Selective disruption of E-cadherin function in early Xenopus embryos by a dominant negative mutant

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SUMMARY

E-cadherin function was disrupted in vivo in developing Xenopus laevis embryos through the expression of a mutant E-cadherin protein lacking its cytoplasmic tail. This truncated form of E-cadherin was designed to act as a dominant negative mutant by competing with the extracellular interactions of wild-type endogenous E-cadherin. Expression of truncated E-cadherin in the early embryo causes lesions to develop in the ectoderm during gastrulation. In contrast, expression of a similarly truncated N-cadherin protein failed to cause the lesions. The ectodermal defect caused by the truncated E-cadherin is rescued by overexpression of wild-type E-cadherin, by co-injection of full-length E-cadherin RNA along with the RNA for the truncated form. Overexpression of full-length C-cadherin, however, is unable to compensate for the disruption of E-cadherin function and can actually cause similar ectodermal lesions when injected alone, suggesting that there is a specific requirement for E-cadherin. Therefore, E-cadherin seems to be specifically required for maintaining the integrity of the ectoderm during gastrulation Xenopus embryo. Differential cadherin expression reflects, therefore, the requirement for distinct adhesive properties during different morphogenetic cell behaviors.

Key words: ectoderm, gastrulation, cell adhesion, Xenopus, E-cadherin

INTRODUCTION

The regulation of cell-cell adhesion is critical for many aspects of development. Appropriate cell contacts are necessary for communication between cells, for cell movements, and for the segregation of groups of cells to form germ layers, tissues and organs. An understanding of the roles of cell adhesion molecules is therefore important in analyzing developmental processes.

The cadherins are a family of Ca²⁺-dependent, homophilic cell-cell adhesion molecules (Takeichi, 1990). Their structure consists of a single transmembrane domain linking a large extracellular domain responsible for binding and recognition between cadherins on a neighboring cell, and a highly conserved cytoplasmic tail responsible for binding proteins known as catenins, which are thought to link cadherins to the actin cytoskeleton (Ozawa et al., 1989, 1990; Hirano et al., 1987). The cytoplasmic tail is required for functional cell adhesion (Ozawa et al., 1989; Nagafuchi and Takeichi, 1988). Different members of the cadherin family have different spatial and temporal expression patterns during embryonic development and in the adult, and changes in expression have often been correlated with morphogenetic events (Takeichi, 1988, 1991; Damjanov et al., 1986; Vestweber et al., 1987; Thiery et al., 1984; Linask, 1992; Pouliot et al., 1990; Levi et al., 1991).

The major cadherins expressed in early embryos of Xenopus laevis are E-cadherin, N-cadherin (Detrick et al., 1990) and a maternal cadherin known as either C-cadherin or EP-cadherin (Choi et al., 1990; McCrea et al., 1993; Ginsberg et al., 1991). U-cadherin (Angres et al., 1991) has properties and an expression pattern similar to C-cadherin, but its molecular relationship to C-cadherin is not clear. C-cadherin is maternally encoded, expressed in oocytes, eggs and through the early stages of development when the embryo is rapidly cleaving and surviving on maternal stores. E-cadherin expression starts when embryonic transcription begins just before gastrulation, a time of complex cell movements, which transform a hollow ball of cells into a three-layered structure, establishing the three germ layers. At these early stages E-cadherin is expressed only in the ectoderm, not in the mesoderm or endoderm (Choi and Gumbiner, 1989; Levi et al., 1991). N-cadherin expression appears as the neural tube begins to form, in restricted areas of ectoderm and mesoderm. In the neural plate, N-cadherin expression appears simultaneously with a decrease in expression of E-cadherin (Detrick et al., 1990).

The distinctive expression patterns of cadherins have led to
suggestions that they play important roles in development. One theory about the function of cadherins in development is that they are involved in selective cell adhesion, resulting in the sorting out of cell types in a developing organism (Takeichi, 1990). Tissue culture cell lines each transfected with a different cadherin will sort out from each other (Nose et al., 1988; Friedlander et al., 1989); there is evidence that a similar sorting out may occur in the formation of the neural tube (Detrick et al., 1990; Fujimori et al., 1990). Another theory about the function of cadherins in development is that different members of the family may exhibit different adhesive properties, which might permit or prevent certain cell movements (Gumbiner and Simons, 1987).

