Regulation of C-Cadherin Function during Activin Induced Morphogenesis of *Xenopus* Animal Caps

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**Abstract.** Treatment of *Xenopus* animal pole tissue with activin results in the induction of mesodermal cell types and a dramatic elongation of the tissue. The morphogenetic movements involved in the elongation appear similar to those in normal gastrulation, which is driven by cell rearrangement and cell intercalations. We have used this system to explore the potential regulation of cell–cell adhesion and cadherin function during morphogenesis.

Quantitative blastomere aggregation assays revealed that activin induction reduced the calcium-dependent adhesion between blastomeres. Activin-induced blastomeres formed smaller aggregates, and a greater proportion of the population remained as single cells compared to uninduced blastomeres. The aggregation was mediated by C-cadherin because C-cadherin was present in the blastomeres during the aggregation assay, and monoclonal antibodies against C-cadherin inhibited the calcium-dependent aggregation of blastomeres. E-cadherin was not detectable until after the completion of the assay and, therefore, does not explain the adhesive differences between induced and uninduced blastomeres.

L cells stably expressing C-cadherin (LC cells) were used to demonstrate that C-cadherin activity was specifically altered after activin induction. Blastomeres induced with activin bound fewer LC cells than uninduced blastomeres. L cells not expressing C-cadherin did not adhere to blastomeres.

The changes in C-cadherin–mediated adhesion occurred without detectable changes in the steady-state levels of C-cadherin or the amount of C-cadherin present on the surface of the cell. Immunoprecipitation of C-cadherin and its associated catenins revealed that the ratio of C-cadherin and the catenins was not altered by activin induction. These results demonstrate that activin decreases the adhesive function of existing C-cadherin molecules on the surface of blastomeres and suggest that decreased cadherin mediated cell–cell adhesion is associated with increased morphogenetic movement.

The regulation of cadherin-mediated cell–cell adhesion is an important component in the morphogenesis of developing tissue. Studies of the regulation of cadherin-mediated adhesion have focused primarily on changes in cadherin gene expression, such as the switch from E-cadherin to N-cadherin expression during neural tube formation (20). Less understood, although probably equally important, is the regulation of cadherin function at the cell surface.

Some evidence for regulated cadherin function has been found. One example of regulated cadherin function is the increase in E-cadherin activity responsible for triggering compaction of the mouse embryo (12). Additional evidence for the regulation of cadherins has been found in cells expressing the viral tyrosine kinase oncogene, v-src (4, 30). Finally, regulation of cadherin function has been found in small cell lung carcinoma cells after exposure to acetylcholine receptor agonists or phorbol esters (42). Despite these examples, the regulation of cadherin function during many morphogenetic processes that occur in developing tissues has not yet been evaluated.

A particularly interesting morphogenetic process that may require the regulation of cadherin function is cell rearrangement (17). Cell rearrangement is a common morphogenetic event occurring in a variety of developing tissues of numerous organisms (25). It has been extensively studied as a major cellular mechanism driving *Xenopus* gastrulation. During cell rearrangement, the cells that form a tissue exchange neighbors and locally reposition themselves, resulting in an overall change in the shape of the tissue. Even though rearranging cells exchange neighbors, they remain closely associated with one another, suggesting the necessity for the regulation of cell adhesion.

To examine the regulation of cell–cell adhesion and cadherin function during tissue morphogenesis, we have begun to analyze cadherin function in the activin-induced *Xenopus* animal cap. The animal cap is an explant of presumptive ectoderm isolated from the animal hemisphere of a blastula.
stage embryo. The animal cap expresses C-cadherin (27) and, eventually, E-cadherin at the beginning of gastrulation (28). When the animal cap is cultured in isolation, it forms a spherical mass of tissue. If, however, the animal cap is treated with activin, a mesoderm-inducing factor of the TGF-β family, the animal cap undergoes extensive morphogenetic movement that results in a dramatic elongation of the tissue (38). The mechanism of the activin-induced tissue movements appears to mimic the cell rearrangements driving normal gastrulation. We hypothesized that cell–cell adhesion and C-cadherin function would be regulated during these movements because the cells that are involved in similar gastrulation movements in the embryo express C-cadherin. To determine if changes in cell–cell adhesion occur after activin induction, a new assay that allows for quantitation of blastomere aggregation was developed. Additionally, an assay was developed to specifically probe C-cadherin activity and to detect possible changes in its function after activin induction.

Materials and Methods

Embryos and Experiments

*Xenopus* eggs and embryos were obtained by standard techniques (24). All manipulations were at room temperature. Eggs were squeezed into 1× MMR (100 mM NaCl, 2 mM KCl, 2 mM CaCl2, 1 mM MgCl2, and 5 mM Hepes, pH 7.4), and embryos were reared in 0.1× MMR. Embryos were dejellied in 2% cysteine HCl (pH 7.8–8.0). Staging of embryos was according to Nieuwkoop and Faber (33). Animal caps were isolated from stage 8 embryos in 1× MMR or in 1× calcium- and magnesium-free medium (CMFM; 88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO3, and 7.5 mM Tris, pH 7.6) if dissociated blastomeres were to be used. Dissociated animal pole blastomeres were isolated by gentle passage through a Pasteur pipette in 1× CMFM. The outer layer of pigmented cells was discarded. Blastomeres were incubated in the presence or absence of 5 ng/ml human recombinant activin A (a gift of Genentech, South San Francisco, CA) in 1× CMFM for 60 min at room temperature. As controls for activin induction, additional animal caps were isolated and cultured in 1× MMR in the presence or absence of 5 ng/ml activin. Induction was assessed by elongation, aggregation, and, eventually, E-cadherin at the beginning of gastrulation (10), and were later confirmed using a specific monoclonal antibody (7). Positive antibody–secreting hybridomas were screened by immunoblotting C-cadherin in extracts of LC cells, using extracts of parental L cells as negative controls. Two independent monoclonal antibodies, S05 and 6B6, were obtained.

