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Cdx mediates neural tube closure through transcriptional regulation of the planar cell polarity gene *Ptk7*

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SUMMARY

The vertebrate Cdx genes (*Cdx1*, *Cdx2* and *Cdx4*) encode homeodomain transcription factors with well-established roles in anteroposterior patterning. To circumvent the peri-implantation lethality inherent to *Cdx2* loss of function, we previously used the Cre-loxP system to ablate *Cdx2* at post-implantation stages and confirmed a crucial role for *Cdx2* function in events related to axial extension. As considerable data suggest that the Cdx family members functionally overlap, we extended this analysis to assess the consequence of concomitant loss of both *Cdx1* and *Cdx2*. Here, we report that *Cdx1-Cdx2* double mutants exhibit a severely truncated anteroposterior axis. In addition, these double mutants exhibit fused somites, a widened mediolateral axis and craniorachischisis, a severe form of neural tube defect in which early neurulation fails and the neural tube remains open. These defects are typically associated with deficits in planar cell polarity (PCP) signaling in vertebrates. Consistent with this, we found that expression of *Ptk7,* which encodes a gene involved in PCP, is markedly reduced in *Cdx1-Cdx2* double mutants, and is a candidate Cdx target. Genetic interaction between Cdx mutants and a mutant allele of *Scrib*, a gene involved in PCP signaling, is suggestive of a role for Cdx signaling in the PCP pathway. These findings illustrate a novel and pivotal role for Cdx function upstream of *Ptk7* and neural tube closure in vertebrates.

KEY WORDS: Cdx, Craniorachischisis, Neural tube, Ptk7, Mouse

INTRODUCTION

The Cdx gene family (*Cdx1*, *Cdx2* and *Cdx4*) encodes homeodomain transcription factors related to the *Drosophila* gene *caudal* (*cad*). In the mouse embryo proper, Cdx genes are sequentially activated beginning at late streak stage (E7.5). *Cdx1* (Meyer and Gruss, 1993) and *Cdx2* (Beck et al., 1995) expression begins in the primitive streak region at E7.5, followed by *Cdx4* expression (Gamer and Wright, 1993) at E8.5, with Cdx1 exhibiting the rostral-most limit of transcript distribution, followed by Cdx2 and Cdx4. This yields a nested, caudal-high, pattern of Cdx transcript distribution that is maintained in the caudal embryo until extinction of expression in the tail bud, with the loss first of Cdx1, followed by Cdx4 and Cdx2. *Cdx1* and *Cdx2* are also expressed in the intestinal hindgut epithelium in the embryo, with expression persisting throughout the life of the animal (Duprey et al., 1988; Meyer and Gruss, 1993; Beck et al., 1995; Chawengsaksophak et al., 1997; Silberg et al., 2000).

In *Drosophila*, *cad* is involved in the specification of the caudal embryo, as well as antero-posterior (AP) patterning (Mlodzik et al., 1985; Macdonald and Struhl, 1986; Mlodzik and Gehring, 1987; Kispert et al., 1994). Functional studies in the mouse have revealed that vertebrate Cdx genes are similarly involved in AP patterning, and that they mediate these effects via direct regulation of Hox gene expression (Shashikant et al., 1995; Subramanian et al., 1995; Epstein et al., 1997; Isaacs et al., 1998; van den Akker et al., 2002; Houle et al., 2003; Chawengsaksophak et al., 2004; Tabaries et al., 2005; Pilon et al., 2007; Savory et al., 2009b; Young et al., 2009).

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More recent work has also demonstrated Hox-independent mechanisms for Cdx function. In this regard, we and others have used conditional mutagenesis approaches to circumvent the peri-implantation lethality associated with the *Cdx2* null mutation. This has led to the finding that *Cdx2* controls crucial aspects of intestinal endoderm patterning and posterior development, and that both of these programs appear to be governed, at least in part, via Hox-independent pathways (Gao et al., 2009; Savory et al., 2009a; Grainger et al., 2010).

Other than the homeodomain, and limited residues involved in protein processing, Cdx members exhibit little sequence similarity, suggesting functional specificity (Marom et al., 1997). However, analysis of compound Cdx mutants (van den Akker et al., 2002; van Nes et al., 2006; Young et al., 2009) as well as gene substitution experiments (Savory et al., 2009b), argue that Cdx members are functionally similar. Given this, and the extensive overlap of expression in the caudal embryo between all three Cdx members, we generated and assessed *Cdx1-Cdx2* double null mutants (referred to hereafter as *Cdx1/2* DKO for simplicity). The phenotype of these mutants suggests previously unsuspected roles for Cdx function in events related to somitogenesis and neural tube closure.

The central nervous system (CNS) develops from the neural tube through the process of neurulation. The neural tube results from the folding and midline fusion of the neural plate. In mammals, this folding occurs sequentially and is nucleated at different levels of the body axis (Copp et al., 2003; Copp and Greene, 2010). Midline fusion is first initiated at the base of the future hindbrain, called closure 1, at around E8.5. Two subsequent de novo closure sites then initiate, with closure 2 at the forebrain/midbrain boundary and closure 3 at the rostral extremity of the forebrain. Continuation of closure between these latter two sites completes cranial closure, whereas the caudal spread of fusion from closure 1, which continues as the embryo elongates, culminates with closure at the posterior neural pore (Copp et al., 2003).

Neural tube defects (NTDs) comprise a group of congenital malformation resulting from a failure in neurulation. NTDs affect around 1 in 1000 pregnancies and are the second most common malformation after congenital heart defects (Mitchell, 2005). The phenotypes of NTDs vary depending on the region of the neural tube affected. For example, incomplete closure of the cranial neural tube leads to anencephaly, whereas disruption of posterior neuropore closure produces spina bifida (Copp et al., 2003; Copp and Greene, 2010). Failure of closure 1 leads to the most severe NTD, craniorachischisis (CRS), in which the neural tube remains open along its entire length. CRS accounts for 10% of all NTDs (Moore et al., 1997), and of the more than 200 mouse mutants with NTDs, 14 genes have been implicated in CRS (Harris and Juriloff, 2007; Harris and Juriloff, 2010). Of these, all but one is implicated in the planar cell polarity (PCP) signaling pathway.

PCP, which dictates the organization of cells within the plane of the epithelium, was first identified in *Drosophila* (Montcouquiol et al., 2006; Jones and Chen, 2007; Simons and Mlodzik, 2008) and is governed by a highly conserved core group of genes, including *frizzled* (*fz*), *flamingo/starry night* (*fmi*; *stan* – FlyBase), *disheveled* (*dsh*), *strabismus* (*stb*)/*Van Gogh* (*vang*) and *prickle* (*pk*). The PCP pathway also includes components of the Wnt signaling pathway (Montcouquiol et al., 2006) but is independent of β-catenin and is therefore considered a non-canonical arm of the Wnt pathway. Orthologs of the *Drosophila* PCP genes are present in vertebrates, and genetic analyses have confirmed that an intact PCP signaling pathway is required for shaping the post-gastrulation embryo, including neural tube closure (Wallingford et al., 2000; Darken et al., 2002; Jessen et al., 2002; Keller, 2002).

Here, we report that *Cdx1/2* DKO mutants exhibit CRS, along with additional defects consistent with a role for Cdx signaling upstream of PCP. In agreement with this, *Cdx1/2* mutants interacted genetically with a *Scrib* mutant allele known to impact PCP. Finally, *Cdx1/2* DKO mutants appear to regulate the expression of *Ptk7* directly, a gene known to be involved in PCP signaling, as evidenced by expression analysis, chromatin immunoprecipitation and transcription assays, suggesting a mechanism by which Cdx impacts on PCP signaling. Together, these finding provide evidence indicative of a novel and crucial role for Cdx members in neural tube closure.

MATERIALS AND METHODS

Generation of mutant mice

Cdx2$^{f/f}$, *Cdx1$^{-/-}$* and *CMV*-β-*actin-Cre-ERT2* (Cre-ERT2) mice have been previously described (Subramanian et al., 1995; Santagati et al., 2005; Savory et al., 2009a). *Cdx1/2* DKO mutants were generated by crossing *Cdx2$^{f/f}$* and *Cdx2$^{f/f}$;Cre-ERT2* lines into the *Cdx1$^{-/-}$* background. *Cdx1$^{-/-}$ Cdx2$^{f/f}$; Cre-ERT2* stud males were mated with *Cdx1$^{-/-}$ Cdx2$^{f/f}$* females, pregnant females were administered a single 2 mg dose of tamoxifen at E5.5 [as described previously (Savory et al., 2009a)] and embryos harvested at E8.5-E10.5 for analysis. Heterozygous *Scrib^{CRC}* males (Murdoch et al., 2001b; Murdoch et al., 2003), obtained from the Jackson Laboratories, were crossed with *Cdx1$^{-/-}$ Cdx2$^{f/f}$; Cre-ERT2* females to generate *Cdx1$^{+/-}$ Cdx2$^{+/f}$; Cre-ERT2 Scrib$^{+/-}$* males, which were mated with *Cdx2$^{f/f}$* or *Cdx1$^{-/-}$Cdx2$^{f/f}$* females. Pregnant females were treated either as above, or with a single 3 mg dose of tamoxifen at E8.5 and embryos collected for analysis at E15.5.

