

β -Catenin controls cell sorting at the notochord–somite boundary independently of cadherin-mediated adhesion

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In *Xenopus laevis*, patterning of the trunk mesoderm into the dorsal notochord and lateral somites depends on differential regulation of Wnt– β -catenin signaling. To study the cellular requirements for the physical separation of these tissues, we manipulated β -catenin activity in individual cells that were scattered within the trunk mesoderm. We found that high activity led to efficient cell sorting from the notochord to the somites, whereas reduced activity led to sorting in the opposite direction. Analysis of individual cells overexpressing β -catenin revealed that

these cells were unable to establish stable contacts with notochord cells but could freely cross the boundary to integrate within the somitic tissue. Interference with cadherin-mediated adhesion disrupted tissue architecture, but it did not affect sorting and boundary formation. Based on these results, we propose that the boundary itself is the result of cell-autonomous changes in contact behavior that do not rely on differences in absolute levels of adhesion.

Introduction

An important aspect of morphogenetic events during embryogenesis is the necessity to establish morphological boundaries between the emerging tissues. These boundaries act as barriers against further cell movements and, thus, ensure the unperturbed progression of development in the respective cell populations. To form and maintain boundaries, cell populations have to acquire differential properties (tissue affinities; Holfreter, 1939) that distinguish them from each other and allow them to physically separate (Steinberg, 1996; McNeill, 2000). These properties were first visualized in mixed aggregates of cells taken from different tissues, in which cells will sort out again according to their original fate (Townes and Holfreter, 1955; Steinberg, 1963). In the embryo, cell populations often first gain differential positional information (or fate), and then a boundary will form where these populations touch. In such a context, the development of differential tissue affinities can be seen as a means to prevent cell populations from mixing rather than as a means to actively sort cells (Tepass et al., 2002). Nevertheless, by manipulating the expression of candidate mole-

cules in some cells within a wild-type cell population, cell sorting can be induced and may allow conclusions to be drawn regarding the role of these molecules during formation of the actual boundary (Xu et al., 1999; Dahmann and Basler, 2000). In this study, we describe the application of such an approach to study the early notochord–somite boundary in *Xenopus laevis*.

The notochord–somite boundary divides the trunk mesoderm into a dorsal-most notochord and lateral somites. In *X. laevis*, the initial pattern of prospective notochord and somitic regions becomes established at the onset of gastrulation, as notochord markers like Xnot and FoxA4 (originally XFD-1 and recently renamed; for review see Pohl and Knochel, 2005) become expressed in the dorsal quadrant of the marginal zone, and markers for somitic muscle like myf-5 and MyoD appear lateral to it (Hopwood et al., 1991, 1992; Knochel et al., 1992; von Dassow et al., 1993). At this stage, the trunk mesoderm still forms a continuous ring around the blastopore. Physical separation occurs later during gastrulation in the involuted part of the mesoderm at a time when it starts to elongate anteroposteriorly to shape the main body axis of the embryo. In scanning electron microscopy preparations and cultured explants, the boundary becomes visible as a fissure between notochord and somitic cells followed by a progressive alignment of cells along each side (Keller et al., 1989, 1992; Wilson and Keller, 1991; Shih and Keller, 1992a).

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Abbreviations used in this paper: CadFL, full-length C-cadherin; CMV, cytomegalo virus; DBD, DNA-binding domain; LEF, lymphoid enhancer factor; TCF, T-cell factor.

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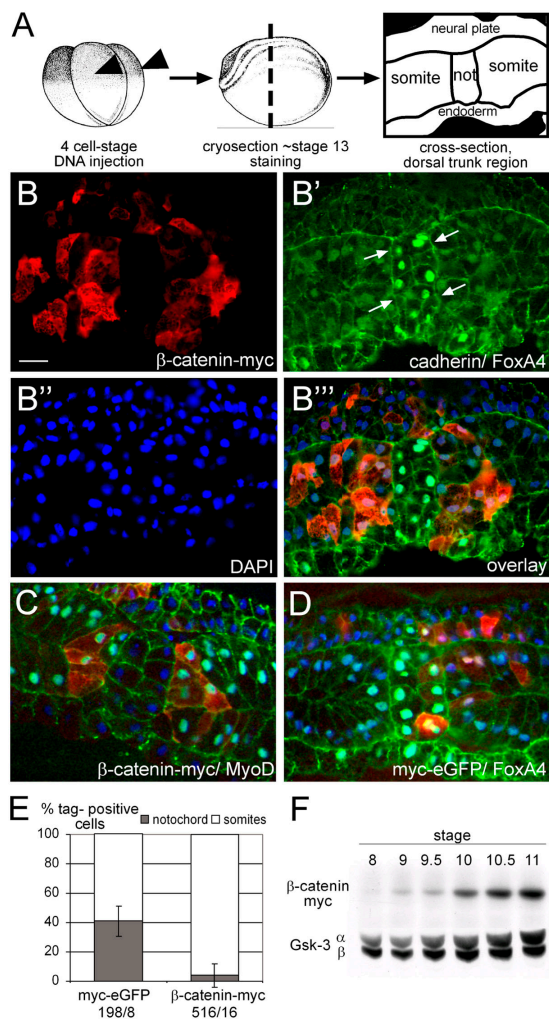


Figure 1. Cells overexpressing β -catenin are excluded from the notochord. (A) Experimental set up and sketch of cross section shown in B. not, notochord. Arrowheads represent injections. (B–B''') Section of a β -catenin-myc-injected embryo stained for myc (B, red), cadherin (plasma membranes), and FoxA4 (notochord nuclear marker; B', both in green) and counterstained with DAPI (B'', blue). (B''') Overlay. Positive cells were found in the somites but not in the notochord. Arrows, notochord–somite boundary. Bar, 50 μ m. (C) Merged image of a section stained for myc (red), cadherin, and the somitic nuclear marker MyoD (both green) and counterstained with DAPI (blue). (D) Merged image of a section from a control myc-eGFP-injected embryo stained as in B. Myc-eGFP-positive cells (red) distributed roughly equally between notochord and somites. (E) Quantitation of the distribution of β -catenin-myc- and myc-eGFP-expressing cells between notochord and somites. Numbers below represent total number of positive cells/number of embryos. Error bars represent SD. (F) Time course of β -catenin-myc expression analyzed by immunoblot (equivalent of one embryo/lane) compared with endogenous Gsk-3 (α and β isoforms).

The Wnt– β -catenin (or canonical Wnt) pathway is essential for early patterning of the trunk mesoderm. Activation of this pathway results in stabilization of cytoplasmic β -catenin, which otherwise is kept at low levels by active degradation. Free β -catenin then enters the nucleus, where it interacts with cofactors of the lymphoid enhancer factor (LEF)/T-cell factor (TCF) family and regulates transcription (Peifer and Polakis, 2000). An early, maternal Wnt– β -catenin signal induces the dorsal organizer and might be involved in early mesoderm induction (Moon and Kimelman, 1998; Schohl and Fagotto,

2003), whereas a second, zygotic signal is required for the formation of somitic muscle tissue and has to be inhibited to allow notochord formation (Christian and Moon, 1993; Hoppler et al., 1996; Yasuo and Lemaire, 2001). Accordingly, the expression domain of zygotic Wnt8 at the gastrula stage extends from ventral to lateral but leaves a gap on the dorsal side (Christian et al., 1991; Smith and Harland, 1991), and a decrease in nuclear β -catenin at early gastrula indicates a transient down-regulation of active Wnt signaling within the prospective notochord region (Schohl and Fagotto, 2002).

