Six3 overexpression initiates the formation of ectopic retina

Felix Loosli, Sylke Winkler, and Joachim Wittbrodt

The homeobox gene sine oculis (so) is essential for visual system formation in Drosophila. A vertebrate member of the so/Six gene family, Six3, is expressed in the developing eye and forebrain. Injection of Six3 RNA into medaka fish embryos causes ectopic Pax6 and Rx2 expression in midbrain and cerebellum, resulting in the formation of ectopic retinal primordia. Injected mouse Six3 RNA initiates ectopic expression of endogenous medaka Six3, uncovering a feedback control of Six3 expression. Initiation of ectopic retina formation reveals a pivotal role for Six3 in vertebrate retina development and hints at a conserved regulatory network underlying vertebrate and invertebrate eye development.

Results

We injected medaka Six3 and mouse Six3 RNA, respectively, into a single blastomere of medaka embryos at the two-cell stage. This led to a widespread and premature expression of Six3 in the injected embryo. In response to both medaka Six3 RNA (38 of 93) and mouse Six3 RNA (19 of 47), injected embryos developed a dramatically enlarged optic vesicle at somitogenesis stages (stage 21; Iwamatsu 1994) suggesting a hyperplasia of retinal tissue (Fig. 1A–C). To unambiguously establish the identity of the ectopic tissue, we examined the expression of the retina-specific homeobox gene Rx2 (Mathers et al. 1997). In wild-type embryos, Rx2 is expressed exclusively in the presumptive neural retina and pigmented retinal epithelium (PRE) within the optic vesicle from the two-somite stage (stage 19) onward and subsequently becomes restricted to the developing neural retina in the optic cup (Fig. 1C). Thus, cells initially expressing Rx2 contribute either to neural retina or to PRE and therefore Rx2 serves as a specific marker for early retinal development. In the Six3 induced enlarged optic vesicles we found an expansion of the Rx2 expression domain, indicating that this tissue is of retinal identity [Fig. 1A,B]. In addition, transverse sections of injected embryos revealed a twofold increase of the cell number in the enlarged optic vesicles [Fig. 3D,E, below], confirming retinal hyperplasia. The severity of retinal hyperplasia was always tightly correlated with the distribution of the coinjected lineage tracer, humanized green fluorescent protein [hGFP], in the injected embryos [Fig. 3F,G, below]. These findings are consistent with the observation that overexpression of Xenopus Six3 RNA leads to an enlarged retina in Xenopus embryos [M. Zuber, M. Perron, and W.A. Harris, pers. comm.]. As additional morphological alterations we observed a broadening of the presumptive midbrain [medaka Six3, 43 of 93; mouse Six3, 15 of 47], which was in some cases associated with retinal hyperplasia.

In vertebrate eye development, the anterior ectoderm gives rise to the retinal primordium in the anterior neuroectoderm and the lens primordia in the lateral head ectoderm. The formation of retinal and lens primordia requires a closely coordinated development involving inductive interactions between these structures [Grainger 1996]. Pax6, a structurally conserved transcription factor is expressed in these primordia and was shown to play a key role in vertebrate and invertebrate eye development [Hill et al. 1991; Ton et al. 1991; Matsu et al. 1993; Quiring et al. 1994; Halder et al. 1995a]. Several other vertebrate and invertebrate genes expressed in the developing eye are also structurally conserved, suggesting an evolutionary conservation of the mechanisms underlying eye development across species lines [Quiring et al. 1994; Halder et al. 1995b; Desplan 1997; Xu et al. 1997]. In Drosophila the development of the larval and adult visual system requires the activity of the homeobox-containing transcription factor sine oculis (so) [Cheyette et al. 1994]. A family of vertebrate homologs has been isolated from a variety of vertebrate species [Oliver et al. 1995a,b; Kawakami et al. 1996a,b; Bovolenta et al. 1998; Kobayashi et al. 1998; Loosli et al. 1998; Seo et al. 1998; Toy et al. 1998]. Of these, Six3 is specifically expressed in the developing eye and ventral forebrain. Recently, in Drosophila a member of the Six subclass of homebox genes, optix, has been isolated, which by sequence comparison of the homeobox appears to be the ortholog of Six3 [Toy et al. 1998]. Functional analysis of optix, however, has not yet been reported.

