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***ato-Gal4* fly lines for gene function analysis: Eya is required in late progenitors for eye morphogenesis**

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Abstract

The Gal4/UAS system is one of the most powerful tools for the study of cellular and developmental processes in *Drosophila*. Gal4 drivers can be used to induce targeted expression of dominant-negative and dominant-active proteins, histological markers, activity sensors, gene-specific dsRNAs, modulators of cell survival or proliferation, and other reagents. We describe here novel *atonal-Gal4* lines that contain regions of the regulatory DNA of *atonal*, the proneural gene for photoreceptors, stretch receptors, auditory organ and some olfactory sensilla. During neurogenesis, the *atonal* gene is expressed at a critical juncture, a time of transition from progenitor cell to developing neuron. Thus, these lines are particularly well suited for the study of the transcription factors and signaling molecules orchestrating this critical transition. To demonstrate their usefulness, we focus on two visual organs, the eye and the Bolwig. We demonstrate the induction of predicted eye phenotypes when expressing the dominant-negative EGF receptor, EGFR^{DN}, or a dsRNA against Notch, Notch^{RNAi}, in the developing eye disc. In another example, we show the deletion of the Bolwig's organ using the proapoptotic factor Hid. Lastly, we investigate the function of the eye specification factor Eyes absent or Eya in late retinal progenitors, shortly before they begin morphogenesis. We show that Eya is still required in these late progenitors to promote eye formation, and show failure to induce the target gene *atonal* and consequent lack of neuron formation.

Keywords

sine oculis; ocelli; Bolwig organ; Johnston organ; chordotonal; sensilla coeloconica

Introduction

Morphogenesis of the *Drosophila* eye begins early in the third and last larval stage (L3) and continues during pupal development. Throughout this process, eye progenitor cells acquire

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specific fates as they come together to form single eye units or ommatidia. The first cell to emerge is the founder R8 neuron, followed by 7 more photoreceptors, 4 lens-secreting cone cells, and lastly multiple pigment and bristle cells. The early stages of this process can be visualized in the L3 eye disc epithelium, when a wave of morphogenesis sweeps across the epithelium from posterior to anterior leaving in its wake clusters of developing neurons (Roignant and Treisman, 2009; Hsiung and Moses, 2002).

The front of this wave (visible as an indentation in the epithelium and called morphogenetic furrow or MF) represents an area of intense signaling and transcriptional reprogramming. Here retinal progenitor cells respond to multiple signals by first transitioning into a proneural state and then selecting the founder R8 neuron of the ommatidium (reviewed in Roignant and Treisman, 2009). Additional rounds of signaling follow, leading to the emergence of additional photoreceptor neurons and eventually the accessory cells of each single eye.

The proneural gene *atonal* (*ato*) is a major player in this process. Its onset marks the transition from proneural progenitors to proneural cells, and its final expression identifies the developing R8 neurons (Sun et al., 1998; Greenwood and Struhl, 1999; Bessa et al., 2002). The *ato* gene is first expressed in a stripe of retinal progenitor cells at the anterior boundary of the MF where it confers neural competence (phase-1 expression; Ato-independent; Fig. 1A). Thereafter, its expression becomes restricted to progressively smaller groups of cells until only one cell per future ommatidial cluster, R8, shows *ato* expression (phase-2 expression; Ato-dependent; Fig. 1A). As neurogenesis sweeps across the L3 disc, this dynamic pattern of *ato* expression is repeated such that the expression domain appears to migrate across the discs from its posterior margin to the anterior boundary of the eye field (Jarman et al., 1994).

