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Diversity of thalamic progenitor cells and postmitotic neurons

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Abstract

The vertebrate thalamus contains multiple sensory nuclei, and relays sensory information to corresponding cortical areas. Moreover, the thalamus actively regulates information transmission to the cortex by modulating the response magnitude, firing mode and synchrony of neurons according to behavioral demands. The thalamus serves many other functions including motor control, learning and memory, and emotion. Such functional importance of the thalamus necessitates a better understanding of its developmental mechanisms. In this review, we will first describe the morphological organization of the developing thalamus. We will then discuss how neuronal diversity is generated and nuclei are formed during thalamic development. The first step in generating neuronal diversity is the formation of spatial diversity of thalamic progenitor cells, which is controlled by locally-expressed signaling molecules such as Sonic hedgehog (Shh), Wnt proteins and Fgf8. Lastly we will describe the roles of several transcription factors in specification of neuronal identity and nuclei formation in the thalamus. Our review will provide a molecular perspective for the organization of the thalamus prior to thalamus–cortex circuit formation.

Introduction

Conscious perception of sensory information typically requires that afferent sensory inputs be relayed to the cerebral cortex via the thalamus. To process the many different type of inputs, the thalamus has evolved to contain a high degree of neuronal diversity, where neurons of a particular subtype, which are generally delegated to perform a particular function, are clustered to form nuclei. Based on patterns of axonal projections to the cortex and gene expression, ~50 thalamic nuclei have been identified (Jones, 2007). Most thalamic nuclei project to the cerebral cortex, but many also project to other regions in the forebrain such as the striatum and amygdala. Nuclei projecting to the cortex can be divided into several classes based on patterns of their afferent and efferent connections with the cortex (Macchi *et al.*, 1996). Of these, the most distinctive class of nuclei, in terms of both morphology and function, are those that project densely onto single cortical areas. This class includes principal sensory nuclei such as the dorsal lateral geniculate (dLG) nucleus, ventral posterior (VP) nucleus and the ventral part of the medial geniculate nucleus (MGv). Neurons in these nuclei relay sensory information from the periphery via topographically organized thalamocortical axons to the primary sensory areas of the neocortex: visual, somatosensory and auditory, respectively (Fig. 1).

A key feature of thalamic development is early regionalization of the diencephalic neuroepithelium along the anterior–posterior (AP) and dorsal–ventral (DV) axes of the forebrain (Fig. 2). Patterning signals released from nearby tissues impose positional information onto neural progenitor cells as they divide in the ventricular zone along the third ventricle through the regulation of a number of transcription factors. Thalamic progenitor cells divide and generate postmitotic neurons, which then migrate to the mantle zone and aggregate into nuclei. Neurons in different thalamic nuclei exhibit distinct morphology and employ specific neurotransmitters. They also connect to different regions of the brain (Jones & Rubenstein, 2004; Yuge *et al.*, 2011).

Insights into how thalamic neurons are assembled from newly generated pronuclear masses into individual nuclei are beginning to be obtained, based on analyses of gene expression and function (Figdor & Stern, 1993; Kitamura *et al.*, 1997; Suzuki *et al.*, 1997; Redies *et al.*, 2000; Nakagawa & O'Leary, 2001; Nakagawa & O'Leary D, 2003; Puelles & Rubenstein, 2003; Jones & Rubenstein, 2004; Puelles *et al.*, 2011b; Suzuki-Hirano *et al.*, 2011; Yuge *et al.*, 2011). Later phases of nuclear differentiation within the thalamus are associated with its innervation by afferent fibres. Specific sets of thalamic nuclei receive inputs from defined afferent pathways and become bidirectionally connected with distinct functional areas of the neocortex (O'Leary *et al.*, 1994). During this period, afferent activity and competition between innervating fibres play an important role in determining the definitive input–output relationships and ultimately the finer grained cytoarchitecture of thalamic nuclei (Sur *et al.*, 1988; Bhide & Frost, 1992; Angelucci *et al.*, 1997; Penn *et al.*, 1998; Hahm *et al.*, 1999; Stellwagen & Shatz, 2002). Thus, correct specification of thalamic nuclei is a prerequisite for these later events and the establishment of functional circuitry of the brain.

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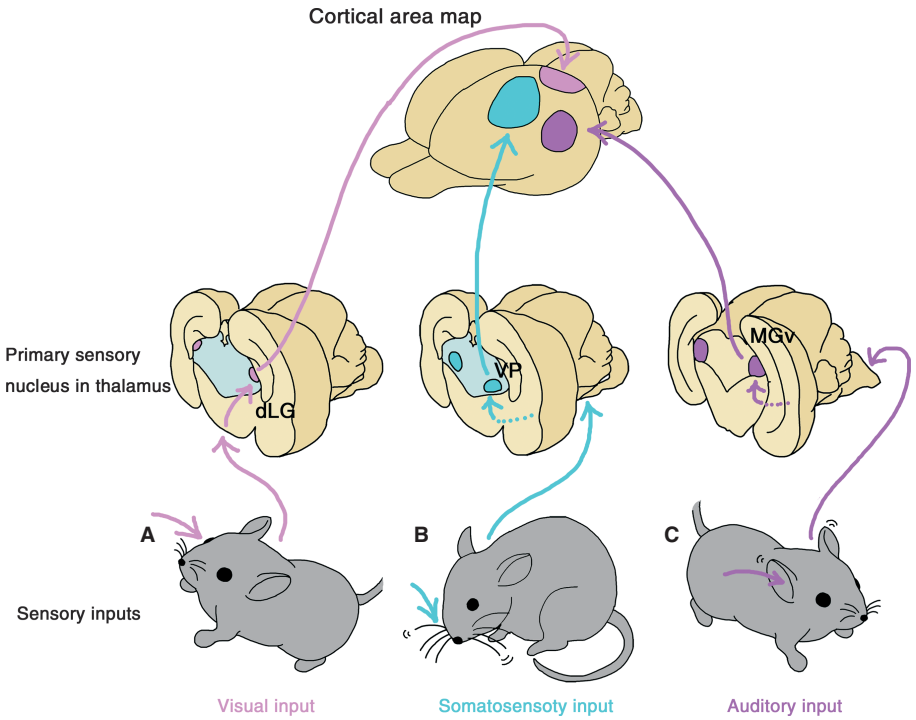