In medaka fish (Oryzias latipes) Six3 is expressed in the anterior-most neuroectoderm at gastrula stages and later in the developing retina [Loosli et al. 1998]. Mosaic misexpression of mouse Six3 in small clones, in response to injected plasmid DNA, resulted in the formation of ectopic lenses in the region of the otic vesicle, suggesting a decisive role for Six3 during vertebrate lens development [Oliver et al. 1996]. Considering the expression of Six3 in the retinal primordia and the essential role of a Drosophila homolog, so, in Drosophila eye development, we investigated a potential role of Six3 in early vertebrate retina development.

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Six3 overexpression results in the formation of ectopic retinal primordia and retinal hyperplasia. Rx2 (blue) in situ analysis in control (C), medaka Six3 (A,D) and mouse Six3 (B,E,F) RNA-injected embryos, respectively, anterior is to the left. Open arrowheads indicate ectopic Rx2 expression; open arrows indicate retinal hyperplasia. (A–C) Dorsal view of six-somite-stage embryos. (D–F) 32-somite-stage embryos. (B) Note the extent of retinal overgrowth (open arrow). (C) Rx2 expression in the optic vesicle of a control embryo. Note that Rx2 is not expressed in the dorsal diencephalon. (Inset) Rx2 expression at 19-somite-stage confined to neural retina. Arrows point to prospective PRE. (D) Transverse section; open arrowhead indicates ectopic optic cup. Note ectopic PRE (arrowhead); arrow indicates wild-type PRE. Lens in wild-type eye is visualized by α-A crystallin expression (red). (E) Lateral view of embryo with optic cup-like structure (open arrowhead) in dorsal midbrain. (F) Transverse section at the level of the eye (open arrowhead in E); note ectopic optic cup (arrowhead); arrow indicates PRE; neural retina adjacent to PRE is free of Rx2 expression. (Inset) Ectopic optic cup at higher magnification; arrowhead indicates ectopic PRE. (ey) Eye; (mb) midbrain; (nr) neural retina; (otv) otic vesicle; (ot) optic tectum; (ov) optic vesicle; (pre) pigmented retinal epithelium.

More importantly, we observed ectopic Rx2 expression in Six3-injected medaka embryos concomitant with the onset of Rx2 expression in the developing wild-type retina (stage 19; Fig. 1; data not shown). In all cases, ectopic Rx2-positive tissue was located exclusively in the midbrain and prospective cerebellum and was associated with a broadening of this region [medaka Six3, 20 of 93; mouse Six3, 10 of 47] (Fig. 1A,B). The widespread distribution of the injected RNA along the anteroposterior axis [Fig. 3F,G, below] indicates that retinal primordia can form in response to Six3 expression at ectopic locations in a competent region of the brain.

At late organogenesis stages (34 somites, stage 29) ectopic Rx2-positive tissue was found to develop ectopic PRE in the dorsal midbrain of injected embryos, abutting ectopic Rx2-positive tissue [3 of 46, Fig. 1D–F]. Transverse sections revealed an optic cup-like structure [Fig. 1, cf. inset in C with D and F]. These ectopic optic cups consist of Rx2-expressing cells in a columnar arrangement [Fig. 1F inset], similar to the morphology of a neuroretina at this stage, surrounded by a single-cell layer of pigmented cells analogous to the PRE of the wild-type eye [Fig. 1D,F]. In some cases, ectopic PRE was detected that was not associated with ectopic Rx2 expression [3 of 46], similar to the wild-type situation in which the central region of the neural retina is free of Rx2 expression [Fig. 1F]. Thus, Six3 overexpression leads to the formation of ectopic retinal primordia located in the region of the midbrain and prospective cerebellum, which have the potential to differentiate into both neural retina and PRE in ectopic optic cup-like structures.

In medaka Six3 RNA-injected embryos, we observed in a few cases (2 of 98) an ectopic lens in the region of the otic vesicle [data not shown] but never in the vicinity of the ectopic retinas, consistent with our results of Six3 plasmid DNA injections [Oliver et al. 1996]. This suggests that the region competent to form ectopic retina in the brain [midbrain and prospective cerebellum] is not adjacent to the region competent to form an ectopic lens in the head ectoderm [level of rhombomere 5/6].

