Available online at www.sciencedirect.com
SCIENCE DIRECT
Developmental Biology 289 (2006) 494 – 506
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Genomes & Developmental Control

Tcf- and Vent-binding sites regulate neural-specific *geminin* expression in the gastrula embryo

Jennifer J. Taylor [a], Ting Wang [b], Kristen L. Kroll [a,*]

[a] *Department of Molecular Biology and Pharmacology, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110, USA*
[b] *Department of Genetics, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110, USA*

Received for publication 22 August 2005, revised 12 October 2005, accepted 14 October 2005
Available online 9 December 2005

Abstract

Vertebrate neural development has been extensively investigated. However, it is unknown for any vertebrate gene how the onset of neural-specific expression in early gastrula embryos is transcriptionally regulated. *geminin* expression is among the earliest markers of dorsal, prospective neurectoderm at early gastrulation in *Xenopus laevis*. Here, we identified two 5′ sequence domains that are necessary and sufficient to drive neural-specific expression during gastrulation in transgenic *Xenopus* embryos. Each domain contained putative binding sites for the transcription factor Tcf, which can mediate Wnt signaling and for Vent homeodomain proteins, transcriptional repressors that mediate BMP signaling. Results from embryos transgenic for constructs with mutated Tcf or Vent sites demonstrated that signaling through the Tcf sites was required for dorsal-specific expression at early gastrulation, while signaling through the Vent sites restricted *geminin* expression to the prospective neurectoderm at mid-gastrulation. Consistent with these results, *geminin* 5′ regulatory sequences and endogenous *Xgem* responded positively to Wnt signaling and negatively to BMP signaling. The two 5′ sequence domains were also conserved among *geminin* orthologs. Together, these results demonstrate that signaling through Tcf and Vent binding sites regulates transcription of *geminin* in prospective neurectoderm during gastrulation.

Keywords: Transcription; Geminin; *Xenopus*; Transgenic; Wnt; BMP; Tcf; Vent; Neural

Introduction

Formation of the vertebrate neural plate is initiated by the process of neural induction, which subdivides ectoderm into prospective neural and non-neural domains. Studies of neural induction began with the demonstration that transplantation of the dorsal blastopore lip (dorsal mesendoderm or organizer) of a gastrula stage newt embryo to the ventral side of another embryo elicited formation of a secondary body axis, with secondary neural tissue induced from the host embryo (Spemann and Mangold, 1924). Structures roughly equivalent to the amphibian organizer in other vertebrates (the shield in zebrafish and the node in chick and mouse) have similar inductive properties (Beddington, 1994; Shih and Fraser, 1996; Waddington, 1930). Numerous studies have since sought to identify the molecular signals that induce neural fate.

Bone morphogenetic protein (BMP) signaling is a prominent candidate regulatory pathway for determining ectodermal fate. Over-expression of dominant negative receptors in *Xenopus* ectoderm results in neural differentiation (Hawley et al., 1995; Hemmati-Brivanlou and Melton, 1992; Xu et al., 1995). *bmp4* mRNA is also downregulated in the future neural ectoderm as the organizer forms (Fainsod et al., 1997; Wilson and Hemmati-Brivanlou, 1995). Additionally, BMP antagonists including Follistatin, Noggin and Chordin, are expressed in the organizer and induce neural tissue (Hemmati-Brivanlou et al., 1994; Lamb et al., 1993; Sasai et al., 1995). Despite the apparent importance of organizer-derived BMP antagonists in specifying neural cell fate, in some cases neural tissue can form when organizer tissue is genetically or surgically ablated (Davidson et al., 1999; Klingensmith et al., 1999; Shih and Fraser, 1996; Smith and Schoenwolf, 1989) and mice mutant for BMP antagonists form neural plates (Bachiller et al., 2000; Matzuk et al., 1995; McMahon et al., 1998). In the early chick embryo, BMP antagonists also appear insufficient to induce neural tissue (Connolly et al., 1997; Levin, 1998; Storey et al., 1992; Streit et al., 1998; Wilson et al., 2000).

* Corresponding author. Fax: +1 314 362 7058.
E-mail address: kkroll@wustl.edu (K.L. Kroll).

0012-1606/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2005.10.047

In contrast, recent studies have re-addressed and confirmed the importance of BMP antagonism for dorsal and neural development in *Xenopus* (Delaune et al., 2005; Khokha et al., 2005; Reversade et al., 2005; Wawersik et al., 2005).

Additional signaling pathways also regulate neural cell fate. In avian and ascidian embryos, FGF (Fibroblast Growth Factor) signaling induces neural markers, and blocking FGF signaling inhibits neural development (Darras and Nishida, 2001; Hudson et al., 2003; Lemaire et al., 2002; Miya and Nishida, 2003; Streit et al., 2000; Wilson et al., 2000). Work in *Xenopus* also suggests that FGF signaling may facilitate neural induction (Delaune et al., 2005; Hongo et al., 1999; Kessel and Pera, 1998; Mason, 1996; Wawersik et al., 2005), but its exact role remains unclear. Deficiencies in neural tissue following inhibition of FGF signaling may reflect a failure of underlying mesoderm to be properly formed, maintained, or patterned during gastrulation. Furthermore, in *Xenopus* and zebrafish, FGF signaling appears more critical for formation of posterior than anterior neural tissue (Haremaki et al., 2003; Koshida et al., 2002; Lamb and Harland, 1995; Launay et al., 1996; Rentzsch et al., 2004; Ribisi et al., 2000).

