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Vestigial-like-2b (VITO-1b) and Tead-3a (Tef-5a) expression in zebrafish skeletal muscle, brain and notochord

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Abstract

The *vestigial* gene has been shown to control skeletal muscle formation in *Drosophila* and the related Vestigial-like 2 (Vgl-2) protein plays a similar role in mice. Vgl-family proteins are thought to regulate tissue-specific gene expression by binding to members of the broadly expressed Scalloped/Tef/TEAD transcription factor family. Zebrafish have at least four Vgl genes, including two Vgl-2s, and at least three TEAD genes, including two Tead3s. We describe the cloning and expression of one member from each family in the zebrafish. A novel gene, *vgl-2b*, with closest homology to mouse and human *vgl-2*, is expressed transiently in nascent notochord and in muscle fibres as they undergo terminal differentiation during somitogenesis. Muscle cells also express a TEAD-3 homologue, a possible partner of Vgl-2b, during myoblast differentiation and early fibre assembly. *Tead3a* is also expressed in rhombomeres, eye and epiphysis regions.

Keywords

muscle; adaxial; zebrafish; vestigial-like; transcription enhancer factor; TEAD domain

1.0 Results and Discussion

1.1 Background

Drosophila vestigial (vg) encodes a 453 amino acid (aa) 46 kd protein (Williams et al., 1991) required for acquisition of muscle fibre identity in a subset of muscle founder cells during embryonic myogenesis and plays a role in conferring indirect flight muscle identity in the pupa (Bate et al., 1993; Bate and Rushton, 1993; Bernard et al., 2003; Sudarsan et al., 2001; Williams et al., 1991). *Vg* also functions elsewhere, most notably to control wing outgrowth where it appears to control cell survival and proliferation (Agrawal et al., 1995; Zider et al., 1996). *Vg* heterodimerizes with Scalloped (*Sd*) protein, and the correct balance of these factors is required for normal wing development. *Vg* has two identified sequence motifs, a *Sd*-interacting domain and an N-terminal domain similar to that of *Paired*, a *Drosophila* homologue of mammalian *Pax3*. In the wing disc, *Sd* promotes *Vg* nuclear accumulation and the complex is thought to target *serum response factor*, *spalt* and other genes (Halder et al., 1998). The targets of *Vg/Sd* in muscle are still unclear.

In mammals, several *vestigial* homologues have been identified. Murine *vestigial-like 2 (vgl-2)*, also known as *VITO1*, is expressed exclusively in skeletal muscle (Mielcarek et al., 2002; Maeda et al., 2002a). *Vgl-4* controls gene expression in heart (Chen et al., 2004b), whereas expression of *vgl-1* and *vgl-3* appears largely restricted to the placenta (Maeda et

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al., 2002a). Vgls interact with vertebrate scalloped homologues, such as Transcriptional enhancer factor-1 (Tef-1, also known as TEAD-1 and belonging to a gene family hereafter called TEADs) (Chen et al., 2004a; Gunther et al., 2004). TEADs bind strongly to MCAT and A/T rich sequences important for muscle-specific expression of certain genes (Azakie et al., 1996; Farrance and Ordahl, 1996; Gunther et al., 2004; Mahoney, et al., 2005). Functional studies have revealed that *vgl-2* is required for normal skeletal myogenesis and loss of function can reduce myoblast terminal differentiation in cell culture. However, over-expression of *vgl-2* alone is not myogenic (Maeda et al., 2002a; Gunther et al., 2004).

1.2 Identification and family analysis of vestigial-like proteins in zebrafish

We searched for zebrafish homologues of vestigial-like and TEAD-like proteins *in silico* using Genbank and the Sanger zebrafish genome assembly (Zv3-Zv5; www.ensembl.org). An EST clone (accession CN504929), named *vgl-2a*, with very strong homology to mouse and human *vgl-2* genes (Fig. 1A) localises to chromosome 12 (Fig. 1B). Additionally, a second locus with a high degree of conservation to both the zebrafish *vgl-2a* and amniote *vgl-2* is found on chromosome 20 of the zebrafish genome (Fig. 1B). Thus, both proteins appear to be homologues/orthologues of *vgl-2* that have arisen by gene duplication (Taylor et al., 2001). To elucidate genomic relationships we compared synteny using neighbouring known genes between fish, human and mouse *vgl-2* loci. *Vgl-2a* shows syntenic conservation with human and mouse by proximity to *Rfxdc1*, *Dcbld1* and *Gopc* genes, whereas *vgl-2b* exhibits a divergence (Fig. 1B). We were unable to find evidence for a genomic duplication of the Vgl-2 locus in either *Fugu rubripes* or *Tetraodon nigroviridis* (data not shown).

