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Xenopus Xlmo4 is a GATA cofactor during ventral mesoderm formation and regulates Ldb1 availability at the dorsal mesoderm and the neural plate

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Abstract

We have identified and functionally characterized the *Xenopus Xlmo4* gene, which encodes a member of the LIM-domain-only protein family. *Xlmo4* is activated at gastrula stages in the mesodermal marginal zone probably in response to BMP4 signaling. Soon after, *Xlmo4* is downregulated in the dorsal region of the mesoderm. This repression seems to be mediated by organizer-expressed repressors, such as Gsc. *Xlmo4* downregulation is necessary for the proper formation of this territory. Increasing *Xlmo4* function in this region downregulates Spemman Organizer genes and suppresses dorsal-anterior structures. By binding to Ldb1, Xlmo4 may restrict the availability of this cofactor for transcription factors expressed at the Spemman Organizer. In the ventral mesoderm, Xlmo4 is required to establish the identity of this territory by acting as a positive cofactor of GATA factors. In the neural ectoderm, *Xlmo4* expression depends on Xiro homeoprotein activity. In this region, Xlmo4 suppresses differentiation of primary neurons and interferes with gene expression at the Isthmic Organizer, most likely by displacing Ldb1 from active transcription factor complexes required for these processes. Together, our data suggest that Xlmo4 uses distinct mechanisms to participate in different processes during development.

Keywords: Xenopus; *XLim-1*; *LMO4*; Ventral mesoderm; iroquois

Introduction

The LIM domain is a cysteine-rich zinc-binding motif present in transcriptional regulators important for development of invertebrates and vertebrates. This domain was originally discovered in proteins containing homeodomains, the so-called LIM homeoproteins (Lhx) (reviewed in Bach, 2000; Dawid et al., 1998; Hobert and Westphal, 2000). During vertebrate development LIM homeoproteins are required for processes such as the correct movement of dorsal mesoderm (Spemann Organizer) cells (Hukriede et al., 2003), maintenance of dorsal mesoderm identity (Kodjabachian et al., 2001; Mochizuki et al., 2000; Shawlot and Behringer, 1995), and the correct specification and differentiation of a subtype of neurons (Pfaff et al., 1996; Sharma et al., 1998; Tanabe et al., 1998; Thaler et al., 2002; Thor et al., 1999).

LIM homeoproteins interact with the LIM-domain-binding proteins Ldb1/NL1/Clim2 and Ldb2/Clim1 (reviewed in Bach, 2000; Dawid et al., 1998; Hobert and Westphal, 2000). Ldb1 contains a dimerization domain and a LIM-interacting region and, in many developmental processes, Ldb1 and Lhx appear to form an active tetramer that contains two molecules of each of these proteins (Milán and Cohen, 1999; Rincón-Limas et al., 2000; Thaler et al., 2002; van Meyel et al., 1999). In addition, Ldb1 also form mul-

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tiprotein complexes with transcription factors of the bHLH, GATA, and Otx families (reviewed in Bach, 2000; Dawid et al., 1998; Hobert and Westphal, 2000). Mutation of *Ldb1* in mice (Mukhopadhyay et al., 2003) or interference with Ldb1 function in zebrafish (Becker et al., 2002) cause multiple developmental defects, such as anterior truncations, partial axis duplication, and alteration of neuronal differentiation.

The functional tetramer of Lhx and Ldb1 proteins was initially described for the *Drosophila* LIM homeoprotein Apterous (Ap) and the Ldb1 homologue Chip (Milán and Cohen, 1999; Rincón-Limas et al., 2000; van Meyel et al., 1999), where it was found to be necessary for defining the dorsal compartment of the imaginal wing discs. The Ap-Chip interaction takes place through the LIM domains of Ap. Moreover, this interaction is regulated by Beadex/dLmo, a member of the Lmo (LIM domain only) family of proteins that are composed almost exclusively of two LIM domains. Beadex, through its LIM domains, binds to and sequesters Chip, thus antagonizing Ap function (Milán and Cohen, 1999; Milán et al., 1998; Shoresh et al., 1998; van Meyel et al., 1999; Weihe et al., 2001; Zeng et al., 1998).

In vertebrates, four Lmo proteins (Lmo1–4) have been identified (Foroni et al., 1992; Grutz et al., 1998; Kenny et al., 1998; Lane et al., 2002; Larson et al., 1995, 1996; Sugihara et al., 1998). In addition to their interaction with Ldb1, vertebrate Lmos also act as positive cofactors of other transcription factors. For instance, during blood development, Lmo2 forms a multiprotein complex with Ldb1, GATA1, and the bHLH factors SCL and E12/E47 (Osada et al., 1995; Vitelli et al., 2000; Wadman et al., 1997). Targeted gene disruption in mice (Yamada et al., 1998) and functional studies in *Xenopus* (Mead et al., 2001) have demonstrated an essential function of Lmo2 in blood formation. In *Xenopus,* Xlmo3 interacts with and increases the transcriptional activity of the neural-specific bHLH protein XHEN-1 during primary neurogenesis (Bao et al., 2000). In addition, mouse Lmo4 can interact with the homologue of the *Drosophila* Deformed epidermal autoregulatory factor 1 (Sugihara et al., 1998).

In this work, we describe the identification and functional analysis of the *Xenopus lmo4 (Xlmo4)* gene. *Xlmo4* was isolated in a screen designed to identify genes regulated by the Xiro homeoproteins, an evolutionary conserved group of transcription factors encoded by the *iroquois (Iro/Irx)* genes. The *Iro/Irx* genes have multiple functions during vertebrate and invertebrate development (reviewed in Cavodeassi et al., 2001), among them the anterior-posterior and the dorsal-ventral subdivision of the neural vertebrate territory (reviewed in Gómez-Skarmeta and Modolell, 2002). Here we show that *Xlmo4* acts, by means of different mechanisms, on several processes during development. Thus, during gastrulation, Xlmo4 is required for acquisition of ventral mesoderm identity, in association with GATA transcription factors, and for the restriction of Ldb1 availability for some Spemman Organizer transcription factors. At neurula stages, Xlmo4 is necessary to delimit primary neuron differentiation and to modulate gene expression at the Isthmus Organizer of the midbrain-hindbrain boundary.

Materials and methods

Isolation of Xlmo4

Twenty animal caps from control or injected (1 ng of *Xirol* mRNA) embryos were excised at stage 10 and cultured until stage 18. Total RNA was extracted and cDNA was synthesized using the SMART PCR cDNA Synthesis Kit (Clontech). The resulting two populations of cDNAs were used to prepare a subtraction library using the Clontech PCR-Select cDNA Subtraction Kit. The library was ligated to the pGEM-T Easy vector (Promega) and transformed into *E. coli*. One of the clones contained a 596-bp cDNA fragment with a full-length ORF that encodes Xlmo4.

Plasmid constructions

The primers 5′-GAC**GAATTC**AATGGTGAATAACCGGAG-3′ and 5′-CTT**CTCGAG**CATCTGGCTCACAGCTC-3′ were used to amplify the *Xlmo4* coding region. The PCR fragment was subcloned in pGEM-T easy, sequenced, excised with *EcoRI* and *XhoI* (in bold in the primer sequences), and cloned in pCS2-MT plasmid (Turner and Weintraub, 1994) to obtain the MT-Xlmo4 construct. To prepare the Xlmo4-MT plasmid, the *Xlmo4* gene was amplified with the primers 5′-GA**GGATC-G**AACGAGGATCAGTAATGG-3′ and 5′-CG**ATC-GAT**CTTTCTGGTCAGGGAGC-3′. The PCR fragment was subcloned in PGEM-T easy, sequenced, excised with *Bam*HI and *Cla*I (in bold in the primer sequences), and cloned in pCS2-MT plasmid. The pCS2-Xlmo4 construct was obtained by removing the carboxy-terminal region that contain the MT domain from pCS2-Xlmo4-MT with *Sac*I (which digest within the *Xlmo4* coding region) and *Xba*I and replacing this fragment by an equivalent *Sac*I-*Xba*I fragment obtained from pCS2-MT-Xlmo4. To prepare the hormone inducible construct, we PCR-amplified the *Xlmo4* coding region using the primers 5′-GAC**GAATTC**AATGGTGAATAACCGGAG-3′ and 5′-CGC**TCGAG**GAGGGGATTCAAGTGG-3′. This fragment was subcloned in pGEM-T easy, sequenced, excised with *Eco*RI and *Xho*I, and cloned in PCS2-MT plasmid. The hormone inducible domain was obtained by PCR using the oligonucleotides 5′-GAGATCCCC**TCTAGA**GGGACC-3′ and 5′-CATCAAAAGTGG**CTCGAG**CCCGCCGCT-3′ from a MyoD-GR plasmid kindly donated by H. Sive. The amplified fragment was cut with *Xba*I and *Xho*I, subcloned in pBluescript, and sequenced. The *Xlmo4* region was excised from pCS2-MT plasmid with *Eco*RI and *Xba*I, and the inducible domain digested with *Xba*I and *Xho*I. These two

fragments were ligated to the pCS2-MT plasmid digested with *Eco*RI and *Xho*I generating the pCS2-MT-Xlmo4-GR construct. The pCMX-Engrailed-Lmo4 vector was generated by isolating a fragment representing full-length mouse Lmo4 from pGBT9Lmo4 (Sugihara et al., 1998) by cutting with *Bam*HI and partially cutting with *Eco*RI, thus allowing in frame fusion between the N-terminally located Engrailed repression domain and Lmo4. The GATA2 constructs were generated by PCR, using the pSP64T-GATA2a plasmid (Zon et al., 1991) as a template and the following oligonucleotides: 5′-AGCTCATATGGAAGTCGCCACTGACCAGCCCCGT-3′ and 5′-AGCTGGATCCCCATAGCCGTCACCATGCTGGAGTGG-3′ (GATA2 full length); 5′-AGCTCATATGGAAGTCGCCACTGACCAGCCCCGT-3′ and 5′-AGCTGGATCCACACTCTCTCCCTTCTGAGCAGGA-3′ (GATA2 aa:1–266); and 5′-AGCTCATATGAAGAGCAGATCCTGCTCAGAAGGGAG-3′ and 5′-AGCTGGATCCCCATAGCCGTCACCATGCTGGAGTGG-3′ (GATA2 aa: 257–452). The PCR products were cut with *Nde*I and *Bam*HI, and cloned into the yeast two-hybrid vector pGADT7 (Clontech). The GATA2 fragments were also cloned into the eukaryotic expression vector pCDNA3 with the in-frame HA tags.

DNA sequencing

DNA sequencing was performed with ABI chemistry in an automatic DNA sequencer. Custom synthesized oligonucleotides were from ISOGEN (Bioscience BV, Maarsesen, The Netherlands).

Whole-mount in situ hybridization, X-Gal, and antibody staining

Antisense RNA probes were prepared from cDNAs using digoxigenin or fluorescein (Roche) as labels. Specimens were prepared, hybridized, and stained as described (Harland, 1991). Double in situ hybridizations were performed as described (Gómez-Skarmeta et al., 1998). X-Gal staining was carried out according to Coffman et al. (1993). Antibody staining was performed (Turner and Weintraub, 1994) after in situ hybridization and bleaching of the embryos using mouse monoclonal anti-Myc from BabCo.

In vitro RNA synthesis

All DNAs were linearized and transcribed as described (Harland and Weintraub, 1985) with GTP cap analogue (New England Biolabs). After DNAse treatment, RNA was extracted with phenol-chloroform, column purified, and precipitated with ethanol. mRNAs for injection were resuspended in water.

