Direct regulatory interaction of the eyeless protein with an eye-specific enhancer in the sine oculis gene during eye induction in Drosophila

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SUMMARY

The Pax-6 gene encodes a transcription factor with two DNA-binding domains, a paired and a homeodomain, and is expressed during eye morphogenesis and development of the nervous system. Pax-6 homologs have been isolated from a wide variety of organisms ranging from flatworms to humans. Since loss-of-function mutants in insects and mammals lead to an eyeless phenotype and Pax-6 orthologs from distantly related species are capable of inducing ectopic eyes in Drosophila, we have proposed that Pax-6 is a universal master control gene for eye morphogenesis. To determine the extent of evolutionary conservation of the eye morphogenetic pathway, we have begun to identify subordinate target genes of Pax-6. Previously we have shown that expression of two genes, sine oculis (so) and eyes absent (eya), is induced by eyeless (ey), the Pax-6 homolog of Drosophila. Here we present evidence from ectopic expression studies in transgenic flies, from transcription activation studies in yeast, and from gel shift assays in vitro that the EY protein activates transcription of sine oculis by direct interaction with an eye-specific enhancer in the long intron of the so gene.

Key words: Drosophila, Eye development, eyeless, sine oculis

INTRODUCTION

Pax-6 genes have been cloned from representatives of eight animal phyla, which to date includes homologs from genomes of human (Ton et al., 1991), mouse (Walther and Gruss, 1991), rat (Matsuo et al., 1993), chicken (Goulding et al., 1993), quail (Martin et al., 1992), Xenopus (Hirsch and Harris, 1997), zebrafish (Krauss et al., 1991; Puschel et al., 1992), ascidian (Glardon et al., 1997), sea urchin (Czerny and Busslinger, 1995), squid (Tomarev et al., 1997), Drosophila (Quiring et al., 1994; Czerny et al., 1999), Caenorhabditis elegans (Chisholm and Horvitz, 1995; Zhang and Emmons, 1995), ribbonworm (Loosli et al., 1996) and planaria (Callaerts et al., 1999). Pax-6 genes encode a transcription factor with two DNA binding domains, a homeodomain and a paired domain, both of which have been highly conserved during evolution. Although the consensus target site for each DNA binding domain has been determined separately by in vitro binding assays (Epstein et al., 1994; Czerny and Busslinger, 1995), only a limited number of target genes of Pax-6 have been identified (Sheng et al., 1997; for review, see Cvekl and Piatigorsky, 1996).

In mammals, congenital diseases known as Aniridia (humans) and Small eye (mice and rats) are caused by loss-of-function mutations of Pax-6 in heterozygotes, whereas homozygous embryos lack eyes and nostrils completely, have brain and spinal cord malformations, and die prior to birth. In Drosophila, loss-of-function mutations in the eyeless (ey) locus, found to encode a Pax-6 homolog (Quiring et al., 1994), also show eye defects (Hoge, 1915). In gain-of-function Drosophila mutants, ectopic eyes are formed on the antennae, legs, wings and halteres of the fly (Halder et al., 1995). Because ectopic expression of Pax-6 homologs from human, mouse, squid and sea squirts (ascidians) are capable of inducing ectopic eyes in Drosophila (Halder et al., 1995; Tomarev et al., 1997; Glardon et al., 1997), we have proposed that Pax-6 may serve as a universal master control gene for eye morphogenesis in metazoa. This implies a monophyletic origin of the eye as a sensory organ and contradicts the previously held view that the various eye types have evolved independent of one another (Salvini-Plawen and Mayr, 1977). Besides Pax-6, which resides at or near the top of the hierarchy or cascade of genes controlling eye morphogenesis, a large number of subordinate genes encoding transcription factors, signaling molecules and structural proteins are hypothesized to be required in this process. To what extent this genetic cascade is evolutionarily conserved in the various eye types is currently unknown. In order to approach this problem, we have set out to identify subordinate target genes of Pax-6. We have found that the Pax-6 genes of Drosophila and the silkworm underwent duplication (Czerny et al., 1999) and that the two paralogs, eyeless (ey) and twin-of-eyeless (toy), are differentially expressed. During embryonic development, toy appears to be expressed prior to ey and is required at least for activation, if not also maintenance, of ey. Downstream of ey, we have identified the...
sine oculis (so) and eyes absent (eya) genes (Halder et al., 1998), but it is not known whether ey interacts directly with these downstream targets. In this paper, we show by the results of experiments performed both in vivo and in vitro that the EY protein activates transcription of so by binding specifically to an eye-specific enhancer in the so gene. The requirement for induction of so by ey is demonstrated with the so\(^1\) mutation, a deletion of 1.3 kb in the eye-specific enhancer region that leaves the coding region intact. In so\(^1\) so\(^1\) mutant flies, ey fails to induce so expression and the formation of ectopic eyes. In contrast, toy induces both ectopic so expression and ectopic eyes in a so\(^1\) so\(^1\) homozygous mutants. We discuss these findings on the genetic control of eye morphogenesis in Drosophila in the context of those obtained in vertebrates.

