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Putative *Krüppel* target gene of novel sequence expressed in *Drosophila* VO5 muscle precursors

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Abstract *Krüppel* (*Kr*), a member of the gap class of *Drosophila* segmentation genes, encodes a zinc finger-type transcription factor. After blastoderm formation, *Kr* is expressed in various spatially and temporally restricted patterns of the developing embryo, including a subset of muscle precursors. By virtue of Krüppel in vitro binding sites, we identified a putative *Kr* target gene, termed *KrT95D*. It encodes a novel protein which contains evolutionarily conserved regions. *KrT95D* is expressed in spatially restricted patterns throughout embryogenesis. *Kr* and *KrT95D* expression overlap in several locations including muscle precursor cells, the tip cell of the Malpighian tubules and the ventral midline cells of the central nervous system. Results from the analysis of the *KrT95D* expression pattern in *Kr* loss-of-fuction and *Kr* gain-of-function embryos suggest that *Kr* activity is not essential for *KrT95D* expression in most locations of the embryo, except in the muscle precursors VO5.

Key words Conserved protein domains · Developmental expression pattern · Drosophila embryo · *Krüppel* · Muscle development

Introduction

The *Drosophila* segmentation gene *Krüppel* (*Kr*) encodes a zinc finger-containing transcription factor (Licht et al. 1990; Sauer and Jäckle 1991; Zuo et al. 1991). Tissue culture as well as in vitro transcription studies revealed that the *Kr* protein (Krüppel) functions as a concentration-dependent activator and repressor of transcription when acting from a single binding site close to a basal promoter (Sauer and Jäckle 1991). The Krüppel monomer causes activation whilst Krüppel dimers, formed at high concentrations, act as repressors (Sauer and Jäckle 1993). In vivo studies indicated that Krüppel functions mainly as a repressor by acting upon far-upstream enhancer elements of other segmentation genes during blastoderm formation (reviewed in Pankratz and Jäckle 1993). During later stages of embryogenesis, *Kr* activity plays multiple roles by acting in the development of the larval visual system (Schmucker et al. 1992), the kidney-like Malpighian tubules (Gaul and Weigel 1991; Hoch et al. 1994), the central nervous system (Romani et al. 1996), the stomatogastric nervous system (Gonzalez-Gaitan and Jäckle 1995) and the muscle pattern of the embryo (Ruiz-Gomez et al. 1997).

Using a molecular approach, we have identified a putative *Kr* target gene, termed *KrT95D*. It encodes a protein of novel sequence which contains two small domains in common with partial sequences of human and mouse cDNAs. *KrT95D* is expressed during oogenesis and in restricted patterns during embryogenesis which in part overlap with *Kr*-expressing cells. In at least one location, the precursor cells of the ventral oblique muscle 5 (VO5), *KrT95D* expression is absent when the precursors fail to express *Kr* due to a mutation. The results indicate that *Kr* activity is necessary to activate *KrT95D* expression in the VO5 muscle precursors, consistent with recent results indicating that *Kr* activity is necessary for the specification of a subset of muscles and their proper innervation during embryogenesis.

Material and methods

Isolation of genomic DNA containing Krüppel-binding sites

DNA fragments containing in vitro binding sites for Krüppel were isolated by whole genome PCR-immunoprecipitation (Kinzler and Vogelstein 1989). For this, 5 μg genomic DNA of Oregon R flies were incubated with the restriction endonuclease HaeIII to yield 70- to 350-bp-long DNA fragments. They were ligated to linkers of the sequence 5'-GAGTAGAATTCTAATATCTC-3'. After XhoI digest (which serves to cleave tandems of the ligated linkers) fol-

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lowed by a phenol extraction, about 200 ng DNA were incubated (30 min on ice) with 10–50 ng bacterially produced, purified *Kr* protein extract (Pankratz et al. 1989) in a total volume of 50 µl binding buffer [150 mM NaCl, 100 mM TRIS pH 7.5, 30 mM KCl, 0.5 mM ethylenedinitrilotetraacetic acid (EDTA), 1 mM Dithiothreitol (DTT), 10% glycerol, 10 µM $ZnSO_4$, 500 µg/ml bovine serum albumin (BSA), 500 ng poly dI/dC]. Twenty microlitres polyclonal rabbit anti-Krüppel antibodies (1: 100 diluted; Gaul et al. 1987) precoupled to protein A sepharose beads were added. After incubation (30 min on ice) DNA fragments bound to the Krüppel-antibody complex were spun down by centrifugation (500 × *g*). Unbound DNA fragments were removed by five washes in 1 ml binding buffer containing 400 instead of 150 mM NaCl (see above). The remaining DNA-containing pellet was treated with 200 µl proteinase K solution [500 µg proteinase K in 1ml 500 mM TRIS pH 9, 20 mM EDTA, 10 mM NaCl, 1% sodium dodecyl sulphate (SDS)] and the DNA was phenol extracted. The purified DNA fragments were amplified by PCR using catch linkers (see above) as primers. One fifth of the PCR reaction was used to repeat the above described immunoprecipitation and DNA purification steps. After five rounds of immunoprecipitation-PCR amplification, the amplified DNA fragments were digested with EcoRI (cleavage site within the catch linkers; see above) and cloned into the pBstKS II vector. DNA fragments were sequenced by the chain termination method (Sanger et al. 1977) using the USB sequencing kit to verify that they contain Krüppel-binding sites.