Regulation of cell–cell adhesion is generally considered to be essential for cell sorting. Cell–cell adhesion is commonly mediated by transmembrane molecules of the cadherin family, and quantitative differences in their expression have been shown to be sufficient for cell sorting in transfected cells (Steinberg and Takeichi, 1994) as well as to be necessary in vivo to sort the oocyte to the posterior end of the *Drosophila melanogaster* ovary (Godt and Tepass, 1998). Because β -catenin, besides its transcriptional activity, also functions by physically connecting cadherins to the actin cytoskeleton, its stabilization might directly increase cell–cell adhesion. Other, more indirect, interrelations between Wnt signaling and cadherin-mediated adhesion have also been proposed. Potentially, such a link between patterning and adhesion could regulate regional differences in cell affinity and mediate sorting (Fagotto and Gumbiner, 1996; Gumbiner, 2000; Nelson and Nusse, 2004).

We manipulated Wnt– β -catenin signal activity in scattered cells within the wild-type trunk mesoderm and evaluated (by immunohistology and time-lapse video microscopy) the cellular effects during formation of the notochord–somite boundary. We show that the Wnt– β -catenin pathway can act as a switch, whereby increased activity leads to cell-autonomous sorting to the somites. In contrast, reduced activity leads to sorting to the notochord. We found evidence that the notochord–somite boundary is not a barrier for cell movement in itself but might be initiated as the result of a cell-autonomous inability of notochord and somitic cells to generate efficient traction on each other. Sorting seems to rely on nuclear Wnt– β -catenin signaling but not on the strength of cadherin-mediated adhesion. In fact, inhibiting cadherin-mediated adhesion revealed a remarkable robustness of sorting activity and boundary formation.

Results

A plasmid-based assay to investigate the distribution, fate, and morphology of cells in a “mosaic” embryo

To study the cellular mechanisms that are involved in formation of the notochord–somite boundary, we devised a protocol to generate a small number of manipulated cells within the wild-type trunk mesoderm. We took advantage of the fact that after injection of a defined amount of plasmid DNA (containing a ubiquitously active cytomegalovirus [CMV] promoter), the exogenous construct is only expressed in a random subset of cells within the injected area. Injections were targeted to the prospective notochord–somite region, and each construct was

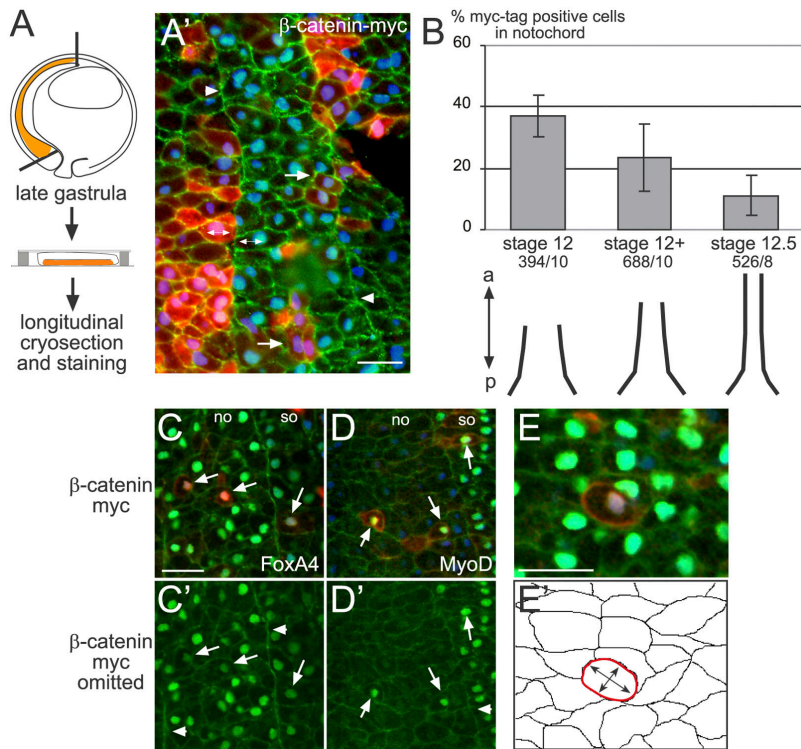


Figure 2. Cells overexpressing β -catenin sort during late gastrulation. (A) Dorsal explants of β -catenin-myc-injected embryos were fixed while flattened under a coverslip. (A') Section of a stage 12.5 explant (anterior to the top) stained as in Fig. 1 B. Several β -catenin-myc-expressing cells can still be found in the notochord (arrows). Double arrows indicate wild-type cells in both tissues that tend to be elongated mediolaterally. (B) The percentage of positive cells in the notochord gradually decreases during late gastrula stages. Numbers below represent total number of positive cells/number of embryos. Error bars represent SD. Sketch depicts approximate changes in notochord shape. Arrow represents the anterior (a) to posterior (p) orientation of the notochord sketch. (C and D) Reduced FoxA4 expression (C and C') and ectopic expression of MyoD (D and D') in β -catenin-myc-expressing cells in the notochord (arrows). Sections (stage 12+) were stained as in Fig. 1 (B and C). (A', C', and D') Arrowheads, notochord-somite boundary. no, notochord; so, somite. (E and E') β -catenin-myc-expressing cell in the notochord shows reduced mediolateral elongation. (E) Stage 12+ section stained for myc (red), FoxA4, cadherin (green), and DAPI (blue). (E') Sketch of E outlining cell shapes. Arrows, length and width of positive cell (red). Bars, 50 μ m.

carefully titrated to achieve a scattered distribution of construct-expressing cells (Fig. 1, A–D).

In contrast to ectopic mRNA, DNA-based expression does not interfere with early maternally controlled events of embryogenesis because transcription only starts at the mid-blastula stage (Newport and Kirschner, 1982). This is particularly important with regard to the Wnt- β -catenin pathway because an early β -catenin signal of maternal origin regulates induction of the dorsal organizer (Moon and Kimelman, 1998). Instead, the products of our DNA constructs reached significant levels at the early gastrula stages (Fig. 1 F), coinciding with the time window in which subdivision of the dorsal mesoderm into axial (notochord) and paraxial (somite) mesoderm occurs.

To identify construct-expressing cells, an epitope tag was appended to all constructs. Embryos were analyzed after gastrulation (stages 13–15) by immunofluorescence on cryosections. In cross sections that were stained for C-cadherin, the notochord appeared well separated from the adjacent somitic mesoderm. Cell membranes around the somites were strongly stained, highlighting the boundary between this tissue and the notochord (Fig. 1, B–D). Additionally, the rather uniform staining of all other cell membranes as well as DAPI-stained nuclei revealed characteristic cell arrangements; the notochord consisted of two parallel rows of cells, whereas the cells of the somitic mesoderm formed an oval-shaped structure. This pattern developed between the end of gastrulation and neurulation, progressing from anterior to posterior. Therefore, it appeared more or less pronounced, depending on the exact stage and on the anteroposterior position of a given section.

In addition to morphological criteria, we also determined notochord and somitic cell fate by using antibodies against two transcription factors: anti-MyoD antibody specifically marked

the somites (Fig. 1 C), and anti-FoxA4 antibody marked the notochord. Note that anti-FoxA4 also marked the endoderm and occasionally marked the dorsal midline of the neural plate (Fig. 1, B' and D). For our purpose, however, it clearly distinguished notochord from somitic tissue. Because both MyoD and FoxA4 showed an exclusively nuclear localization and cadherin was found only at plasma membranes, we used antibodies against cadherin and FoxA4 or MyoD simultaneously.