The divergence between *vgl-2a* and *vgl-2b* is also reflected in the extent of conservation of the protein sequence: Vgl-2a is more similar than Vgl-2b to mammalian Vgl-2s (Fig. 1C). Whereas mouse and human sequences are ~85% identical, zebrafish Vgl-2a is ~57% identical to both human and mouse Vgl-2 proteins at the amino acid level. By contrast, Vgl-2b is only ~35% identical to mouse and human Vgl-2 and only slightly more (37%) identical with zebrafish Vgl-2a. However, strong homology was observed in the Tef-interacting domain (also known as TDU motif; Vaudin et al., 1999), which is completely conserved between human, mouse and zebrafish (Fig. 1A). We also observed strong homology in the N-terminal region, which acts as a signature to distinguish Vgl-sub-family members (Fig. 1A and data not shown). Searching the Pfam and SMART databases for additional motifs only identified the Tef-interaction domain. The paired domain is not readily detected in the fish Vgl-2s. Despite the low overall identity of Vgl-2b and other Vgl-2s, conservation in specific regions is sufficient to assign orthology to Vgl-2.

Mouse Vgl-2 is known to shuttle in and out of the nucleus in cultured muscle cells according to differentiation status (Maeda et al., 2002a). In zebrafish, a proposed nuclear export signal (NES) is retained in Vgl-2b, but not in Vgl-2a (Fig. 1A, underlined; Maeda et al., 2002a). However, the putative nuclear localisation sequence (NLS) KRRRE in the mouse sequence (Maeda et al., 2002a) is absent from human and zebrafish Vgl-2 family members (Fig. 1A, bold). We focused on *vgl-2b* because *vgl-2a* is being analysed by others (X. Cousin, personal communication).

1.3 Expression of *vgl-2b* in somites

In situ hybridisation of staged embryos revealed that *vgl-2b* mRNA is undetectable during gastrulation (data not shown) and becomes detectable in somitic adaxial cells adjacent to the notochord at the ~7 somite stage (7s) (Fig. 2A,B). This timing and the absence of signal in adaxial cells of presomitic mesoderm indicate that expression commences with the first terminal differentiation of slow muscle fibres (Fig. 2C,I; Devoto et al., 1996). Co-expression

of *vgl-2b* with myosin heavy chain (MyHC) at 14s supports this view (Fig. 2D-F). Subsequently, *vgl-2b* is expressed in the region where slow fibres are differentiating as each somite forms (Fig. 2G-J,M,R).

To confirm that *vgl-2b* is expressed in slow fibres, we examined mutant fish lacking slow fibre formation. Hedgehog (Hh) signalling is required for slow myogenesis (Blagden et al., 1997; Barresi et al., 2000). Prevention of Hh signalling either in the signalling pathway mutant *smoothened* or by application of the Smoothened inhibitor cyclopamine ablated somitic *vgl-2b* mRNA at 15s (Fig. 3A-C,J-M). However, *u-boot* (*ubo* = *prdm1*; Baxendale et al., 2004) mutants or embryos injected with *prdm1* morpholino, which undergo adaxial myogenesis, but ultimately fail to produce slow muscle, show *vgl-2b* expression similar to wild type (Fig. 3D-F). Similarly, *fused somites* (*fss* = *tbx24*; van Eeden et al., 1996; Nikaido et al., 2002) and *after eight* (*aei* = *deltaD*; van Eeden et al., 1996) mutants which have slow fibres, despite defective somite border formation, retain adaxial *vgl-2b* expression (Fig. 3G,H). Thus, early somitic *vgl-2b* expression is in differentiating adaxial muscle fibres.