Embryo collection and analysis

Animals were mated overnight and noon of the day of detection of a vaginal plug was considered as E0.5. Pregnant females were treated with tamoxifen by oral gavage as above, embryos dissected in PBS, yolk sacs collected for genotyping and embryos fixed overnight in 4% paraformaldehyde (PFA) at 4°C. Whole-mount in situ hybridization was performed as described previously (Houle et al., 2000; Pilon et al., 2007; Savory et al., 2009a) using probes generated from previously described plasmids: *Wnt3a* (Takada et al., 1994), *T* (Wilkinson et al., 1990), *Cyp26a1* (Abu-Abed et al., 2003), *Uncx4.1* (*Uncx* – Mouse Genome Informatics) (Mansouri et al., 1997), *Sox2* (Kamachi et al., 1998), *Pax3* (Goulding et al., 1991), *Mox1* (*Meox1* – Mouse Genome Informatics; ATCC EST, IMAGE 3984366), *Paraxis* (*Tcf15* – Mouse Genome Informatics; ATCC EST, IMAGE 5143248), *Vangl2* (ATCC EST, IMAGE 6509008), *Dvl1* (ATCC EST, IMAGE 3583393), *Dvl2* (ATCC EST, IMAGE 6402000), *Scrib* (ATCC EST, IMAGE 5721250) and *Ptk7* (ATCC EST, IMAGE 30619280). In all cases, embryos to be compared were processed in parallel with control for variations in signal intensity.

Morphometric analysis

Embryos were dissected in PBS, fixed in 4% PFA overnight and then washed and photographed in PBS. Stage-matched embryos were measured at identical magnification, using a minimum of three embryos of each genotype.

Chromatin immunoprecipitation (ChIP) analysis

ChIP assays from embryonic material were performed as described previously (Pilon et al., 2006; Savory et al., 2009a) using polyclonal antiserum to Cdx2 (Savory et al., 2009b). Rabbit pre-immune serum and an anti-Gal4 antibody (Santa Cruz) were employed as negative controls. PCR was performed using the primers: 5′-TGACCCGCCTCCCA-CACCCC-3′ and 5′-GCCCCCTCCTCTCCGCCTGC-3′.

Transfection analysis

An 883 bp fragment 5′ to the Ptk7 translation start site was PCR amplified from mouse genomic DNA, verified by sequencing and subcloned into pXP2 (Nordeen, 1988; Jung et al., 2002) to generate a luciferase-based reporter construct. The effect of Cdx on the activity of this promoter was assessed by transfection analyses in P19 embryo carcinoma cells as previously described (Pilon et al., 2006; Savory et al., 2009a).

Semi quantitative RT-PCR

Semi-quantitative RT-PCR was performed as previously described (Savory et al., 2009a). Primers for Cdx1, Cdx2, Cdx4 and β-actin have been described previously (Savory et al., 2009b). The Ptk7 fragment was amplified using the primers: 5′-GGAGATCTCAAACAGTTCC-3′ and 5′-GCCGGTCCTTGGGGTTCG-3′.

RESULTS

Ablation of *Cdx1* and *Cdx2* leads to loss of *Cdx4* expression

To generate embryos lacking both *Cdx1* and *Cdx2*, *Cdx2$^{f/f}$* (Savory et al., 2009a) and *Cdx2$^{f/f}$; Cre-ERT2* lines (Santagati et al., 2005; Savory et al., 2009a) were crossed into the *Cdx1$^{-/-}$* background (Subramanian et al., 1995). Pregnant females from *Cdx1$^{-/-}$Cdx2$^{f/f}$; Cre-ERT2* × *Cdx1$^{-/-}$Cdx2$^{f/f}$* matings were administered a single 2 mg dose of tamoxifen at E5.5 by oral gavage; this treatment results in complete loss of Cdx2 protein (Savory et al., 2009a). *Cdx1/2* double mutants (*Cdx1/2* DKO) were recovered at E8.5-E10.5 at the expected ratio.

We, and others, have reported reduction of *Cdx4* expression in *Cdx2*-null mutants (Chawengsaksophak et al., 2004; Savory et al., 2009a). As cross- and autoregulation have been reported among Cdx family members (Lorentz et al., 1997; Trinh et al., 1999; Prinos et al., 2001; Beland et al., 2004; Chawengsaksophak et al., 2004), we assessed the expression of Cdx4 by whole-mount immunohistochemistry in *Cdx1/2* DKO, and found that Cdx4 was lost in the *Cdx1/2* DKO mutants at E8.5 (Savory et al., 2011). This suggests that the *Cdx1/2* DKO phenotype described below may be due to loss of all Cdx function.

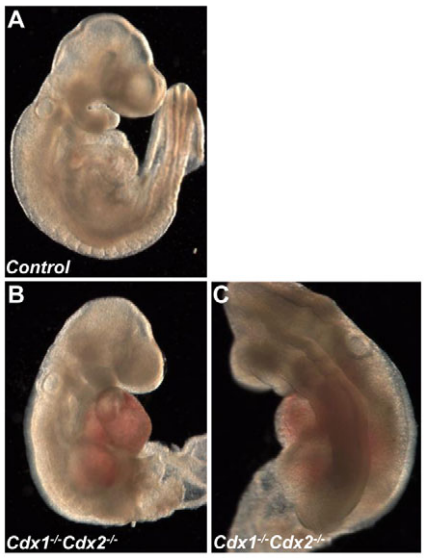

Fig. 1. *Cdx1/2* DKO mutant phenotype. (**A-C**) Control (A), and lateral (B) and dorsal (C) views of the *Cdx1/2* DKO mutants showing the axial truncation (B) and craniorachischisis (CRS) (C). CRS is 100% penetrant.

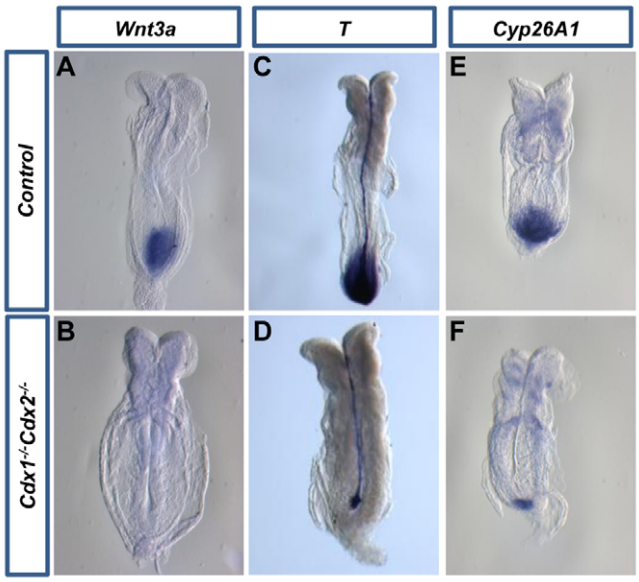

Fig. 2. Expression of genes involved in paraxial mesoderm specification is compromised in *Cdx1/2* DKO mutants. (**A-F**) In situ hybridization analysis of E8.5 control (A,C,E) and *Cdx1/2* DKO mutant (B,D,F) embryos. Expression of *Wnt3a, T* and *Cyp26a1* is markedly reduced in the mutant tailbud, while notochordal expression of *T* is unaffected.

Cdx1/2 DKO mutant embryos exhibit severe axial elongation defects

Cdx1/2 DKO mutants died at midgestation (E10.5), and exhibited a foreshortened AP axis and CRS, a severe NTD in which the entire neural tube remains open (Fig. 1B,C). Relative to *Cdx2* conditional null mutants, *Cdx1/2* DKO offspring exhibited a more severe truncation with axis elongation ending rostral to the forelimb bud region (Fig. 1B) (Savory et al., 2009a). This is consistent with the co-expression of Cdx family members in the posterior embryo, and their previously demonstrated functional overlap (van den Akker et al., 2002; Davidson and Zon, 2006; van Nes et al., 2006; Faas and Isaacs, 2009). However, none of the previously described Cdx mutants reported to date exhibit neural tube defects (Subramanian et al., 1995; Chawengsaksophak et al., 2004; Savory et al., 2009a; van den Akker et al., 2002; van Nes et al., 2006; Young et al., 2009), suggesting previously unsuspected roles for Cdx function (see below).

Axis truncation in $Cdx2^{-/-}$ conditional null embryos is associated with reduced expression of several Cdx target genes required for presomitic mesoderm (PSM) ontogenesis, including *Wnt3a*, *T* and *Cyp26a1* (Savory et al., 2009a). We therefore assessed the expression of these targets to determine whether the increased severity of the axial defect in *Cdx1/2* DKO manifested through convergent transcriptional regulation of these pathways. *Wnt3a* is expressed in the tailbud and PSM, and functions to maintain a pool of paraxial mesoderm precursor cells and to specify paraxial mesoderm in conjunction with *T* and *Tbx6* (Chapman et al., 1996; Dunty et al., 2008; Galceran et al., 1999; Greco et al., 1996; Herrmann et al., 1990; Takada et al., 1994; Yamaguchi et al., 1999; Yoshikawa et al., 1997; Aulehla et al., 2007). *Cyp26a1* mediates retinoic acid catabolism in the tailbud, establishing a region deplete of retinoid signaling believe to be necessary to prevent precocious differentiation (Abu-Abed et al., 2003; Sakai et al., 2001). Both *Wnt3a* and *T* were essentially completely extinguished in the tailbud of E8.5 *Cdx1/2 DKO* (Fig. 2B,D), in contrast to the *Cdx2*-null mutant background, where they were only modestly affected at this stage (Savory et al., 2009a). *T* expression was also maintained in the notochord of the mutants (Fig. 2D), consistent with *Cdx1/2* function exclusively in the tailbud. *Cyp26a1* expression was also severely compromised at E8.5 in the *Cdx1/2* DKO background (Fig. 2F), although it is also significantly impacted by loss of *Cdx2* alone at this stage (Savory et al., 2009a).

Tbx6 is expressed in the primitive streak and nascent paraxial mesoderm and is downregulated upon somite formation (Chapman et al., 1996; Chapman and Papaioannou, 1998), while *Msgn1* is expressed in the PSM and is necessary for its maturation (Yoon and Wold, 2000). Both genes were correctly expressed in the PSM of E8.5 *Cdx1/2* DKO embryos, but at markedly reduced levels (data not shown). Thus, PSM appears to be correctly specified in the double mutants, but as with the $Cdx2^{-/-}$ mutants, its production appears to be prematurely terminated (Savory et al., 2009a). These data are consistent with the functional overlap between *Cdx1* and *Cdx2*, leading to an increased severity of axial truncation owing to reduced expression of previously characterized target genes, including *T*, *Wnt3a* and *Cyp26a1*.

