Molecular and Cellular Neuroscience 52 (2013) 128–139

Contents lists available at SciVerse ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Pleiotropic and isoform-specific functions for *Pitx2* in superior colliculus and hypothalamic neuronal development ☆

Mindy R. Waite [a], Jennifer M. Skidmore [b], Joseph A. Micucci [c], Hidetaka Shiratori [d], Hiroshi Hamada [d], James F. Martin [e,f,g], Donna M. Martin [a,b,h,*]

[a] Cellular and Molecular Biology Graduate Program, 2966 Taubman Medical Library, University of Michigan, Ann Arbor, MI 48109-0619, USA
[b] Department of Pediatrics, 3520A MSRB I, University of Michigan, Ann Arbor, MI 48019-5652, USA
[c] Department of Biological Chemistry, 3520A MSRB I, University of Michigan, Ann Arbor, MI 48019-5652, USA
[d] Developmental Genetics Group, Graduate School of Frontier Bioscience, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565-0871, Japan
[e] Department of Molecular Physiology and Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
[f] Texas Heart Institute, Houston, TX 77030, USA
[g] Program in Developmental Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
[h] Department of Human Genetics, 3520A MSRB I, University of Michigan, Ann Arbor, MI 48019-5652, USA

ARTICLE INFO

Article history:
Received 17 April 2012
Revised 1 October 2012
Accepted 2 November 2012
Available online 10 November 2012

Keywords:
Migration
Transcription factor
Midbrain
Isoform
Differentiation
Axon

ABSTRACT

Transcriptional regulation of gene expression during development is critical for proper neuronal differentiation and migration. Alternative splicing and differential isoform expression have been demonstrated for most mammalian genes, but their specific contributions to gene function are not well understood. In mice, the transcription factor gene *Pitx2* is expressed as three different isoforms (PITX2A, PITX2B, and PITX2C) which have unique amino termini and common DNA binding homeodomains and carboxyl termini. The specific roles of these isoforms in neuronal development are not known. Here we report the onset of *Pitx2ab* and *Pitx2c* isoform-specific expression by E9.5 in the developing mouse brain. Using isoform-specific *Pitx2* deletion mouse strains, we show that collicular neuron migration requires PITX2AB and that collicular GABAergic differentiation and targeting of hypothalamic projections require unique *Pitx2* isoform dosage. These results provide insights into *Pitx2* dosage and isoform-specific requirements underlying midbrain and hypothalamic development.

Introduction

Gene expression is a tightly controlled process known to direct critical aspects of neuronal migration and differentiation (Briscoe and Novitch, 2008; Dessaud et al., 2008; Wilson and Maden, 2005). Alternative splicing adds an additional layer of gene regulation, wherein a single gene gives rise to multiple protein isoforms with distinct functions, greatly increasing functional capacity. Splicing occurs in up to 98% of human genes with multiple exons (Dessaud et al., 2008; Pan et al., 2008; Wang et al., 2008). Recent data on mouse gene splicing is not available, but previous studies found that the mouse genome undergoes slightly less splicing than the human genome (Chacko and Ranganathan, 2009; Kim et al., 2007; Modrek and Lee, 2003). Organs with increased cellular and functional complexity, such as the central nervous system (CNS), utilize gene splicing (Modrek et al., 2001; Yeo et al., 2004), nonetheless, there are few detailed studies of protein isoform functions in the developing brain. The morphogen *fibroblast growth factor 8* (*Fgf8*) gene is expressed as eight unique isoforms with variable receptor binding properties and roles in midbrain/hindbrain development (Guo et al., 2010). Several transcription factor genes expressed in the brain, including the forkhead-domain containing gene *FOXP2* and the basic helix–loop helix domain containing gene *TCF4* (mutated in human Pitt–Hopkins syndrome) exhibit alternative splicing, but the specific roles of individual isoforms for these two genes in neuronal development are also unclear (Santos et al., 2011; Sepp et al., 2011). A critical unanswered question is whether different transcription factor isoforms also exhibit unique functions during brain development.