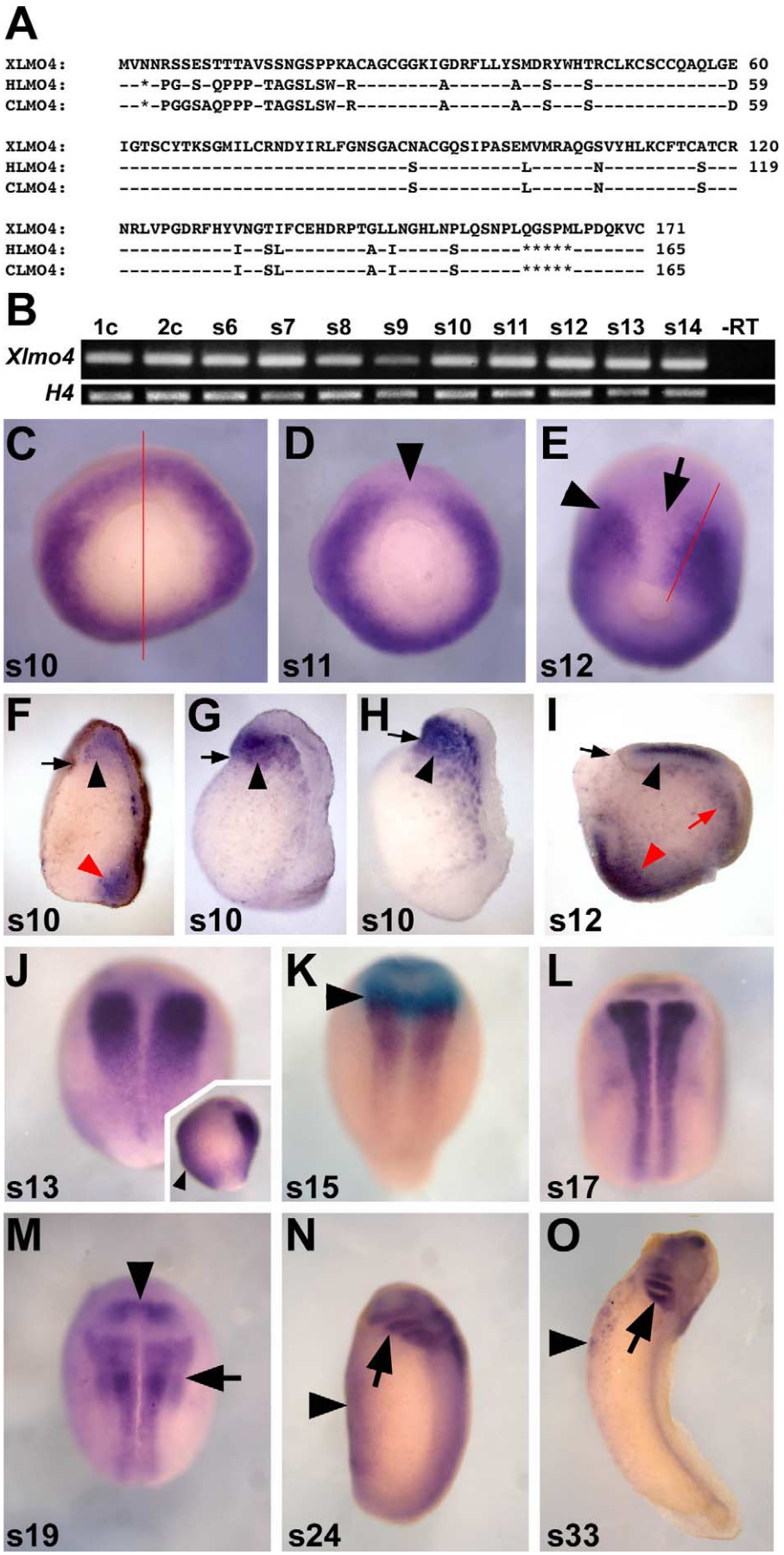
RT-PCR analysis

Embryos were injected with the corresponding mRNA in the animal region at one-cell stage, caps were explanted at stage 9, and aged until stage 19–20 when RNA was extracted. The RNA was treated with DNAse and processed for RT-PCR as described (Gawantka et al., 1995).

Protein-protein interaction by GST pulldown and coimmunoprecipitations

The GST pulldown assays were performed as previously described (Sugihara et al., 2001). Briefly, GST protein or GST-Lmo4 fusion protein were incubated with ^{35}S-labeled in vitro translated GATA proteins under previously described conditions except that incubation was carried out at room temperature for 30 min. After washing three times, the glutathione-agarose beads were resuspended in SDS sample buffer, boiled, and analyzed on 10% SDS-polyacrylamide gels. Coimmunoprecipitations, using ^{35}S-labeled in vitro translated proteins, were performed as previously described (Sugihara et al., 1998), using Myc and HA antibodies recognizing the tagged proteins. Coimmunoprecipitations in extracts from HEK293T cells were performed as previously described, using Myc antibody recognizing tagged Lmo4 and HA antibody recognizing tagged Gata2 constructs (Sugihara et al., 2001).

Fig. 1. Protein encoded by *Xlmo4* and expression pattern of this gene during *Xenopus* development. (A) Amino acid sequence alignment of *Xenopus*, human, and chick Lmo4 proteins (accession numbers AJ511277, U24576, and AF532926, respectively). Identical residues are indicated by dashes. Asterisks correspond to gaps inserted to maximize alignment. (B) *Xlmo4* mRNA expression (upper panel) at different developmental stages examined by RT-PCR and compared with the control *Histone H4* mRNA (lower panel). 1–2c, one–two-cell stage; s6–14, stages 6–14. Vegetal views (C and D), dorsal views (E, and J–M), sagital views (F–1), and lateral views (J inset, N, and O) of embryos showing the expression of *Xlmo4* (C–F and I–O), *Xlim1* (G), or *Otx2* (H). (C) At early gastrula stage (stg), *Xlmo4* is expressed in the mesodermal region. (D) At mid gastrula, *Xlmo4* expression in the dorsal mesoderm starts to decline (arrowhead). (E) At the end of gastrulation and beginning of neurulation, *Xlmo4* mRNA is not longer detected in the dorsal mesoderm (arrow) and accumulates in two symmetrical patches of the neural ectoderm (arrowhead). (F–H) Sagittal sections through a stage 10 embryo (as shown in C by the red line) indicate that *Xlmo4* is expressed in the dorsal mesendodermal cells (F, black arrowhead) that also express *Xlim1* and *Otx2* (G and H, respectively; arrowheads). At this stage, *Xlmo4* mRNA is also present in the ventral mesodermal cells (F, red arrowhead). Arrows point at the blastopore: (I) Sagittal sections through a stage 12 embryo (as shown in E by the red line) indicate that *Xlmo4* is expressed in the neuroectoderm, anterior endomesodermal cells (red arrow), and ventral mesoderm (red arrowhead), but not in the notochord (black arrowhead). (J–L) The *Xlmo4* neural expression domains persist during all neurula stages. From late gastrula onward, *Xlmo4* expression is also detected in the ventral epidermis (inset in J; N, and O, arrowheads). A double staining of *Xlmo4* (purple) and *Otx2* (cyan) indicate that the anterior limit of the domain of *Xlmo4* expression is within the caudal *Otx2* territory (K). (M) At late neurula stage, *Xlmo4* mRNA becomes detectable in an anterior band within the forebrain (arrowhead), and in the cephalic neural crests (arrow). (N and O) At later stages, *Xlmo4* transcripts are also present in migrating neural crest cells (arrows), in the brain, and in the ventral epidermis (arrowhead).


A
XLMO4: MVNNRSSESTTTAVSSNGSPPKACAGCGGKIGDRFLLYSMDRYWHTRCLKCSCCQAQLGE 60
HLMO4: --*-PG-S-QPPP-TAGSLSW-R--------A------A--S---S----------D 59
CLMO4: --*-PGGSAQPPP-TAGSLSW-R--------A------A--S---S----------D 59
XLMO4: IGTSCYTKSGMILCRNDYIRLFGNSGACNACGQSIPASEMVMRAQGSVYHLKCFTCATCR 120
HLMO4: ----------S----------L------N---------S--- 119
CLMO4: ----------S----------L------N---------S---
XLMO4: NRLVPGDRFHYVNGTIFCEHDRPTGLLNGHLNPLQSNPLQGSPMLPDQKVC 171
HLMO4: ----------I--SL--------A-I-----S------*****------- 165
CLMO4: ----------I--SL--------A-I-----S------*****------- 165
B
1c 2c s6 s7 s8 s9 s10 s11 s12 s13 s14 -RT
Xlmo4
H4
C
s10
D
s11
E
s12
F
s10
G
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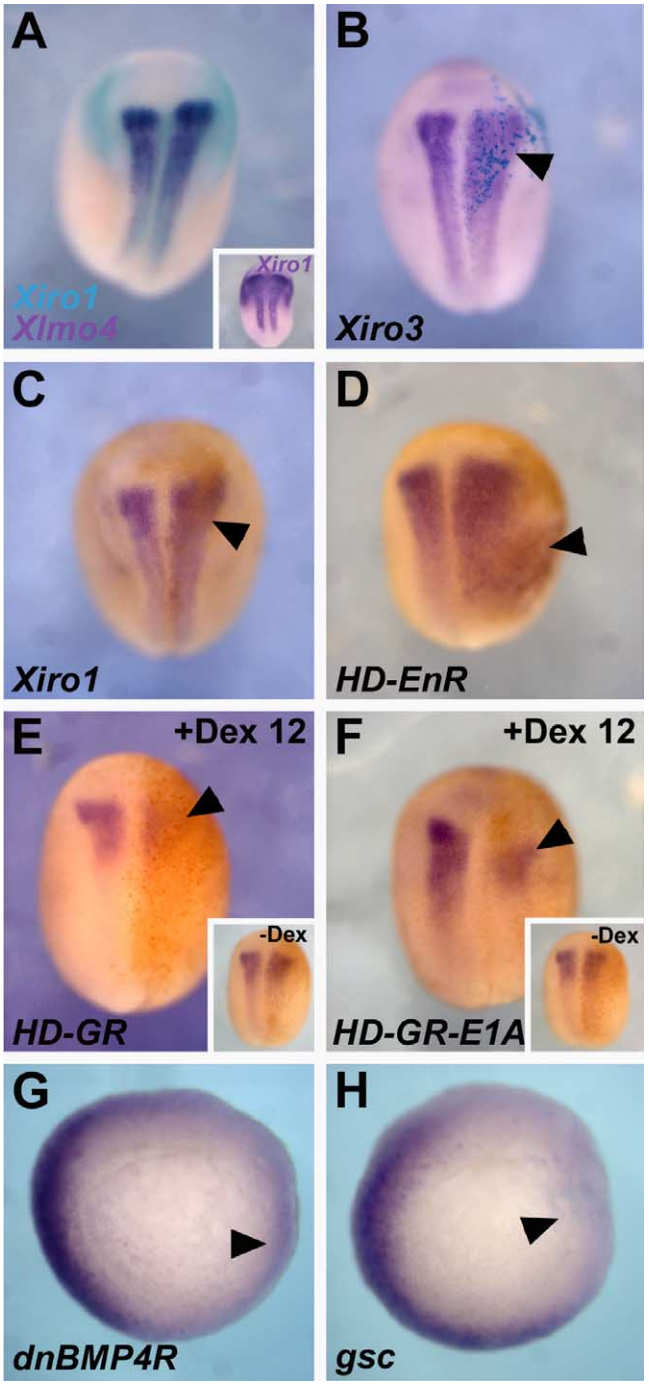


Fig. 2. Regulation of *Xlmo4* expression. Dorsal views (A–F) of midneurula and vegetal views of gastrula (G and H) in control and injected embryos. Injection side was determined by X-Gal staining (B) or by anti-myc staining (C–F). (A) Embryo doubly hybridized for *Xiro1* (cyan) and *Xlmo4* (purple). Note that *Xlmo4* expression in the neural plate is coincident within the *Xiro* bilateral bands. Inset shows a single hybridization for *Xiro1*. (B and C) Embryos injected with 100 or 500 pg of *MT-Xiro3* or *MT-Xiro1* mRNAs respectively. In the injected side (arrowheads), the domain of *Xlmo4* expression is expanded. (D) Similar ectopic expression of *Xlmo4* is detected in embryos injected with 500 pg of *HD-EnR* mRNA (arrowhead). (E and F) In embryos injected with 500 pg of *HD-GR* (E) or *HD-GR-E1A* (F) mRNAs, *Xlmo4* is downregulated (arrowheads) when Dex was added at stage 12. These effects are not observed in the absence of Dex (insets). (G) Embryo injected with *dnBMP4R* mRNA (125 pg). *Xlmo4* expression is reduced (arrowhead). (H) Ectopic ventral expression of *gsc* mRNA (150 pg) downregulates *Xlmo4* (arrowhead).

Embryos, explants, microinjection of mRNA and MO oligonucleotides, and hormone treatment

Xenopus embryos were obtained and animal caps were prepared as described (Gómez-Skarmeta et al., 1998) and staged according to Niewkoop and Faber (1967). Synthetic mRNAs were injected into embryos at the one- or two-cell stage using 8–12 nl. 10–50 ng of an *Xlmo4* antisense morpholino (MO) oligonucleotide (5′-ACTCGGAGATC-CGGTTATTCACCAT-3′) was injected into embryos at the one- or two-cell stage using 8–12 nl. To activate the hormone-inducible Xlmo4 quimeric protein, injected embryos were incubated at different stages with dexamethasone (10 μg/ml).