Altogether, these steps describe a simple protocol to obtain mosaic embryos and to simultaneously visualize (on a single section) construct-expressing cells (tag), tissue organization (cadherin and DAPI), cell morphology (cadherin), and tissue identity (FoxA4 or MyoD).

Cells overexpressing β -catenin are excluded from the notochord

To test for cell-autonomous effects of the Wnt- β -catenin pathway on cell behavior in the notochord-somite region, we activated the pathway by injecting a plasmid-encoding myc-tagged β -catenin. To ensure that the expression level of β -catenin was not affected by endogenous Wnt activity, we used a mutated form ($\Delta\beta$ cat-myc, hereafter referred to as β -catenin) that was insensitive to Gsk-3-mediated degradation (Yost et al., 1996). In parallel, we injected control embryos with a plasmid-encoding myc-eGFP.

Comparing the distribution of these construct-expressing cells revealed a striking difference. Although myc-eGFP-positive cells were evenly distributed within the notochord and adjacent somitic region (41% in the notochord; Fig. 1, D and E), β -catenin-myc-positive cells were almost completely excluded from the notochord (4% in the notochord; Fig. 1, B, C, and E). Instead, these cells accumulated on the somitic side of the noto-

chord–somite boundary. They expressed the somitic marker MyoD and were incorporated into the proper cell arrangement of the somites. Note that the section selected in Fig. 1 B contains an unusually high number of positive cells to illustrate the efficiency of sorting. The high density of β -catenin–expressing cells disturbed the normal tissue organization (compare with Fig. 1 D) and, in the most extreme cases, led to a reduced notochord that was formed by wild-type cells and surrounded by β -catenin–myc-positive cells expressing MyoD (not depicted). These results show that elevated levels of β -catenin are sufficient to exclude a cell from the notochord and to shift it toward somitic fate.

Cells overexpressing β -catenin are sorted during elongation of the notochord field

To determine the time frame in which sorting of β -catenin–myc-expressing cells occurs, we analyzed injected embryos during late gastrulation (stages 12–12.5). At stage 12, a significant amount of prospective notochord–somite tissue has already involuted, and the boundary has formed. Nevertheless, the tissue is still relatively thin in the dorsoventral dimension and is not very well defined (Keller et al., 1989; unpublished data). To improve the analysis of these stages, we sectioned dorsal explants along the anteroposterior axis, which provided sections containing large stretches of the notochord–somite region and allowed better visualization of cell morphology patterns and boundary structure (Fig. 2).

At these stages, the notochord region was already marked by the expression of FoxA4, and the somitic region was marked by MyoD (Fig. 2, A', C, and D). Cadherin staining revealed the characteristic cell alignment that defines the notochord–somite boundary (Fig. 2, A', C', and D'; arrowheads). The notochord field changed its shape from stages 12–12.5, narrowing in the mediolateral axis and correspondingly elongating in the anteroposterior axis (Fig. 2 B). At all stages, the most posterior involuting part of the notochord was wider, and characteristic alignment of the cells was not yet apparent (not depicted). For quantitative analysis, β -catenin–positive cells within this posterior field were omitted when their position could not be clearly defined.

We found that 37% of positive cells were still located in the notochord at stage 12 (Fig. 2 B). This distribution was close to the distribution in control myc–eGFP–injected embryos at stage 13 (Fig. 1 E), suggesting that little sorting had yet occurred at stage 12. The percentage decreased to 23% at stage 12+ and to 11% at stage 12.5. Thus, the majority of cells expressing β -catenin–myc sorted during late gastrulation at a time when the notochord–somite boundary was already established.

β -Catenin–overexpressing cells in the notochord can display signs of somitic fate

An analysis of explants also showed that β -catenin–myc-expressing cells in the notochord tended to display reduced FoxA4 staining (Fig. 2 C). At all stages that were analyzed, roughly half of positive cells displayed such a reduction (stage

Table I. β -Catenin–expressing cells in the notochord show reduced cell elongation

Cell type	Tissue	No. of cells measured	Percentage of cells with length/width ratio		
			<1.5	1.5–2.5	>2.5
β -Catenin positive	notochord	55	56	36	8
	somites	51	2	51	47
Wild-type	notochord	239	5	54	41
	somites	253	8	59	33

Explants from β -catenin–myc-injected embryos were fixed at stages 12–12.5, sectioned, and stained as in Fig. 2 A. The length/width ratio of myc-positive cells or control cells was measured by determining the length of cells in the mediolateral axis and their width perpendicular to it (11 explants/3 experiments).

12, 54% [10 explants]; stage 12+, 44% [10 explants]; and stage 12.5, 62% [8 explants]). In comparison, FoxA4 staining of wild-type cells in the notochord was only reduced in 3% (12 explants) of nuclei. In addition, although wild-type notochord cells never expressed MyoD, we found β -catenin–myc-positive cells in the notochord that showed strong MyoD expression (Fig. 2 D). These results suggest that at least a fraction of the cells overexpressing β -catenin acquire somitic fate cell autonomously before sorting occurs.

β -Catenin–overexpressing cells in the notochord display reduced mediolateral elongation

During gastrulation, cells in the notochord and in somitic regions intercalate in the mediolateral axis, thereby driving the anteroposterior elongation of these tissues. Intercalating cells are mediolaterally elongated, with protrusions restricted to both ends. This polarized, protrusive activity is thought to allow cells to exert traction and to migrate between each other (Keller et al., 2000). Cells bordering the emerging notochord–somite boundary form stable protrusions only on neighboring cells within the same tissue but not across the boundary. These cells adopt a “triangular” shape (Fig. 2 A', double arrows) with a flat surface along the boundary and an elongated side pointing away from the boundary (Keller et al., 1992; Shih and Keller, 1992a,b). Because cell shape (elongated within the tissues and triangular at the boundary) seems to reflect the geometry of pulling forces between neighboring cells, we reasoned that the morphology of β -catenin–myc-expressing cells might allow us to draw conclusions about their interactions with surrounding notochord or somitic cells.

To measure cell elongation, we first identified the β -catenin–myc-expressing cells that were not touching the boundary and that were only surrounded by wild-type cells (Fig. 2 E). The length/width ratio of each of these cells was determined with respect to the mediolateral axis (Fig. 2 E'). As a control, the length/width ratio of wild-type cells in the vicinity of each positive cell was also determined. The cells were classified into three categories: not or weakly elongated (ratio <1.5), moderately elongated (ratio between 1.5 and 2.5), and strongly elongated (ratio >2.5). The results summarized in Table I reveal

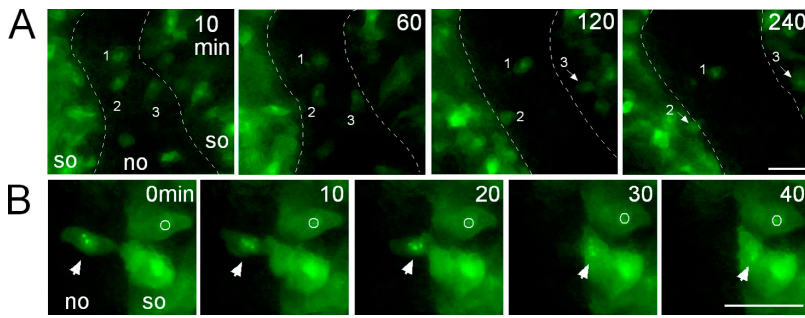


Figure 3. Cells overexpressing β -catenin move from the notochord to the somites. Selected frames from time-lapse videos of dorsal explants with β -catenin-eYFP-expressing cells that were cultured from late gastrula on. (A) Cells 2 and 3 cross the boundary (dashed lines). (B) Higher magnification of a β -catenin-eYFP-expressing cell moving into the somitic mesoderm (arrowheads). Positive cell at the somitic side of the boundary (circles) shows no net movement. no, notochord; so, somitic mesoderm. Bars, 50 μ m. See Videos 1 and 3, available at <http://www.jcb.org/cgi/content/full/jcb.200503009/DC1>.

striking tissue-dependent differences in the morphology of β -catenin-myc-expressing cells. When located in the somitic tissue, positive cells showed length/width ratios that were similar to neighboring wild-type cells (47 and 33% strongly elongated, respectively; and 2 and 8% not elongated, respectively). In contrast, in the notochord, only 8% of β -catenin-myc-expressing cells were strongly elongated (compared with 41% of wild-type notochord cells) and 56% were not or weakly elongated (5% for wild-type notochord cells).

