The Xenopus homologue of the Drosophila gene tailless has a function in early eye development

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SUMMARY

Genetic circuits responsible for the development of photoreceptive organs appear to be evolutionarily conserved. Here, the Xenopus homologue Xtll of the Drosophila gene tailless (tll), which we find to be expressed during early eye development, is characterized with respect to its relationship to vertebrate regulators of eye morphogenesis, such as Pax6 and Rx. Expression of all three genes is first detected in the area corresponding to the eye anlagen within the open neural plate in partially overlapping, but not identical, patterns. During the evagination of the optic vesicle, Xtll expression is most prominent in the optic stalk, as well as in the distal tip of the forming vesicle. In tadpole-stage embryos, Xtll gene transcription is most prominent in the ciliary margin of the optic cup. Inhibition of Xtll function in Xenopus embryos interferes specifically with the evagination of the eye vesicle and, in consequence, Xpax6 gene expression is severely reduced in such manipulated embryos. These findings suggest that Xtll serves an important regulatory function in the earliest phases of vertebrate eye development.

Key words: Xenopus laevis, Eye development, tailless, Pax6, Rx

INTRODUCTION

A set of genes has been identified that is critically involved in the regulation of eye development. One prime example of such a regulatory activity is the role that the Pax6 gene plays in eye development in both vertebrate and invertebrate systems. Loss of Pax6 (eyeless) function in Drosophila prevents eye formation. Most interestingly, ectopic expression of Pax6 in imaginal discs destined to become wings, legs or antennae can lead to the formation of supernumerary eyes (Quiring et al., 1994; Halder et al., 1995). More recently, similar activities have been attributed to dachshund, as well as to eyes absent, both genes encoding nuclear proteins in Drosophila (Shen and Mardon, 1997; Bonini et al., 1997). Dominantly inherited eye malformations in man (aniridia) and mouse (small eye) can be caused by mutations that reduce Pax6 dosage. In mice, Pax6 is also necessary for normal development of nose and brain (Hogan et al., 1986; Glaser et al., 1990; Jordan et al., 1992; Ton et al., 1991; Walther et al., 1991a,b). Ectopic Pax6 has been reported to result in the formation of ectopic lenses in Xenopus embryos, as was evident from the induction of the lens-specific marker βB1-crystallin (Altmann et al., 1997), but it is not sufficient to promote ectopic eye development in vertebrates. In addition to Pax6, a newly identified vertebrate homeobox gene, termed Rx, has been found to be required for vertebrate eye morphogenesis; misexpression of Rx in Xenopus embryos leads to the formation of ectopic retina. Murine Rx null mutant embryos exhibit severe truncations of forebrain and midbrain structures, as well as a complete failure to form an optic vesicle (Mathers et al., 1997).

We report here on the expression and function of a gene which appears to be involved in Xenopus eye formation. This gene encodes an orphan nuclear receptor termed tailless (tll) and is expressed during the development of the central nervous system in insect and vertebrate embryos. During Drosophila embryogenesis, the tll gene is first expressed at the two termini and later in the developing brain, as well as transiently in the peripheral nervous system (Pignoni et al., 1990). Early expression is required for normal pattern formation in the termini, including the establishment of the anlage for the posterior portion of the brain. The role of late tll expression in the nervous system is unclear, and so far there is no direct evidence that tll function might be connected to eye development in the fly. Tailless homologues have been described in two different vertebrate systems. In chicken embryos, tailless (Tlx) expression is first detected in primary optic vesicles, and later predominantly in the eye and forebrain (Yu et al., 1994). Similarly, in mouse embryos, tailless (mtll) is first expressed in the developing forebrain, and later in the dorsal midbrain as well as in the eye and in the nose (Yu et al., 1994; Monaghan et al., 1995). The fact that tll gene structure as well as elements of its expression characteristics during vertebrate development have been so highly conserved makes it interesting to study tll function during early vertebrate embryogenesis, in particular its possible involvement in eye morphogenesis.

