The *Drosophila* *eyes absent* gene directs ectopic eye formation in a pathway conserved between flies and vertebrates

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**SUMMARY**

The fly *eyes absent* (*eya*) gene which is essential for compound eye development in *Drosophila*, was shown to be functionally replaceable in eye development by a vertebrate *Eya* homolog. The relationship between *eya* and that of the *eyeless* gene, a *Pax-6* homolog, critical for eye formation in both flies and man, was defined: *eya* was found to be essential for eye formation by *eyeless*. Moreover, *eya* could itself direct ectopic eye formation, indicating that *eya* has the capacity to function as a master control gene for eye formation. Finally, we show that *eya* and *eyeless* together were more effective in eye formation than either gene alone. These data indicate conservation of the pathway of *eya* function between flies and vertebrates; they suggest a model whereby *eya/Eya* gene function is essential for eye formation by *eyeless/Pax-6*, and that *eya/Eya* can in turn mediate, via a regulatory loop, the activity of *eyeless/Pax-6* in eye formation.

Key words: eye development, *eyes absent*, *eyeless*, *Drosophila*

**INTRODUCTION**

The *Drosophila* eye, although structurally distinct from the vertebrate eye, shows striking parallels at the molecular level. Many genes that function in eye formation in the fly have homologs that are expressed during vertebrate eye development (Quiring et al., 1994; Zuker, 1994; Oliver et al., 1995). Among these is the *Drosophila eyes absent* (*eya*) gene which encodes a nuclear protein that, in the fly, functions prior to the first notable differentiation event – morphogenetic furrow formation – in eye progenitor cell development (Bonini et al., 1993; Leiserson et al., 1994). *eya* is the founding member of a class of vertebrate *Eya* genes, with homologs showing expression in the eye and other tissues (Abdelhak et al., 1997; Duncan et al., 1997; Xu et al., 1997; Zimmerman et al., 1997). Human mutations at the *Eya1* locus have been identified that result in defects in organ formation (Abdelhak et al., 1997).

*eya* functions at a similar time and place as the fly *eyeless* gene. *eyeless* is a counterpart of the human *ANIRIDIA* and mouse *Sey* (*Small eye*) genes, which are *Pax-6* family members containing a paired-box and homeobox and likely function as transcription factors (Quiring et al., 1994). Mutated *eyeless* results in loss of the fly eye (Quiring et al., 1994); similarly, mutation of the human or mouse counterparts leads to eye malformation and reduction of the eye in the extreme, and cataracts in mild forms (Hogan et al., 1986; Hill et al., 1991; Ton et al., 1991; Glaser et al., 1992; Jordan et al., 1992; see Hanson and van Heyningen, 1995). Expression of the *eyeless* cDNA in the fly using tissue-specific elements can direct the formation of ectopic eyes in the antennae, legs and wings (Halder et al., 1995a). The mouse *Sey* cDNA, when introduced into the fly, can similarly direct ectopic *Drosophila* eye development, suggesting potential conservation of fundamental molecular features by which these homologs act in eye formation. *Sey* mouse mutants have reduced expression of the *Eya1* and *Eya2* genes in eye progenitor tissue (Xu et al., 1997), suggesting that *Eya* genes might be mediators of *Pax-6* function for eye formation in vertebrates.

Since the vertebrate *Eya* homologs show expression in eye tissue, it is tantalizing to speculate that the vertebrate and fly genes are functional homologs. Here, we address the level of functional conservation between fly *eya* and a vertebrate *Eya* homolog. We then used the fly to define the relationship between *eya* and *eyeless* that was suggested by vertebrate work: that the *eya* gene may be a conserved mediator of *eyeless* function in eye formation. We found that not only is *eya* essential for *eyeless* function, but that the *eya* gene itself can serve as a master control gene for eye formation. These data indicate striking functional conservation of the genetic pathway of eye formation between flies and vertebrates, and suggest a model of combinatorial gene functions for eye formation.