MATERIALS AND METHODS

Fly strains
Flies were reared on standard medium at 25°C. Embryos of the yellow white strain (y ac w\(^{118}\)) were used as recipients for DNA injection for generation of transgenic lines. The stock carrying so-lacZ line (Cheyette et al., 1994) was kindly provided by Larry Zipursky. Flies carrying the dpp\(^{blanks}\);Gal4 transgene (line C40.6, Staeling-Hampton et al., 1994) were gift from Michael Hoffmann. UAS-ey and UAS-toy, ey\(^2\) and so\(^1\) have been described in Quiring et al. (1994), Cheyette et al. (1994) and Halder et al. (1995). The molecular lesions of the ey\(^2\) and so\(^1\) mutants were described previously by Quiring et al. (1994) and Cheyette et al. (1994), respectively.

Construction of so5-lacZ and so10-lacZ and generation of transgenic flies
All so genomic fragments were cloned in the same orientation upstream of the truncated hsp70 promoter in a P-element lacZ reporter plasmid, pCE (kindly provided by Markus Affolter). The so5 fragment was isolated as a 398 bp EcoRI-BamHI restriction fragment from a so genomic DNA (Cheyette et al., 1994). The 398 bp fragment was subcloned into pCE with the EcoRI-BamHI linker. The so10 fragment was isolated as a 428 bp EcoRI-Asp718 fragment from a so genomic DNA. The fragment was subcloned into the Asp718 site of pCE with the EcoRI-Asp718 linker. At least ten transformant lines of each construct were generated and analyzed as described by Rubin and Spradling (1982).

Ectopic eyeless expression and β-galactosidase detection
Virgin females homozygous for HS-ey (Halder et al., 1995) were crossed with males homozygous for the lacZ reporter transgenes. Eggs laid during 1 hour were collected and incubated at 25°C. Heat-shock cycles were applied starting at 81 hours after egg laying. One cycle consisted of a heat shock at 38°C for 45 minutes followed by recovery at 25°C for 3 hours 15 minutes. The heat-shocked larvae were allowed to recover for 3 hours prior to dissection. Detection of β-galactosidase was carried out in 0.5 ml of 10 mM NaH\(_2\)PO\(_4\)/H\(_2\)O/Na\(_2\)HPO\(_4·2\)H\(_2\)O (pH 7.2), 150 mM NaCl, 1 mM MgCl\(_2·6\)H\(_2\)O, 3.1 mM K\(_2\)Fe\(_3\)(CN)\(_6\), 3.1 mM K\(_3\)Fe\(_2\)(CN)\(_6\) and 0.3% Triton X-100 and 12.5 μl of 8% X-gal in DMSO; subsequently, the dissected larvae were washed several times in PBS and the discs were mounted in glycerol (Dakopatts) for microscopic examination. All lines derived from the same DNA construct gave similar patterns and intensities of staining.

In situ hybridization
Digoxigenin-labeled DNA probes were prepared from full length so cDNA (Cheyette et al., 1994) and hybridized to whole-mount imaginal discs. Hybridization was carried out in 50% formamide, 5x SSC, 100 μg/ml of sonicated salmon sperm DNA, 10 μg/ml of tRNA, 50 μg/ml of heparin and 0.1% Tween 20 overnight at 48°C. After washing several times with PBS-0.1% Tween 20, discs were incubated with an anti-digoxigenin antibody coupled with alkaline phosphatase and the staining reaction allowed to proceed for 2 hours.