In order to address this question, we first performed a comparative analysis of tll, Pax6 and Rx gene expression during Xenopus embryogenesis. These three genes are found to be
expressed in dynamic, but only partially overlapping patterns and they can serve as molecular markers for the earliest events in eye differentiation. The function of _Xtll_ was assayed by ectopic expression of a fusion protein that contains the DNA binding domain of _Xtll_ fused to the engrailed repressor domain. This treatment results in the specific inhibition of eye vesicle evagination and reduces _Xpax6_ expression.

**MATERIALS AND METHODS**

Cloning and sequencing of _Xpax6_ and _Xtll_

RNA from oocytes and embryos and from tissues of adult frogs was extracted as described (Döring and Stick, 1990). In order to remove contaminating DNA, the RNA was incubated with 50 i.u. DNase I (Boehringer-Mannheim) in DNase buffer (40 mM Tris/HCl, pH 7.9, 10 mM NaCl, 6 mM MgCl₂, 0.1 mM CaCl₂) at 37°C. The RNA was reextracted with phenol/chloroform, precipitated with 1 M ammonium acetate and 2.5 volumes of ethanol, washed with 80% ethanol and resuspended in water at a final concentration of 1 µg/µl.

RT-PCR analysis was carried out using the Gene Amp RT-PCR kit from Perkin-Elmer Cetus. First-strand cDNA was synthesized from 1 µg RNA from embryonic stages (stage 6, 9, 13, 17, 22 and 36) with random hexanucleotides as primers, following the manufacturer's protocol. The degenerated primers that were used to amplify _xpl_–related sequences were as follows: primer P1, corresponding to amino acids 44-51, 5’ AARCAYGGGTITAYGCGT and primer P2, corresponding to amino acids 210-217, 5’ TTIACYTCTRTGIGG-RTAYTIGG. The degenerated primers that were used to amplify _xpl_–related sequences were as follows: primer P7, corresponding to amino acids 1-9, 5’ ATGCAARYGYGAYWSIGGIGT and primer P8, corresponding to amino acids 391-400, 5’ CATIGGYTGGATTCATRTGITYGTYGCTAT.

Initial denaturation was performed for 3 minutes at 95°C, followed by 40 amplification cycles (1 minute, 95°C; 1.5 minutes, 52°C; 1.5 minutes, 72°C) and a final polymerization step (10 minutes, 72°C). For each of _Xtll_ and _Xpax6_, a single PCR product was obtained, gel-purified, T/A-cloned into pGEM-5T (Promega) and sequenced.

A random-primed λ-ZAP express cDNA library was constructed from 10 µg poly(A)+ RNA prepared from 1000 isolated heads of stage-32 embryos following the manufacturer's protocol (Stratagene). 1.9x10⁶ and 4.9x10⁶ primary phages were obtained from cDNA fraction 1 and cDNA fraction 2, respectively. Of each fraction about 2x10⁸ phages were amplified in samples containing 50000 pfu each; each sample was eluted with 15 ml SM-buffer and stored over chloroform. Double-stranded sequencing was done using the ABI cycle sequencing kit (United States Biochemical Corp.).

Screening of amplified cDNA libraries was performed as described (Israel, 1993). 35 samples of the head cDNA library containing 1.75x10⁶ pfu were screened by PCR, which was carried out in a final volume of 25 µl with 2.5 µl of phage stocks as template using the Gene Amp PCR kit (Perkin-Elmer Cetus). Oligonucleotides used as primers were deduced from the partial cDNA sequence generated by RT-PCR. Of one of six positive cDNA library samples, the minimum volume of phage stock that is positive in the PCR was determined (as described above). This volume was rescreened in 6.4 ml liquid cultures that were sampled in 100 µl using a 8x8 matrix of a 96-well microtiter plate. 4x10⁶ pfu of a single pool were plated and screened by non-radioactive filter hybridization. Of the positive clones, three were plaque-purified. All three contained identical inserts. Screening of amplified cDNA libraries for _Pax6_ was done following the same procedure (GenBank accession numbers are _Xtll_, U67886 and _Xpax6_, U67887).

