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N- and E-cadherins in *Xenopus* are specifically required in the neural and non-neural ectoderm, respectively, for F-actin assembly and morphogenetic movements

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Transmembrane cadherins are calcium-dependent intercellular adhesion molecules. Recently, they have also been shown to be sites of actin assembly during adhesive contact formation. However, the roles of actin assembly on transmembrane cadherins during development are not fully understood. We show here, using the developing ectoderm of the *Xenopus* embryo as a model, that F-actin assembly is a primary function of both N-cadherin in the neural ectoderm and E-cadherin in the non-neural (epidermal) ectoderm, and that each cadherin is essential for the characteristic morphogenetic movements of these two tissues. However, depletion of N-cadherin and E-cadherin did not cause dissociation in these tissues at the neurula stage, probably owing to the expression of C-cadherin in each tissue. Depletion of each of these cadherins is not rescued by the other, nor by the expression of C-cadherin, which is expressed in both tissues. One possible reason for this is that each cadherin is expressed in a different domain of the cell membrane. These data indicate the combinatorial nature of cadherin function, the fact that N- and E-cadherin play primary roles in F-actin assembly in addition to roles in cell adhesion, and that this function is specific to individual cadherins. They also show how cell adhesion and motility can be combined in morphogenetic tissue movements that generate the form and shape of the embryonic organs.

KEY WORDS: *Xenopus*, Actin, Cadherin, Morphogenetic movement, Neurulation

INTRODUCTION

Morphogenetic tissue movements control both the overall shape of the embryo and the shapes of its constituent organ primordia. Tissue movements are distinguished from movements of individual cells by the fact that the cells are held together, so that the individual cell movements collectively generate a characteristically shaped structure. Several kinds of movement may occur in the same developing tissue. Within the vertebrate neural ectoderm, for example, cells undergo convergent extension movements, which lengthen the forming tube (Keller et al., 1992; Keller et al., 2008; Schoenwolf and Alvarez, 1989), while at the same time undergoing folding movements, which are maximal at specific hinge regions, by apical constriction of the cells (Colas and Schoenwolf, 2001; Schoenwolf and Franks, 1984) (reviewed by Wallingford, 2005). One of the major puzzles about morphogenetic tissue movements is the mechanism by which cell adhesion and motility are coordinated. Recent work on the assembly of cortical actin on transmembrane cadherins suggests that this might provide a basic mechanism linking cell adhesion to cell motility.

Cadherins are a large family of calcium-dependent cell-cell adhesion proteins. Since their discovery (Gallin et al., 1983; Peyrieras et al., 1983; Yoshida and Takeichi, 1982), more than 100 proteins have been assigned to the cadherin family. C-, E- and N-cadherin are Type I sub-family members, characterized by five extracellular cadherin (EC) domains, with an HAV sequence in the most distal one (reviewed by Suzuki and Takeichi, 2008). The full range of functions of the cadherin family is not yet known. During vertebrate morphogenesis, different cadherin family members are expressed to different extents in different tissues. In *Xenopus*, C-cadherin is the major cadherin expressed during the egg to blastula stage (Choi et al., 1990; Ginsberg et al., 1991), and is absolutely required for cell adhesion in the blastula (Heasman et al., 1994b). The animal cells of the blastula give rise to the ectoderm, which activates the expression of E-cadherin. At the end of gastrulation, the ectoderm becomes segregated into the dorsal neural ectoderm, which activates expression of N-cadherin, and turns off E-cadherin, and the more ventral non-neural ectoderm, which retains expression of E-cadherin, and becomes the epidermis. C-cadherin continues to be expressed in both tissues throughout early development. Many such examples of combinatorial expression of the cadherins have been identified (reviewed by Takeichi, 1988). The functional consequences of combinatorial expression of cadherins during development are not fully understood, although functional differences have been shown between different cadherins expressed in the same cell. For example, Cadherin-11 and N-cadherin, expressed in neurons, have different adhesive properties and Ca^{2+} affinities (Heupel et al., 2008).

Attachment of transmembrane cadherins to the cortical actin of the cell through α-, β- and γ-catenin is known to be essential for strong intercellular adhesion (Hirano et al., 1987; Matsuzaki et al., 1990; Nagafuchi and Takeichi, 1988; Ozawa et al., 1989). However, this story has become more complex recently, firstly by the finding that α-catenin does not bind simultaneously to both β-catenin and actin (Drees et al., 2005; Yamada et al., 2005), and secondly by the discovery that cadherins themselves can be sites of actin assembly (Ehrlich et al., 2002; Jamora and Fuchs, 2002; Kovacs et al., 2002a; Kovacs et al., 2002b). Actin nucleation proteins such as Arp2/3 and formin 1 are associated with nascent cadherin-mediated adhesive contacts (Kobielak et al., 2004; Kovacs et al., 2002b; Verma et al.,

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2004), as are nucleation-promoting proteins such as Ena/Vasp and cortactin (Helwani et al., 2004; Scott et al., 2006; Vasioukhin et al., 2000). These data show that cadherins play an active part in assembling actin, rather than merely attaching to a pre-existing actin network.

It is therefore of major interest to identify whether cadherin-mediated actin assembly plays a major role during development in vivo, and in particular, to find out whether differential expression of cadherin family members leads to different types of actin assembly and different types of tissue movement. In previous work, we showed that expression of C-cadherin in the *Xenopus* blastula is essential for cortical actin assembly (Tao et al., 2007), and that the level of C-cadherin expression is controlled, in turn, by at least two G protein-coupled receptor (GPCR)-mediated pathways. One is activated by the receptors for the signaling lipid lysophosphatidic acid (LPA) (Lloyd et al., 2005). The other is activated by Xflop, an orphan GPCR (Tao et al., 2005). In addition, the catenins plakoglobin/γ-catenin (Kofron et al., 2002; Kofron et al., 1997) and p120 (Tao et al., 2007) are both required for cadherin-based cortical actin assembly.

In this paper, we use the developing neural and non-neural ectoderm to test the hypothesis that differential expression of cadherins in these tissues controls assembly of actin required for characteristic morphogenetic movements of these tissues. First we show that at post-gastrula stages of ectodermal differentiation, C-, E- and N-cadherin each has a specific regional expression pattern on the cells that express them. Second, we show that both N- and E-cadherin are capable of directing cortical actin assembly, by replacing C-cadherin with each of them in the blastula. Third, we show that each is required, in the neural plate and epidermis, respectively, for assembly of both cortical and cytoplasmic actin, and when each is depleted, using a morpholino oligo, the characteristic actin-based tissue movements of each tissue fail to take place. Fourth, we show that when N-cadherin is depleted in the neural plate, or E-cadherin is depleted in the non-neural ectoderm, the activated motor protein phosphorylated myosin light chain (P-MLC) is reduced in the corresponding tissues. Lastly, we show that these functions are cadherin-type-specific. E- and N-cadherin cannot replace each other in their respective tissues. These data show that cadherin-based cortical actin assembly is a general mechanism during development, and is required for the tissue-wide movements of morphogenesis.

MATERIALS AND METHODS

Oocytes and embryos

All *Xenopus laevis* animals used in this study were obtained through Nasco (Fort Atkinson, WI). Mature (St. VI) oocytes used in host transfer experiments were obtained using ovaries from 2-year-old female frogs and manual defolliculation. Defolliculated oocytes were cultured in oocyte culture medium (OCM) (Tao et al., 2007) and microinjected with antisense oligos or mRNA as described in the text. In C-cadherin replacement experiments, mRNA was injected into cultured oocytes 24 hours after their injection with antisense oligos. Oocytes were matured and fertilized using the host transfer method described previously (Holwill et al., 1987). All embryos were cultured in 0.1×MMR. Embryos were dejellied in a 2% cysteine solution made in 0.1×MMR (pH 7.8). For microinjecting mRNA and/or morpholino oligos into 8- or 16-cell-stage embryos, 2% Ficoll solution made in 0.2×MMR was used. Morpholino oligos were injected together with Rhodamine lysine dextran (RLDX) and mis-targeted embryos were eliminated from the experiments using the fluorescent signal at early neurula stages. In MO + mRNA experiments, morpholinos were injected first followed by a second injection of the mRNA into the same cell.

