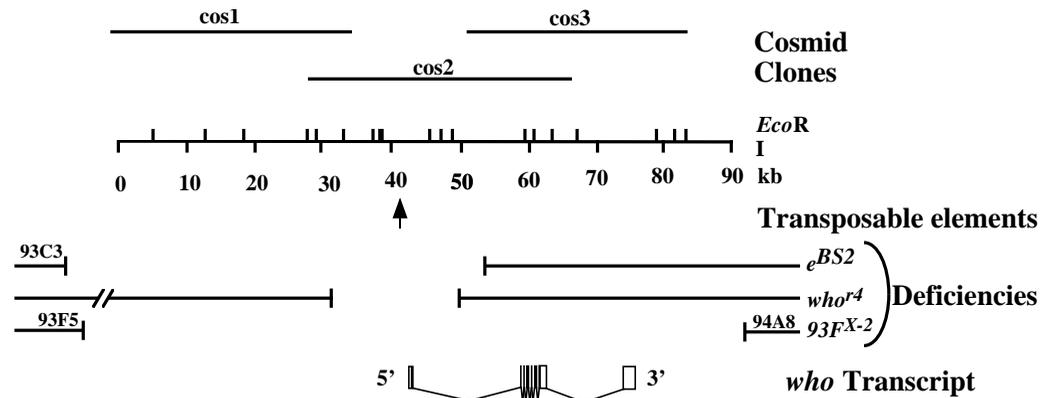
ated and fused myotubes as indicated by staining with antibodies directed against myosin heavy chain, but these cells display a disorganized pattern and are round-shaped compared to cells of wild-type animals (Fig. 1C-E). The expressivity of this phenotype is variable – the myotubes of some embryos appear to migrate but are disorganized and don't possess the proper pattern of attachment (Fig. 1D), while the myotubes of other embryos show no signs of migration and actually appear to have fewer myoblasts (Fig. 1E). *who* function appears to be specific to muscle, however, since analyses of other tissues have not revealed any defects.

Partial loss-of-*who*-function results in lethality during the metamorphosis from a larva to an adult. Most (90%) *who^{r17}* homozygotes die during metamorphosis with their head stuck in their thorax (Fig. 2A,B). If *who^{r17}* is combined with either *who^{e44}* or a deletion in this region all animals die during metamorphosis. A small number of *who^{r17}* homozygotes escape as adults that do not fold their wings properly and, thus, possess the phenotype for which this gene was named (Fig. 2C,D). These flies also have blisters on their wings (Fig. 2D). *who^{x3}* homozygotes are viable, but also possess defects in wing position. When either *who^{x3}* or the *2612* P-element insertion are combined with stronger alleles the resulting progeny possess partial pupal lethality and more severe wing defects, indicating that these mutations form an allelic series. Therefore, the strength of the these alleles is ordered *who^{e44}*, *who^{r17}*, *who^{x3}*, and the *2612* P-element from strongest to weakest.

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)e^{BS2}* deficiency deletes from 93B to 93F14, the *Df(3R)who^{r4}* deficiency spans approximately 18 kb, while the *Df(3R)93F^{x2}* 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).

*Eco*RI 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, *who^{r17}* possesses an aberration in the size of this RNA transcript (data not presented), and *who^{r17}* fails to complement the *who^{e44}* null allele. Most significantly, *who^{e44}* 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,F).

Previous studies indicated that *who* transcription increases at the onset of metamorphosis. During studies of the ecdysone-regulated *E93* gene, we detected two RNAs with genomic probes from the 93F locus that increased in transcription at the onset of metamorphosis (Baehrecke and Thummel, 1995). We now know that the smaller (4.0 kb) transcript encodes the *who*

gene. We were interested in determining if *who* is expressed in a similar pattern during embryogenesis and metamorphosis, 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 *who*^{e44} null allele, the exons containing coding sequence of homozygous mutant and the parental chromosome that was used for this mutagenesis were sequenced. *who*^{e44} 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* (Siomi 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

Fig. 4. *who* is transcribed in presumptive muscle and muscle attachment site cells during *Drosophila* embryogenesis as detected by whole-mount embryonic RNA in situ hybridization (dark staining). (A) *who* transcription is first detected at the onset of gastrulation in the mesodermal precursor cells on the ventral side of the embryo (arrow). (B) As development proceeds to the germ band extended stage, *who* transcription continues in mesoderm precursors. (C) When mesodermal cell lineages divide, *who* is detected in both somatic (arrowhead) and visceral (arrow) muscle cell precursors. (D) As dorsal closure occurs, *who* transcription is detected in 2 patches of pharyngeal cells in the anterior end of the embryo, cardiac precursors (arrowhead), and muscle attachment sites (arrow). (E) *who* transcript is detected in the heart when dorsal closure is complete (arrow). (F) Late in embryogenesis, *who* is transcribed in epidermal muscle attachment sites (arrow). All embryos are oriented with the anterior end to the left. A-C and F, lateral views; D and E, dorsal views.