Cloning of the *KrT95D* gene and in situ hybridization

A 150-bp DNA fragment containing three in vitro Krüppel-binding sites was used to isolate genomic phages from an λFix genomic *Drosophila* DNA phage library (Canton S; Stratagene). Whole-mount in situ hybridizations (Tautz and Pfeifle 1989) to wild-type embryos were done with digoxygenin-labelled DNA fragments or by the double-labelling technique described by Hartmann and Jäckle (1995). cDNAs were isolated from a λgt 11 cDNA phage library prepared from 0 to 15-h-old embryos and from a λZap cDNA phage library (2- to 14-h-old embryos; Stratagene). Further genomic DNA fragments from the *KrT95D* transcription unit (see Fig. 1B) were isolated from an EMBL4 genomic *Drosophila* DNA phage library and from a cosmid library (Hoheisel et al. 1991). Localization of the *KrT95D* gene in polytene chromosomes, mapping of the cloned DNA, location of the Krüppel-binding DNA-fragment within the transcription unit and the structural analysis of the *KrT95D* gene and its transcripts (see Fig. 1) were done as described by Wimmer et al. (1993). The DNA sequence has been submitted to GenBank (accession number AF001796). Northern blot analysis with 10 µg poly(A)+ RNA of 0- to 16-h-old embryos prepared as described (Sambrook et al. 1989) was done according to Farrell (1993) using a 5.8-kb Xba genomic DNA fragment covering the open reading frame of the *KrT95D* gene as a probe.

Results and discussion

Isolation, localization, structure and expression of a putative *Kr* target gene

In order to identify putative target genes of Krüppel, we performed a search by employing the DNA binding properties of the Krüppel zinc finger domain. To this end, *Drosophila* genomic DNA was digested with Hae III, ligated to polymerase chain reaction (PCR) primers, incubated with recombinant Krüppel and co-immunoprecipitated with anti-Krüppel antibodies. After five rounds of repeated PCR amplifications and immunoprecipitations, the DNA fragments (between 70 and 350 bp in length) were cloned. They were examined for the presence of Krüppel-binding sites of the consensus sequence $AAAA^{C}/_{G}GGGTTAA$ (Pankratz et al. 1992; Small and Levine 1991; Stanojevic et al. 1989; Treisman and Desplan 1989) by sequencing (for details see Materials and methods).

