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# *Neurogenin 1 (Neurog1)* expression in the ventral neural tube is mediated by a distinct enhancer and preferentially marks ventral interneuron lineages

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ABSTRACT

The bHLH transcription factor Neurog1 (Ngn1, Neurod3, neurogenin 1) is involved in neuronal differentiation and cell-type specification in distinct regions of the developing nervous system. Here, transgenic mouse models were developed that use a Bacterial Artificial Chromosome (BAC) containing 208 kb flanking the *Neurog1* gene to efficiently drive expression of GFP and Cre in all *Neurog1* domains. Two characteristics of *Neurog1* gene regulation were uncovered. First, a 4 kb region previously shown to be sufficient for driving expression of a reporter gene to a subset of the *Neurog1* pattern in the developing midbrain, hindbrain, and spinal cord is required uniformly for high levels of expression in all *Neurog1* domains, even those not originally identified as being regulated by this region. Second, a 0.8 kb enhancer was identified that is sufficient to drive *Neurog1*-like expression specifically in the ventral neural tube. Furthermore, *Neurog1* progenitor cells in the ventral neural tube are largely fated to interneuron lineages rather than to motoneurons. These studies provide new tools for directing tissue specific expression in the developing neural tube, define *Neurog1* lineages in the spinal cord, and further define the complex genomic structure required for obtaining the correct levels and spatial restriction of the neuronal differentiation gene *Neurog1*.



## Introduction

Proper neural function depends on development of the correct number of cells with the correct identity for accurate assembly of neuronal circuits. Neural basic helix-loop-helix (bHLH) transcription factors are known regulators of neuronal differentiation and neuronal sub-type specification. A subset of neural-bHLH transcription factors, including Neurog1, is transiently expressed in proliferating cells, and expression is lost as these cells become postmitotic and differentiate into more mature neural cell types (Cau et al., 2002; Fode et al., 2000; Gowan et al., 2001; Lee, 1997; Ma et al., 1996; Schuurmans et al., 2004). Overexpression studies have shown that Neurog1 is sufficient to induce neuronal differentiation in mouse embryonic carcinoma P19 cells, cortical progenitors in mouse, and neural tube in chick, Xenopus and zebrafish (Blader et al., 1997; Farah et al., 2000; Gowan et al., 2001; Ma et al., 1996; Sun et al., 2001). Furthermore, Neurog1 has been shown to play a role in specifying neuronal subtype in neural crest derivatives where ectopic expression induced sensory neuron-appropriate markers in non-sensory crest derivatives, and in chick dorsal neural tube where Neurog1 induced excess dI2 dorsal interneurons at the expense of neighboring dI1 and dI3 interneurons (Gowan et al., 2001; Perez et al., 1999). Loss-of-function studies in mouse have shown that Neurog1 is required for the formation of olfactory neurons and cranial sensory ganglia (Andermann et al., 2002; Cau et al., 2002; Ma et al., 1998, 1999), and along with the related factor Neurog2 (Ngn2, Math4A, neurogenin 2), is required for the proper development of dorsal root ganglia, dorsal interneuron population dI2 in the developing neural tube, and cerebral cortex (Gowan et al., 2001; Kriks et al., 2005; Ma et al., 1999; Nieto et al., 2001). Taken together these studies show that Neurog1 can induce general neuronal differentiation and specify neuronal subtype in the peripheral and central nervous systems. Hence, understanding how *Neurog1* expression is regulated during neurogenesis is an important part of identifying the mechanisms involved in generating the correct numbers and types of neurons necessary for the accurate assembly of neuronal circuits.

Previous studies in mouse that tested regions of *Neurog1* flanking sequence across a 15 kb region identified multiple intergenic regions sufficient to direct expression of reporter genes to a subset of the *Neurog1* expression domain (Blader et al., 2004; Gowan et al., 2001; Murray et al., 2000; Nakada et al., 2004). However, these regulatory regions were not sufficient to recapitulate the entire *Neurog1* pattern. Here we use a modified Bacterial Artificial Chromosome (BAC) and transgenic mice to demonstrate 208 kb flanking the *Neurog1* gene is sufficient to direct expression to all *Neurog1* domains. For efficient levels of *Neurog1*-like expression, the BAC requires sequences that fall