While cadherins clearly have the potential to mediate various developmental processes, less is known of their actual function in an embryo. In order to address the question of the function of E-cadherin during early development in Xenopus, a truncated form of E-cadherin lacking the cytoplasmic domain was constructed to disrupt function in a dominant negative fashion. Although the cytoplasmic tail is essential for cell-cell adhesion (Ozawa et al., 1989; Nagafuchi and Takeichi, 1988), there is evidence that the extracellular domain alone is able to interact with normal cadherins on the cell surface, and to disrupt adhesion in vitro (Wheelock et al., 1987). It was therefore predicted that the truncated form of E-cadherin, if present in high amounts in embryos, could disrupt E-cadherin-mediated adhesion in vivo, yielding information about the roles of E-cadherin during development.

MATERIALS AND METHODS

cDNA cloning, plasmids and RNA
A cDNA clone encoding Xenopus E-cadherin was isolated from a λgt10 stage 17 (early neurulae) cDNA library (Kintner and Melton, 1987) by probing with a portion of the chicken N-cadherin cDNA under conditions of low stringency hybridization (Hatta et al., 1988). Positive phage clones were placed into groups by cross hybridization, and those groups corresponding to Xenopus N-cadherin and C-cadherin were identified by sequence analysis. One remaining group consisted of cDNA clones encoding E-cadherin. The E-cadherin cDNA was inserted into the 64T vector under the SP6 promoter for in vitro transcription of RNA (64T12C). This plasmid also contains β-globin 5′ and 3′ sequences for efficient translation in Xenopus embryos.

The dominant negative form of E-cadherin (Etrunc) was constructed using PCR to insert two consecutive stop codons and an EcorV site immediately following the predicted transmembrane sequence; a 370 bp fragment extending upstream to a BglII site was amplified and cloned back into the original vector (64T12C) containing a deletion extending from the BglII site to an EcoRV site in the 3′ untranslated region (Fig. 1). This results in a deletion of the E-cadherin sequence consisting of the entire cytoplasmic domain and about 500 b of 3′ untranslated sequence. The amplified region was sequenced using the Sequenase kit (USB) and found to contain only one point mutation which conserved the amino acid sequence. To make the N-cadherin truncation mutant, a similar mutation was introduced into the Xenopus N-cadherin cDNA using PCR.

The Xenopus C-cadherin was cloned and sequenced by Chung-Hyun Lee and B. Gumbiner (unpublished data); comparison of nucleotide sequences showed that it is virtually identical to EP-cadherin (Ginsberg et al., 1991), with only 4 amino acid differences between the two proteins. The C-cadherin cDNA was inserted into a modified 64T vector from Enrique Amaya (pSP36T) for synthesizing mRNA in vitro using an SP6 promoter.

RNA for injections into embryos was in vitro transcribed using the MEGAscript kit (Ambion) and quantitated using an ethidium bromide spot test, absorbance at 260 nm, or incorporation of trace radiolabelled ribonucleotides before being diluted to appropriate concentrations in DEPC-treated water.

Handling of Xenopus eggs and embryos
Production of eggs, fertilization and removal of the jelly coat were done as described (Newport and Kirschner, 1982). Injections were performed (using a Xenopus oocyte injector from Sutter Instruments) in 1× MMR (0.1 M NaCl, 2 mM KCl, 1 mM MgSO4, 2 mM CaCl2, 5 mM Heps pH 7.8, 0.1 mM EDTA) containing 6% Ficol and penicillin/streptomycin. After several hours of incubation to allow healing, the embryos were transferred to 0.1× MMR+ 6% Ficol and usually incubated overnight at 14°C. Embryos were staged according to the normal table of Xenopus laevis development (Nieuwkoop and Faber, 1967).