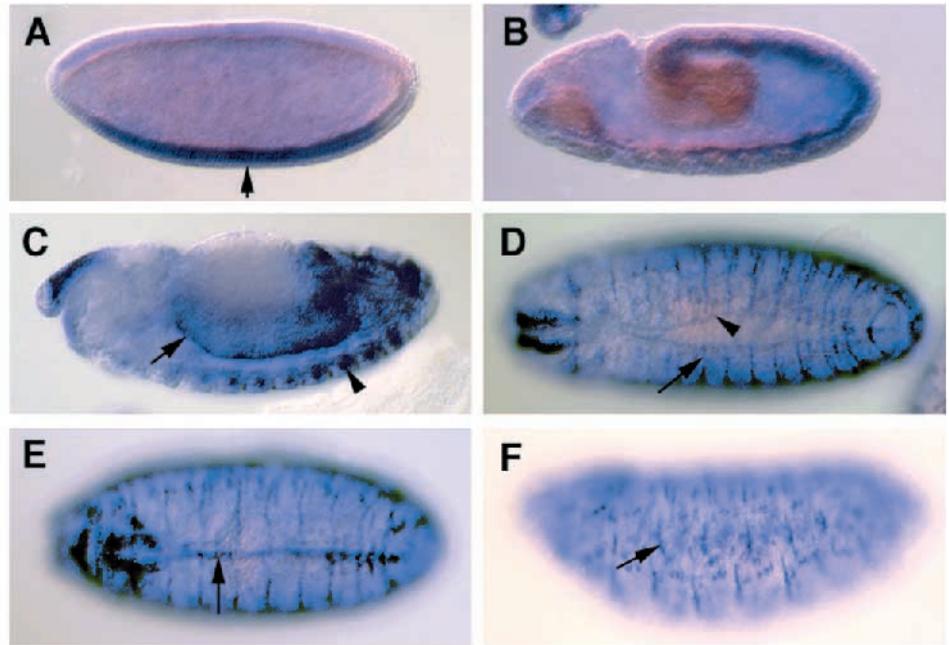
al., 1996). Like *who*, both the integrins and *stripe* are expressed in a similar population of cells when adult muscle development is occurring during metamorphosis (Fernandes et al., 1996).

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
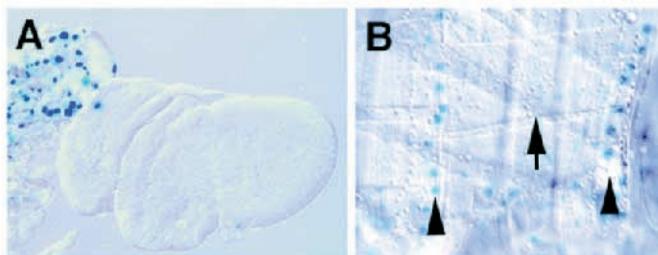

Fig. 5. *who* is transcribed in presumptive muscle and muscle attachment site cells during *Drosophila* metamorphosis. The spatial pattern of *who* expression was analyzed at the onset of metamorphosis by staining a *who* enhancer-trap line for β-galactosidase activity (blue staining). *who* expression is detected (A) in muscle cells attached to structures that will become the adult wing, and (B) in parallel rows of adult muscle attachment site cells (arrowheads) lining the body wall and oriented perpendicular to muscle fibers (arrow).
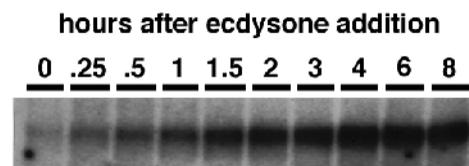
Fig. 6. *who* is induced by ecdysone at the onset of metamorphosis. Third larval instar *Drosophila* were isolated prior to the increase in hormone titer, cultured for 8 hours, and 5.5×10^{-6} M ecdysone was added to each culture for various periods of time as listed above each lane (therefore, the 0 hour lane is actually tissues that were cultured for 8 hours, but never had ecdysone added and is a negative control). Following culture, *who* transcription was analyzed by northern blot hybridization. Note that *who* transcription increases with duration of exposure to hormone, even though equal amounts of RNA are loaded/time point (data not presented). This northern blot has been used for the analysis of numerous ecdysone-regulated genes (Karim and Thummel, 1992).