These results show that β -catenin-myc-expressing cells display reduced mediolateral elongation as a specific reaction to surrounding notochord cells and suggest that intercalation behavior is impaired under these conditions.

Analysis of cell sorting in live explants

To directly visualize cell behavior during sorting, we explanted the dorsal region of β -catenin-eYFP-injected embryos at late gastrula (stages 12–12.5) and exposed the already involuted notochord–somite region by removing the endodermal epithelium. As expected, at the time we began our time-lapse recordings, eYFP fluorescent cells had already accumulated at the somitic side of the boundary. This allowed us to clearly locate the notochord–somite interface and to follow the remaining dispersed β -catenin-eYFP-expressing cells in the notochord (Fig. 3, A and B). 21 of a total of 33 explants still had positive cells in the notochord at the beginning of the recordings. Out of these cells, 85% (71 cells) crossed the boundary and moved into the somitic tissue within the next 1–4 h, whereas 15% (13 cells) remained in the notochord until the end of the recordings (Fig. 3 A and Videos 1 and 2, available at <http://www.jcb.org/cgi/content/full/jcb.200503009/DC1>). It should be noted that all β -catenin-eYFP-expressing cells that had disappeared from the notochord by the end of the experiment could be traced to the somites, ruling out selective cell death or down-regulation of expression. Boundary crossing was specific to these cells because, in additional explants (using epiluminescence to observe all cells), we never found wild-type cells moving across the boundary (unpublished data). These experiments demonstrate that the absence of β -catenin-overexpressing cells in the notochord was a result of efficient sorting to the somites.

β -Catenin-eYFP-expressing cells in the notochord formed random, unstable protrusions and underwent frequent shape changes. These cells did not appear to be polarized, and when cell movement was observed, it was also random (Fig. 3 A, cell 1; and Videos 1 and 2). Once a cell touched the boundary,

it translocated irreversibly to the somitic side (Fig. 3 B and Video 3, available at <http://www.jcb.org/cgi/content/full/jcb.200503009/DC1>). Sorting at the boundary appeared to be very efficient. In fact, none of the 13 positive cells remaining in the notochord had any obvious contact with the boundary during the experiment. Once in the somites, β -catenin-eYFP-expressing cells ceased to show random protrusive activity and adopted an elongated morphology. This suggests that the pattern of protrusive activity depends on the cellular environment and is not a cell-intrinsic property.

The state of Wnt- β -catenin signaling determines the directionality of sorting

Because overexpression of β -catenin was sufficient to sort cells to the somites, we tested if upstream activation or inhibition of the Wnt- β -catenin pathway would also induce sorting. To achieve cell-autonomous activation, we used an extracellular truncated form of LRP5, an essential coreceptor of the Wnt receptor frizzled. Expression of this LRP5 mutant is known to constitutively activate the pathway and to stabilize β -catenin (Mao et al., 2001). In LRP5-injected embryos, only 20% of positive cells localized in the notochord in comparison with 54% in myc-eGFP-injected embryos (Fig. 4, A and C). Thus, although less efficient than direct overexpression of β -catenin, upstream activation of the Wnt- β -catenin pathway is sufficient to sort cells to the somites. To achieve cell-autonomous inhibition, we expressed a mutated version of axin (Ax12-810; Fagotto et al., 1999). 89% of cells expressing this construct were found in the notochord (Fig. 4, B and C), showing that inhibition of the Wnt- β -catenin pathway leads to sorting from somites to the notochord. These results indicate that the intracellular state of the Wnt- β -catenin pathway determines whether a cell sorts to the notochord or to somites.

The signaling function of β -catenin is sufficient for cell sorting

Wnt-induced up-regulation of β -catenin might directly activate signaling and increase adhesion in parallel (Yap et al., 1997a; Nelson and Nusse, 2004). Thereby, a high Wnt- β -catenin signal in somitic cells could directly lead to increased adhesion, whereas a low signal in notochord cells could translate into lower adhesion, providing a source for differential affinity and cell sorting. To be able to manipulate Wnt signaling without influencing β -catenin levels and, thus, avoiding possible direct effects on adhesion, we used constructs in which the DNA-

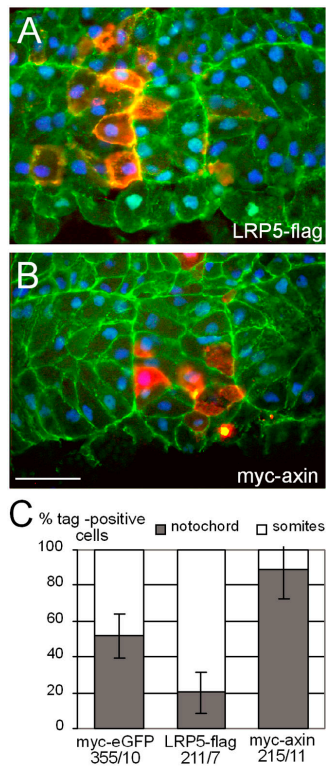


Figure 4. The activity of Wnt signaling determines the direction of sorting. The pathway was activated using a constitutively active truncated LRP5 (A) or was inhibited using an axin variant (B). Sections (stage 13) were stained as in Fig. 1 B. Cells expressing LRP5-Flag sorted largely to the somitic mesoderm, whereas cells expressing myc-axin sorted to the notochord. Bar, 50 μ m. (C) Quantitation of positive cell distributions. Numbers below represent total number of positive cells/number of embryos. Error bars represent SD.

binding domain (DBD) of LEF1 was fused to either an engrailed repressor domain or a VP16-activating domain. These constructs do not interact with β -catenin and modify transcription of target genes autonomously (Vlaminckx et al., 1999). Also, whereas the TCF/LEF family consists of several members, the minimal constructs that were used in this study should be promiscuous in their activity because the DBD is highly conserved and all proteins bind the same DNA consensus sequence (Clevers and van de Wetering, 1997).

Expression of the dominant active form LEFVP16 resulted in very efficient sorting of positive cells to the somites (98%; Fig. 5, A and D). These cells were incorporated into the normal somitic tissue architecture and showed the appropriate expression of tissue-specific markers. Occasionally, when many positive cells accumulated, LEFVP16 overexpression led to a partial loss of notochord tissue (unpublished data). Therefore, LEFVP16 phenocopied β -catenin overexpression, indicating that the TCF/LEF-mediated signaling function of β -catenin is sufficient to confer to a cell all the attributes needed to become part of the somites. The opposite experiment, however, did not yield the expected result. Although inhibiting β -catenin activity by mutated axin (Fig. 4, B and C) or cadherin constructs (see Fig. 6) led to sorting to the notochord, the expression of LEFeng had no effect on cell sorting (54% in the notochord; Fig. 5, B and D) compared with myc-eGFP-injected embryos (46% in the notochord; Fig. 5 D). Nevertheless, LEFeng-expressing cells in somites showed cell-autonomous expression of the notochord marker FoxA4 (Fig. 5 B') and a loss of MyoD expression (not depicted). In addition, these cells showed strong expression of brachyury, which, at this stage, specifically marks the notochord (unpublished data). Cells expressing LEFeng were frequently not properly incorporated into the tissue architecture of either notochord or somites but were found in small clusters at the periphery of the tissues on either side of the notochord–somite boundary (unpublished data).