FIG. 1. Cartoon schema of sensory input pathway in CNS. (A) Visual information is conveyed to the dLG in the thalamus and is then relayed to the primary visual cortex. (B) Somatosensory information from rodent whiskers and the skin is sent to the VP nuclear complex via the brainstem and the spinal cord, and reaches the primary somatosensory cortex. (C) Auditory information reaches the inferior colliculus of the midbrain via the cochlear nuclei and superior olivary complex of the brainstem. This information then reaches the MGv in the thalamus and is relayed to the primary auditory cortex.

In this review, we will focus on the current knowledge on how neuronal diversity is generated in the thalamus and the molecular mechanisms that determine the formation of distinct nuclei. A recently published article has provided an excellent overview of thalamic development, mainly from embryological and evolutionary perspectives (Scholpp & Lumsden, 2010).

Spatial organization of thalamic progenitor cells

Due to the curvature of the neural tube, the long-dominant columnar models of the forebrain organization had poor definition of the longitudinal axis, which confused the axial nomenclature of the diencephalon including the thalamus (reviewed in Keyser, 1973; Puelles & Rubenstein, 1993; Shimamura *et al.*, 1995). Neuromeric models, on the other hand, defined this axis and identified the zona limitans intrathalamica (ZLI) as the rostral border of the thalamus (Fig. 2A and B). Puelles and Rubenstein used patterns of gene expression [especially for *Sonic hedgehog* (*Shh*) and *Nkx2.2*] in addition to morphology to define the AP axis of the forebrain, and proposed their prosomere model (Puelles & Rubenstein, 1993, 2003; Puelles *et al.*, 2011a). In this model, the progenitor region of the diencephalon is subdivided into three transverse domains along the AP axis: prosomere 1 (p1), prosomere 2 (p2) and prosomere 3 (p3) (Fig. 2A). The pretectum, thalamus and prethalamus are derived from the alar plate of p1, p2 and p3, respectively (Fig. 2B). The alar plate of p2 also contains the dorsally located habenula. Thus, in the prosomeric model, the terms 'thalamus' and 'prethalamus' are appropriately defined according to their relative positions along the AP axis, where the thalamus is posterior to the ZLI and the prethalamus is anterior to the ZLI (Puelles & Rubenstein, 2003). Some literature still uses 'dorsal thalamus' for the thalamus and 'ventral thalamus' for the prethalamus; these terms are consistent with the columnar model of forebrain organization and not the prosomere model.

Based on the fundamental concept of neural development that positional identity of neural progenitor cells plays a crucial role in the specification of neuronal identity (Jessell, 2000), Vue *et al.* (2007) set out to describe such heterogeneity in the embryonic mouse thalamus (Fig. 2C). It was found that thalamic progenitor cells are marked by the expression of the basic helix-loop-helix (bHLH) transcription factor Olig3 (Fig. 2E), and two distinct domains of progenitor cells were further identified. The smaller, anteroventral, domain expressed the bHLH transcription factor Ascl1 (=Mash1) and homeodomain transcription factor Nkx2.2, and was named pTH-R [rostral (=anterior) progenitor domain of the thalamus; Fig. 2C and E]. Subsequent studies showed that *Gsx1*, *Tal1* and *Tal2* (Bucher *et al.*, 2000; Kataoka & Shimogori, 2008; Jeong *et al.*, 2011) are also expressed in this progenitor cell domain. This domain is also known as the rim domain (Kataoka & Shimogori, 2008), rT (Scholpp & Lumsden, 2010) and the caudal shell of the ZLI (Garcia-Calero *et al.*, 2006; also Fig. 2C). The larger posterior domain, pTH-C (C is for caudal, which is the same as posterior) or cT (Scholpp & Lumsden, 2010), expressed bHLH factors Neurog1 (Neurogenin 1), Neurog2 (Neurogenin 2) and Olig2, as well as Dbx1 (Fig. 2C and E). Olig2 showed higher expression anteroventrally within the pTH-C domain, while Dbx1 showed the oppositely graded pattern (Fig. 2E; Vue *et al.*, 2007). Suzuki-Hirano *et al.* (2011) did a large-scale gene expression analysis in embryonic mouse thalamus and found a number of other genes that are expressed in pTH-C progenitor cells but not in pTH-R, including *Barhl2*, *Ddc* and *D2R*, of which *Ddc* also showed a expression gradient within pTH-C similar to that of Olig2. The domain-specific patterns of gene expression appear as early as at embryonic day (E)10.5, soon after the formation of the ZLI, and are most prominent

