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Ethanolamine kinase controls neuroblast divisions in *Drosophila* mushroom bodies

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Abstract

The *Drosophila* mushroom bodies (MBs), paired brain structures composed of vertical and medial lobes, achieve their final organization at metamorphosis. The *alpha lobe absent* (*ala*) mutant randomly lacks either the vertical lobes or two of the median lobes. We characterize the *ala* axonal phenotype at the single-cell level, and show that the *ala* mutation affects *Drosophila* ethanolamine (Etn) kinase activity and induces Etn accumulation. Etn kinase is overexpressed in almost all cancer cells. We demonstrate that this enzymatic activity is required in MB neuroblasts to allow a rapid rate of cell division at metamorphosis, linking Etn kinase activity with mitotic progression. Tight control of the pace of neuroblast division is therefore crucial for completion of the developmental program in the adult brain.

Keywords: Ethanolamine kinase; *alpha lobes absent*; Mushroom body; *Drosophila*; Neuroblast; Mitosis

Introduction

In all species, organogenesis entails a precisely regulated temporal and spatial pattern of cell proliferation. In this respect, the question of how a neural progenitor cell can generate different types of neurons and glia is an outstanding problem in developmental biology. Two sets of determinative factors, external cues and internal cell-autonomous responses, interplay to define cell fate. Thus, the position and time of birth of a neuron in the central nervous system allows it to receive specific and transient signals from surrounding cells (Edlund and Jessell, 1999). The mushroom bodies (MBs) are insect brain structures highly relevant to this issue, as their highly specialized organization is elaborated in several discrete developmental steps.

Adult MB cells (Kenyon cells) send their dendrites into the calyx, where they receive input from the antennal lobes. Their axons extend anteriorly and ventrally into the peduncle and terminate in one of several groups of lobes that are composed of several classes of neurons (Strausfeld et al., 2003). Three of these, γ, α′/β′, and α/β neurons, have been particularly well studied (Crittenden et al., 1998; Lee et al., 1999). The MBs receive multimodal sensory information and have been implicated in higher-order brain functions, including olfactory learning and short-term memory (de Belle and Heisenberg, 1994; Heisenberg, 1998; Roman and Davis, 2001), olfactory long-term memory (Isabel et al., 2004; Pascual and Preat, 2001), courtship behavior (Ferveur et al., 1995; McBride et al., 1999; O'Dell et al., 1995), and elementary cognitive functions, such as visual context generalization (Liu et al., 1999). The individual MB lobes are functionally specialized. In particular, specific lobes have been implicated in short-term memory (Zars et al., 2000), while the vertical MB lobes play a role in long-term memory (Isabel et al., 2004; Pascual and Preat, 2001). How this neural diversity is generated during development remains poorly understood.

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Four neuroblasts (Nbs) give rise to each MB. These progenitor cells are among the first to delaminate from the procephalic embryonic ectoderm, and they begin to proliferate from embryonic stage 9 onward (Noveen et al., 2000). During embryogenesis, the four MB Nbs give rise to between 100 and 300 γ neurons (Armstrong et al., 1998; Ito and Hotta, 1992; Technau and Heisenberg, 1982), whose axons branch to form a medial and a dorsal lobe (Armstrong et al., 1998). Most other embryonic Nbs stop dividing transiently in the late embryo. However, MB Nbs continue proliferating through the postembryogenic stages, and they are actively dividing at the time of larval hatching (Prokop and Technau, 1991; Truman and Bate, 1988). About 12 h after hatching, some scattered Nbs in the central brain resume division. Neurogenesis proceeds at an accelerating rate in the central brain through the remainder of larval life and puparium formation. Nb proliferation ceases about 20 to 30 h after puparium formation (APF) (White and Kankel, 1978) except for the MB Nbs, which continue to divide almost until the end of metamorphosis. Thus, MB Nbs are distinctive in that they divide continuously throughout development (Ito and Hotta, 1992; Prokop and Technau, 1994; Truman and Bate, 1988).

During metamorphosis, many larva-specific neurons are definitively removed by programmed cell death, while most of the remaining cells withdraw larva-specific projections and extend new processes. Some immature neurons differentiate during metamorphosis to produce adult-specific networks (reviewed by Truman, 1990). Clonal analysis (Lee et al., 1999) has demonstrated that all MB neurons generated from the time of larval hatching until the mid third-instar larval stage give rise to branched γ neurons. In mid third-instar larvae, the progeny of the MB Nbs undergo a sharp change in cell fate and start to generate branched α′/β′ neurons. The larval projections of these neurons remain relatively unchanged during metamorphosis. In contrast, γ projections undergo pruning by glial cells at metamorphosis to give rise to adult γ lobes that project only medially (Awasaki and Ito, 2004; Lee et al., 1999). Finally, all MB neurons born after puparium formation are α/β neurons.