Results

Isolation and expression of Xlmo4

A *Xenopus* subtraction library enriched in cDNA fragments of genes putatively activated by *Xiro1* overexpression was prepared by subtracting the population of cDNAs from animal caps injected with *Xiro1* mRNA from those obtained from uninjected caps (see the materials and methods section). One cDNA (596 bp) from this enriched library encoded a *Xenopus* homologue of the LIM-domain-only protein Lmo4 (Fig. 1A). Its sequence was 83% identical (90% considering conservative substitutions) to that of human and chick Lmo4 proteins. Hence, the corresponding gene was named *Xlmo4*.

We first performed RT-PCR analyses to determine the presence of *Xlmo4* mRNA at different developmental stages. During early development, *Xlmo4* mRNA was present at similar levels from the one-cell to the neurula stage, with only a slight reduction of *Xlmo4* mRNA accumulation during the midblastula transition (Fig. 1B). This indicates the presence of both maternal and zygotic *Xlmo4* mRNA. We then examined the spatial distribution of *Xlmo4* mRNA by whole-mount in situ hybridization. At the beginning of gastrulation (stage 10), *Xlmo4* mRNA is found throughout most of the marginal zone (Fig. 1C). Slightly later (stage 11), *Xlmo4* expression is downregulated in the dorsal most region (Fig. 1D, arrowhead) and finally disappears from this territory (Fig. 1E, arrow). By this stage, *Xlmo4* expression extends toward neuroectodermal cells (Fig. 1E, arrowhead). Section analyses of gastrulating embryos indicate that at the beginning of gastrulation *Xlmo4* mRNA is present, although at low levels, in dorsal mesendodermal cells that also express both *Xlim1* and *Otx2* (Fig. 1F–H, black arrowheads) and, at higher levels, in ventral mesodermal cells (Fig. 1F, red arrowheads). Section analyses of late gastrula embryos (Fig. 1I) show that *Xlmo4* is found in the neuroectoderm, anterior endodermal cells (red arrow) and ventral mesodermal cells (red arrowhead), but not in dorsal mesodermal cells (black arrowhead). During

neurulation, neuroectodermal cells expressing *Xlmo4* become localized in bilateral patches of the neural plate (Fig. 1J–L). In addition, from late gastrula onwards, *Xlmo4* mRNA is also detected in the ventral epidermis (Fig. 1J, arrowhead in inset). Double staining indicates that the anterior limit of the *Xlmo4* domain partially overlap with the caudal part of the *Otx2* domain (Fig. 1K, arrowhead). At late neurula stages, *Xlmo4* mRNA is also found in a more rostral territory (Fig. 1M, arrowhead) and in the anterior neural crests (Fig. 1M, arrow). At later stages, *Xlmo4* transcripts are found in the brain, in the migrating neural crests (Fig. 1N and O, arrows), and in the ventral epidermis (Fig. 1N and O, arrowheads). The expression pattern of *Xlmo4* in the neuroectoderm is largely similar to that of the zebrafish *Lmo4* gene. However, in contrast to *Xlmo4*, zebrafish *Lmo4* is not expressed in the marginal zone of gastrulating embryos. In addition, zebrafish *Lmo4*, but not *Xlmo4*, is detected in the developing somites (Lane et al., 2002).

Xlmo4 expression in the neural plate requires Xiro activity, while that in the mesoderm depends on BMP-4 signaling

Since we found *Xlmo4* in a subtraction library enriched in genes putatively activated by *Xiro* overexpression, we examined whether *Xlmo4* expression requires the activity of *Xiro*. In contrast to *Xiro1* (Gómez-Skarmeta et al., 1998), *Xlmo4* is not expressed in the prospective neural plate of the early gastrula. However, *Xlmo4* is expressed in this territory of the neurula embryo and its expression domain coincides with the bilateral stripes of *Xiro1* (Fig. 2A). Moreover, the expression of both genes has the same anterior limit. This temporal and spatial coincidence is consistent with Xiro regulating *Xlmo4*. Indeed, injection of either *Xiro1, 2,* or *3* mRNA expands the territory of expression of *Xlmo4* (50–73%, $n = 32$–30; Fig. 2B and C, arrowheads, and not shown). It is known that Xiro1 acts as a repressor whose function can be mimicked or antagonized by constructs containing the Xiro1 homeodomain fused to the Engrailed repressor (HD-En) or to the E1A activator (HD-E1A) domains, respectively (Gómez-Skarmeta et al., 2001). Consistently, we find that injection of *HD-En* mRNA activates *Xlmo4* ectopically (60%, $n = 28$; Fig. 2D, arrowhead). In contrast, interference with Xiro1 function accomplished by expressing either one of two inducible dominant negative constructs (*HD-GR* and *HD-GR-E1A*) downregulated *Xlmo4* in the presence (56–57%, $n = 28$–32; Fig. 2E and F, arrowheads), but not in the absence (Fig. 2E and F, insets), of the hormone dexamethasone (Dex). These results indicate that Xiro1 is required for the expression of *Xlmo4* in the neural plate.

We also examined the regulation of *Xlmo4* in the mesoderm. Expression of many genes in the ventral mesoderm depends on BMP-4 signaling. This was also the case for *Xlmo4*. Interference with BMP-4 signaling, accomplished by expressing a dominant negative BMP-4 receptor (*dn-BMPR4* RNA, 250 pg), significantly reduced *Xlmo4* expression (78%, $n = 55$; Fig. 2G). We have seen that during gastrulation *Xlmo4* was progressively downregulated at the dorsal mesoderm (Fig. 1D and E). Thus, we examined whether misexpression of a dorsal mesoderm gene that encodes a repressor, *goosecoid (gsc),* alters *Xlmo4* expression. Ventral misexpression of *gsc* mRNA (150 pg) downregulated *Xlmo4* expression (84%, $n = 63$; Fig. 2H). Taken together these data suggest that *Xlmo4* is activated in the ventral mesoderm by BMP-4 signaling and downregulated in the dorsal mesoderm by Organizer repressors, among them, Gsc.

Xlmo4 can interfere with dorsal structures

We analyzed the function of Xlmo4 during development by overexpressing it in different tissues. We used an *MT-Xlmo4* construct, which contains an Myc epitope fused in-frame to Xlmo4. Injection of 2–4 ng of *MT-Xlmo4* mRNA in the prospective ectoderm or in the ventral mesoderm did not induce any visible alteration in tailbud embryos (data not shown). However, a similar injection in the dorsal mesoderm impaired formation of dorsal-anterior structures (63%, $n = 72$; Fig 3A and B). Similar results were found with embryos injected with an inducible form of *Xlmo4 (MT-Xlmo4-IND,* 0.5 ng). In the absence of Dex no phenotype was observed (100%, $n = 42$; Fig. 3C); but its addition, before the start of gastrulation (stage 6), caused strong suppression of dorsal-anterior structures (76%, $n = 58$; Fig. 3D). Phenotypes were progressively milder when Dex addition was delayed to later stages (42–9%, $n = 48$–64; Fig. 5E and F, respectively). These data indicate that Xlmo4 can interfere with dorsal mesoderm formation at early gastrula stages. Similar results were obtained by overexpressing 2–4 ng of a nontagged *Xlmo4* mRNA (Fig. 3G).

Lmo proteins are known to sequester the cofactor Ldb1 from its complexes with different transcription factors, thus interfering with the activity of these complexes (Milán and Cohen, 1999; Milán et al., 1998; Shoresh et al., 1998; Thaler et al., 2002; van Meyel et al., 1999; Weihe et al., 2001; Zeng et al., 1998). Thus, we examined the effect of interfering with Ldb1 by means of an Ldb dominant negative construct (*DN-Ldb;* Bach et al., 1999). Injection of *DN-Ldb* mRNA (2 ng) in the dorsal mesoderm, similarly to the overexpression of *Xlmo4*, strongly impaired the development of dorsal-anterior structures (68%, $n = 44$; Fig. 3H). Recently, it has been shown that mice mutant for *Ldb1* display defective head structures, probably as a consequence of disrupting the early Otx2 and Lim1 pathways (Mukhopadhyay et al., 2003). In these mutant embryos, the expression of Wnt antagonists is abrogated. Thus, it has been proposed that suppression of head structures in Ldb1 mutant embryos is the consequence of increased Wnt signaling due to the impairment of early Otx2 and Lim1 pathways, which are required for the expression of Wnt antagonists (Mukhopadhyay et al., 2003). During *Xenopus* development, *Otx2*

and *Xlim1* are expressed and required for dorsal mesoderm fomation. Thus, we have examined the effect of *Xlmo4* overexpression in these and other dorsal mesoderm genes. We observed similar effects in embryos injected with *MT-Xlmo4* mRNA (2–4 ng; not shown) or *MT-Xlmo4-IND* (0.5 ng) mRNA in the presence (Fig. 4), but not in the absence (insets in Fig. 4), of the hormone Dex. Overexpression of these forms of *Xlmo4* mRNAs downregulated the dorsally expressed transcription factors (*gsc* and *Otx2*) and the antagonists of the Bmp4 (*chordin, chd*) and the Wnt [*cerberus (cer)* and *Frizzled-b (Frzb)*] pathways (57–64%, $n = 36–63$; Fig 4A–E). These repressions were not associated with the activation of ventral markers, such as *Xvent1* and *Xwnt8* (100%, $n = 35–48$; Fig. 4F and G). In addition, these genes were not affected by ventral overexpression of *Xlmo4* mRNAs (not shown). Double staining of *Xlim1* and *Xlmo4* at early gastrula stages indicates that both genes are coexpressed before *Xlmo4* is downregulated in the dorsal-most mesoderm (Fig. 4H). Thus, before its expression is removed from the dorsal mesoderm, *Xlmo4* may be restricting the availability of Ldb1 for organizer-expressed transcription factors.

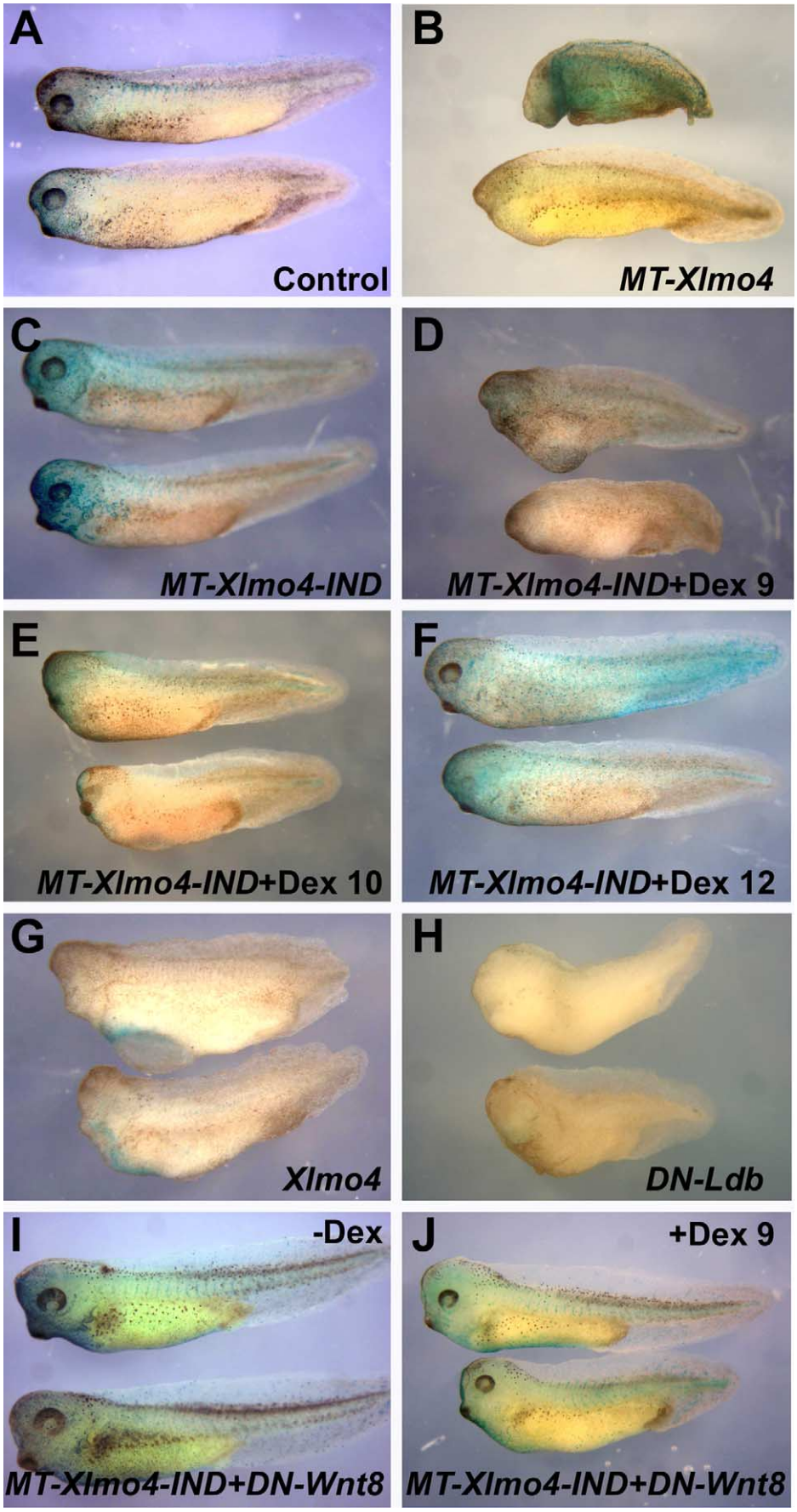
We have also examined whether the suppression of head structures observed upon *Xlmo4* overexpression is caused by the downregulation of Wnt inhibitors, and thus it is an indirect consequence of the increased activity of the Wnt pathway in the dorsal mesoderm. To test this, we coexpressed in the dorsal mesoderm *MT-Xlmo4-IND* (0.5 ng) mRNA and an mRNA encoding a dominant negative form of *Wnt8 (DN-Wnt8*, 0.5 ng), which suppresses Wnt signaling (Hoppler et al., 1996). The DN-Wnt8 restored the dorsal anterior structures (Fig. 3I and J), indicating that the suppression of these structures in *Xlmo4*-injected embryos is probably due to the reduction of Wnt inhibitors and the concomitant increased Wnt signaling.