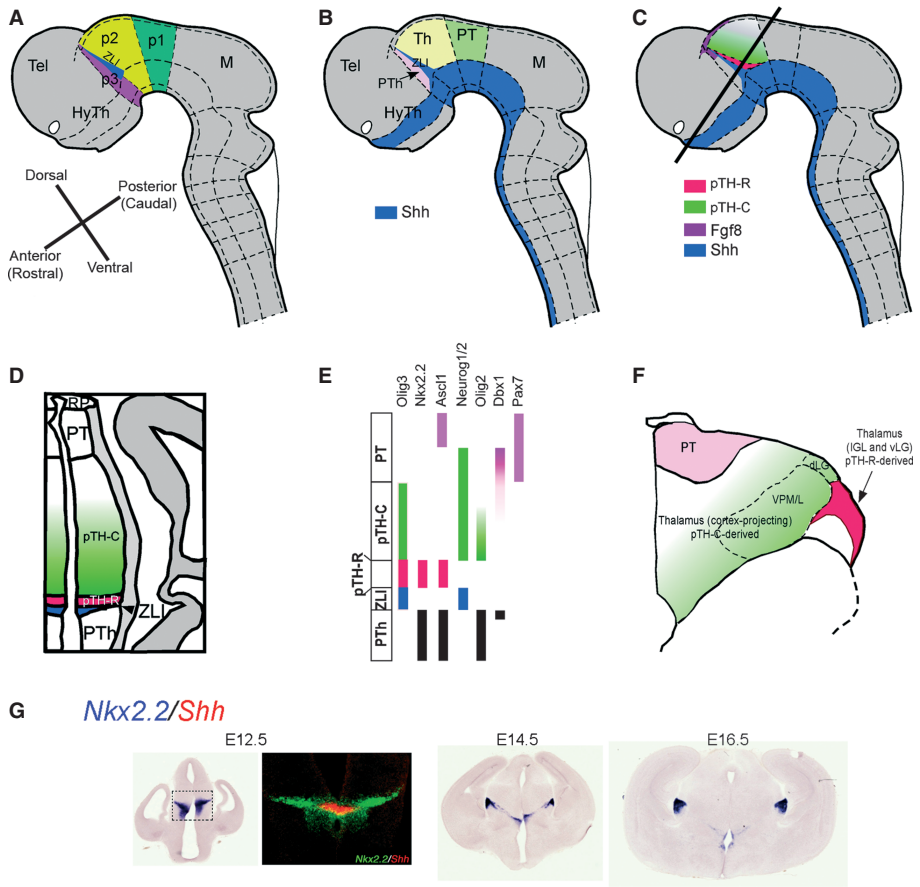

FIG. 2. Progenitor domains of developing diencephalon. (A) Lateral view of embryonic mouse forebrain. The diencephalon is subdivided into transverse domains p1, p2 and p3, from posterior to anterior. AP and DV axes around the thalamus are also shown. (B) The pretectum (PT), thalamus (Th) and prethalamus (PTh) are located in the alar plate of p1, p2 and p3, respectively. The ZLI forms a transverse boundary in the alar plate between the thalamus and the prethalamus. (C) The thalamic progenitor domain is further divided into two subdomains, pTH-R and pTH-C. pTH-R is located anteroventrally, close to the ZLI and the basal plate, where Shh is expressed. pTH-C is a larger domain located more posterodorsally. The gradient in green within the pTH-C domain exhibits graded patterns of various genes, indicating its heterogeneity shown in E. For example, Olig2 is highly expressed in the dark green region and Dbx1 is highly expressed in the light green region. Tel, telencephalon; HyTh, hypothalamus; M, midbrain. The black line in C indicates the horizontal plane that is used to cut the schematic section shown in D. (D–F) Differential expression of transcription factors in discrete progenitor domains of the embryonic thalamus (E) and the findings of genetic lineage tracing of thalamic progenitor cells (F). (G) Expression patterns of *Nkx2.2* in the thalamus and prethalamus at E12.5, E14.5 and E16.5. At E12.5, *Nkx2.2* is initially expressed in progenitor cells in the thalamic pTH-R domain and the prethalamus, as indicated by a double *in situ* hybridization with *Shh*. As progenitor cells differentiate into neurons, expression of *Nkx2.2* persists in these cells, which later populate the IGL and vLG.

during thalamic neurogenesis. Collectively, these studies demonstrate that thalamic progenitor cells are spatially heterogeneous and such heterogeneity is likely to contribute to the vast diversity of thalamic neurons.

Cell lineages in the developing thalamus

In order to understand how patterning of the thalamus and the spatial heterogeneity of thalamic progenitor cells contribute to the generation of neuronal diversity and distinct nuclei, it is crucial to understand the lineage relationship between each of the thalamic progenitor cell populations and their postmitotic progeny. Earlier studies took advantage of replication-incompetent retrovirus and labeled diencephalic progenitor cells of chick embryos to trace the migratory behavior of newly born thalamic neurons (Golden & Cepko, 1996; Golden *et al.*, 1997). They found that about three-quarters of the identified clones were composed of radially arranged neurons, whereas the rest of the clones showed evidence of tangential migration. Analysis of cell cohorts at later stages found that two or more postmitotic thalamic nuclei were often populated by neurons derived from single clones. These studies indicate the presence of postmitotic mechanisms that serve to specify and sort neurons into particular nuclei. The studies also implied that some progenitor cells may divide more than once to generate neurons that are destined to contribute to different nuclei.

More recently, genetic approaches have been used in mice to analyze the postmitotic fates of progenitor cells that express specific genes (summarized in Fig. 2D–F). For example, *Neurog2-EGFP* knock-in mice and *Neurog1-EGFP* BAC transgenic mice were used as short-term lineage tracers to demonstrate that pTH-C progenitor cells later contribute to all of the cortex-projecting thalamic nuclei (Vue *et al.*, 2007). Kim *et al.* (2011) used *Neurog1-CreER* mice to confirm the above results with a more permanent method of lineage tracing. In addition, two markers that show graded patterns of expression within the pTH-C domain were used to test the hypothesis that progenitor cells at different locations within this domain give rise to specific sets of postmitotic thalamic nuclei. Analysis of *Olig2-EGFP* knock-in mice showed that anteroventrally located pTH-C progenitor cells preferentially contribute to principal sensory nuclei, which are located anteriorly or ventrally in the mature thalamus (Vue *et al.*, 2007;

Puelles *et al.*, 2011b). In addition, expression of β-galactisidase in *Dbx1-LacZ* knock-in mice indicated that posterodorsal pTH-C progenitor cells generate nuclei that are located more posterodorsally than those derived from Olig2-expressing progenitor cells (Vue *et al.*, 2007). These results suggest that positions of progenitor cells provide a broad map for their descendent neurons, but other mechanisms may regulate the precise sorting of neurons into nuclei.