Quantitative RT-PCR analysis

Quantitative RT-PCR was carried out using the Gene Amp RT-PCR kit from Perkin-Elmer Cetus following the manufacturer’s protocol. First-strand cDNA was synthesized from 0.5 µg RNA using random hexanucleotides as primers. Amplification was done in a 100 µl reaction mixture containing the appropriate primer mix for _Xtll_ (P3, corresponding to amino acids 161-177, 5’ CAGCCAAACCC-AAAATCCTCTP4, corresponding to amino acids 275-281, 5’ CCACTAGCGAAGGTGTCGAA, resulting in a DNA product 364 nucleotides long), _Xpax6_ (P9 and P10, as described above) and histone H4 (P11, corresponding to amino acids 24-31, 5’ CGGGATAACATTACGGATACCT 3’ and P12, corresponding to amino acids 79-86, 5’ ATCCATGGGTAACGTTCTCTC 3’, resulting in a DNA product 189 nucleotides long), respectively. The linear range of amplification was determined by carrying out PCR with 0.5 µg of RNA of stage-32 embryos with increasing numbers of PCR cycles. Amplification was linear up to cycle number 28. RT-PCR was carried out as described above, except that 1 µCi of [α-32P]dCTP was included in the PCR. Amplification of _Xtll_ transcripts was done for 76 cycles, amplification of _Xpax6_ transcripts was done for 25 cycles and amplification of histone H4 transcripts for 23 cycles. One tenth of each PCR product was analyzed on a 6% polyacrylamide gel under denaturing conditions. α-32P-end-labelled 1-kb ladder (Gibco) was used as a size marker. Dried gels were analyzed using a PhosphorImager and the ImageQuant 2.0 program (Molecular Dynamics).

Whole-mount in situ hybridization analysis

Whole-mount in situ hybridization was done in principle as described by Harland (1991). For double-staining analyses, digoxigenin-UTP and fluorescein-UTP-labeled RNA probes were used. After the first staining with NBT/BCIP, the enzyme reaction was stopped by soaking the embryos for 2 minutes in methanol. The staining for the second transcript was as for the first one, but used Fast Red as a dye (Boehringer). For the preparation of vibratome sections, stained and fixed embryos were equilibrated in PBS containing 4.4 mg/ml gelatin, 0.27 g/ml BSA, 0.18 g/ml sucrose. After equilibration embryos were mounted by crosslinking the solution with glutaraldehyde. 30 µm sections were obtained with a Vibratome 1000 (Technical Products International Inc.) and mounted onto gelatin-coated slides. Photographs were taken on a Zeiss Axiohot using Nomarski interference optics.

Embryo microinjection procedures

A full-length _Xtll_ expression plasmid, termed _Xtll-WT_, was constructed in the expression vector pCS2+MT (Rupp et al., 1994). A PCR was carried out making use of primers P13, corresponding to amino acids 2-7 (5’ GCGGAATTCAGCAAAACCGGCTAGCTAC) and P14, corresponding to amino acids 381-386 (5’ CCGCTCGAGTTTCGATGTCACTCGATTGTAC). The PCR product was digested with EcoRI and XhoI and inserted into the vector. The _Xtll-ZF-engR_ fusion protein was generated as follows: a fragment encoding amino acids 2-299 of the _Drosophila_ engrailed (en) gene was generated by PCR using primers P15 (5’ CCTTTTGCTAGCCCGTTCGGATCCTGC) and P16 (5’ CTTTGGTACGTATCAGTCGGGTTCACGATAGCAGAGA) from a partial en gene clone (Han and Manley, 1993). The resulting fragment was digested by XbaI and SnaBI and inserted into _XbaI-SnaBI_ of pCS2+NLS+MT. In order to clone the _Xtll_ DNA-binding domain in front of engR, an EcoRI-XbaI restriction fragment corresponding to amino acids 2-217 was isolated from the plasmid _Xtll-WT_ and inserted into the _EcoRI-XbaI_ of pCS2+NLS+MT+engR to give _Xtll-ZF-engR_. Truncated or mutated versions of _Xtll-WT_ were generated by PCR from the pCS2+MT-Xtll-WT. All constructs were checked by double-stranded sequencing and by comparing the SDS-PAGE migration of the proteins synthesized from these constructs in vitro (coupled Transcription/Translation TNT Promega kit).