**MATERIALS AND METHODS**

*Drosophila* strains and P-element-mediated transformation

Fly strains were grown on standard molasses, yeast and cornmeal medium at 25°C. *UAS-eya* transgenics were made by subcloning the full-length *Drosophila* *eya* typeI cDNA into the pUAST vector (Brand and Perrimon, 1993). *UAS-Eya2* mouse transgenes were made by subcloning a predicted full length mouse *Eya2* subclone (Zimmerman et al., 1997) into the pUAST vector. Flies were transformed using standard transgenic techniques (Rubin and Spradling, 1982). *eyeless-
GAL4 was made by subcloning a fragment which reports eyeless staining in eye progenitor cells (Quiring et al., 1994), into the GAL4 vector (Brand and Perrimon, 1993), and transforming the construct into flies. Other GAL4 lines were obtained from Drosophila Stock Centers: eye mutant alleles are as previously described (Bonini et al., 1993); eyeless2 mutant strain was provided by courtesy of Dr Walter Gehring. GAL4 lines used included T59, T155 and dpp-GAL4, all of which express in the eye portion of the eye-antennal disc. dpp-GAL4 is expressed in the imaginal discs in an expression pattern similar to that of dpp (Staehling-Hampton et al., 1994; see also Shen and Mardon, 1997). eyeless-lacZ lines are as described by Quiring et al. (1994); UAS-eyeless lines are as described by Halder et al. (1995a).

**Immunohistochemistry**

Tissue preparations were fixed with 2% paraformaldehyde in TBS, permeabilized with 0.5% Triton X-100, and stained in primary antibody overnight. Primary antibodies were anti-Eya (Bonini et al., 1993) and anti-Glass (Ellis et al., 1993). After rinsing in TBS, tissue was stained with secondary antibodies conjugated to fluorescein or cyanine-3 (Jackson Immunoresearch Laboratory), rinsed in TBS, then mounted in PDA-glycerol, as previously described (Bonini et al., 1993). Staining with β-galactosidase for eyeless-lacZ expression pattern was performed as described by Quiring et al. (1994). For viewing flies by scanning electron microscopy, flies were critical point dried and scanned at 5 kV. For sections of eye tissue, flies were fixed in glutaraldehyde, embedded in epon and thick sectioned (Bonini et al., 1993).

**RESULTS**

A vertebrate Eya homolog is able to functionally replace the fly gene

To test potential functional homology between the vertebrate and fly eya genes, we asked whether a vertebrate Eya homolog was capable of replacing the fly eya gene in the eye developmental pathway. To do this, we required functional replacement of eya activity in the eya2 mutant background. The eya2 mutant is a viable, eye-specific null for eye activity in eye progenitor cells anterior to the furrow (Bonini et al., 1993). These mutant flies are completely eyeless due to complete loss of eye progenitor cells by cell death (Fig. 1A). Thus, any survival and development of ommatidia to the adult eye in this mutant background would indicate functional replacement of fly eya gene activity.

To express the vertebrate gene, the GAL4-UAS system of tissue-specific targetting was used (Brand and Perrimon, 1993). UAS-Eya2 transgenic animals were made using a mouse Eya2 cDNA that is predicted to encode a full-length protein (Zimmerman et al., 1997). The protein encoded by this cDNA that is predicted to encode a full-length protein is expressed in the eye-antennal disc. dpp-GAL4 is expressed in the imaginal discs in an expression pattern similar to that of dpp (Staehling-Hampton et al., 1994; see also Shen and Mardon, 1997). eyeless-lacZ lines are as described by Quiring et al. (1994); UAS-eyeless lines are as described by Halder et al. (1995a).