DNA constructs and mRNA

By blasting the *X. tropicalis* N-cadherin coding sequence, an IMAGE clone containing the full-length *X. tropicalis* N-cadherin cDNA (IMAGE clone ID:7760787) was identified and purchased (Open Biosystems, Huntsville, AL). The 5′ and 3′ untranslated regions were removed by PCR amplifying the open reading frame (ORF) from this clone and re-cloned into pCS 107 using the following primers and restriction enzyme sites: forward primer (*Not*I-Xt-N-cad), 5′-TCGAGCGGCCGCACCATGTGCCGGAAAGA-GCC-3′; reverse primer (Xt-N-cad-Myc-*Xho*I), 5′-CGACTCGAGTC-ATTCATTCAAGTCCTCTTCAGAAATGAGCTTTTGCTCCATGTCG-TCGCTTCCGCCGTA-3′. A single Myc tag was introduced onto the 3′ end using the reverse primer. *Nsi*I restriction enzyme was used to linearize this construct and capped mRNA was synthesized using the Sp6 mMESSAGE mMACHINE Kit (Ambion). mRNA coding for full-length *X. laevis* E-cadherin-HA was synthesized using a construct (XE-cad/pCS2+C-3HA) kindly provided by Pierre McCrea (University of Texas M.D. Anderson Cancer Center). XE-cadherin is cloned to the *Stu*I (5′) and *Xho*I (3′) sites of the pCS2+C-HA vector, which includes three HA tags in the C-terminal end. *Not*I restriction enzyme was used to linearize the construct and capped mRNA was synthesized using the Sp6 mMESSAGE mMACHINE Kit. A QuikChange Site-Directed Mutagenesis Kit (Stratagene) was used for making a morpholino resistant E-cadherin construct. The following primer pair was used to introduce eight mismatches to the E-cadherin morpholino recognition sequence in the original construct: forward primer, 5′-CGAATTCAAGGCCTATGGGCCTCAAACGACCTTGGTTACTTGG-TGCTGTCGTGTTG-3′; reverse primer: 5′-CAGCACCAAGTAAC-CAAGGTCGTTTGAGGCCCATAGGCCTTGAATTCGAATCGA-3′. The mutated sequence is as follows: 5′-C*T*[ATG]GG*CC*T*CAA*A*C*G*A*CC*T*-TGGTT-3′ (the start codon is indicated by the square brackets and the mutated nucleotides are indicated in italics). The E-cadherin amino acid sequence was not changed by the mutagenesis.

Oligonucleotides

The following antisense oligodeoxynucleotides (designated AS below) or morpholino oligonucleotides (designated MO below) were used: C-cad AS, 5′-C*C*T*CTCCAGCTCCCT*A*C*G-3′ (Heasman et al., 1994b) (asterisks indicate phosphorothioate-modified residues); N-cad-MO (translation blocker), a generous gift from Richard Lang (CCHMC): 5′-GAAGGGCTCTTTCCGGCACATGGTG-3′; E-cad-MO (translation blocker), 5′-AACCAGGGCCTCTTCAACCCCATTG-3′. All antisense morpholinos were purchased through Genetools, LLC.

F-actin and immunostaining

For F-actin staining of animal caps or dorsal plates, embryos were dissected in 1×MMR and immediately fixed in FG fixative (Tao et al., 2007) for 10 minutes followed by washing in PBSTw (PBS+0.1% Tween 20) for 30 minutes (3×10 minutes). Samples were then stained with Alexa-488-conjugated Phalloidin (5 U/ml in PBSTw) for 4 hours at room temperature (RT) or at 4°C overnight and washed with PBSTw for an equal time period. C-cadherin immunostaining was done using the 6B6 monoclonal antibody as previously described (Tao et al., 2007). For E-cadherin and myosin light chain staining, dissected tissues were fixed in 2% TCA for 30 minutes and washed with PBSTx (PBS+0.3% TritonX 100) for 30 minutes. Samples were then blocked in 10% normal goat serum (NGS) for 1 hour at room temperature and incubated with the following concentrations of primary antibodies. E-cadherin monoclonal antibody (5D3, Developmental Studies Hybridoma Bank, Iowa City) was used at 2.5 mg/ml, and rabbit polyclonal antibody for pMLC (Abcam: 2480) was used at 3 mg/ml concentration in 10% NGS. For N-cadherin immunostaining, dissected tissues were fixed in 3.7% formaldehyde (made in PBSTw) for 30 minutes and a rabbit polyclonal antibody (Detrick et al., 1990; Heasman et al., 1994a) was used at a 1:100 dilution in 10% NGS. For Myc and HA tag immunostaining, tissues were fixed in FG fixative and 1:200 dilutions of rabbit polyclonal Myc antibody (Cell Signaling: 2272) or a rat monoclonal HA antibody 3F10 (Roche) was used. For β-catenin staining a rabbit polyclonal antibody H-102 (Santa Cruz: 7199) was used at a 1:300 dilution on FG fixed tissues. After primary antibody incubation all immunostaining samples were washed with PBST× for 3 hours and incubated with Cy5-conjugated secondary antibodies at 1:300 dilutions. Goat anti-mouse

cy5 for C- and E-cadherin, goat anti-rabbit cy5 for P-MLC, N-cadherin, β-catenin and Myc tag staining, and goat anti-rat for HA tag staining (Jackson Laboratory) was used. Samples were then extensively washed with PBST× before imaging with confocal microscopy. For dorsal and ventral ectoderm cross-sections, fixed tissues were carefully sectioned by hand using a sharp razor blade.

Confocal imaging

All confocal imaging was carried out using a Zeiss LSM 510 inverted confocal microscope. For imaging fine F-actin structures in animal caps an LD C-Apochromat 40×/1.1 W korr UV-vis IR water objective was used. For dorsal and ventral ectoderm images a Fluar UV/20× NA0.75 objective was used for higher power or a PlanApo 10× NA 0.45 objective was used for lower power images. For higher power images of dorsal plate cross-sections a C-apochromat 63×/1.2 W Korr objective was used.

Quantitation

For analysis of cortical actin in animal caps, five to ten caps were examined from each experimental group. Pixel intensity of the entire cap was used to quantitate F-actin levels as previously described (Tao et al., 2007). For analysis of cortical actin in treated dorsal plates, five to ten samples were examined in each experimental group. Pixel intensities and apical surface areas were quantitated by outlining individual cells using the Zeiss LSM 510 software on en-face images. Readings from 10-15 cells were quantitated for each treated or untreated clone of cells from a single sample. Bar charts in results represent the mean values of five to ten samples.

For measuring the average thickness of the ventral ectoderm, the Overlay tool of the Zeiss LSM 510 software was used to analyze 20× *z*-stack images. The average of three to four readings from a single sample was taken and the average of five to ten samples from each group is represented in the bar charts. For measuring the membrane expression level of E- and N-cadherins, the Profile tool of the Zeiss LSM 510 software was used to measure the linear pixel intensity across individual cell membranes. The average pixel intensity for a 3 μm-wide area was taken for each reading. A minimum of 20 readings was taken from each experimental group from five to ten samples and averaged for generating the bar charts. All results are expressed as means (±s.d.). Statistical significance was analyzed by Student's *t*-test (*P<0.05, **P<0.01, ***P<0.001 in figures).

Real-time RT-PCR

For RT PCR analysis cDNA was synthesized using oligo dt primers and total RNA extracted from two embryos at each stage as previously described by Zhang et al. (Zhang et al., 1998). Real-time RT-PCR was carried out using the LightCycler system as previously described by Kofron et al. (Kofron et al., 2002) and using the following primer combinations: ODC forward primer 5′-GCCATTGTGAAGACTCTCTCCATTC-3′, reverse primer 5′-TTCGGGTGATTCCTTGCCAC-3′; C-cadherin forward primer 5′-AGGAAGGTGGAGGAGAGGAG-3′, reverse primer 5′-GAGAGTCATATGGGGGAGCA-3′; E-cadherin forward primer 5′-CGAAGATGTAAACGAAGCC-3′, reverse primer 5′-GCCATTTCCAGTGACAATC-3′; N-cadherin forward primer 5′-CAGGGACCAGTTGAAGCACT-3′, reverse primer 5′-TGCCGTGGCCTTAAAGTTAT-3′.

RESULTS

Expression patterns of C-, E- and N-cadherin in the *Xenopus* ectoderm

The *Xenopus* post-gastrula ectoderm was selected for this study, firstly because it is on the surface of the embryo, and thus easily studied, and second because it segregates into two components, the neural and non-neural ectoderm, each of which expresses a different cadherin and undergoes a different type of morphogenetic movement. Fig. 1A shows by qPCR the temporal patterns of expression of C-, E and N-cadherin from the egg to the tailbud stage. C-cadherin was found to be expressed throughout early development (yellow bar in Fig. 1A), confirming previously published data (Levi et al., 1991). E-cadherin expression started at the beginning of gastrulation (St. 10.5, blue bar in Fig. 1A), whereas N-cadherin expression started at the end of gastrulation (St. 13, red bar in Fig. 1A). Ornithine decarboxylase (ODC, green bar) is shown as a loading control. The spatial patterns of expression in the ectoderm of the three cadherins were assayed by immunocytochemistry. E-cadherin was found, using the 5D3 monoclonal antibody (Choi and Gumbiner, 1989), to be expressed initially in all the animal cells of the gastrula that give rise to ectoderm (St. 11 in Fig. 1B), but became rapidly excluded from the dorsal ectoderm, and limited to the non-neural ectodermal cells by the early neural plate stage (St. 13 in Fig. 1B). In the non-neural ectoderm (presumptive epidermis), E-cadherin was expressed predominantly on the lateral and basal surfaces of the superficial cells, and not on the apical surfaces (right-hand panel, Fig. 1B). C-cadherin distribution was examined using the 6B6 monoclonal antibody (Developmental Studies Hybridoma Bank, Iowa). It was found ubiquitously in the embryo at St. 13 in the neural ectoderm (Fig. 1C). It was expressed predominantly laterally and basally in the superficial cells, whereas in the non-neural ectoderm, it was expressed at high concentrations apically (right-hand panels, Fig. 1C). N-cadherin expression was initiated only in the dorsal ectoderm and remained restricted to the neural plate cells (Fig. 1D). In cross-sections of the neural plate, N-cadherin expression was seen to be concentrated in the apical cytoplasm (Fig. 1D), similar to mouse and chicken embryos (Bronner-Fraser et al., 1992; Hatta et al., 1987; Hatta and Takeichi, 1986), but unlike zebrafish (Hong and Brewster, 2006). The antibody used for this was a rabbit anti-*Xenopus* N-cadherin polyclonal antibody, previously characterized by western blotting (Detrick et al., 1990; Heasman et al., 1994a). To check its specificity by immunocytochemistry, early larval sections were stained (Fig. 1E). Cross-reaction with CNS, peripheral nerves, notochord and heart, showed that the antibody specifically stains tissues reported previously to express N-cadherin (Takeichi et al., 1990). This antibody also showed some background staining of cell nuclei. However, this did not interfere with analysis of the cell surface staining. Ectopically expressed Myc-tagged *X. tropicalis* N-cadherin also showed strong apical staining, thus confirming the apical staining pattern observed for N-cadherin using this antibody (see Fig. S1A in the supplementary material). β-catenin staining is seen in all regions of the cell membranes, supporting the fact that cadherins are also seen throughout the cell membranes (see Fig. S1C,D in the supplementary material). Thus, in both the neural and non-neural ectoderm, cadherins are differentially localized apically and basolaterally. However, the cadherins localized to these regions of the cells differ in each region. C-cadherin, although expressed in both regions of the ectoderm, is differentially localized. It is predominantly apical in the epidermis, but basolateral in the neural plate.