Yeast one-hybrid system
DNA-binding analysis by the yeast one-hybrid system was carried out essentially as described by Mastick et al. (1995). ey embryonic cDNA (Quiring et al., 1994) was inserted into an activator plasmid (pBM258T; Mastick et al., 1995) under the control of the pGAL1 promoter, which is activated by galactose and repressed by glucose. Fragments of the so genomic gene were inserted into a reporter plasmid (pHR307a; Mastick et al., 1995). EY induced by galactose, bound to its target site, in turn induces HIS3 expression, which allows for growth on medium lacking histidine. URA3 and TRP3 were used for the selection of the activator and reporter constructs, respectively. As a control, two or four copies of a consensus PRD binding site (5'-TTCCAGCATAGTTCCT-3') were inserted into the reporter plasmid pHR307a.

Gel shift assay
EY protein was synthesized using a coupled in vitro transcription translation kit (Promega) with the T7 primer and ey embryonic cDNA as template (Quiring et al., 1994). Crude protein extracts were prepared from heat-induced and control (non-induced) HS-ey embryos. Genomic fragments depicted in Fig. 3A were divided into subfragments (approximately 120 bp each) by PCR and tested for EY binding activity by gel shift assay. For so5, four subfragments 1-120, 101-220, 201-320 and 301-428 were similarly generated for so10 probes. Purified PCR products were radioactively labeled with poly nucleotide kinase and [γ-\(^{32}\)P]ATP. For super shift experiments, 1 μl of rabbit anti-EY antiserum (kindly provided by Uwe Waldorf) was added. Binding reactions were carried out in 20 μl of 10 mM Tris-HCl pH 8.0, 50 mM KCl, 4 mM dithiothreitol, 10% glycerol and incubated at room temperature for 15 minutes and analyzed by electrophoresis through 6% native polyacrylamide gels followed by autoradiography. Both binding and electrophoresis were performed at room temperature.

RESULTS

Heat-shock eyeless induces ectopic expression of sine oculis
Although sine oculis (so) has been shown to be required during normal and ectopic eye development and to act downstream of ey (Halder et al., 1998), whether ey controls so directly or indirectly remains to be determined. If so is under the direct control of ey, ectopic expression of so should be induced as an immediate-early response to the ectopic expression of ey. To determine whether such an immediate-early response is induced, a so-lacZ enhancer trap line that reflects the expression pattern of the endogenous so gene (Fig. 1; Cheyette et al., 1994) and a heat shock-eyeless (HS-ey) line were crossed and the transheterozygous larvae tested for ectopic lacZ expression after application of several cycles of heat shock. After 2 cycles of heat shock, ectopic expression of β-galactosidase in the so-lacZ line was barely detectable in wing and leg imaginal discs (Fig. 2A). However, ectopic expression was strongly induced after 3 cycles of heat shock (Fig. 2B, arrow). The site of ectopic expression of β-galactosidase in the so-lacZ discs corresponds to the location of the ectopic eyes in
the adult flies (data not shown). In contrast, when the so-lacZ line was crossed to y ac w1118 flies as a control, no ectopic expression of β-galactosidase was detected upon heat shock (Fig. 2D-F). This result indicates that the expression of so is induced immediately upon induction of ey expression. Interestingly, the capacity to induce ectopic eyes was much reduced in HS-ey lines as compared to Gal4 driver lines. Under the conditions used, no ectopic eyes were formed after 2 or 3 cycles of heat shock. However, after 6 cycles of heat shock, small ectopic eyes were detected on wings and legs (data not shown).

**eyless** protein binds to an eye-specific enhancer and the promoter region of **sine oculis** and activates transcription in a yeast one-hybrid system

The time course of induction of so by ey in the heat-shock experiments suggests, but does not prove, a direct interaction.