Somite defects in *Cdx1/2 DKO* mutants

Somites are derived from segmentation of the PSM in the caudal embryo. Once formed, somites differentiate into different compartments along the dorsoventral and mediolateral axes; dermal precursor cells and skeletal muscles are derived from the dorsally located dermamyotome, whereas the ventrally located sclerotome gives rise to bone and cartilage. Each somite is further subdivided into rostral and caudal compartments (Dubrulle and Pourquie, 2004). In the *Cdx1/2* DKO mutants, somites appeared to be poorly condensed and irregularly shaped, as well as wider and

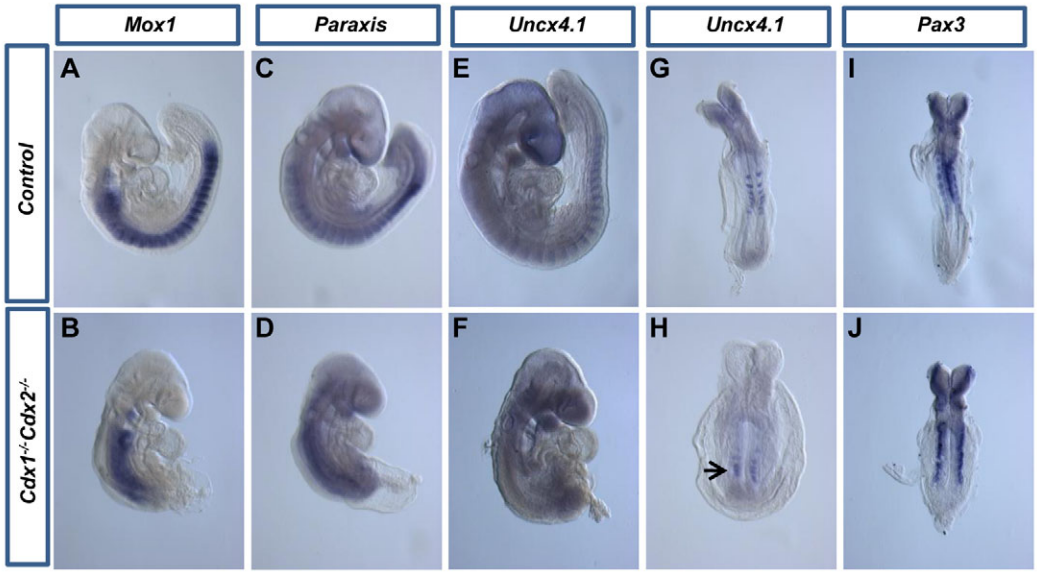

Fig. 3. *Cdx* function is required for somite patterning. (**A-J**) Expression of *Mox1*, *Paraxis* (*Tcf15*), *Uncx4.1* (*Uncx*) and *Pax3* in control (A,C,E,G,I) and *Cdx1/2* DKO mutant (B,D,F,H,J) embryos at E9.5 (A-F) and E8.5 (G-J). Clear somite boundaries are lacking in the mutants (B,D,F,H,J).

foreshortened with intersomitic boundaries being indistinct at E9.5 (Fig. 3 and see below); similar defects have not been reported for *Cdx1* (Subramanian et al., 1995) or *Cdx2* (Chawengsaksophak et al., 2004; Savory et al., 2009a) single mutants.

To further characterize somite defects, we used in situ hybridization to examine the expression of *Mox1* and *Paraxis*, which mark somitic mesoderm. In control embryos, *Mox1* and *Paraxis* were similarly expressed in the somites as well as in the anterior PSM (Fig. 3A,C). By contrast, both genes were expressed at very low levels and with indistinct boundaries in *Cdx1/2* DKO mutants (Fig. 3B,D).

We next examined whether somite patterning was affected by loss of *Cdx*. In control embryos, *Uncx4.1* was expressed as regular reiterations in the caudal somite compartment at E9.5 and E8.5 (Fig. 3E,G). By contrast, expression was markedly reduced in *Cdx1/2* DKO at E9.5 (Fig. 3F). Moreover, although *Uncx4.1* was detected in the double mutants at E8.5, its expression was lost in anterior somites, whereas transcripts in posterior somites exhibited an irregular and often fused appearance (Fig. 3H, arrow). Finally, *Pax3* is expressed in the dorsal neural tube and in the dermamyotome of condensed somites at E8.5 (Fig. 3I). By contrast, *Pax3* was expressed as a single, bilateral, stripe in the mutants embryos (Fig. 3J). Taken together, these data indicate that paraxial mesoderm is specified in *Cdx1/2* DKO mutants, although somite segmentation and rostral-caudal somite pattering is defective.

Cdx function is essential for neural tube closure

Neural tube closure normally initiates at three sites along the rostrocaudal axis, with failure of the initial event at the midbrain-hindbrain boundary resulting in CRS (Copp et al., 2003). Neural tube closure is preceded by the extension of the AP axis with concomitant narrowing of the mediolateral axis (convergence). These morphogenetic movements are essential for neural tube closure, and are believed to rely, at least in part, on PCP signaling. During this process, the reshaping of the neural plate brings the neural folds in close juxtaposition to facilitate fusion. Impairment of these movements leads to an embryo that is typically shorter and wider, with the consequence being that the neural folds are spaced more widely apart, impinging on events necessary for neural tube closure (see Copp and Greene, 2010; Copp, 2003).

To determine whether *Cdx1/2* DKO mutants exhibited defects characteristic of lesions in PCP-dependent morphogenetic movements, we measured the length and width of embryos over the window (four to eight somites) immediately preceding neural tube closure (Fig. 4A-C). Consistent with other CRS mutants (Garcia-Garcia et al., 2008; Wang et al., 2006a; Ybot-Gonzalez et al., 2007; Wallingford et al., 2002), we found a significant reduction in the length-to-width ratio (LWR) in *Cdx1/2* DKO mutants relative to controls. Although control embryos from four to seven somites exhibited a continuous increase in LWR, no such increase was observed in the mutants (Fig. 4A,C). Whereas a foreshortened AP axis in the mutants may be ascribed, in part, to the premature termination of axis elongation, as seen in *Cdx2*$^{-/-}$ embryos, neither a concomitant widening of the mediolateral axis nor an open neural tube is seen in these single null mutants (Savory et al., 2009a).

CRS mouse mutants typically exhibit an abnormal broadening of the midline, which has been proposed to be due to defects in convergence-extension movements in some instances (see Ybot-Gonzalez et al., 2007; Yen et al., 2009). We performed a histological examination of transverse sections of *Cdx1/2* DKO at E8.5 to assess midline morphology. In control embryos, a sharp bend in the midline of the neural plate at the median hinge point is evident in the caudal embryo (Fig. 4D, arrow). By contrast, in *Cdx1/2* DKO mutants two apparent hinge points were evident on either side of a broadened midline (Fig. 4E arrows). In addition, the mutant neuroepithelium appeared thicker and both the neuroectoderm and mesoderm appeared disorganized, with an absence of the dorsal aorta and hindgut diverticulum (Fig. 4E).

The domain of genes expressed in the floor plate, such as *Shh* and *T* are broadened in a number of mutants exhibiting CRS, such as *Dvl1/2* double mutants and *Vangl2* mutants (Greene et al., 1998; Wang et al., 2006a). Although the neural plate appeared wider in the *Cdx1/2* DKOs, both *Shh* and *T* were expressed in the midline

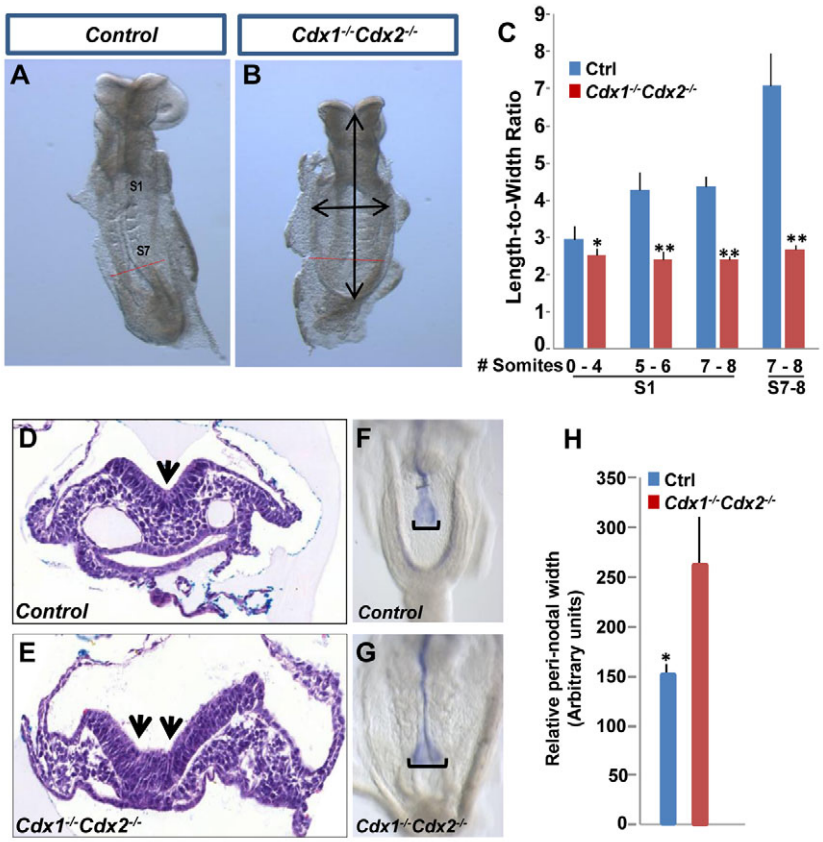

Fig. 4. *Cdx1/2* DKO mutants exhibit aberrant length-to-width ratio and a broadened midline. (**A**,**B**) Dorsal view of representative control (A) and *Cdx1/2* DKO mutant (B) littermates at E8.5. S1 and S7 mark the first and seventh somite, respectively. Arrows in B mark the embryonic length and width as measured on photomicrographs of embryos viewed from the dorsal side. At the seven- to eight-somite stage, width measurements were taken at the level of the first (S1) and last formed (S7 or S8) somites. (**C**) *Cdx1/2* DKO mutants displayed significant reduction of the length-to-width ratio (LWR) at the level of the first and last formed somites. (**D**,**E**) Histological analysis of control (D) and a representative *Cdx1/2* DKO (**E**) showing a broadened midline characteristic of the mutant background. There are two hinge points in the mutant compared with control (arrows). Broken red lines indicate the axial level of the sections in D and E. (**F**,**G**) In situ hybridization of *Shh* expression at E8.5 in control (F) and mutant (G) embryos. Brackets indicate the peri-nodal region measured and quantified in H. (**H**) Quantitative analysis illustrating a broadening of the peri-nodal domain of *Shh* expression in the DKO mutants relative to controls. In C,H, error bars represent s.d. from the mean of a minimum of three independent stage-matched samples. *P<0.08. **P<0.0009.