Capped pCS2+MT-Xtll-WT and mutant versions mRNAs were transcribed using SP6 RNA polymerase as described (Kintner and...
Melton, 1987). RNAs were injected in a volume of 5 nl at a concentration of 5-200 pg/μl into one single blastomere of embryos at the two-cell stage, as described previously (Coffman et al., 1990). In some experiments, LacZ mRNA was coinjected as a marker. Injection of LacZ mRNA alone was done as a control. Histochemical staining for β-galactosidase was performed to visualize the distribution of injected mRNAs. Embryos were collected at the indicated stages and subjected to in situ hybridization as described above.

RESULTS

Xtll and Xpax6 cDNA cloning and analysis of developmental expression characteristics

Full-length Xtll and Xpax6 transcripts encoding complementary DNAs were isolated by reverse transcriptase-polymerase chain reaction from a stage-32 head-specific cDNA library (see Materials and methods). The predicted protein products are highly homologous to the corresponding vertebrate and Drosophila sequences. Overall amino acid identities are 91% between Xtll and mouse tll and 39% in a comparison with Drosophila tll. For Pax6, overall amino acid identities are 95% between the corresponding Xenopus and mouse proteins and 58% in a comparison with Drosophila eyeless (data not shown). With these cDNAs as tools at hand, we then performed a detailed comparative expression analysis of the two genes during early Xenopus embryogenesis.

RT-PCR analysis was utilized in order to establish the temporal pattern of expression of Xtll (Fig. 1). Zygotic transcripts are detected in RNA preparations from neurula (stage 17) Xenopus embryos, whereas Xpax6 gene transcription can be detected significantly earlier with late gastrula/early neurula stages (stage 13) of development. Both Xtll and Xpax6 transcripts persist until the latest stages of embryogenesis analyzed (stage-46 tadpoles). In adult organs/tissues, Xtll transcription is restricted to neural derivatives such as brain and eye; the only other organ that expresses Xtll is the testis. Xpax6 expression is detected in the same organs, as well as in the spinal cord and weakly also in RNA preparations from intestine and stomach.

Spatial and temporal patterns of Xtll and Xpax6 expression were further analyzed by whole-mount in situ hybridization with Xenopus embryos from stages 12.5 to 23, and also compared with the Rx gene (Fig. 2). Xtll expression is first detected in open neural fold stage (stage 16) embryos within an area that is located in the anterior, prechordal portion of the neural plate. Two groups of cells lateral to the most anterior portion of the median groove exhibit Xtll-specific transcripts. These two patches of cells are connected by a thin line of Xtll-positive cells over the median midline (Fig. 2G). Lineage analysis of the same cells demonstrated that they will give rise to the eyes and chiasmatic ridge of early tadpoles (Eagleson and Harris, 1990). The corresponding area within the developing neural plate of other amphibians was mapped, earlier this century by Hans Spemann, as being part of the eye anlage (Spemann, 1936). Xpax6 exhibits overlapping expression characteristics but is activated significantly earlier than Xtll, as early as stage 12.5 (Fig. 2M). The primary Xpax6 expression domain in these and in stage-16 embryos is similarly found to overlap with the eye anlage; additional,
more posterior signals can be detected in an area corresponding to the neural folds. Double-staining analysis reveals that the two areas as defined by Xpax6 and Xttl expression overlap but are not identical. The expression domain of Xpax6 extends more anteriorly, whereas the posterior boundaries of the same two genes are indistinguishable (Fig. 2J). A comparative analysis of horizontal sections generated from stage-17 embryos confirms this overlap of Xttl and Xpax6 expression domains at this early stage of development (Fig. 2Q,S). Double-staining analysis making use of Xttl- and Xrx-specific probes suggests a more exclusive pattern of expression for these two genes in early neurula (stage 16) embryos (Fig. 2A). Xrx-specific signals are primarily detected anterior to the Xttl-expressing cells. This is supported by double-staining analysis of Xttl and en2 in slightly later stages of development (stage 19) (Fig. 2B); Xttl is expressed anterior and adjacent to en2, defining a territory that does not express Xrx (Mathers et al., 1997) and which is likely to correspond to most of the putative midbrain.