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CGTTGCTGTCGGGTTCAGTGGTAGGAAAATTAGGAGGAATCGTTCAGTAAAGTCCCGAGATCCGTTCGCTATTCCCCGACTCGAATAAAA          90
TCCATATACACATATATCAAATAAACAAAGGATAGGAGTCCTCAGAGAAGAAAATAACGTGTTAAAAGTAGCAAAAACAAATAAAAAAGA         180
AGTGTGCACCAAGTAAAGAAAGCAGAGACCCAAGGAAAGTTGAAAGTTGAAAACCAGAATCTCGTCGAGAACAAAACAAAAAGTTCCCAA         270
AAACCCACACATCACACACGACACGCAACAAAATGAGTGTCTGTGAGAGCAAAGCCGTTGTGCAACAGCAACTGCAGCAGCACTTGCAGCA         360
                                       M  S  V  C  E  S  K  A  V  V  Q  Q  Q  L  Q  Q  H  L  Q  Q      20
ACAGGCAGCCGCAGCAGTTGTTGCGGTCGCGCAACAGCAGCAGGCTCAAGCCCAAGCCCAGGCTCAGGCTCAGGCACAGCAGCAGCAACA         450
  Q  A  A  A  A  V  V  A  V  A  Q  Q  Q  Q  A  Q  A  Q  A  Q  A  Q  A  Q  A  Q  Q  Q  Q  Q         50
GGCGCCGCAGGTGGTGGTCCCCATGACCCCGCAGCACTTGACCCCACAGCAGCAGCAGCAGAGCACACAGAGCATCGCCGACTATCTGGC         540
  A  P  Q  V  V  V  P  M  T  P  Q  H  L  T  P  Q  Q  Q  Q  Q  S  T  Q  S  I  A  D  Y  L  A         80
                                                              gtaagtcgct...V...atcttttcag
CCAGTTGCTCAAGGACCGCAAGCAACTGGCCGCCTTCCCCAACGTCTTCACCCACGTCGAACGCCTGCTGGACGAAGAAATTGCACGCGT         630
  Q  L  L  K  D  R  K  Q  L  A  A  F  P  N  V  F  T  H  V  E  R  L  L  D  E  E  I  A  R  V        110
GCGCGCCTCACTGTTCCAGATCAATGGGGTCAAGAAGGAGCCGCTCACTCTGCCCGAACCCGAGGGCTCTGTGGTG̲A̲C̲G̲A̲T̲G̲A̲A̲C̲G̲A̲G̲A̲A̲         720
  R  A  S  L  F  Q  I  N  G  V  K  K  E  P  L  T  L  P  E  P  E  G  S  V  V  T  M  N  E  K        140
                              gtaagtgttc...V...ttcattccag
GGTTTATGTGCCAGTCCGCGAGCATCCAGATTTCAACTTTGTCGGTCGCATTTTGGGACCCCGTGGCATGACCGCCAAGCAATTGGAACA         810
  V  Y  V  P  V  R  E  H  P  D  F  N  F  V  G  R  I  L  G  P  R  G  M  T  A  K  Q  L  E  Q        170
                                                     gtgagtgcat...V...ccgttttcag
GGAGACCGGCTGCAAGATCATGGTCCGAGGCAAGGGTTCCATC[G]GCGACAAGAAGAAGGAGGACGCCAACCGTGGCAAGCCTAACTGGGA         900
  E  T  G  C  K  I  M  V  R  G  K  G  S  M  [R] D  K  K  K  E  D  A  N  R  G  K  P  N  W  E        200
GCATCTGTCCGATGACCTGCATGTCCTGATAACCGTCGAGGACACCGAGAACCGTGCCACAGTGAAGTTGGCCCAGGCCGTCGCCGAAGT         990
  H  L  S  D  D  L  H  V  L  I  T  V  E  D  T  E  N  R  A  T  V  K  L  A  Q  A  V  A  E  V        230
                 gtaagtgttt...V...gtactaacag
ACAGAAGTTGCTCGTGCCGCAAGCCGAAGGCGAAGATGAGCTAAAGAAACGTCAACTCATGGAATTGGCGATTATTAATGGCACTTATAG        1080
  Q  K  L  L  V  P  Q  A  E  G  E  D  E  L  K  K  R  Q  L  M  E  L  A  I  I  N  G  T  Y  R        260
                 gtatgtcaac...V...tatcccgcag
GGACACAACAGCGAAATCTGTCGCAGTGTGCGATGAGGAGTGGCGCCGCCTGGTTGCCGCCTCTGATAGCCGCCTGCTGACATCCACCGG        1170