Fast muscle also expresses *vgl-2b*. Fast muscle differentiation begins around 16s, when most slow fibres move from their medial location to the superficial surface of the myotome (Devoto et al., 1996). By 25s, *vgl-2b* mRNA is detected in the lateral somite and transverse sections reveal expression in most, if not all, muscle, including fast fibres (Fig. 2N-P). Somitic *vgl-2b* mRNA is retained until at least 24 hour post fertilization (hpf), after which it declines rapidly (Fig. 2Q-S and data not shown). Thus, fast muscle fibres express *vgl-2b* as they differentiate. *Fgf8* (*acerebellar*, *ace*) mutants lack a proportion of fast fibres and express reduced levels *vgl-2b* mRNA (Fig. 3N-P data not shown; Groves et al., 2005). Mouse Vgl-2 can bind to Mef2 proteins (Maeda et al., 2002a; Maeda et al., 2002b). In zebrafish, the expression of *vgl-2b* in the myotome coincides with the expression of *mef2c* mRNA and protein (Ticho et al., 1996; Thisse and Thisse, 2004; Hinitz and Hughes, 2007). Overall, *vgl-2b* mRNA is detected in terminally differentiated muscle fibres.

1.4 Expression of *vgl-2b* in notochord

Beginning just after slow fibres express MyHC, *vgl-2b* transcript is transiently detected in developing notochord (Fig. 2A-N,P). Initially, the posterior limit of notochord expression parallels slow muscle, but anteriorly it extends beyond somites into the hindbrain region (Fig. 2D-H,J-L). Subsequently, expression declines in the maturing notochord at rostral somitic levels as slow fibres begin lateral migration (Fig. 2J,L). Hindbrain level notochord loses *vgl-2b* expression by 25s (Fig. 2N,P). *Vgl-2b* expression remains high in the posterior notochord, adjacent to nascent somites (Fig. 2P-S). Notochord expression is confirmed by the loss of *vgl-2b* expression at 15s in *floatinghead*, a mutant lacking notochord and early slow muscle (Fig. 3I). Notochord *vgl-2b* expression is unaffected in the Hh signalling and somite border mutants examined (Fig. 3A-H). At the end of somitogenesis (24 hpf), notochordal *vgl-2b* mRNA is restricted to the tail tip and subsequently disappears (Fig. 2R,S). Thus, all regions of the notochord express *vgl-2b* transiently for a period of a few hours.

1.5 Identification and phylogenetic analysis of TEAD-like proteins in zebrafish

Vgl proteins frequently function as transcription factors together with TEAD proteins. ENSEMBL and Genbank database searches with a consensus sequence derived from the human and mouse TEA DNA binding domain (TEAD) motif identified three complete members of the TEAD family: one homologue of *Tea-1* localising to chromosome 25 and two *Tea-3* (*Tef-5*) homologues (Fig. 4A). *Tea-3a* is the more closely related to human and mouse *Tea-3*, sharing 80% and 78% protein identity, respectively, and preliminary synteny analysis positions it in a poorly assembled genomic region but, as in amniotes, next to *Tulp1*

(Fig. 4B). *Tead-3b* on chromosome 7 is marginally more distantly related to human and mouse *Tead-3*, being 78% and 76% identical at the protein level, respectively, but apparently lacks synteny (Fig. 4b). Future assemblies will be needed to verify this. *Tead-3a* and *Tead-3b* are 87% identical at the protein level and each retains the identifiable N-terminal signature of *Tead-3* proteins (Fig. 4F).

We used degenerate primers and RT-PCR to amplify and clone a region either side of the TEA domain of zebrafish *Tead-3a* (Fig. 4C). This sequence confirmed several interesting aspects of *TEAD* gene structure. As previously reported (Jacquemin et al., 1999), the exon structure of *TEAD-3* homologues is extremely well conserved in all species examined, both in terms of the size and number of coding exons (Fig. 4C,D). The first coding exon in zebrafish and mammals is replaced, in the chicken, by three exons separated by short introns that together appear to encode for the equivalent polypeptide (Fig. 4D; data not shown). Comparison of our cDNA sequence with ESTs and genomic sequence revealed alternative splicing of two near-identical 63 bp exons 3 and a 12 bp exon 4. Our cDNA contains exon 3a, which is separated from exon 3b by a short intron. Other species show the same genomic structure (Fig. 4D,E). Although no ESTs contain both exons, transcripts with each exon separately exist in various species (data not shown). As exon 3a and 3b encode nearly identical sequences located at the C-terminal end of the TEA domain (Fig. 4D, bar in 4E), it is unlikely that they will co-exist (functionally) in the same mRNA transcript (Jacquemin et al., 1999). The alternate exons 3 encode either a single tyrosine (Y) or two serine (S) residues that may serve as phosphorylation sites. After searching the exon sequences on the Scansite program (Obenauer et al., 2003), exon 3a showed no consensus phosphorylation sites whereas we confirmed that exon 3b contains a putative PKA phosphorylation site the mutation of which does not change DNA binding activity (Gupta et al., 2000). Exon 4 is a 12 bp in-frame exon encoding AMNL located close to the C-terminus of the TEA domain (Fig. 4C). ESTs with and without this exon exist in other species (Jacquemin et al., 1997). Our amplified zebrafish cDNA lacks exon 4, but it is retained in the alignment for ease of comparison (Fig. 4F). Overall, gene structure is highly conserved between fish and amniotes.