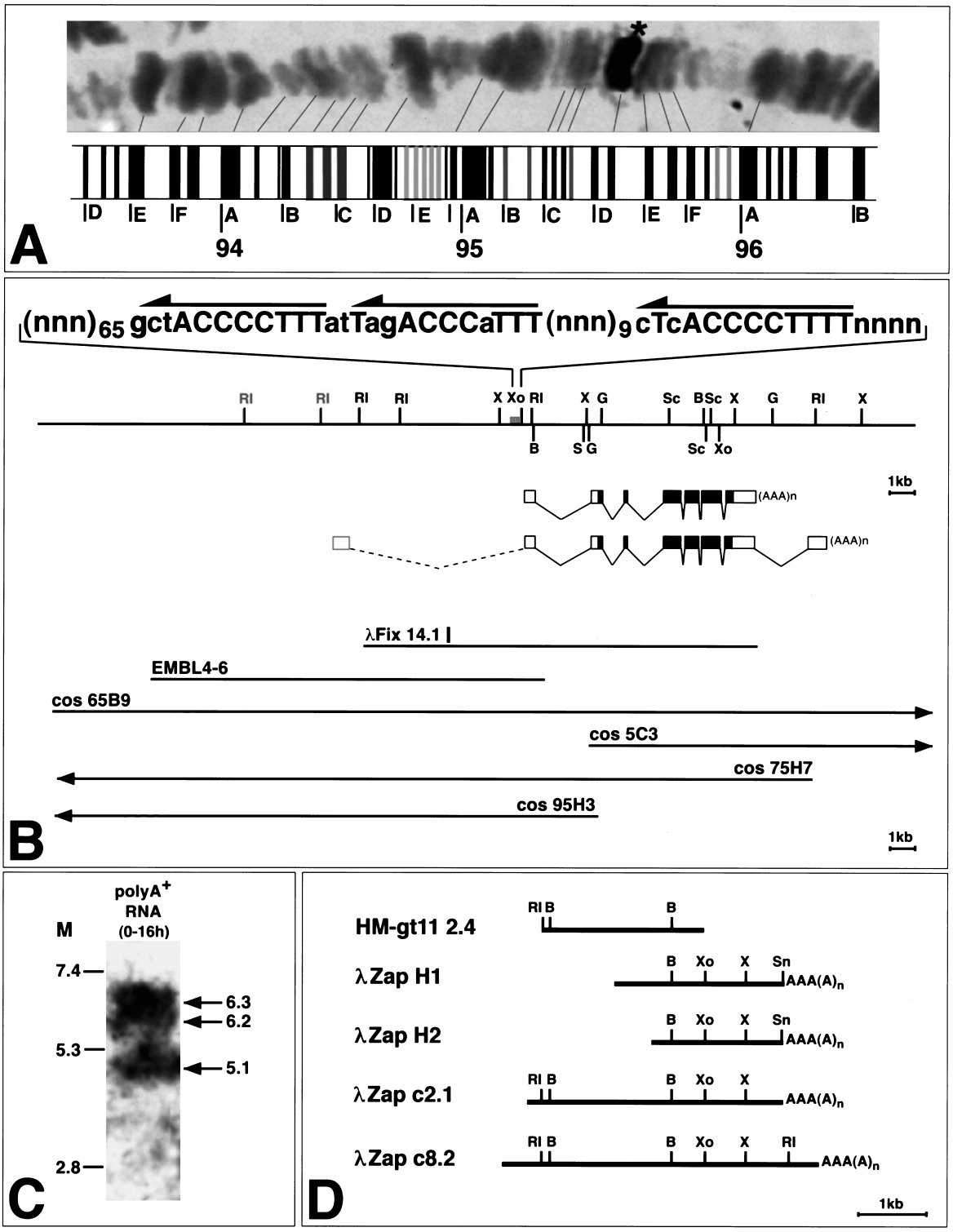
Starting from a 150-bp immunoprecipitated DNA fragment which contains three in vitro Krüppel-binding sites (see Fig. 1), we isolated more than 50 kb of overlapping genomic DNA fragments covering a novel transcription unit, termed *KrT95D*, in chromosome region 95D on the right arm of chromosome 3 (Fig. 1A). In addition, we isolated a total of five cDNA clones corresponding to three splicing variants of the *KrT95D* transcript. The structure of *KrT95D*, the size of three embryonically expressed transcripts and the characterization of two of the spliced transcripts which code for the same open reading frame (see below) are summarized in Fig. 1B–D.

The embryonic expression patterns of *KrT95D* were examined by whole-mount in situ hybridization to ovaries and embryos at different stages of development (Fig. 2). *KrT95D* is expressed in nurse cells of stage-9 egg chamber and the transcripts are transported into the oocytes (Fig. 2A). After egg deposition, the maternal *KrT95D* transcripts appear homogeneously distributed (Fig. 2B). From gastrulation onwards, zygotic *KrT95D* expression is observed in various locations of the embryo including the anterior and posterior midgut anlage, the tip cells of the Malpighian tubules (Figs. 2C, 3A), the neuroectoderm and visceral mesoderm (Fig. 2D), precursors of the dorsal, lateral and ventral body wall muscle (Fig. 2E) and the proventriculus (Fig. 2F). Furthermore, *KrT95D* expression continues in the developing central nervous system (CNS), in a subset of the myotubes (Fig. 2G, H), in the ventral midline cells (Fig. 2I) and remains in the dorsal organ, the dorsopharyngial apodemes and throughout the CNS of the mature embryo (Fig. 2J).

Kr is necessary for the activation of *KrT95D* in VO5 muscle precursors

At stage 14, when the different precursors can be identified by their shape and position, *KrT95D* expression is

Fig. 1A–D Chromosomal localization, genomic structure and transcripts of the *KrT95D* transcription unit. **A** In situ hybridization of genomic λFix 14.1 insert DNA to polytene chromosome indicating that the KrT95D gene is localized in region 95D on the right arm of the third chromosome (*asterisk*). **B** Physical map of the genomic structure of the *KrT95D* transcription unit (*B* BamHI, *G* BglII, *RI* EcoRI, *S* SalI, *Sc* SacI, *X* XbaI, *Xo* XhoI). The *gray bar* indicates the localization of the immunoprecipitated DNA fragment containing the three Krüppel-binding sites. The sequence of the in vitro Krüppel-binding sites (confirmed by footprinting analysis, not shown) is shown *above*. Nucleotides matching the Kr consensus sequence are indicated by *capital letters*. All three Kr-binding sites are arranged in tandem and show reverse orientation. The exon-intron structures of two alternative spliced *KrT95D* transcripts differing in the 5' and 3' untranslated regions are indicated *below*. They consist of at least seven or nine exons, respectively spanning a genomic region of about 20 kb. ▶