**eya function is essential for eyeless activity**

Given this striking level of conservation of eya function, we were interested to determine the relationship between eyeless gene activity and eya gene activity suggested by expression studies in vertebrates. To determine whether eya gene expression occurred upon eye formation directed by the eyeless gene, we generated ectopic eyes with eyeless background, genotype eya2 UAS-Eya2 eyeless-GAL4. The eye shows a pattern of ommatidial units with bristle cells similar to the normal fly compound eye. (C) Tangential section of a fly eye generated by the mouse Eya2 gene, genotype eya2 UAS-Eya2 eyeless-GAL4 fly. Photoreceptor cells have developed in a pattern typical of the compound eye (compare to Fig. 3C). In this section, it is possible to see all photoreceptor cells: to the right, ommatidia with R1-R6, plus R7 are present; to the left, ommatidia with R1-R6, plus R8 are seen. The ommatidia are surrounded by a pigment lattice, and the equator can be seen running through the eye. Bar, 50 µm for A,B; 3.5 µm for C.

**Table 1. eya function is essential for ectopic eye formation by the eyeless gene**

<table>
<thead>
<tr>
<th>Structure with ectopic eye formation</th>
<th>UAS-eyeless/ dpp-GAL4</th>
<th>eya2; UAS-eyeless/ dpp-GAL4*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antennae</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Legs</td>
<td>100%;†</td>
<td>0%;†</td>
</tr>
<tr>
<td>Wing</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Haltere</td>
<td>24%</td>
<td>0%</td>
</tr>
</tbody>
</table>

50 animals scored for each data point; ectopic eye development is scored by the presence of ommatidia with pigment.

*Animals are completely eyeless in addition to lacking ectopic eye formation.

†Legs truncated and abnormally shaped (see Fig. 2C-E).
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data not shown). Of these imaginal tissues, Eya is normally expressed only in eye and ocellar progenitor cells of the eye-antennal disc, and the peripodial membrane of the wing disc; Eya is not normally expressed in cells of the antennal, leg or wing disc proper (Fig. 2H,K, data not shown; Bonini et al., 1993). Activation of eya gene expression by eyeless suggested that eya may indeed be required for formation of eyes by eyeless, similar to the requirement for eya function during normal compound eye development.

To test whether eya gene activity was essential for eyeless-driven ectopic eye formation, we attempted to induce ectopic eyes with UAS-eyeless crossed to dpp-GAL4, but now in the eya2 mutant background. As noted, the eya2 mutant is completely eyeless and null for the early eye function of the eya gene. If eye formation by eyeless were dependent upon eya gene activity, then ectopic eye formation should fail in the eya2 mutant background. Eye formation indeed failed in all tissues of the fly where ectopic eyes had previously developed: the legs, wings and antennal segments of the head (Table 1, Fig. 2B,D). In the imaginal tissues, as anticipated, no ectopic Eya protein expression was detectable in the antennal, leg or wing discs of animals bearing UAS-eyeless in trans to dpp-GAL4 in the eya2 mutant background (Fig. 2G,J). These experiments also demonstrated that UAS-eyeless was not able to restore normal eye formation to the eya2 mutant (Fig. 2B), indicating that eyeless gene activity cannot replace or substitute for the function of eya in eye development. Taken together, these data clearly indicate that eya gene function is essential for eyeless to form eyes; the eya gene thus appears to be an essential biological target of eyeless gene activity in eye formation.

The eya gene directs ectopic eye formation

Given the high conservation demonstrated above of the eya pathway at the functional level between flies and vertebrates, and given the essential role of eya for eyeless function that may well extend between flies and vertebrates, we were interested
to determine what were potential effects of the eya gene itself for eye formation. Could eya, like eyeless, mediate eye formation? Previously, we had expressed the eya gene in the fly with a heat shock promoter (Bonini et al., 1993); whereas such expression could restore the eyes to eya mutants that lacked eye formation, no other consistent effects were observed in the rescued animals. Nevertheless, we attempted to express eya at higher levels using the GAL4-UAS system (Brand and Perrimon, 1993), to determine whether it was possible to induce dominant phenotypes that would yield clues to the function of the gene.

