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Bhlhb5::flpo allele uncovers a requirement for Bhlhb5 for the development of the dorsal cochlear nucleus

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ABSTRACT

Auditory information is initially processed in the cochlear nuclei before being relayed to the brain. The cochlear nuclei are subdivided into dorsal, anterior ventral, and posterior ventral domains, each containing several subtypes of neurons that are thought to play discrete roles in the processing of sound. However, the ontogeny of these neurons is poorly understood, and this gap in knowledge hampers efforts to understand the basic neural circuitry of this nucleus. Here, we reveal that Bhlhb5 is expressed in both excitatory (unipolar brush cells) and inhibitory neurons (cartwheel cells) of the DCN during development. To gain genetic access to Bhlhb5-expressing neurons in the DCN, we generated a *Bhlhb5::flpo* knockin allele. Using an intersectional genetic strategy, we labeled cartwheel cells, thereby providing proof of concept that subpopulations of Bhlhb5-expressing neurons can be genetically targeted. Moreover, fate-mapping experiments using this allele revealed that *Bhlhb5* is required for the proper development of the DCN, since mice lacking *Bhlhb5* showed a dramatically diminished number of neurons, including unipolar brush and cartwheel cells. Intriguingly, the *Bhlhb5::flpo* allele also genetically labels numerous other regions of the nervous system that process sensory input, including the dorsal horn, the retina, and the nucleus of the lateral olfactory tract, hinting at a more general role for Bhlhb5 in the development of neurons that mediate sensory integration.

1. Introduction

The dorsal cochlear nucleus (DCN) is a brainstem auditory nucleus where acoustic information is first processed and integrated with somatosensory and cochlear input. One of the functions of this nucleus is to help localize sound in the vertical plane so that an organism can determine whether the acoustic source is from above or below, thereby helping an organism evade predators or locate prey. In addition, the DCN may also contribute to our experience of painfully loud sound, an aversive sensation that evolved to protect hearing from acoustic assault. When acoustic damage occurs repeatedly, this trauma can cause ringing in the ear due to the false perception of sound, a pathological condition known as tinnitus (Axelsson and Ringdahl, 1989; Shargorodsky et al., 2010; Maes et al., 2013). Importantly, recent studies suggest that tinnitus is caused, at least in part, by hyperactivity within the dorsal cochlear nucleus due to decreased inhibition (Wang et al., 2009, 2011; Middleton et al., 2011). These physiological and pathophysiological roles underscore the importance of understanding the neural circuitry of the DCN, which remains poorly defined. In this regard, the development of tools that allow cell type-specific genetic control would be extremely useful.

Elegant fate-mapping studies have revealed that the DCN arises largely from progenitors within rhombomere 5 of the auditory lip (Farago et al., 2006; Nichols and Bruce, 2006). These neurons migrate to form a laminar structure comprised of three layers: the molecular layer, the fusiform cell layer, and the deep layer (Osen,

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1969; Ryugo and Willard, 1985; Hackney et al., 1990). Within these layers, there are eight major morphological classes of neurons that have been identified. Previous work has revealed that the excitatory subtypes (granule cell, unipolar-brush cells, giant cells, and fusiform cells) arise from Atoh1-expressing progenitors, whereas the inhibitory subtypes (golgi cells, superficial-stellate cells, cartwheel cells and tuberculo-ventral cells) arise from Ptf1a-expressing progenitors (Fujiyama et al., 2009). However, the transcriptional programs within the DCN that mediate terminal differentiation and neuronal connectivity are poorly understood.

To determine what role these different cell types play in the function of the DCN, we need tools that allow cell-type specific genetic manipulation. We find that a key factor in the development of the DCN is Bhlhb5 (also known as Bhlhe22), a basic helix-loop-helix transcription factor that is related to Atonal (Ross et al., 2003). In many regions of the nervous system, Bhlhb5 is selectively expressed in early post-mitotic neurons as they undergo terminal differentiation and establish synaptic connections (Ross et al., 2010). Using genome-wide CHIP-seq analysis, we previously showed that Bhlhb5 binds to a specific DNA consensus motif