Fates of the pTH-R progenitor cells were studied in *Olig3-EGFP*, *Ascl1-EGFP* and *Tal1-CreER* mice, which collectively showed that the pTH-R domain contributes to the intergeniculate leaflet (IGL) and part of the ventral lateral geniculate (vLG) nucleus (Vue *et al.*, 2007; Jeong *et al.*, 2011). On the other hand, other parts of vLG nucleus are derived from the prethalamic lineage expressing Dlx2-Dlx5/6 (Jeong *et al.*, 2011), demonstrating the multiple embryonic origins of this nuclear complex. *Neurog1-EGFP, Neurog2-EGFP*, *Shh-Cre* and *Pitx2-Cre* mice provided evidence that the ZLI probably contribute to the cell population that is located between the thalamus and the prethalamus, which could be called the external medullary lamina or nuclei of the zona limitans (Vue *et al.*, 2007; Jeong *et al.*, 2011; Suzuki-Hirano *et al.*, 2011). Interestingly, the nuclei that are derived from pTH-R and the ZLI all consist of GABAergic neurons, and Ascl1-expressing progenitor cells contribute not only to IGL and vLG, but also to scattered cells within dLG and further along the lateral surface of the thalamus, leading to a population of cells between the thalamus and the habenula (Vue *et al.*, 2007). This region is also rich in GABAergic neurons (Y. Nakagawa, unpublished observations). Therefore, it is possible that pTH-R progenitor cells broadly contribute to most of the GABAergic neurons within the entire thalamic territory, which fits with the observed tangential migration in chick embryos (Golden & Cepko, 1996; Golden *et al.*, 1997). However, because Ascl1 is also expressed in the prethalamus and the pretectum, more studies are needed to restrict the lineage labeling in genetic tracing experiments. Results of genetic lineage tracing of pTH-R and pTH-C progenitor domains are summarized in Fig. 2F. These results are consistent with studies of gene expression at different embryonic stages. For example, Kitamura *et al.* (1997) showed the time-course of the expression of *Shh*, *Brx1* (=*Pitx2*), *Nkx2.2*, *Arx* and *Dlx1* from E8.5 to E18 in mice and described the relationship between early and late structures in the thalamus, ZLI and prethalamus. Of these genes, *Nkx2.2* is initially expressed in progenitor cells of the pTH-R domain but later found in postmitotic nuclei of vLG and IGL, which corresponds to the cell lineage determined by genetic tracing methods (Fig. 2G; also Kataoka and Shiomogori, 2008; Suzuki-Hirano *et al.*, 2011).

Results of the genetic lineage tracing studies in mice highlight intriguing coincidences with studies in chick embryos. Earlier studies examined a specific cell lineage within the chick thalamus that may generate anteroventrally located neuronal groups (Uchikawa *et al.*, 1999; Redies *et al.*, 2000; Yoon *et al.*, 2000; Martinez-de-la-Torre *et al.*, 2002; Hashimoto-Torii *et al.*, 2003; Garcia-Lopez *et al.*, 2004). This cell lineage expresses Cad6B, *Sox21*, *Sox14* and *Nkx2.2*, and sequential analysis of gene expression and fate mapping with quail-chick grafts indicated its relationship with postmitotic nuclei such as the internal nucleus of the optic tract (ITO) and perirotundic area (ApR) in the chick thalamus. Both *Sox14* and *Nkx2.2* are also expressed in pTH-R cell lineage in mice (Kataoka & Shimogori, 2008), and both the pTH-R domain in mice and the anteroventral cell lineage in chick contribute to nuclei that receive non-topographic retinal projections and project to the midbrain (IGL in mice, ITO and ApR in chick). ITO and ApR are also located between the chick equivalents of dLG and vLG (Martinez *et al.*, 1991; Martinez-de-la-Torre *et al.*, 2002). Thus, despite the apparent difference in nuclear organization of the thalamus (Vieira *et al.*, 2010; Puelles *et al.*, 2011b), the pTH-R–anteroventral thalamic lineage contributes to a molecularly and anatomically conserved set of postmitotic neuronal populations in mice and chick. Additionally, Scholpp *et al.* (2009) showed that a similar domain also exists in zebrafish, demonstrating the conserved organization of thalamic progenitor domains among many vertebrate species.

Signaling molecules and thalamic patterning

Recent studies have identified a number of molecules that may control the patterning of the diencephalon in mice (Miyashita-Lin *et al.*, 1999; Fode *et al.*, 2000; Nakagawa & O'Leary, 2001; Suda *et al.*, 2001; Nakagawa & O'Leary D, 2003; Suzuki-Hirano *et al.*, 2011; Yuge *et al.*, 2011), monkey (Jones & Rubenstein, 2004) and chick (Kobayashi *et al.*, 2002; Lim & Golden, 2007). It has been suggested that the ZLI is a physical boundary that separates the alar plate of p3 from the alar plate of p2 (Larsen *et al.*, 2001), and may also function as a secondary organizer (Vieira *et al.*, 2005). Shh is expressed in both the ZLI and the basal plate, and is a key signaling molecule that patterns the thalamus in mice (Ishibashi & McMahon, 2002), chick (Hashimoto-Torii *et al.*, 2003; Kiecker & Lumsden, 2004; Vieira *et al.*, 2005; Zeltser, 2005; Lim & Golden, 2007) and zebrafish (Scholpp *et al.*, 2006; also Fig. 2B and C). Additionally, Wnt expression in the thalamus is also required for normal development, especially for the establishment of the regional thalamic identity (Braun *et al.*, 2003; Zhou *et al.*, 2004). Finally, *Fgf8* controls AP polarity of the thalamus (Kataoka & Shimogori, 2008). The contributions of each of these signaling molecules to thalamic development are further described in the sections below.