Both N- and E-cadherin can assemble cortical actin at the blastula stage

To test whether all three classical cadherins share the property of cortical actin assembly, cadherin replacement experiments were carried out at the blastula stage. The maternal pool of C-cadherin mRNA was depleted by injection of 5 ng anti-C-cadherin antisense oligo (Heasman et al., 1994b; Tao et al., 2007) into cultured oocytes. These were subsequently injected with either N-cadherin (350 pg) or E-cadherin (350 pg) mRNA, and fertilized by the oocyte transfer method (Holwill et al., 1987). Animal caps were dissected from these embryos at the late blastula stage, and stained for both cortical actin and for the appropriate cadherin, tagged with either Myc or HA (Fig. 2). C-cadherin-depleted animal caps had dramatically reduced levels of C-cadherin on their surfaces, were partly dissociated, and lacked a dense cortical actin network, as reported previously (Tao et al., 2007). Both N-cadherin and E-cadherin rescued both cell

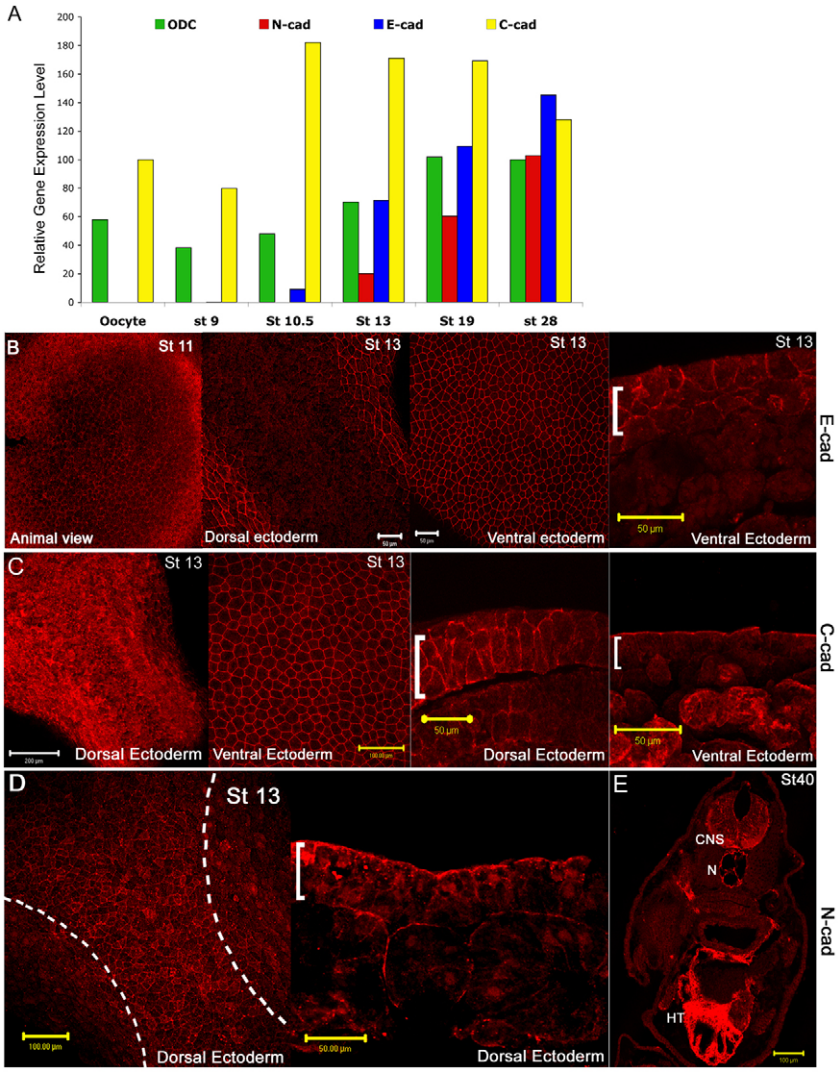

Fig. 1. Temporal and spatial expression patterns of classical E-, C- and N-cadherins. (**A**) Real-time RT-PCR analysis of cDNA isolated from *Xenopus* embryos at different stages showing the temporal expression pattern of N- (red), E- (blue) and C- (yellow) cadherin. Green bars show levels of the loading control, ODC. (**B**) E-cadherin is expressed at st. 11 in animal cells at low levels (left panel), is lost from the dorsal (neural) ectoderm, but is expressed strongly in the ventral (non-neural) ectoderm at st. 13 (center two panels), and is expressed primarily in the lateral and basal regions of ventral ectoderm cells (right-hand panel). (**C**) C-cadherin is expressed in both neural and non-neural ectoderm of the neurula (left two panels), and is expressed primarily in the lateral and basal regions of the neural plate, but in the apical regions of the non-neural ectoderm (right-hand two panels). (**D**) N-cadherin is expressed in the neural, but not non-neural, ectoderm at st. 13 (left panel), primarily in the apical cytoplasm (right-hand panel). Dashed line demarcates junction between neural and non-neural ectoderm. (**E**) The distribution of N-cadherin in sections of a st. 40 embryo. White brackets in B-D indicate the ectoderm layer in each cross-section. CNS, central nervous system; HT, heart; N, notochord. Scale bars: 50 μm, except 200 μm in C (left) and 100 μm in C (right), D (left) and E.

adhesion and cortical actin assembly in these embryos (Fig. 2A-C). These data show that classical cadherins as a group have the ability to promote actin assembly at the cell surface. In order to test whether this property is required in the post-gastrula tissues in which they are normally expressed, morpholino oligos were used to target their expression in descendants of specific blastomeres.

N-cadherin is required for F-actin assembly in the apical cytoplasm of the neural plate

Single dorsal animal cells at the 16-cell stage (arrowed in Fig. 3A) were injected with 12.5 ng morpholino oligo against N-cadherin (see Materials and methods for details), together with the lineage tracer RLDX. This generated clones of cells in the neural plate (Fig. 3B). When the embryos reached the neurula stage, the neural plate, dorsal mesoderm and dorsal archenteron roof was dissected as a single piece, the 'dorsal plate' (Fig. 3C), fixed immediately in FG fixative, and stained for either N-cadherin or for F-actin as previously described (Kofron et al., 2002; Lloyd et al., 2005; Tao et al., 2005; Tao et al., 2007). Fig. 3D shows a neural plate stained for F-actin (green). The clone of cells descended from the injected blastomere fluoresces red owing to the RLDX lineage tracer. Regions containing both uninjected and injected cells (illustrated by the box in Fig. 3D) were examined under higher magnification. The morpholino-containing cells showed a dramatic loss of N-cadherin staining compared with adjacent untreated cells (Fig. 3E), although non-specific nuclear staining can still be seen. Transverse sections showed that the apical membrane staining of N-cadherin seen in Fig. 1 had been lost (see Fig. S1B in the supplementary material). C-cadherin staining was unaffected by the N-cadherin depletion (Fig. 3F). Phalloidin staining for F-actin showed in en face views (Fig. 3G) a reduction of levels of F-actin in the morpholino-containing cells, as well as their apparent enlargement, compared with adjacent untreated cells. A transverse section showing three N-cadherin-depleted cells is shown in Fig. 3I. Here it can be seen that the apparent increase in size seen in en face pictures is due to a radial expansion of the apical end of each N-cadherin depleted cell (Fig. 3I), which also lacked F-actin. Each N-cadherin-depleted cell had a normal-looking basal F-actin assembly (yellow arrows in Fig. 3I). It also had a circumferential cortical actin belt surrounding it, seen in the grazing section of one cell, and in cross-section through the center of another (white arrows in Fig. 3I). However, the F-actin normally seen in the apical cytoplasm was reduced. Quantitation of this was carried out by measuring the pixel intensities of *z*-stacks from en face views extending apically from approximately half way from the basal aspect of the cells. In this way, the F-actin in the basal cytoplasm was excluded from the measurement (see Materials and Methods for