Therefore, we examined whether EY binds directly to regulatory regions of so in a yeast one-hybrid system and thereby activates transcription. For expression of the one-hybrid fusion protein, ey cDNA was inserted downstream of the pGAL1 promoter of the yeast activator plasmid (see Materials and Methods). We first tested two promoter regions and a putative eye-specific enhancer region of so. Four fragments from these regions (Fig. 3A, upper row) were inserted upstream of HIS3 in the yeast reporter plasmid. The binding activity of EY to the so genomic fragments was assayed by growth of yeast on medium lacking histidine and containing galactose. Two out of four fragments (so fragments 2 and 4) were positive in the first test (Fig. 3B). These two positive fragments were subdivided (Fig. 3A, middle and lower rows) and tested again by the same method. Ultimately, two EY binding regions, fragments 5 and 10, were identified (Fig. 3B). Each fragment is approximately 400 bp in length and contains a putative PRD binding site (Fig. 4A,B, shaded boxes). The
sequences of the putative sites are at maximum 88% homologous (fragment 5 site) and 70% (fragment 10 site) with the consensus PRD binding site sequence reported by Czerny and Busslinger (1995) (Fig. 4C). As a further control, we tested the PRD consensus binding site in the yeast one-hybrid system and found that EY does indeed recognize this sequence (Fig. 3B). These results demonstrate that EY binds directly to the so locus (fragments 5 and 10), putatively at PRD consensus sequences and activates reporter gene transcription in a yeast one-hybrid system.

**eyeless protein selectively binds a subfragment of the eye-specific enhancer in *sine oculis* in vitro**

To narrow down further the EY binding region, fragments 5 and 10 were subdivided each into four subfragments approximately 120 bp in length and analyzed for EY binding activity by gel shift assay with crude protein extracts from heat-induced HS-ey embryo. In these assays, only one subfragment (Fig. 4 underlined) of genomic fragment 10, which was derived from the so eye-specific enhancer, revealed a strong reduction in migration rate consistent with the formation of a specific protein-DNA complex (Fig. 5, lane 6), but the four subfragments of genomic fragment 5, derived from the so promoter region, failed to bind protein in the extract even though the so promoter region was positive in the yeast one-hybrid system (data not shown). The apparent complex was observed with heat-shocked extracts from HS-ey embryos (compare lanes 6 and 7) but not with extracts from heat-shocked y ac w1118 control embryos (data not shown). Competition with unlabeled oligonucleotide (lane 8) revealed that the complex was specific. To confirm that the complex observed with crude HS-ey extracts was due to binding of the EY protein, band shifts were performed with EY protein produced by in vitro transcription and translation. A shifted complex was observed to co-migrate with the heat-shock-induced species (compare lanes 2 and 6), demonstrating conclusively that with heat-shock-induced extracts the specific complex was formed by EY. Furthermore, addition of antiserum against EY to binding reactions with both crude and in vitro synthesized protein prevented formation of the complex (lanes 2 and 6). These results indicate that EY binds directly to the so eye-specific enhancer region in vitro. Because the fragment that is bound by EY contains a putative PRD consensus binding sequence, we tested a PRD consensus binding site fragment (Czerny and Busslinger, 1995) by gel shift assay and again observed specific complex formation with heat-shock-induced extracts.

**eyeless induces ectopic expression of *sine oculis* by interaction with the eye-specific enhancer region in vivo**

To determine whether EY binds to so genomic fragments 5 and 10 in vivo, transgenic reporter lines so5-lacZ and so10-lacZ were generated with a P-element vector. Although β-galactosidase expression of the so-lacZ control

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**Fig. 3.** EY protein binds to the so enhancer and promoter regions and activates reporter gene transcription in a yeast one-hybrid system. (A) Genomic map of so. Exons are depicted as boxes with the protein coding region shown in black. The dotted box represents an exon transcribed from a second internal promoter. The extent of the so′ deletion is indicated below the map. The bars above represent genomic DNA fragments tested in the one-hybrid assay (see below). The five′ fragments 1, 2, 3, 5 and 6 encompass two promoter regions and the more 3′ fragments 4, 7, 9 and 10 are clustered in a so′-enhancer region, which is deleted in the so′ mutation. (B) Yeast one-hybrid analysis. a and b were carried out on galactose medium, which induces EY protein expression, c and d on glucose medium in which EY is not induced. Both media lack uracil, tryptophan and histidine. (a) Reporter gene expression allowing growth is observed with two promoter fragments (2 and 5) and with three enhancer fragments (4, 7 and 10). No growth is observed without an ey producer plasmid (b) or without EY expression in glucose medium (c,d). The consensus PRD binding sequence also allows EY protein to bind and to activate transcription. PRD binding sequence 1 contains four repeats of the consensus sequence and 2, two repeats.
The putative PRD binding site in the eye-specific enhancer of eyeless (Czerny and Busslinger 1995). Nucleotides indicated in bold in A and B match the consensus PRD and 379-395 in which were found to be positive in the yeast one-hybrid system.