  D  T  T  A  K  S  V  A  V  C  D  E  E  W  R  R  L  V  A  A  S  D  S  R  L  L  T  S  T  G        290
CCTGCCCGGCCTTGCCGCCCAGATCCGTGCACCCGCCGCCGCCCCGCTTGGCGCCCCATTGATCCTGAATCCCCGGATGACCGTCCCCAC        1260
  L  P  G  L  A  A  Q  I  R  A  P  A  A  A  P  L  G  A  P  L  I  L  N  P  R  M  T  V  P  T        320
AACGGCGGCCAGCATATTGTCCGCCCAGGCCGCTCCGACAGCCGCCTTCGACCAGACCGGCCATGGAATGATCTTCGCACCGTACGATTA        1350
  T  A  A  S  I  L  S  A  Q  A  A  P  T  A  A  F  D  Q  T  G  H  G  M  I  F  A  P  Y  D  Y        350
                             gtgggttgtt...V...tatattttag
TGCGAACTACGCCGCCCTAGCCGGCAATCCTCTGCTCACGGAATATGCTGATCATAGCGTAGGCGCCATTAAGCAGCAGCGTCGTTTGGC        1440
  A  N  Y  A  A  L  A  G  N  P  L  L  T  E  Y  A  D  H  S  V  G  A  I  K  Q  Q  R  R  L  A        380
GACTAACCGCGAGCATCCCTATCAGCGAGCAACGGTCGGAGTGCCAGCCAAGCCGGCGGGTTTCATTGAGATACAGTAAATACCATGCAT        1530
  T  N  R  E  H  P  Y  Q  R  A  T  V  G  V  P  A  K  P  A  G  F  I  E  I  Q                       405
AGTGAATCGAGAAATTCGAAGCACTATAACTCAGAAAGTAACACTCTAAAATCACACCATATCGGCGAAAGCAACTTTATAACTTTAAAT        1620
CAATAATGTACGAAAGAAGAAAAGAAACCATTAAGAACAACGGATAACGAACGATTGCGTTTGCCTGCCCTTTGATTGCGTACGCCAGGC        1710
AGCCGATCACTTTAGTTCAATTATTTTAAAATTTTGCTATTTATTTTGATACAAATGCTGCTCATAACTGACCATCCCCAATTCCAATCC        1800
ACACTGTATGTTCGATGAAACCTGACCGGAATCGTTGGCCTGGCCTGGCCGCTCTGCCCTGATCAGGGTTCTGAAAAAGAATTTTCAAGC        1890
           gttagttgtg...V...cctgccgtag
GCACCGCCTCCACAGATGAGCACGGAATCAGAGCAGCAGCAGCATCCCAGCCAGGCCGAAAGCAGAGCCACGTGTGTGTTGTGAATGTGC        1980
TTACTTGAAGCTAACAAAAAAGCGTAACCAAAAGTCAGATATCCTGAGCCACCACATGTCACTTACACAACTACTAAAACACTGAACCCC        2070
TCCGAACGCCAAACTCCAAACTCTGAACCTATTTGCCAAATGAGCAGCAGATGCGGCACATTCAACACCAGCAGCATCACAACATCAACA        2160
GCAAAAGCAAAAGCAAAAGCAACAGCAACAGCAAACGAACAGCAGCAACATCAGTATGTATTGAATCTTGTGGCCATTAAACTGTTACGT        2250
TTTGCCAGCAAACAAAACAAGGCAATTAAGCAAACGATTGATAAGAGAATCAAACATAGAGCTTAGTTTAATTATGATAATCTAAAGGAA        2340
AATACAAGTTAGTTCTTAGTGTAAAAGTTCAACGAACAAAAACATACTTAATGCAAGCATATACATATATATATATATTTAAATATATAT        2430
ACATCCAAATGCATATACAAGTCTGTATATAATAACCCCAAAAGCCCCTAAGCACAAAAAATAAGGAAAATCATGTTAACGGAATCTCAT        2520
CCAAGAAACTCTACTCAACCAACCAAAACTGTTTTAATTTTTTTAAAAATCTGCACTTTTAACTCAAATTGCAAAGATGAATCTTTACAC        2610
TTTTCATTGGATTGTGCATGGAAGTCTATTTAAATCCATATTGTGTCGGATATAGACTATCCACCCTTCGATTAAGCATAATCTATTTTT        2700
GTTAAACTGACGCTAATTCTTGTTAATGAAGTATTTTTTTGATTTGTTATTGCATATCTATATTTTATGTATAGATTACGTACCCCACTTC        2790
AAATCGATCGTGTGATGAGTAAGTGGGGGCGAAACAGAAACAACATATTAAAACACATTTAACAGCCATATTTAGTTAATGAAACAAACA        2880
TATATTATGATAATAGCAGCAAGCATTCATAATGTACAACTAAACGCTAAACTTAAATTATTTTTAATATTTTAAAGCTAATGTATTATA        2970