1.6 Expression of *tead-3a* in muscle precursors

Like, *vgl-2b*, *tead-3a* expression is not observed during gastrulation and is first seen at low levels in 3s embryos in the head (see below) and in the adaxial slow muscle cells (Fig. 5A-B). As somitogenesis proceeds, a narrow band of expression adjacent to either side of the notochord is observed (arrowheads in Fig. 5D, F, G, I, K, L) extending into the presomitic mesoderm of the tail region (Fig. 5F). Somitic expression remains at a low level and restricted to regions adjacent to the notochord until ~18s, when slow precursors begin migrating laterally. At this time the *tead-3a* signal increases to include the bulk of the somite, including regions of fast fibre formation (Fig. 5N-T). In particular, stronger expression is observed in the ventral and posterior domain of each somite (Fig. 5O, S), gradually forming refined bands at the somite borders as somitogenesis finishes at 24 hpf (Fig. 5U-W). After this stage, somitic expression of *tead-3a* declines to lower levels by 48 hpf (Fig. 5X). We detected no expression of zebrafish *tead-3a* in developing heart, unlike expression of *tead-3* in mice (Jacquemin, 1997; Maeda, 2002).

1.7 Expression of *tead-3a* in the developing central nervous system and elsewhere

Expression of *tead-3a* in the developing eye, epiphysis and presumptive hindbrain is observed from the earliest stages of somitogenesis (Fig. 5A, C, E, G, H) and is more heavily expressed in the dorsal, than the ventral, retina. At 10s stage, retinal expression persists, and a band of signal appears in the region of the epiphysis in the dorsal forebrain directly between the eyes (Fig. 5J, L, M). *Tead-3a* is also expressed in the hindbrain in two of the

rhombomeres, probably rhombomeres 3 and 5 (Fig. 5J, M, P, Q). Epiphysis expression declines by 22s, whereas rhombomere expression persists until the end of somitogenesis. Expression of *tead-3a* in the eye also gradually declines during this period, being lost first from the more ventral retinal region and lastly, the dorsal retina where the signal was originally most intense (Fig. 5P, Q, U, V). At 48 hpf, little eye expression remains, though low levels of signal appear in the head branchial region (Fig. 5X).

1.8 Conclusion

Using bioinformatics, we have identified duplications of both *vgl-2* and *tead-3* genes in zebrafish. Syntenic and protein phylogenetic relationships identified the designated 'a' homologue of each gene pair as the closest relative to the amniote counterpart. *Vgl-2b* and *Tead-3a* are co-expressed during muscle differentiation throughout zebrafish somitogenesis. Elsewhere in the organism these genes have distinct expression patterns. As Vgl proteins usually function with a Tead protein, Vgl-2b presumably acts in combination with other Tead proteins in regions where *tead-3a* is not expressed. Tead-3a may function alone or with other myogenic proteins, including other Vgls, SRF or Mef2 (Gupta et al., 2001; Maeda et al., 2002b).

2.0 Experimental procedures

2.1 Zebrafish lines and maintenance

All embryos were King's wild type background unless stated otherwise. Staging and husbandry were as described (Westerfield, 2000; Kimmel et al., 1995).