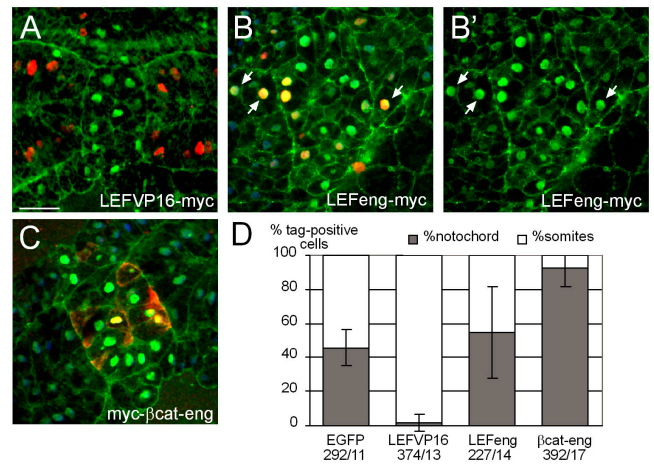


Figure 5. Signaling downstream of β -catenin is sufficient for sorting. Sections from stage 13 embryos were stained as in Fig. 1 B. (A) Cells expressing LEFVP16-myc sort to the somites. (B) Cells expressing LEFeng-myc do not sort but activate FoxA4 ectopically in the somites (arrows). (B') Same section as B, only with FoxA4/cadherin staining. (C) Cells expressing myc- β -catenin-eng sort to the notochord. Bar, 50 μ m. (D) Quantitation of positive cell distributions. Numbers below represent total number of positive cells/number of embryos. Error bars represent SD.

Another efficient inhibitor of Wnt- β -catenin signaling is β -catenin-eng, a β -catenin variant in which the COOH-terminal transactivation domain has been replaced by the engrailed repressor domain. This construct still associates with cadherins and α -catenin and seems to function normally in adhesion. In the nucleus, however, β -catenin-eng antagonizes Wnt- β -catenin signaling (Montross et al., 2000). In contrast to LEFeng, cells expressing this construct sorted very efficiently to the notochord and incorporated into the normal cell arrangement (92%; Fig. 5, C and D). Although we cannot satisfactorily explain the effects of the LEFeng construct, together, the efficient sorting activities of LEFVP16 and β -catenin-eng indicate that sorting is mediated by transcriptional activation/inactivation of Wnt target genes rather than by the adhesive function of β -catenin.

Sorting to the notochord can be induced by cadherin constructs that bind β -catenin

In a complementary approach to dissect the signaling and adhesive functions of β -catenin, we overexpressed full-length C-cadherin (CadFL) or a cadherin deletion mutant lacking the extracellular domain (Cad Δ E). The rationale was that both molecules antagonize Wnt signaling by sequestering β -catenin to

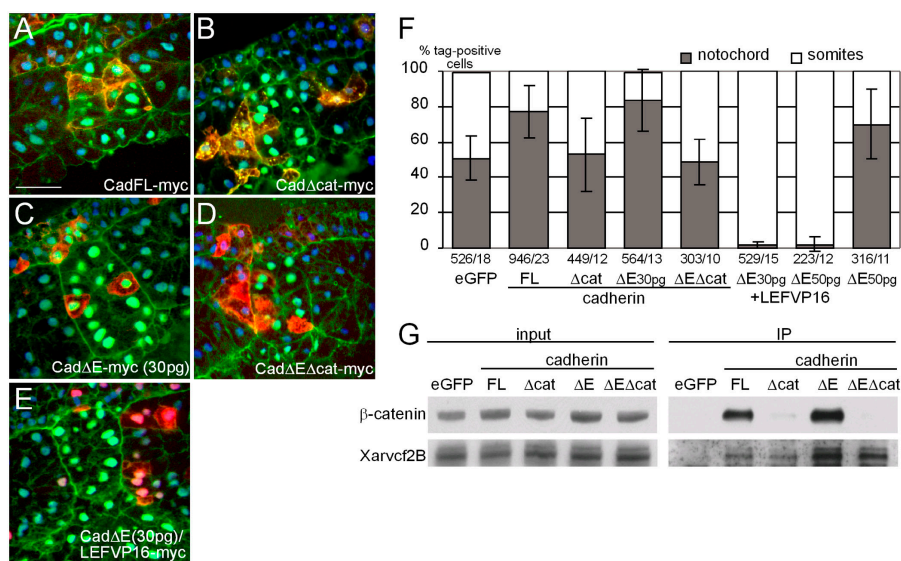


Figure 6. Sorting to the notochord can be induced by expression of cadherin constructs. Sections of stage 13 embryos expressing the following myc-tagged constructs were stained as in Fig. 1 B: full-length C-cadherin (CadFL; A); CadΔcat, β-catenin-binding domain deleted (B); CadΔE, extracellular domain deleted (30 pg/injection; C); CadΔEΔcat, extracellular and β-catenin-binding domains deleted (D); and CadΔE (30 pg) and LEFVP16 (E). (A–D) Cells expressing cadherin constructs with β-catenin binding (A and C) sorted to the notochord. Bar, 50 μm. (E) Cells expressing both CadΔE (plasma membrane) and LEFVP16 (nuclear) sorted to the somites. (F) Quantitation of positive cell distributions, including experiments with higher amounts (50 pg) of CadΔE; see Fig. 7. Numbers below represent total number of positive cells/number of embryos. Error bars represent SD. (G) β-catenin binding of cadherin constructs; myc-eGFP (negative control) or cadherin constructs (all myc tagged) were immunoprecipitated from stage 11 extracts with anti-myc antibody. Precipitates were analyzed for β-catenin and Xarvcf by Western blotting. Input, equivalent of one embryo/lane. Immunoprecipitate (IP), equivalent of 20 embryos/lane.

the plasma membrane (Heasman et al., 1994; Fagotto et al., 1996), whereas their respective effects on cell adhesion should be opposite of one another; CadFL should increase adhesion, whereas CadΔE should act as a dominant negative (Kintner, 1992). To control for possible β-catenin-independent effects, we used a variant of each construct in which the β-catenin-binding site was deleted (CadΔcat and CadΔEΔcat, respectively). Coimmunoprecipitation confirmed that Cad and CadΔE, but not CadΔcat or CadΔEΔcat, bound β-catenin. All constructs contained a functional juxtamembrane domain, as shown by the interaction with Xarvcf2B (Fig. 6 G).