TGCAGAAATAAAACACAAACTTACTTTTTTTAAACGAATCATTGTCCTTTGAATGCGATTGCCACAACAAAATTGAATTCTTTAATTTAA        3060
AATAAATATCACGCCGCCAGTAATGGCAAAAATGTATCTATTTTATAATAGTGAATGAAATAAACAAAATGCAGAAAAATTAGCACGGAA        3150
CAAAACTAGAAAGCGAAAGTAAATTGCATTTAATTTATGGTACAAAGCAATCAACGCAAAGTTGTTGCAAACTTTATTTTAAAATTAAAGA        3240
ATTTGCATTAAATTTTGTTGACAAAAAATAAATCTTATCAATATGTGCCTTAAACAAATTGTATAATTGGCACCAATCAAATGAAAATT        3330
AAATATAAAACAATGAAAAGTATACAGAAACTCTGTTGAATTATTCTGCGAAATGTGAGACTTATACTCAGAAACAAAACGATGTTCAAC        3420
CACATGGGAGTTGACTAGTCTTTTAATGTGAATAAACTAATCATAATGGATAATTTTTATTGACAATGACGGAGATCGTGCAACGTAGCA        3510
AACTTTGCTTATGATGAGGTTCCGGATAAAATTTTATCCCGATGGAAAGGGATCCTTCGGTAATTGGGGACGGCAAACGGCAACCGTAGC        3600
AAACTTTGCTTATGATGAGTCAGAATTAATTTTATCACGATGAAATGTAACCTTTCGATAATTGAATAACAGAAAGTAAACGATCGTCCG        3690
TTTTATTCACATTACATTAACGTATGCTGTGCCCCTAACATCGCATAATAAACATCATTCATTCATTGCCAGCATTGTATAATTTTAACT        3780
TCATAATTTGAGAAAGAAATCATTGATTTTTTGTGAGATAATCCATATGTTCAAAACCTAATAATATAAATCTAAAGCAAACGCACATGT        3870
AATTAAATGCATATGTACGAGCGTGTGCACATATGTACGATTATGTATAACTTTTTGCATAAATAAATGTTTATAAAAAAAAAAAAAAAA        3960
AAAAAAAAAAAAAAA                                                                                  3970
```

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*[e44] 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.
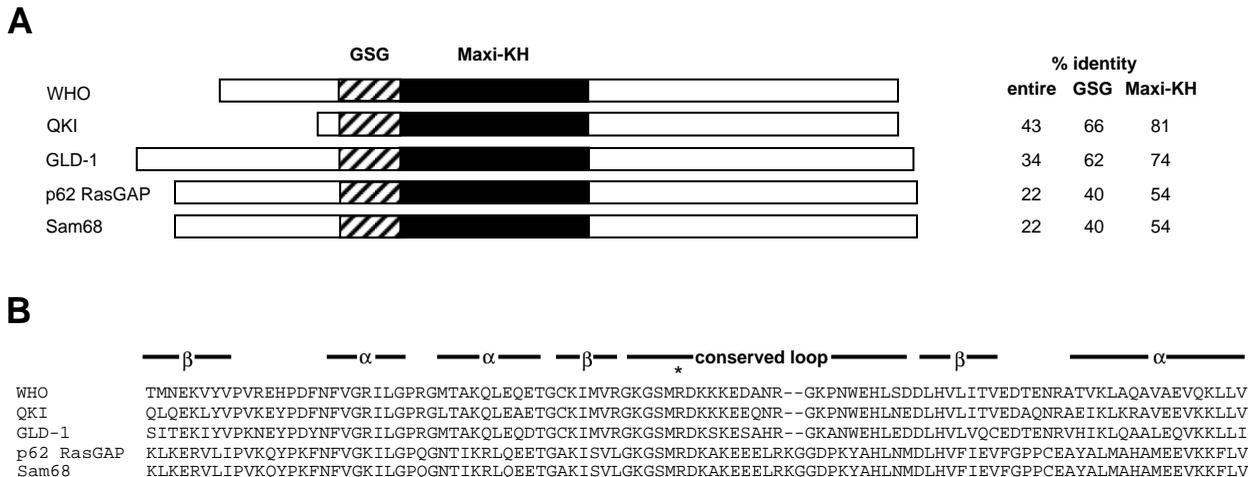
A

B


```
                β            α            α        β       *conserved loop           β                α
WHO       TMNEKVYVPVREHPDFNFVGRILGPRGMTAKQLEQETGCKIMVRGKGSMRDKKKEDANR--GKPNWEHLSDDLHVLITVEDTENRATVKLAQAVAEVQKLLV
QKI       QLQEKLYVPVKEYPDFNFVGRILGPRGLTAKQLEAETGCKIMVRGKGSMRDKKKEEQNR--GKPNWEHLNEDLHVLITVEDAQNRAEIKLKRAVEEVKKLLV
GLD-1     SITEKIYVPKNEYPDYNFVGRILGPRGMTAKQLEQDTGCKIMVRGKGSMRDKSKESAHR--GKANWEHLEDDLHVLVQCEDTENRVHIKLQAALEQVKKLLI
p62 RasGAP KLKERVLIPVKQYPKFNFVGKILGPQGNTIKRLQEETGAKISVLGKGSMRDKAKEEELRKGGDPKYAHLNMDLHVFIEVFGPPCEAYALMAHAMEEVKKFLV
Sam68     KLKERVLIPVKQYPKFNFVGKILGPQGNTIKRLQEETGAKISVLGKGSMRDKAKEEELRKGGDPKYAHLNMDLHVFIEVFGPPCEAYALMAHAMEEVKKFLV
```

Fig. 8. WHO 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.

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 *E74* gene (Fletcher et al., 1995). Also, the wings held out *who* mutant phenotype represents perturbation of adult development during metamorphosis. The similarity of defects in mutants of the steroid regulated *BR-C* gene (Restifo and White, 1992) and the muscle cell migration and attachment phenotypes of *stripe* mutants (Costello and Wyman, 1986; Frommer et al., 1996) also indicate that ecdysone regulates adult muscle cell development during metamorphosis.