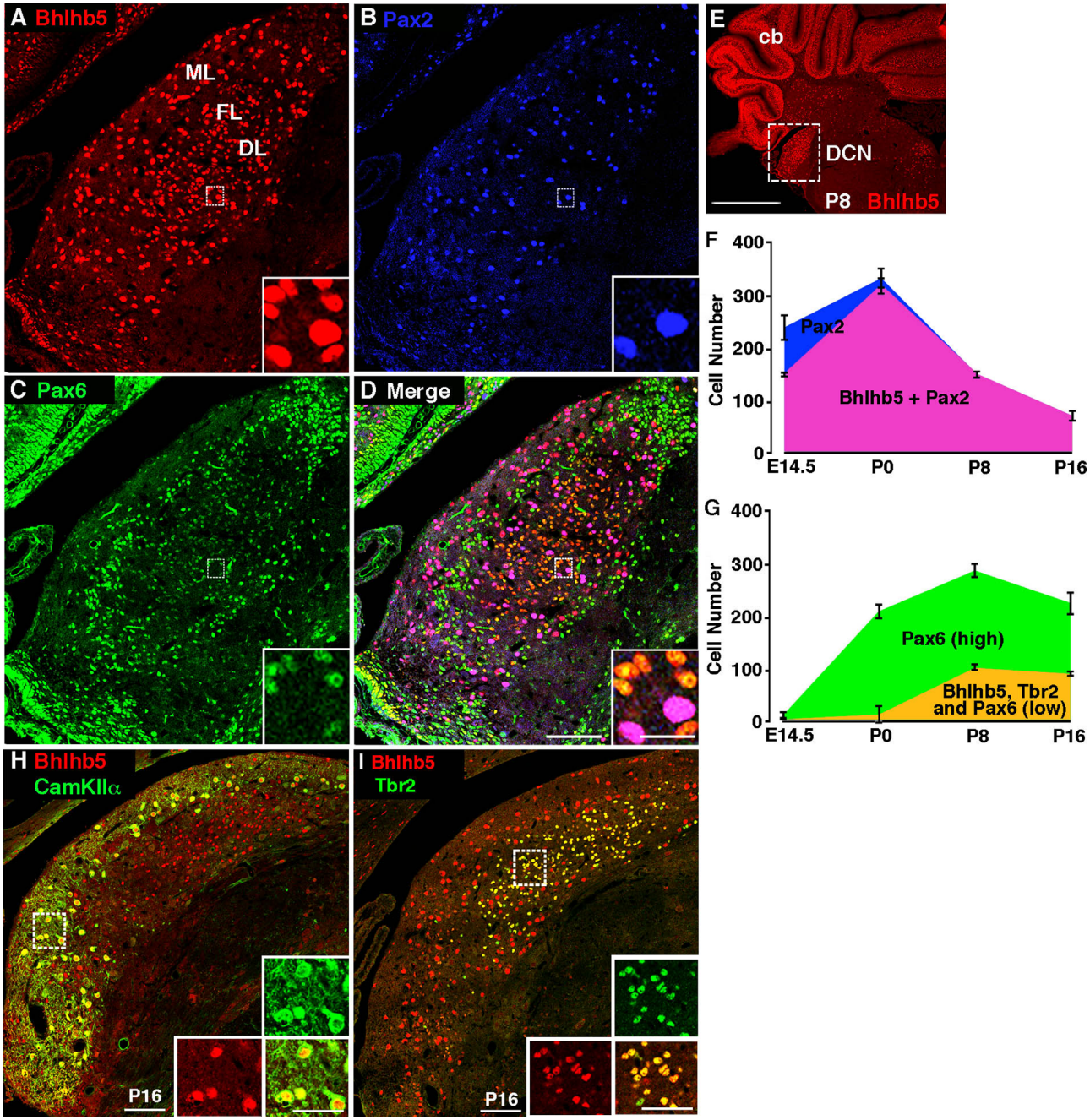

Fig. 1. Bhlhb5 is expressed in the dorsal cochlear nucleus during development. (A-D) Coronal section from mouse at P8 immunostained with antibodies directed against Bhlhb5 (red), Pax2 (blue) and Pax6 (green), as indicated. All Bhlhb5-expressing cells either express either Pax2 or Pax6. Scale bar =100 μm; inset scale bar =20 μm; ML, molecular layer; FL, fusiform layer; DL deep cell layer. (E) Coronal brain hemi-section stained with Bhlhb5; boxed inset of DCN is shown in A-D. Scale bar =500 μm; cb, cerebellum. (F) Quantification of the number of Pax2-expressing cells that do (pink) or do not (blue) co-express Bhlhb5 from E14.5 – P16 (data are average +/− SEM, n =3). (G) Quantification of the number of Pax6-expressing cells in the DCN that do (orange) or do not (green) co-express Bhlhb5 from E14.5–P16. Bhlhb5-expressing cells showed a relatively low level of Pax6 expression and also co-expressed Tbr2 (data are average +/- SEM, n =3). (H-I) Coronal section from mouse at P16 immunostained with antibodies directed against Bhlhb5 (red), CamKIIα (green) and Tbr2 (green) as indicated. Boxed area is shown in insets (H: molecular layer; I: fusiform-deep cell layers). Scale bar =100 μm; inset scale bar =50 μm. All images are single confocal optical sections.