Note that the exact localization of the 5' exon of the second transcript is not determined. The open reading frame (ORF) is indicated by the *black bars*, the untranslated 5' and 3' regions are shown by *open bars*. Genomic DNA from the *KrT95D* locus present in phages (λFix and *EMBL4*) and cosmids (*cos*; Hoheisel et al. 1991) is shown *below*. *Arrowheads* indicate the direction in which the cosmid clones are extending. **C** Northern blot showing that the *KrT95D* transcription unit encodes three transcripts (5.1, 6.2 and 6.3 kb; probe: a 5.8-kb XbaI genomic DNA fragment covering the ORF of *KrT95D*). **D** Physical maps of five different cDNA clones isolated from two different cDNA libraries (*B* BamHI, *RI* EcoRI, *Sn* SnaBI, *X* XbaI, *Xo* Xho). Note that the two cDNA clones λZap H1 and H2 contain a SnaBI restriction site close to the polyA-tail which is not present in the cDNA clone λZap c2.1 suggesting that these two cDNA clones represent a third splicing variant of the KrT95D transcription unit. The isolated cDNA clones differ in their length (λZap c2.1, 3.7 kb; λZap c8.2, 4.6 kb) from that of the transcripts identified by northern blot analysis suggesting that there are 5' untranslated sequences missing