Xlmo4 is required for ventral mesoderm identity

Xlmo4 expression in the ventral mesoderm suggests a function of this gene in this territory. It is known that Lmo proteins act as positive cofactors of several transcription factors of the bHLH and GATA classes (Bao et al., 2000; Mead et al., 2001; Osada et al., 1995; Sugihara et al., 1998; Vitelli et al., 2000; Wadman et al., 1997) and that GATA factors participate in formation of the ventral mesoderm (Sykes et al., 1998). Hence, we first attempted to interfere with Xlmo4 function by means of a morpholino antisense oligonucleotide designed to bind to the Xlmo4 translation start site. Although injection of 10 ng of this morpholino effectively suppresses translation of 2 ng of an Xlmo4 construct bearing an Myc epitope at the carboxy-terminus (Xlmo4-MT), we did not detect a phenotypic effect in embryos injected with up to 50 ng of such morpholino (not shown). Thus, it is possible that this morpholino is unable to block translation of the endogenous gene, or alternatively, that other Lmo proteins rescue the loss of function of Xlmo4.

On the other hand, if Lmo4 acts as a positive cofactor of some transcription factor(s), a chimeric fusion of Lmo4 with the Engrailed repressor domain, which behaves as a dominant negative in cell culture transcription assays (Andersen, B., unpublished data), might interfere with this function. Thus, we tested the effects of this chimera and compared them with those of a dominant negative GATA construct *(Gata-En). Gata-En* interferes with several GATA factors, which are required for ventral mesoderm formation during gastrulation (Sykes et al., 1998). Overexpression of *Lmo4-En* mRNA (2 ng) in ventral mesoderm dorsalized the injected embryos (48%, $n = 92$; Fig. 5A). In contrast, coinjection of *Lmo4-En* mRNA and *MT-Xlmo4* mRNA essentially abolished this effect (94%, $n = 100$, Fig. 5B). This indicated that Lmo4-En interferes specifically with the function of Xlmo4. The dorsalization caused by *Lmo4-En* was similar, although weaker, to that produced by ventral overexpression of *Gata-En* mRNA (0.2 ng; Fig. 5C). Interference with Xlmo4 function ectopically activated the dorsal gene *gsc* (75%, $n = 54$), but not other dorsal markers such as chordin *(chd), cerberus*, or *XlimI* (100%, $n = 46$; Fig 5G, and not shown). In addition, the ventral markers *Xvent1* (75%, $n = 34$; Fig. 5J) and *Xwnt8* (50%, $n = 40$; Fig. 5M), but not *Xvent2* (not shown), were repressed. Consistent with the partial dorsalization observed, at early neurula stages, the dorsal mesoderm genes *MyoD* and *sonic hedgehog (shh)* were not expressed ectopically in the ventral side of these injected embryos (not shown). Coexpression of *Lmo4-En* and *MT-Xlmo4* mRNAs rescued these effects (Figs. 5E, K and N). Thus, ectopic *gsc* was observed in only 10% of the coinjected embryos (Fig. 5E) and repression of *Xvent1* and *Xwnt8* was detected in only 17% and 10% of the embryos ($n = 36–40$, Fig. 5K and N, respectively). Other *Xenopus Lmo* genes were not tested in this rescue assay since they are not expressed at gastrula stages in the ventral mesoderm. As previously shown (Sykes et al., 1998), ventral interference with GATA function is also accompanied by repression of *Xvent1* (63%, $n = 16$; Fig. 5L) and *Xwnt8* (60%, $n = 20$; Fig. 5O) but not of *Xvent2* (not shown). However, in em-

Fig. 3. *Xlmo4* prevents dorsal mesoderm formation. Lateral views of stage 35 embryos injected with the corresponding mRNAs. These mRNAs were coinjected with *lacZ* mRNA (300 pg). (A) Control embryo injected only with *lacZ* mRNA. (B) Embryos injected with 4 ng of *MT-Xlmo4* mRNA; dorso-anterior structures are missing. (C) Embryos injected with 500 pg of *MT-Xlmo4-IND* mRNA; they are normal in the absence of the hormone. (D–F) Embryos injected as in (C) and Dex added before gastrulation, at early gastrula or at late gastrula, respectively; the dorso-anterior structures are strongly, mid, or not suppressed. (G and H) Embryos injected with nontagged *Xlmo4* (4 ng, H) or *DN-Ldb* (2 ng, G) mRNAs showed similar suppression of dorso-anterior structures. (I and J) Embryos coinjected with *MT-Xlmo4-IND* and *DN-Wnt8* mRNAs (500 pg) show enlarged heads both in the absence (I) or in the presence (J) of Dex.


A
Control
B
MT-Xlmo4
C
MT-Xlmo4-IND
D
MT-Xlmo4-IND+Dex 9
E
MT-Xlmo4-IND+Dex 10
F
MT-Xlmo4-IND+Dex 12
G
Xlmo4
H
DN-Ldb
I
-Dex
MT-Xlmo4-IND+DN-Wnt8
J
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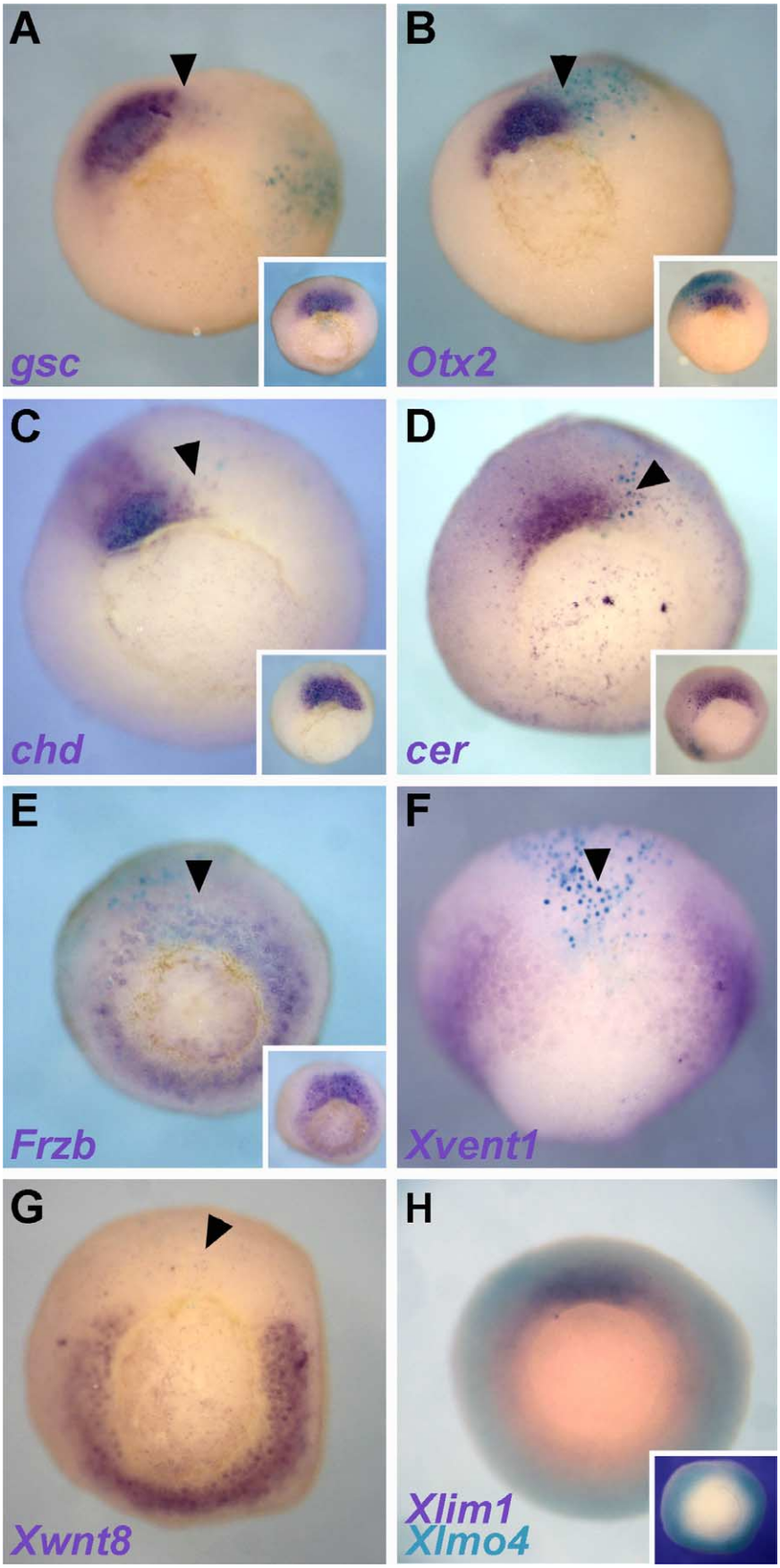
MT-Xlmo4-IND+DN-Wnt8


A
gsc
B
Otx2
C
chd
D
cer
E
Frzb
F
Xvent1
G
Xwnt8
H
Xlim1
Xlmo4

bryos injected with *Gata-En* mRNA, *chd* but not *gsc* was ectopically activated (80–100%, $n = 20–18$; Fig. 5I and F, respectively). These effects were not rescued by coexpression of *MT-Xlmo4* mRNA (not shown). This indicated that interference with Xlmo4 and GATA function caused similar although not identical effects.

As mentioned above, Lmo2 binds to and acts as a cofactor of GATA1 during blood formation (Mead et al., 2001; Osada et al., 1995; Vitelli et al., 2000; Wadman et al., 1997). Therefore, it seemed possible that Lmo4 might also bind to and act as a cofactor of GATA factors. In fact, *Gata2*, *4*, *5*, and *6*, but not *Gata1*, are expressed at and required for ventral mesoderm formation during gastrulation (Bertwistle et al., 1996; Kelley et al., 1994; Sykes et al., 1998; Walmsley et al., 1994). By means of coimmunoprecipitation assays we have detected a physical interaction between Lmo4 and GATA2 similar to that observed between Lmo4 and Ldb1 (Fig. 6A). Moreover, GST-pulldown experiments have allowed us to confirm that this interaction is direct and to determine that Lmo4 interacts with the N-terminal region of GATA2 (amino acids 1–266), which contains the activation domain, but not with the C-terminal region (amino acids 257–452), which contains the zinc-finger region (Fig. 6B). The same results were observed in coimmunoprecipitation assays performed with transfected cells (not shown). To further test whether GATA2 and Xlmo4 act in the same pathway during ventral mesoderm formation, we examined in coinjection experiments whether they have synergistic ventralizing effects. Embryos injected with suboptimal concentrations of *Gata2* (100 pg) or *MT-Xlmo4* (500 pg) mRNAs developed with little or no sign of ventralization (Fig. 6C and D; 100–96%, $n = 45–52$, respectively). However, embryos injected with both mRNAs showed clear ventralization phenotypes (Fig. 6E, 34%, $n = 53$). We conclude that, similarly to Lmo2, Lmo4 is a positive cofactor of GATA's during ventral mesoderm formation.

