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A temporal mechanism that produces neuronal diversity in the *Drosophila* visual center

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ABSTRACT

The brain consists of various types of neurons that are generated from neural stem cells; however, the mechanisms underlying neuronal diversity remain uncertain. A recent study demonstrated that the medulla, the largest component of the *Drosophila* optic lobe, is a suitable model system for brain development because it shares structural features with the mammalian brain and consists of a moderate number and various types of neurons. The concentric zones in the medulla primordium that are characterized by the expression of four transcription factors, including Homothorax (Hth), Brain-specific homeobox (Bsh), Runt (Run) and Drifter (Drf), correspond to types of medulla neurons. Here, we examine the mechanisms that temporally determine the neuronal types in the medulla primordium. For this purpose, we searched for transcription factors that are transiently expressed in a subset of medulla neuroblasts (NBs, neuronal stem cell-like neural precursor cells) and identified five candidates (Hth, Klumpfuss (Klu), Eyeless (Ey), Sloppy paired (Slp) and Dichaete (D)). The results of genetic experiments at least explain the temporal transition of the transcription factor expression in NBs in the order of Ey, Slp and D. Our results also suggest that expression of Hth, Klu and Ey in NBs trigger the production of Hth/Bsh-, Run- and Drf-positive neurons, respectively. These results suggest that medulla neuron types are specified in a birth order-dependent manner by the action of temporal transcription factors that are sequentially expressed in NBs.

Introduction

For successful functional brain development, a large number of various types of neurons must be generated at the optimal time and location. The molecular mechanisms underlying neuronal diversity and the spatio-temporal regulation of neurogenesis are largely unknown because the brain is too complex to elucidate its entire developmental mechanisms. Our previous study revealed that the medulla, the largest component of the *Drosophila* optic lobe, is a suitable model system for brain development (Hasegawa et al., 2011). The medulla has similar structural features to the mammalian brain, such as layer and columnar structures and contains at least 60 types of 40,000 neurons (Fischbach and Dittrich, 1989; Hofbauer and Campos-Ortega, 1990). Thus, the medulla is genetically tractable and sufficiently complex to be considered as a model of brain development. The developing medulla is subdivided into concentric zones that are characterized by the expression of the genes encoding conserved transcription factors *homothorax* (*hth*), *brain-specific homeobox* (*bsh*), *runt* (*run*) and *drifter* (*drf*), which are collectively called concentric genes (Hasegawa et al., 2011). This type of subdivision also exists in the developing mammalian spinal cord (Jessell, 2000), telencephalon and eye (Lupo et al., 2006). Thus, the developmental mechanism of the medulla could be highly similar to that of the mammalian central nervous system.

During the development of the central nervous system, neural stem cells generate a variety of neuronal cells depending on spatial and temporal information. In mammals, neural stem cells generate neurons and then glia in a stereotypical order that is determined by the temporal restriction of the precursor cell fate. The transition from neurogenesis to gliogenesis is controlled by extrinsic and intrinsic factors (Cepko, 1999). The cell-intrinsic mechanism that restricts the competence of stem cells has been well investigated in the developing embryonic central nervous system of *Drosophila*. Neuroblasts (NBs), stem cell-like multipotent precursors, divide asymmetrically to produce a ganglion mother cell (GMC) and

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a NB in a self-renewal fashion (Bossing et al., 1996). The GMC further divides into two post-mitotic neurons that are specified upon the birth of their mother cells (Doe and Goodman, 1985). The GMC birth-order identity is determined by the expression of heterochronic transcription factors, including Hunchback (Hb), Krüppel (Kr), Pdm1/Pdm2 (Pdm), Castor (Cas) (Isshiki et al., 2001; Kambadur et al., 1998) and Granyhead (Grh) (Almeida and Bray, 2005; Chen et al., 2012). These transcription factors are expressed sequentially in each NB and are maintained in their daughter GMC to contribute to specifying the neuronal identity of their progeny (Isshiki et al., 2001). Although the sequential expression of various transcription factors in NBs elicits temporal neuronal specification in the embryonic central nervous system, the mechanisms underlying neuronal diversity during adult brain development are poorly understood.

Medulla NBs are located on the cortical surface and generate neurons in both a linear and radial orientation. Thus, a single NB generates many medulla neurons of various identities that are characterized by the expression of each concentric gene (Hasegawa et al., 2011). The expression of these genes correlates with the birth order of the medulla neurons, suggesting that the medulla neurons are also specified in a birth order-dependent manner as observed in the embryonic central nervous system. Additionally, the wave of differentiation called 'proneural wave' progresses in medial-to-lateral orientation and induces the transition of neuroepithelia (NE) into medulla NBs (Fig. 1A). As a result, early-born NBs are situated medially while later-born NBs are situated laterally on the surface of the larval medulla primordium. An advantage of using the medulla as a model could be that NBs of different ages can be observed and compared at the same time (Yasugi et al., 2008). A group of genes that are transiently and sequentially expressed in the medulla NBs may be involved in temporal neuronal specification in the medulla.