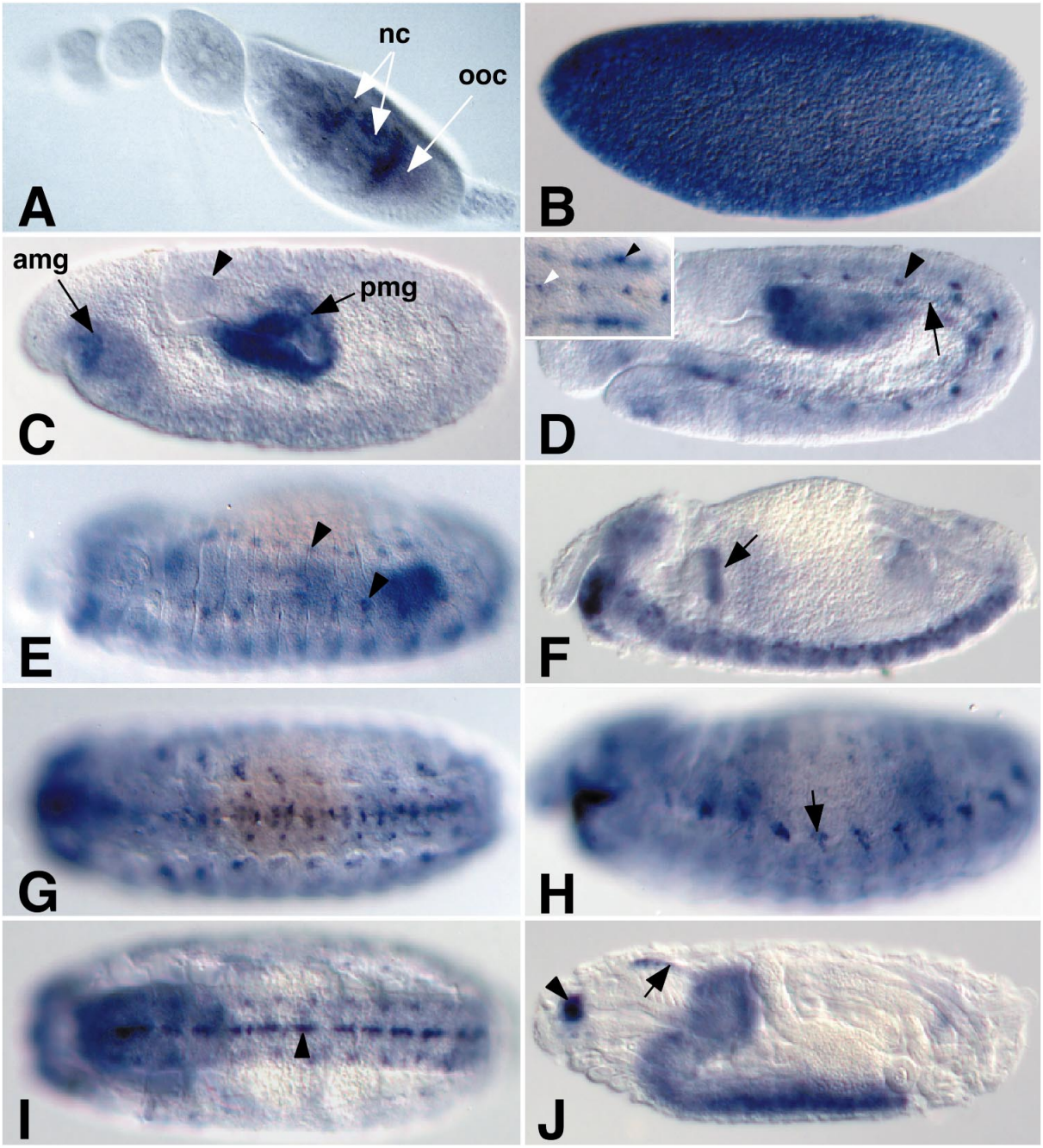


Fig. 2A–J Expression pattern of *KrT95D* during oogenesis and embryogenesis examined by in situ hybridizations. **A** Ovariole from a wild-type fly showing *KrT95D* expression in the nurse cells (*nc*) and in the oocyte (*ooc*) at stage 9. **B** Homogeneous distribution of maternal *KrT95D* transcripts at the syncytial blastoderm stage. **C** Zygotic *KrT95D* expression in the anterior (*amg*) and posterior mid gut primordium (*pmg*) at embryonic stage 9. Weak *KrT95D* expression can also be detected in the region where the tip cells of the Malpighian tubles are formed (*arrowhead*). **D** *KrT95D* expression in a segmentally repeated pattern of cells (*arrowhead*) and in the visceral mesoderm (*arrow*) at stage 10. The segmentally repeated expression belongs to cells of the neuroectoderm (*white arrowhead*) and the somatic mesoderm (*black arrowhead*; *inset* showing a dorsal view of a late stage 10 embryo). **E** *KrT95D* expression in dorsal, lateral and ventral muscle precursor cells (*arrowheads*) at stage 13. **F** *KrT95D* expression in the central nervous system (brain and ventral nerve cord) and in the region of the proventriculus (*arrow*). **G** Ventral view of a stage 14 embryo showing *KrT95D* expression in the ventral cord and in ventral muscles. **H** Lateral view of a stage 14 embryo showing *KrT95D* expression in two ventral muscle precursors, VO2 and VO5 (*arrow*). **I** Ventral view of a stage 16 embryo showing strong *KrT95D* expression in cells of the midline (*arrowhead*) of the ventral cord. **J** *KrT95D* expression in the dorsal organ of the antenna-maxillary complex (*arrowhead*), in two parallel stripes of cells, the dorsopharyngial apodeme cells (*arrow*) and throughout the CNS at stage 17