Xlmo4 participates in neural plate regionalization

Since we identified *Xlmo4* as a gene positively regulated by Xiro1, we examined its function during neural development by overexpressing *Xlmo4* mRNA. To prevent early mesodermal effects during gastrulation, we injected *MT-Xlmo4-IND* mRNA (0.5 ng) and added Dex at the end of gastrulation (stage 12). In these embryos, we observed a downregulation of the proneural gene *Xngnr1* (67%, $n = 43$; Fig. 7A, arrow) and a reduction of the number of primary neurons, as determined by *N-tubulin* expression (58%, $n = 43$; Fig. 7D; arrow). No effects were observed in the absence of Dex (Fig. 7A and D, insets). Injection of *Lmo4-En* mRNA also reduced expressions of *XngnrI* and *N-tubulin* (80–60%, $n = 40–30$; Fig. 7B and E, arrows, respectively). These results suggest that overexpression of either Xlmo4 or Lmo4-En similarly prevents primary neuron differentiation. Thus, in this context, Lmo4-En does not behave as a dominant negative molecule. It is possible that, as it occurs in the dorsal mesoderm, Xlmo4 withholds Ldb1 from transcription factors that are necessary for neuronal differentiation. This would be consistent with Lmo4-En not acting as a dominant negative molecule, since, despite its repression domain, this chimeric protein should still sequester Ldb1. In support of this interpretation, we have observed that, in the dorsal mesoderm, Lmo4-En does not interfere with Xlmo4 function and behaves like this protein in preventing dorsal mesoderm development (not shown). If Xlmo4 or Lmo4-En do sequester Ldb1 in the neural plate, it would be expected to find similar effects upon interference with Ldb1 function. To test this, we examined the effect of overexpressing a dominant negative form of Lbd protein *(DN-Ldb)*. *DN-Ldb* mRNA (1 ng) downregulated both *Xgnr1* and *N-tubulin* (60–62%, $n = 25–32$; Fig. 7C and F, arrows, respectively).

Impairment of Ldb1 function, both in mice mutant for this gene or by overexpression of *DN-Ldb* mRNA in zebrafish, disrupts gene expression at the Isthmus Organizer, which is located at the midbrain-hindbrain (M/H) boundary (Becker et al., 2002; Mukhopadhyay et al., 2003). Thus, we examined the effect of overexpressing *MT-Xlmo4-IND* (0.5 ng) in this territory at the end of gastrulation. *Otx2* was either not affected or slightly shifted posteriorly (50%, $n = 36$; Fig. 7G, compare black and red arrows in the injected and control sides, respectively). In contrast both *Gbx2* and *En2* were downregulated (78–58%, $n = 54–36$; Fig. 7J and M, arrows, respectively). These effects were not observed in the absence of the hormone (Fig. 7G, J, and M; insets). Similar effects were observed in embryos injected with *Lmo4-En* mRNA (2 ng) (64–82%, $n = 33–42$; Fig. 7H, K, and N, black arrows) or in embryos injected with *DN-Ldb* mRNA (1 ng) (55–57%, $n = 33–35$; Fig. 7I, L, and O, black arrows). These data indicate that Xlmo4 regulates Ldb1 availability for transcription factors required for both primary neurogenesis and Isthmus Organizer formation.

Fig. 4. *Xlmo4* overexpression downregulates gene expression at the dorsal mesoderm. (A–G) Vegetal views of early gastrula embryos injected with *MT-Xlmo4-IND* mRNA (500 pg) treated with Dex at stage 6. In these embryos the dorsal genes *gsc* (A), *otx2* (B), *chd* (C), *cer* (D), and *Frzb* (E) are repressed, but the ventral gene *Xvent1* (F) and *Xwnt8* (G) are not ectopically activated in the dorsal side. Repression of dorsal gene expression was not observed in similar injected embryos not treated with the hormone (A–E, insets). (H) Vegetal view of an early gastrula embryo showing the expression of *Xlmo4* (cyan) and *Xlim1* (purple). Note that both genes are coexpressed in the dorsal mesoderm. Inset shows the same embryo after the first chromogenic reaction to detect *Xlmo4* mRNA (cyan).

Discussion

Xlmo4 is a GATA cofactor required for ventral mesoderm formation and a negative modulator of the activity of Ldb1-dependent organizer genes

During gastrulation *Xlmo4* is initially expressed both at the dorsal and ventral mesoderm while later its expression is downregulated in the dorsal-most mesoderm. The expression of *Xlmo4* in the mesoderm seems to depend on positive factors such as Bmp4 and negative regulators such as Gsc, which may mediate dorsal downregulation of *Xlmo4* as gastrulation proceeds. In the ventral mesoderm, we find that decreasing the function of Xlmo4 with an Lmo4-En chimeric protein, which in this context acts as a dominant negative molecule, promotes partial dorsalization of the embryos. This is evidenced by the repression of the ventral mesoderm markers *Xvent1* and *Xwnt8* and the activation of the dorsal gene *gsc*. Other dorsal markers assayed (*chd*, *cerberus*, *Xlim1*, *MyoD*, and *shh*) are not ventrally activated. This could explain the weak dorsalization observed in these embryos. We do not know why the ectopic activation of *gsc* does not promote a full dorsalization program by activating other dorsal mesoderm genes. One possibility is that the levels of ectopic *gsc* promoted by interference with Xlmo4 function are insufficient for the activation of other dorsal mesoderm genes.

The LIM only protein Lmo2 has been shown to form a multiprotein complex with Ldb1, GATA1, and the bHLH factors SCL and E12/E47, which are required for development of the blood tissue in *Xenopus* and the hematopoietic system in other vertebrates (Mead et al., 2001; Osada et al., 1995; Pevny et al., 1991; Vitelli et al., 2000; Wadman et al., 1997). The blood derives from the most ventral mesoderm. In *Xenopus*, *Gata1* and *Xlmo2* are expressed during late neurula in territories in which blood will form (Kelley et al., 1994; Mead et al., 2001). However, *Gata1* is not expressed in the ventral mesoderm at early gastrula stages. So its interaction at this stage with *Xlmo4* is most unlikely. In contrast, *Gata2*, *4*, *5*, and *6* are all expressed during gastrulation in the ventral mesoderm and the function of these genes is required for the correct development of this territory (Bertwistle et al., 1996; Kelley et al., 1994; Sykes et al., 1998; Walmsley et al., 1994). Indeed, interference with their function partially dorsalizes embryos and generates axis duplications as a result of the downregulation of ventral mesoderm genes and the ectopic ventral activation of some dorsal genes (Sykes et al., 1998). Thus, it is possible that during ventral mesoderm formation Xlmo4 acts as a positive cofactor of some of these GATA factors. We have found, by means of GST-pulldown and coimmunoprecipitation assays, that there is indeed an interaction between Lmo4 and GATA2. We have mapped this interaction within the N-terminal region of GATA2, in which the activation domain is located. Moreover, we have found that coinjection of *Gata2* and *Xlmo4* act synergistingly to promote ventral fates. These results strongly suggest that Xlmo4 is a cofactor of GATA proteins during early ventral mesoderm formation. Ldb1 may also participate in this complex. Indeed, mice mutant for Ldb1 show a posterior axis duplication (Mukhopadhyay et al., 2003), and we have observed a partial dorsalization of embryos ventrally injected with a dominant negative Ldb1 construct (not shown). Thus, it is likely that, similar to the multiprotein complex required for blood formation, a related complex formed by Xlmo4, GATA factors, and probably Ldb1 is required for ventral mesoderm formation. Other *Xenopus* Lmo proteins may also interact with GATA factors during ventral mesoderm formation. However, neither *Xlmo2* nor *Xlmo3* are expressed in this territory during gastrulation (Bao et al., 2000; Mead et al., 2001). This indicates that Xlmo4 is the main candidate to act as cofactor for GATA molecules present in the ventral mesoderm during gastrulation. However, overexpression of *Xlmo4* cannot rescue the dorsalization caused by interference of GATA function by *Gata-En* mRNA. It is likely that the GATA-En chimeric protein interferes with GATA activity by binding to GATA sites in the regulatory region of downstream genes. The chimeric protein would then repress these downstream genes. This repression would not be affected by overexpression of Xlmo4 since GATA-En does not contain the Xlmo4 binding domain. Interestingly, interference with Xlmo4 function in the ventral mesoderm promotes the ectopic expression of *gsc*, but not that of other dorsal genes, while that with GATA factors causes ventral ectopic expression of *chd* but not of *gsc*. Thus, it is possible that, in the ventral mesoderm, Xlmo4 acts also as a cofactor for other ventral transcription factor distinct from those of the GATA family.

As mentioned above, at the beginning of gastrulation *Xlmo4* is present all around the mesoderm and thus it is coexpressed with organizer genes. However, dorsal *Xlmo4* expression soon disappears, probably by repression mediated by some organizer transcription factors such as Gsc. This downregulation seems to be important for the proper development of the dorsal mesoderm, since overexpression of *Xlmo4* in the dorsal mesoderm represses many organizer genes. Lmo proteins are known to interfere with LIM homeoproteins, and possible other transcription factors, by binding and sequestering the cofactor Ldb1 from it complexes with those factors (Milán and Cohen, 1999; Milán et al., 1998; Shoresh et al., 1998; Thaler et al., 2002; van Meyel et al., 1999; Weihe et al., 2001; Zeng et al., 1998). One possibility is that Xlmo4 may regulate Ldb1 availability for dorsally expressed transcription factors. Two candidates are Xlim1 and Otx2, both of which are required for dorsal mesoderm formation (reviewed in Bach, 2000; Dawid et al., 1998; Hobert and Westphal, 2000). Indeed, overexpression of *Xlmo4* in the dorsal mesoderm cause similar effects that those observed in mice mutant for *Ldb1* (our data and Mukhopadhyay et al., 2003). In these embryos Wnt inhibitors are downregulated, and probably as a consequence of increased Wnt signaling, dorso-anterior structures