The sorting activity of these constructs is summarized in Fig. 6 F. Control myc-eGFP-expressing cells distributed evenly between notochord and somites (51% in the notochord). In contrast, cells expressing CadFL or CadΔE were found mostly in the notochord (77% and 84%; Fig. 6, A and C). In both cases, sorting was dependent on the β-catenin-binding domain because the Δcat variants distributed evenly again (CadΔcat, 53% in the notochord; CadΔEΔcat, 48% in the notochord; Fig. 6, B and D). Therefore, sorting of cells to the notochord seemed to rely on the depletion of cytosolic β-catenin. As LEFVP16 causes complete sorting to the somites (Fig. 5 A), we used it to antagonize the sorting activity of CadΔE. In these coinjection experiments, all cells expressing both constructs were found in the somites (98%; Fig. 6 E), demonstrating that activation of the Wnt-β-catenin pathway downstream of β-catenin can overcome the effect of CadΔE.

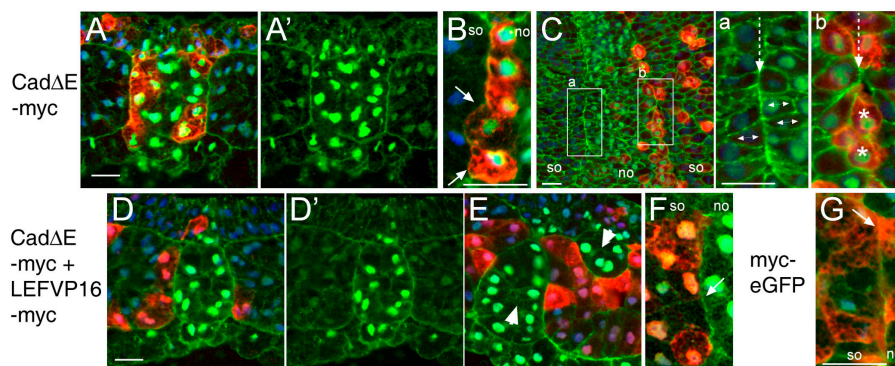
Reduction of cadherin-mediated adhesion affects cell morphology and tissue architecture but not cell sorting

CadΔE is known to be a potent inhibitor of β-catenin-dependent adhesion. For instance, injection of CadΔE mRNA blocks cell-cell adhesion completely in early gastrula embryos, caus-

ing cells to round up and become loose. Because the cytoplasmic domain of classical cadherins is highly conserved and all cadherins of this type mediate adhesion via β-catenin, CadΔE acts as a general inhibitor regardless of the type of classical cadherin that is expressed in a tissue (Kintner, 1992; Broders and Thiery, 1995). In the above experiment, CadΔE indeed seemed to affect cell contacts, as CadΔE-expressing cells in the notochord as well as CadΔE- and LEFVP16-expressing cells in the somites tended to be less elongated than wild-type cells (Fig. 6, C and E). Because regulation of cadherin-mediated adhesion is thought to be essential for convergence extension (Brieher and Gumbiner, 1994; Zhong et al., 1999), we were quite surprised by the efficiency with which these CadΔE-expressing cells sorted.

To further challenge the sorting process, we increased the concentration of CadΔE plasmid from 30 to 50 pg per injection. At this high concentration, the morphology of cells expressing CadΔE or CadΔE + LEFVP16 was strongly affected. Instead of being elongated, these cells were rounded or oval shaped. In both cases, the shape and arrangement of neighboring wild-type cells was also disrupted (Fig. 7, A–F). In fact, already at late gastrula stage, cells expressing high CadΔE were rounded rather than mediolaterally elongated (Fig. 7 C). These observations indicate that high expression of CadΔE disrupts cell-cell interactions. Nevertheless, CadΔE-expressing cells sorted to the notochord, although with lower efficiency (70%; Fig. 6 F), and coexpression of high CadΔE with LEFVP16 still led to complete sorting to the somites (98%; Fig. 6 F). Moreover, embryos completed gastrulation, and the notochord-somite boundary formed even when strongly positive, rounded cells were directly apposed to it (Fig. 7, A and D). In fact, cells expressing CadΔE + LEFVP16 never violated the boundary, and cells expressing high CadΔE only occasionally protruded into the somitic field (Fig. 7, B and F; and Table II). Coinjec-

Figure 7. High CadΔE affects cell and tissue morphology. Embryos were injected with higher amounts of CadΔE-myc (50 pg/injection) alone (A–C) or together with LEFVP16-myc (D–F). All were stage 13 except for C (stage 12+). Staining as in Fig. 1 B. (A' and D') Stained only for FoxA/cadherin (green). (A and B) CadΔE-expressing cells are rounded, and the notochord morphology is perturbed (compare A with Fig. 1 D), but most positive cells are in the notochord and respect the boundary. Arrows in B point to two cells breaking the boundary. (C) Stage 12+ dorsal explant. On the left side, a normal boundary (inset a, dashed arrow) is surrounded by wild-type cells aligned mediolaterally (double arrows). On the right side, the boundary (inset b, dashed arrow) is disrupted by CadΔE-expressing cells, and the cell alignment is lost (asterisks). (D) Cells expressing CadΔE + LEFVP16 locate to the somites and respect the boundary. (E) Split notochord (arrowheads) surrounded by CadΔE/LEFVP16-positive cells. (F) Higher magnification of boundary (arrow). Positive cells are rounded but sort to the somites and respect the boundary. (G) Control, same magnification as F. myc-eGFP cells locate on both sides of the boundary (arrow) and have an elongated morphology. no, notochord; so, somite. Bars, 50 μm.



tion of high CadΔE + LEFVP16 still affected the general formation of notochord and somitic tissue because in 44% of the embryos, positive cells divided the notochord region, which resulted in a partially split notochord (Fig. 7 E). This phenotype was never observed after myc-eGFP, CadΔE, or LEFVP16 injections (Table II). These results show that high expression levels of CadΔE have severe effects on cell morphology and tissue organization. Nevertheless, these cells sorted, and a boundary can form between the notochord and somites.

Discussion

Boundary formation has been studied in different model systems by producing manipulated cells within a wild-type environment (Xu et al., 1999; Dahmann and Basler, 2000). We show that such an approach, when applied to *X. laevis* embryos, can induce very efficient cell sorting at the notochord–somite boundary and allows a detailed analysis of underlying cell behavior. The results presented in this study suggest that the formation of the boundary is based on cell-autonomous differences between notochord and somitic cells that seem to be independent of the strength of cadherin-mediated adhesion.

Wnt-β-catenin signaling and the boundary

To establish the initial pattern of notochord and somites, Wnt-β-catenin signaling has to be blocked in the most dorsal region but has to be active lateral to it (Yasuo and Lemaire, 2001). Using a variety of constructs, we manipulated different steps of the pathway (from the receptor at the plasma membrane to the transcription factor in the nucleus) and demonstrated that it can act as a cell-autonomous switch in the trunk mesoderm. Stimulation of the signal is sufficient to sort cells to the somites, and inhibition leads to sorting to the notochord. All constructs that were employed are in keeping with this conclusion with the exception of LEFeng. Cells expressing this construct seemed to adopt notochord fate, but, in contrast to cells expressing β-catenin-eng, they nevertheless did not sort to the notochord. LEFeng-positive cells were often not properly incorporated into either notochord or somitic tissue architecture, giving the impression that they were in an “intermediate” state. The dif-

ferent outcome of β-catenin-eng and LEFeng expression could possibly be explained by recent data suggesting that β-catenin and LEF/TCF can regulate transcription independently of each other by interacting with other cofactors (Hikasa and Sokol, 2004; Sinner et al., 2004). The above observation that cells expressing FoxA4 (and losing MyoD) did not inevitably sort to the notochord also raises the question of how closely linked sorting and expression of tissue-specific markers are.