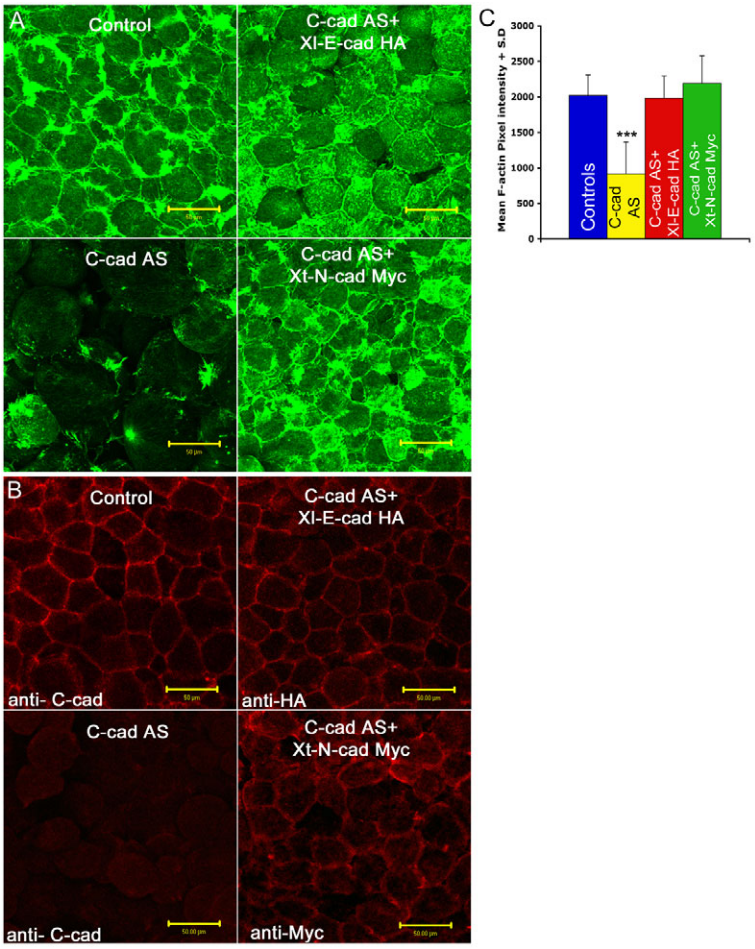

Fig. 2. E- and N-cadherin can replace C-cadherin to assemble cortical actin during the blastula stage. (**A**) Cortical F-actin levels in cells of the inner surfaces of dissected *Xenopus* animal caps from embryos that were untreated (Control), depleted of maternal C-cadherin mRNA (C-cad AS) or depleted of C-cadherin mRNA and subsequently injected with either HA-tagged E-cadherin mRNA (C-cad AS+Xl-E-cad HA) or Myc-tagged N-cadherin mRNA (C-cad AS+Xt-N-cad Myc). Expression of either E- or N-cadherin rescues the loss of cortical actin caused by C-cadherin depletion. (**B**) Cadherin levels in animal caps from the same experiment. The left-hand panels show the degree of depletion of C-cadherin protein (compare Control with C-cad AS, stained with anti-C-cadherin antibody). The right-hand panels show that both E-cadherin and N-cadherin, stained using anti-HA and anti-Myc, respectively, were expressed at the cell surface. (**C**) Mean F-actin pixel intensity quantified for each group shown in A. ***Statistically significant difference between control and C-cad AS levels of phalloidin staining (P<0.001). Scale bars: 50 μm.

details). Fig. 3H shows the reduction of apical F-actin, and the increase in cross-sectional area (taken from the same *z*-stacks) in the apical cytoplasm of N-cadherin-depleted and untreated cells.

This result correlated well with the distribution of N-cadherin on the apicolateral surface of neural plate cells (Fig. 1D), and suggests that the restriction of the effect of N-cadherin depletion to the apical cytoplasm is due to actin polymerization on other cell surface proteins (C-cadherin for example) in the lateral and basal cytoplasm. The increase in apical surface area and the outward protrusion of the cells suggest that these cells have lost apical cell surface tension and contractility owing to the loss of the actin skeleton in the apical region. Cell adhesion in N-cadherin-depleted cells was not obviously affected at the neurula stage, probably because C-cadherin continued to be expressed in N-cadherin-depleted neural plate cells (Fig. 3F). By microinjecting 54 pg of *Xenopus tropicalis* N-cadherin mRNA (which is not recognized by the N-cad MO designed to target the *laevis* transcript) into the same cell injected with N-cad MO, the actin cytoskeleton could be rescued to a normal level (Fig. 3J).

N-cadherin in the neural plate is required for the morphogenic movements of neurulation

In most vertebrates, including humans, mice, frogs and chickens, the formation of the central nervous system starts with the invagination of the flat sheet of epithelial cells of the neural plate into a hollow tube. In humans, defects in this process lead to spina bifida (reviewed by Detrait et al., 2005). The thick apical actin belt in the superficial cells of the neural plate has been implicated in regulation of invagination, convergent extension and the proper closing of the neural tube (reviewed by Wallingford, 2005). To test the hypothesis that N-cadherin is also required for morphogenetic movements of neurulation, N-cadherin was depleted throughout the entire neural plate by microinjecting 20 ng N-cadherin MO into the each of the two dorsal animal cells of the 8-cell-stage embryo (Fig. 4A), and time-lapse movies were made of neurulation. Frames from one movie are shown in Fig. 4B (see Movie 1 in the supplementary material). The neural plates in these embryos failed to undergo the normal invagination movements. Interestingly, the N-cadherin-depleted embryos formed distinct lip-like structures in the edges of the neural plate (Fig. 4B, arrows). Movies show that this is caused by pushing forces applied by the non-neural ectoderm on the sides of the neural plate. These movements are unaffected by N-cadherin depletion. However, paralyzing the movements of the neural plate allowed us to observe the degree to which the movements of the non-neural ectoderm assist in pushing the neural folds together in the trunk region. Examination of later-stage embryos confirmed that the defect observed in these embryos was not simply a delay in neurulation, but a complete abrogation of this morphogenic movement, which led to spina bifida at the larval stage (Fig. 4C).

Apical localization of activated myosin light chain does not occur in neural plate cells depleted of N-cadherin

Myosin-mediated apical constriction has been previously demonstrated to regulate neurulation in mammalian and chicken embryos (Lee et al., 1983; Lee and Nagele, 1985; Smedley and Stanisstreet, 1986). In order to assess whether N-cadherin-based