of mutants in a manner similar to controls (Fig. 2C,D; Fig. 5A,B) at E8.5 and E9.5. However, the expression domain of *Shh* was markedly broader in the peri-nodal region in *Cdx1/2* DKO mutants relative to controls (Fig. 4F-H). The expression of the neural markers, *Sox2* and *Hes5*, were also medially expanded (Fig. 5C-F), consistent with the broadened neural plate in the mutants, and confirming that neuroectoderm was specified.

Cdx1/2 DKO mutants and PCP signaling

Of the 14 known genes that cause CRS in mouse, all but one encode members involved in PCP signaling (see Harris and Juriloff, 2007; Harris and Juriloff, 2010). Such PCP mutants also typically exhibit broadened and foreshortened somites (Lu et al., 2004; Curtin et al., 2003; Murdoch et al., 2001a; Murdoch et al., 2003; Wang et al., 2006a; Wang et al., 2006b), wider neural plates with a broadened midline (Greene et al., 1998; Lu et al., 2004; Wang et al., 2006a; Ybot-Gonzalez et al., 2007), a shortened and widened body axis as well as disruption of the stereociliary bundle orientation in the organ of Corti (Lu et al., 2004; Montcouquiol et al., 2003; Wang et al., 2006b).

Although the early lethality of *Cdx1/2* DKO offspring precluded examination of the stereociliary bundle orientation in the organ of Corti, which is likely to be unaffected given the expression domain of Cdx members, the phenotypic commonalities between *Cdx1/2* DKO mutants and known PCP mouse mutants is consistent with a crucial role for Cdx genes upstream of PCP signaling. In agreement with this, Cdx genes and many PCP components exhibit overlapping expression domains in the caudal embryo (Kibar et al., 2001; Klingensmith et al., 1996; Lohnes, 2003; Lu et al., 2004; Murdoch et al., 2003; Sussman et al., 1994). To begin to determine whether Cdx function impacted on transcription of PCP members, we compared the expression of several PCP genes between *Cdx1/2* DKO and control embryos. Core PCP components, defined as those that are required for PCP in multiple tissues, include *Vangl2*, *Dvl1/2*, *Ptk7* and *Celsr1*; several lines of evidence also indicate that *Scrib* plays key roles in PCP signaling (Coubard et al., 2009; Kallay et al., 2006; Montcouquiol et al., 2003; Moncouquiol et al., 2006b; Murdoch et al., 2001) as does *Ptk7* (Lu et al., 2004; Paudyal et al., 2010; Shnitsar and Borchers, 2008).

Vangl2 is expressed throughout the developing nervous system and in the hindgut diverticulum (Kibar et al., 2001). *Scrib* is expressed in the neuroepithelium with lower but detectable levels in presomitic, somitic and lateral plate mesoderm (Murdoch et al., 2003). *Dvl1* and *Dvl2* are broadly expressed with stronger expression in the neural folds (Klingensmith et al., 1996; Sussman et al., 1994), while *Ptk7* has a broad and dynamic expression pattern during neurulation with the highest levels of expression in the posterior embryo (Lu et al., 2004).

At E8.5 the expression levels of *Vangl2* and *Scrib* were modestly reduced in the caudal region of *Cdx1/2* DKO mutants relative to controls (Fig. 6A,B,G,H). By contrast, expression of *Ptk7*, *Dvl1* and *Dvl2* was almost absent in the posterior of mutant embryos (Fig. 6C-F). These results are consistent with Cdx function being necessary for the expression of several key PCP genes.

Ptk7 is a Cdx target gene

In *Vangl2*-null mutants, axis elongation is normal prior to neural tube closure (Gerrelli and Copp, 1997), while *Dvl1/2* double mutants have a foreshortened body axis but relatively normal somite morphology (Wang et al., 2006a). By contrast, *Ptk7*-null mutants have defects in both axis elongation and in somite morphology by the five- to seven-somite stage (Lu et al., 2004),

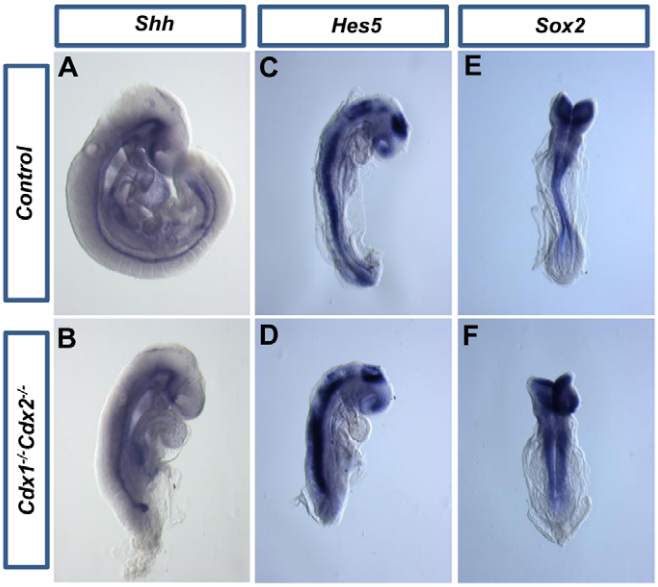

Fig. 5. Neuroectoderm is specified in *Cdx1/2* DKO mutants. (**A**,**B**) Lateral view showing expression of *Shh* in E9.5 control (A) and *Cdx1/2* DKO mutant (B) embryos. (**C-F**) Expression of the neural marker *Hes5* (C,D) and *Sox2* (E,F) in control (C,E) and mutant (D,F) embryos. The neuroectoderm domain is broader in the mutants (D,F).

and is therefore the PCP mutant which most closely phenocopies the *Cdx1/2* DKO phenotype. Moreover, unlike *Dvl1/2* and *Vangl2* mutants, neither *Cdx1/2* DKO nor *Ptk7*-null embryos exhibit broadened floor plate gene expression patterns (Lu et al., 2004). In addition, *Ptk7* expression was downregulated at E7.5 in *Cdx1/2* DKO mutants, preceding initiation of neural tube closure (see Fig. S1 in the supplementary material), and consistent with in situ hybridization analysis at E8.5 (Fig. 6J)

Ptk7 interacts genetically with *Vangl2* (see Lu et al., 2004; Paudyal et al., 2010) and is an essential regulator of neural tube closure in the mouse (Yen et al., 2009). Binding site algorithms identified several Cdx response elements in an 883 bp region of 5′ flanking sequence (Fig. 7A), which has previously been shown to be functional as a promoter for human *PTK7* (Jung et al., 2002). This sequence is 100% conserved between mouse and human. Consistent with this, ChIP analysis revealed occupancy of this promoter region by Cdx2 in E8.5 embryos (Fig. 7B). A functional relationship is further supported by transfection assays in P19 cells, which illustrated that these promoter sequences respond to both Cdx1 and Cdx2 (Fig. 7C). Taken together, these data are entirely consistent with *Ptk7* as a direct Cdx target, and suggest a mechanistic basis by which Cdx regulates neural tube closure.

Cdx and *Scrib* interaction

Scrib loss-of-function mutants exhibit CRS and other defects typical of PCP signaling mutants (Murdoch et al., 2001b) and have been shown to interact genetically and physically with other members of the PCP pathway (Montcouquiol et al., 2003; Montcouquiol et al., 2006b; Wansleeben et al., 2010). We therefore investigated the relationship of *Cdx* and PCP via analysis of an allelic series of *Cdx-Scrib* mutants.

Following tamoxifen-mediated deletion of *Cdx2* at E5.5, $Cdx1^{+/-}Cdx2^{+/-}Scrib^{+/-}$, $Cdx1^{-/-}Cdx2^{+/-}Scrib^{+/-}$ and $Cdx1^{+/-}Cdx2^{-/-}Scrib^{+/-}$ E9.5 offspring were found to have modestly shortened AP axes, but did not display CRS (data not shown). LWR measurements at the seven-somite stage likewise revealed no significant difference in the *Cdx-Scrib* backgrounds relative to controls (see Fig. S2A in the supplementary material). It is possible that the single copy of Cdx1, and concomitant partial recovery of Cdx4 activity in these mutants (see Fig. S2B,C in the supplementary material), elicits sufficient *Ptk7* expression to effect neural tube closure in the face of reduced *Scrib* function.

