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Zebrafish *colgate/hdac1* functions in the non-canonical Wnt pathway during axial extension and in Wnt-independent branchiomotor neuron migration

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Abstract

Vertebrate gastrulation involves the coordinated movements of populations of cells. These movements include cellular rearrangements in which cells polarize along their medio-lateral axes leading to cell intercalations that result in elongation of the body axis. Molecular analysis of this process has implicated the non-canonical Wnt/Frizzled signaling pathway that is similar to the planar cell polarity pathway (PCP) in *Drosophila*. Here we describe a zebrafish mutant, *colgate (col)*, which displays defects in the extension of the body axis and the migration of branchiomotor neurons. Activation of the non-canonical Wnt/PCP pathway in these mutant embryos by overexpressing Δ*Ndishevelled, rho kinase2* and *van gogh-like protein 2 (vangl2)* rescues the extension defects suggesting that *col* acts as a positive regulator of the non-canonical Wnt/PCP pathway. Further, we show that *col* normally regulates the caudal migration of nVII facial hindbrain branchiomotor neurons and that the mutant phenotype can be rescued by misexpression of *vangl2* independent of the Wnt/PCP pathway. We cloned the *col* locus and found that it encodes *histone deacetylase1 (hdac1)*. Our previous results and studies by others have implicated *hdac1* in repressing the canonical Wnt pathway. Here, we demonstrate novel roles for zebrafish *hdac1* in activating non-canonical Wnt/PCP signaling underlying axial extension and in promoting Wnt-independent caudal migration of a subset of hindbrain branchiomotor neurons.

Keywords: Wnt signaling; Histone deacetylase; Convergent extension; Migration; PCP; Zebrafish

1. Introduction

The basic vertebrate body plan consists of the three germ layers that emerge during gastrulation. Carefully orchestrated movement of groups of cells relative to each other culminates in the transformation of an unstructured mono-layered blastula into a gastrula with germ layers. Cell intercalations result in the elongation of the body axis. An important driving force for these cell movements is a process known as convergent-extension (CE). Studies suggest that CE in zebrafish has at least two distinct components (Kane and Warga, 1994; Solnica-Krezel et al., 1995; Wallingford et al., 2000). The first involves directed migration of cells towards the dorsal side of the gastrula, termed dorsal convergence. Convergence is a migratory event not involving cell rearrangements. This is followed by cellular rearrangements where cells converging at the dorsal midline become polarized along the medio-lateral axis resulting in cell intercalations and elongation of the body axis.

The dissociation of convergence and medio-lateral intercalation and extension is evident from zebrafish mutants affecting CE movements differently. For example, in *silberblick (slb)* mutants, both convergence and extension movements are defective (Heisenberg et al., 2000), whereas in *no tail (ntl)* and *somitabun (sbn)* mutants convergence is

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significantly affected with extension occurring almost normally (Myers et al., 2002; Solnica-Krezel et al., 1996). In *knypek (kny)* mutants, mediolateral intercalations that underlie CE movements are impaired (Topczewski et al., 2001).

The molecular basis for CE movements in vertebrates is incompletely understood. The polarization of cells within the plane of tissues undergoing CE in vertebrate embryos is akin to the polarization of epithelial cells in the insect cuticle. In *Drosophila*, the orientation of cells in a plane, planar cell polarity (PCP), is regulated by a non-canonical Wnt signaling cascade. As in the case of the canonical Wnt signaling pathway, this pathway also uses the Frizzled receptor and Dishevelled (Dsh). Other proteins, such as Inversin, (Simons et al., 2005), Diversin, (Schwarz-Romond et al., 2002), Naked and Casein Kinase 1 (Yan et al, 2001; McKay et al., 2001), all of which either interact directly with Dsh or with Dsh-associated proteins, have been shown to regulate both Wnt pathways. However, downstream of Dsh, PCP signaling recruits a different set of molecules including Van gogh-like protein 2, Prickle, and JNK (Shulman et al, 1998; Boutros and Mlodzik, 1999; Adler and Lee, 2001).