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within a previously identified enhancer and are conserved across multiple species including zebrafish (Blader et al., 2004; Gowan et al., 2001; Nakada et al., 2004). Even with the enhancer deleted, the BAC retains activity for low levels of tissue specific expression suggesting the presence of an autoregulatory element or a redundant secondary enhancer at a distinct location. Furthermore, we identify a 0.8 kb region that directs transgene expression specifically to the ventral *Neurog1* domain, identifying an enhancer that is distinct from the previously defined dorsal neural tube enhancer for *Neurog1* (Nakada et al., 2004), or the specific enhancers identified in zebrafish (Blader et al., 2004, 2003). Finally, we use the *Neurog1* regulatory locus for in vivo genetic fate mapping using a Cre-flox system to demonstrate that a majority of *Neurog1* progenitors in the ventral neural tube are preferentially fated to become ventral interneurons rather than motoneurons.

## Materials and methods

### *Targeted modification of bacterial artificial chromosomes and generation of transgenic mice*

*Neurog1*$^{457}$*-nGFP* and *Neurog1*$^{457}$*-Cre* (herein called *N1*$^{457}$*-nGFP* and *N1*$^{457}$*-Cre*) were developed using the RP23 457E22 BAC obtained from BACPAC Resources Center (BPRC) at Children's Hospital Oakland Research Institute in Oakland, CA. This BAC contains a genomic insert of 208 kb with the *Neurog1* coding sequence located centrally. Homologous recombination in bacteria (Yang et al., 1997) was used to replace the *Neurog1* coding region precisely with coding sequence for *EGFP* (Clontech) with a nuclear localization signal (Lumpkin et al., 2003) or for Cre recombinase. The *N1*$^{457}$*-nGFP* BAC was further used to delete the regions for *N1*$^{457}$*-nGFPΔR1*, *N1*$^{457}$*-nGFPΔR2*, and *N1*$^{457}$*-nGFPΔR3* using BAC recombineering strategies (Lee et al., 2001). In each case the targeting constructs for the BAC recombineering contained 100–350 bp homology arms and deleted sequences from 2.5 to 4.0 kb (see Fig. 1 and Table 1). TgN1-16 was generated from *N1*$^{457}$*-nGFP* using the BAC retrieval method (Liu et al., 2003). TgN1-15 and TgN1-15vnt were generated by cloning the region of interest by PCR into BgnGFP reporter cassette (Lumpkin et al., 2003). TgN1-15vnt$^{mgli}$ is TgN1-15vnt with two candidate gli consensus sites mutated. The sequence TGGGTGTTCAGCCCCTGCTGGAAAAAGGCTCGGTGGGTGGG with the possible gli sites underlined was mutated from TGGGT in each case to GTATA. TgN1-2 and TgN1-13dnt have been described previously (Nakada et al., 2004, TgN1-13). Table 1 lists the positions on chromosome 13 for the transgene ends or deletion regions used in this study (using the mouse mm9 assembly July 2007).

**Table 1**
Mouse chromosome positions for transgenes.

| Name | Mouse[a] chromosome position (deleted regions in ΔR1, ΔR2, ΔR3) | Deletion or transgene size (kb) |
|---|---|---|
| Neurog 1 coding[b] | chr13: 56,352,559–56,353,294 | 0.735 |
| *N1*$^{457}$*-nGFP* | chr13: 56,245,586–56,454,497 | 208 |
| *N1*$^{457}$*-nGFPΔR1* | chr13: 56,354,675–56,357,162 | 2.5 |
| *N1*$^{457}$*-nGFPΔR2* | chr13: 56,357,364–56,361,332 | 4.0 |
| *N1*$^{457}$*-nGFPΔR3* | chr13: 56,361,620–56,365,378 | 3.8 |
| *TgN1-16* | chr13: 56,345,580–56,362,076 | 16.5 |
| *TgN1-2* | chr13: 56,356,824–56,364,454 | 7.6 |
| *TgN1-13dnt* | chr13: 56,359,269–56,360,099 | 0.830 |
| *TgN1-15* | chr13: 56,361,901–56,364,454 | 2.5 |
| *TgN1-15vnt* | chr13: 56,363,312–56,364,118 | 0.806 |
| LATE[c] | chr13: 56,359,328–56,359,715 | 0.387 |
| ANPE[c] | chr13: 56,360,975–56,361,111 | 0.136 |
| LSE[c] | chr13: 56,361,733–56,361,970 | 0.237 |

[a]Mouse mm9 assembly from July 2007 used to determine locations.
[b]Transcribed in the reverse orientation.
[c]Regulatory regions conserved with *D. rerio* (Blader et al., 2003, 2004).