Genetic interaction between *Ptk7* and *Vangl2* has been suggested by an increase in the incidence of spina bifida, which manifests at later stages of development (Lu et al., 2004). However, we were unable to assess such NTD due to the lethality evoked by *Cdx2* loss at mid-gestation (Chawengsaksophak et al., 2004; Savory et al., 2009a). We therefore treated pregnant females at E8.5 with a single suboptimal dose of tamoxifen to circumvent lethality. This regimen reduced the severity of the Cdx2 mutant phenotype (Savory et al., 2009a), and offspring examined at E15.5 (n=36) did not exhibit spina bifida. However, most (90%) of the $Cdx1^{+/-}Cdx2^{-/-}Scrib^{+/-}$

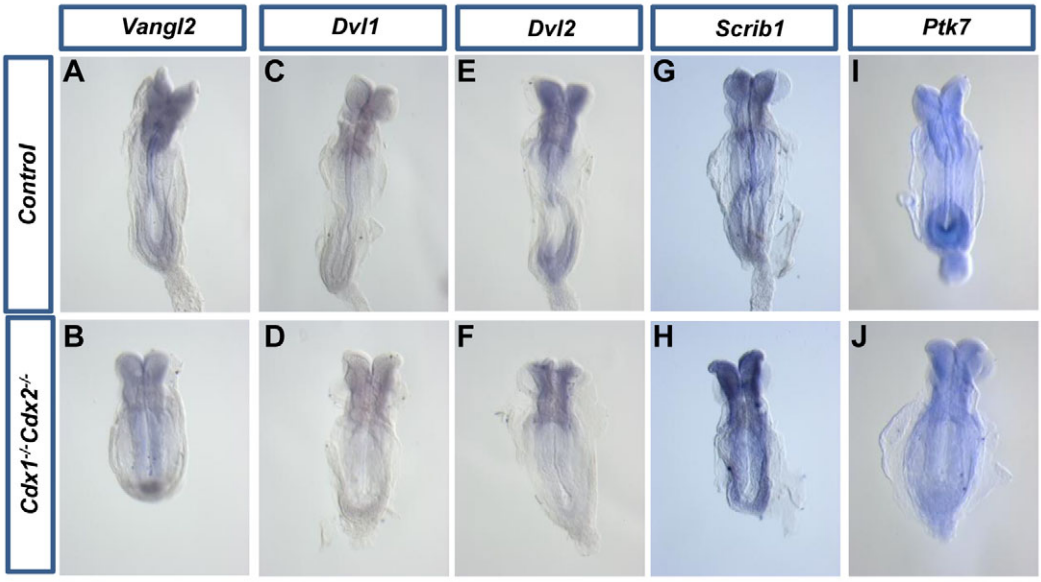

Fig. 6. Expression of PCP genes in *Cdx1/2* DKO mutants. (**A-J**) In situ hybridization analysis of E8.5 control (A,C,E,G,I) and *Cdx1/2* DKO mutant (B,D,F,H,J) embryos showing reduced expression of *Dvl1, Dvl2* and *Ptk7* in the mutants relative to controls.

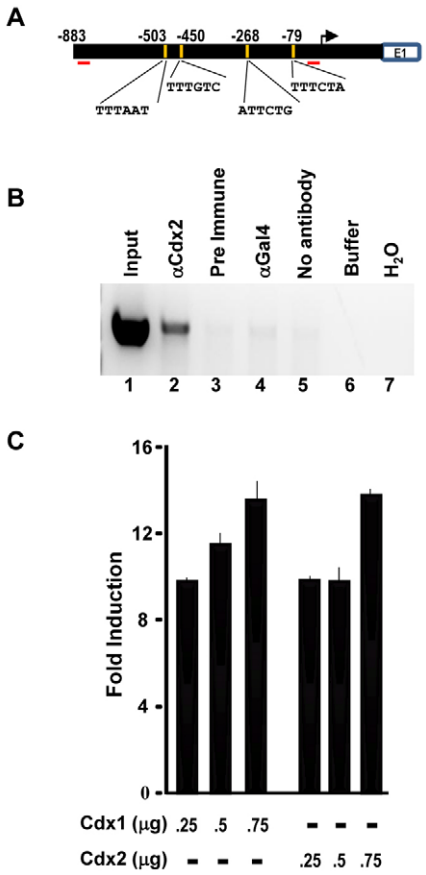

Fig. 7. Regulation of the *Ptk7* promoter by Cdx members. (**A**) Schematic representation of the 883 bp 5′ proximal region of the *Ptk7* promoter showing the positions of putative CDREs (yellow lines) identified by binding site algorithms. Numbers indicate the position of the first base pair in each of the putative CDREs relative to the transcription start site (arrow), with the sequence of each motif indicated below. E1 represents exon 1 of the *Ptk7* gene, while red bars indicate the relative positions of the oligonucleotides used in the ChIP analysis. (**B**) ChIP analysis of the *Ptk7* promoter from E8.5 embryos demonstrating occupancy by Cdx2. (**C**) The *Ptk7* promoter responds to Cdx1 and Cdx2. A luciferase-based reporter vector derived from the 883 bp 5′ proximal region of the *Ptk7* promoter was transfected in the absence or presence of Cdx expression vectors. Activity was normalized relative to a co-transfected β-galactosidase vector. Error bars indicate s.d. from the mean in independent triplicate samples.

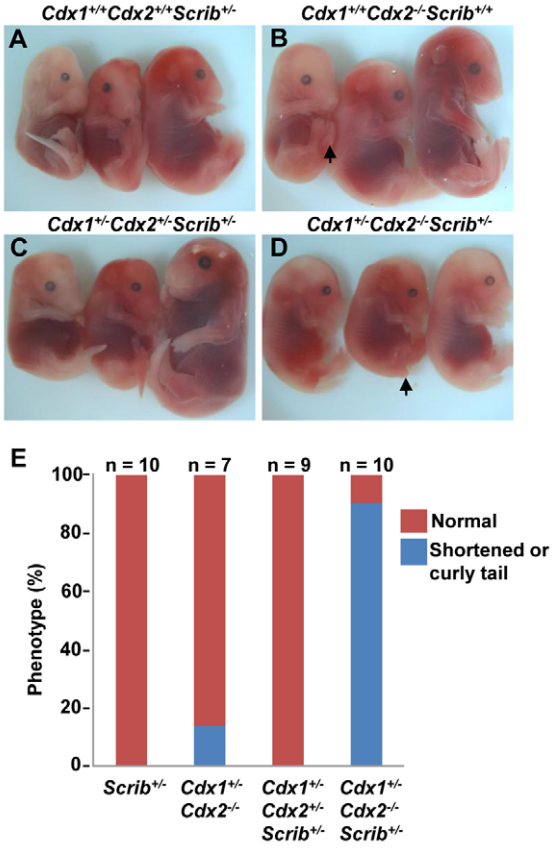

Fig. 8. Genetic interaction between *Cdx* and *Scrib*. (**A-D**) Lateral and ventral views of single and compound heterozygous embryos showing the caudal phenotype in *Cdx1*$^{+/-}$*Cdx2*$^{-/-}$*Scrib*$^{+/-}$ mutants (arrow in D). (**E**) Quantification of the caudal phenotype. The number of embryos for each of the four genotypes is indicated at the top.

embryos exhibited a shortened or curly tail (e.g. arrow in Fig. 8D). This phenotype was seen in only one of seven *Cdx1*$^{+/-}$*Cdx2*$^{-/-}$ mutants (arrow in Fig. 8B), and in none of the *Scrib* heterozygotes. Although the suboptimal tamoxifen treatment at E8.5 probably accounts for the relatively mild phenotype in the *Cdx1*$^{+/-}$*Cdx2*$^{-/-}$*Scrib*$^{+/-}$ mutants, nevertheless, these data are consistent with an interaction between Cdx function and the PCP pathway.

DISCUSSION

Central effectors in planar cell polarity are crucial to several fundamental events in vertebrate development, including neural tube closure (Montcouquiol et al., 2006a; Simons and Mlodzik, 2008). Although considerable information has emerged regarding PCP pathway members, our understanding of the nature of upstream transcriptional regulators of these core constituents is lacking. We used the Cre-loxP system to delete *Cdx2* in the context of the *Cdx1*-null mutant background, and found that the resulting *Cdx1/2* DKO offspring exhibited features consistent with an impact on PCP signaling, including CRS associated with a foreshortened and broader axis. Additional analyses suggest that the PCP member *Ptk7* is a direct Cdx target gene, suggesting a novel role for Cdx function in regulating neural tube closure.

Cdx members functionally overlap

Functional overlap among Cdx members has been suggested by the finding that compound mutants typically exhibit more pronounced vertebral patterning phenotypes than seen in the corresponding single mutant backgrounds (van den Akker et al., 2002; Young et al., 2009). In addition, gene substitution approaches have shown that *Cdx2* can fulfill *Cdx1* function in regulating Hox gene expression and cervical vertebral patterning (Savory et al., 2009b). The phenotype of the *Cdx1/2* DKO mutants described herein further highlights the functional overlap among Cdx family members, as neither cognate single mutant exhibits NTD (Chawengsaksophak et al., 2004; Savory et al., 2009a; Subramanian et al., 1995). This functional commonality is probably due to convergent regulation of common target genes, as evidenced by the marked reduction of expression of the Cdx targets *T* and *Wnt3a* in the double mutants, compared with *Cdx2* single mutants.

This supposition is also consistent with ChIP studies which place all three Cdx members on these, and other, target promoters in vivo (Savory et al., 2009a).

Cdx genes exhibit extensive overlapping in expression in the caudal embryo. Given that *Cdx4* expression is essentially lost in the *Cdx1/2* DKO background, it is possible that the *Cdx1/2* DKO phenotype is reflective of complete loss of Cdx function. However, *Cdx4*-null mutants appear morphologically normal and Cdx4 function is only apparent in the context of compound mutant backgrounds (van Nes et al., 2006). Thus, the contribution of *Cdx4* to the *Cdx1/2* DKO phenotype may be relatively modest.