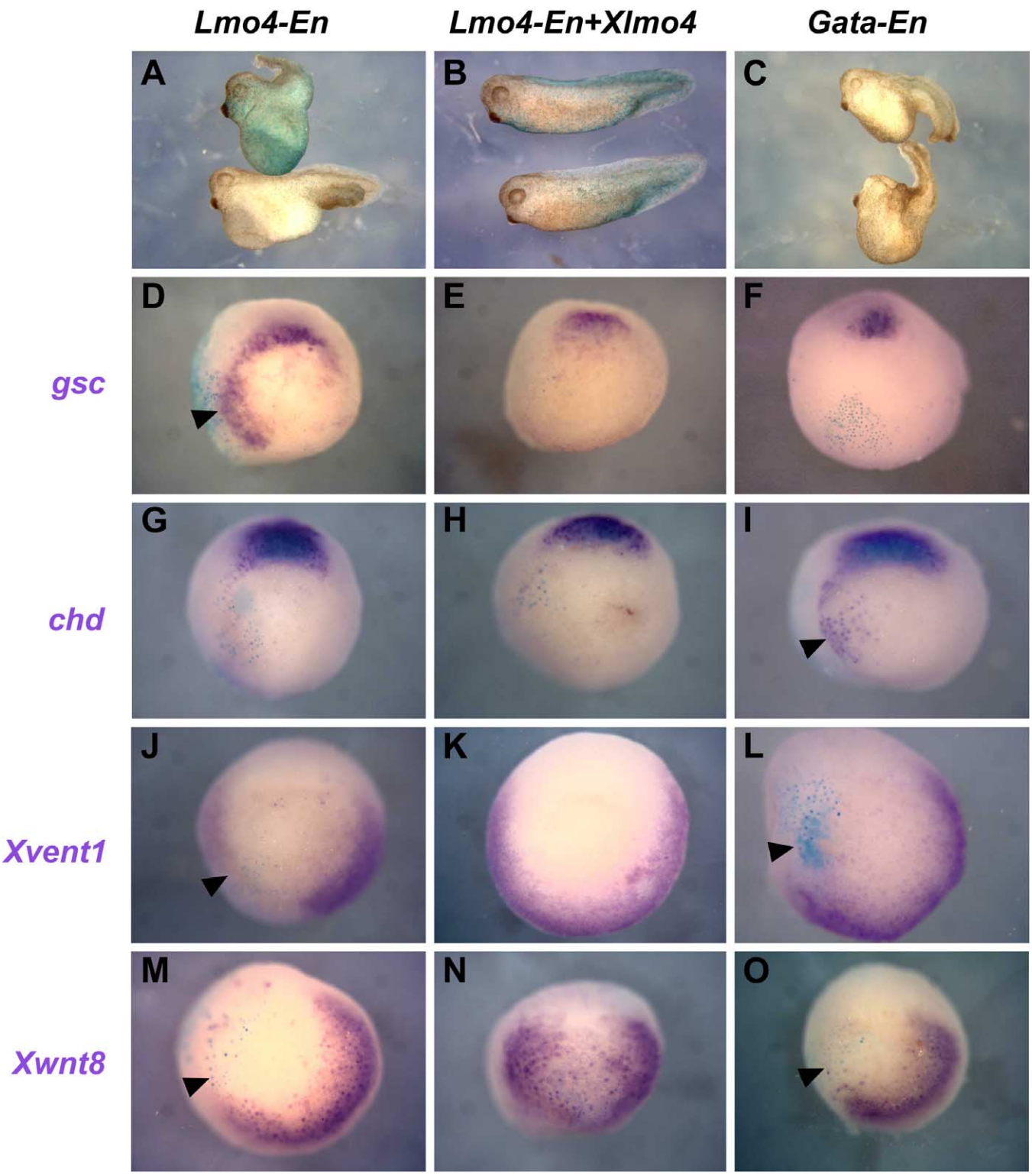


Fig. 5. *Xlmo4* participates in ventral mesoderm identity. Lateral views of stage 35 (A and C) or vegetal views of stage 10.5 (D–O) embryos injected with the indicated mRNAs together with *lacZ* mRNA (300 pg). The injection side was determined by X-Gal staining. (A, D, G, J, and M) Embryos injected with 2 ng of *Lmo4-En* mRNA are dorsalized (A) probably as a result of the ectopic ventral expression of *gsc* (D, arrowhead) and the repression of *Xvent1* (J, arrowhead) and *Xwnt8* (M, arrowhead) genes. The dorsal gene *chd* was not activated in the ventral region of these injected embryos (G). (B, E, H, K, and N) Coinjection of 2 ng of MT-Xlmo4 suppressed the effects observed in *Lmo4-En* injected embryos. (C, F, I, L, and O) Ventral overexpression of 200 pg of *Gata-En* mRNA causes dorsalization of the embryos (C). In these embryos, the dorsal gene *chd* is ectopically activated in the ventral mesoderm (I, arrowhead), while the ventral markers *Xvent1* (L, arrowhead) and *Xwnt8* (O, arrowhead) are suppressed. Note that *gsc* is not activated in the ventral mesoderm of these injected embryos (F).

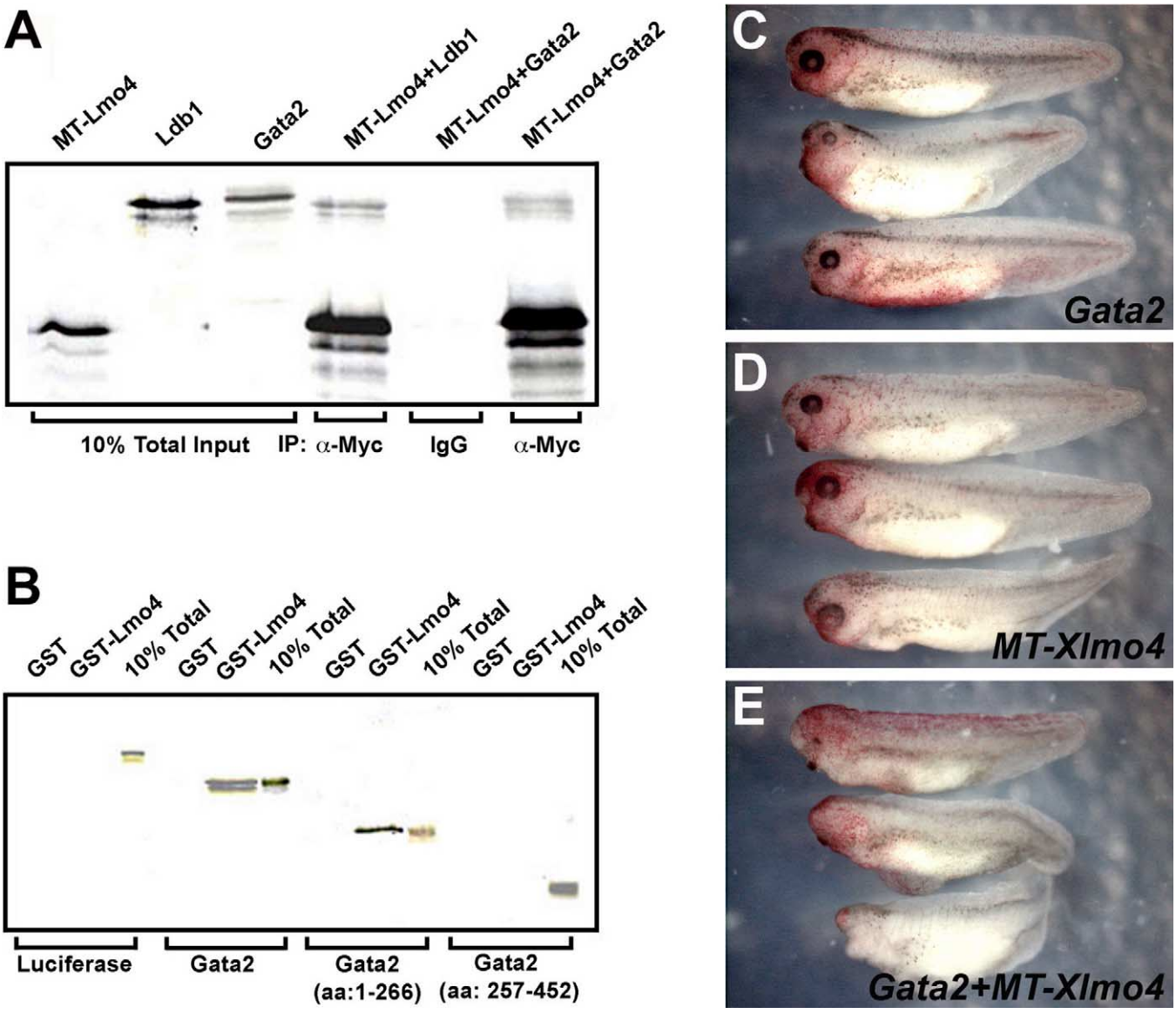


Fig. 6. Lmo4 and GATA2 physically interact and synergize in promoting ventralization. (A) The indicated in vitro translated ^{35}S-labeled GATA2 and Ldb1 proteins were coimmunoprecipitated with MT-Lmo4, using anti-Myc antibody or IgG. In a mixture of MT-Lmo4 and Ldb1 (lane 4) and MT-Lmo4 and GATA2 (lane 6), anti-Myc antibody precipitated Ldb1 and GATA2, respectively, indicating that LMO4 interacts with Ldb1 and GATA2. IgG does not precipitate these proteins, indicating that the effect is specific. The first three lanes show 10% of the proteins used for the immunoprecipitations. (B) GST-pulldown assays to determine Lmo4 interaction with GATA2. The indicated in vitro translated ^{35}S-labeled proteins were incubated either with GST protein beads alone or GST-Lmo4 protein beads. A negative control radiolabeled Luciferase is not pulled down by GST-Lmo4 (lane 2). In contrast, radiolabeled GATA2 is specifically precipitated by GST-Lmo4 (lane 5). A fragment of GATA2 containing the N-terminal region (aa: 1–266) but not the C-terminal domain (aa: 257–452) interacts with GST-Lmo4, mapping the interaction domain to the N-terminus of GATA2. (C–E) Lateral views of stage 35 embryos injected with with *lacZ* mRNA (300 pg) and the indicated mRNAs. Embryos injected with either *Gata2* or *MT-Xlmo4* mRNAs (100 or 500 pg, respectively) showed no sign of ventralization. In contrast, coinjection of both mRNAs promoted a clear ventralization phenotype.

are impaired (our data and Mukhopadhyay et al., 2003). Similarly, we find that suppression of head structures upon *Xlmo4* overexpression is rescued by interfering with Wnt signaling. Thus, before its expression is downregulated from the dorsal mesoderm, Xlmo4 may negatively modulate the activity of Xlim1 and Otx2 homeoproteins. In addition, Xlmo4, which is later restricted to the ventral mesoderm, may also restrict Ldb1 availability for some dorsal homeoproteins.

In summary, at early gastrula stages, it seems that Xlmo4 binds Ldb1 in all the marginal zone. However, while in the ventral mesoderm Xlmo4 acts in concert with Ldb1 and GATA to promote ventral identity, in the dorsal mesoderm the Xlmo4-Ldb1 interaction sequesters Ldb1 and, consequently, it negatively regulates the complexes formed with this cofactor and some organizer homeoproteins. We conclude that at the same developmental time Xlmo4 positively and negatively regulates ventral and dorsal mesoderm formation, respectively.

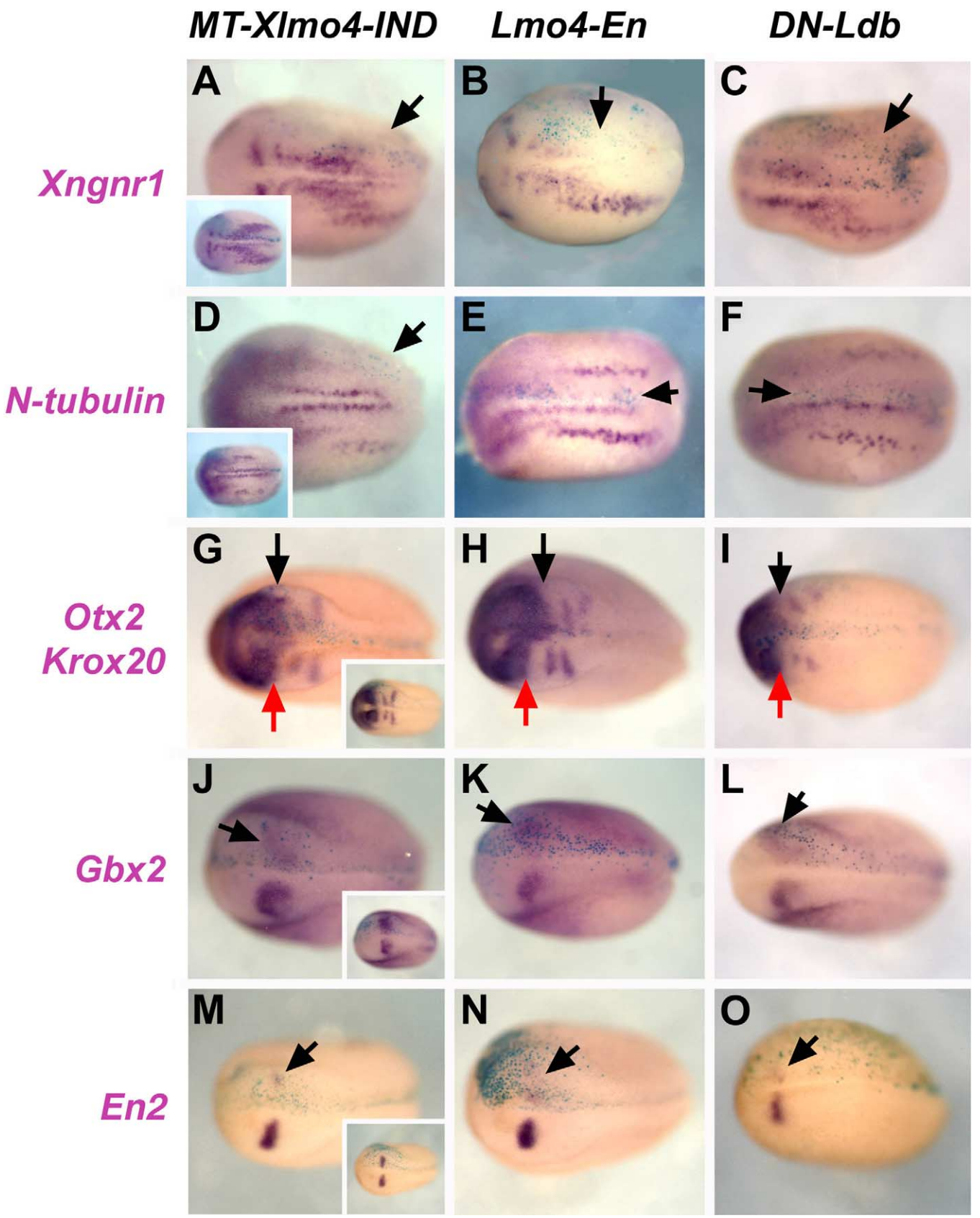


Fig. 7. *Xlmo4* participate in neural development. All panels show dorsal views of neurula embryos injected with the indicated mRNAs together with *lacZ* mRNA (300 pg). The injection side was determined by X-Gal staining. (A, D, G, J, and M) Embryos injected with 500 pg of *MT-Xlmo4-IND* mRNA and treated with Dex at stage 12. In these embryos *Xngnr1* (A), *N-tubulin* (D), *Gbx2* (J), and *En2* (M) are downregulated (arrows). In some of these embryos, the expression domains of *Otx2* and *Krox20* are slightly shifted toward posterior (G, compare black and red arrows in injected and control sides). Insets in these panels show similarly injected embryos not treated with Dex. Similar results were observed in embryos injected with 2 ng of *Lmo4-En* (B, E, H, K, and N; arrows) or 1 ng of *DN-Ldb* (C, F, I, L, and O; arrows) mRNAs.