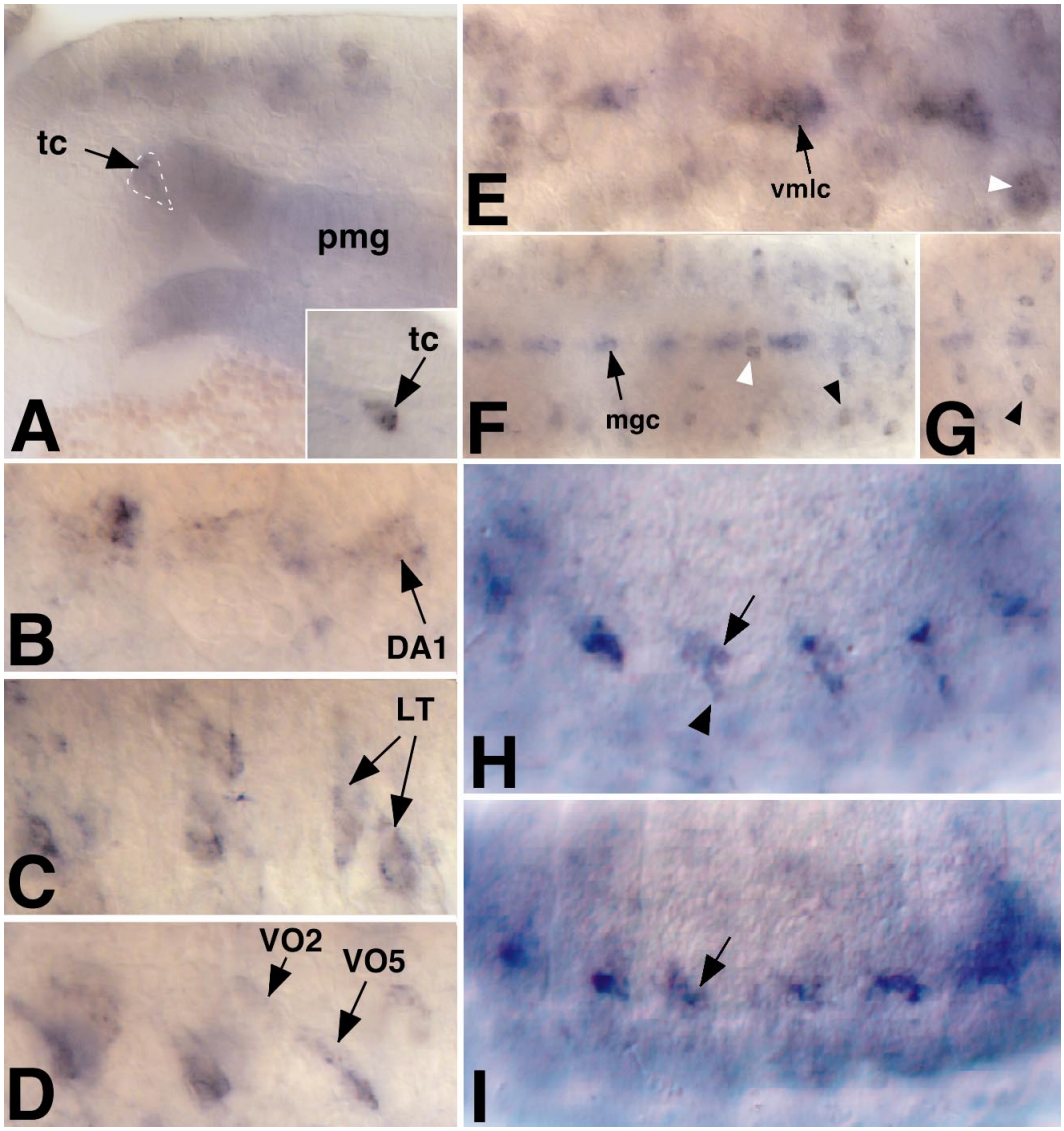


Fig. 3A–I Co-expression of *KrT95D* and *Kr* in different tissues during embryogenesis and loss of *KrT95D* expression in the precursor cells of muscle VO5 in embryos lacking Krüppel activity. **A–G** Whole-mount in situ hybridizations using a biotinylated *Kr*-DNA probe (*brown*) and a digoxygenin-labelled *KrT95D*-DNA probe (*blue*) to visualize co-expression of both genes. **A** Co-expression of *KrT95D* and *Kr* in the tip cell (*tc*) of the Malpighian tubule at stage 10 and stage 16 (see *inset*). **B–D** Co-expression of the two genes in different muscle precursor cells at late stage 14: **B** In the precursor of the dorsal-acute muscle DA1; **C** in two lateral transverse muscles, LT2 and LT4; **D** in the ventral-oblique muscles VO2 and VO5. **E** Dorsal view of a stage 9 embryo showing co-expression of *KrT95D* and *Kr* in cells of the ventral midline (*vmlc*). **F** Ventral cord of a stage 16 embryo showing expression of *KrT95D* in midline glia cells (*mgc*), of *Kr* in a pair of cells located posterior of the posterior connectives (*white arrowhead*) and co-expression of both genes in a cell located laterally in the ventral cord (*black arrowhead*). **G** Co-expression of *KrT95D* and *Kr* in a cell located ventrally above the longitudinal axons. **H** Wild-type *KrT95D* expression in the precursor cells of the two ventral-oblique muscles VO2 (*arrow*) and VO5 (*arrowhead*) at stage 14. **I** *KrT95D* expression in $Kr^{CD+}Kr^{1}$ embryos showing that the *KrT95D* expression is lost in the precursor cells of muscle VO5

found in a subset of muscle precursor cells including DA1, LT2, LT4, VO2 and VO5 (not shown; for a description of *Drosophila* muscle pattern and nomenclature see Bate 1993). During muscle development, its putative regulator, Krüppel, is expressed in a subset of precursors which give rise to muscles DA1, DO1, LL1, LT1, LT2, LT4, VL2-4, VA2, VO2, VO5 and DT1 (Ruiz-Gomez et al. 1997). Double staining experiments show that *Kr* and *KrT95D* expression indeed overlaps in the precursors of muscles DA1, LT2, LT4, VO2 and VO5 (Fig. 3B–D). Furthermore, overlapping expression of the two genes is observed in the tip cell of the Malphigian tubules (Fig. 3A; see also Hoch et al. 1994), in cells of the ventral midline and in segmentally repeated, laterally located groups of neuroectodermal cells (Fig. 3E–G).

In order to investigate whether *Kr* activity is required for the control of *KrT95D* expression in these locations, we examined the *KrT95D* expression pattern in embryos which lack *Kr* activity due to a *Kr* mutation. However, *Kr* lack-of-function alleles cause strong segmentation defects early in development and, thus, any phenotypic de-