Among the Six family members the formation of retinal primordia is a specific feature of Six3, as another member of the vertebrate Six homeobox gene family, mouse Six2, had no effect on injection of the mRNA under conditions in which Six3 initiates ectopic retina formation. In mouse Six2 RNA-injected embryos, morphology, and expression of Rx2 [0 of 114], Pax6 [0 of 140] and Six3 [0 of 113], respectively, were not affected. This is consistent with the finding that Six2 is expressed in the neural retina and PRE of the adult mouse only [Oliver et al. 1995a,b; Kawakami et al. 1996a,b; Ohno et al. 1998], whereas Six3 is expressed in the embryonic retinal primordia. Furthermore, in vitro binding studies indicate that Six3 binds to a sequence unrelated to that of Six2 [Kawakami et al. 1996b].

Overexpression of Six3 in zebrafish embryos results in
Ectopic *Emx2* expression, indicating an enlargement of the rostral forebrain [Kobayashi et al. 1998]. To examine whether overexpression of *Six3* in medaka embryos similarly affects *Emx2* expression in injected embryos, we performed whole-mount in situ analysis. In the wild-type embryo *Emx2* is expressed dorsally in the anlage of the telencephalon and ventrally in the hypothalamus at the two-somite stage [Fig. 4C, below]. *Six3* overexpression results in an enlarged *Emx2* expression domain [8 of 60], indicating an expansion of the forebrain in these embryos [Fig. 4D, below].

*Pax6* expression in the developing retina has been shown to be required for retinal development [Hill et al. 1991; Hogan et al. 1986; Quinn et al. 1996]. We therefore asked whether *Pax6* expression was induced upon *Six3* RNA injection. In wild-type embryos *Pax6* is expressed in an anterior domain, comprising the optic vesicles and diencephalon, and in a posterior domain that includes the posterior hindbrain and spinal cord at neurula stages; the midbrain and prospective cerebellum do not express *Pax6* [Fig. 2A; Loosli et al. 1998]. Whole mount in situ hybridization of medaka *Six3* RNA-injected embryos revealed an expansion of the anterior *Pax6* expression domain and ectopic *Pax6* expression in the prospective midbrain and cerebellum at the late neurula stage [Fig. 2B,C; stage 18, 72 of 102], thus preceding ectopic *Rx2* expression in this region. At the six-somite stage [stage 21], ectopic *Pax6* expression covers the entire enlarged optic vesicles and extends into the midbrain and anterior hindbrain [medaka *Six3*; 58 of 79; mouse *Six3*, 24 of 29; data not shown]. Isolated ectopic *Pax6*-expressing tissue was found within the midbrain and prospective cerebellum [Fig. 2D]. This observation is consistent with the ectopic *Rx2* expression in this region. *Pax6* expression was not affected in the posterior hindbrain and spinal cord. Thus, *Six3* overexpression causes ectopic *Pax6* expression in a competent region corresponding to the midbrain and prospective cerebellum, preceding the formation of ectopic retinal primordia in this region.

To investigate whether *Pax6* is also expressed in the ectopic optic cups—as in the wild-type eye—we analyzed *Pax6* expression in *Six3*-injected embryos at the 34-somite stage. In 11 of 88 embryos ectopic PRE and closely associated *Pax6* expression was detected [Fig. 2E,F]. The distribution of *Pax6* expression relative to the PRE in these structures was highly reminiscent of the wild-type retina at this stage [Fig. 2F and inset].

In *Drosophila*, it has been suggested that expression of a *Six3* homolog, so, is controlled by a regulatory feedback loop [Cheyette et al. 1994]. In medaka embryos, injection of medaka or mouse *Six3* RNA equivalently resulted in the formation of ectopic retinal primordia and preceding marker gene expression [Figs. 1, cf. A with B, D with F, and 2, cf. B with C]. To determine whether in vertebrates *Six3* functions in a regulatory feedback loop, we examined medaka *Six3* expression in embryos that were injected with mouse *Six3* RNA. In wild-type embryos, *Six3* is expressed in the forebrain and optic vesicles at neurula and somitogenesis stages [Fig. 3C, Loosli et al. 1998]. At the late neurula stage in mouse *Six3* RNA-injected embryos, the endogenous *Six3* expression domain is expanded dramatically and endogenous *Six3* is expressed ectopically in the region of the midbrain and prospective cerebellum (31 of 40; data not shown). Also at the two-somite stage, ectopic medaka *Six3* expression was found in the midbrain and the prospective cerebellum (39 of 49) but not in the posterior hindbrain and spinal cord [Fig. 3A–C]. Thus, ectopic medaka *Six3* expression is activated in the region where *Six3* misexpression results in ectopic *Pax6* expression [cf. Figs. 3B with 2, B and C].