Xlmo4 is a Xiro-regulated gene that modulates Ldb1 activity in the neural plate

We have identified *Xlmo4* in a screen designed to identify genes activated by *Xiro*. Consistently, we have found that, during neurulation, *Xiro* and *Xlmo4* are expressed in coincidental domains symmetrically located at each side of the midline, that these domains comprise part of the neurogenic ectoderm, and that their anterior limits extend up to that of *En2* within the Otx2 territory. These neural domains appear after the onset of *Xiro* expression (Gómez-Skarmeta et al., 1998). Moreover, since an increase or a reduction of *Xiro* function respectively activates or downregulates *Xlmo4*, it is clear that the expression of *Xlmo4* in the neural plate is downstream of Xiro. However, *Xiro* does not directly activate *Xlmo4*, since the Xiro proteins participate in this process as repressors. This seems to be also the case for many other Xiro-dependent processes (de la Calle-Mustienes et al., 2002a,b; Glavic et al., 2002; Gómez-Skarmeta et al., 2001).

Our data suggest that, in the neural plate, Xlmo4 negatively modulates the availability of Lbd1 for transcription factors that participate in primary neurogenesis and midbrain/hindbrain (M/H) boundary formation, probably by sequestering this cofactor from active complexes. LIM homeoproteins are known to participate in neuron differentiation (reviewed in Bach, 2000; Dawid et al., 1998; Hobert and Westphal, 2000). Thus, it is possible that Xlmo4 interferes with the complexes formed by Ldb1 and LIM homeoproteins. However, although *Xlim1* and *3* are respectively expressed in the intermediate and medial stripes of primary neurons, their expression occurs at relatively late stages, when these neurons have already been singularized (Taira et al., 1992,1993). Thus, these homeoproteins are probably implicated in the acquisition of neuronal fate but not in the initial differentiation of these neurons. This suggests that Xlmo4 may negatively regulate primary neuron differentiation through a different mechanism. In *Drosophila*, the GATA factor Pannier (Pnr) is required for the activation of proneural genes (Garcia-Garcia et al., 1999). The Ldb1 protein Chip interacts with Pnr and proneural proteins, and this multiprotein complex seems to be required for neuronal differentiation (Ramain et al., 2000). Moreover, overexpression of *Drosophila Lmo* removes Pannier-dependent sensory organs (Ramain et al., 2000). It is thus possible that a similar complex may be required for proneural gene expression and primary neurogenesis in *Xenopus*. Xlmo4 might negatively modulate such a complex by sequestering Ldb1. On the other hand, in *Xenopus*, Xlmo3 binds to and acts as a positive cofactor of bHLH proneural proteins (Bao et al., 2000). Thus, an additional possibility, that is not incompatible with the previous one, is that Xlmo4 may compete with Xlmo3 and generate inactive complexes with proneural proteins. Consistently, we find that *Xlmo4* expression coincides with that of *Xiro1* and neurons arise at the border of *Xiro1* domains (de la Calle-Mustienes et al., 2002a). Thus, although *Xlmo4* expression overlaps with that of the proneural gene *Xngnr1*, neurons arise within *Xngnr1* domains devoid of *Xlmo4*. The exact mechanism by which Xlmo4 prevents primary neurogenesis will require identification of other proteins that may interact with this cofactor.

It is of interest to note that Xiro proteins seem to help define the territories in which neurons differentiate by means of several parallel mechanisms. First, Xiro proteins prevent primary neuron differentiation by indirectly activating the neuronal repressor *XZic2* and *XHairy2A* (de la Calle-Mustienes et al., 2002a). Second, Xiro proteins repress *XGadd45-γ*, which seems to facilitate differentiating neurons to exit from the cell cycle (de la Calle-Mustienes et al., 2002a). And third, by activating *Xlmo4* Xiro interferes with transcription factors required for primary neurogenesis in the neural plate.

We have also found that Xlmo4 participates in the specification of the M/H organizer. Thus, *Xlmo4* is expressed in this territory and its increased activity downregulates *Gbx2* and *En2*. *Otx2* is shifted caudally, probably as a consequence of the *Gbx2* repression. These effects are likely due to the displacement of Ldb1 from active complexes formed by this cofactor and different transcription factors. Candidates are several LIM homeoproteins that are expressed in this territory (Bach et al., 1997). Consistently with our results, mice mutant for Ldb1 (Mukhopadhyay et al., 2003) and zebrafish or *Xenopus* embryos in which Ldb1 function is impaired by a dominant negative form of Ldb1 (Becker et al., 2002), and our results) display a downregulation of M/H genes.

Interestingly, *Xiro1* is also required for the correct specification of the M/H organizer (Glavic et al., 2002). At least part of this requirement may be effected by its downstream gene *Xlmo4*.

If, as suggested, the overexpression of *Xlmo4* in many cases reduces the availability of Ldb1 for other transcription factors, we would expect that increasing the levels of Ldb1 would rescue the effects associated with *Xlmo4* overexpression. However, we have been unable to observe such rescue (not shown). Moreover, embryos coinjected with *Ldb1* and *Xlmo4* displayed phenotypes stronger that embryos injected with *Xlmo4* alone. It has been found in *Drosophila* that the stoichiometry of the Lhx protein Ap and the Ldb1 homologue Chip, which together form complexes containing two Ap and two Chip proteins, is critical for the formation of the dorsal compartment in the wing disc (Milán and Cohen, 1999; Rincón-Limas et al., 2000; van Meyel et al., 1999; Weihe et al., 2001). Thus, Chip overexpression causes similar defects to Chip loss of function, probably by the ability of Chip to form multimeres that alter that precise stoichiometry (Fernández-Fúnes et al., 1998). A similar critical stoichiometry may occur in *Xenopus* for those complexes formed by Ldb1 and different homeoproteins. Xlmo4, by binding to Ldb1, would probably regulate this stoichiometry in vivo and, consequently, overexpression of *Xlmo4* and/or *Ldb1* may alter it in a way incompatible with wild-type

function. It has been recently shown in *Xenopus* that the RING finger protein Rnf12/RLIM is required in the Organizer to degrade the excess of Ldb1 that is not bound to Xlim1. This degradation is important for dorsal mesoderm formation since extra Ldb1 can alter the proper stoichiometry of Xlim1/Lbd1 complexes. Indeed, overexpression of Ldb1 downregulates *Xlim1* target genes (Hiratani et al., 2003). Thus, to rescue the effects of *Xlmo4* overexpression by increasing the levels of *Ldb1* it would be necessary to restore the exact stoichiometry of these complexes, a feat difficult to achieve with mRNA injections. *Rnf12/RLIM* is expressed at similar levels at the dorsal and the ventral mesoderm. Thus, since at the dorsal mesoderm Xlim1 prevents Rnf12/RLIM-mediated Ldb1 degradation, Ldb1 should be at lower levels in the ventral than in the dorsal region. However, this is not the case and similar levels of Ldb1 protein are found in all mesodermal territory. Hence, it is possible that Xlmo4 binding to Ldb1 in the ventral mesoderm prevents within this region its degradation by Rnf12/RLIM (Hiratani et al., 2003). In contrast to the complexes of dorsal mesoderm transcription factors and Ldb1, which require a precise stoichiometry, those formed in the ventral mesoderm by GATA, Ldb1, and Xlmo4 seem not to be affected by overexpression of Xlmo4, as judged by phenotypic effects and the expression of molecular markers. This might result from an incapacity of Xlmo4 to multimerize and form alternative inactive complexes with Ldb1 and GATA.

In summary, Xlmo4 appears to participate in several processes during development, both by acting as a positive cofactor of transcription factors, such as those of the GATA family, or by regulating the precise stoichiometry of complexes formed by Ldb1 with other transcription factors.

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References

Bach, I., 2000. The LIM domain: regulation by association. Mech. Dev. 91, 5–17.

Bach, I., Carriere, C., Ostendorff, H.P., Andersen, B., Rosenfeld, M.G., 1997. A family of LIM domain-associated cofactors confer transcriptional synergism between LIM and Otx homeodomain proteins. Genes Dev. 11, 1370–1380.

Bach, I., Rodriguez-Esteban, C., Carriere, C., Bhushan, A., Krones, A., Rose, D.W., Glass, C.K., Andersen, B., Izpisua Belmonte, J.C., Rosenfeld, M.G., 1999. RLIM inhibits functional activity of LIM homeodomain transcription factors via recruitment of the histone deacetylase complex. Nat. Genet. 22, 394–399.

Bao, J., Talmage, D.A., Role, L.W., Gautier, J., 2000. Regulation of neurogenesis by interactions between HEN1 and neuronal LMO proteins. Development 127, 425–435.

Becker, T., Ostendorff, H.P., Bossenz, M., Schluter, A., Becker, C.G., Peirano, R.I., Bach, I., 2002. Multiple functions of LIM domain-binding CLIM/NLI/Ldb cofactors during zebrafish development. Mech. Dev. 117, 75–85.

Bertwistle, D., Walmsley, M.E., Read, E.M., Pizzey, J.A., Patient, R.K., 1996. GATA factors and the origins of adult and embryonic blood in Xenopus: responses to retinoic acid. Mech. Dev. 57, 199–214.

Cavodeassi, F., Modolell, J., Gómez-Skarmeta, J.L., 2001. The Iroquois family of genes: from body building to neural patterning. Development 128, 2847–2855.

Coffman, C.R., Skoglund, P., Harris, W.A., Kintner, C.R., 1993. Expression of an extracellular deletion of Xotch diverts cell fate in Xenopus embryos. Cell 73, 659–671.

Dawid, I.B., Breen, J.J., Toyama, R., 1998. LIM domains: multiple roles as adapters and functional modifiers in protein interactions. Trends Genet. 14, 156–162.

de la Calle-Mustienes, E., Glavic, A., Modolell, J., Gómez-Skarmeta, J.L., 2002a. Xiro homeoproteins coordinate cell cycle and primary neuron fomation by upregulating neuronal-fate repressors and downregulating the cell-cycle inhibitor XGadd45-γ. Mech. Dev. 119, 69–80.

de la Calle-Mustienes, E., Modolell, J., Gómez-Skarmeta, J.L., 2002b. The Xiro-repressed gene CoREST is expressed in Xenopus neural territories. Mech. Dev. 110, 209–211.

Fernández-Fúnes, P., Lu, C.H., Rincón-Limas, D.E., García-Bellido, A., Botas, J., 1998. The relative expression amounts of apterous and its co-factor dLdb/Chip are critical for dorso-ventral compartmentalization in the Drosophila wing. EMBO J. 17, 6846–6853.

Foroni, L., Boehm, T., White, L., Forster, A., Sherrington, P., Liao, X.B., Brannan, C.I., Jenkins, N.A., Copeland, N.G., Rabbitts, T.H., 1992. The rhombotin gene family encode related LIM-domain proteins whose differing expression suggests multiple roles in mouse development. J. Mol. Biol. 226, 747–761.