Shh functions in thalamic patterning

Shh is a secreted protein that plays numerous roles in development and disease. During early stages of embryonic development, *Shh* is expressed in the axial mesoendoderm underlying the neural plate. This early non-neural expression later induces neural expression of *Shh* in the ventral part of the brain and the spinal cord, including the basal plate and the floor plate. Many studies have demonstrated that graded Shh signaling plays a crucial part in DV patterning of the entire central nervous system (CNS; Jessell, 2000; Hebert & Fishell, 2008).

During thalamic development in mice, *Shh* is expressed initially in the notochord and then in the basal plate, followed by the induction in the newly formed ZLI by E10.5 (Shimamura *et al.*, 1995; Fig. 2B and C). This temporal and spatial pattern of *Shh* expression makes it a likely candidate molecule in many aspects of thalamic development. *In ovo* electroporation and grafting studies in chick showed that ectopic expression of Shh in the caudal diencephalon and mesencephalon induces the expression of *Gbx2* and reduces *Pax6* (Kiecker & Lumsden, 2004; Vieira *et al.*, 2005). Conversely, inhibition of Shh signaling by a dominant negative form of the Shh receptor Ptch1 reduced *Nkx2.2, Ptch1* and *Gbx2* expression (Kiecker & Lumsden, 2004). Together, these studies establish the importance of Shh signaling in the global regionalization of the diencephalon, particularly its role in specifying the identity of the thalamus as a whole. Germline mutation of *Shh* in mice caused a lack of the thalamic marker genes *Gbx2* and *Dbx1*, indicating the early role of Shh signaling in the formation of the thalamic anlage (Ishibashi & McMahon, 2002). These studies, however, did not clearly address whether Shh signaling plays a role in specifying positional identities of progenitor cells after the thalamic identity is established and the ZLI is formed. During thalamic neurogenesis, which occurs mainly between E10.5 and E12.5, Shh signaling as revealed by the expression of its downstream target genes *Ptch1* and *Gli1* shows a graded pattern across thalamic progenitor

domains (Vue *et al.*, 2009). It is strong in the pTH-R domain and gradually attenuates caudally and dorsally within pTH-C (Fig. 3A and C). Recent studies described below used conditional genetic manipulations in mice in order to address the roles of Shh signaling in thalamic development in more specific temporal and spatial contexts.

Szabo *et al.* (2009) used the *Foxb1-Cre* allele to conditionally knock out *Shh*. *Foxb1* is expressed broadly in caudal forebrain including p1 to p3 by E10.0. As a result, these mutants lost *Shh* expression in the diencephalic basal plate by E10.0. Expression of *Nkx2.2* and *Ptch1*, the direct target genes of Shh signaling, were completely missing in the thalamus, and there was no indication that the ZLI was formed in *Foxb1-Cre/*Shh mutant mice. Despite this decrease in Shh signaling, the thalamus was partially specified and expressed *Gbx2* and *Dbx1* in reduced domains at E12.5. At E18.5, postmitotic thalamic nuclei were grossly abnormal in their size and gene expression, although medially located nuclei such as the paraventricular, mediodorsal, centromedial and centrolateral were less affected. In addition to these phenotypes, axonal projections from the thalamus to the cortex were completely missing in the conditional *Shh* mutant mice.

Vue *et al.* (2009) used the *Nestin-Cre* allele to conditionally reduce or enhance the level of Shh signaling starting at E10.5, after the regional thalamic identity is established and the ZLI is formed. They showed that high Shh signaling specifies the fate of pTH-R and the anteroventral part of the pTH-C domain. For instance, markers for the pTH-R domain and the anteroventral part of the pTH-C domain were completely missing in the conditional *Shh* mutant mice, whereas

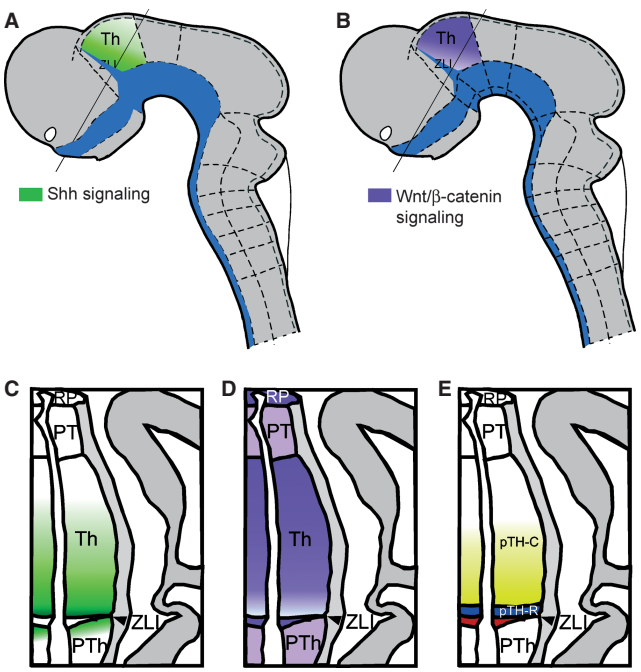

FIG. 3. Differential Shh and Wnt–*β*-catenin signaling in thalamic progenitor cells. (A) Lateral view of embryonic mouse forebrain. The gradient in green shows Shh signaling as indicated by expression of Shh target genes *Ptch1* and *Gli1* (Vue *et al.*, 2009). Approximate section plane in C is indicated by a black line. (B) The gradient shown in purple shows Wnt–*β*-catenin signaling as indicated by expression of its target gene *Axin2* and *Bat-gal* transgene activity (Bluske *et al.*, 2009). The signal is stronger in the posterodorsal part of the thalamus. Approximate section plane in D is indicated by a black line. (C) Graded Shh signaling within the thalamus on near-horizontal section shown by a black line in A. (D) Graded Wnt–*β*-catenin signaling within the thalamus on near-horizontal section shown by a black line in B. (E) The same section plane as C and D, indicating the positions of two progenitor domains, pTH-R and pTH-C. RP, roof plate; PT, pretectum.