Dissection of this process requires a level of temporal control of gene/protein perturbation that is not provided by analysis in mutant organisms or in clones of mutant tissue (these reflect states that are either permanent or induced too early in L1 or L2). In absence of the rare temperature-sensitive allele, targeted-expression of a dsRNA or dominant negative/active protein offers the best method to study these events, but unfortunately, suitable Gal4 drivers have been lacking. The recent dissection of the control of *ato* expression offered the opportunity to generate new Gal4 lines ideal for this purpose (Zhang et al., 2006; Tanaka-Matakatsu et al., 2008; Zhou et al., 2014). The drivers described here are expressed in *ato*-related patterns in the eye and other *ato*-dependent sensory organs, including the ocelli, the Bolwig organ (larval eye), the auditory Johnston's organ, the chordotonal stretch receptors and the olfactory sensilla coeloconica (Jarman et al., 1993; Jarman et al., 1994; Jarman et al., 1995; Gupta and Rodrigues, 1997). Hence, these reagents are ideal to investigate the transition from progenitor to developing neuron in these systems. We report here on their expression in the eye and demonstrate their use in eye and Bolwig.

Results and Discussion

Expression pattern of novel *atonal-Gal4* drivers in the *Drosophila* eye

We describe below the pattern of Gal4-driven *UAS-GFP* expression at the mRNA level (*in situ* hybridization) and at the protein level (anti-GFP Ab). Differences in GFP expression at the mRNA and protein levels are expected and reflect the efficiency of transcription/translation of the *UAS-GFP* transgene used and the stability of its mRNA and protein products. We used endogenous Senseless, which is transcriptionally induced by Ato in the R8 cells (Nolo et al., 2000), to identify expressing cells behind the MF and to pinpoint the timing of GFP protein detection behind the MF. We used the expression pattern of endogenous Eyes absent (Eya), a pan-retinal marker (progenitors through developing retinal cells), to assess expression relative to the MF and the eye progenitor field (Bonini et al., 1993).

ato^{5'EYE}-Gal4 and *ato^{5'FL}-Gal4* contain the 5' full length or the distal 5' eye genomic region, respectively (Fig. 1A–B). The 5' eye region directs phase-2 expression of endogenous *ato* in the proneural clusters and single R8 neurons emerging posterior to the MF (Fig. 1A). This phase-2 expression is autoregulatory and depends on the previous Ato expression, induced by the 3' regulatory elements (Sun et al., 1998; Zhang et al., 2006). In *ato^{5'EYE}-Gal4 UAS-GFP* discs, *GFP* mRNA is seen in late proneural clusters along the posterior border of the MF, and in smaller groups of cells and then single cells posterior to it (Fig. 1C). At the protein level, strong GFP expression is delayed compared to the mRNA (Fig. 1C, and D–D'). The protein is clearly present in the second row of Sens-positive R8 neurons (Fig. 1E–E''). One or two weakly positive cells occasionally flank the R8 neuron (arrowheads in Fig. 1E–E') and may reflect weaker expression at an earlier stage. Once present, the GFP protein, but not the mRNA, persists throughout the posterior region of the eye disc due to GFP stability (Fig. 1D'). GFP expression driven by the *ato^{5'FL}-Gal4* line is essentially identical to *ato^{5'EYE}-Gal4* (not shown).

ato^{3'FL}-Gal4 contains the full-length 3' regulatory region responsible for phase-1, Ato-independent expression (Fig. 1A–B) (Sun et al., 1998). This construct drives the *UAS-GFP* mRNA in a dorso-ventral stripe that appears to migrate from posterior to anterior across the eye disc epithelium (Fig. 1F). As expected, GFP protein detection is delayed compared to expression of the GFP mRNA, starting from the posterior edge of the MF (visible in the staining for the eye factor Eyes absent, Eya) (Fig. 1G–G''). Once present, the GFP protein (but not the mRNA) persists throughout the posterior region of the eye disc reflecting GFP stability (Fig. 1G').

