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Distinct regulation of *atonal* in a visual organ of *Drosophila*: Organ-specific enhancer and lack of autoregulation in the larval eye

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ABSTRACT

Drosophila has three types of visual organs, the larval eyes or Bolwig's organs (BO), the ocelli (OC) and the compound eyes (CE). In all, the bHLH protein Atonal (Ato) functions as the proneural factor for photoreceptors and effects the transition from progenitor cells to differentiating neurons. In this work, we investigate the regulation of *ato* expression in the BO primordium (BOP). Surprisingly, we find that *ato* transcription in the BOP is entirely independent of the shared regulatory DNA for the developing CE and OC. The core enhancer for BOP expression, ato^{BO}, lies ~6 kb upstream of the *ato* gene, in contrast to the downstream location of CE and OC regulatory elements. Moreover, maintenance of *ato* expression in the neuronal precursors through autoregulation—a common and ancient feature of *ato* expression that is well-documented in eyes, ocelli and chordotonal organs—does not occur in the BO. We also show that the ato^{BO} enhancer contains two binding sites for the transcription factor Sine oculis (So), a core component of the progenitor specification network in all three visual organs. These binding sites function *in vivo* and are specifically bound by So *in vitro*. Taken together, our findings reveal that the control of *ato* transcription in the evolutionarily derived BO has diverged considerably from *ato* regulation in the more ancestral compound eyes and ocelli, to the extent of acquiring what appears to be a distinct and evolutionarily novel *cis*-regulatory module.

1. Introduction

Higher Diptera, like *Drosophila*, utilize three sense organs to navigate the visual environment: the compound eyes (CE) and ocelli (OC) of the adult and the Bolwig's organs (BO) of the larva (also known as larval eyes; Fig. 1A-A"). While structurally very diverse, comparative evidence leads to the conclusion that the CE, OC, and BO descended from a single precursor visual organ present in an ancient invertebrate lineage that predated the diversification of arthropods (Friedrich, 2006; Friedrich et al., 2013). Their shared evolutionary history is reflected in the common use of many signaling molecules and transcription factors during development, from the time of primordium specification through to the terminal differentiation stage (Friedrich, 2006). One such common factor is the master regulator of neurogenesis Atonal (Ato), a bHLH protein that functions as the proneural factor for photoreceptor neurons (Jarman et al., 1994, 1995).

The acquisition of neurocompetence and the selection of neuronal precursors are key steps of neurogenesis that are controlled by Ato and are directly reflected in its pattern of expression. In all three visual organs, Ato is first detected in a broad domain that defines a field of neurocompetent progenitors; soon after, it becomes restricted to a set of 'founder' or 'primary' neuronal precursors (Fig. 1B). As shown most clearly for the CE and the BO, these founder neurons in turn promote recruitment of secondary photoreceptors, in an Ato-independent but EgfR-dependent manner (Freeman, 1994; Tio et al., 1994; Daniel et al., 1999; Yang and Baker, 2001). The dynamic pattern of Ato expression plays a central role in this process. As this pattern is controlled at the level of transcription, uncovering the *cis*-regulatory logic of *ato* gene expression is essential for understanding the progenitor-to-neuron transition characteristic to each of the visual organs.

The *cis*-regulation of *ato* is best understood for the developing CE, where *ato*'s dynamic expression reflects two mechanistically distinct phases of transcription. The first phase is dependent on upstream factors (initiation) and the second phase on positive feedback regulation by Ato (maintenance) (Fig. 1B) (Jarman et al., 1995; Baker et al., 1996; Sun et al., 1998). In the CE, the initiation phase is controlled