Embryonic and adult muscles develop from a distinct sets of precursor cells (Bate et al., 1991). Cells that are fated to become adult muscles use many of the genes that function in embryonic muscle and muscle attachment site development during metamorphosis (Fernandes et al., 1996). Like *myospheroid* and *stripe*, *who* functions during the development of adult muscles and is expressed in both muscle and muscle attachment site cells at this time. What regulates this stage- and cell-specific expression? Northern blot analyses indicate that *who* transcription increases at the onset of metamorphosis, and that this transcription is inducible by ecdysone in culture. This finding raises the possibility that other genes that function in muscle development during metamorphosis could be steroid-regulated. Conversely, some genes could be selectively induced by ecdysone to regulate critical events in the initiation of larval to adult changes. The specificity of the pattern of *who* expression indicates the presence of a combination of stage- and cell-specific regulatory mechanisms. Understanding the mechanisms of *who* regulation should explain how steroids regulate such specific responses.

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.

REFERENCES

Abmayr, S. M., Erickson, M. S. and Bour, B. A. (1995). Embryonic development of the larval body wall musculature of *Drosophila melanogaster*. *Trends Genet.* **11**, 153-159.

Arning, S., Gruter, P., Bilbe, G. and Kramer, A. (1996). Mammalian splicing factor SF1 is encoded by variant cDNAs and binds to RNA. *RNA* **2**, 794-810.

Baehrecke, E. H. and Thummel, C. S. (1995). The Drosophila E93 gene from the 93F early puff displays stage- and tissue-specific regulation by 20-hydroxyecdysone. *Dev. Biol.* **171**, 85-97.

Bate, M. (1990). The embryonic development of larval muscles in *Drosophila*. *Development* **110**, 791-804.

Bate, M. (1993). The mesoderm and its derivatives. In *The development of Drosophila melanogaster*, **II** (ed. M. Bate and A. Martinez Arias), pp. 1013-1090. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Bate, M., Rushton, E. and Currie, D. A. (1991). Cells with persistent *twist* expression are the embryonic precursors of adult muscles in *Drosophila*. *Development* **113**, 79-89.

Bier, E., Vaessin, H., Sheperd, S., Lee, K., McCall, K., Barbel, S., Ackerman, L., Carretto, R., Uemura, T., Grell, E., Jan, L. Y. and Jan, Y. N. (1989). Searching for pattern and mutation in the *Drosophila* genome with a P-*lacZ* vector. *Genes Dev.* **3**, 1273-1287.

