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Eye-specification genes in the bacterial light organ of the bobtail squid *Euprymna scolopes*, and their expression in response to symbiont cues

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ABSTRACT

The squid *Euprymna scolopes* has evolved independent sets of tissues capable of light detection, including a complex eye and a photophore or 'light organ', which houses the luminous bacterial symbiont *Vibrio fischeri*. As the eye and light organ originate from different embryonic tissues, we examined whether the eye-specification genes, *pax6*, *eya*, *six*, and *dac*, are shared by these two organs, and if so, whether they are regulated in the light organ by symbiosis. We obtained sequences of the four genes with PCR, confirmed orthology with phylogenetic analysis, and determined that each was expressed in the eye and light organ. With *in situ* hybridization (ISH), we localized the gene transcripts in developing embryos, comparing the patterns of expression in the two organs. The four transcripts localized to similar tissues, including those associated with the visual system ~1/4 into embryogenesis (Naef stage 18) and the light organ ~3/4 into embryogenesis (Naef stage 26). We used ISH and quantitative real-time PCR to examine transcript expression and differential regulation in postembryonic light organs in response to the following colonization conditions: wild-type, luminescent *V. fischeri*; a mutant strain defective in light production; and as a control, no symbiont. In ISH experiments light organs showed down regulation of the *pax6*, *eya*, and *six* transcripts in response to wild-type *V. fischeri*. Mutant strains also induced down regulation of the *pax6* and *eya* transcripts, but not of the *six* transcript. Thus, luminescence was required for down regulation of the *six* transcript. We discuss these results in the context of symbiont-induced light-organ development. Our study indicates that the eye-specification genes are expressed in light-interacting tissues independent of their embryonic origin and are capable of responding to bacterial cues. These results offer evidence for evolutionary tinkering or the recruitment of eye development genes for use in a light-sensing photophore.

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1. Introduction

Research on visual systems has revealed remarkable conservation of eye-associated genes throughout much of the animal kingdom. Among such genes are *pax6* (paired box gene 6), *eya* (eyes absent), *six* (sine oculis), and *dac* (dachshund); transcription factors that interact in a regulatory network (Fig. 1A) and are critical for eye morphogenesis (Donner and Maas, 2004). Among other locations, these genes are expressed in both simple and complex eyes of diverse taxa, and are often referred to as "eye-specification genes" (e.g., Kumar and Moses, 2001). For example, the well-studied gene *pax6*, is found in animals with simple pigment-cup eyes (e.g., *Platynereis dumerilii*, Arendt et al., 2002), invertebrate compound eyes (e.g., *Drosophila* sp., Halder et al., 1995; Gehring and Ikeo, 1999), and vertebrate camera eyes (e.g., *Mus musculus*, Donner and Maas, 2004). Although eyes have been studied at the level of gene expression and in an evolutionary context (e.g., see Spady et al., 2005; Harzsch et al., 2006; Porter et al., 2012), few such studies have focused on photophores, light-emitting organs that have ocular attributes (but see Tong et al., 2009; Schnitzler et al., 2012).

The Hawaiian bobtail squid, *Euprymna scolopes* (Fig. 1B), is a model invertebrate species with complex eyes and a photophore or 'light organ' (Fig. 1C) that houses the luminous bacterial symbiont *Vibrio fischeri*. The light emitted by *V. fischeri* matches down-welling moonlight and starlight, and camouflages the squid while active at night in an anti-predatory phenomenon called counter-illumination (McFall-Ngai and Ruby, 1991; Jones and Nishiguchi, 2004; see also Johnsen et al., 2004). The bacterial symbionts, which are harvested anew each generation, enter through pores on either side of the light organ, and ultimately reside along the apical surfaces of polarized epithelia in the crypt spaces (Fig. 1D). Striking anatomical, biochemical, molecular, and physiological similarities exist between the eye and light organ. These similarities include a lens with crystallin proteins (Montgomery and McFall-Ngai, 1992), an analog of the tapetum with 'reflectin' proteins (Crookes et al., 2004), genes and proteins involved in phototransduction (Tong et al., 2009), and the physiological ability to respond to light (Tong et al., 2009). In addition, the ink sac, which surrounds a portion of the light organ, functions as both an iris and a choroid (McFall-Ngai and Montgomery, 1990). Such features in the light organ are thought to enable *E. scolopes* to detect and, in turn, control the light emitted by *V. fischeri*.

Although the eyes and light organ have notable similarities, they also have key differences. The eyes and light organ are not homologous, developing from ectoderm and mesendoderm, respectively (Montgomery and McFall-Ngai, 1993). The organs also develop at slightly different stages during embryogenesis (Table 1). In addition, light stimuli for the eyes and light organ come from different sources, environmental light and the luminous bacterial symbiont, respectively. Light stimulus is important in the development of both sets of tissues. Environmental light contributes to maturation of the vertebrate eye (e.g., see Grün, 1979; Tian,

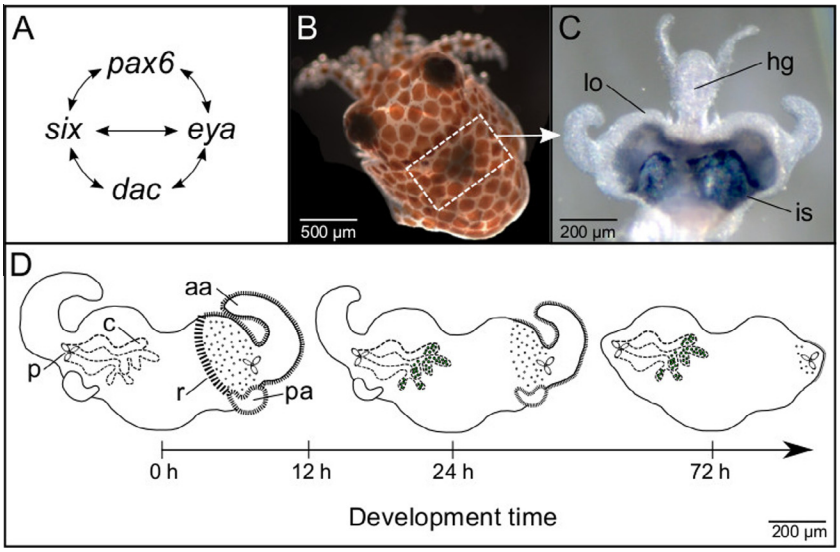

Fig. 1 – Study system. (A) Regulatory network of the eye-specification genes (adapted from Donner and Maas, 2004). (B) Juvenile *E. scolopes*. The light organ is located within the white-dash boxed region and is associated with the ink sac, which is visible as a slightly darkened area. (C) Juvenile light organ (lo) surrounded by the ink sac (is) and attached to the hindgut (hg). (D) Early postembryonic development of the juvenile light organ. The juvenile light organ has three pores (p) that enable bacterial symbiont *V. fischeri* to enter the internal crypt spaces (c) (left side of light organ). *V. fischeri* are shown as green dots in the crypt spaces at 24 and 72 h of development. The surface tissues of the juvenile light organ include the anterior (aa) and posterior appendages (pa), and the ciliated ridges (r) (right side of light organ), all of which regress during the first several days of development. The developmental time of 12 h post hatching marks the point at which regression of the appendages is no longer reversible and can proceed in the absence of wild-type *V. fischeri*.