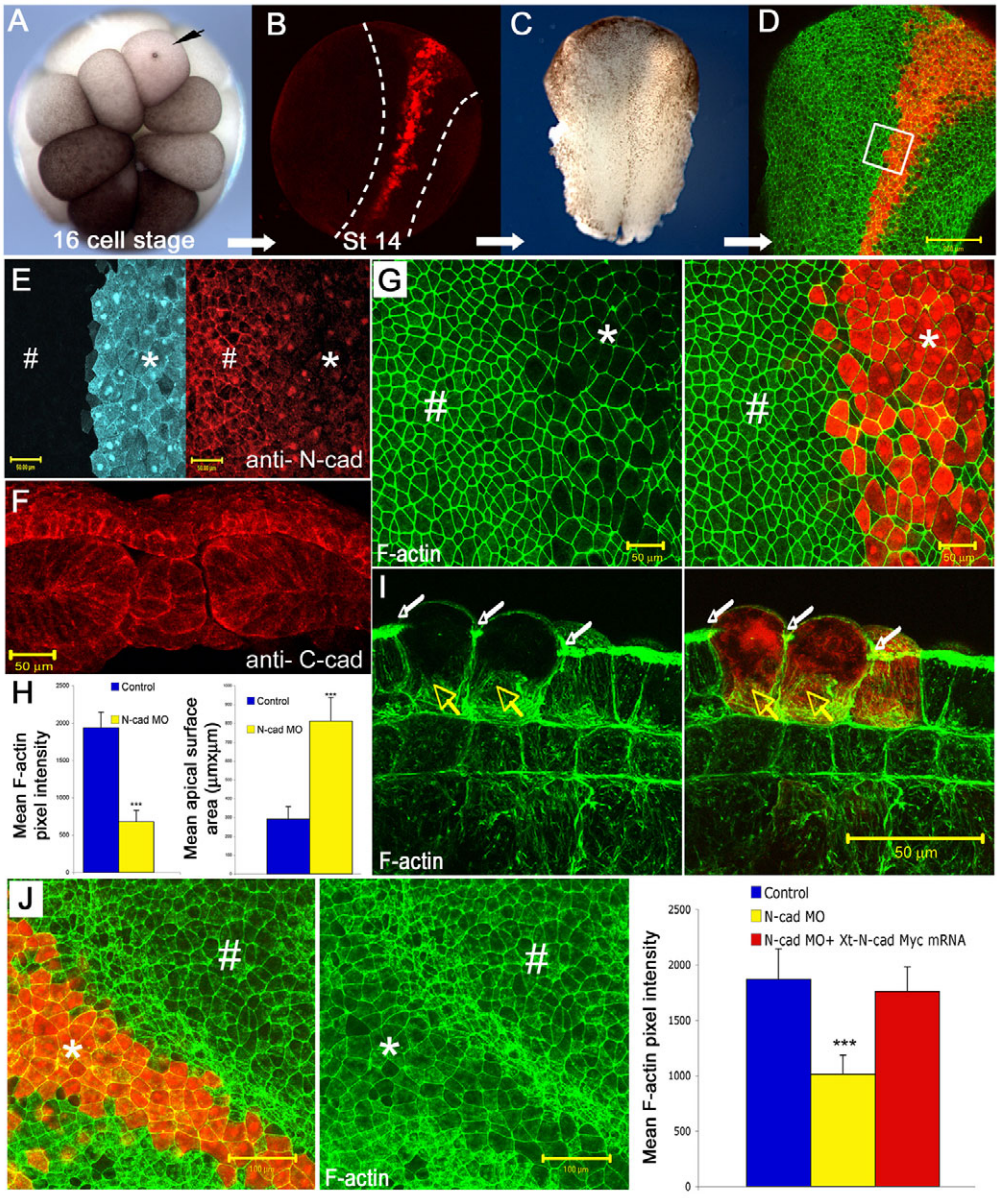

Fig. 3. N-cadherin is required to assemble F-actin in the apical cytoplasm of neural plate cells. (**A-D**) Injection of a 16-cell embryo (A; the arrow indicates the injected cell) gives rise to labeled clones in the neural plate at st. 14 (B; the dashed line indicates boundaries of the neural plate). Neural plates (C) were stained for F-actin (D). (**E**) The boundary between N-cadherin-depleted (*) and uninjected (#) cells in a neural plate stained with N-cadherin antibody to show the reduction in N-cadherin protein in the injected cells. (**F**) Transverse section of a neural plate showing that C-cadherin continues to be expressed in N-cadherin-depleted embryos. (**G**) The dramatic reduction in F-actin in N-cadherin-depleted cells (red) as compared with untreated neighboring cells of a neural plate. (**H**) Quantitation of staining intensity for F-actin and of mean apical surface area of injected as compared with untreated cells. (**I**) Transverse section through the neural plate in G, to show that only the apical regions of the neural plate cells are affected by N-cadherin depletion. White arrows indicate circumferential cortical actin belt. Yellow arrows indicate F-actin assembly is retained in the basal cytoplasm of N-cadherin-depleted neural plate cells. (**J**) Injection of *Xenopus tropicalis* N-cadherin mRNA, which does not include sequence complementary to the MO, rescues the F-actin expression in N-cadherin-depleted cells. The left-hand panel shows the RLDX lineage tracer (red); the center panel shows only the F-actin staining in the same specimen. Asterisk indicates the treated cells; #, the untreated region. The right-hand panel shows quantitation of the cortical actin staining from the pixel intensity. ***Statistically significant difference in phalloidin staining between control and N-cadherin-depleted neural plate cells ($P<0.001$). Scale bars: 50 µm, except 100 µm in J and 200 µm in D.

cortical actin assembly is necessary for apical myosin assembly during *Xenopus* neurulation, we carried out immunostaining using an antibody specific to the phosphorylated form of myosin light chain, P-MLC (Lee and Harland, 2007). High levels of P-MLC were observed in the apical surface of untreated neural plate cells undergoing neurulation (Fig. 5A,B). However, this apical localization was lost when N-cadherin was depleted in the neural plate cells (Fig. 5C,D). These data show that N-cadherin-mediated actin assembly is required for the apical localization of activated myosin light chain.

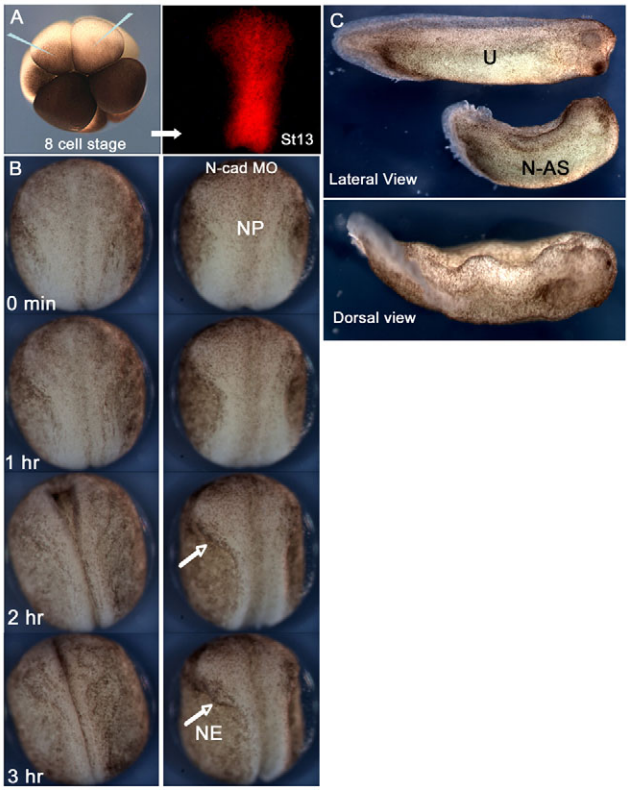

Fig. 4. N-cadherin depletion in the neural plate blocks neural fold closure and causes spina bifida. (**A**) The two dorsal animal cells injected with anti-N-cadherin MO (arrows), and the clone of cells derived from this at st. 13 (right-hand panel). (**B**) Frames from a movie showing failure of folding of the neural plate in MO-injected *Xenopus* embryos (right-hand panels) as compared with untreated embryos (left-hand panels). The arrow indicates a distinct lip in the neural plate (NP) caused by the pushing movement of the non-neural ectoderm (NE). (**C**) Spina bifida in N-cadherin-depleted as compared with untreated embryos. N-AS, N-cadherin-depleted embryos; U, untreated embryos.

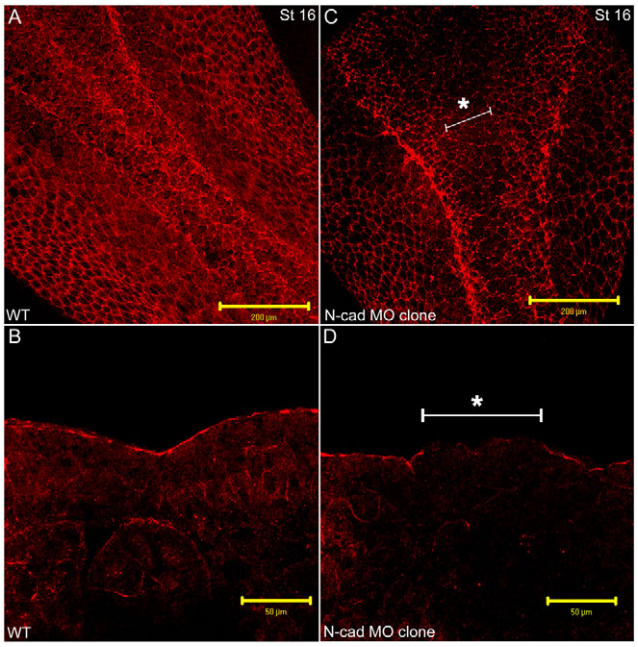

Fig. 5. N-cadherin depletion causes loss of activated myosin light chain in the apical cytoplasm in neural plate cells. (**A**,**B**) En face and transverse sectional views, respectively, of an untreated *Xenopus* embryo, stained at st. 16 with anti-phosphorylated myosin light chain antibody. Staining is intense in the apical cytoplasm of the center of the folding neural plate. (**C**,**D**) Corresponding views of an embryo with a clone of N-cadherin-depleted cells in the neural plate; in the region of the clone (asterisk with bracket), staining is lost in the apical cytoplasm of the neural plate cells. Scale bars: 200 μm in A,C; 50 μm in B,D.