PITX2 is a bicoid-like homeodomain transcription factor gene. Heterozygous *PITX2* mutations in humans result in Rieger syndrome, characterized by developmental defects in the eyes, teeth, umbilicus, heart, and brain (Amendt et al., 2000; Childers and Wright, 1986; Cunningham et al., 1998; Idrees et al., 2006; Semina et al., 1997). Mouse models for *Pitx2* deficiency exhibit ocular, tooth, and brain phenotypes similar to humans with *PITX2* mutations, but the underlying molecular mechanisms of these defects are only partially understood (Gage et al., 1999; Kitamura et al., 1999; Lin et al., 1999; Liu et

☆ This work was supported by R01 NS054784 to DMM.

* Corresponding author at: 3520A Medical Science Research Building I, University of Michigan Medical Center, Ann Arbor, MI 48109-5652, USA. Fax: +1 734 763 9512.
E-mail addresses: mrwaite@umich.edu (M.R. Waite), camelot@umich.edu (J.M. Skidmore), jmicucci@umich.edu (J.A. Micucci), shiratori@fbs.osaka-u.ac.jp (H. Shiratori), hamada@fbs.osaka-u.ac.jp (H. Hamada), jmartin@ibt.tamhsc.edu (J.F. Martin), donnamm@umich.edu (D.M. Martin).

1044-7431/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.mcn.2012.11.007

al., 2003; Lu et al., 1999; Martin et al., 2004; Skidmore et al., 2012; Waite et al., 2011). In the mouse CNS, *Pitx2* is expressed in discrete populations of neurons in the hypothalamus, midbrain, rhombomere 1, and spinal cord. In the hypothalamus, *Pitx2* is necessary for formation of the mammillothalamic tract (MTT) and midbrain *Pitx2* is critical for neuronal migration and GABAergic differentiation (Skidmore et al., 2012; Waite et al., 2011). In the midbrain, *Pitx2* is expressed downstream of a GABAergic cell-fate signaling cascade involving *Helt* and *Gata2* (Cazorla et al., 2000; Miyoshi et al., 2004; Nakatani et al., 2007). In vitro studies have shown that *Pitx2* is capable of activating *Gad1* expression for GABA synthesis (Chen et al., 2011; Westmoreland et al., 2001), suggesting *Pitx2* may act indirectly or directly as a terminal GABAergic differentiation factor.

In chick, mouse, and rat, *Pitx2* gives rise to three unique isoforms (PITX2A, PITX2B, and PITX2C) that arise from alternative promoter usage and exon splicing. These isoforms have distinct N-termini which are necessary for modulation of gene expression and exhibit dosage and tissue-specific requirements (Kioussi et al., 2002; Simard et al., 2009). In mouse, PITX2C (but not PITX2AB) is required for left-sided morphogenesis of the heart, lungs, and ovaries, as well as for looping of the gut (Guioli and Lovell-Badge, 2007; Liu et al., 2001, 2002). Conversely, PITX2A is the only isoform expressed in and required for heart development in zebrafish (Essner et al., 2000). In vitro, PITX2C is necessary for retention of myoblasts in an undifferentiated state and for continued proliferation (Martinez-Fernandez et al., 2006), whereas PITX2A regulates actin–myosin changes in HeLa cells to promote cell spreading and migration (Wei and Adelstein, 2002). Interestingly, no unique in vivo requirements for PITX2A or PITX2B have been identified in the mouse, although PITX2AB appears to be sufficient for tooth development (Liu et al., 2003).

All three *Pitx2* isoforms appear to be equally expressed in the mature rodent brain (Smidt et al., 2000). Therefore, we hypothesized that PITX2 isoforms may have unique functions during brain development. To test this hypothesis, we characterized the onset of *Pitx2* isoform expression in the brain and the effects of global, conditional, or isoform-specific *Pitx2* deficiency on hypothalamic and midbrain neuronal development. Our results suggest the presence of brain-region, dosage, and isoform-specific roles for *Pitx2* in neuronal migration, differentiation, and axon tract formation.