In early tailbud (stage 23) embryos, Xttl, Xpax6 and Xrx are found to be expressed in the evaginating eye vesicle (Fig. 2C, I, L, P). However, in comparison with Xpax6 and Xrx, Xttl-staining of the eye vesicle is only relatively weak. Examination of horizontal sections reveals that Xttl expression is most prominent in the optic stalk and, albeit weaker, in the distal portion of the evaginating optic vesicle. In contrast, Xpax6...
gene transcription is prominent in the entire optic vesicle (Fig. 2R,T). The differential expression of *Xtll* and *Xpax6* in the developing eye becomes even more pronounced after invagination of the optic vesicle in tadpole (stage 36) embryos (Fig. 3). At this stage of eye development, *Xtll* transcripts are specific to the distal tips of the eye cup, referred to as the ciliary margin; these cells will give rise to all major cell types in the frog retina (Wetts and Fraser, 1988) and they also define the primary site of *Xrx* expression at this stage of development (Mathers et al., 1997).

*Xpax6* expression is detected predominantly in the neural retina as well as in the lens, where *Xtll* is not expressed. Expression characteristics in fore- and midbrain are similarly exclusive in a comparative analysis of *Xtll* and *Xpax6* (Fig. 3H). Furthermore, staining of brains isolated from tadpole (stage 34) embryos defines different expression boundaries along the anterior/posterior body axis (Fig. 3B,E,F). *Xtll*-encoding mRNA is detected from the rostral tip of the forebrain up to the midbrain/hindbrain boundary, whereas the *Xpax6* expression domain begins in a more caudal position within the forebrain and ends more anteriorly to the midbrain/hindbrain boundary.

To summarize, at early neurula stages of *Xenopus* embryogenesis, *Xtll, Xpax6* and *Xrx* are expressed in overlapping but not identical patterns defined by cells that will give rise to the embryonic eye. This early expression of *Xtll* overlaps with *Xpax6*, but is exclusive in comparison with *Xrx*. Conversely, as development proceeds, *Xtll* and *Xpax6* expression in the embryonic eye becomes almost exclusive, whereas *Xrx* gene transcription, which is now restricted to the ciliary margin of the optic cup, is found to be vastly overlapping with *Xtll*. Similarly, *Xpax6* is strongly expressed in the lens, but not *Xtll* or *Xrx*.

### Table 1. Phenotypic effects resulting from manipulation of *Xtll* protein activity in *Xenopus* embryos

<table>
<thead>
<tr>
<th>Sample</th>
<th>RNA injected (pg)</th>
<th>N</th>
<th>n (%)</th>
<th>Reduced eye (%)</th>
<th>Normal eye (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Xtll</em>-ZF-engR</td>
<td>800</td>
<td>4</td>
<td>131</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>7</td>
<td>218</td>
<td>36</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6</td>
<td>209</td>
<td>24</td>
<td>47</td>
</tr>
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<td></td>
<td>100</td>
<td>5</td>
<td>185</td>
<td>18</td>
<td>42</td>
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<td>10</td>
<td>2</td>
<td>73</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Xtll</em>-WT</td>
<td>800</td>
<td>4</td>
<td>165</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>4</td>
<td>178</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Xtll</em>-ZF-engR+</td>
<td>1000</td>
<td>3</td>
<td>81</td>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

**Xtll-WT (1:1)**

ZF, zinc finger; engR, engrailed repressor domain; WT, wild type.