Preparation of samples and immunoblotting
Embryos were extracted in 1% NP-40 in solution A (10 mM Heps pH 7.4, 150 mM NaCl, 1.5 mM EDTA) supplemented with protease inhibitors (1 mM PMSF, 0.5 mM iodoacetamide, 1 µg/ml pepstatin A, 2 µg/ml leupeptin, 4 µg/ml aprotinin, 10 µg/ml antipain, 50 µg/ml benzamidine, 10 µg/ml soybean trypsin inhibitor, 1 mM EGTA) as described (Choi and Gumbiner, 1989) but modified for a smaller number of embryos. Between 1 and 10 embryos were homogenized in 40 µl extraction buffer by repeated pipetting. After centrifugation at 13,000 revs/minute for 25 minutes at 4°C in a microcentrifuge, the supernatant was removed from the insoluble pellet and from the lipid material on the surface using a gel-loading pipet tip, and quickly frozen on dry ice before storage at −20°C. Proteins were separated on 8% SDS-polyacrylamide gels, electrophoretically transferred to nitrocellulose and immunoblotted using standard procedures. Blocking was carried out in PBS with 5% milk and 0.2% Triton X-100. To detect E-cadherin, the primary antibody was a mixture of four monoclonal antibodies against Xenopus E-cadherin (19A2, 5D3, 31D2, 8C2; Choi and Gumbiner, 1989). To detect C-cadherin, the primary antibody was a mixture of two monoclonal antibodies against Xenopus C-cadherin (5D5 and 6B6; McCrea et al., 1993). The secondary antibodies were horseradish peroxidase-conjugated goat anti-mouse IgG, detected using an enhanced chemiluminescence kit (ECL; Amersham).

Cell surface labelling
Cell surface labelling of embryos with biotin was carried out as described (Choi et al., 1990). Labeling was done on ice for 30 minutes in 1 mg/ml NHS-LC-Biotin (sulfosuccinimidy l-6-(biotinamido) hexanoate; Pierce Chemical Co.) in either 0.1× MMR or 0.1× CMFM (Ca2+, Mg2+-free MMR). Labeling was quenched by rinsing embryos three times in 10 mM glycine (in 0.1× MMR). To open the tight junctions and allow access of the labeling reagent to the internal basolateral cell surfaces, one set of embryos was preincubated in 0.1× CMFM for 1 hour at room temperature. As a control, another set of embryos was lysed directly into 1 mg/ml NHS-LC-biotin in 0.1× CMFM by repeated pipetting. The glycine solution was then added (rinsing was not possible). Embryos were extracted as above. One-
Disruption of Xenopus E-cadherin

half of each sample was immunoprecipitated by incubating with a mixture of two monoclonal antibodies to E-cadherin (19A2 and 5D3; Choi and Gumbiner, 1989) followed by precipitation with protein A-agarose. The other half was precipitated with avidin-agarose. SDS-PAGE and electrotransfer to nitrocellulose were done as described previously. The immunoprecipitated samples were blotted with avidin and biotin-conjugated horseradish peroxidase (Vectorstain kit). Immunoblotting was done as above. In both cases proteins were detected using the ECL system (Amersham).

Histology

Embryos were fixed, stained for β-galactosidase, embedded in paraffin, sectioned and counterstained with hemotoxylin-eosin as described previously (Detrick et al., 1990).

RESULTS

Cloning and sequence of Xenopus E-cadherin cDNA

The cDNA clone encoding Xenopus E-cadherin was isolated from a λgt10 stage 17 (early neurulae) cDNA library (Kintner and Melton, 1987) by probing with a portion of the chicken N-cadherin cDNA under conditions of low stringency hybridization (Hatta et al., 1988). Positive phage clones were placed into groups by cross hybridization, and those groups corresponding to Xenopus N-cadherin and C-cadherin were identified by sequence analysis. One remaining group consisted of cDNA clones encoding E-cadherin based on several criteria. First, RNA encoded by these cDNAs first appear in Xenopus embryos at the beginning of gastrulation where it is expressed in ectoderm undergoing epidermogenesis, in agreement with the antibody staining pattern reported for E-cadherin antibodies (Choi and Gumbiner, 1989). Second, embryos injected with synthetic RNA transcripts produced from these cDNAs and analyzed by western analysis before gastrulation were found to express a polypeptide recognized by the E-cadherin antibodies (Fig. 3A).