A UAS-eya construct was made with the eya cDNA. To determine whether the construct was functional, we attempted rescue of the eya2 mutant phenotype using various GAL4 lines that express in the eye progenitor field prior to furrow formation. A number of GAL4 lines were tested, including eyeless-GAL4, dpp-GAL4, T59, and T155. These GAL4 insertions, when crossed to a UAS-eya insert, could restore to the eya2 mutant eyes up to three quarters of normal size. Given that rescue was partial, we attempted to increase eye size by increasing gene dosage of the UAS-eya and GAL4 insertions. In doing this, we found that UAS-eya lines in trans to GAL4 insertion lines were lethal in two doses of the transgenes; for those lines that were lethal at the late pupal stage (dpp-GAL4, T59), the homozygous animals could be observed by dissection of the pupae. This analysis showed that these lines displayed not only rescue of the eye, but also ectopic eye formation in other regions of the animals where GAL4 was expressed with these constructs. The chromosomes bearing the UAS-eya and GAL4 inserts were then crossed out of the eya mutant background and into a normal background for additional analysis. We focused on expression of UAS-eya driven by dpp-GAL4, since this combination led to late pupal lethals that could be readily observed; in these animals, GAL4 expression occurs in the imaginal disc expression pattern of dpp, in the eye and antennal portions of the eye-antennal disc, the leg and wing discs, among other tissues (Blackman et al., 1991).

In a wild-type background, UAS-eya dpp-GAL4 in single copy generated rare examples of ectopic eyes, which resembled normal eyes, on the antennal segment (10% of the animals, Fig. 3A,B). Tangential sections of the ectopic eyes formed indicated that photoreceptor cells developed in a pattern similar to that of the normal compound eye (Fig. 3C). With two copies of UAS-eya dpp-GAL4, ectopic eye formation was induced in the antennal region of the head in almost all animals (96% ectopic eye formation on antennae); 80% also showed ommatidial array formation on the legs, and occasionally on the wings. Glass, a photoreceptor-specific protein, was used as a marker to detect development of retinal tissue in the larval imaginal discs. Glass expression is normally restricted to the eye portion of the eye-antennal disc and does not occur in the antennal portion or other imaginal discs (Ellis et al., 1993). Ectopic expression of Glass was seen in the antennal and leg imaginal discs; in these tissues, rosettes of developing photoreceptor clusters similar to the normal pattern were seen (Fig. 3D,E). These data indicate that eya has the capacity to function as a master regulatory gene for eye formation.

**Requirement for eyeless in ectopic eyes produced by eya**

These observations raised questions regarding the relationship between the eya and eyeless gene functions during eye formation. Since eya was essential for ectopic eye formation by eyeless (see Table 1; Fig. 2), was eyeless gene function essential for ectopic eye formation by eya? To address this, we first asked whether eyeless gene expression was induced during ectopic eye formation directed by the eya gene. Normally, eyeless expression is restricted to the eye portion of the eye-antennal imaginal disc (Fig. 4A; Quiring et al., 1994). In UAS-eya dpp-GAL4 animals, expression of eyeless occurred ectopically in the antennal region of the eye-antennal disc, in the region where eya directed ectopic eye formation (Fig. 4B). Although eya also directed ectopic eye formation in the leg discs, ectopic eyeless expression was not detectable in that tissue upon eya expression (Fig. 4C); eyeless was capable of autoregulation in leg discs when eyeless itself was ectopically expressed (Fig. 4D). This suggested that eyeless might be required for eya activity to form eyes in some tissues, but dispensable in others. We also determined that eyeless remained expressed in the eye progenitor cells of the eya2 mutant (Fig. 4F).