2.2 Bioinformatics and cloning

We used the protein family (Pfam) database on Sanger (Bateman et al., 2004; Bateman and Haft, 2002); the SMART (Simple Modular Architecture Research Tool) database at EMBL, Heidelberg (Letunic et al., 2002; Schultz et al., 1998; Letunic et al., 2002), Scansite program (Obenauer et al., 2003) and the Lasergene MegAlign program (DNA Star Inc, Madison, USA) and ClustalW and X (Thompson et al., 1994) to perform sequence alignments and domain searches. The latter was also used to create the phylogenetic trees for use in Phylip (Felsenstein, 1989). Where possible, for simplicity, we used the largest isoform or isoforms confirmed by publication or that we deduced following multiple species comparison. The following Genbank sequences were used for Vestigial family analysis: *Drosophila melanogaster* (DM) Vg: S72379; *Danio rerio* (DR) Vgl-1: XM_681743, DR Vgl-2: XM_679778; DR Vgl-4: XM_685490; *Gallus gallus* (GG) Vgl-1: XM_420234; GG Vgl-4: NM_001030593; *Homo sapiens* (HS) Vgl-1: NM_016267; HS Vgl-2: NM_182645; HS Vgl-3: NM_016206; HS Vgl-4: NM_014667; *Mus musculus* (MM) Vgl-1: NM_133251; MM Vgl-2: NM_153786; MM Vgl-4: NM_177683. ENSEMBL sequences used for Vgl family analysis were: *Takifugu rubripes* (FR) Vgl-1: GENSCAN00000017397; FR Vgl-2: SINFRUG00000138532; FR Vgl-4: SINFRUG00000128809; GG Vgl-2: ENSGALG00000014917; *Xenopus tropicalis* (XT) Vgl-2 E1: ENSXETG00000014041; XT Vgl-2 E2: ENSXETG00000021503; XT Vgl-2 BC: BC063357; XT Vgl-4: ENSXETG00000007712. The following Genbank sequences were used for TEAD family analysis: DM Sd (PA): NM_167465; *Apis mellifera* (honey bee) (AM) TEAD-1: XM_392157; HS TEAD-1 (Tef-1): NM_021961; HS TEAD-4 (Tef-3): NM_201443; HS TEAD-2 (Tef-4): NM_003598; HS TEAD-3 (Tef-5): NM_003214; MM TEAD-1: NM_009346; MM TEAD-5: NM_011567; MM TEAD-2: NM_011565; MM TEAD-3: NM_011566; GG TEAD-1: XM_420962; GG TEAD-4: NM_204771. ENSEMBL sequences used for TEAD family analysis were DR TEAD-1: ENSDARG00000028159; DR Tead-3a: ENSDARG00000062559; DR TEAD-3b: ENSDARG00000059834.

RT-PCR from total RNA was performed as described (Mann et al., 2006). Primers used to amplify zebrafish TEAD-3a were forward 5'-ATCCTCTCAGATGAAGGAAAG-3' and reverse 5'-TCCAGKACGCTGTTCATCATG-3' whereas some sequence of the Vgl-2b EST clone was obtained with the primer 5'-ACTTTACCGGCTCCCCTTGG-3' (TAGN, Newcastle-upon-Tyne, UK). All amplified and commercial EST sequences were confirmed by automated sequencing.

2.3 In situ mRNA hybridisation and microscopy

Staged embryos were fixed in 4% paraformaldehyde, stored in methanol at -20°C for at least 24 hours and processed for *in situ* mRNA hybridisation as described (Jowett and Yan, 1996; Thisse et al., 2004; Westerfield, 2000; Jowett and Yan, 1996; Thisse et al., 2004).

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Figure 1. Family relationship, sequence and structural comparison of Vgl-2b (Vito-1b)
(A) Clustal W alignment of the translation product of the zebrafish *vgl-2a* and *vgl-2b* gene with respect to human and mouse *vgl-2* gene products. Amino acids conserved between human, mouse and the two zebrafish homologues are shown in grey. Boxed area shows the putative Tef-interaction domain (TDU), whereas the black bar shows the site of a putative nuclear export signal (NES; Maeda *et al.*, 2002). Downstream of this in the mouse sequence is a putative nuclear localisation sequence (NLS, bold). **(B)** The syntenic organisation of *vgl-2* genes in zebrafish, human and mouse, demonstrating the proximity of zebrafish *vgl-2a*, but not *vgl-2b*, to the *Rfxdc1*, *Dcbld1* and *Gopc* genes, suggesting an ancient duplication of this locus in zebrafish. **(C)** A phylogenetic tree showing the evolutionary distance among predicted vestigial and Vgl proteins. Branch length is proportional to divergence. The Vgl families are clearly defined whereas *Drosophila* *vg* appears to be more closely related to the Vgl-4 family. For species and gene abbreviations see Experimental Procedures.