The nucleotide sequence for the coding region of one E-cadherin cDNA was determined and the corresponding amino acid sequence was aligned to Xenopus N-cadherin and C-cadherin as shown (Fig. 2). The alignment shows that E-cadherin shares 60% amino acid identity with Xenopus C-cadherin and 40% identity with Xenopus N-cadherin. In addition, the amino acid sequence of Xenopus E-cadherin is slightly more related to that of mouse E-cadherin (59% identity) than to that of mouse P-cadherin (53% identity). The results of these alignments indicated that Xenopus E-cadherin, like Xenopus C-cadherin, belongs to a subgroup of the cadherin family that is related to both E-cadherin and P-cadherin in higher vertebrates. These cadherins, however, are markedly more divergent compared to N-cadherin from Xenopus, mouse and chick, which share 85% amino acid identity.

Expression of the truncated E-cadherin in Xenopus embryos

In vitro transcribed RNA (2 ng) for the truncated E-cadherin (Fig. 1) was injected into both cells of embryos at the 2-cell

Fig. 2. Amino acid sequence of Xenopus E-cadherin aligned with the sequences of Xenopus C-cadherin (EP-cadherin) and Xenopus N-cadherin. Xenopus E-cadherin shares 60% amino acid identity with Xenopus C-cadherin and 40% identity with Xenopus N-cadherin. Xenopus E-cadherin shares 59% identity with mouse E-cadherin and 53% identity with mouse P-cadherin. Sig, signal sequence; Pre, precursor portion; EC1-EC5, cadherin extracellular domains; TM, transmembrane domain; CYTO, cytoplasmic domain.
stage. Western blotting of embryos extracted at various stages demonstrates that the injected RNA is translated into protein, detectable as early as half an hour after injection (Fig. 3A). At early times both the precursor and proteolytically processed sized polypeptides are detected, but like the endogenous E-cadherin, the processed form accumulates with time. The amount of protein increases so that at the time endogenous E-cadherin is detectable (early gastrulation), the level of the truncated form is much higher than the endogenous form; this level is maintained at least through late neurulation.

Cell surface localization of the truncated E-cadherin was analyzed by labeling with the membrane impermeable protein biotinylating reagent, NHS-LC-biotin (Fig. 4). Embryos were labeled at stage 11, before endogenous full-length E-cadherin reaches detectable levels, either with or without opening the tight junctions by incubation in Ca\(^{2+}\)-free medium. Embryos were then extracted with detergent solutions and samples were either precipitated with avidin and blotted with antibody to E-cadherin (Fig. 4A), or precipitated with antibody to E-cadherin and blotted with avidin (Fig. 4B). Labeling of the truncated E-cadherin was detected using both procedures and increased amounts were observed when the junctions were opened, allowing access to the basolateral membrane. This demonstrates that the truncated E-cadherin is expressed on the surface of embryos, and that some of it is present on the basolateral surfaces between blastomeres. As a control, embryos were intentionally lysed in the labelling medium (lane 3 in both Fig. 4A,B); this did not result in detectable biotinylation of the truncated E-cadherin, presumably due to the excess of cytoplasmic proteins. Therefore, the labelling observed was not due to labelling of intracellular E-cadherin or to artifactual labeling after cell lysis.