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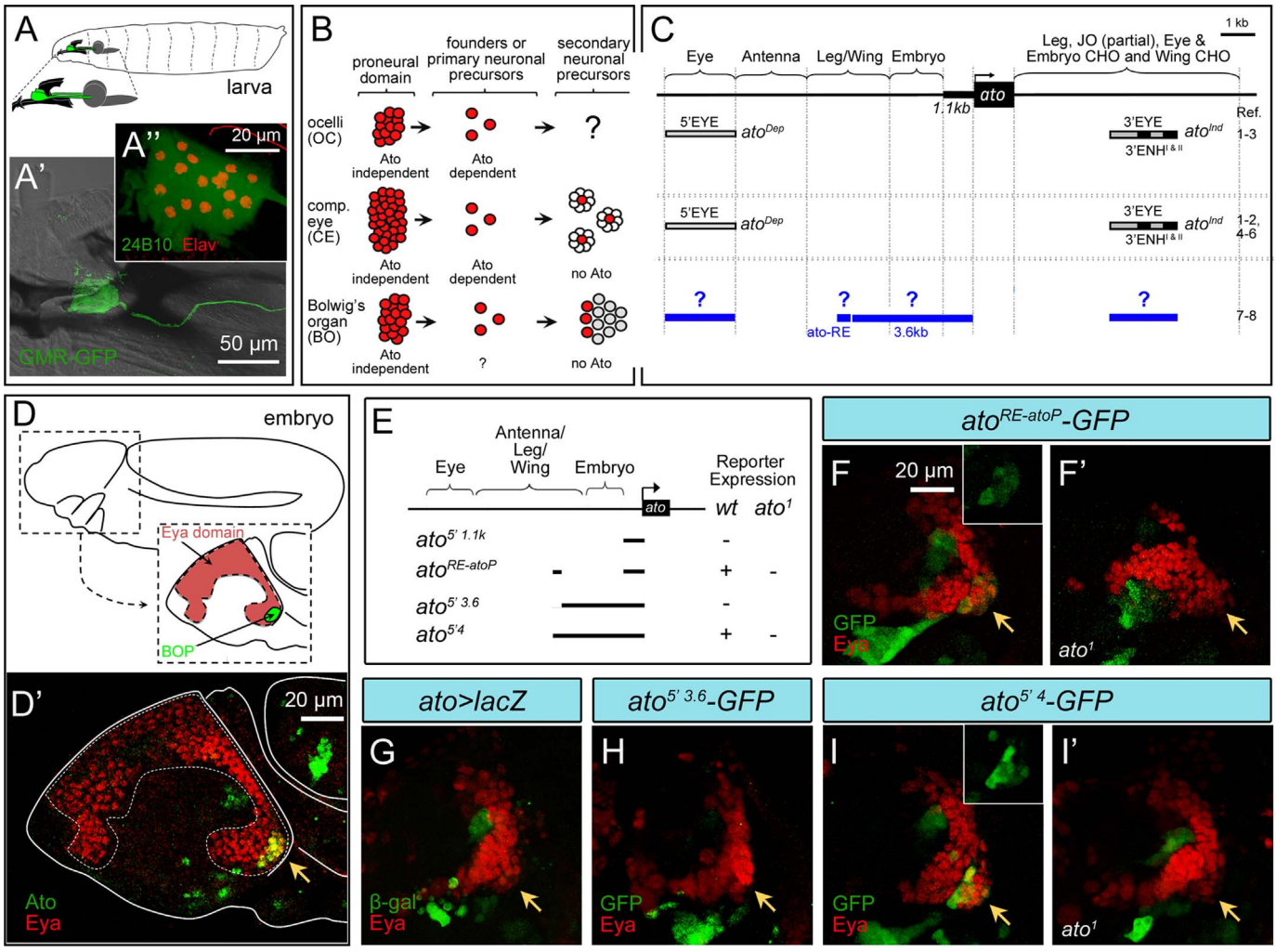