Cdx function and neural tube closure

The importance of PCP signaling in neural tube closure, and in other developmental paradigms, has become increasingly clear with the characterization of loss-of function mutants in various vertebrate models, including the mouse (Curtin et al., 2003; Darken et al., 2002; Jessen et al., 2002; Lu et al., 2004; Murdoch et al., 2003; Wang et al., 2006a; Wang et al., 2006b), *Xenopus* (see Tada and Smith, 2000; Wallingford et al., 2000) and zebrafish (see Jessen et al., 2002; Marlow et al., 1998). In both *Xenopus* (Darken et al., 2002; Tada, 2000; Wallingford et al., 2002) and zebrafish (see Hammerschmidt et al., 1996; Jessen et al., 2002; Marlow et al., 1998; Sepich et al., 2000; Solnica-Krezel et al., 1996), the PCP pathway impacts on convergent extension (CE), leading to CE-related defects such as a wider and shorter axis, a wider notochord and CRS. Although the morphogenic movements that create the elongated AP axis in the mouse are not as well characterized, the phenotypic similarities between these CE mutants and those of PCP mouse mutants suggest a similar mechanism at play. Indeed the wider midline in *Vangl2* mutants has been attributed to a defect in CE (Ybot-Gonzalez et al., 2007), while Ptk7 mutants have been suggested to have a lesion in mesodermal CE (Yen et al., 2009).

A significant reduction in LWR is associated with neural tube closure defects in both *Xenopus* (Wallingford et al., 2002; Wallingford and Harland, 2002) and in several mouse PCP mutants (Wang et al., 2006a; Ybot-Gonzalez et al., 2007). A marked reduction in LWR was also observed in *Cdx1/2* DKO embryos prior to normal initiation of neural tube closure. Although a decrease in the length of the DKO mutants may be due to impaired axial elongation, the increase in width cannot be explained by such a deficiency. In this regard, *Cdx2* mutants exhibit precocious termination of axial extension, but no discernable medial widening or neural tube defects (Savory et al., 2009a). The reduced LWR in the *Cdx1/2* DKO mutants is thus consistent with disruption of PCP function, and is entirely congruent with the other phenotypic commonalities shared by *Cdx1/2* DKO and mouse PCP mutants, including a broadened midline and foreshortened somites (Garcia-Garcia et al., 2008; Greene et al., 1998; Lu et al., 2004).

Cdx and PCP interactions

Non-allelic non-complementation is typically taken as evidence for functional interaction between two gene products operating in a common process, and has been widely employed to position putative PCP components (Lu et al., 2004; Montcouquiol et al., 2003; Qian et al., 2007; Satoh et al., 2008; Wang et al., 2006a). We further assessed the relationship between Cdx and PCP by generation and analysis of *Cdx1/2* and *Scrib* compound mutants (Murdoch et al., 2003); the Scrib mutant has been shown to interact with the *Vangl2* mouse mutant in similar assays (Montcouquiol et al., 2003; Murdoch et al., 2001b).

We found a marked and highly significant increase in the incidence of caudal defects in *Cdx-Scrib* mutant offspring relative to individual Cdx or *Scrib* mutant backgrounds. Although the phenotype of the *Cdx-Scrib* mutants is relatively modest, owing to the necessity to effect late *Cdx2* ablation, it nonetheless clearly illustrates an interaction between Cdx and a gene that interacts with core PCP members, and is comparable to the previously described interactions between *Scrib* and *Vangl2* mutants. It is also apparent that minimal Cdx function suffices for neural tube closure, as CRS and other PCP-related defects were not observed in $Cdx1^{+/-}$ $Cdx2^{-/-}$ offspring, nor in any other Cdx compound mutant reported to date.

Cdx-dependent regulation of PCP pathway members

PCP is governed by a set of conserved ‘core’ proteins as well as a number of upstream effectors, including non-canonical Wnts (Montcouquiol et al., 2006; Simons and Mlodzik, 2008). We surveyed the expression of a number of these genes in *Cdx1/2* DKO embryos, and found that the transcript levels of *Dvl1, Dvl2* and *Ptk7* were all reduced in the mutant background, whereas the expression of *Vangl2* and *Scrib* appeared to be unperturbed. The close phenocopy between the *Cdx1/2* DKO and *Ptk7* mutants prompted further investigation, leading to our finding that Cdx2 occupied the *Ptk7* promoter in vivo, and that this promoter could be transcriptionally regulated by Cdx members in transfection assays. Although *Ptk7* is not considered to be a core PCP gene, axis elongation fails in *Ptk7* mutants, and the genetic interaction between *Ptk7* and *Vangl2* mutants indicate that they function in a common genetic pathway to regulate PCP (Lu et al., 2004). Taken together, these findings are entirely consistent with Cdx impacting on PCP, at least in part, via regulation of *Ptk7*.

Novel roles for Cdx in the mouse

Cdx transcription factors are well documented to affect vertebral patterning through regulation of Hox genes. Recent work has also identified Hox-independent functions for Cdx genes in both intestinal patterning and hindgut specification (Gao et al., 2009; Grainger et al., 2010) as well as in the ontogenesis of presomitic mesoderm and axial extension (Savory et al., 2009a). Our results reveal an additional novel role for Cdx members upstream of PCP in the mouse. Morpholino knockdown of Cdx genes in *Xenopus* results in posterior defects, but such manipulations have not been reported to impact on PCP (Faas and Isaacs, 2009), nor has a role for Cdx genes in PCP signaling been reported in zebrafish. These findings suggest either that a role for Cdx in PCP signaling is unique to the mouse or, more likely, that sufficient Cdx function remains in these other models to support PCP. Taken together with prior findings, our present observations place Cdx in a hierarchy wherein they coordinate the development and patterning of caudal mesodermal and endodermal derivatives, as well as PCP-dependent morphogenesis necessary for neural tube closure.

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Competing interests statement

The authors declare no competing financial interests.

Supplementary material

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http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.056622/-/DC1

References

Abu-Abed, S., Dolle, P., Metzger, D., Wood, C., MacLean, G., Chambon, P. and Petkovich, M. (2003). Developing with lethal RA levels: genetic ablation of Rarg can restore the viability of mice lacking Cyp26a1. *Development* **130**, 1449-1459.

Aulehla, A., Wiegraebe, W., Baubet, V., Wahl, M. B., Deng, C., Taketo, M., Lewandoski, M. and Pourquie, O. (2007). A beta-catenin gradient links the clock and wavefront systems in mouse embryo segmentation. *Nat. Cell Biol.* **10**, 186-193.

Beck, F., Erler, T., Russell, A. and James, R. (1995). Expression of Cdx-2 in the mouse embryo and placenta: possible role in patterning of the extra-embryonic membranes. *Dev. Dyn.* **204**, 219-227.

Beland, M., Pilon, N., Houle, M., Oh, K., Sylvestre, J. R., Prinos, P. and Lohnes, D. (2004). Cdx1 autoregulation is governed by a novel Cdx1-LEF1 transcription complex. *Mol. Cell. Biol.* **24**, 5028-5038.

Chapman, D. L. and Papaioannou, V. E. (1998). Three neural tubes in mouse embryos with mutations in the T-box gene Tbx6. *Nature* **391**, 695-697.

Chapman, D. L., Agulnik, I., Hancock, S., Silver, L. M. and Papaioannou, V. E. (1996). Tbx6, a mouse T-Box gene implicated in paraxial mesoderm formation at gastrulation. *Dev. Biol.* **180**, 534-542.

Chawengsaksophak, K., James, R., Hammond, V. E., Kontgen, F. and Beck, F. (1997). Homeosis and intestinal tumours in Cdx2 mutant mice. *Nature* **386**, 84-87.

Chawengsaksophak, K., de Graaff, W., Rossant, J., Deschamps, J. and Beck, F. (2004). Cdx2 is essential for axial elongation in mouse development. *Proc. Natl. Acad. Sci. USA* **101**, 7641-7645.

Copp, A. J. and Greene, N. D. (2010). Genetics and development of neural tube defects. *J. Pathol.* **220**, 217-230.

Copp, A. J., Greene, N. D. and Murdoch, J. N. (2003). The genetic basis of mammalian neurulation. *Nat. Rev. Genet.* **4**, 784-793.

Curtin, J. A., Quint, E., Tsipouri, V., Arkell, R. M., Cattanach, B., Copp, A. J., Henderson, D. J., Spurr, N., Stanier, P., Fisher, E. M. et al. (2003). Mutation of Celsr1 disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse. *Curr. Biol.* **13**, 1129-1133.

Darken, R. S., Scola, A. M., Rakeman, A. S., Das, G., Mlodzik, M. and Wilson, P. A. (2002). The planar polarity gene strabismus regulates convergent extension movements in Xenopus. *EMBO J.* **21**, 976-985.

Davidson, A. J. and Zon, L. I. (2006). The caudal-related homeobox genes cdx1a and cdx4 act redundantly to regulate hox gene expression and the formation of putative hematopoietic stem cells during zebrafish embryogenesis. *Dev. Biol.* **292**, 506-518.

Dubrulle, J. and Pourquie, O. (2004). Coupling segmentation to axis formation. *Development* **131**, 5783-5793.

Dunty, W. C., Jr, Biris, K. K., Chalamalasetty, R. B., Taketo, M. M., Lewandoski, M. and Yamaguchi, T. P. (2008). Wnt3a/beta-catenin signaling controls posterior body development by coordinating mesoderm formation and segmentation. *Development* **135**, 85-94.

Duprey, P., Chowdhury, K., Dressler, G. R., Balling, R., Simon, D., Guenet, J. L. and Gruss, P. (1988). A mouse gene homologous to the Drosophila gene caudal is expressed in epithelial cells from the embryonic intestine. *Genes Dev.* **2**, 1647-1654.