ato³⁴⁸-Gal4 contains the 348 bp core region (3'ENH^{II} enhancer; Zhang et al., 2006) that drives expression of *ato* in the phase-1 pattern (Fig. 1A–B), but only in anterior progenitors and starting some distance away from the posterior margin (Zhou et al., 2014). Like the related *ato^{3'FL}-Gal4* line, the *ato³⁴⁸-Gal4* induces *UAS-GFP* mRNA expression in a dorso-ventral stripe but at a lower level (Fig. 1H). This driver differs from endogenous *ato* in that expression begins precociously. As shown in Fig. 1I–I'', GFP is clearly detectable anterior to the MF, in the proneural region where retinal progenitors have not yet activated the *ato* gene (nor *ato^{3'FL}-Gal4*) (Greenwood and Struhl, 1999; Bessa et al., 2002). The reporter

protein then persists in cells posterior to the MF past the point where transcripts can be detected, and falls below detection level somewhat variably from cell-to-cell (Fig. 11'). This driver can selectively query the progression phase of morphogenesis without affecting the ommatidia laid out early along the posterior margin, and it can do so anterior to the endogenous *ato* domain within the G1-arrested, proneuronal progenitor cells.

In the experiments below, we also use two previously described *ato*-related drivers: 1) *ato^{5'EYE3'}-Gal4*, which contains both *ato^{5'EYE}* and *ato^{3'FL}* regulatory regions (Fig. 1B) and displays the full pattern of *ato* expression in the eye (phase-1 and phase-2; Fig. 1A) (Yu et al., 2012); and 2) *ato-Gal4* (Fig. 1A–B), originally used for its expression at the embryonic stage and in the larval brain (Hassan et al., 2000). It is important to note that *ato-Gal4* does not express in imaginal discs (Fig. 1J–K).

Induction of *EGFR* and *Notch* mutant phenotypes using *atonal-Gal4* lines

To assess the usefulness of these drivers, we first induced well-known loss-function-phenotypes using UAS transgenes that encode the dominant negative Epidermal Growth Factor Receptor protein (*EGFR^{DN}*) or an RNAi reagent against the Notch receptor (*Notch^{RNAi}*). *EGFR* and *Notch* are pleiotropic genes with many functions in the eye and other tissues, and have been extensively studied using loss- and gain-of-function approaches. Since null alleles of these loci are embryonic lethal, later functions have been analyzed using temperature sensitive alleles (Cagan and Ready, 1989; Freeman 1996; Kumar et al., 1998).

During eye neurogenesis, the EGF receptor is required for the initial recruitment of the R1–R7 photoreceptors and for the differentiation (not the establishment) of the R8 neuron; thus, inactivation of *EGFR* function in the early stages of eye morphogenesis results in absence of R1–R7 neurons and loss of differentiated characteristics in R8 (Freeman 1996; Kumar et al., 1998). Consistent with these roles, we detected loss or delay of photoreceptor neurons recruitment (using the pan-neuronal marker *Elav*) but normal presence of the R8 neuron (as per the R8-specific marker *Boss*), when *EGFR^{DN}* was expressed in the phase-1 (*ato^{3'FL}-Gal4*) or phase-2 (*ato^{5'EYE}-Gal4*) patterns of *ato* expression (Fig. 2A–A'). Interestingly, the driver that combines the two regulatory regions, *ato^{5'EYE3'}-Gal4* induced a much stronger phenotype; *ato^{5'EYE3'}-Gal4 UAS-EGFR^{DN}* discs displayed only a few *Elav*-positive *Boss*-negative neurons near the posterior margin of the disc (Fig. 2A', right most panel). This phenotype is expected for a more severe loss of *EGFR* signaling, and it reflects a severe failure of R1–R7 recruitment and a loss of R8 differentiation characteristics (e.g. loss of *Boss* expression; Kumar et al., 1998). The paucity of neurons is also expected, because the induction of only R8 cells would be insufficient to sustain eye morphogenesis, a process dependent on the production of the secreted factor Hedgehog by the array of differentiating cells posterior to the MF (Heberlein et al., 1993; Ma et al., 1993; Roignant and Treisman, 2009).