E-cadherin is required for F-actin assembly in the presumptive epidermal cells

Does E-cadherin play an equivalent role in actin polymerization in the non-neural ectoderm? To test this an antisense morpholino was designed that blocks the translation of E-cadherin (see Materials and methods). Twenty-two nanograms of the MO was injected into single ventral animal cells of the 8-cell embryo (arrowed in Fig. 6A), thus generating large clones of E-cadherin-depleted cells in the non-neural ectoderm (lower panel in Fig. 6A). Immunostaining showed that the E-cadherin protein was extensively and uniformly depleted in morpholino-injected clones (asterisk in Fig. 6B,C). When stained for F-actin, the E-cadherin-depleted clones had significantly reduced F-actin networks, compared with adjacent, untreated cells (Fig. 6C). Cross-sections through these clones revealed that the actin skeleton was reduced throughout the cells, including the apical regions (Fig. 6D). Interestingly, these clones of cells, despite the fact that E-cadherin protein is reduced to an undetectable level, maintained some cortical actin, and did not show the increase in apical cell surface area seen in the neural plate depleted of N-cadherin. This suggests that other cadherins might be present in the non-neural ectoderm. This is supported by the fact that the cells did not disaggregate. Staining with anti-C-cadherin antibody showed this to be the case (Fig. 6E). Morphogenetic movements of E-cadherin-depleted non-neural ectoderm were also affected. The spreading movements that push the trunk region of the neural folds together did not occur, resulting in a significant delay in neurulation (Fig. 6F, and see Movie 2 in the supplementary material). This experiment, in which the neural plate is normal, but the non-neural ectoderm has reduced spreading behavior, confirms that movements of the non-neural ectoderm are important in the timing of neural fold closure, and that these movements require E-cadherin-mediated cortical actin assembly. The effect of the E-cadherin MO was shown to be specific by rescuing the reduction in cortical actin after MO injection by subsequent injection of 100 pg of morpholino resistant E-cadherin mRNA (see Materials and methods) into the same blastomere at the 8-cell stage (Fig. 6G).

The thickness of the epidermis was compared in control and E-cadherin-depleted embryos at stage 19, when neural fold closure, and the initial spreading movements of the non-neural ectoderm, were complete. Transverse sections showed that the epidermis was significantly thicker than in the controls (Fig. 7A-C) and contained more than the usual two cell layers. These data show that defective spreading is due to failure of the normal movements of the presumptive epidermal cells, and not to overall loss of tissue.

To test whether E-cadherin controls the distribution or quantity of P-MLC, control (Fig. 7D) and E-cadherin-depleted (Fig. 7E) embryos were co-stained with anti-P-MLC (green in Fig. 7) and anti-E-cadherin (red in Fig. 7). Fig. 7E shows that P-MLC levels are controlled by the amount of E-cadherin at the cell surface. Cells lacking E-cadherin (no red staining) have reduced levels of P-MLC

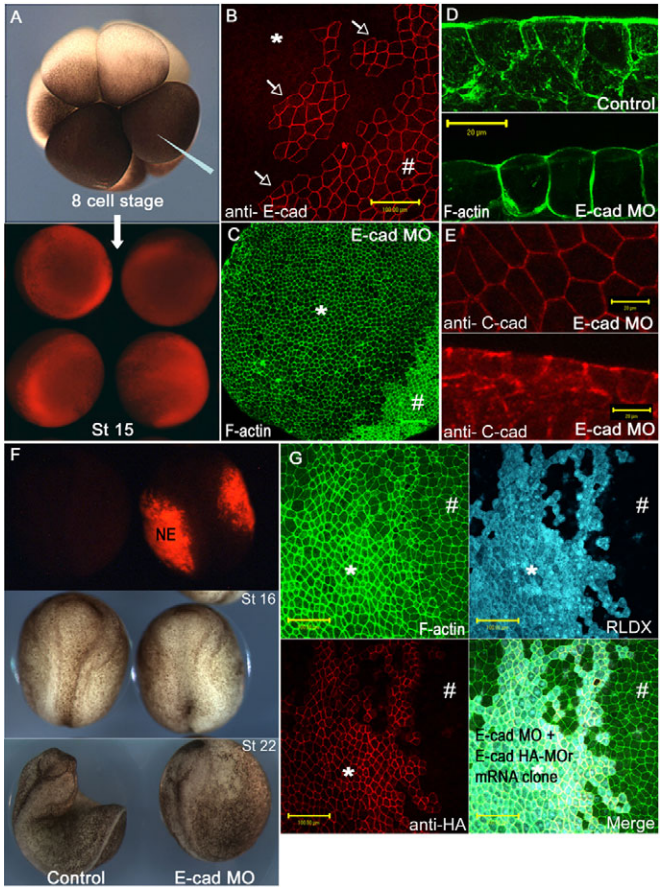

Fig. 6. Depletion of E-cadherin in the ventral (non-neural) ectoderm causes reduction of F-actin. (**A**,**B**) Injection of E-cadherin MO into single ventral animal cells (arrow in A) gives rise to large clones of cells depleted of E-cadherin protein (lower panel in A, B). Arrows in B indicate boundaries between injected cells (*) and uninjected cells (#), which express E-cadherin. (**C**) Reduction of F-actin in E-cadherin-depleted cells (*) as compared with uninjected cells (#). (**D**,**E**) Reduction, but not absence, of F-actin in E-cadherin-depleted cells (D, lower panel), probably because of continued expression of C-cadherin, as shown en face and in cross-section in E. (**F**) Most of the non-neural ectoderm is labeled by injection of both ventral animal cells at the 8-cell stage. The same embryo is shown in the center and lower panels during neural fold closure, which is delayed compared with untreated embryo (left) owing to reduced pushing movements of the non-neural ectoderm (NE). (**G**) Rescue of the cortical actin skeleton by injection of an MO-resistant form of E-cadherin mRNA into the cell injected with E-cadherin MO. The asterisk marks the treated cells; #, adjacent untreated cells. Four images are shown of the same field of view. The RLDX (blue) and anti-HA (red) staining show the expression of the HA-tagged mRNA in the same cells as those injected with the MO (co-injected with RLDX), whereas the Phalloidin staining (green) shows increased assembly of F-actin in the injected cells (compare with C, where actin staining is reduced in the MO-injected cells). Scale bars: 100 μm in B,C,G; 20 μm in D,E.

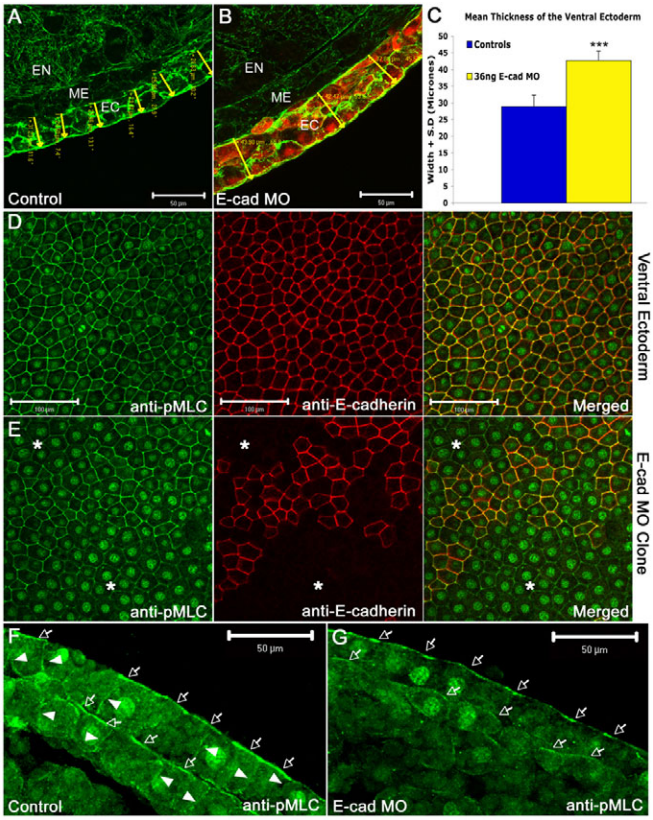

Fig. 7. Depletion of E-cadherin in the non-neural ectoderm leads to increased thickness and number of cell layers in the epidermis, and to reduction of phosphorlyated myosin light chain in the non-neural ectoderm cells. (**A-C**) Transverse sections of control and E-cadherin-depleted epidermis at st. 19. The epidermis is thicker and contains increased numbers of cell layers after E-cadherin depletion. Red, MO-injected cells; green, F-actin; EN, endoderm; ME, mesoderm; EC, ectoderm. Thickness is quantitated in C. ***Statistically significant difference in thickness of ectoderm between control and E-cadherin-depleted ectoderm ($P<0.001$). (**D**,**E**) En face views of the non-neural ectoderm in control (D) *Xenopus* embryos, and in embryos with a large clone of E-cadherin-depleted cells (E). Green, P-MLC; red, E-cadherin. The cells that lack E-cadherin also have reduced levels of P-MLC. Asterisk indicates MO-containing cells. (**F**,**G**) Transverse sections through embryos from the same experiment. P-MLC is distributed on all surfaces of the non-neural ectoderm cells in controls (F), but only remains apically in E-cadherin-depleted cells (G). White arrowheads indicate P-MLC on lateral cell membranes; arrows indicate apical P-MLC. Scale bars: 50 μm in A,B,F,G; 100 μm in D,E.

at the membrane (reduced green staining). However, P-MLC levels are not reduced to zero in E-cadherin-depleted cells. In the neural ectoderm, P-MLC is localized predominantly apically, and is lost after depletion of N-cadherin, which is also apically localized. In the non-neural ectoderm, C-cadherin is apically localized, and remains present when E-cadherin is depleted. We therefore hypothesized that the remaining P-MLC in E-cadherin-depleted epidermis would be apically localized, and controlled by C-cadherin. Fig. 7F,G shows that this is the case. Transverse sections of the E-cadherin-depleted non-neural ectoderm showed that P-MLC normally found laterally at the surface (white arrowheads in Fig. 7E) is missing in E-cadherin-depleted embryos, whereas apical P-MLC (arrows in Fig. 7F,G) is retained apically, making it likely that this is controlled by C-cadherin.