**Effect of expressing the truncated E-cadherin on development**

Embryos injected with the truncated E-cadherin RNA show no observable effects until gastrulation, when they exhibit a specific defect not seen with injections of a control β-galactosidase RNA (Fig. 5). The embryos develop rips in the ectoderm at around mid-gastrulation; holes appear in the pigmented outer layer of the animal hemisphere. There is a range of severity of this phenotype; those embryos with only small disruptions continue development, usually retaining a small patch where unpigmented inner cells are visible. Embryos that lose the integrity of large areas of ectoderm are unable to complete gastrulation. When RNA is injected into both cells at the 2-cell stage, in order to yield widespread expression, this phenotype is observed with 2 ng of RNA injected into each embryo, but not with lower amounts. In five separate experiments under these conditions, of 139 embryos injected, 67% developed this lesion (Table 1). Since the defects occur in the ectoderm, injections were then performed into one animal cell at the 8-cell stage to concentrate expression in a more restricted area of the ectoderm. Under these conditions the lesions are observed with 1 ng RNA. Of 102 embryos injected in three separate experiments, 78% showed the defects (Table 1). The lesions occur around the injection site, presumably where the highest levels of the truncated form are expressed. This was demonstrated by co-injecting the RNA with fast green dye into albino embryos; the lesions colocalize with the green color (data not shown).

To analyze the defects caused by the truncated E-cadherin on a more detailed level, embryos exhibiting the lesions were fixed and sectioned (Fig. 6B). The embryos were co-injected with β-galactosidase mRNA to identify the region of high expression by histochemical staining. The disruption in the outer ectodermal layer is apparent, with cells becoming entirely separated from one another (compare to control with β-galactosidase mRNA alone, Fig. 6A). There is also a disturbance of the underlying cells, but it is not clear if this is a direct result of expression of the truncated E-cadherin or an indirect result of the lack of an occluding epithelial layer above.

Expression of the truncated form of E-cadherin in *Xenopus* embryos therefore seems to result in a defect in the ectoderm during gastrulation, consistent with the hypothesis that it specifically disrupts E-cadherin mediated cell-cell adhesion in the ectoderm.

**Rescue of the defect with wild-type E-cadherin**

The truncated E-cadherin is postulated to bind the extracellular domain of E-cadherin on a more detailed level, embryos exhibiting the lesions were fixed and sectioned (Fig. 6B). The embryos were co-injected with β-galactosidase RNA to identify the region of high expression by histochemical staining. The disruption in the outer ectodermal layer is apparent, with cells becoming entirely separated from one another (compare to control with β-galactosidase mRNA alone, Fig. 6A). There is also a disturbance of the underlying cells, but it is not clear if this is a direct result of expression of the truncated E-cadherin or an indirect result of the lack of an occluding epithelial layer above.

Expression of the truncated form of E-cadherin in *Xenopus* embryos therefore seems to result in a defect in the ectoderm during gastrulation, consistent with the hypothesis that it specifically disrupts E-cadherin mediated cell-cell adhesion in the ectoderm.
to have a distinct effect on the embryo. To test the selectivity of expression of a different cadherin with the same deletion ought cadherin disrupts endogenous E-cadherin function selectively, other cadherins expressed in the embryo. If the truncated E-interfere selectively with endogenous E-cadherin relative to mechanism, the truncated E-cadherin mutant is postulated to Cadherin selectivity of dominant negative effects (Table 2).

those injected with the truncated form alone; 55% showed no 123 embryos were co-injected, none showed large lesions like ectoderm. In three different experiments, in which a total of rips; some display a small, very contained disruption in the latter. None of the co-injected embryos develop substantial deficit observed, it should be possible to rescue the phenotype by increasing the amount of expression of full-length E-cadherin. Embryos were co-injected with 1 ng truncated E-cadherin RNA and 1 ng full-length E-cadherin RNA, into 1 animal cell of 8-cell embryos. Injections of the full-length E-cadherin RNA alone had no obvious effects on the embryos, when observed intact (data not shown). Comparison of the embryos that received truncated E-cadherin alone (Fig. 7A) and those that received both truncated and full-length E-cadherin (Fig. 7B) reveals a dramatic rescue of the lesion in the latter. None of the co-injected embryos develop substantial lesions like those injected with the truncated form alone; 55% showed no surface defects at all and 45% exhibited a small residual lesion (Table 2).