A

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MDAVLQKSMDMIIELTASGKNGRPGTVVACLRAERVSSIPVDHDNKNNNS      50
VLLADRVAEYSDEDEEAEFSSGEFNDEANELGLIRGYDPKDPRDYNHPAK     100
HDMRKYRNKLQRSGIEDCALVGHHPGSIQHHHPGVVVDSDSEFEMKDKSS     150
SRAKFSRTISLQQRNFKQKIVALLKRFKVSEELEGESGHRGTAALRGERD     200
LDALFQELESLSCCEGDDSGPDMDSISVGSTPKPSLRPFFTNSRIMLHDN     250
ITGNGGDLSQLVGGGLGPGTGTAGNSERRSSDKSDQLTNSSYNLENNKNQ     300
KCIHLTNNNNSATTPDRGGNDSSGNEGNAGYTDGQNSDPQNSPPRDKDYL     350
RLQQMQQQQLTPVSSVAASMGSGSVITPAQTEKRSRLFRTSSNTPANAGS     400
GGNSVSAITRSGGGKRKHTLSLSAEPRSVLEACLSPTNVEPRKLLLDQLS     450
RVFAGEDSAIPEVVTIISPPEALGGSALLAKLVTLFANSFKPAFVPQNTA     500
EVKAVLQALMAKIQKYCNSNAKPPHTVKVLLIGGDWLQGATLRHYVELMG     550
VRPPDWLNHLRFYLVPVGGSCGSVARHLSQMDQAYAVMFGSDNWTQLCER     600
AAATAAAVSAVTTVNATALTTNLADAAGVAKSDIAELVQRIQRYLLAAGP     650
CTQIPIAEAMVNYKDEDSCQIFVPFVSDVRIGYLDAQASLDLEENAAGSN     700
AVGSGLGSGSASSSAIPIGSQSSPNVHGVVSGSPPQQQSLGRISPPLQTP     750
PSSASSHRERNTSESLSTPSSVQQQSFSGALAAAEAVELQVDYWPLVRPG     800
EGHAKESKGGLSKGSDAGGKNSIKSTFRNLQVWRLPQHAQQLGDMFNGLT     850
VSFATKEKKQKQIMRLGKKKDKERDLEKEQCVEGVARLICSPKQSHPVPL     900
RVYIDGTEWTGVKFFQVSSQWQTHVKNFPIALIGCTPCPVLNYPSHFKPR     950
PHTGHSQLP                                               959
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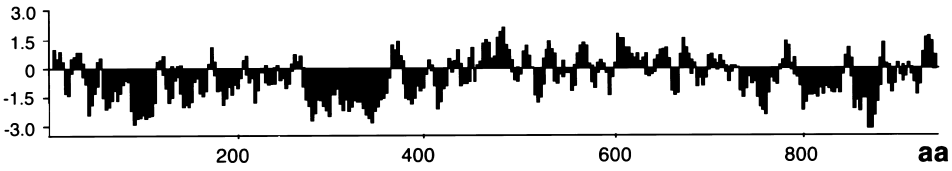
B



C

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KRT95D          KQKIVALLKRFKVSEELEGESGHRGTAALRG-ERDLDALFQELESLSCCE
emb[Z44004]     KQKFVALLKRFKVSDEVGFGLEHVSREQIREVEEDLDELYDSLEMYNPS-

KRT95D          GDDSGPDMDSISVG-STPKPSLRPFFTNSRIMLHDNITGNGGDLSQLVGG
emb[Z44004]     --DSGPEMEETESILSTPKPKLKPFFEGMSQSSSQTEIGSLNSKGSLGKD

KRT95D          GLGPGTGTAGNSERRS
emb[Z44004]     TTSPMELAALEKIKST

KRT95D          KEKKQKQIMRLGKKKDKERDLEKEQCVEGVARLICSPKQSHPVPLRVYID
gb[W81731]      KNKK---VMFLPKKTKDKEVESKSQCIEGISRLICTAKHQQNM-LRVLID
gb[H24655]                                                  VLID
gb[R50269]                                                  LRVSID

KRT95D          GTEWTGVKFFQVSSQWQTHVKNFPIALIGC
gb[W81731]      GVECSDVKFFQLAAQWSSHVKHFPICIFGH
gb[H24655]      GVECSDVKFFQLAAQWSSHVKHFPICIFG
gb[R50269]      GVEWSDIKFFQLAAQWPTHVKHFPVGL
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Fig. 4A–C Amino acid sequence, hydrophobicity blot and similarities to the putative proteins encoded by human and mouse cDNA clones. **A** Amino acid sequence of *KrT95D* deduced from the common open reading frame of the two different splicing variants (see Fig. 1B). The eight putative N-glycosylation sites of the KRT95D protein are indicated by *bold letters*. The *boxed regions* indicate conserved regions in the amino- and carboxy-terminal part of the KRT95D (see **C**). **B** Hydrophobicity analysis of the KRT95D amino acid sequence after Kyte-Doolittle (+, hydrophobic; –, hydrophilic) showing that the KRT95D protein is mainly hydrophilic (*aa* amino acids). **C** Alignment of the amino-terminal (aa 167–280) amino acid sequence of KRT95D with the putative protein encoded by a human partial cDNA clone (gene bank accession number Z44004) and of the carboxy-terminal (aa 856–935) amino acid sequence with the putative proteins encoded by the mouse EST clone (gene bank accession number W81731) and the two human EST clones (gene bank accession numbers H24655 and R50269), respectively, revealed two conserved regions in the KRT95D protein. Conserved amino acids between the proteins are highlighted by the *blue*, similar amino acids by the *yellow* color