**N**, number of experiments; **n**, number of embryos.

### A function for *Xtll* in eye development

The *Xtll* gene product is an orphan nuclear receptor that is thought to bind to its cognate DNA binding site as a monomer and to serve as a transcription activator (Mangelsdorf and Evans, 1995). Following an idea that was first applied to the analysis of c-Myb in transgenic T-cells and most recently to the functional characterization of two different transcription activators in *Xenopus* embryos (Badiani et al., 1994; Conlon et al., 1996; Belfroid et al., 1996), we designed an artificial transcription repressor that has the DNA binding specificity of *Xtll*. To do so, we fused the *Xtll* zinc finger domain to the engrailed repressor domain; the resulting construct is termed *Xtll-ZF-engR* (Fig. 4E). This fusion protein was expressed in...
Xenopus embryos by means of microinjection of the corresponding mRNA into one cell of a two-cell-stage embryo. This treatment is found to interfere specifically with the formation of the eye (Fig. 4 and Table 1). Phenotypic effects were evident in tadpole (stage 34) embryos as the absence or reduced size of the pigmented embryonic eye in the injected half of the embryo (Fig. 4A,B). The developmental defects were found to be dose-dependent. Low doses (up to 100 pg RNA injected) resulted in a significant reduction of the eye diameter, whereas higher doses (up to 800 pg) could lead to the complete absence or more severe reduction of the embryonic eye structure. The specificity of these effects was confirmed by a number of control injections. Firstly, neither ectopic expression of the engrailed repressor domain alone nor of the \textit{Xtll} zinc finger domain alone affected eye development. Secondly, neither ectopic expression of full-length \textit{Xtll}, nor of a mutant version of \textit{Xtll} that has the zinc finger domain deleted or the zinc fingers connected to the E1A activation domain, had obvious phenotypic effects. Thirdly and most importantly, coinjection of equal amounts of \textit{Xtll}-ZF-engR together with the wild-type version of \textit{Xtll} resulted in a partial rescue of the developmental defects observed with the same amount of \textit{Xtll}-ZF-engR alone (Table 1). Furthermore, if manipulated embryos were allowed to grow to the equivalent of stage 56, no other developmental defects in addition to the absence of one eye on the injected side of the embryo were detected (Fig. 4C,D). These observations show that ectopic expression of the artificial \textit{Xtll}-derived transcription repressor in \textit{Xenopus} embryos specifically interferes with eye development. We assume that this effect is a consequence of a competitive inhibition of the endogenous \textit{Xtll} transcription activator.

The developmental defects in eye development observed as a consequence of ectopic \textit{Xtll}-ZF-engR expression could be either the result of an inhibition of a primary event, i.e. evagination of the eye vesicle, or they could be caused by a secondary degeneration of a properly formed eye vesicle prior to the invagination of the eye cup. In order to distinguish between these two possibilities, and also in order to establish the correlation between \textit{Xtll} function and \textit{Xpax6} expression, we analyzed the \textit{Xpax6} mRNA distribution in \textit{Xtll}-ZF-engR injected embryos. Whole-mount in situ hybridization with \textit{stage-19} and \textit{stage-23} embryos revealed that the \textit{Xpax6} expression domain corresponding to the evaginating eye vesicle is absent from the microinjected side of the embryo (Fig. 5C,E). \textit{Xpax6} expression characteristics in earlier stages of development (stages 14 and 16) were not found to be affected by \textit{Xtll}-ZF-engR injection (data not shown).
Horizontal sections from the same embryos further demonstrate that this is a consequence of the absence of the corresponding structure in such manipulated embryos (Fig. 5D,F). *Xpax6* expression within the anterior portion of the neural tube appears to be more diffuse and defined a slightly broadened area, as if the cell population which would normally form the eye vesicle was arrested in its morphogenetic movements. Moreover, the neural tube in the injected half of the embryo seems to be slightly expanded. The situation in tadpole stage (stage 32/34) embryos with respect to this loss of a *Xpax6* expression domain in the area that would normally contain the eye was even more drastic (Fig. 5A,B). Transverse sections prepared from such manipulated embryos reemphasized the loss of the embryonic eye that correlates with a severe reduction in *Xpax6* expression. Residual, ectopic *Xpax6* gene transcription in the rostral neural tube may identify the cells that have failed to evaginate in the context of eye vesicle formation. Thus, these experiments demonstrate that ectopic expression of *Xtll-ZF-engR* leads to an inhibition of eye vesicle evagination as well as to a severe reduction of *Xpax6* gene expression.