On the contrary, the Notch signaling pathway plays an opposite role in eye neurogenesis; it prevents the recruitment of supernumerary neurons through a mechanism called lateral inhibition. Hence, inactivation of *Notch* at the time of photoreceptor recruitment results in the addition of extra neurons to the clusters, sometime generating a lawn of neurons (Cagan and Ready, 1989). Consistent with this role, expression of a *Notch* dsRNA (*UAS-*

Notch^{RNAi} under control of *ato^{5EYE3}-Gal4* led to the formation of a lawn of neurons (Elav-positive) throughout the developing eye field (Fig. 2B). This loss of lateral inhibition was also observed in *ato³⁴⁸-Gal4 UAS-Notch^{RNAi}* discs (Fig. 2B'–B''), even though this driver's expression begins in more anterior progenitors (at a time when Notch function is required in a proneural role and its loss prevents neurogenesis). The likely cause for this effect is the presence of residual Notch protein due to the lower expression from the *ato³⁴⁸-Gal4* driver, and the lower requirement for Notch function at the proneural stage than during lateral inhibition (Baker and Yu, 1997).

Thus, all four Gal4 lines can be used to induce loss of function in and around the MF as predicted from their pattern of expression in the developing L3 eye disc.

***atonal-Gal4* drivers can be used to delete sensory organs**

To explore the use of these lines in other *ato*-dependent sensory organ, we chose the Bolwig's Organ (BO), the visual organ of the *Drosophila* larva. In the BO, as in the eye, *ato* gene expression is first detected in a broader domain (phase-1, Ato-independent) and then becomes restricted to 3 or 4 cells, the founder neurons (phase-2, Ato-dependent). Additional neurons are then recruited by the founders through an EGFR-dependent mechanism (Suzuki and Saigo, 2000; Daniel et al., 1999; Sprecher et al. 2007).

To generate larvae that completely lacked the larval eye, we decided to drive expression of the proapoptotic protein Hid in the founder neurons of the Bolwig. Whereas control of *ato* in the developing eye disc has been extensively dissected (Sun et al., 1998; Tianyi et al., 2006; Tanaka-Matakatsu et al., 2008; Distefano et al., 2012; Tanaka-Matakatsu et al., 2014; Zhou et al. 2014), regulation in other organs has not been equally explored. However, the control elements driving phase-1 expression in the BO (though still unidentified) most likely map to genomic DNA on the 5'-side of *ato* (Fig. 1A), possibly within the region present in *ato-Gal4* (Fig. 1B) (as suggested by Sprecher et al., 2007). We therefore choose to drive expression of a *UAS-hid* transgene using two drivers, *ato-Gal4* (Hassan et al., 2000) and *ato^{5FL}-Gal4*. We predicted that, as both lines should express in founder neurons, both would induce a complete loss of the larval eye. Surprisingly, *ato^{5FL}-Gal4 UAS-hid* larvae, but not *ato-Gal4 UAS-hid*, completely lacked the BO (Fig. 2C). This finding strongly suggests that, although the regulation of phase-1 expression is located 5' to the *ato* transcription unit, it reside at least in part outside the 'embryo' region included in the *ato-Gal4* construct (Fig. 1A–B).

Thus, as shown here for the BO, these *ato*-related Gal4 drivers can be used to explore the development or function of, or the regulation of *ato* in, other Ato-dependent sensory organs.