Epstein, M., Pillemer, G., Yelin, R., Yisraeli, J. K. and Fainsod, A. (1997). Patterning of the embryo along the anterior-posterior axis: the role of the caudal genes. *Development* **124**, 3805-3814.

Faas, L. and Isaacs, H. V. (2009). Overlapping functions of Cdx1, Cdx2, and Cdx4 in the development of the amphibian Xenopus tropicalis. *Dev. Dyn.* **238**, 835-852.

Galceran, J., Farinas, I., Depew, M. J., Clevers, H. and Grosschedl, R. (1999). Wnt3a$^{-/-}$-like phenotype and limb deficiency in Lef1$^{-/-}$Tcf1$^{-/-}$ mice. *Genes Dev.* **13**, 709-717.

Gamer, L. W. and Wright, C. V. (1993). Murine Cdx-4 bears striking similarities to the Drosophila caudal gene in its homeodomain sequence and early expression pattern. *Mech. Dev.* **43**, 71-81.

Gao, N., White, P. and Kaestner, K. H. (2009). Establishment of intestinal identity and epithelial-mesenchymal signaling by Cdx2. *Dev. Cell* **16**, 588-599.

Garcia-Garcia, M. J., Shibata, M. and Anderson, K. V. (2008). Chato, a KRAB zinc-finger protein, regulates convergent extension in the mouse embryo. *Development* **135**, 3053-3062.

Gerrelli, D. and Copp, A. J. (1997). Failure of neural tube closure in the loop-tail (Lp) mutant mouse: analysis of the embryonic mechanism. *Brain Res. Dev. Brain Res.* **102**, 217-224.

Goulding, M. D., Chalepakis, G., Deutsch, U., Erselius, J. R. and Gruss, P. (1991). Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. *EMBO J.* **10**, 1135-1147.

Grainger, S., Savory, J. G. and Lohnes, D. (2010). Cdx2 regulates patterning of the intestinal epithelium. *Dev. Biol.* **339**, 155-165.

Greco, T. L., Takada, S., Newhouse, M. M., McMahon, J. A., McMahon, A. P. and Camper, S. A. (1996). Analysis of the vestigial tail mutation demonstrates that Wnt-3a gene dosage regulates mouse axial development. *Genes Dev.* **10**, 313-324.

Greene, N. D., Gerrelli, D., Van Straaten, H. W. and Copp, A. J. (1998). Abnormalities of floor plate, notochord and somite differentiation in the loop-tail (Lp) mouse: a model of severe neural tube defects. *Mech. Dev.* **73**, 59-72.

Hammerschmidt, M., Pelegri, F., Mullins, M. C., Kane, D. A., Brand, M., van Eeden, F. J., Furutani-Seiki, M., Granato, M., Haffter, P., Heisenberg, C. P. et al. (1996). Mutations affecting morphogenesis during gastrulation and tail formation in the zebrafish, Danio rerio. *Development* **123**, 143-151.

Harris, M. J. and Juriloff, D. M. (2007). Mouse mutants with neural tube closure defects and their role in understanding human neural tube defects. *Birth Defects Res. A Clin. Mol. Teratol.* **79**, 187-210.

Harris, M. J. and Juriloff, D. M. (2010). An update to the list of mouse mutants with neural tube closure defects and advances toward a complete genetic perspective of neural tube closure. *Birth Defects Res. A Clin. Mol. Teratol.* **88**, 653-669.

Herrmann, B. G., Labeit, S., Poustka, A., King, T. R. and Lehrach, H. (1990). Cloning of the T gene required in mesoderm formation in the mouse. *Nature* **343**, 617-622.

Houle, M., Prinos, P., Iulianella, A., Bouchard, N. and Lohnes, D. (2000). Retinoic acid regulation of Cdx1: an indirect mechanism for retinoids and vertebral specification. *Mol. Cell. Biol.* **20**, 6579-6586.

Houle, M., Sylvestre, J. R. and Lohnes, D. (2003). Retinoic acid regulates a subset of Cdx1 function in vivo. *Development* **130**, 6555-6567.

Isaacs, H. V., Pownall, M. E. and Slack, J. M. (1998). Regulation of Hox gene expression and posterior development by the Xenopus caudal homologue Xcad3. *EMBO J.* **17**, 3413-3427.

Jessen, J. R., Topczewski, J., Bingham, S., Sepich, D. S., Marlow, F., Chandrasekhar, A. and Solnica-Krezel, L. (2002). Zebrafish trilobite identifies new roles for Strabismus in gastrulation and neuronal movements. *Nat. Cell Biol.* **4**, 610-615.

Jones, C. and Chen, P. (2007). Planar cell polarity signaling in vertebrates. *BioEssays* **29**, 120-132.

Jung, J. W., Ji, A. R., Lee, J., Kim, U. J. and Lee, S. T. (2002). Organization of the human PTK7 gene encoding a receptor protein tyrosine kinase-like molecule and alternative splicing of its mRNA. *Biochim. Biophys. Acta* **1579**, 153-163.

Kamachi, Y., Uchikawa, M., Collignon, J., Lovell-Badge, R. and Kondoh, H. (1998). Involvement of Sox1, 2 and 3 in the early and subsequent molecular events of lens induction. *Development* **125**, 2521-2532.

Keller, R. (2002). Shaping the vertebrate body plan by polarized embryonic cell movements. *Science* **298**, 1950-1954.

Kibar, Z., Vogan, K. J., Groulx, N., Justice, M. J., Underhill, D. A. and Gros, P. (2001). Ltap, a mammalian homolog of Drosophila Strabismus/Van Gogh, is altered in the mouse neural tube mutant Loop-tail. *Nat. Genet.* **28**, 251-255.

Kispert, A., Herrmann, B. G., Leptin, M. and Reuter, R. (1994). Homologs of the mouse Brachyury gene are involved in the specification of posterior terminal structures in Drosophila, Tribolium, and Locusta. *Genes Dev.* **8**, 2137-2150.

Klingensmith, J., Yang, Y., Axelrod, J. D., Beier, D. R., Perrimon, N. and Sussman, D. J. (1996). Conservation of dishevelled structure and function between flies and mice: isolation and characterization of Dvl2. *Mech. Dev.* **58**, 15-26.

Lohnes, D. (2003). The Cdx1 homeodomain protein: an integrator of posterior signaling in the mouse. *BioEssays* **25**, 971-980.

Lorentz, O., Duluc, I., Arcangelis, A. D., Simon-Assmann, P., Kedinger, M. and Freund, J. N. (1997). Key role of the Cdx2 homeobox gene in extracellular matrix-mediated intestinal cell differentiation. *J. Cell Biol.* **139**, 1553-1565.

Lu, X., Borchers, A. G., Jolicoeur, C., Rayburn, H., Baker, J. C. and Tessier-Lavigne, M. (2004). PTK7/CCK-4 is a novel regulator of planar cell polarity in vertebrates. *Nature* **430**, 93-98.

Macdonald, P. M. and Struhl, G. (1986). A molecular gradient in early Drosophila embryos and its role in specifying the body pattern. *Nature* **324**, 537-545.

Mansouri, A., Yokota, Y., Wehr, R., Copeland, N. G., Jenkins, N. A. and Gruss, P. (1997). Paired-related murine homeobox gene expressed in the developing sclerotome, kidney, and nervous system. *Dev. Dyn.* **210**, 53-65.

Marlow, F., Zwartkruis, F., Malicki, J., Neuhauss, S. C., Abbas, L., Weaver, M., Driever, W. and Solnica-Krezel, L. (1998). Functional interactions of genes mediating convergent extension, knypek and trilobite, during the partitioning of the eye primordium in zebrafish. *Dev. Biol.* **203**, 382-399.

Marom, K., Shapira, E. and Fainsod, A. (1997). The chicken caudal genes establish an anterior-posterior gradient by partially overlapping temporal and spatial patterns of expression. *Mech. Dev.* **64**, 41-52.

Meyer, B. I. and Gruss, P. (1993). Mouse Cdx-1 expression during gastrulation. *Development* **117**, 191-203.

Mitchell, L. E. (2005). Epidemiology of neural tube defects. *Am. J. Med. Genet. C Semin. Med. Genet.* **135C**, 88-94.