García-García, M.J., Ramain, P., Simpson, P., Modolell, J., 1999. Different contributions of pannier and wingless to the patterning of the dorsal mesothorax of Drosophila. Development 126, 3523–3532.

Gawantka, V., Delius, H., Hirschfeld, K., Blumenstock, C., Niehrs, C., 1995. Antagonizing the Spemann organizer: role of the homeobox gene Xvent-1. EMBO J. 14, 6268–6279.

Glavic, A., Gómez-Skarmeta, J.L., Mayor, R., 2002. The homeoprotein Xiro1 is required for midbrain-hindbrain boundary formation. Development 129, 1609–1621.

Gómez-Skarmeta, J.L., de la Calle-Mustienes, E., Modolell, J., 2001. The Wnt-activated Xiro1 gene encodes a repressor that is essential for neural development and downregulates BMP4. Development 128, 551–560.

Gómez-Skarmeta, J.L., Glavic, A., de la Calle-Mustienes, E., Modolell, J., Mayor, R., 1998. Xiro, a Xenopus homolog of the Drosophila Iroquois complex genes, controls development of the neural plate. EMBO J. 17, 181–190.

Gómez-Skarmeta, J.L., Modolell, J., 2002. Iroquois genes: genomic organization and function in vertebrate neural development. Curr. Opin. Genet. Dev. 12, 403–408.

Grutz, G., Forster, A., Rabbitts, T.H., 1998. Identification of the LMO4 gene encoding an interaction partner of the LIM-binding protein LDB1/NLI1: a candidate for displacement by LMO. Oncogene 17, 2799–2803.

Harland, R., 1991. In situ hybridization: an improved whole mount method for Xenopus embryos. Methods Cell Biol. 36, 685–695.

Harland, R., Weintraub, H., 1985. Translation of mRNA injected into Xenopus oocytes is specifically inhibited by antisense RNA. J. Cell Biol. 101, 1094–1099.

Hiratani, I., Yamamoto, N., Mochizuki, T., Ohmori, S.-y., Taira, M., 2003. Selective degradation of excess Ldb1 by Rnf12/RLIM confers proper Ldb1 expression levels and Xlim-1/Ldb1 stoichiometry in *Xenopus* organizer functions. Development 130, 4161–4175.

Hobert, O., Westphal, H., 2000. Functions of LIM-homeobox genes. Trends Genet. 16, 75–83.

Hoppler, S., Brown, J.D., Moon, R.T., 1996. Expression of a dominant-negative Wnt blocks induction of MyoD in Xenopus embryos. Genes Dev. 10, 2805–2817.

Hukriede, N.A., Tsang, T.E., Habas, R., Khoo, P.L., Steiner, K., Weeks, D.L., Tam, P.P., Dawid, I.B., 2003. Conserved requirement of Lim1 function for cell movements during gastrulation. Dev. Cell 4, 83–94.

Kelley, C., Yee, K., Harland, R., Zon, L.I., 1994. Ventral expression of GATA-1 and GATA-2 in the Xenopus embryo defines induction of hematopoietic mesoderm. Dev. Biol. 165, 193–205.

Kenny, D.A., Jurata, L.W., Saga, Y., Gill, G.N., 1998. Identification and characterization of LMO4, an LMO gene with a novel pattern of expression during embryogenesis. Proc. Natl. Acad. Sci. USA 95, 11257–11262.

Kodjabachian, L., Karavanov, A.A., Hikasa, H., Hukriede, N.A., Aoki, T., Taira, M., Dawid, I.B., 2001. A study of Xlim1 function in the Spemann-Mangold organizer. Int. J. Dev. Biol. 45, 209–218.

Lane, M.E., Runko, A.P., Roy, N.M., Sagerstrom, C.G., 2002. Dynamic expression and regulation by Fgf8 and Pou2 of the zebrafish LIM-only gene, lmo4. Gene Expr. Patterns 2, 207–211.

Larson, R.C., Lavenir, I., Larson, T.A., Baer, R., Warren, A.J., Wadman, I., Nottage, K., Rabbitts, T.H., 1996. Protein dimerization between Lmo2 (Rbtn2) and Tal1 alters thymocyte development and potentiates T cell tumorigenesis in transgenic mice. EMBO J. 15, 1021–1027.

Larson, R.C., Osada, H., Larson, T.A., Lavenir, I., Rabbitts, T.H., 1995. The oncogenic LIM protein Rbtn2 causes thymic developmental aberrations that precede malignancy in transgenic mice. Oncogene 11, 853–862.

Mead, P., Deconinck, A.E., Huber, T.L., Orkin, S.H., Zon, L.I., 2001. Primitive erythropoiesis in the Xenopus embryo: the synergistic role of LMO-2, SCL and GATA-binding proteins. Development 128, 2301–2308.

Milán, M., Cohen, S.M., 1999. Regulation of LIM homeodomain activity in vivo: a tetramer of dLDB and apterous confers activity and capacity for regulation by dLMO. Mol. Cell. 4, 267–273.

Milán, M., Díaz-Benjumea, F.J., Cohen, S.M., 1998. Beadex encodes an LMO protein that regulates Apterous LIM-homeodomain activity in Drosophila wing development: a model for LMO oncogene function. Genes. Dev. 12, 2912–2920.

Mochizuki, T., Karavanov, A.A., Curtiss, P.E., Ault, K.T., Sugimoto, N., Watabe, T., Shiokawa, K., Jamrich, M., Cho, K.W., Dawid, I.B., Taira, M., 2000. Xlim-1 and LIM domain binding protein 1 cooperate with various transcription factors in the regulation of the goosecoid promoter. Dev. Biol. 224, 470–485.

Mukhopadhyay, M., Teufel, A., Yamashita, T., Agulnick, A.D., Chen, L., Downs, K.M., Schindler, A., Grinberg, A., Huang, S.P., Dorward, D., Westphal, H., 2003. Functional ablation of the mouse Ldb1 gene results in severe patterning defects during gastrulation. Development 130, 495–505.

Niewkoop, P.D., Faber, J., 1967. Normal Table of Xenopus laevis. North Holland, Amsterdam.

Osada, H., Grutz, G., Axelson, H., Forster, A., Rabbitts, T.H., 1995. Association of erythroid transcription factors: complexes involving the LIM protein RBTN2 and the zinc-finger protein GATA1. Proc. Natl. Acad. Sci. USA 92, 9585–9589.

Pevny, L., Simon, M.C., Robertson, E., Klein, W.H., Tsai, S.F., D'Agati, V., Orkin, S.H., Costantini, F., 1991. Erythroid differentiation in chimaeric mice blocked by a targeted mutation in the gene for transcription factor GATA-1. Nature 349, 257–260.

Pfaff, S.L., Mendelsohn, M., Stewart, C.L., Edlund, T., Jessell, T.M., 1996. Requirement for LIM homeobox gene Isl1 in motor neuron generation reveals a motor neuron-dependent step in interneuron differentiation. Cell 84, 309–320.

Ramain, P., Khechumian, R., Khechumian, K., Arbogast, N., Ackermann, C., Heitzler, P., 2000. Interactions between Chip and the Achaete/Scute-Daughterless heterodimers are required for Pannier-driven proneural patterning. Mol. Cell 6, 781–790.

Rincón-Limas, D.E., Lu, C.-H., Canal, I., Botas, J., 2000. The level of DLDB/CHIP controls the activity of the LIM homeodomain protein Apterous: evidence for a functional tetramer complex in vivo. EMBO J. 19, 2602–2614.

Sharma, K., Sheng, H.Z., Lettieri, K., Li, H., Karavanov, A., Potter, S., Westphal, H., Pfaff, S.L., 1998. LIM homeodomain factors Lhx3 and Lhx4 assign subtype identities for motor neurons. Cell 95, 817–828.

Shawlot, W., Behringer, R.R., 1995. Requirement for Lim1 in head-organizer function. Nature 374, 425–430.

Shoresh, M., Orgad, S., Shmueli, O., Werczberger, R., Gelbaum, D., Abiri, S., Segal, D., 1998. Overexpression Beadex mutations and loss-of-function heldup-a mutations in Drosophila affect the 3′ regulatory and coding components, respectively, of the Dlmo gene. Genetics 150, 283–299.

Sugihara, T.M., Bach, I., Kioussi, C., Rosenfeld, M.G., Andersen, B., 1998. Mouse deformed epidermal autoregulatory factor I recruits a LIM domain factor, LMO-4, and CLIM coregulators. Proc. Natl. Acad. Sci. USA 95, 15418–15423.

Sugihara, T.M., Kudryavtseva, E.I., Kumar, V., Horridge, J.J., Andersen, B., 2001. The POU domain factor Skin-1a represses the keratin 14 promoter independent of DNA binding. A possible role for interactions between Skn-1a and CREB-binding protein/p300. J. Biol. Chem. 276, 33036–33044.

Sykes, T.G., Rodaway, A.R., Walmsley, M.E., Patient, R.K., 1998. Suppression of GATA factor activity causes axis duplication in Xenopus. Development 125, 4595–4605.

Taira, M., Hayes, W.P., Otani, H., Dawid, I.B., 1993. Expression of LIM class homeobox gene Xlim-3 in Xenopus development is limited to neural and neuroendocrine tissues. Dev. Biol. 159, 245–256.

Taira, M., Jamrich, M., Good, P.J., Dawid, I.B., 1992. The LIM domain-containing homeo box gene Xlim-1 is expressed specifically in the organizer region of Xenopus gastrula embryos. Genes Dev. 6, 356–366.

Tanabe, Y., William, C., Jessell, T.M., 1998. Specification of motor neuron identity by the MNR2 homeodomain protein. Cell 95, 67–80.

Thaler, J.P., Lee, S.K., Jurata, L.W., Gill, G.N., Pfaff, S.L., 2002. LIM factor Lhx3 contributes to the specification of motor neuron and interneuron identity through cell-type-specific protein-protein interactions. Cell 110, 237–249.

Thor, S., Andersson, S.G., Tomlinson, A., Thomas, J.B., 1999. A LIM-homeodomain combinatorial code for motor-neuron pathway selection. Nature 397, 76–80.

Turner, D.L., Weintraub, H., 1994. Expression of achaete-scute homolog 3 in Xenopus embryos converts ectodermal cells to a neural fate. Genes Dev. 12, 1434–1447.

van Meyel, D.J., O'Keefe, D.D., Jurata, L.W., Thor, S., Gill, G.N., Thomas, J.B., 1999. Chip and apterous physically interact to form a functional complex during Drosophila development. Mol. Cell. 4, 259–265.

Vitelli, L., Condorelli, G., Lulli, V., Hoang, T., Luchetti, L., Croce, C.M., Peschle, C., 2000. A pentamer transcriptional complex including tal-1 and retinoblastoma protein downmodulates c-kit expression in normal erythroblasts. Mol. Cell. Biol. 20, 5330–5342.

Wadman, I.A., Osada, H., Grutz, G.G., Agulnick, A.D., Westphal, H., Forster, A., Rabbitts, T.H., 1997. The LIM-only protein Lmo2 is a bridging molecule assembling an erythroid, DNA-binding complex which includes the TAL1, E47, GATA-1 and Ldb1/NLI proteins. EMBO J. 16, 3145–3157.

Walmsley, M.E., Guille, M.J., Bertwistle, D., Smith, J.C., Pizzey, J.A., Patient, R.K., 1994. Negative control of Xenopus GATA-2 by activin and noggin with eventual expression in precursors of the ventral blood islands. Development 120, 2519–2529.

Weihe, U., Milán, M., Cohen, S.M., 2001. Regulation of Apterous activity in Drosophila wing development. Development 128, 4615–4622.

Yamada, Y., Warren, A.J., Dobson, C., Forster, A., Pannell, R., Rabbitts, T.H., 1998. The T cell leukemia LIM protein Lmo2 is necessary for adult mouse hematopoiesis. Proc. Natl. Acad. Sci. USA 95, 3890–3895.

Zeng, C., Justice, N.J., Abdelilah, S., Chan, Y.M., Jan, L.Y., Jan, Y.N., 1998. The Drosophila LIM-only gene, dLMO, is mutated in Beadex alleles and might represent an evolutionarily conserved function in appendage development. Proc. Natl. Acad. Sci. USA 95, 10637–10642.

Zon, L.I., Mather, C., Burgess, S., Bolce, M.E., Harland, R.M., Orkin, S.H., 1991. Expression of GATA-binding proteins during embryonic development in Xenopus laevis. Proc. Natl. Acad. Sci. USA 88, 10642–10646.