Studies have also implicated Wnt signaling in defining presumptive neurectoderm, in both positive and negative regulatory roles. In *Xenopus*, Wnt ligands (*xWnt8*, *xWnt3a*, and *mWnt8*), the receptor *xFrz8*, or intracellular components that potentiate Wnt signaling (*xDsh*, *dnGSK3*, or a constitutively active *beta-catenin*) can induce neural tissue and suppress *bmp4* expression (Baker et al., 1999). Early Wnt signaling also correlates with expression of the BMP antagonists *noggin* and *chordin* in the dorsal animal and marginal zone tissues of blastulae (De Robertis and Kuroda, 2004; Wessely et al., 2001). In zebrafish, Beta-catenin suppresses *bmp2b* through activation of *bozozok,* and *bozozok* mutants have defects in the anterior neurectoderm (Fekany-Lee et al., 2000; Leung et al., 2003). Therefore, suppression of BMP signaling by the Wnt pathway may be a conserved mechanism mediating dorsal and/or neural development. Conversely, in avian embryos Wnt signaling inhibits neural fates (Wilson et al., 2001). These apparently conflicting roles of Wnt signaling in *Xenopus* and avian embryos may reflect temporal differences in the roles of Wnt signaling during development. In *Xenopus*, Wnt signaling prior to the mid-blastula transition (MBT) directs dorsal development, but post-MBT activity promotes ventral development (Hamilton et al., 2001; Roel et al., 2002; Schneider et al., 1996; Stern, 2005). Alternatively, *Xenopus* and avian embryos may specify neural fate using differential mechanisms.

Understanding the transcriptional regulation of neural gene expression may clarify the roles of the BMP, FGF, or Wnt signaling pathways in neural development. The development of transgenic approaches for manipulating the *Xenopus* embryo and identification of genes that mark the early formation of prospective neurectoderm now enable us to examine this issue. One such gene, whose expression marks presumptive neural tissue at early gastrulation, is *geminin*. *geminin* encodes a novel coiled-coil protein with orthologs in vertebrates, *Drosophila*, and *C. elegans*. The roles of Geminin in regulating both neural cell fate and the fidelity of DNA replication were originally defined in *Xenopus* (Kroll et al., 1998; McGarry and Kirschner, 1998). Functional studies in *Xenopus* and *Drosophila* have shown that Geminin is required for neural and/or neuronal cell fate specification (Kroll et al., 1998; Quinn et al., 2001). Recently, we also demonstrated that Geminin is essential for maintaining the neuronal progenitor state and controlling the timing of neuronal differentiation (Seo et al., 2005).

Expression of *geminin* is among the earliest molecular markers of prospective neural tissue in *Xenopus*. During cleavage and blastula stages, maternal *geminin* RNA and protein are distributed throughout the animal hemisphere (Kroll et al., 1998). At late blastula through early gastrula stages, *geminin* is upregulated in dorsal ectoderm, while ventral transcripts are rapidly lost, generating a domain of expression by the onset of gastrulation (st. 10− to 10+) that marks the future neural plate. How the onset of neural-specific gene expression at gastrulation is transcriptionally regulated was previously unknown for any vertebrate gene. Here, we used transgenic and computational approaches to determine sequences required for regulating *geminin* transcription in the prospective neurectoderm during gastrulation.

Materials and methods

Plasmid construction

Sequences 5′ of the transcription start site of the human *geminin* (*hgem*) locus were amplified by polymerase chain reaction (PCR) with Advantage 2 Polymerase (Clontech). PAC Clone RP3-369A17 containing the *hgem* genomic locus, was used as template (Sanger Centre) to amplify 5.1 kb of upstream sequence. The PCR product was cloned into pRAREeGFP (Davis et al., 2001).

Using [−4694 to +436] as template, plasmids containing 5′ sequence subdomain combinations or deletions were created by PCR with high fidelity polymerase. PCR products were digested and ligated into pRAREeGFP, pGL3basic (Promega), or pRAREsCMV. The pRARE vectors (Davis et al., 2001) are derived from pCS2 (Rupp et al., 1994), by removal of the sCMV IE94 promoter and replacement with a polylinker, followed by eGFP (Clontech) and the SV40 virus late polyadenylation signal. pRAREsCMV additionally contains the simian cytomegalovirus minimal promoter (sCMV) and was used for constructs lacking the *hgem* sequences adjacent to the transcription start site. pGL3basic is a promoterless vector driving expression of firefly *luciferase*. Numerous pRAREeGFP- and pGL3basic-based constructs were tested and the same pattern of reporter expression was obtained regardless of vector. Each empty vector yielded few embryos showing any spatially-restricted reporter expression upon introduction into transgenic embryos (pRAREeGFP shown in Figs. 2J, K). The few false positives may have resulted from non-specific trapping of in situ probe in the archenteron or may reflect effects of a particular chromosomal integration site on the expression pattern in an individual transgenic embryo. Subcloning products were verified by DNA sequencing.

Mutagenesis primers changed CAAAG to TGCCT (for Tcf), AGA-CAAATTCC to GTGAGGGCGAA (for XVent1), or CATTAGTAAT to TGCGGACCCG (for XVent2). Using construct [−3783 to −2920] + [−2425 to −1580] as template, PCR was performed with mutagenesis primers containing the above changes. Mutagenesis was performed using the Quick Change Site-directed Mutagenesis kit protocol (Stratagene). Mutations were verified by DNA sequencing.

All primers are available upon request.

Transgenic X. laevis embryos

Transgenic embryos were generated as described (Kroll and Amaya, 1996) with modifications. For each reaction, approximately 6.25×10^5 *X. laevis* sperm nuclei were incubated with 1 μg linearized DNA in a total volume of 10 μl for 5