Mlodzik, M. and Gehring, W. J. (1987). Expression of the caudal gene in the germ line of Drosophila: formation of an RNA and protein gradient during early embryogenesis. *Cell* **48**, 465-478.
Mlodzik, M., Fjose, A. and Gehring, W. J. (1985). Isolation of caudal, a Drosophila homeo box-containing gene with maternal expression, whose transcripts form a concentration gradient at the pre-blastoderm stage. *EMBO J.* **4**, 2961-2969.
Montcouquiol, M., Rachel, R. A., Lanford, P. J., Copeland, N. G., Jenkins, N. A. and Kelley, M. W. (2003). Identification of Vangl2 and ScrbScrib1 as planar polarity genes in mammals. *Nature* **423**, 173-177.
Montcouquiol, M., Crenshaw, E. B., 3rd and Kelley, M. W. (2006a). Noncanonical Wnt signaling and neural polarity. *Annu. Rev. Neurosci.* **29**, 363-386.
Montcouquiol, M., Sans, N., Huss, D., Kach, J., Dickman, J. D., Forge, A., Rachel, R. A., Copeland, N. G., Jenkins, N. A., Bogani, D. et al. (2006b). Asymmetric localization of Vangl2 and Fz3 indicate novel mechanisms for planar cell polarity in mammals. *J. Neurosci.* **26**, 5265-5275.
Moore, C. A., Li, S., Li, Z., Hong, S. X., Gu, H. Q., Berry, R. J., Mulinare, J. and Erickson, J. D. (1997). Elevated rates of severe neural tube defects in a high-prevalence area in northern China. *Am. J. Med. Genet.* **73**, 113-118.
Murdoch, J. N., Doudney, K., Paternotte, C., Copp, A. J. and Stanier, P. (2001a). Severe neural tube defects in the loop-tail mouse result from mutation of Lpp1, a novel gene involved in floor plate specification. *Hum. Mol. Genet.* **10**, 2593-2601.
Murdoch, J. N., Rachel, R. A., Shah, S., Beermann, F., Stanier, P., Mason, C. A. and Copp, A. J. (2001b). Circletail, a new mouse mutant with severe neural tube defects: chromosomal localization and interaction with the loop-tail mutation. *Genomics* **78**, 55-63.
Murdoch, J. N., Henderson, D. J., Doudney, K., Gaston-Massuet, C., Phillips, H. M., Paternotte, C., Arkell, R., Stanier, P. and Copp, A. J. (2003). Disruption of scribble (ScrbScrib1) causes severe neural tube defects in the circletail mouse. *Hum. Mol. Genet.* **12**, 87-98.
Nordeen, S. K. (1988). Luciferase reporter gene vectors for analysis of promoters and enhancers. *Biotechniques* **6**, 454-458.
Pilon, N., Oh, K., Sylvestre, J. R., Bouchard, N., Savory, J. and Lohnes, D. (2006). Cdx4 is a direct target of the canonical Wnt pathway. *Dev. Biol.* **289**, 55-63.
Pilon, N., Oh, K., Sylvestre, J. R., Savory, J. G. and Lohnes, D. (2007). Wnt signaling is a key mediator of Cdx1 expression in vivo. *Development* **134**, 2315-2323.
Prinos, P., Joseph, S., Oh, K., Meyer, B. I., Gruss, P. and Lohnes, D. (2001). Multiple pathways governing Cdx1 expression during murine development. *Dev. Biol.* **239**, 257-269.
Qian, D., Jones, C., Rzadzinska, A., Mark, S., Zhang, X., Steel, K. P., Dai, X. and Chen, P. (2007). Wnt5a functions in planar cell polarity regulation in mice. *Dev. Biol.* **306**, 121-133.
Sakai, Y., Meno, C., Fujii, H., Nishino, J., Shiratori, H., Saijoh, Y., Rossant, J. and Hamada, H. (2001). The retinoic acid-inactivating enzyme CYP26 is essential for establishing an uneven distribution of retinoic acid along the anterio-posterior axis within the mouse embryo. *Genes Dev.* **15**, 213-225.
Santagati, F., Minoux, M., Ren, S. Y. and Rijli, F. M. (2005). Temporal requirement of Hoxa2 in cranial neural crest skeletal morphogenesis. *Development* **132**, 4927-4936.
Satoh, W., Matsuyama, M., Takemura, H., Aizawa, S. and Shimono, A. (2008). Sfrp1, Sfrp2, and Sfrp5 regulate the Wnt/beta-catenin and the planar cell polarity pathways during early trunk formation in mouse. *Genesis* **46**, 92-103.
Savory, J. G., Bouchard, N., Pierre, V., Rijli, F. M., De Repentigny, Y., Kothary, R. and Lohnes, D. (2009a). Cdx2 regulation of posterior development through non-Hox targets. *Development* **136**, 4099-4110.
Savory, J. G., Pilon, N., Grainger, S., Sylvestre, J. R., Beland, M., Houle, M., Oh, K. and Lohnes, D. (2009b). Cdx1 and Cdx2 are functionally equivalent in vertebral patterning. *Dev. Biol.* **330**, 114-122.
Savory, J. G., Mansfield, M., St Louis, C. and Lohnes, D. (2011). Cdx4 is a Cdx2 target gene. *Mech. Dev.* **128**, 41-48.
Sepich, D. S., Myers, D. C., Short, R., Topczewski, J., Marlow, F. and Solnica-Krezel, L. (2000). Role of the zebrafish trilobite locus in gastrulation movements of convergence and extension. *Genesis* **27**, 159-173.
Shashikant, C. S., Bieberich, C. J., Belting, H. G., Wang, J. C., Borbely, M. A. and Ruddle, F. H. (1995). Regulation of Hoxc-8 during mouse embryonic development: identification and characterization of critical elements involved in early neural tube expression. *Development* **121**, 4339-4347.
Silberg, D. G., Swain, G. P., Suh, E. R. and Traber, P. G. (2000). Cdx1 and cdx2 expression during intestinal development. *Gastroenterology* **119**, 961-971.
Simons, M. and Mlodzik, M. (2008). Planar cell polarity signaling: from fly development to human disease. *Annu. Rev. Genet.* **42**, 517-540.
Solnica-Krezel, L., Stemple, D. L., Mountcastle-Shah, E., Rangini, Z., Neuhauss, S. C., Malicki, J., Schier, A. F., Stainier, D. Y., Zwartkruis, F., Abdelilah, S. et al. (1996). Mutations affecting cell fates and cellular rearrangements during gastrulation in zebrafish. *Development* **123**, 67-80.
Subramanian, V., Meyer, B. I. and Gruss, P. (1995). Disruption of the murine homeobox gene Cdx1 affects axial skeletal identities by altering the mesodermal expression domains of Hox genes. *Cell* **83**, 641-653.
Sussman, D. J., Klingensmith, J., Salinas, P., Adams, P. S., Nusse, R. and Perrimon, N. (1994). Isolation and characterization of a mouse homolog of the Drosophila segment polarity gene dishevelled. *Dev. Biol.* **166**, 73-86.
Tabaries, S., Lapointe, J., Besch, T., Carter, M., Woollard, J., Tuggle, C. K. and Jeannotte, L. (2005). Cdx protein interaction with Hoxa5 regulatory sequences contributes to Hoxa5 regional expression along the axial skeleton. *Mol. Cell. Biol.* **25**, 1389-1401.
Tada, M. and Smith, J. C. (2000). Xwnt11 is a target of Xenopus Brachyury: regulation of gastrulation movements via Dishevelled, but not through the canonical Wnt pathway. *Development* **127**, 2227-2238.
Takada, S., Stark, K. L., Shea, M. J., Vassileva, G., McMahon, J. A. and McMahon, A. P. (1994). Wnt-3a regulates somite and tailbud formation in the mouse embryo. *Genes Dev.* **8**, 174-189.
Trinh, K. Y., Jin, T. and Drucker, D. J. (1999). Identification of domains mediating transcriptional activation and cytoplasmic export in the caudal homeobox protein Cdx-3. *J. Biol. Chem.* **274**, 6011-6019.
van den Akker, E., Forlani, S., Chawengsaksophak, K., de Graaff, W., Beck, F., Meyer, B. I. and Deschamps, J. (2002). Cdx1 and Cdx2 have overlapping functions in anteroposterior patterning and posterior axis elongation. *Development* **129**, 2181-2193.
van Nes, J., de Graaff, W., Lebrin, F., Gerhard, M., Beck, F. and Deschamps, J. (2006). The Cdx4 mutation affects axial development and reveals an essential role of Cdx genes in the ontogenesis of the placental labyrinth in mice. *Development* **133**, 419-428.
Wallingford, J. B. and Harland, R. M. (2002). Neural tube closure requires Dishevelled-dependent convergent extension of the midline. *Development* **129**, 5815-5825.
Wallingford, J. B., Rowning, B. A., Vogeli, K. M., Rothbacher, U., Fraser, S. E. and Harland, R. M. (2000). Dishevelled controls cell polarity during Xenopus gastrulation. *Nature* **405**, 81-85.
Wallingford, J. B., Fraser, S. E. and Harland, R. M. (2002). Convergent extension: the molecular control of polarized cell movement during embryonic development. *Dev. Cell* **2**, 695-706.
Wang, J., Hamblet, N. S., Mark, S., Dickinson, M. E., Brinkman, B. C., Segil, N., Fraser, S. E., Chen, P., Wallingford, J. B. and Wynshaw-Boris, A. (2006a). Dishevelled genes mediate a conserved mammalian PCP pathway to regulate convergent extension during neurulation. *Development* **133**, 1767-1778.
Wang, Y., Guo, N. and Nathans, J. (2006b). The role of Frizzled3 and Frizzled6 in neural tube closure and in the planar polarity of inner-ear sensory hair cells. *J. Neurosci.* **26**, 2147-2156.
Wilkinson, D. G., Bhatt, S. and Herrmann, B. G. (1990). Expression pattern of the mouse T gene and its role in mesoderm formation. *Nature* **343**, 657-659.
Yamaguchi, T. P., Takada, S., Yoshikawa, Y., Wu, N. and McMahon, A. P. (1999). T (Brachyury) is a direct target of Wnt3a during paraxial mesoderm specification. *Genes Dev.* **13**, 3185-3190.
Ybot-Gonzalez, P., Savery, D., Gerrelli, D., Signore, M., Mitchell, C. E., Faux, C. H., Greene, N. D. and Copp, A. J. (2007). Convergent extension, planar-cell-polarity signalling and initiation of mouse neural tube closure. *Development* **134**, 789-799.
Yen, W. W., Williams, M., Periasamy, A., Conaway, M., Burdsal, C., Keller, R., Lu, X. and Sutherland, A. (2009). PTK7 is essential for polarized cell motility and convergent extension during mouse gastrulation. *Development* **136**, 2039-2048.
Yoon, J. K. and Wold, B. (2000). The bHLH regulator pMesogenin1 is required for maturation and segmentation of paraxial mesoderm. *Genes Dev.* **14**, 3204-3214.
Yoshikawa, Y., Fujimori, T., McMahon, A. P. and Takada, S. (1997). Evidence that absence of Wnt-3a signaling promotes neuralization instead of paraxial mesoderm development in the mouse. *Dev. Biol.* **183**, 234-242.
Young, T., Rowland, J. E., van de Ven, C., Bialecka, M., Novoa, A., Carapuco, M., van Nes, J., de Graaff, W., Duluc, I., Freund, J. N. et al. (2009). Cdx and Hox genes differentially regulate posterior axial growth in mammalian embryos. *Dev. Cell* **17**, 516-526.