Bogaert, T., Brown, N. and Wilcox, M. (1987). The *Drosophila* PS2 antigen is an invertebrate integrin, that like the fibronectin receptor, becomes localized to muscle attachments. *Cell* **51**, 929-940.

Brown, N. H. (1994). Null mutations in the α_{PS2} and β_{PS} integrin subunit genes have distinct phenotypes. *Development* **120**, 1221-1231.

Callahan, C. A., Bonkovsky, J. L., Scully, A. L. and Thomas, J. B. (1996). *derailed* is required for muscle attachment site selection in *Drosophila*. *Development* **122**, 2761-2767.

Callahan, C. A., Muralidhar, M. G., Lundgren, S. E., Scully, A. L. and Thomas, J. B. (1995). Control of neural pathway selection by a *Drosophila* receptor protein-tyrosine kinase family member. *Nature* **376**, 171-174.

Chen, T., Bunting, M., Karim, F. D. and Thummel, C. S. (1992). Isolation and characterization of five *Drosophila* genes that encode an *ets*-related DNA binding domain. *Dev. Biol.* **151**, 176-191.

Costello, W. J. and Wyman, R. J. (1986). Development of indirect flight muscle in a muscle-specific mutant of *Drosophila melanogaster*. *Dev. Biol.* **118**, 247-258.

Driever, W., Thoma, G. and Nusslein-Volhard, C. (1989). Determination of the spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the bicoid morphogen. *Nature* **340**, 363-367.

Ebersole, T. A., Chen, Q., Justice, M. J. and Artzt, K. (1996). The *quaking* gene product necessary in embryogenesis and myelination combines features of RNA binding and signal transduction proteins. *Nature Genet.* **12**, 260-265.

Fernandes, J. J., Celniker, S. E. and VijayRaghavan, K. (1996). Development of the indirect flight muscle attachment sites in *Drosophila*: role of the PS integrins and the *stripe* gene. *Dev. Biol.* **176**, 166-184.

Fletcher, J. C., Burtis, K. C., Hogness, D. S. and Thummel, C. S. (1995). The *Drosophila E74* gene is required for metamorphosis and plays a role in the polytene chromosome puffing response to ecdysone. *Development* **121**, 1455-1465.

Francis, G. R., Barton, M. K., Kimble, J. and Schedl, T. (1995). *gld-1*, a tumor suppressor gene required for oocyte development in *C. elegans*. *Genetics* **139**, 579-606.

Frommer, G., Vorbruggen, G., Pasca, G., Jackle, H. and Volk, T. (1996). Epidermal egr-like zinc finger protein of *Drosophila* participates in myotube guidance. *EMBO J.* **15**, 1642-1649.

Halfon, M. S., Hashimoto, C. and Keshishian, H. (1995). The *Drosophila Toll* gene functions zygotically and is necessary for proper motoneuron and muscle development. *Dev. Biol.* **169**, 151-167.

Jones, A. R. and Schedl, T. (1995). Mutations in *gld-1*, a female germ cell-specific tumor suppressor gene in *Caenorhabditis elegans*, affect a conserved domain also found in Src-associated protein Sam68. *Genes Dev.* **9**, 1491-1504.

Karim, F. D. and Thummel, C. S. (1992). Temporal coordination of regulatory gene expression by the steroid hormone ecdysone. *EMBO J.* **11**, 4083-4093.

Kiehart, D. P. and Feghali, R. (1986). Cytoplasmic myosin from *Drosophila melanogaster*. *J. Cell. Biol.* **103**, 1517-1525.

Lamka, M. L., Boulet, A. M. and Sakonju, S. (1992). Ectopic expression of UBX and ABD-B proteins during *Drosophila* embryogenesis: competition, not a functional hierarchy, explains phenotypic suppression. *Development* **116**, 841-854.

Lee, J. C., VijayRaghaven, K., Celniker, S. E. and Tanouye, M. A. (1995). Identification of a *Drosophila* muscle development gene with structural homology to mammalian early growth response transcription factors. *Proc. Natl. Acad. Sci. USA* **92**, 10344-10348.

Leffers, H., Dejgaard, K. and Celis, J. E. (1995). Characterization of two major cellular poly(rC)-binding proteins each containing three K-homologous (KH) domains. *Eur. J. Biochem.* **230**, 447-453.

Leptin, M., Bogaert, T., Lehmann, R. and Wilcox, M. (1989). The function of PS integrins during *Drosophila* embryogenesis. *Cell* **56**, 401-408.

Lewis, E. B. and Bacher, F. (1968). Methods of feeding ethyl methane sulfonate (EMS) to *Drosophila* males. *Dros. Info. Service* **43**, 193.