Cadherin selectivity of dominant negative effects
Because cadherins interact by a homophilic binding mechanism, the truncated E-cadherin mutant is postulated to interfere selectively with endogenous E-cadherin relative to other cadherins expressed in the embryo. If the truncated E-cadherin disrupts endogenous E-cadherin function selectively, expression of a different cadherin with the same deletion ought to have a distinct effect on the embryo. To test the selectivity of inhibition, the same deletion was introduced into N-cadherin, which was then expressed in the early embryo by RNA injection. Introduction of this mRNA resulted in the expression of large amounts of a polypeptide recognized by the N-cadherin antiserum, but, as expected, smaller than the endogenous N-cadherin polypeptide (data not shown). In contrast to truncated E-cadherin, expression of truncated N-cadherin did not result in ectodermal rips during gastrulation (Fig. 6C). This mutant did, however, interfere with normal neural tube development (Fig. 6D), probably by interfering with endogenous N-cadherin function in the developing neural tube. Thus, the two cadherin truncation mutants exhibit selective disruptive effects on the embryo, which are consistent with their acting as dominant negative mutants via a homophilic binding mechanism.

Lack of rescue by C-cadherin
The ability of increased levels of full-length E-cadherin to compensate for the presence of the truncated E-cadherin and rescue the lesions implies that the truncated E-cadherin causes its effects through a disruption of normal E-cadherin function, which appears to be required in the ectoderm during gastrulation. The question then arises whether this requirement is specifically for E-cadherin, or simply for an adequate amount of an adhesion molecule in the ectoderm. C-cadherin is also present in the ectoderm at this stage of development (Ginsberg et al., 1991; Fagotto and Gumbiner, unpublished data), so the endogenous level of C-cadherin is unable to compensate for the disruption in E-cadherin function.

In order to determine whether increased levels of another cadherin could satisfy the adhesive requirement of the ectoderm during gastrulation, C-cadherin RNA was co-injected with the truncated E-cadherin RNA. Western analysis of embryos injected into one animal blastomere at the 8-cell stage with 2 ng of C-cadherin RNA, either alone or in combination with 1 ng of truncated E-cadherin RNA, demonstrated that the injected C-cadherin RNA is translated to yield a level of protein substantially higher than endogenous protein during gastrulation (Fig. 3B). The presence of increased levels of C-cadherin is unable to rescue the defects caused by the truncated E-cadherin. In fact, injections of full-length C-cadherin RNA alone result in ectodermal lesions similar to those produced by the truncated E-cadherin (Table 3), suggesting that increased expression of normal full-length C-cadherin can interfere with endogenous E-cadherin.

**DISCUSSION**

The experiments presented provide evidence for the experimental disruption of E-cadherin function in *Xenopus* embryos by overexpression of a truncated form of E-cadherin. The truncated E-cadherin mutant is postulated to act in a dominant negative fashion. The dominant negative approach has previously been used successfully in *Xenopus* (Amaya et al., 1991; Kintner, 1992). It is particularly useful in studying the function of specific molecules in *Xenopus* development, where the embryological data are extensive but genetic manipulations are very difficult. The truncated form of E-cadherin used in the present experiments contains the entire extracellular and transmembrane domains, but lacks the cytoplasmic tail which is

![Fig. 4](image-url)
required for functional activity. Since the extracellular domain is responsible for recognition and homophilic binding to another molecule of E-cadherin on the surface of the neighboring cell, it is postulated that the truncated form binds to endogenous E-cadherin, competing with the normal interaction between two full-length E-cadherin molecules. If the concentration of the truncated form is sufficiently higher than that of the full-length E-cadherin, a substantial proportion of the complexes formed will contain the truncated form.

