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ScienceDirect
Developmental Biology 302 (2007) 345–355
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Gli2 and *Gli3* play distinct roles in the dorsoventral patterning of the mouse hindbrain

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Received for publication 29 March 2006; revised 1 August 2006; accepted 2 August 2006
Available online 9 August 2006

Abstract

Sonic Hedgehog (Shh) signaling plays a critical role during dorsoventral (DV) patterning of the developing neural tube by modulating the expression of neural patterning genes. Overlapping activator functions of Gli2 and Gli3 have been shown to be required for motoneuron development and correct neural patterning in the ventral spinal cord. However, the role of Gli2 and Gli3 in ventral hindbrain development is unclear. In this paper, we have examined DV patterning of the hindbrain of $Shh^{-/-}$, $Gli2^{-/-}$ and $Gli3^{-/-}$ embryos, and found that the respective role of *Gli2* and *Gli3* is not only different between the hindbrain and spinal cord, but also at distinct rostrocaudal levels of the hindbrain. Remarkably, the anterior hindbrain of $Gli2^{-/-}$ embryos displays ventral patterning defects as severe as those observed in $Shh^{-/-}$ embryos suggesting that, unlike in the spinal cord and posterior hindbrain, Gli3 cannot compensate for the loss of Gli2 activator function in Shh-dependent ventral patterning of the anterior hindbrain. Loss of Gli3 also results in a distinct patterning defect in the anterior hindbrain, including dorsal expansion of *Nkx6.1* expression. Furthermore, we demonstrate that ventral patterning of rhombomere 4 is less affected by loss of Gli2 function revealing a different requirement for Gli proteins in this rhombomere. Taken together, these observations indicate that *Gli2* and *Gli3* perform rhombomere-specific function during DV patterning of the hindbrain.

Keywords: Sonic hedgehog; Gli transcription factors; Neural tube; Hindbrain; Dorsoventral patterning; Rhombomere; Mouse

Introduction

During development of the vertebrate nervous system, neural progenitors are specified according to their positions along the antero-posterior (AP) and dorsoventral (DV) axes of the neural tube. The DV axis of the neural tube is established by dorsally derived signals, such as Bone Morphogenetic Proteins (BMPs), and ventrally derived signals, including Sonic hedgehog (Shh). In the ventral neural tube, Shh signal modulates the expression of several patterning genes (Figs. 1A, B). The current model suggests that the class I patterning genes, such as *Pax6* and *Irx3*, are negatively regulated by Shh and their expression is excluded from the ventral parts of the neural tube. On the other hand, the class II patterning genes, including *Nkx6.1* and *Olig2*, are positively regulated by Shh and are expressed in the ventral region of the neural tube. It was also suggested that pairs of class I and class II genes repress each other, leading to sharp expression borders. The profile of expression of these patterning genes forms a combinatorial code that leads to the specification of distinct progenitor domains within the ventral neural tube. For example, $Pax6^{+}$, $Nkx6.1^{+}$, $Olig2^{+}$ and $Irx3^{-}$ neural progenitors will become motoneurons while $Pax6^{+}$, $Nkx6.1^{+}$, $Olig2^{-}$ and $Irx3^{+}$ progenitor cells will form V2 interneurons (reviewed in Wilson and Maden, 2005). Consistent with this model, $Shh^{-/-}$ neural tube showed no expression of class II patterning genes, which results in a complete absence of motoneurons as well as V2 and V3 interneurons (Chiang et al., 1996).

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0012-1606/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2006.08.005

In *Drosophila*, Hedgehog (Hh) signal is transduced by a single bi-functional Gli transcription factor, Cubitus interruptus (Ci) (Méthot and Basler, 2001). In the mouse, Hh signaling is mediated by the combinatorial activity of three Gli transcription factors, Gli1, Gli2 and Gli3. Biochemical studies have shown that Gli2 and Gli3 each contain both activation and repression domains, although Gli2 is a stronger activator than repressor while Gli3 is a stronger repressor than activator (Sasaki et al., 1999). In contrast, Gli1 contains only activation domains and appears to be exclusively a transcriptional activator (Dai et al., 1999; Ruiz i Altaba, 1999). Genetic analysis of *Gli* mutant mice showed that Gli1 does not play a primary role in Shh signaling during development (Park et al., 2000). Gli2 plays an activator role in the ventral spinal cord; *Gli2*$^{-/-}$ mice lack a floor plate and most V3 interneurons, two structures requiring the highest Shh response for their specification (Ding et al., 1998; Matise et al., 1998). *Gli3*$^{-/-}$ mice do not exhibit defect in the ventral spinal cord but its repressor function is required for the correct specification of the ventro-lateral progenitor domains (p0, p1 as well as progenitors of dl4 to dl6 dorsal interneurons) (Persson et al., 2002; reviewed in Jacob and Briscoe, 2003). Mutant analysis of compound mutant mice has revealed a functional redundancy between *Gli2* and *Gli3* in the patterning of the ventral spinal cord. Overlapping functions of *Gli2* and *Gli3* are required for the correct organization of the ventral spinal cord as well as the formation of motoneurons (Motoyama et al., 2003; Bai et al., 2004; Lei et al., 2004). Furthermore, the knock in of *Gli3* into the *Gli2* locus produced a substantial, though incomplete, rescue of the *Gli2*$^{-/-}$ phenotype (Bai et al., 2004). Together, these experiments established that *Gli3* could function as an activator in ventral neural tube patterning when *Gli2* function is eliminated. On the other hand, removal of *Gli3* rescues the specification of several ventral neural cell types in *Shh*$^{-/-}$ mice, indicating that a critical function of Shh is to suppress *Gli3* repressor function in the ventral neural tube. In *Shh*$^{-/-}$ mice, the repressor form of Gli3 is not restricted anymore to the dorsal neural tube and is present in the ventral neural tube, which suppresses the formation of ventral cell types (Litingtung and Chiang, 2000; Wijgerde et al., 2002).