ectopic expression of the constitutively active mutant form of the transmembrane signal transducer Smoothened (SmoM2) resulted in the expansion of the pTH-R domain and anteroventral pTH-C identity. In addition to *Nestin-Cre* mice, Vue *et al.* (2009) used *Olig3-Cre* mice to temporally and spatially enhance Shh signaling specifically in thalamic progenitor cells and the ZLI. When SmoM2 was ectopically expressed only in the thalamus using *Olig3-Cre* mice or by *in utero* electroporation, the anteroventral identity of the pTH-C domain was over-represented throughout the thalamic ventricular zone at the expense of posterodorsal identity. The opposite change was observed when the Shh or Smo gene was deleted with either the *Nestin-Cre* or the *Olig3-Cre* line. Consistent with the lineage tracing studies described in the previous section, alteration of the positional identity of thalamic progenitor cells resulted in corresponding changes in their descendent nuclei at later stages of development. For example, expansion or shrinkage of the pTH-R domain caused a change in the sizes of IGL and vLG nuclei, whereas changes in the anteroventral part of pTH-C was accompanied by alteration in the sizes of principal sensory nuclei, particularly dLG.

Jeong *et al.* (2011) investigated the specific roles of Shh expressed in the diencephalic basal plate by taking advantage of their previous discovery (Jeong *et al.*, 2006) of a distinct 525-bp intronic sequence named Shh brain enhancer-1 (*SBE1*). This sequence mediates *Shh* expression in the caudal diencephalic basal plate. Jeong and colleague generated a mouse line with targeted deletion of *SBE1* and found that *Shh* transcription was initiated in the p2 basal plate but was not maintained, yet its expression in the ZLI was unaffected (Jeong *et al.*, 2011). In the absence of basal plate Shh, pTH-R showed a fate switch to pTH-C. Postmitotic derivatives of pTH-R were also depleted in *SBE1*-deleted mice. This and other studies (Kiecker & Lumsden, 2004; Vieira & Martinez, 2006) collectively indicate that, at least in mice and chick (see Scholpp *et al.*, 2006 for the difference in zebrafish), Shh from both the ZLI and the basal plate is required for the correct specification of the pTH-R progenitor domain.

Although the above studies demonstrated the critical roles of Shh signaling in thalamic regional identity and patterning, many important questions still remain. For example, the precise roles of ZLI-derived Shh in AP patterning of the thalamus have not clearly demonstrated. A study in chick (Vieira & Martinez, 2006) used a micro-barrier to physically separate the thalamus from the ZLI and showed that anteriorly located thalamic nuclei often failed to form without the influences of factors derived from the ZLI or the prethalamus. However, as discussed below, ZLI and the neighboring prethalamus express other signaling molecules such as Wnts and Fgfs, which have also been shown to have important roles for diencephalic development. Thus, specific deletion of *Shh* in the ZLI will be needed to clearly demonstrate its functions. In addition, we do not know whether the ZLI-derived Shh regulates the AP patterning in broader diencephalic domains such as the prethalamus and the pretectum. Lastly, two studies discussed above (Szabo *et al.*, 2009; Jeong *et al.*, 2011) proposed that Shh plays a role in postmitotic thalamic neurons for their specification, migration or aggregation into nuclei or axonal projections to the cortex. These functions will need to be directly tested by more restricted manipulations of Shh signaling in postmitotic neurons.

Wnt function in thalamic patterning

Wnts are a family of secreted proteins that have important roles in many tissues including the developing central nervous system. Many Wnt ligands are expressed in the dorsalmost part of the brain and spinal cord, and Wnt signaling, through its downstream effector *β*-catenin, plays a role in dorsalizing the neural tissue and antagonizes the ventralizing effects of Shh signaling (Ulloa & Marti, 2010).

Wnts and their intracellular signaling components are expressed in the diencephalon in discreet spatial and temporal patterns throughout embryogenesis. During early forebrain development in the chick, two Wnt ligands, *Wnt11* and *Wnt8c*, are expressed in the caudal paraxial mesoderm underlying the prospective caudal neural plate (Nordstrom *et al.*, 2002). Explant cultures and *in ovo* electroporation in chick embryos showed that Wnt–β-catenin signaling is critical for establishing the identity of the caudal diencephalon including the thalamus (Braun *et al.*, 2003). Mice with germline deletion of *Lrp6*, a Wnt co-receptor gene, showed transformation of thalamic progenitor cells into those of the prethalamus, as well as a lack of ZLI formation (Zhou *et al.*, 2004). These results indicate the importance of Wnt–β-catenin signaling in the initial establishment of the thalamic identity.

As the thalamus is specified, Wnt ligands are now induced within the thalamus itself and the ZLI. For example, *Wnt3* is induced in the entire thalamus in both chick and mice (Roelink & Nusse, 1991; Braun *et al.*, 2003). *Wnt3a* has a broad dorsal expression that extends in a wedge-shaped pattern ventrally towards the ZLI at E10.5 in mice (Roelink & Nusse, 1991; Louvi *et al.*, 2007). *Wnt7b* shows an expression pattern that is complementary to that of *Wnt3a*. By E11.5, *Wnt3a* expression is restricted to the dorsal midline and the ZLI (Bluske *et al.*, 2009). These *Wnt* genes also show dynamic expression patterns in chick (Quinlan *et al.*, 2009). Studies by Quinlan *et al.* (2009) and Bluske *et al.* (2009) analyzed expression patterns of Wnt receptors, inhibitors and transcription factors that mediate Wnt–β-catenin signaling in thalamic progenitor cells at different embryonic stages in chick and mouse embryos respectively. Their expression showed spatially and temporally dynamic patterns, which suggests that Wnt–β-catenin signaling plays multiple roles in different aspects of thalamic development after the establishment of thalamic identity. Analysis of β-galactosidase expression in *BAT-gal* transgenic mice and *Axin2* expression, both of which reflect activity of Wnt–β-catenin signaling, demonstrates differential patterns within the thalamus during neurogenesis: high in the dorsal and low in the ventral region of the pTH-C domain, and also low in the pTH-R domain (Fig. 3B and D). This pattern Wnt–β-catenin signaling predicts its roles in thalamic development (Bluske *et al.*, 2009). Detailed functional studies in chick and mice are in progress. Activity of Wnt–β-catenin and Shh signaling pathways shows partially reciprocal patterns in the thalamus (Bluske *et al.*, 2009), raising the possibility that these two pathways antagonize each other. A recent report by Quinlan shows that Shh signal represses the expression of one of the Wnt ligands, *Wnt4*, in the chick thalamus (Quinlan *et al.*, 2009), while elevating or reducing Shh signaling in mouse thalamus did not significantly change the pattern of *Bat-gal* transgene expression (Bluske *et al.*, 2009). Further studies are needed to delineate the interactions of Shh and Wnt signaling at the molecular level (Ulloa & Marti, 2010).