Previous evidence suggests that this truncated E-cadherin protein is likely to have at least a partially functional extracellular domain capable of interacting with other E-cadherin molecules, but an inability to mediate adhesion. E-cadherin lacking a cytoplasmic tail fails to mediate adhesion between tissue culture cells, unlike the full-length molecule (Nagafuchi and Takeichi, 1988; Ozawa et al., 1990). However, cytoplasmic tail deletion mutants of E-cadherin were found to be expressed on the cell surface when transfected into L cells and to have a normal extracellular domain as measured by resistance to trypsin in the presence of Ca^{2+}. Also, purified N-cadherin absorbed on plastic serves as a substratum for N-

<table>
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<th>Exp.</th>
<th>No. embryos</th>
<th>No. rips</th>
<th>% rips</th>
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</tr>
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<td>Uninjected (total)</td>
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Experiments 1-5: 1 ng RNA injected into each cell of a 2-cell embryo (2 ng total).
Experiments 6-8: 1 ng RNA injected into one cell of an 8-cell embryo (1 ng total).

Fig. 5. Effect of truncated E-cadherin on gastrulating embryos. (A) Stage 11.5 embryos injected with 2 ng β-galactosidase RNA at the 2-cell stage. (B) Stage 11.5 embryos injected with 2 ng truncated E-cadherin RNA at the 2-cell stage.

Fig. 6. Histology of defect in embryos injected with truncated E-cadherin or truncated N-cadherin mRNAs. All embryos were co-injected with RNA encoding β-galactosidase and sections were stained for β-galactosidase activity (blue). (A) Injection of β-galactosidase RNA alone, section through ectoderm of gastrula stage embryo. (B) Injection of truncated E-cadherin RNA, section through ectoderm of gastrula stage embryo. (C) Injection of truncated N-cadherin RNA, section through ectoderm of gastrula stage embryo. (D) Injection of truncated N-cadherin RNA, section through neural tube of neurula stage embryo.
Disruption of *Xenopus* E-cadherin (Bixby and Zhang, 1990) demonstrating that cytoplasmic interactions are not essential for extracellular binding. Furthermore, the soluble extracellular domain of cell-CAM 120/80, the human homologue of E-cadherin, was found to disrupt cell-cell adhesion when present in the culture medium of epithelial cells, providing evidence that the extracellular domain alone can interact with other cadherin molecules (Wheelock et al., 1987). This experiment is analogous to those presented here, demonstrating that an excess of a truncated cadherin lacking the cytoplasmic tail can disrupt cadherin-mediated adhesion, presumably by competing with the interactions between normal cadherin molecules.

Since the cadherins seem to interact primarily by a homophilic mechanism (Nose et al., 1988), and the binding specificity is localized in the extracellular domain (Nose et al., 1990), the truncated E-cadherin was expected to interact only with E-cadherin, and not to interfere with the functioning of the other cadherins. Our findings are consistent with homophilic specificity. The truncated E-cadherin causes no defects until mid-gastrulation despite the presence of C-cadherin throughout the embryo during early development. Even when the truncated form is injected into both cells of 2-cell embryos in order to generate expression in all three germ layers, the defects still appear to be localized to the ectoderm. Also, in embryos that managed to develop to tadpole stage no obvious defects were observed in neural tube formation, although N-cadherin is expressed there and the levels of the truncated E-cadherin protein remain high through neurulation. Furthermore, overexpression of a similar truncated N-cadherin molecule failed to cause the ectodermal lesion even though it did interfere with neural tube formation. Thus, expression of a mutant cadherin lacking its cytoplasmic domain seems to interfere selectively with the function of the normal endogenous cadherin by a homophilic mechanism. Therefore, the ectodermal disruptions produced by the truncated E-cadherin are very likely to be due to selective interference with E-cadherin function in the early embryo.

The apparent specificity of the disruption for E-cadherin can be contrasted to the results obtained by expression in *Xenopus* of a dominant negative cadherin construct consisting of the highly conserved cytoplasmic tail of N-cadherin and the transmembrane domain, but lacking almost all of the extracellular domain (Kintner, 1992). Expression of this molecule in

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**Table 2. Rescue of effect of truncated E-cadherin by overexpression of full-length E-cadherin**

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<th>RNA injected</th>
<th>No. embryos</th>
<th>No. rips</th>
<th>No. residual rips</th>
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<td></td>
</tr>
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<tr>
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<tr>
<td>Total</td>
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<td>55 (45%)</td>
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Experiments 1-2: 1 ng truncated E-cadherin RNA + 1 ng full-length E-cadherin RNA, into one cell of an 8-cell embryo.