**Figure 2.** Ectopic *Pax6* expression in response to *Six3* overexpression. *Pax6* in situ analysis in control [A], medaka *Six3* [C–F], and mouse *Six3* [B] RNA-embryos. [A,B] Dorsal view of late neurula stage; [C] two-somite-stage embryos; [D] six-somite-stage embryos. [E,F] Transverse section of 34-somite stage embryos at the level of the midbrain. Open arrowheads indicate ectopic *Pax6* expression; open arrows retinal hyperplasia. [B] The embryonic axis is highlighted by yellow dotted lines. [B,D] Unilateral expansion of *Pax6* expression (open arrows) occurs in the optic vesicle. [C,D] Ectopic *Pax6* expression in the midbrain [open arrowhead]. [E,F] Ectopic *Pax6* expression (open arrowhead) is abutting ectopic PRE [solid arrowhead] in the midbrain. [F] Only part of the ectopic retina expresses *Pax6*, similar to the wild-type situation [inset]. (de) Diencephalon; (hb) hindbrain.
Ectopic expression of the endogenous Six3 gene in response to mouse Six3 expression is indicative of a regulatory feedback loop involving the medaka Six3 gene and suggests that this regulatory feature is conserved between vertebrates and invertebrates.

Ectopic Pax6, Six3, and the formation of retinal primordia in response to Six3 overexpression was detected exclusively in the midbrain and prospective cerebellum. To investigate whether cells ectopically expressing Pax6 and Six3, respectively, contribute to the ectopic retinal primordia as visualized by the expression of Rx2, we performed double labeling whole-mount in situ analysis. Similar to the wild-type situation in the developing retina, ectopic Rx2 expression partially overlaps with both ectopic Pax6 and Six3 expression, respectively, at the six-somite stage [stage 21; Fig. 4]. In all cases in which ectopic Rx2 expression was detected, it was associated and partially overlapping with ectopic Pax6 expression [11 of 47] and ectopic Six3 expression [13 of 58].

Discussion

Our results provide evidence that Six3 functions in vertebrate retina development. Overexpression of Six3 causes an enlargement of the normal retina. Retinal hyperplasia is preceded by an expansion of the expression domains of Six3 and Pax6, respectively. Consistent enlargement of structures derived from the retinal primordium in response to Six3 overexpression was also observed in Xenopus (retinal hyperplasia, M. Zuber, M. Perron, and W.A. Harris, pers. comm.) and zebrafish (enlargement of the optic stalk region; Kobayashi et al. 1998). In addition to retinal hyperplasia we observed at a lower frequency an expansion of the forebrain in Six3 RNA-injected embryos, which is in agreement with the findings in zebrafish [Kobayashi et al. 1998].

More importantly we show that early and widespread expression of Six3 results in the formation of retinal primordia at ectopic locations in the midbrain and prospective cerebellum, involving a regulatory interaction of Six3 and Pax6. Similar to the wild-type situation in the developing eye, Pax6 and Six3 are expressed in the region where the ectopic retinal primordia will subsequently form. These ectopic retinal primordia have the potential to develop into optic cups, as visualized by morphology and marker gene expression. The higher frequency of ectopic retinal primordia at early somitogenesis stages compared to ectopic optic cups formed at the 34-somite stage (Kobayashi et al. 1998).

Figure 3. Mouse Six3 expression reveals regulatory feedback control of the medaka Six3 gene. Six3 in situ (A–E) and lineage tracer [F,G] analysis in control [C], mouse [A,B,D], and medaka [F,G] Six3 RNA-injected embryos. Dorsal views [A–C,F,G] and transverse sections at the level of the optic vesicle [D,E] of twosomite-stage embryos, [A–C,F,G] Anterior is to the left. Open arrowhead indicates ectopic Six3 expression; open arrow indicates retinal hyperplasia. [A] The embryonic axis is highlighted by yellow dotted lines. [F,G] Lineage tracer analysis reveals a correlation of the severity of retinal hyperplasia with an unequal distribution of the injected RNA in the two body halves. Arrows point to enlarged optic vesicle, which is strongly fluorescent. [G] Widespread distribution of the lineage tracer along the entire anteroposterior axis.