Results

Pitx2 isoforms and alleles

The mouse *Pitx2* gene is composed of two promoters and six exons (Fig. 1A). Alternative splicing and promoter usage generates three different *Pitx2* isoforms, PITX2A, PITX2B, and PITX2C (Fig. 1A,B). All three isoforms have unique N-termini, but share the same C-terminus composed of exons 5 and 6. Exon 5 contains the homeodomain which is required for proper DNA binding, specificity, and transactivation potential of *Pitx2* (Amendt et al., 1998; Saadi et al., 2001). PITX2C is the largest isoform at 324 amino acids due to the large size of exon 4, whereas PITX2A is the smallest with 271 amino acids.

To determine the functions and expression patterns of *Pitx2* isoforms in the developing mouse brain, we used various combinations of mouse *Pitx2* alleles (Fig. 1C). *Pitx2*$^{\Delta ab}$ is a *Pitx2ab*-specific

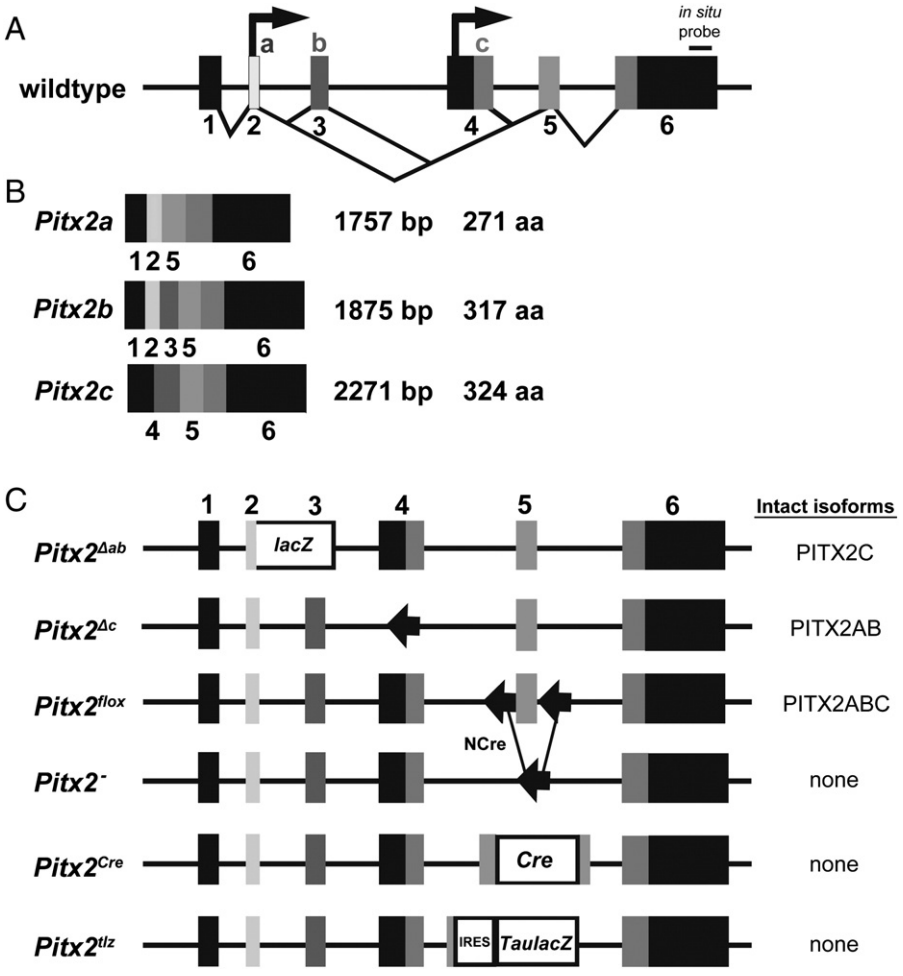

Fig. 1. *Pitx2* isoforms and alleles. (A) Map of the *Pitx2* gene showing exons, introns, and isoforms. Arrows indicate alternate transcription start sites. (B) Summary of exon usage and size of *Pitx2* isoforms. (C) List of mouse *Pitx2* alleles used to generate unique *Pitx2* deficient embryos. *Pitx2* isoforms that remain intact are listed on the right.