Lindsley, D. L. and Zimm, G. G. (1992). The genome of *Drosophila melanogaster*. San Diego: Academic Press.

Liu, K. and Hanna, M. M. (1995). NusA contacts nascent RNA in *Escherichia coli* transcription complexes. *J. Mol. Biol.* **247**, 547-558.

Macdonald, P. M., Ingham, P. W. and Struhl, G. (1986). Isolation, structure, and expression of *even skipped*: a second pair-rule gene of *Drosophila* containing a homeobox. *Cell* **47**, 721-734.

Mount, S. M., Burks, C., Hertz, G., Stormo, G. D., White, O. and Fields, C. (1992). Splicing signals in *Drosophila*: intron size, information content, and consensus sequences. *Nucl. Acids Res.* **20**, 4255-4262.

Musco, G., Stier, G., Joseph, C., Morelli, M. A. C., Nilges, M., Gibson, T. J. and Pastore, A. (1996). Three dimensional structure and stability of the KH domain: molecular insights into fragile X syndrome. *Cell* **85**, 237-245.

Nandabalan, K., Price, L. and Roeder, G. S. (1992). Mutations in U1 snRNA

bypass the requirement for a cell type-specific RNA splicing factor. *Cell* **73**, 407-415.

O'Kane, C. J. and Gehring, W. J. (1987). Detection in situ of genomic regulatory elements in *Drosophila. Proc. Natl. Acad. Sci. USA* **84**, 9123-9127.

Perrimon, N., Noll, E., McCall, K. and Brand, A. (1991). Generating lineage-specific markers to study *Drosophila* development. *Dev. Genet.* **12**, 238-252.

Regnier, P., Grunberg-Manago, M. and Portier, C. (1987). Nucleotide sequence of the pnp gene of *E. coli* encoding polynucleotide phosphorylase. *J. Biol. Chem.* **262**, 63-68.

Restifo, L. L. and White, K. (1992). Mutations in a steroid hormone-regulated gene disrupt the metamorphosis of internal tissues in *Drosophila*: salivary glands, muscle, and gut. *Wilhelm Roux's Arch. Dev. Biol.* **201**, 221-234.

Richard, S., Yu, D., Blumer, K. J., Hausladen, D., Olszowy, M. W., Connelly, P. A. and Shaw, A. S. (1995). Association of p62, a multifunctional SH2- and SH3-domain binding protein, with *src* family tyrosine kinases, Grb2, and phospholipase C gamma-1. *Mol. Cell. Biol.* **15**, 186-197.

Riddiford, L. M. (1993). Hormones and *Drosophila* development. In *The development of Drosophila melanogaster*, **II** (ed. M. Bate and A. Martinez Arias) pp. 899-940. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Robertson, C. W. (1936). The metamorphosis of *Drosophila melanogaster*, including an accurately timed account of the principal morphological changes. *J. Morphol.* **59**, 351-399.

Siomi, H., Siomi, M. C., Nussbaum, R. L. and Dreyfuss, G. (1993). The protein product of the fragile X gene, *FMR1*, has characteristics of an RNA-binding protein. *Cell* **74**, 291-298.

Spradling, A. C., Stern, D. M., Kiss, I., Roote, J., Laverty, T. and Rubin, G. M. (1995). Gene disruptions using P transposable elements: an integral component of the *Drosophila* genome project. *Proc. Natl. Acad. Sci. USA* **92**, 10824-10830.

Tautz, D. and Pfeifle, C. (1989). A non-radioactive method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback. Chromosoma* **98**, 81-85.

Volk, T. and VijayRaghaven, K. (1994). A central role for epidermal segment border cells in the induction of muscle patterning in the *Drosophila* embryo. *Development* **120**, 59-70.

Wilson, C., Pearson, R. K., Bellen, H. J., O'Kane, C. J., Grossniklaus, U. and Gehring, W. J. (1989). P-element-mediated enhancer detection: an efficient method for isolating and characterizing developmentally regulated genes in *Drosophila. Genes Dev.* **3**, 1301-1313.

Wong, G., Muller, O., Clark, R., Conroy, L., Moran, M. F., Polakis, P. and McCormick, F. (1992). Molecular cloning and nucleic acid binding properties of the GAP-associated tyrosine phosphoprotein p62. *Cell* **69**, 551-558.

Zusman, S., Patel-King, R. S., ffrench-Constant, C. and Hynes, R. O. (1990). Requirements for integrins during *Drosophila* development. *Development* **108**, 391-402.