In this study, we examine the molecular mechanism that produces neuronal diversity in the developing medulla. Among four concentric transcription factors that are expressed in medulla neurons, Hth expression is also detected in NEs and newly differentiated NBs, suggesting that Hth expression is inherited from NB to neurons (Hasegawa et al., 2011; Reddy et al., 2010). Hth might also be one of the heterochronic transcription factors expressed in the medulla NBs. Additionally, we searched for transcription factors that are transiently expressed in medulla

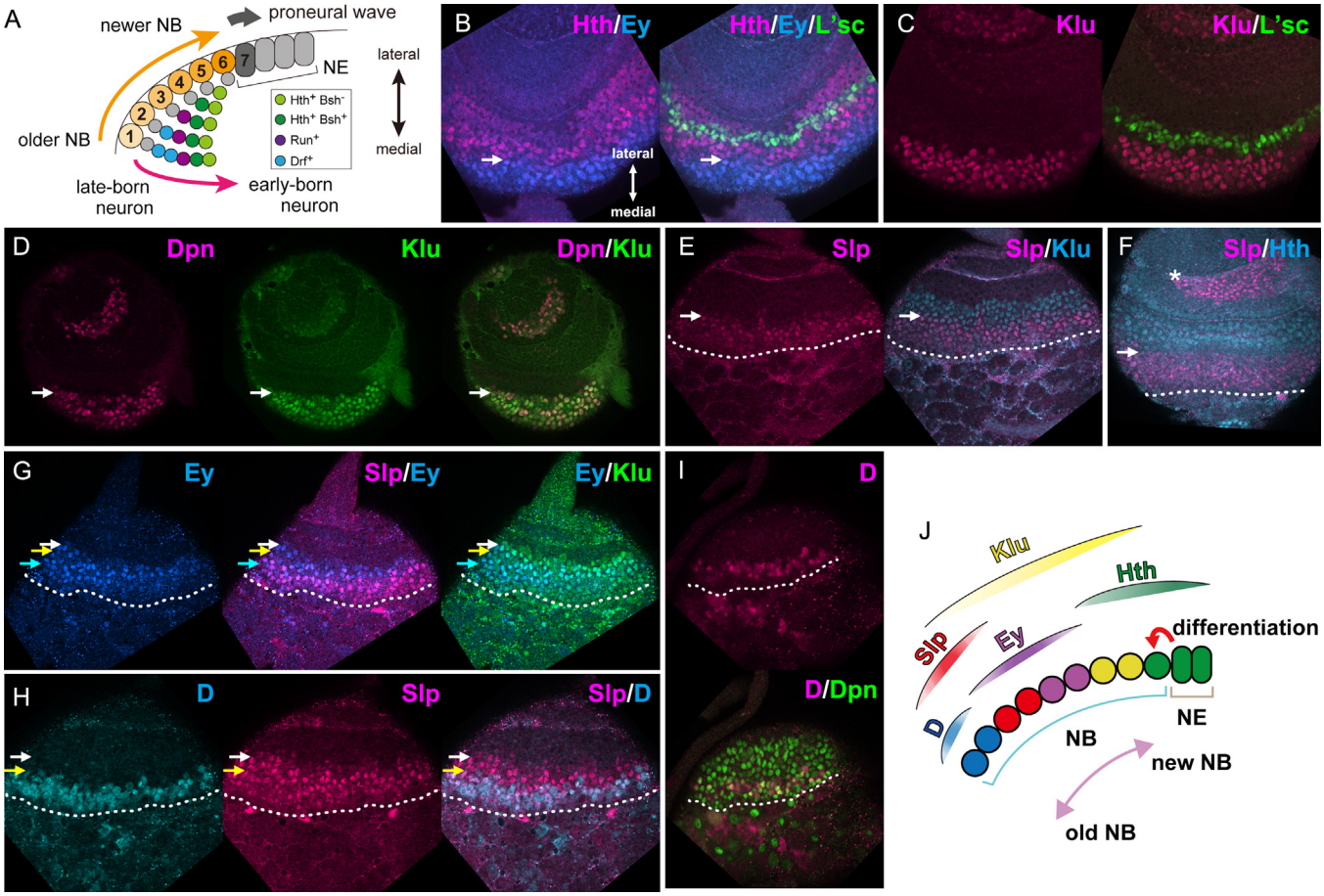

Fig. 1. Hth, Klu, Ey, Slp and D are temporally expressed in the medulla NBs. (A) Schematic model of NB production in the medulla primordium. NBs are numbered in a numerical order: the NB1 is firstly differentiated from NE and the NB6 is the last. NE7 is the NE cell that is just differentiating to NB. NBs sequentially produce medulla neurons in the order of cells labeled in light green (Hth+Bsh−), dark green (Hth+Bsh+), magenta (Run+) and blue (Drf+). (B–I) The cortical surface of medulla primordium in frontal views at wandering late third larval instar. Lateral to the top, medial to the bottom as indicated in (B). (E–I) White dots indicate the borders between the medulla and central brain. (B) Hth expression (magenta) is observed in L'sc expressing NEs (green) and lateral NBs. Ey (blue) expression does not overlap Hth. Arrows indicate the border between Hth and Ey domains. (C) Klu expression (magenta) is observed in NBs adjacent to L'sc-positive NEs (green). (D) Klu (green) is strongly expressed in the lateral-most NBs (Dpn; magenta, arrow). (E) Slp (magenta) is expressed in medial NBs compared to Klu-positive NBs (blue). Slp is weakly expressed in Klu-positive NBs. Arrows indicate the lateral borders of Slp domain. (F) Hth (blue) is not expressed in Slp-positive NBs (magenta). Slp expression in the lamina is indicated by asterisk. Arrow indicates the border between Hth and Slp domains. (G) Ey (blue) is expressed in Klu-positive NBs (green) except for the most lateral NBs. Ey and Slp (magenta) expression partially overlaps. White, yellow, and blue arrows indicate the lateral borders of the Klu, Ey and Slp domains respectively. (H) Slp (magenta) and D (blue) are expressed in intermediate and medial NBs, respectively. Slp and D expression partially overlaps. White and yellow arrows indicate the lateral borders of Slp and D domains, respectively. (I) D (magenta) is expressed in medial NBs (Dpn; green). (J) Schematic model illustrating the expression domains of Hth, Klu, Ey, Slp and D in medulla NBs.