FGF8 function in thalamic patterning

Fgf8 expression in the diencephalon starts at E10.5 close to the dorsal midline of p2, which extends towards the ZLI (defined as F11 in chick; Crossley *et al.*, 2001). Direct comparison of *Shh* and *Fgf8* expression in the p2–p3 region revealed that the *Fgf8* expression toward ZLI was exclusive and slightly anterior to Shh expression (Fig. 4A and B). *Fgf17* and *Fgf18*, members of the Fgf8 subfamily with similar receptor affinities and functions in other systems, are also expressed in the diencephalon in similar manners, which suggests that multiple ligands might contribute to FGF activity in the developing diencephalon. Analysis of *Fgf8* hypomorphic mouse lines with reduced levels of *Fgf8* (*Fgf8*$^{neo/neo}$ and *Fgf8*$^{null/neo}$; Meyers *et al.*, 1998) demonstrated

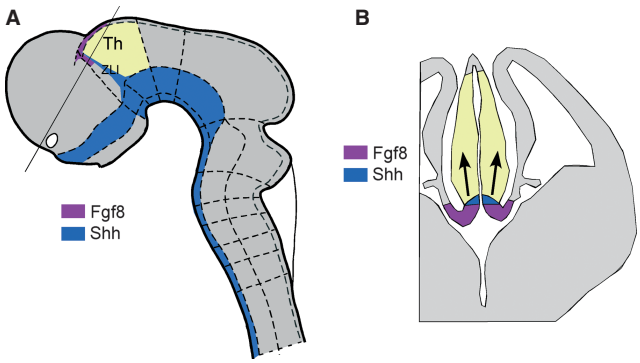

FIG. 4. Positional relationship between Fgf8 and Shh near the thalamus. (A) Lateral view of embryonic mouse forebrain. Fgf8 is expressed in an L-shaped region surrounding p2. Fgf8 expression (shown in pink) is found immediately anterior to the ZLI in the dorsal region of the prethalamus and the dorsalmost part of p2. Shh expression is shown in blue. Approximate section plane in B is indicated by a black line. (B) Location of Fgf8 and Shh expression on near-horizontal section through the dorsal p2. Fgf8 and Shh are expressed in adjacent and mutually exclusive domains.

that normal expression of *Fgf8* is necessary for the development of the habenula and pineal gland in a dose-dependent manner (Martinez-Ferre & Martinez, 2009). Ectopic Fgf8 expression in the p2 domain converts the diencephalon into midbrain and hindbrain in chick (Crossley *et al.*, 2001) and mouse (Lee *et al.*, 1997; Liu *et al.*, 1999). Therefore, it is peculiar that endogenous Fgf8 in the dorsal midline of p2 controls growth of the habenula but not does not convert it into midbrain and hindbrain. Testing expression patterns of Fgfs downstream genes, such as *sprouty1*, *ERK* and ETS transcription factors, revealed that there is no strong Fgfs activity in the habenular region (A. Suzuki-Hirano and T. Shimogori, unpublished data). It is possible that the habenular region has region-specific competence in responding to Fgf8 activity.

To test the function of Fgf8 expressed just anterior to the ZLI, focal *in utero* electroporation was employed to manipulate Fgf8 activity only in the p3 region (Shimogori & Ogawa, 2008). Overexpression of Fgf8 to increase Fgf8 activity expanded the pTH-R domain and shrank the pTH-C region (Kataoka & Shimogori, 2008). Introducing truncated Fgfr3 construct, to inhibit locally expressed endogenous *Fgf8*, caused shrinkage of the pTH-R domain and expanded the remaining pTH-C. Expansion of Fgf8 activity shifted the thalamic sensory nuclei, including the VP, dLG and other nuclei such as vLG and the external medullary lamina, which are derived from the pTH-R domain, along the AP axis in the postnatal brain (Kataoka & Shimogori, 2008). These results suggest that Fgf8 activity originating in p3 controls the AP pattern of thalamic nuclei; however, its downstream pathway is still unknown.

Transcription factors regulate neuronal identity in the thalamus

In mice lacking functional orthodenticle homolog (Otx2) there was ectopic activation of Pax3, Pax7, Ascl1 and Lhx1 within the thalamic pTH-C domain at the expense of Neurog2, and this gave rise to a marked increase in proliferating activity of thalamic progenitors and the formation of hyperplastic cell masses (Puelles *et al.*, 2006). This fate-switch of progenitor cells was accompanied by the induction of GABAergic neurons which replaced glutamatergic neurons, indicating the normal functions of Otx2 in preventing the GABAergic fate. It is currently unknown how Otx2 interacts with signaling pathway(s) of patterning molecules of the thalamus.

However, it is suggested that proper assignment of identity and fate of neuronal precursors in the thalamus occurs through alternative differentiation programs.