Recent studies have revealed that the orthologs of PCP pathway molecules control CE during gastrulation in *Xenopus* and zebrafish (Park and Moon, 2002; Kibar et al., 2001; Carreira-Barbosa et al., 2003). Mutant versions of Dsh have implicated the PCP signaling pathway as a regulator of CE movements in vertebrates (Heisenberg et al., 2000; Tada and Smith, 2000; Wallingford et al., 2000). A construct of Dsh that specifically disrupts PCP signaling in *Drosophila*, but does not affect the canonical Wnt pathway was able to block CE movements in both *Xenopus* and zebrafish (Wallingford et al., 2000; Heisenberg et al., 2000). Conversely, deletion constructs of Dsh that are unable to activate the canonical Wnt pathway were shown to rescue CE in *silberblick,* a zebrafish *wnt11* mutant, as well as the overexpression of a dominant-negative form of *wnt11* in *Xenopus* embryos (Tada and Smith, 2000; Heisenberg et al, 2000). In addition to *dsh,* other PCP genes also have homologs in vertebrates. For example, the zebrafish *trilobite* mutant is defective in the homolog of the *van gogh-like protein 2* gene and is expressed in cells undergoing CE (Park and Moon, 2002). Two homologs of *prickle* that regulate gastrulation movements in zebrafish have been identified recently (Veeman et al., 2003; Carreira-Barbosa et al., 2003). Additionally, other CE genes specific to vertebrates have been isolated, including the *formin* morphology protein *daam-1, knypek/glypican 4/6* and Wnt ligands *wnt5/pipetail* and *wnt11/silberblick* (Hammerschmidt et al., 1996; Heisenberg et al., 2000; Jessen et al., 2002; Kilian et al., 2003; Rauch et al., 1997; Solnica-Krezel et al., 1996; Topczewski et al., 2001).

Chromatin modifications play a key role in regulating eukaryotic gene expression (Jenuwein and Allis, 2001). Histones have numerous sites where post-translational modifications occur, and the pattern of modification encodes the expression status of a gene (Strahl and Allis, 2000; Rice and Allis, 2001). The silencing of gene expression has been found to be associated with deacetylation, whereas acetylation of histones is associated with activation of gene expression (Allfrey, 1966). Histone deacetylases (HDACs) are primarily nuclear enzymes involved in removing acetyl groups from histone lysine tails (de Ruijter et al., 2003; Marks et al., 2003). A role for Hdac1 in repressing the expression of canonical Wnt target genes has been shown in *Drosophila* and vertebrates (Chen et al., 1999; Billin et al., 2000; Brantjes et al., 2001; Yamaguchi et al., 2005). Hdac1 has been shown to exert its repressive function via association with Groucho and LEF1 in the nucleus (Chen et al., 1999; Brantjes et al., 2001; Billin et al., 2000). Roles for zebrafish *hdac1* in *notch* and *sonic hedgehog* signaling have also been reported (Yamaguchi et al., 2005; Cunliffe, 2004).

We have shown that the zebrafish mutant *colgate (col)* displays defects in early dorso-ventral and brain patterning (Nambiar and Henion, 2004) that can exclusively be rescued by overexpression of canonical Wnt pathway antagonists (Nambiar and Henion, 2004). Here, we show that *col* mutants also display defects both in axial extension and the migration of a subset of hindbrain branchiomotor neurons that can be selectively and differentially rescued by overexpressing molecules of the non-canonical Wnt/PCP signaling pathway. We have cloned the *col* locus and found that it encodes *histone deacetylase 1 (hdac1)*. In this study we demonstrate novel roles for Hdac1 in the non-canonical Wnt/PCP pathway during axial extension as well as in Wnt/PCP-independent neuronal migration, functions not previously attributed to *hdac1*.

2. Results

2.1. Defects in axial extension contribute to the col mutant phenotype

col mutant embryos are shorter (Table 1) and have a downward curved body compared to wildtype embryos. The somites of 48 hpf *col* mutants appear rounded, unlike chevron-shaped wildtype somites (Fig. 1A and B). There are also fewer somite pairs in *col* mutants than in wildtype by 27 hpf, although no significant difference is apparent at 16 hpf (Table 2). The 1–2 somite pair deficit in *col* mutants at 27 hpf persists at 48 hpf (Table 2). Compared to wildtype embryos, *col* mutants also have abnormally wide

Table 1
Statistical analysis of mean length of wildtype and *col/hdac1* embryos at 25, 48 and 72 hpf

Hours post fertilization	Mean length of embryos in mm ±1 SD		*P* value
	Wildtype	*col/hdac1*	
25	2.115 ± 0.085 $n = 20$	1.943 ± 0.058 $n = 20$	$P < 0.001$
48	2.934 ± 0.054 $n = 10$	2.710 ± 0.052 $n = 10$	$P < 0.001$
72	3.466 ± 0.067 $n = 10$	2.684 ± 0.184 $n = 10$	$P < 0.001$