Figure 4. Ectopic Rx2 expression partially overlaps with ectopic Pax6 and Six3 expression, respectively; expanded Emx2 expression indicates enlarged forebrain. [A,B] Dorsal views of six-somite-stage embryos injected with medaka Six3 RNA (A) and mouse Six3 RNA (B), respectively. The focal plane is dorsal at the level of the optic tectum. [A] Rx2 expression [blue] partially overlaps with Pax6 expression [red] in the ectopic retinal primordium [open arrowhead] as in the wild-type eye. [B] Rx2 expression [blue] partially overlapping with Six3 expression [red] in the ectopic retinal primordium [open arrowhead] and the enlarged optic vesicle [open arrow], respectively. [C,D] Lateral views of control (C) and medaka Six3-injected embryos (D). Note expanded Emx2 expression domain in D.
stage indicates that not all ectopic retinal primordia develop into an optic cup. Thus, Six3 initiates, but not fully implements, later stages of retinal development.

Our results indicate that the region competent to form ectopic retinal primordia corresponds to the midbrain and prospective cerebellum, where Pax6 is normally not expressed. In this region of competence ectopic Six3 can activate Pax6 expression. It is interesting to note that the region in which wild-type and ectopic retinal primordia develop lies within the expression domain of the Otx genes [Simeone et al. 1992; Li et al. 1994; Mori et al. 1994; Loosli et al. 1998], which based on their expression at early embryogenesis stages and function [Acampora et al. 1995; Matsuo et al. 1995; Ang et al. 1996] could play a role in providing competence for retinal development.

Ectopic lenses are formed in the region of the otic vesicle in response to Six3 overexpression both by RNA as well as plasmid DNA injection. On the other hand, ectopic retinal primordia formation was observed exclusively in response to Six3 RNA injection. This correlates well with the finding that overexpression of Six3 by RNA but not DNA [Oliver et al. 1996] injection induces Pax6 expression. Thus, early and widespread overexpression of Six3 at high levels is sufficient for the induction of Pax6 expression, whereas Six3 expression in small clones starting at mid-blastula transition is not. The correlation of ectopic Pax6 expression and retinal primordia formation suggests that Pax6 expression is a prerequisite for ectopic retina formation, supported by the essential role of Pax6 in eye development of vertebrates and invertebrates [Hill et al. 1991; Ton et al. 1991; Matsuo et al. 1993; Quiring et al. 1994; Halder et al. 1995a].

In Drosophila the Six3 homolog so when coexpressed with eyes absent, can ectopically activate a Pax6 homolog eyeless [ey], and cause ectopic eye formation in a Pax6-dependent manner [Pignoni et al. 1997]. This hints at an evolutionary conserved interaction of homologous genes in vertebrates and invertebrates. In addition, our results demonstrate a regulatory feedback loop of Six3 in developing retinal primordia, a mechanism that was suggested for so in Drosophila.

Thus, in addition to a structural conservation of the genes, their genetic interactions appear also to be conserved between vertebrates and invertebrates. It has been argued that in Drosophila an interactive network of eye specification genes, including so and ey, serves to implement and maintain the retinal cell fate program [Chen et al. 1997; Desplan 1997, Pignoni et al. 1997; Halder et al. 1998]. Our experiments reveal that in vertebrates this developmental program involves evolutionary conserved interactions of Six3 and Pax6, homologs of so and ey.

Materials and methods

Medaka stocks

Wild-type O. latipes from a closed stock at the MPI for Biophysical Chemistry were kept as described [Köster et al. 1997].

Isolation of partial Rx2 and Emx2 cDNA

A 560-bp fragment encoding medaka Rx2 and a 540-bp fragment encoding medaka Emx2 were amplified by RT-PCR from total RNA isolated from early neurula-stage (stage 17; Iwamatsu 1994) or early somitogenesis-stage (stage 19–21; Iwamatsu 1994) embryos, respectively, using degenerate PCR primers specific for Rx2 [up, 5′-GACGACGACCARC- RCCAACAAAAARCA; low, 5′-YRRHYTGDATRTGTYCTTTCYC- TTTCAT] or Emx [up, 5′-AATCGGWSYYTTICGGIIAAR; low, 5′-CYGTTYTGAAACCAATTTYACGTY]. PCR conditions were 5 cycles at 94°C for 1 min, 48°C for 2 min, and 72°C for 4 min, followed by 30 cycles (35 cycles for Emx2) with annealing at 53°C. The resulting PCR products were cloned into the TopoTA vector [Invitrogen] and sequenced. The accession numbers in the EMBL database are AJ007939 and AJ132403.