![Fig 3. The eya gene directs ectopic eye formation.](image-url)
Fig 4. Ectopic eye formation by eya turns on eyeless gene expression. (A) Expression of eyeless in eye progenitor cells of a normal eye-antennal imaginal disc. eyeless expression is detected with a β-galactosidase reporter construct (Quiring et al., 1994). Arrow indicates position of the morphogenetic furrow. (B,C) Ectopic expression of eyeless directed by the eya gene. In animals of genotype UAS-eya dpp-GAL4, ectopic eyeless expression occurred in the antennal region of the eye-antennal imaginal disc (B, arrowhead; small arrow indicates the position of the morphogenetic furrow). In the leg discs (C), however, ectopic eyeless is not detectable upon eya expression. (D) When eyeless itself is ectopically expressed, eyeless expression is detectable in leg discs (D) as well as in the antennal portion of the eye-antennal disc (not shown), consistent with autoregulation of the eyeless gene (Glardon et al., 1997). Animals of genotype eyeless-lacZ; UAS-eyeless in trans to dpp-GAL4, (E) eyeless expression is present in the eye progenitor field of eya mutant discs. The eye portion of the disc is reduced in size due to loss of the eye progenitor cells by programmed cell death, no morphogenetic furrow is present (Bonini et al., 1993). Anterior to the right. Bar 50 μm.

4E), suggesting that eya gene function is not essential for the normal expression pattern of eyeless.

To address a functional requirement for eyeless, we investigated whether there were detectable genetic interactions between the eyeless and eya genes. Such experiments are limited by the mutants of eyeless currently available – there are no null mutants for the eye function of eyeless which would allow us to remove eyeless gene activity completely (Quiring et al., 1994). Nevertheless, we attempted to induce ectopic eye formation with eya in a background of reduced eyeless gene activity, by using the eyeless mutant allele. eyeless2 mutant flies show a range of reduced eye phenotypes, with about 30% of the flies missing at least one eye completely. Directed expression of the eye gene in the eyeless background, did not result in ectopic eye formation in the antennal segments of the head, or the legs (data not shown). These data indicated a dependence on eyeless gene activity in the ability of eya to direct eye development both in the head and in the legs. Thus, eya appears to function both downstream and upstream of eyeless gene activity in eye formation.

Potentiation between eyeless and eya in eye formation

This regulatory relationship between the two genes prompted us to ask whether we could detect additional interactions between the genes. To do this, we examined the ability of eyeless to direct eye formation when combined with additional doses of eya gene activity. Animals bearing UAS-eya in trans to dpp-GAL4 show limited dominant effects (see above and Table 2); in animals bearing UAS-eyeless in trans to dpp-GAL4, Eya protein is already highly expressed (see Fig. 2). Nevertheless, ectopic eye formation by eyeless was dramatically enhanced when additional eya gene activity was provided (Table 2, Fig. 5). The ectopic eyes were larger and formed with higher penetrance than with eyeless or eya alone, and eye formation now occurred on the genitalia, a condition never previously observed in individuals with either gene alone (Table 2 and Fig 5B,E). This effect did not appear additive (Table 2). Rather, these data suggest functional synergy between eyeless and eya gene activities in eye formation.

Table 2. Functional synergy between eya and eyeless in ectopic eye formation

<table>
<thead>
<tr>
<th>Structures with ectopic eye formation</th>
<th>UAS-eya</th>
<th>UAS-eyeless</th>
<th>UAS-eyeless + eya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antennae</td>
<td>10%</td>
<td>95%</td>
<td>100%</td>
</tr>
<tr>
<td>Proboscis</td>
<td>0%</td>
<td>13%</td>
<td>91%</td>
</tr>
<tr>
<td>Legs</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Wing</td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Haltere</td>
<td>0%</td>
<td>33%</td>
<td>93%</td>
</tr>
<tr>
<td>Genitalia</td>
<td>0%</td>
<td>0%</td>
<td>55%</td>
</tr>
</tbody>
</table>

110 animals scored for antennal data point, 50-55 animals scored for other data points. Ectopic eye development is scored by the presence of ommatidia with pigment. Expression of the UAS constructs driven with dpp-GAL4.

DISCUSSION

Our data reveal an active role of the eya gene in eye formation, and suggest a model of gene regulatory interactions between eyeless and eya in eye formation in the fly that may extend to their mammalian counterparts.