The roles of N- and E-cadherin in actin assembly in the neural and non-neural ectoderm are cadherin-type-specific

Neural and non-neural ectoderm cells express different classical cadherins, which are expressed in different parts of the cell, which undergo different kinds of movement. To ask whether specificity lies

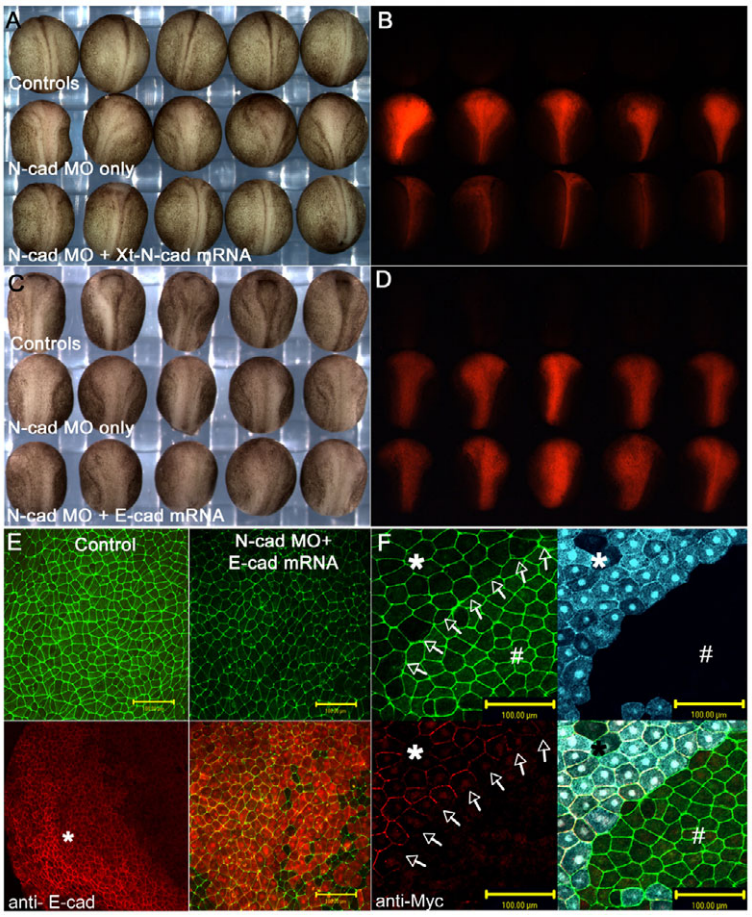

Fig. 8. N- and E-cadherin cannot replace each other in the neural and non-neural ectoderm. (**A**,**B**) The same field of view in brightfield and fluorescence. Injection of N-cadherin MO + RLDX into both dorsal animal cells at the 8-cell stage, thus depleting N-cadherin in most or all of the neural plate, blocks neural fold closure (compare upper row of untreated embryos with middle row of MO-injected embryos). This is rescued by subsequent injection of *X. tropicalis* (MO-resistant) N-cadherin mRNA into the same cells (lower row of embryos). (**C**,**D**) Equivalent pictures to show that injection of E-cadherin mRNA does not rescue neural fold closure. The lower row of embryos has received MO + E-cadherin mRNA. (**E**) A higher magnification field of view to show that F-actin is not rescued by E-cadherin mRNA (compare control to N-cad MO + E-cad mRNA panels), although the E-cadherin protein is expressed (stained red in the cross-section view with anti-E-cadherin antibody). (**F**) Four images of the same en face field of view of a clone of cells (asterisk, blue in right-hand panels) depleted of E-cadherin, and subsequently injected with Myc-tagged N-cadherin mRNA. The N-cadherin protein is expressed (stained red with anti-Myc antibody), but does not rescue the loss of F-actin (green) caused by E-cadherin depletion. The cells along the border between untreated cells (#; which express E-cadherin) and cells in which E-cadherin has been replaced by N-cadherin (arrows), do not express N-cadherin on the cell surface adjacent to E-cadherin-expressing cells. Scale bars: 100 μm.

in the cadherins themselves, or in their different cellular environments, we carried out cadherin exchange experiments by depleting one cadherin and injecting rescuing doses of another. First, we carried out a homotypic rescue by injecting *X. tropicalis* N-cadherin mRNA (50-100 pg/cell), which is not recognized by the MO, into both dorsal animal cells at the 8-cell stage, immediately after injection of 20 ng/cell N-cadherin MO. The embryos were photographed when controls were at the closed neural tube stage (Fig. 8A,B). In N-cadherin-depleted embryos (middle row), the neural plates were still open. This can be seen more easily by the distribution of the fluorescent lineage tracer (Fig. 8B). Injection of the N-cadherin mRNA rescued this effect. In embryos injected with both MO and mRNA, the neural folds had closed (lower row in Fig. 8A,B). Fig. 8C,D show that injection of E-cadherin mRNA did not rescue the effects of N-cadherin depletion. The neural folds remained open in embryos injected with MO alone, or MO+E-cadherin mRNA. Fig. 8E shows that E-cadherin protein was expressed in the neural plate in this experiment but did not rescue the cortical actin skeleton. The reciprocal experiment, in which E-cadherin in the epidermis was replaced by N-cadherin, showed the same result: the thinning movements of the ventral ectoderm were not rescued by the expression of N-cadherin in the ventral ectoderm (see Fig. S2 in the supplementary material). In this experiment, N-cadherin protein was expressed in the epidermis, but did not rescue the loss of cortical actin caused by E-cadherin depletion (Fig. 8F). Interestingly, the cells at the boundaries of cadherin-exchanged clones of epidermal cells, which expressed N-cadherin, but not E-cadherin, did not insert N-cadherin into the membranes that abutted the E-cadherin-expressing cells (arrowed in Fig. 8F), suggesting that assemblies of heterotypic N-E cadherin interactions were prevented. It also implies that cells must receive and interpret information on the type of cadherin being expressed by adjacent cells.

In these mutual rescue experiments, mRNA-only injections showed that E-cadherin in doses of up to 200 pg did not cause any defect in the neural plate, and N-cadherin did not cause any defect in the non-neural ectoderm, of control embryos (data not shown).

In order to exclude the possibility that failure of rescue of one cadherin by the other was due to low translation levels in ectopic locations, we carried out a careful dose range, and quantitated the amount of cadherin protein using cadherin-type-specific antibodies, instead of antibodies against the protein tags. This enabled us to compare the amount of N-cadherin expressed in the ventral ectoderm in rescue experiments with the normal levels found in the neural plate, as well as the same comparison for E-cadherin. The results are shown in Fig. 9. Fig. 9A,B show the degree of cortical actin reduction, measured by pixel intensity after Phalloidin staining, in neural plates injected at the 8-cell stage with N-cadherin MO only, and subsequently injected with E-cadherin mRNA (Fig. 9A), and in non-neuronal ectoderm injected with E-cadherin MO only, and subsequently injected with N-cadherin mRNA (Fig. 9B). In neither case is the cortical actin level rescued by the other cadherin. Fig. 9C,D show the amounts of E-cadherin and N-cadherin proteins, respectively, in the same experiment, compared with the amount of E- and N-cadherins expressed in the endogenous tissues. In each experiment, there is at least as much protein found in the ectopic tissue as is normally found in the endogenous tissue. These data show that failure to rescue is not due to defective translation in the ectopic tissues. To measure protein