With the aim of identifying genes involved in brain metamorphosis, we screened enhancer trap lines displaying specific patterns of expression in the central brain at the third-instar larval stage (Boquet et al., 2000a,b). This work led to the recovery of six mutants showing central brain defects in the adult. One of these, *alpha lobes absent* (*ala*) presents a peculiar MB phenotype. *ala* MBs completely lack α′ and α or β′ and β lobes in a random pattern (Pascual and Preat, 2001). In contrast, γ lobes appear normal. This phenotype proved useful in ascribing to dorsal MB lobes a role in *Drosophila* long-term memory (Isabel et al., 2004; Pascual and Preat, 2001).

Here, we show that *ala* corresponds to *easily shocked* (*eas*), a previously described gene that encodes ethanolamine (Etn) kinase, the first enzyme of the Kennedy pathway (Pavlidis et al., 1994). We show that *eas* mutants display a brain phenotype similar to that of *ala* mutants. We also report that Etn kinase is expressed in MB Nbs, where it controls the rapid mitoses that occur just before and during metamorphosis.

Materials and methods

Drosophila stocks

Drosophila were maintained on a 12:12 dark/light cycle on standard cornmeal-yeast agar medium at 25°C and 50% relative humidity. The wild-type strain was *Canton-Special* (CS). The *Df(1)4b18* (spanning14B08; 14C01), *UAS-mCD8::GFP* (Lee et al., 1999), *hs-FLP, w^{1118}; Adv^{1}/CyO* and *FRT^{19A}; ry^{506}* lines were all provided by the Bloomington stock center. The *w, eas^{2}, f* and *hs-eas^{+}* stocks were obtained from the collection of Mark A. Tanouye (University of California, Berkeley). The *FRT^{G13}, UAS-mCD8::GFP; Gal4-OK107* and *FRT^{19A}, tubP-Gal80, hs-FLP; UAS-mCD8::GFP; Gal4-OK107* stocks were provided by Liqun Luo (Stanford University, Stanford). The *w^{1118}, eas^{alaP}* allele was induced by *P(GawB)* mutagenesis (Boquet et al., 2000b), and the *w^{1118}, eas^{alaE13}* allele, which behaves as a strong hypomorphic allele (Boquet et al., 2000b), was obtained by excision of the P element from *eas^{alaP}* flies. All *eas* chromosomes carry the *w^{1118}* mutation, although not explicitly stated within the text. For MARCM analysis, *eas^{alaE13}, FRT^{19A}* and *eas^{alaE13}, hs-FLP; FRT^{G13}, tubP-Gal80* stocks were generated.

MARCM analysis of eas MB neurons

To generate MB clones in *eas* pupae using the MARCM system, white puparia of the appropriate genotypes (see table legends) were collected, heat shocked at 37°C for 30 min and returned to 25°C. Adults were processed for paraffin inclusion and sectioned. Clones were detected by immunostaining with an anti-GFP antibody (1:500; Roche, Germany).

To detect two-cell/single-cell MB clones in *eas* flies, white puparia of the appropriate genotypes (control clones: *hs-FLP/Y; FRT^{G13}, tubP-Gal80/FRT^{G13}, UAS-mCD8::GFP; Gal4-OK107/+*; *eas* clones: *eas^{alaE13}, hs-FLP/Y; FRT^{G13}, tubP-Gal80/FRT^{G13}, UAS-mCD8: GFP; Gal4-OK107/+*) were collected and heat shocked once at 37°C for 30 min at different time points during the first 48 h of the pupal stage. Adult brains were dissected and processed as described (Pascual and Preat, 2001).

To generate *eas* homozygous clones in an *eas*/+ background *eas^{alaE13}, FRT^{19A}* females were mated to *FRT^{19A}, tubP-Gal80, hs-FLP; UAS-mCD8::GFP; Gal4-OK107* males. The progeny were heat shocked once at 37°C for 30 min at different time points during the overall course of development (first-, second-, and third-instar larval and 48-h pupal stages). Offspring females were processed for paraffin