Conservation of eya function between vertebrates and flies

We found that a vertebrate homolog of eya, the mouse Eya2 gene, can functionally replace the fly gene in eye formation. These data suggest that the role of the eya gene in eye formation has been conserved through evolution, between flies and vertebrates, despite dramatic differences in eye structure between the two (see Zuker, 1994). Such functional homology has been shown for various Pax-6 homologs of the eyeless gene (Halder et al., 1995a; Glardon et al., 1997): we have extended those studies to eya and its homologs, a second gene of the eye developmental pathway. The vertebrate Eya homologs identified to date are all expressed in the developing or adult eye, suggesting all homologs may function in aspects of vertebrate eye formation and maintenance (Duncan et al., 1997; Xu et al., 1997; Zimmerman et al., 1997). For the Eya2 homolog, we demonstrate here a homologous role in the eye developmental pathway.

Relation of eya gene activity to eyeless

We have addressed and clarified the relationship of eya gene
activity to that of the *eyeless* gene. Previous data indicate that, normally, *eyeless* expression precedes that of *eya* in eye progenitor cells. Whereas *eyeless* is expressed in the eye primordium from embryonic stages (Quiring et al., 1994), *eya* expression is initiated during the mid-larval stages (Bonini et al., 1993). We found that *eya* gene activity was essential for eye formation by *eyeless*; these data, along with the observation that *eyeless* remains expressed in fly *eya* mutants, suggest that *eya* is downstream of *eyeless* as data indicate that expression of *eyeless* (Quiring et al., 1994) occurs prior to expression of *eya* (Bonini et al., 1993) in normal eye development. However, a loop between *eya* and *eyeless* is proposed because results suggest that not only is *eya* activity essential for *eyeless* function, but also *eyeless* function is essential for *eya* function. Since *eya* and *eyeless* together are more effective in eye formation than either gene alone, *eya* and *eyeless* may function in at least partially distinct pathways (curved arrow to the left and the pathway through *eya*), both of which are critical for eye formation. We propose these same gene interactions may exist for the mammalian counterparts, given the conservation of function of *eyeless* with mouse *Sey* shown previously (Halder et al., 1995a), and functional conservation between mammalian Eya2 and fly *eya* shown here.

**eya as a master control gene for eye formation**

We found that *eya* shares with *eyeless* the capacity to function as a ‘master control gene’ for eye formation. By this term, we refer to the fact that *eya* has the capacity to direct the appropriate genetic program of the many genes required for eye development (Halder et al., 1995a). Using loss-of-function *eyeless* mutants, we found evidence that *eyeless* activity is
required foreya to form eyes – similar to the requirement for
eya gene activity in the proper eyless function in eye
formation. Thus, the activities of the eya and eyless genes
appear connected by a regulatory loop, with each functionally
required by the other in eye formation (Fig. 6). One qualifica-
tion of these conclusions is that in leg discs, we were unable
to detect eyless expression upon ectopic eya activity. Never-
theless, genetic studies in the eyless mutant background
indicated that eye formation by eya in legs appeared dependent
on eyless gene activity. Thus, we suggest that eyless function
is required for ectopic eye formation by the eya gene. In Fig.
6, we place eya downstream of, but connected back to, eyless
gene function. Genetically, there is little to argue which gene
is first; however, the normal expression patterns of the genes
indicate that eyless expression temporally precedes that of eya
during normal eye formation (Bonini et al., 1993; Quiring et
al., 1994). In ectopic eye formation, the genes are each
essential for the others’ function, thus are interchangeable
in placement.