Eya function is required in late retinal progenitors for eye morphogenesis

In addition to serving as marker for all retinal cells (including progenitors in L2 and L3, and MF and post-MF developing cells in the L3 disc), the transcriptional co-activator Eya is a critical component of the Retinal Determination (RD) network, a cascade of transcription factors that together confer eye fate and thus specify the retinal primordium in the L2 eye disc (Treisman, 2013). Indeed, as the first eye-primordium factor to be deployed, Eya is required during L2 to initiate expression of the other RD genes, beginning with its own partner *sine oculis* (*so*) (Bonini et al., 1993; Pignoni et al., 1997; Kenyon et al, 2003). As

apparent in animals homozygous for the eye-specific mutant allele *eya*² or with *ey-Gal UAS-eya^{RNAi}* genotype, the Eya-null condition triggers the complete loss of eye in the adult (Fig. 3A, *ey-Gal4*; Bonini et al., 1993). This outcome is presaged by a failure to initiate neurogenesis (no Ato) and the consequent absence of photoreceptor neurons (no Elav) in the L3 eye disc (Fig. 3C–D, *ey-Gal4*). Importantly, these effects are the consequence of an early failure of retina primordium formation at the L2 stage. However, Eya continues to be broadly expressed in the L3 eye disc, in both retinal progenitors and the developing eye field (Bonini et al., 1993). Posterior to the MF, Eya appears to be dispensable for photoreceptor specification and patterning, but is required for R-cell axon targeting and formation of lens and pigment cells (Fig. 3C–E, *GMR-Gal4*; Karandikar et al., 2014; Xiong et al., 2009). Demonstrating a requirement for Eya after the establishment of the eye primordium but before eye morphogenesis is considerably more difficult, as it requires knockdown of *eya* in progenitor cells specifically in L3 (i.e. anterior to the MF but well after onset of RD factors expression in L2). A slow down of the MF without loss of photoreceptor neurons was observed when Eya function was partially reduced in L3 progenitors (with a synthetic mutant allele; Karandikar et al., 2014), suggesting a role for Eya in the progression of the MF across the disc. In addition, a likely target of Eya at the L3 stage is *ato* itself. Eya, in complex with So, is thought to bind an So binding site located in an eye enhancer for phase-1 expression (Zhang et al., 2006; Zhou et al., 2014). However, whether this actually reflects activity of Eya in the proneural domain remains to be shown. Thus, although Eya plays a role in the morphogenetic process, we do not know whether Eya function is still essential in late progenitors for photoreceptor neuron formation.

To address this question, we used *ato*³⁴⁸-*Gal4* to drive *UAS-eya^{RNAi}* expression after initiation of eye morphogenesis. As described above, *ato*³⁴⁸-*Gal4* drives expression in a modified *ato* pattern due to the absence of both positive and negative regulatory elements (Zhang et al., 2006). Specifically, expression starts some distance away from the posterior margin (leaving this part of the disc and the earlier L2 stage unperturbed) and it is induced in cells anterior to the normal *ato* domain (in late stage progenitor cells just ahead of the MF) (Fig. 1I–I''). This allowed us to probe a requirement for Eya in these preproneural cells (Greenwood and Struhl, 1999; Bessa et al., 2002). Interestingly, neurogenesis was severely impaired in *ato*³⁴⁸-*Gal4 UAS-eya^{RNAi}* discs, showing a striking absence of photoreceptor neurons particularly on the ventral half of the disc (Fig. 3A, *ato*³⁴⁸-*Gal4*), where this *Gal4* line drives stronger expression (Fig. 1I–I''). Since neurons formed normally when Eya was removed behind the MF (as in *GMR-Gal4 UAS-eya^{RNAi}* discs; Fig. 3C, *GMR-Gal4*), the lack of developing photoreceptors in *ato*³⁴⁸-*Gal4 UAS-eya^{RNAi}* uncovered a requirement for Eya function shortly before and/or in the MF.