Fig. 1. Role and regulation of *ato* in the *Drosophila* larval eye. In all figures, embryos or larvae are shown with anterior to the left and dorsal up; yellow arrows point to the BO primordium (BOP). (A-A") The larval eye of *Drosophila*. (A) Schematic representation of a *Drosophila* larva showing the position of the Bolwig's organ (green) and the central nervous system (gray) in relation to the cephalopharyngeal skeleton (black). (A'-A") Visualization of the fully formed BO. (A') BO from L3 larva marked by GMR-GFP expression (green). (A") BO stained with the pan-photoreceptor marker Chaoptin (24B10 Ab, green) and the pan-neuronal nuclear marker Elav (red). (B) Schematic drawing of the *ato* expression pattern with genetic requirements for *ato* transcription in the developing visual organs; the drawings symbolically represent the resolution of the broader proneural expression domain of Ato into single Ato-expressing cells, the primary neuronal precursors; the number and spatial arrangement of these cells is specific to each organ and no attempt has been made to reflect the natural pattern. Similarly, the recruitment of the secondary neuronal precursor is represented in schematic fashion. In the CE and OC primary neuronal precursors, *ato* expression is maintained through autoregulation; thereafter, recruitment of the secondary neuronal precursorsoccurs without inducing *ato* expression but requires activation of EgfR signaling (Daniel et al., 1999). The process is poorly understood in the OC, but recent work suggests that it shares similarities with the CE (Zhou et al., 2016). (C) Diagram of *cis*-regulatory regions for *ato* transcription in the developing visual organs. Previously identified DNA regions for *ato* regulation in developing adult sense organs and the embryonic CHOs are marked above. The 3'ENH^{I} and 3'ENH^{II} sequences are marked by black boxes within the 3'EYE regulatory sequences. *ato^{Dep}*=Ato-dependent, maintenance phase; *ato^{Ind}*=Ato-independent, initiation phase. References: 1=Sun et al., 1998; 2=Zhang et al., 2006; 3=Zhou et al., 2016; 4=Tanaka-Matakatsu and Du., 2008; 5=Tanaka-Matakatsu et al., 2014; 6=Zhou et al., 2014; 7=zur Lage et al., 2004; 8=Sprecher et al., 2007. (D-D') Visualization of the BO during embryonic development. (D) Schematic representation of a stage ~10–11 embryo and enlargement of the head region showing the position of the BOP in relation to the domain of Eya expression. (D') Ato protein (green) is expressed in the BOP, which is located at the posterior ventral corner of the cephalic domain of Eya expression (red). (E) Diagram of genomic regions tested for enhancer activity and summary of results in wt and *ato* mutant embryos. Here and in other similar diagrams, previously identified DNA regions for *ato* regulation in developing adult sense organs and the embryonic CHOs are marked above. (F-I') Confocal images from embryos stained for the reporter proteins GFP (F-F' and H-I') or β-Galactosidase (G) (green) and Eya (red); insets show the green channel for the BO region. (F-F') *ato$^{RE\text{-}atoP}$-GFP* embryos stained for GFP (green) and Eya (red). BOP expression is seen in the wt (F) but not in the *ato^{1}* mutant (F') embryo. (G-H) Neither *ato > lacZ (=ato-Gal4 UAS-lacZ)* (G) nor *ato$^{5'3.6}$-GFP* (H) show any reporter protein expression in the BOP. (I-I') GFP from *ato$^{5'\,4}$-GFP* is expressed in the wt (I) but not in the *ato^{1}* mutant (I') BOP, similarly to *ato$^{RE\text{-}atoP}$-GFP*.

through ~2 kb of genomic DNA located downstream of the *ato* transcription unit (3'EYE region in Fig. 1C). Recent progress has identified components of the Retinal Determination Network (RDN), a transcription factor cascade that specifies eye identity, as direct inducers of *ato* in the developing CE. In retinal progenitor cells, the Six1/2-type protein Sine oculis (So) and the Pax6-type factor Eyeless (Ey) directly bind to sites in two enhancers, 3'ENH^{I} and 3'ENH^{II}, located within 3'EYE (Fig. 1C) (Zhang et al., 2006; Tanaka-Matakatsu and Du, 2008; Zhou et al., 2014). The subsequent autoregulation of *ato* during the maintenance phase depends on enhancer(s) located between ~7.5 and ~10.5 kb upstream of the *ato* gene (within the 5'EYE region in Fig. 1C). This region drives expression in small groups of cells that resolve into single primary precursors, the founder R8 neurons (Fig. 1B) (Baker et al., 1996; Sun et al., 1998). As the precise *cis*-elements for Ato-dependent control have not been identified, it is still unknown whether feedback regulation is direct or indirect. The subsequent recruitment of secondary neuronal precursors by the founder R8 proceeds without further involvement of Ato, which is neither expressed nor required in the emerging secondary photoreceptors R1-R7 (Yang and Baker, 2001). Of note, studies in the silkworm moth *Bombyx mori* suggest that the pattern of *ato* expression may be conserved in insect compound eye development (Yu et al., 2012).