Reduced Wnt-β-catenin signaling, which is indicated by a loss of nuclear β-catenin, occurs in the prospective notochord region only during a short time window at early gastrula (Schohl and Fagotto, 2002). Although this event seems to be sufficient to set off the notochord program, additional fate-determining factors also seem to be active. The existence of such signals has been shown by grafting cells from different regions of the embryo into the notochord or somites. Even at late gastrulation, these grafts were incorporated into the host tissue and changed fate accordingly (Domingo and Keller, 2000). We found that cells with a manipulated Wnt-β-catenin signal sorted very efficiently and could change fate even before sorting. Thus, strongly reducing or increasing β-catenin generates a signal that dominates other notochord or somitic determinants, whereas in the endogenous situation, such determinants may be needed to reinforce the initial spatial pattern set by the Wnt-β-catenin pathway.

Cell sorting and the boundary

Cells at either side of the boundary essentially never cross to the other side or detach to move back deeper into their tissue (Shih and Keller, 1992a,b). Therefore, the boundary can be seen as a barrier and a “trap” for intercalating cells, which is a mechanism that has been referred to as “boundary capture” (Keller et al., 2000; Wallingford et al., 2002). We show that cells overexpressing β-catenin progressively sort from the notochord to somitic tissue after the boundary has already formed, clearly demonstrating that it does not constitute a barrier to cell movement itself. Thus, trapping of cells at the boundary must rely on other mechanisms.

Wild-type cells of both the notochord and the somite region display an elongated morphology and mediolaterally ori-

Table II. Effects induced by high levels of CadΔE

Injected plasmid	Affected embryos/total embryos	
	Split notochord	Boundary disruption ^a
myc-eGFP	0/12 (0%)	0/12 (0%)
CadΔE (50 pg)	0/18 (0%)	11/18 (61%)
LEFVP16	0/12 (0%)	0/12 (0%)
CadΔE (50 pg) + LEFVP16	7/16 (44%)	0/12 (0%)

^aAt least 1 section out of ~14 sections/embryo showed a boundary defect.

ented protrusions. β-Catenin–overexpressing cells in the somites also show these attributes, whereas they show a rounded shape and unstable, nonpolarized protrusions as long as they are still located in the notochord. This suggests that cell polarization depends on tissue-specific cues and is not cell autonomous. We also noted that notochord cells adjacent to cells overexpressing β-catenin were often diverted from their normal mediolateral path and seemed to attach preferentially to other notochord cells (Fig. 2 E). Thus, it appears that β-catenin–overexpressing cells and wild-type notochord cells are not able to form stable protrusions on each other, which then would result in inefficient cell traction. This resembles the behavior of notochord and somitic cells at the boundary, suggesting that this aspect of boundary behavior can be expressed at the cell–cell level and does not require a higher order boundary structure. In the simplest scenario, all cells of the notochord and somites have this distinguishing attribute. Alternatively, an inhibitory signal might be generated only when notochord and somitic cells touch. The rounded cell shape of β-catenin–overexpressing cells might be a secondary effect of such a loss of traction, although we cannot rule out that other signals regulate cell shape independently of protrusive activity. We propose that cells first become determined according to their position in the trunk mesoderm and the boundary then forms, because notochord and somitic cells do not form stable protrusions and, therefore, cannot pull on each other.

The above observations imply that cells overexpressing β-catenin in the notochord cannot intercalate efficiently in the mediolateral axis. How do these cells then reach the boundary to undergo sorting? During late gastrula, when most sorting occurred, wild-type notochord cells converged mediolaterally, and the notochord narrowed until it formed a two-cell–layer-wide structure. Therefore, essentially all cells in the notochord, including cells overexpressing β-catenin, will touch the boundary by the end of gastrulation. Because we show that β-catenin–overexpressing cells crossed inevitably to the somitic side once they reached the boundary, sorting can satisfactorily be explained as the result of “chance” encounters with the boundary without the need for any directional movement. We would like to emphasize that sorted β-catenin–overexpressing cells behaved like unmanipulated somitic cells and became trapped at the boundary, showing that boundary capture is still effective.

The role of cadherins in structuring the trunk mesoderm

CadΔE proved to be a very useful tool to investigate the mechanisms underlying cell sorting at the notochord boundary.

Expression of CadΔE has two effects. On one hand, by sequestering β-catenin to the plasma membrane, it is an efficient inhibitor of Wnt signaling. On the other hand, CadΔE also strongly inhibits cadherin-mediated cell–cell adhesion, most likely by competing for β-catenin and, thereby, disrupting the link between endogenous cadherins and the actin cytoskeleton (Kintner, 1992; Fujimori and Takeichi, 1993). Because Wnt–β-catenin signaling appeared to act as a binary switch to determine the direction of sorting at the notochord–somite boundary, expression of CadΔE alone or in conjunction with LEFVP16 produced two cell types, both with decreased adhesion but with opposite directions of sorting. It is important to note that, under these experimental conditions, the manipulated cells retained enough adhesive activity to remain at least partially integrated in the tissues. Stronger cadherin inhibition would have caused loose cells to be expelled from the tissues (Kintner, 1992; unpublished data).

Transmission of contractile forces from cell to cell via cadherin-based contacts is considered to be the motor for mediolateral intercalation (Keller et al., 2000). This model is supported by the fact that global interference with cadherin function causes strong gastrulation defects (Broders and Thiery, 1995; Lee and Gumbiner, 1995; Kuhl et al., 1996) and is now further strengthened by our observations on single cells (in particular the loss of polarized, elongated shape). The difference between weaker sorting to the notochord by high CadΔE alone and complete sorting to the somites by CadΔE + LEFVP16 could then simply rely on the fact that cells mislocalized in the somites require active mediolateral intercalation to converge toward the boundary, whereas cells mislocalized in the notochord will all eventually contact the boundary as the notochord narrows.

The robustness of sorting in the presence of CadΔE is a very surprising observation that is difficult to explain. It is certainly not consistent with the classical model of differential adhesion. In this model, weak adherent cells could, in principle, be simply extruded from one tissue, and, provided that adhesion is similarly weaker in neighboring tissue, they could become integrated. In our experiments, however, CadΔE-expressing cells could sort in either direction, depending only on the status of β-catenin signaling. Judging from their morphology, cells expressing high levels of CadΔE should be less adhesive than both notochord and somitic cells. Yet, even when manipulated cells were obviously quite loose and not properly integrated in the tissue, they respected the boundary in most cases.

We propose that sorting does not depend on differential levels of adhesion. Instead, we suggest the existence of signals that would regulate cell–cell interactions independently of absolute levels of adhesion. An example for such a mechanism is the role of ephrins and their ephrin receptors that mediate cell sorting at rhombomeric boundaries (Xu et al., 1999). Other good candidates for cell–cell signals include members of the protocadherin family. In *X. laevis*, they are differentially expressed in the trunk mesoderm and can induce homotypic cell sorting (Kim et al., 1998; Kuroda et al., 2002). Although initially considered to be adhesion molecules, protocadherins ac-

tually lack obvious connection to the actin cytoskeleton and are rather thought to act by activating intracellular signals (Medina et al., 2004; Unterseher et al., 2004). The method presented in this study provides tools to further investigate the role of these molecules and other intracellular effectors.

Materials and methods

Frogs and embryos

Embryos were obtained and injections were performed as described previously (Reintsch and Hausen, 2001). Injections were performed at the four-cell stage by injecting 2×5 nl into two dorsal blastomeres $\sim 30^\circ$ off the dorsal cleavage and about midway from the animal pole to the equator.