Transgenic mice were generated by pronuclear injection using fertilized eggs from B6SJLF1 (C57BL/6J × SJL/J) crosses using standard procedures (Hogan et al., 1986) in the UTSW Core Transgenic Facility. Qiagen purified BAC DNA (two independent clones per BAC deletion) was injected at 0.3–1 ng/μl in 10 mM Tris pH 7.5, 0.1 mM EDTA, 100 mM NaCl. Non-BAC transgenes were isolated from the vector backbone and injected at 1–3 ng/μl in the injection buffer above lacking the NaCl. Transgenic animals were identified by PCR using

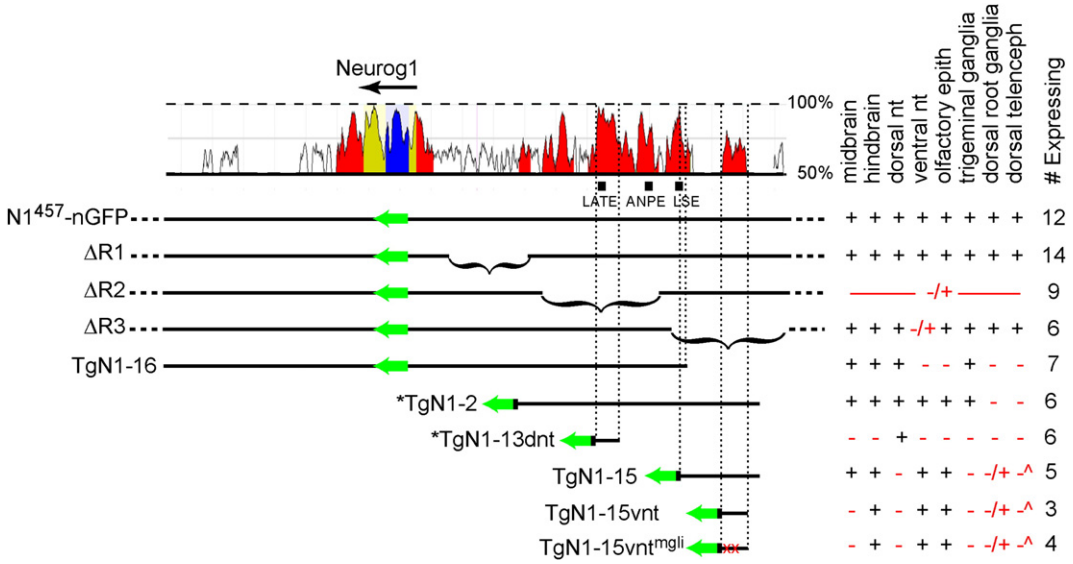


**Fig. 1.** Diagram and summary of activity of Neurog1-GFP transgenes. Comparison of mouse and human genomes surrounding the *Neurog1* coding sequence on mouse chromosome 13 reveals extensive conservation (shown 50–100%) in non-coding regions using ECR browser (Ovcharenko et al., 2004). Colors indicate over 70% conservation in sequence where blue is *Neurog1* coding, yellow is UTR, and red is intergenic. Black blocks below the ECR diagram indicate sequence conserved to *D. rerio* that have been identified in functioning enhancers (LATE, ANPE, LSE) (Blader et al., 2004, 2003). The BAC transgene *N1*$^{457}$*-nGFP* (modified BAC RP23 457E22) is shown with the location of deletions in ΔR1, ΔR2, ΔR3 indicated by brackets. The relative location of the deletions and the sequences tested in the transgenes are diagramed below the ECR browser image to highlight the conserved regions included in each. Precise coordinates in the genome are given in Table 1. Green boxes indicate the GFP reporter coding sequence. # Expressing indicates the number of independent transgenic founder embryos at E11.5 that had detectable GFP and was examined for expression in the tissues listed. Expression pattern was consistent across embryos with the same transgene and representative images are shown in Figs. 2 and 4. Black +/- indicate similarity to wildtype while red +/- highlight expression different from wildtype. The asterisks on TgN1-2 and TgN1-13dnt indicate they were reported in Nakada et al. (2004) and are shown here for comparison. ^ indicates these transgenes aberrantly directed ventral telencephalon (vt) expression rather than dorsal. vnt, ventral neural tube; dnt, dorsal neural tube.