Plasmids

A 1.3-kb Apal–HindIII fragment of the medaka Six3 cDNA [Loosli et al. 1998] and a 0.75-kb fragment of hGFP [Clontech], respectively, were cloned into the pCS2 vector. The entire cDNA of the mouse Six3 cDNA (1.4 kb, Oliver et al. 1995a) and the mouse Six2 cDNA (2.2 kb, Kawakami et al. 1996b), respectively, were cloned into the pCS2 vector. The resulting vectors were analyzed by digestions with restriction enzymes and the correct orientation was verified by sequence analysis. Expression of a protein of the expected size was confirmed by in vitro translation.

RNA injections

Linearized pCSmouseSix3, pCSmedakaSix3, pCSGFP, and pCSmouseSix2 plasmid DNA was in vitro transcribed [Ambion SP6 mRNA Machine Kit]. The purified RNA [Qiagen RNeasy Kit] was injected in 1× Yamamoto ringer [Yamamoto 1975] into one blastomere at the two-cell stage as described [Köster et al. 1997]. Injected embryos were raised as described at 28°C [Köster et al. 1997]. An aliquot of the injection solution was analyzed by agarose gel electrophoresis to verify the concentration and integrity of the RNA. We have examined the effects of Six3 overexpression over a wide concentration range (35–200 ng/μl both for medaka and mouse Six3 RNA). We observed formation of ectopic retinal primordia and retinal hyperplasia over the entire concentration range. For the analysis presented, medaka Six3 RNA and mouse Six3 RNA, respectively, were injected at 100 ng/μl. Medaka Six3 RNA was co-injected at 100 ng/μl with 30 ng/μl hGFP as a lineage tracer. The distribution of the GFP-protein was analyzed at the two somite stage by microscopy of live embryos. The morphology of the injected embryos was unaffected in 22 of 93 [medaka Six3] and 19 of 47 [mouse Six3], respectively. Mouse Six2 RNA was co-injected at 200 and 500 ng/μl with 60 ng/μl hGFP RNA, respectively. The integrity of the injected RNAs was verified by fluorescence microscopy of live embryos at neurula stages. For controls, hGFP RNA was injected at 200 and 400 ng/μl, respectively. To test the coding capacity of the injected RNA, fluorescence was examined at neurula stages by microscopy of live embryos. Fluorescent embryos (>5%) were fixed for whole-mount in situ hybridization.

Whole-mount in situ hybridization and vibratome sectioning

Whole-mount in situ hybridization was performed using digoxigenin- and fluorescein-labeled RNA riboprobes as described [Loosli et al. 1998]. The entire cloned cDNAs of Pax6, Six3, Rx2, Emx2, and a-crystallin, respectively were transcribed for the RNA riboprobes. Vibratome sections were done following standard procedures [Boher et al. 1994]. Retinal hyperplasia was quantified by counting cell numbers in transverse sections at the comparable levels of mouse Six3 RNA injected (n = 3; 182 cells) and control embryos (n = 4; 84 cells), respectively.

In vitro transcription/translation

Medaka Six3, mouse Six3, and mouse Six2 RNA, respectively, were in vitro translated to test their coding capacity. In vitro transcription and translation was performed according to the manufacturer’s instructions [Promega]. One microgram of pCSmouseSix3, pCSmedakaSix3, and pCSmouseSix2 plasmid, respectively, SP6 RNA polymerase [Boehringer], rabbit reticulocyte lysate [Promega], and [35S]methionine were used, products were separated by SDS–PAGE, and visualized by autoradiography. Proteins of the expected size were detected for medaka Six3, mouse Six3, and mouse Six2, respectively.

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GENES & DEVELOPMENT 653
Loosli et al.

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Note added in proof

While this manuscript was in press, we were informed by W.A. Harris that the isolated Xenopus gene mentioned in the text is the Optx2 homolog.

References


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