The staining pattern of the so 1 mutant in which this region is deleted (Fig. 6A). Expression of so10-lacZ was confined to the eye imaginal disc (Fig. 6D) and its pattern very similar to the endogenous so expression pattern in the eye disc, i.e. both anterior and posterior to the morphogenetic furrow of the eye disc, indicating that this 428 bp region harbors an eye-specific enhancer of so responsible for the selective expression of so in the eye disc. This result is in agreement with the eye-specific phenotype (no eyes or eyeless) of the so 1 mutant in which this region is deleted (Cheyette et al., 1994). We also determined whether HS-ey can induce ectopic expression of β-galactosidase in the so5-lacZ and the so10-lacZ lines. The staining pattern of so5-lacZ line was unaltered after 2 cycles of heat shock (Fig. 6B) and additional cycles of heat shock failed to induce ectopic expression of β-galactosidase (Fig. 6C). In contrast, ectopic expression of β-galactosidase was detected after 2 cycles and 3 cycles of heat shock in both wing and leg discs from the so10-lacZ line (Fig. 6E,F), demonstrating clearly that ectopic expression of EY by heat shock induces ectopic expression of the so10 reporter gene. These results show that EY controls so expression through the eye-specific enhancer in the so gene in vivo.

The eyeless protein is unable to induce so expression in homozygous so 1 enhancer deletion mutants

If EY protein interacts exclusively with the eye-specific enhancer in so, induction of so expression by EY should be abolished in homozygous so 1/so 1 deletion mutants that lack the enhancer (cf. Fig. 3). Indeed, as shown by in situ hybridization, EY is not capable of inducing so expression (Fig. 7B,D). This finding is consistent with the observation that EY cannot induce ectopic eyes in so 1/so 1 mutants (Halder et al., 1998 and this work), but in marked contrast to results with twin of eyeless (toy), a second Pax-6 homolog found in Drosophila and other higher insects (Czerny et al., 1999). Even though EY and TOY share 90% and 95% amino acid sequence identity in their paired and homeodomains, respectively, the two proteins differ significantly in their DNA binding properties. TOY can ectopically activate so transcription in so 1/so 1 mutants (Fig. 7C) and also induces ectopic eyes (Fig. 7E). This indicates that EY and TOY do not interact with the same regulatory regions in so.
DISCUSSION

Several target genes of Pax-6 in vertebrates have already been identified: mouse and chicken Α-crystallin, chicken δ1-crystallin, guinea pig ζ-crystallin and mouse N-CAML (Cvekl et al., 1994, 1995a,b; Richardson et al., 1995; Chalepakis et al., 1994). In Drosophila, rhodopsin is a candidate target gene (Sheng et al., 1997), although the evidence for direct interaction is inconclusive. Interestingly, all of these target genes encode protein products for terminal differentiation in eye morphogenesis. However, Pax-6 acts on the developing eye primordia from the earliest stages of morphogenesis, it may not only regulate genes involved in terminal differentiation, but also transcription factors functioning earlier to regulate further downstream genes. This includes Pax-6 itself which was shown to autoregulate its own promoter (Plaza et al., 1995). We have shown here that ey directly regulates so, which therefore functions downstream in the eye morphogenetic pathway.

The so gene encodes a homeodomain protein required for the development of the entire visual system, including the compound eye, the ocelli, the optic lobe of the brain and the...