fects observed in *Kr* embryos could be a consequence of the abnormal segmentation process. Furthermore, *Kr* mutant embryos fail to develop Malpighian tubules (Gloor 1954; Hoch, et al. 1994). To circumvent this difficulty in assessing the role of *Kr* for the expression of *KrT95D*, we examined *KrT95D* expression in the recently described "$Kr^{CD+}Kr^{1}$ embryos" (Romani et al. 1996). Such embryos, which are homozygous for the Kr^{1} lack-of-function mutation, carry a *Kr* transgene providing early *Kr* expression corresponding to the *Kr* segmentation function. Provision of the *Kr* transgene specifically rescues the segmentation defect of *Kr* lack-of-function mutants (Romani et al. 1996) and thereby permits the study of segmentation-unrelated aspects of *Kr* requirement, except for the Malpighian tubules which fail to form in such embryos.

In $Kr^{CD+}Kr^{1}$ embryos, we found that the transient *KrT95D* expression in the muscle precursors DA1, LT2 and LT4 is variably affected (not shown). However, recent examination of the muscle pattern has shown that DA1, LT2 and LT4 are also morphologically affected in $Kr^{CD+}Kr^{1}$ embryos, i.e. they are either lost or transformed into muscles of different specification (Hartmann 1996; Ruiz-Gomez et al. 1997). Thus, the alterations of *KrT95D* expression in the muscle precursors DA1, LT2 and LT4 are likely to be a secondary effect of the lack of Kr activity in those muscles. In contrast, *KrT95D* constantly fails to be expressed in the muscle VO5 precursors while it is still expressed in the muscle VO2 (Fig. 3I). Thus, the lack of *Kr* activity affects *KrT95D* expression in all four muscle precursor cells where the two genes are co-expressed. However, only in the case of the VO5 precursors, can *Kr* requirement be demonstrated directly. Nevertheless, the variable lack of *KrT95D* expression in the other muscles can be explained if *Kr* functions in these precursors as a component of a partially redundant activator system.

VO5 is not among the muscles which are affected by the absence of *KrT95D* expression in $Kr^{CD+}Kr^{1}$ embryos and, thus, the absence of *KrT95D* expression is not due to an early transformation or the lack of VO5 precursor cells. Therefore, *Kr* activity is necessary for *KrT95D* expression by acting upstream of the *KrT95D* gene. The *cis*-acting control region of *KrT95D*, which contains three clustered binding sites for the *Kr* protein, is able to drive reporter gene expression in *KrT95D*-corresponding patterns (Hartmann 1996). This observation and the finding that *Kr* activity is necessary for *KrT95D* expression in VO5 precursor cells suggest that the transcription factor Krüppel participates directly in the control of *KrT95D* expression. We note, however, that ectopic expression of *Kr* under the control of an inducible heat-shock promotor (Romani et al. 1996) does not induce or affect *KrT95D* expression (data not shown). Thus, *Kr* activity by itself is not sufficient for the activation of *KrT95D* expression. This implies that the Krüppel acts in concert with one or several other transcription factor(s) required for the cell-specific activation of the *KrT95D* gene. This leaves the question of whether the absence of *Kr* or *KrT95D* expression causes a mutant phenotype. VO5 precursor cells which lack *Kr*, and consequently also *KrT95D*, expression develop into VO5 muscles as judged by their normal size, shape and position. Thus, neither *Kr* nor *KrT95D* activities contribute to a morphological function that is apparent in the *Kr* mutant muscle pattern.

KrT95D encodes a novel protein containing conserved protein regions

In the absence of a morphologically apparent phenotype, we reasoned that the protein sequence of the *KrT95D* product may provide a hint towards the function of the gene and, therefore, we sequenced the cDNA clones λFix c8.2 and λFix c2.1. Sequencing revealed a single open reading frame of 2,832 bp which codes for a 959-amino acid protein of a calculated molecular weight of 103 kD which is mostly hydrophilic (Fig. 4A, B). No diagnostic protein motif has been found. However, sequence comparison with known polypeptide sequences revealed two relatively short stretches of conserved sequences which are encoded by partial cDNA clones of human and mouse transcripts (Fig. 4C). The observation that the conserved sequences were found in the N- and C-terminal region of the *KrT95D* protein, respectively, leave the possibility that the two partial sequences of the human cDNAs derive from the same transcript, which would imply that the *KrT95D* gene is in fact conserved in humans. Alternatively, or in addition, the conserved protein regions may represent functional domains of different proteins which are conserved in evolution. In this context it is interesting to note that no corresponding domains have been found in the yeast genome. This argues that the conserved regions of the *KrT95D* protein may serve functions not required in lower eucaryotes.

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