Moreover, we found that eya and eyless displayed func-
tional synergy in eye formation – the same dosage of eyless
was potentiated when combined with additional eya gene
function. This synergy was observed with a dpp enhancer
construct, and whether other regulatory elements will mediate
a similar level of synergy remains to be determined. However,
that eyless has functions in addition to activating eya activity
is also suggested by the severely affected leg morphology
observed upon eyless expression in the eya mutant back-
ground (see above). These data suggest that eyless and eya
may function in at least partially distinct pathways for eye
formation (Fig. 6). By such a model, expression of either gene
alone, if expressed strongly enough, will eventually drive both
pathways because they form part of a regulatory loop.
However, when both genes are expressed strongly, both
pathways will be strongly driven, leading to an enhancement
of eye formation compared to that with either gene alone.

Taken together, these data suggest that early events of eye
formation proceed not by a simple linear pathway, but rather
by a combinatorial code of gene function. Thus, there may be
no single ‘master control gene’ for eye formation, but a
complex regulatory network of gene activities required to
trigger the biological event of eye development. These initial
events of eye formation include additional genes, such as dac
and sine oculis. The position of dac in this regulatory pathway
will be of interest, as dac has also been shown able to direct
eye formation (Shen and Mardon, 1997). Given the increase in
eyless expression upon dac-directed eye formation (Shen
and Mardon, 1997), dac likely also requires eyless gene activity
to form eyes. This suggests that, minimally, dac and eya are
connected through common regulation of, and regulation by,
eyless gene activity.

The relationship between eya, eyless and other genes
central to eye formation conserved in both flies and vertebrates,
such as sine oculis/Six-3 (Cheyette et al., 1994; Serikaku and
O’Tousa, 1994; Oliver et al., 1995), are also of great interest.
Moreover, how these early events of eye determination subse-
quently merge with pattern formation events of furrow
movement (Heberlein and Moses, 1994) and cell cycle regula-
tion (Thomas and Zipursky, 1994), are key aspects of gener-
ating a patterned neural structure like an eye. Somehow, these
different aspects of the eye developmental process must be

triggered by the eye differentiation pathway and coordinately
regulated, to achieve this exquisitely organized neural center.

The role of the eya gene

The eya gene has additional roles in development. In flies
mutations in eya can be embryonic lethal (Nüsslein-Volhard et
al., 1984; Bonini et al., 1993; Leiserson et al., 1994), or result
in defects in gonad formation (Boyle et al., 1997), whereas
humans mutant in EYA1 show developmental defects of the
branchial arches, ear and kidney (Abdelhak et al., 1997). Thus,
the eya gene has roles in development of the animal in addition
to a function in eye formation. It is thus of interest to determine
whether expression of eya has consequences over and above
ectopic eye formation. Expression in other tissues or at other
times in development may lead to elucidation of additional
roles of the gene, as well as insight into the specificity of
expression for eye formation. Toward this end, it is rather sur-
prising that genes like eyless, eya and dac, with roles in the
animal in addition to eye formation, should induce eye develop-
ment when ectopically expressed. What leads to this speci-
cicity is of particular interest. With respect to the role of eya in
eye formation, loss-of-function eya mutants show death of eye
progenitor cells (Bonini et al., 1993).

Taken together, these data indicate that, although the
function of eya in eye differentiation is coupled to both differ-
entiation and survival, the most dramatic effects of the gene
upon strong expression are in the differentiation pathway.
Thus, we anticipate that the relationship of eya activity with
cell death will be indirect, through an effect on the pathway of
differentiation. What gene activities might be altered in eya
mutants, such that the cells become directed down a death
pathway, remain to be defined.

With respect to the eye developmental pathway, the biologi-
cal activities of eyless and eya in eye formation extend to their
mammalian counterparts Pax-6 and Eya. The mouse Sey gene
has been shown to function in the fly (Halder et al., 1995a) and
we have shown that a mouse homolog of eya has the ability to
functionally replace the endogenous fly gene in eye develop-
ment. These data indicate a remarkable level of conservation
of gene function in eye formation between flies and mammals.
These data lend support to the idea (Quiring et al., 1994; Halder
et al., 1995a,b; see Zuker, 1994) that common genetic
pathways may be used for the formation of eyes of widely
divergent structure in organisms as evolutionarily distant as
flies and man.

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