Another crucial transcription factor in thalamic development, Gbx2, is a homeobox transcription factor expressed in early diencephalon and has an important role in regulating the formation of lineage-restriction boundaries of the thalamus (Chen *et al.*, 2009). *Gbx2*-expressing neurons in mouse diencephalon initially contribute to the entire thalamic nuclear complex. However, later in development, *Gbx2* is downregulated in anteriorly and laterally located nuclei including dLG and VP. *Gbx2*-expressing postmitotic neurons form sharp lineage-restriction boundaries delineating the thalamus from the pretectum, habenula and prethalamus, revealing multiple compartmental boundaries within the mouse diencephalon. Without *Gbx2*, cells originating from the thalamus abnormally contribute to the habenula and pretectum (Chen *et al.*, 2009). Chimeric and genetic mosaic analysis has demonstrated that Gbx2 plays a non cell-autonomous role in controlling the segregation of postmitotic thalamic neurons from the neighboring brain structures that do not express *Gbx2* (Chen *et al.*, 2009). Based on these results, Chen and colleagues speculated that expression of *Gbx2* allows the thalamus as a whole to be segregated from the neighboring structures that do not express *Gbx2*, while within the thalamus the dynamic and differential expression of *Gbx2* may lead to segregation of *Gbx2*-positive neurons into nuclei. In addition to these roles, *Gbx2* is required for survival of thalamic neurons (Szabo *et al.*, 2009) and projections of thalamocortical axons to the cortex (Miyashita-Lin *et al.*, 1999).

Finally, Pax6 is expressed broadly in diencephalic progenitor cells at E10.5; it is thereafter downregulated, especially in anteroventrally located thalamic progenitor cells. Mice lacking functional *Pax6* (small-eye homozygotes: *Sey*/Sey) exhibit defects in thalamocortical axons and abnormalities of thalamic patterning (Pratt *et al.*, 2000). The pTH-R marker *Nkx2.2* is expanded in the *Sey*/Sey thalamus, and *Shh* expression is also expanded in the ZLI (Pratt *et al.*, 2000). Furthermore, in these mice, increased *Fgf8* expression is detected in the diencephalon, and this might be causing the expansion of pTH-R (A. Kataoka and T. Shimogori, unpublished data). Thus, Pax6 may play a role in patterning the thalamus by controlling the expression of signaling molecules such as Shh and Fgf8.

Temporal diversity of thalamic progenitor cells

Classical studies by Angevine (1970) and Altman & Bayer (1979, 1988a,b,c, 1989a,b,c) used thymidine autoradiography to determine the birthdates of neurons that later populated distinct thalamic nuclei in mice and rats. In general, neurons of the principal sensory nuclei are generated early (E10.5-11.5 in mice), while neurons in other nuclei are born towards the end of thalamic neurogenesis, with a peak at E12.5 (Angevine, 1970). Although the generation of thalamic nuclei does not follow a strict spatial–temporal order, it is possible that the same progenitor cell could generate neurons with two sequential rounds of cell division and possibly contribute to different nuclei. Other regions in the central nervous system such as the neocortex and the retina use this strategy to generate neuronal diversity (Pearson & Doe, 2004; Lui *et al.*, 2011).

In addition to the different timing of cell division, the neocortex could also generate neuronal diversity by employing neural progenitor cells that exhibit different modes of division (Pontious *et al.*, 2008; Lui *et al.*, 2011). For example, radial glial cells divide asymmetrically at the ventricular surface and generate one neuron with each division, whereas intermediate progenitor cells divide away from the lateral ventricle and generate a pair of neurons (Haubensak *et al.*, 2004; Miyata *et al.*, 2004; Noctor *et al.*, 2004). It would be interesting to address whether these two progenitor populations contribute to distinct neuronal types in the neocortex. The thalamus was also long known to exhibit cell divisions away from the third ventricle (Smart, 1972), and Wang *et al.* (2011) recently described detailed gene expression and cell cycle characteristics of these basal progenitor cells in the thalamus (Wang *et al.*, 2011). The thalamic basal progenitor cells partially share molecular markers with their neocortical counterpart but express distinct set of genes and also show different requirement of Pax6 and neurogenins for their generation and/or maintenance. Whether the thalamic radial glia and basal progenitor cells also differentially contribute to neuronal diversity remains to be clarified.

Summary

Locally expressed signaling molecules in developing CNS are critical for spatial patterning in the developing neuroepithelium. These signaling molecules control the expression of downstream transcription factors that provide spatial information appropriate for the location of neural progenitor cells along the AP and DV axes. Such transcription factors in turn control the expression of signaling molecules, thus forming a signaling network essential for the establishment of spatial diversity in neural progenitor cells.

Recent studies have shown that such a general concept of early neural development applies to thalamic patterning as well. The thalamus further provides a unique system that allows us to study how a complex array of neuronal types that form distinct nuclei are generated from spatially and temporally heterogeneous neural progenitor cells within the p2 alar plate. From the patterning perspective, Shh, Fgfs and Wnts are presented in unique three-dimensional patterns surrounding the early thalamic tissue, and the emerging data suggest that they all play critical and distinct roles in thalamic development. Further studies are needed to reveal how these signaling pathways interact with each other and regulate downstream genes. In addition, our knowledge is limited as to how the diversity of thalamic progenitor cells later gives rise to the distinct neuronal populations that form both distinct and continuous neural maps with other brain regions, especially with the neocortex. Thus, future studies on early thalamic development may open the door to our understanding of mechanisms of brain wiring and how such mechanisms differ between species and between normal and pathological conditions.

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Abbreviations

AP, anterior–posterior; CNS, central nervous system; dLG, dorsal lateral geniculate; DV, dorsal–ventral; E, embryonic day; IGL, intergeniculate leaflet; MGv, ventral part of the medial geniculate nucleus; p1, prosomere 1; p2, prosomere 2; p3, prosomere 3; pTH-C, caudal progenitor domain of the thalamus; pTH-R, rostral progenitor domain of the thalamus; Shh, Sonic hedgehog; vLG, ventral lateral geniculate; VP, ventral posterior; ZLI, zona limitans intrathalamica.

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