Figure 2. Developmental expression of *vgl-2b*

In situ mRNA hybridisation for *vgl-2b* (blue/purple) shown in lateral (A,E,K,M,Q,R) or dorsal (B,F,L,N,S) wholemount, dorsal flatmount (C,D,G,H,I,P; anterior to top) and transverse cryosection (I,O; dorsal to top). **A-C.** Expression of *vgl-2b* begins at low levels at ~7s in adaxial cells (arrowheads) as they begin to differentiate, but is absent from notochord (n). **D-I.** *Vgl-2b* mRNA co-localises with slow MyHC-antibody staining at 14 som (arrowheads, D-I). Shown in F are the co-expression of *vgl-2b* mRNA and MyHC around similar-shaped nuclei (arrowheads) in 3.5-fold magnifications of the boxed regions in D (at left) and E (at right, reversed). Notochord expression projects rostrally beyond the first somite (arrows in D,G,H) and extends caudally adjacent to slow muscle. **J-L.** At 20 som, signal diminishes in maturing notochord but persists in the most nascent posterior regions where somitogenesis is still occurring and adjacent to the hindbrain (arrows). **M-Q.** Anterior notochord expression is lost by 25 som, but continues in the tail tip (arrowheads; M,P,Q,R). *Vgl-2b* mRNA persists in muscle tissue, including fast muscle (O). **R,S.** At 24 hpf, as somitogenesis concludes, *vgl-2b* expression in muscle declines. (nt) neural tube; (n) notochord.

Figure 3. Hedgehog signalling is required for appropriate regulation of *vgl-2b*

In situ mRNA hybridisation for *vgl-2b* (blue/purple) shown anterior to top in dorsal (A-I, L, M) or lateral (J, K) flatmount or dorsal (N) or lateral (O, P) wholemount. Arrowheads indicate lack of *vgl-2b* mRNA in adaxial cells. **A-C, J-M.** *Smoothed* (siblings, A, J, L; *smo*, B, K, M) and cyclopamine-treated (*cycl*; C) embryos in which loss of hedgehog (Hh) signalling prevents adaxial slow myogenesis. Notochord expression is unaffected. **D-F.** Neither *prdm1* mutation (*ubo*, D) nor *prdm1* morpholino injection (E, F) noticeably alters *vgl-2b* expression. **G, H.** Mutations in *deltaD* (*aei*; G) or *tbx24* (*fss*; H) affect somite borders, but *vgl-2b* expression persists in slow fibres (arrowheads), although poorly segmented. **I.** *Floatinghead* (*flh*) mutants have an absence of notochord due to mutations in the homologue of *Xenopus Xnot* and lack both notochord and early adaxial expression of *vgl-2b*. **N-P.** Fast fibres are reduced (brackets) at 24 hpf by loss of *fgf8* in *acerebellar* (*ace*) mutants, but *vgl-2b* expression is otherwise present.

Figure 4. Family relationship, sequence and structural comparison of *tead-3a* (*Tef-5a*)

A. A phylogenetic tree showing the evolutionary distance among predicted scalloped and TEAD/Tef-family proteins. Branch length is proportional to divergence. The TEAD families are clearly defined whereas *Drosophila* and honey bee (*Apis mellifera*; AM) Sd/Tef-1 appear to be more closely related to the Tef-4 family. **B.** Syntenic relationship suggests that the *tead-3a* locus is more closely related to the human and mouse *TEAD-3* loci due to proximal localisation of the *tulp1* gene, despite incomplete assembly of the zebrafish loci. **C.** **D.** The conserved exon structure and size of *tead-3*. **E.** The sequences of two third exons are conserved across species. **F.** Amino acid sequence alignment of Tead-3. Exon 3a (black bar) overlaps the end of the TEA domain (grey box). Exon (white box). Note also the isoleucine non-standard start codon typical of TEADs. No other functional domains were identified in database searches.

Figure 5. Developmental expression of *tead-3a*

In situ mRNA hybridisation for *tead-3a* in lateral (A,C,J,M,Q,U,X) and dorsal (B,D,K,N,R,V) views of wholemount (A-F,J,K,M,N,Q,R,U,V,X), flatmount (G-I,L,O,P,S,W) or cryosection (T, dorsal to top). **A-L.** Somitic expression begins at 3 som in narrow adaxial bands (arrowheads) on either side of the notochord (n) that extend into the anterior presomitic mesoderm (I). Strong retinal expression is evident from 5 som, with epiphysis (epi) and rhombomeres (Rh) expression apparent by 10 som. **M-T.** Adaxial expression persists until differentiation of fast muscle begins at 18 som, when signal is detected more broadly throughout the somite (T), but strongest in a posterior-ventral domain (S). Epiphysis and rhombomere expression declines. **U-X.** By the end of somitogenesis, *tead-3a* mRNA is concentrated at the somite borders and in the branchial region.