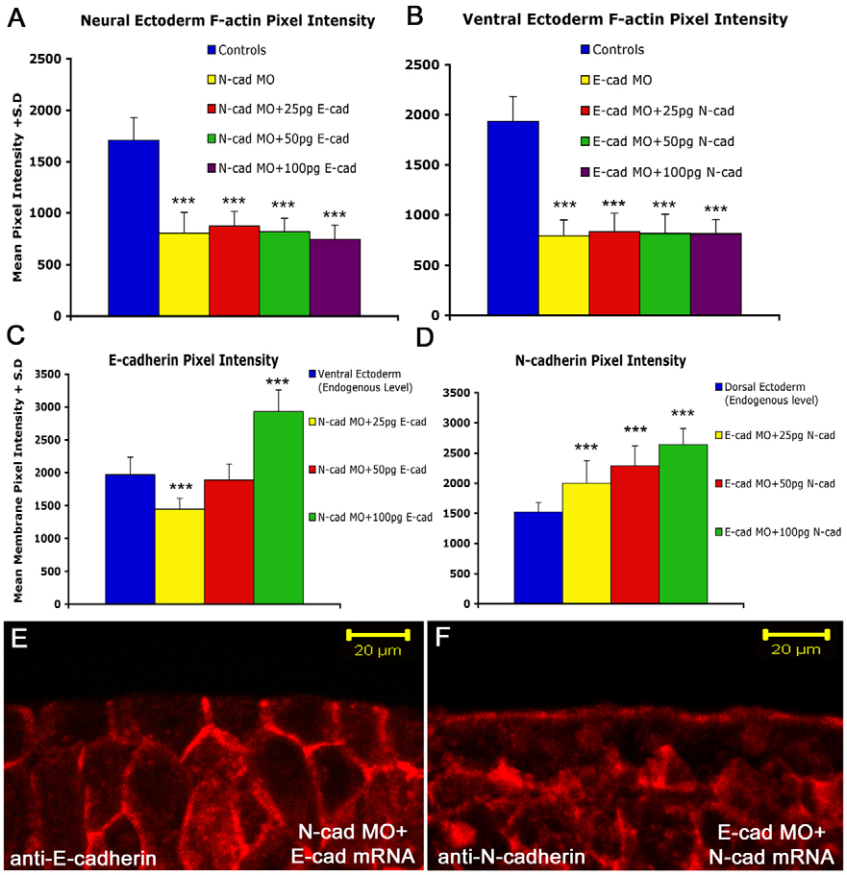

Fig. 9. N- and E-cadherin may not rescue each other because they localize to different regions of the ectodermal cells. (**A**) Levels of cortical F-actin in the neural ectoderm in controls, N-cadherin-depleted and N-cadherin-depleted followed by three different doses of E-cadherin mRNA. Cortical actin is not rescued by any dose of E-cadherin mRNA. (**B**) The converse experiment compares the cortical actin levels in non-neural ectoderm of control, E-cadherin-depleted and N-cadherin depleted followed by three doses of N-cadherin mRNA. Cortical actin is not rescued by any dose of N-cadherin mRNA. (**C**,**D**) The levels of E-cadherin and N-cadherin proteins in *Xenopus* embryos from the same experiment. In each case, translation is efficient and generates at least as much protein as found in the control tissues. ***Statistically significant difference between the control (blue) and labeled bar ($P<0.001$). (**E**,**F**) The location of E-cadherin expressed in N-cadherin-depleted neural ectoderm (E), and N-cadherin expressed in E-cadherin-depleted non-neural ectoderm (F). E-cadherin is expressed basolaterally in the neural ectoderm, whereas N-cadherin is concentrated apically in the non-neural ectoderm. Scale bars: 20 µm.

levels, pixel intensities were assayed using the confocal microscope after antibody staining with E- and N-cadherins, respectively (see Materials and methods).

An alternative possibility for cadherin specificity in cortical actin assembly is segregation of the cadherin proteins to different regions of the cell surface. To test this, we examined transverse sections of neural plates depleted of N-cadherin and 'rescued' with E-cadherin, and non-neural ectoderm depleted of E-cadherin and 'rescued' with N-cadherin. In the neural plate, E-cadherin was found basolaterally, but not apically, localized (Fig. 9E), whereas in the non-neural ectoderm, N-cadherin was expressed predominantly apically (Fig. 9F). In both cases, the failure to rescue could therefore be that the ectopic cadherin did not become localized to the same region of cytoplasm as the endogenous protein. As a control for ectopic N-cadherin staining in the ventral ectoderm, ventral ectoderm injected with E-cadherin MO only did not show any specific staining with the N-cadherin antibody (see Fig. S3 in the supplementary material).

DISCUSSION

During development, and in cancers, changes in expression of cadherins take place, which lead to changes in cell behavior. In the *Drosophila* embryo, DE-cadherin expression is replaced by DN-cadherin in the mesoderm (Oda et al., 1998), and the equivalent change (E- to N-cadherin) takes place during mesoderm formation in the chicken gastrula (Hatta et al., 1987). In each case, a change in cell behavior takes place. During cancer progression, changes in cadherin subtype expression also accompany changes in tumor aggressiveness (Islam et al., 1996; Shimazui et al., 1996). The mechanism by which a cadherin switch mediates changing cell behavior is unknown. In a previous paper, we showed that C-cadherin expressed at the blastula stage is essential for the cortical actin skeleton that controls shape and rigidity of the whole embryo (Tao et al., 2007). Here we show that as cadherin switching takes place during early lineage specification in the *Xenopus* embryo, this function of cadherins in cortical actin assembly is maintained, and controls at least some of the specific properties of motility in these tissues.

Several novel findings are presented here. First, N-, E- and C-cadherins have different distributions in the cell, and the sub-cellular distribution of C-cadherin is tissue-specific. Second, N- and E-cadherin have primary functions, in addition to their roles in cell adhesion, in the control of assembly of the actin skeleton. Neither is essential for intercellular adhesion at the neurula stage, presumably because C-cadherin expression is maintained in both tissues when N- and E-cadherin are depleted. Previous reports have suggested a primary role for cadherins in cell adhesion, based on the effects of dominant-negative constructs, the expression of which caused tissue dissociation (Dufour et al., 1994; Fujimori and Takeichi, 1993; Levine et al., 1994). The most likely explanation of this is that the dominant negative cadherin interferes with the functions of both N- and C-cadherins in the neural plate. Alternatively, C-cadherin could play a primary role in cell adhesion, whereas N-cadherin plays a more specific role in localized actin assembly and tissue movement. This could be tested by depleting C-cadherin and N-cadherin simultaneously. However, we were unable to find a morpholino oligo that efficiently depleted the zygotic synthesis of C-cadherin. Third, the actin assembly controlled by N- and E-cadherin in their respective tissues is essential for the specific morphogenetic movements of these early embryonic tissues (although we have not ruled out the alternative explanation that some other property of N- and E-cadherin, in addition to their roles in F-actin assembly, is required for the morphogenetic

movements affected when they are removed). In the case of N-cadherin, this function is localized to the apical cytoplasm, and its depletion causes loss of the folding movements of the neural plate. It is most likely that this is due to the failure of assembly of specific motor proteins, or their associated signaling molecules, in the apical cytoplasm. This is supported by the fact that the localization of activated myosin light chain in the apical cytoplasm is lost from N-cadherin-depleted neural plate cells. Several actin assembly proteins shown to be required for neurulation movements are also localized apically, including *Xenopus* enabled (Xena), (Roffers-Agarwal et al., 2008), vinculin (Xu et al., 1998), Mena/VASP (Lanier et al., 1999; Menzies et al., 2004), Shroom (Hildebrand and Soriano, 1999), Marcks (Stumpo et al., 1995) and Abl (Koleske et al., 1998). Planar cell polarity proteins required for neurulation are also apically localized, including Strabismus/van gogh (Darken et al., 2002; Kibar et al., 2001; Ybot-Gonzalez et al., 2007), Celsr1/flamingo (Curtin et al., 2003) and disheveled (Wallingford et al., 2002; Wallingford and Harland, 2001). It will be important to show whether N-cadherin is required for localization of these important components, in addition to activated myosin light chain.

It is interesting to compare the results seen here, in which N-cadherin has been targeted in the *Xenopus* neural plate, with results of null N-cadherin mutations in the mouse embryo (Radice et al., 1997). In these embryos, initial development of the neural tube is relatively normal, resulting in closure of the neural folds and a radial arrangement of cells in transverse sections. However, the neural tube became undulated later on, possibly owing to defects in surrounding structures, including the somites, and the embryos die shortly after this due to cardiac defects. It is possible that other cadherins can substitute for N-cadherin in the mouse (Radice et al., 1997).

Little is known of the mechanism underlying the spreading movements of the non-neural ectoderm. Data presented here show that actin assembly on E-cadherin is absolutely required for this process. As E-cadherin is not expressed apically in the non-neural ectoderm, we would expect to find motor proteins associated with E-cadherin accumulated on lateral and basal membranes, and this will be the basis of a future study. It is clear from E-cadherin-depleted embryos that spreading movements of the non-neural ectoderm play a role in closing the neural tube in the trunk region. This supports previously published data (Colas and Schoenwolf, 2001; Lawson et al., 2001; Sausedo et al., 1997).

It is particularly interesting to note the degree of specificity in actin-based functions of N- and E-cadherin. Different kinds of tissue movement are affected by their individual depletions. One possibility is that each protein is localized to a different region in the cell. In the neural plate, N-cadherin is localized apically, whereas in the non-neural ectoderm, E-cadherin is expressed basolaterally. Furthermore, expression of E-cadherin mRNA in N-cadherin-depleted neural plates resulted in the E-cadherin protein occupying a basolateral position in neural plate cells, i.e. it occupied the position it would have occupied in its normal tissue. The same proved to be true of N-cadherin expressed in non-neural ectoderm depleted of E-cadherin. It is likely, therefore, that localization mechanism is an important factor in functional specificity of cadherins in these tissues. However, localization might be only one functional difference. Alternatives could be due to differences in availability of actin assembly proteins, motor proteins or signals that activate these in the two tissues. Future studies will address these issues.

In conclusion, this work has identified a major function of cadherins in actin assembly during morphogenesis, and shown that tissue-restricted expression of cadherins is an essential component of the mechanism leading to different types of morphogenetic tissue movements. There are many examples of cadherin-expressing tissues undergoing morphogenetic movements, including branching morphogenesis of exocrine glands, the lungs and the kidneys. It will be extremely interesting to see the extent to which the cadherins expressed in these tissues are required for their specific patterns and extents of tissue movement.

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Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/136/8/1327/DC1

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