Shh signal is required for the patterning of the ventral neural tube at all rostrocaudal levels, from forebrain to spinal cord (Ericson et al., 1995a,b). Genes involved in the patterning of ventral spinal cord, such as *Nkx2.2*, *Nkx6.1* and *Pax6*, are known to play equally important functions in the patterning of the ventral hindbrain. In the hindbrain, the p3 progenitor domain gives rise to several types of neurons, among them visceral and branchial motoneurons as well as serotonergic neurons. *Nkx2.2* is required for the specification of these neurons (Briscoe et al., 1999; Pattyn et al., 2003a,b, 2004) as well as the specification of V3 interneurons in the spinal cord (Briscoe et al., 1999). Similarly, *Nkx6.1*, *Pax6* and *Olig2* are involved in the specification of somatic motoneurons in the hindbrain (Ericson et al., 1997; Osumi et al., 1997; Pattyn et al., 2003a; Takahashi and Osumi, 2002) and in the spinal cord (Ericson et al., 1997; Lu et al., 2002; Sander et al., 2000; Takebayashi et al., 2002; Zhou and Anderson,

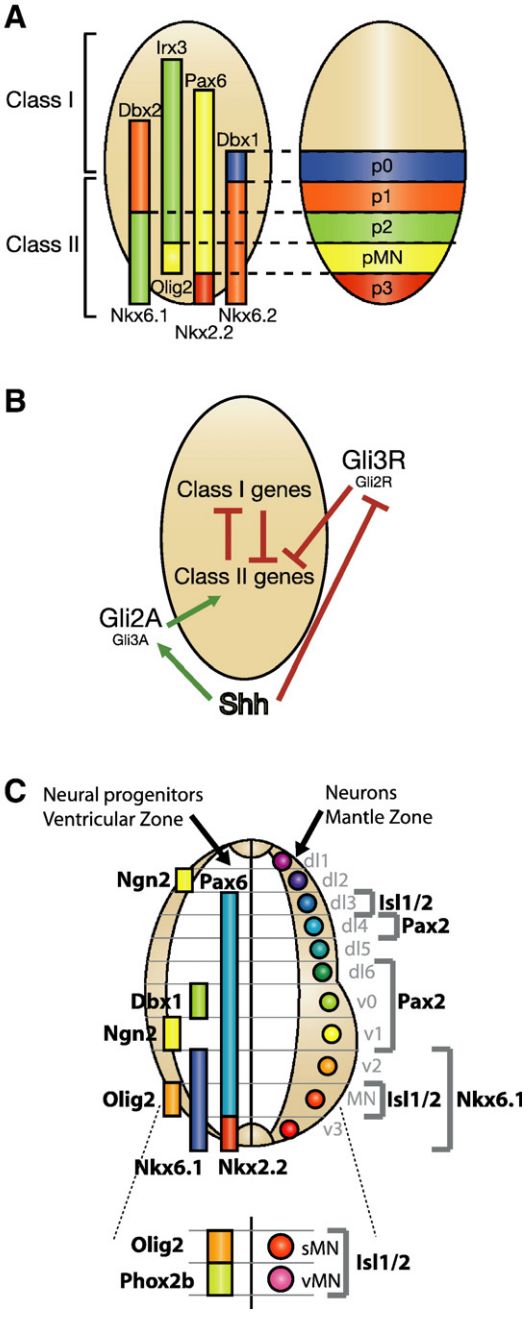

Fig. 1. The expression of patterning genes is controlled by Shh through Gli2 and Gli3 transcription factors. (A) Expression of class I (*Pax7*, *Dbx2*, *Irx3*, *Pax6* and *Dbx1*) and class II (*Nkx6.1*, *Olig2*, *Nkx2.2* and *Nkx6.2*) patterning genes in the neural tube forms a combinatorial code leading to the specification of progenitor domains in the ventral neural tube. pMN, progenitor domain of motoneurons; p0 to p3: progenitor domains of V0 to V3 ventral interneurons. (B) Shh modulates the expression of class II genes by either directly activating the expression of class II genes in the ventral neural tube trough Gli2 activator (Gli2A) and Gli3 activator (Gli3A) function or by inhibiting the repressor function of Gli3 and Gli2 (Gli3R, Gli2R) in the ventral neural tube, thereby allowing the expression of class II genes. Mutual repression between class I and class II genes limits the expression of class I genes to the medial part of the neural tube. (C) Patterning genes and terminal markers examined in this study. Neural progenitors located in the ventricular zone express different combination of patterning genes, as illustrated at the left. After the progenitors exit the cell cycle, they migrate to the mantle zone and express specific terminal marker, as illustrated at the right. Olig2 and Phox2b play specific role in specification of the somatic and visceral motoneurons in the ventral spinal cord and hindbrain.