Plasmid constructs

The following plasmids were used: pCS-myc-eGFP (gift from T. Joos, Natural and Medical Sciences Institute, Tuebingen, Germany), pCS- $\Delta\beta$ -catenin-myc (NH₂-terminal aa 47 deleted; gift from R.T. Moon, Howard Hughes Medical Institute, Seattle, WA), pCS-myc- β -catenin-eng (gift from P. McCrea, MD Anderson Cancer Center, Houston TX), pCS-Cad Δ E-myc (pCS-Cad Δ E-myc, extracellular domain deleted; gift from P. Hausen, Max-Planck-Institut für Entwicklungsbiologie, Tuebingen, Germany), pCMV-C2LRP5-flag (gift from D. Wu, University of Connecticut, Farmington, CT), and pCS-myc-axinmut (Ax12-810). For pCS- $\Delta\beta$ -catenin-myc-eYFP, eYFP (CLONTECH Laboratories, Inc.) was PCR amplified and inserted downstream and in frame with $\Delta\beta$ -catenin-myc. For pCS-LEFeng and pCS-LEFVP16, the DBD of LEF1 (aa 1–6 and 265–394) was cut from LEF1 Δ N β CTA (gift from A. Hecht, Max-Planck-Institute of Immunobiology, Freiburg, Germany), and BamHI-Clal was inserted into pCS2 + myc to obtain pCS2-LEF1-DBD-myc. The engrailed repressor domain and the VP16 activator domain were PCR amplified from pBSRN3-engXnotHD and pBSRN3-VP16XnotHD, respectively (gift from P. Lemaire, Institut de Biologie du Développement de Marseille, Marseille, France). The fragments were inserted downstream into pCS-LEF1-DBD-myc. For pCS-C-cad-myc (CadFL), the $2 \times$ myc tag of the original clone (gift from B.M. Gumbiner, University of Virginia, Charlottesville, VA) was replaced by the $6 \times$ myc tag of pCS2 + myc. For pCS-Cad Δ cat-myc, the COOH terminus of C-cadherin-myc was PCR amplified from an internal HindIII site to aa 810, eliminating the last 70 aa (β -catenin-binding domain). Then, the COOH-terminal end of CadFL-myc was replaced by the PCR fragment. For pCS-Cad Δ E Δ cat-myc, a fragment of pCS2-Cad Δ E-myc corresponding to the signal sequence followed by aa 627–810 of C-cadherin was PCR amplified and inserted into pCS2 + myc. The plasmid concentrations for injections (pg/nl) were as follows: pCS-myc-eGFP, 6; pCS- $\Delta\beta$ -catenin-myc, 8; pCS-myc- β -catenin-eng, 20; pCS- $\Delta\beta$ -catenin-myc-eYFP, 10; pCMV-C2LRP5-flag, 10; pCS-myc-axinmut, 14; pCS-C-cad-myc, 10; pCS-Cad Δ cat-myc, 10; pCS-Cad Δ E-myc, 6 (low) and 10 (high); pCS-Cad Δ E Δ cat-myc, 10; pCS-LEFeng-myc, 10; and pCS-LEFVP16-myc, 4.

Antibodies

The following antibodies were used in this study: rabbit anti-C-cadherin antibody (gift from B.M. Gumbiner; Yap et al., 1997b), mouse anticadherin mAb 6D5 (gift from P. Hausen), mouse anti-MyoD mAb D7F2 (gift from J. Gurdon, Wellcome Trust/Cancer Research UK Gurdon Institute, Cambridge, UK; Hopwood et al., 1992), mouse anti-myc tag mAb 9E10, rabbit anti-myc tag antibody (gift from T. Joos), mouse anti-Flag mAb M2 (Sigma-Aldrich), rabbit antibrachyury antibody (gift from J. Smith, Wellcome Trust), rabbit anti- β -catenin antibody P14L (gift from P. Hausen), rabbit anti-Xarvcf2B antibody (gift from P. McCrea), and mouse anti-Gsk3 mAb 4G-1E (Upstate Biotechnology). A rabbit polyclonal antiserum against FoxA4 was raised by using the full-length protein as an antigen. For this purpose, FoxA4 (XFD1; gift from W. Knochel, University of Ulm, Ulm, Germany) was cloned into pET32b (Novagen).

Histology and immunofluorescence

Histology was performed as described previously (Fagotto et al., 1999). In brief, embryos were fixed first in formaldehyde and then in DMSO/methanol. After embedding in fish gelatin, 10- μ m serial cryosections were prepared. Antibodies were diluted in 5% milk/PBS. Secondary antibodies were goat anti-mouse and goat anti-rabbit antibodies, respectively, coupled to AlexaFluor488 or 546 (Molecular Probes). Nuclei were counterstained with 0.5 μ g/ml DAPI, and yolk was stained with 0.2% eriochrome.

Explants

For cryosectioning, dorsal regions were explanted at different gastrula stages, transferred into 4% formaldehyde/PBS, and immediately flattened under a silicone-supported coverslip. After prefixation, they were fixed in DMSO/methanol, sectioned longitudinally, and stained.

For live imaging, dorsal explants were cut at stages 12–12.5. The endodermal epithelium covering the axial/paraxial mesoderm was removed, and the explants were flattened on 1 μ g/ml fibronectin-coated glass (blocked with 2% BSA), secured with a silicon-supported coverglass, and cultured in "Danilchik's for Amy" medium (Davidson et al., 2002) at RT.

Data acquisition

All pictures and time-lapse videos were obtained using an inverted microscope (model TV135; Carl Zeiss MicroImaging, Inc.) with a $25 \times$ N.A. 0.8 water immersion objective (Carl Zeiss MicroImaging, Inc.) and a CCD video camera (model DXC-950P; Sony). Images were acquired and processed with AnalySIS software (Soft Imaging System GmbH). Images of AlexaFluor488, 546, and DAPI stainings were acquired separately and were subsequently merged. Large high resolution images were obtained by collating pictures of adjacent regions (Schohl and Fagotto, 2002). For time-lapse videos, the movement of β -catenin-eYFP-expressing cells was recorded in intervals of 5–10 min for up to 6 h.

Quantitation of cell distribution

Three to four sections from different positions along the anteroposterior axis were evaluated for each embryo. For explants, every second section was used. In these sections, we counted the number of construct-expressing cells in the notochord and in the adjacent first four cell rows of the somites. For each construct, at least three independent experiments were performed. To control for normal distribution, embryos that were injected with pCS-myc-eGFP were included in all experiments.

Determining mediolateral cell elongation

The mediolateral axis of explants was defined as being perpendicular to the anteroposterior axis of the notochord. Maximal cell length and width were determined based on the orientation of a cell with respect to the mediolateral axis; the maximum axis of elongation was defined as length if it fell within 45° of the mediolateral axis and as width if the angle exceeded 45° . The elongation of cells was then expressed as a ratio between length and width.

Immunoprecipitations

Embryos were injected at the four-cell stage with mRNA of different myc-tagged cadherin constructs (500 pg/injection) and were extracted at stage 11 with 0.5% NP-40, 120 mM NaCl, 1 mM MgCl₂, 1 mM CaCl₂, and 50 mM Tris-HCl, pH 7.4, supplemented with protease inhibitors. Precipitation was performed with mAb anti-myc 1 (Oncogene Research Products) bound to protein G-Sepharose beads (Sigma-Aldrich).

Online supplemental material

Time-lapse videos supplement Fig. 3. Videos 1 and 2 show β -catenin-eYFP-expressing cells during sorting from the notochord to somites. Video 3 shows one positive cell crossing the notochord-somite boundary. Online supplemental material is available at <http://www.jcb.org/cgi/content/full/jcb.200503009/DC1>.

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