Since induction of *ato* expression is a critical step at this stage of eye development and the Eya transcriptional co-activator is thought to directly regulate this gene target, in a complex with the homeodomain transcription factor Sine oculis (Zhang et al., 2006; Zhou et al., 2014), we probed *ato*³⁴⁸-*Gal4 UAS-eya^{RNAi}* discs for expression of its direct target *ato*. Consistent with the observed lack of neurons (Elav staining), *ato*³⁴⁸-*Gal4 UAS-eya^{RNAi}* discs showed a clear loss of Ato expression where Eya was most severely reduced (Fig. 3D, *ato*³⁴⁸-*Gal4*). As expected, knock-down of Eya from a slightly later stage, in *GMR-Gal4 UAS-eya^{RNAi}* discs, did not have any effect on Ato (Fig. 3D, *GMR-Gal4*). Therefore, the

failure of neurogenesis observed in *ato*³⁴⁸-*Gal4* *UAS-eya*^{RNAi} discs is likely due, at least in part, to a failure of *ato* induction.

In conclusion, Eya is still required in retinal progenitor cells at the proneural stage promoting their ability to undergo neurogenesis.

Conclusion

In conclusion, we have constructed several Gal4 lines that express in the eye disc in *ato*-related patterns and are particularly well suited for analysis of neurogenesis in the eye disc. As for any Gal4 driver, the impact of the proteins or RNAs expressed will depend on their rate of synthesis, minimum concentration required for activity, and perdurance. Moreover, since these lines make use of fragments across the entire 5' and 3' regulatory DNA of *ato*, each construct depicted in Fig. 1B will drive expression in a subset of Ato-dependent sensory organs (eyes, ocelli, Bolwig, Johnston's organ, chordotons and sensilla coeloconica) permitting analysis of the onset of neurogenesis in all these systems.

Methods

Cloning and transgenesis

Transgenic lines *ato*^{5'FL}-*Gal4*, *ato*^{3'FL}-*Gal4*, *ato*³⁴⁸-*Gal4* and *ato*^{5'EYE}-*Gal4* were generated in our lab by site-directed (*ato*^{5'FL}-*Gal4*) or P-element transformation (all others) (Spradling and Rubin, 1982; Bischof et al., 2007). In all but one construct (*ato*³⁴⁸-*Gal4*; Brás-Pereira et al., 2011), the 1.1 kb *ato* genomic fragment containing 1 kb of 5'-flanking DNA and 74 bp of 5' UTR precedes an *hsp70* basal promoter just ahead of the Gal4 ORF. All transgenic lines are homozygous viable; cloning information for all constructs and related transgenic stocks are available upon request.

Genetics

Flies were raised on standard fly food at 25°C. Other stocks used: *ato*^{5'EYE3'}-*Gal4* (Yu et al., 2012); *ato*-*Gal4* (Hassan et al., 2000); *UAS-GFP.nls* (BDSC.4776); *UAS-EGFR^{DN}* (BDSC.5364); *UAS-Notch^{RNAi}* (TRiP.JF01053); *UAS-eya^{RNAi}* (TRiP.JF03160); *UAS-dicer2* (VDRC.60008); *UAS-hid* (Zhou et al., 1997).

Histology

Ab used: rat anti-Elav (1:100; DSHB); mouse anti-Eya (1:200; DSHB); guinea pig anti-Sens (1:1000) (Nolo et al., 2000); sheep anti-Ato (1:1000) (Holohan et al., 2006); mouse anti-Boss (1:1000) (Krämer et al. 1991); rabbit anti-GFP (1:1000; Upstate Biotech); mouse anti-Chaoptic or 24B10 (1:200; DSHB). Anti-mouse, anti-rat, anti-rabbit, anti-guinea pig and anti-sheep Cy2-, Cy3- or Cy5-conjugated (Jackson Immuno Research Laboratories) secondary Ab were used at 1:200 dilution. Confocal microscopy was performed using a Leica DM5500 Q. *In situ* probes were generated using the DIG RNA Labeling Kit (Roche).