Fig. 6. β-galactosidase expression in eye-antennal (left), wing (center) and leg (right) discs of late third instar larvae carrying so5-lacZ (A–C) and so10-lacZ (D–F) transgenes. Detection of β-galactosidase activity in control (no heat shock) so5-lacZ (A) and so10-lacZ (D) discs. Two cycles (B,E) and three cycles (C,F) of heat shock. The staining pattern in the eye disc of so10-lacZ (D) is similar to that of the enhancer trap line so-lacZ (cf. Fig. 1). The staining pattern of so5-lacZ (A) is unrelated to that of so-lacZ and does not reflect so gene expression (B,C). The so5-lacZ construct was not inducible by HS-ey, whereas ectopic expression of β-galactosidase was clearly detected in wing and leg discs of so10-lacZ after 2 cycles of heat shock (E) and became more intense with three cycles.

Fig. 7. Analysis of so transcription in homozygous so1/so1 mutants. (A–C) In situ hybridizations with a so cDNA probe in leg imaginal discs. (A) Wild type. Ectopic expression of ey (B) did not lead to any detectable ectopic expression of so. Ectopic expression of toy (C) produces a single spot of ectopic so expression. Ectopic eyes are induced by ectopic expression of toy (E, arrowhead), but not of ey (D) in a so1/so1 homozygous mutants. ey and toy were expressed ectopically with dpp^block-Gal4 in the Gal4/UAS system (Brand and Perrimon, 1993).
larval photoreceptor designated as Bolwig’s organ (Cheyette et al., 1994; Serikaku and O’Tousa, 1994; Pignoni et al., 1997).

so acts downstream of ey (Halder et al., 1998) and physically interacts with another transcription factor encoded by eyes absent (eya) to form a protein complex that feeds back on ey expression and is also capable of inducing ectopic eyes (Bonini et al., 1993, 1997; Pignoni et al., 1997). We have identified so and eya as mediators of the eye-inducing activity of ey (Halder et al., 1998). Both so and eya are induced by ey and the expression of both genes is required independently during ectopic eye induction, but not vice versa. We have proposed a model in which ey induces the initial expression of both so and eya in the eye disc, after which so and eya participate in a positive feedback loop that regulates the expression of all three and possibly more genes.

In this paper, we present evidence for a direct interaction between EY and the eye-specific enhancer of so, which lends support to the above model. The evidence is based upon the yeast one-hybrid system in which EY protein is expressed by a driver plasmid and tested for its ability to bind a putative DNA binding site and activate reporter gene transcription. DNA fragments from both the eye-specific enhancer and the promoter of so were recognized as target sites. These results were confirmed for the eye-specific enhancer by gel shift assays in vitro, which in combination with the yeast one-hybrid system, allowed us to narrow down the binding site to a fragment of 120 bp containing a putative Pax-6 paired domain binding sequence. The functional importance of the eye-specific enhancer of so is demonstrated in vivo by means of the so1 mutant, which deletes a 1.3 kb region including the enhancer, but leaves the coding sequences intact. In so1 homozygous flies, ey is neither capable of inducing so transcription nor can it induce ectopic eyes. In contrast to ey, its paralog toy induces both ectopic so transcription and ectopic eyes in a so1 mutant background. This indicates that ey and toy regulate so by different mechanisms.

Sine oculis homologs have been identified in humans (Boucher et al., 1996), chicken (Bovolenta et al., 1996) mouse (Oliver et al., 1995), zebrafish (Seo et al., 1998; Kobayashi et al., 1998) and medaka fish (Loosli et al., 1998) as well as in planarians (P. Callaerts and E. Salo, personal communication). However, because several paralogs have been identified both in mammals and Drosophila that form a small gene family, which genes are true orthologs and which are paralogs is not clear in all cases. Nevertheless, several members of the vertebrate so/Six family are expressed during eye morphogenesis and their functional importance in eye development is clearly illustrated by the experiments of Oliver et al. (1996) who showed that ectopic expression of the mouse Six3 gene in medaka fish embryos can induce ectopic lens formation. Therefore, at least some of the sine oculis homologs play an important role in eye morphogenesis, but the exact evolutionary relationships between the genes and their functions remains to be elucidated. Since recent studies of Bonini et al. (1997) indicate that the role of eya in eye development has also been conserved between flies and vertebrates, it seems that several important circuits of gene regulation have been conserved during evolution of the eye developmental pathway even though different types of eyes are formed in insects and vertebrates.

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REFERENCES


Conservation of Pax-6 in a lower chordate, the ascidian Phallusia mammillata. Development 124, 817-825.