**DISCUSSION**

A *Xenopus* gene that is structurally homologous to the *Drosophila* gene *tailless*, and hence termed *Xtll*, is an early molecular marker for eye development. It is first expressed at the open neural plate stage in two groups of cells that will give rise to the embryonic eyes. Ectopic expression of a putative dominant negative version of *Xtll*, which was generated by fusion of the *Xtll* zinc fingers to the engrailed repressor domain, inhibits eye vesicle evagination and reduces the *Xpax6* expression domain. We conclude that the vertebrate homologue of the Drosophila gene *tailless* has an important function in early steps of vertebrate eye development.

Embryonic expression patterns for three vertebrate homologues of *Drosophila tailless* from chicken, mouse (Yu et al., 1994; Monaghan et al., 1995) and *Xenopus* reveal very characteristic common features. They are all expressed in early fore- and midbrain structures and in the developing eye. We have not observed any obvious morphological defects of the embryonic brain upon counteracting the activity of the endogenous *Xtll* protein by means of the *Xtll-ZF-engR* construct in *Xenopus*. The most recent analysis of *tlx*-deficient mice has failed to reveal defects in embryonic eye development (Monaghan et al., 1997). In contrast, disruption of the *tlx* locus has been found to result in impaired development of a specific subset of different forebrain-derived structures. One explanation for these apparently conflicting results obtained in mouse and *Xenopus* upon inhibition of *tailless* gene function comes from consideration of the different experimental strategies that have been employed. While functional redundancy may explain the absence of an eye phenotype in the mouse, the ectopic expression of a dominantly negative acting artificial repressor in *Xenopus* embryos should also inhibit other activators that may operate on the same target genes as *Xtll*.

Conversely, it has been demonstrated that mouse embryos carrying a null allele of the *Rx* homeobox gene do not form optic cups (Mathers et al., 1997), and thus exhibit a phenotype that is similar in this respect to what we have observed in *Xenopus* embryos upon injection of *Xtll-ZF-engR*. Both genes *Xtll* and *Xrx* exhibit dynamic patterns of expression during embryonic eye development. They are transcribed in adjacent, mostly exclusive territories at the open neural plate stage (Fig. 2), and they both become expressed in vastly overlapping areas in the optic vesicle of tailbud-stage embryos, as well as in the ciliary margin of the optic cup in tadpole-stage embryos (Mathers et al., 1997 and this study). The similarity of effects on eye development observed in loss of function type experiments with both genes, as well as the overlapping expression characteristics in middle and late stages of eye morphogenesis, seem to suggest that these two genes might be functionally connected. However, *Xrx* is not expressed in the optic stalk of tailbud-stage embryos, which is the most prominent site of *Xtll* gene transcription in this phase of eye development.

The comparative analysis of *Xtll* and *Xpax6* expression during eye development has revealed an extensive overlap at early stages of eye development, i.e. at the open neural plate stage. As development proceeds, expression domains segregate; *Xpax6* expression in the optic stalk was not as pronounced as observed with *Xtll*, and expression of these two genes in the optic cup was almost exclusive. The suggested function for *Xtll* in eye vesicle evagination is apparently distinct from the one that has been ascribed to the *Pax6* gene in mice and man. In mouse embryos homozygous for mutations in the *Pax6* gene, evagination of the optic vesicle is not inhibited, but the vesicle formed appears to fail to constrict proximally (Grindley et al., 1995). The failure of lens formation/induction in the overlying, contacted ectoderm, and hence the lack of reciprocal communication between the corresponding groups of cells, is probably one reason why eye vesicle development does not continue to progress normally in the same mice. Conversely, the failure of *Xtll-ZF-engR*-manipulated *Xenopus* embryos to form a proper lens is most likely due to the absence of an underlying, contacting eye vesicle. One signaling molecule that could be critically involved in the communication between lens and eye vesicle is BMP-7. In mice lacking BMP-7, optic cup formation appears to proceed relatively normally, but later development is characterized by rapid and extensive deterioration of the eye and optic nerve. Therefore, BMP-7 appears to be important in maintaining the integrity of optic structures (Dudley et al., 1995; Luo et al., 1995), rather than instructing early events of eye development such as the evagination of the eye vesicle.

For the future, it will be interesting to analyze the effects that ectopic expression of combinations of the different genes which are or appear to be important regulators of vertebrate eye development might have in *Xenopus* embryos.

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