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Figure 1. Expression patterns of the new 'atonal-Gal4' lines

All L3 eye discs (C–J) are shown with posterior to the left and dorsal up; (C–I'') solid triangles along bottom mark the position of the MF. (A) Diagram of the *atonal* gene including the transcription unit (small arrow) and its 5' and 3' regulatory regions (adapted from Sun et al., 1998). A schematic drawing of the *ato* expression pattern in and around the MF of the eye disc is shown below the gene map; color-code relates the Ato-independent phase-1 domain and the Ato-dependent phase-2 domain of expression to the relevant regulatory fragment. (B) Structure of all constructs. All fragments originate from *ato* genomic DNA as shown by color; *hsp70* basal promoter is in black. *Eye expression is described in Yu et al. (2012); **Embryonic and larval brain expression is described in Hassan et al. (2000). (C–E'') *ato*^{5EYE}-Gal4 UAS-GFP L3 eye discs: expression of GFP mRNA (C) and protein (D–E''). GFP is shown in green and Eya (D–D'') or Sens (E–E'') in red. Panels E–E'' show the central region of the disc in D–D'' at higher magnification; arrowheads point to weaker expression in one or two cells next to R8. (F–G'') *ato*^{3FL}-Gal4 UAS-GFP L3 eye discs: expression of GFP mRNA (F) and protein (G–G''); green=GFP; red=Eya. (H–I'') *ato*³⁴⁸-Gal4 UAS-GFP L3 eye discs: expression of GFP mRNA (H) and protein (I–I''); green=GFP; red=Eya. White dotted-line in I' marks the posterior margin of the disc. (J–K) *ato*-Gal4 line (from Hassan et al., 2000) driving expression of UAS-lacZ: β-

Galactosidase is not expressed in eye, antennal or leg L3 discs (J–K), but it is present in the larval brain (K).

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Figure 2. Use of 'atonal-Gal4' drivers in the developing eye and the Bolwig's organ

In the panels (A–C), genotypes are as marked above and to the left; L3 eye discs and diagrams (A–B) or larval heads (C) are oriented posterior to the left and dorsal up; and diagrams (A–B) show patterns of Gal4 expression in the eye disc. (A–A') Effect of EGFR^{DN} expression on eye neurogenesis. Solid triangles at bottom mark position of MF. Row of panels (A') shows eye discs stained for the pan-neural marker Elav (green) and the R8 marker Boss (red). (B–B'') Effect of *Notch^{RNAi}* expression on eye neurogenesis. Eye disc stained for the pan-neural marker Elav (green). Notice that development of the neuronal clusters is normal in the most posterior region of the disc (B''), where *ato³⁴⁸-Gal4* is not expressed. For WT controls, see Elav staining in A', WT and Fig. 3C, WT. (C) Effect of the proapoptotic protein Hid on the BO. Panels show anterior region of the L3 larva. The BO is visualized by staining for the photoreceptor-specific marker Chaoptic (24B10 Ab). The *ato-Gal4* line is from Hassan et al. (2000).

Figure 3. Eya function is still required in late progenitors for eye morphogenesis

For each A–E column, genotypes are as marked above; adult heads (A), Gal4 expression diagrams (B) or eye discs (C–E) are oriented posterior to the left and dorsal up; solid triangles along bottom (C–E) mark the position of the MF. (A–E) Effect of preventing onset of Eya expression in all cells of the eye field from the L2 stage (*ey-Gal4 UAS-eya^{RNAi}*) or starting from some neuronal cluster away from the MF (*GMR-Gal4 UAS-eya^{RNAi}*). In *ato³⁴⁸-Gal4 UAS-eya^{RNAi}* disc, Eya expression is downregulated starting in the proneural domain through several rows of differentiating neuronal clusters, but not in the posterior of the disc where this Gal4 line is not expressed. Effect of *UAS-eya^{RNAi}* expression is shown at the adult (A) and L3 (C–E) stages. Eye discs are stained for Eya (red) and one other factor (green); Elav (C) or Ato (D). All top-right insets show single channel of Eya expression (grayscale). Bottom-right inset in D, *ato³⁴⁸-Gal4* shows Ato expression (grayscale). Arrow points to same region of severe loss of expression in the larger panel and inset.