Experiment 3: 2 ng truncated E-cadherin RNA + 1 ng full-length E-cadherin RNA, into one cell of an 8-cell embryo.

Rips are defined as large gaps in the pigmented ectoderm surface layer of cells of the embryo, revealing the underlying unpigmented cell layers as shown in figures 5B and 7A.

Residual rips are defined as the very small discrete spots that remain on the surface of the embryos (indicated by white arrows in figure 7B) at the sites of microinjection, but do not form gaps in the pigmented ectodermal surface layer.

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**Table 3. Effect of ectopic expression of C-cadherin in embryos**

<table>
<thead>
<tr>
<th>RNA injected</th>
<th>No. embryos</th>
<th>No. rips</th>
<th>% rips</th>
</tr>
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<td>3</td>
<td>28</td>
<td>24</td>
<td>86</td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
<td>55</td>
<td>53</td>
</tr>
</tbody>
</table>

Experiments 1-2: 2 ng RNA into one cell of an 8-cell embryo.

Experiment 3: 1.5 ng RNA into one cell of an 8-cell embryo.
embryos resulted in a much more widespread disruption of almost all cell contacts in the early embryo. The embryos developed ectodermal lesions after the internal cells dissociated. Moreover, this mutant completely stopped gastrulation movements. Since the effects occur before N-cadherin is expressed, they must be caused by disruption of other cadherins, and most likely by disruption of both E- and C-cadherin. It is an intriguing possibility that disruption of E-cadherin by this mutant is responsible for the ectodermal lesion, as observed here, and that its disruption of C-cadherin causes internal cell dissociation.

The disruption of many different cadherins by overexpression of the tail is thought to occur by competition for the cytoplasmic factors that interact with the cytoplasmic tail and are required for functional cell-cell adhesion. A similar competition could account for the disruption of the ectoderm by overexpression of C-cadherin. High levels of C-cadherin could compete for cytoplasmic factors without providing adequate adhesive properties. A very similar ectodermal disruption has also been observed with ectopic expression of N-cadherin (Detrick et al., 1990; Kintner, 1992). The hypothesis, originally suggested with respect to ectopic N-cadherin (Detrick et al., 1990), is that the other cadherins can compete with the endogenous E-cadherin for factors binding to the tail, presumably in a similar manner to overexpression of the tail domain alone. This is supported by the finding that the cytoplasmic tail without the extracellular domain disrupts cadherin-mediated adhesion of transfected cells (Fujimori and Takeichi, 1993).

It is striking that increased expression of another cadherin in the ectoderm is not able to compensate for the disruption of endogeneous E-cadherin by the truncated E-cadherin, but instead causes a disruption of cell-cell adhesion. This strongly suggests that there is a specific requirement for E-cadherin on ectodermal cells during the process of epiboly, in order to maintain the integrity of the ectoderm. Epiboly is characterized by spreading of the cells as a sheet over the outside of the embryo, presumably involving force on the cells and the necessity of strong intercellular adhesion in order to maintain an epithelial sheet. E-cadherin expression in many systems correlates with stationary groups of cells which strongly adhere to each other and have a restriction of cell movements with respect to each other. C-cadherin, on the other hand, is expressed on cells undergoing convergence and extension during gastrulation, which involves extensive cell rearrangements. The present results provide in vivo evidence that the different cadherins mediate different adhesive qualities; it appears that E-cadherin-mediated adhesion is required for ectodermal integrity during epiboly, and simply increasing the amount of other adhesive molecules is not sufficient. Cadherins are apparently not interchangeable molecules. Although differential cadherin expression may be important for sorting out different cell types from each other, it also appears to reflect the need for distinct adhesive properties during different morphogenetic behaviors.

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REFERENCES


Disruption of Xenopus E-cadherin


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