Cell Culture

L cells and L cells stably expressing C-cadherin (LC cells) were cultured in DME, 10% supplemented calf serum, and 2 mM glutamine at 37°C in 5% CO2. Cells were split 1:5 and used 3 d later for L cell–blastomere binding assays or L cell aggregation assays.

Generation of the LC Cell Line by Transfection

A cDNA containing the complete C-cadherin coding sequence was subcloned into the mammalian cell expression vector pCMVdi2SK obtained from M. Walter and W. Rutter at the University of California at San Francisco. L cell fibroblasts (Ltr cells obtained from D. Littman, University of California at San Francisco, CA)-cells obtained from D. Littman, University of California at San Francisco) were cotransfected with this vector and the pUCHSG plasmid (obtained from P. Bates and H. Varmus, University of Washington, Seattle, WA) containing a hygromycin resistance gene. Transfected cells were selected for hygromycin resistance by hygromycin, by the calcium phosphate precipitation method (2). Colonies resistant to 400 μg/ml hygromycin were picked, expanded, and screened for expression of C-cadherin by immunoblotting with an antibody to a highly conserved cadherin cytoplasmic domain (10), and were later confirmed using a specific mAb to C-cadherin (see below). The LC cell line, which expressed the highest levels of C-cadherin, was cloned twice by limiting dilution.

Generation of Anti-C Cadherin Monoclonal Antibodies

Mice were immunized with a bacterially produced fusion protein containing a portion of glialtuhione-S-transferase fused to the amino terminus of a large portion of the C-cadherin extracellular domain (containing about two thirds of domain EC2 and the region from EC2 to just before the transmembrane domain). To produce the fusion protein, the BamHI-Hind3 fragment encoding residues 924–2,089 of the C-cadherin coding region was ligated into XhoI and SmaI inframe with the glutathione–S-transferase coding sequence into the pGEX-11N vector from AMRAD (Victoria, Australia). Although most of the fusion protein produced was incorporated into insoluble inclusion bodies, the smaller soluble fraction was purified on a glutathione-Sepharose column (Sigma Immunochemicals, St, Louis, MO) and used for immunization (5 μg/mouse per injection). Immunization and production, growth, and limiting dilution subcloning of the hybridomas were done by standard methods (7). Positive antibody–secreting hybridomas were screened by immunoblotting C-cadherin in extracts of LC cells, using extracts of parental L cells as negative controls. Two independent monoclonal antibodies, S05 and 6B6, were obtained.

Extract Preparation, SDS-PAGE, and Immunoblotting

*Xenopus* eggs, embryos, explants, or L cells were extracted with 1% NP-40 in solution A (10 mM Hepes, 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, and 2 mM EDTA) as described previously (9). Glycoproteins from L cell extracts were enriched by binding to concanavalin A-Sepharose-4B (Sigma Immunochemicals) as described (31). Samples were boiled in SDS sample buffer for 5 min and the proteins separated on 8% polyacrylamide gels under denaturing conditions. Proteins were electrophoretically transferred to nitrocellulose and blotted for C-cadherin with a mixture of mAbs S05 and 6B6, for E-cadherin with mAb 5D3 (9), or for α- or β-catenin using rabbit polyclonal antisera (32). HRP-conjugated goat anti-mouse or goat anti-rabbit (Bio Rad Laboratories, Richmond, CA) were used as secondary antibodies to detect mouse and rabbit antibodies, respectively, by chemiluminescence using the enhanced chemiluminescence detection kit (Amersham Corp., Arlington Heights, IL).

Immunoprecipitation

Blastomere aggregates were lysed in 0.5 ml 0.5% NP-40 in solution A, clarified by centrifugation at 14,000 rpm for 10 min at 4°C, and the supernatant was immunoprecipitated overnight at 4°C for C-cadherin with 3 μg of mAb 6B6 or 3 μg of mouse nonimmune IgG. Antibody–antigen complexes were precipitated by 1-h incubation with 30 μl protein G Sepharose (Sigma Immunochemicals) and washed three times in 0.5% NP-40 in solution A. Proteins were eluted from the beads by boiling in SDS sample buffer and separated by SDS-PAGE. After transferring to nitrocellulose, α-catenin or β-catenin were detected by immunoblotting as described above with anti-α-catenin antisera or anti-β-catenin antisera, respectively.

Aggregation Assays

To label blastomers for aggregation assays, dissociated, activated, or uninduced blastomeres were pelleted in 1.5-ml centrifuge tubes at 75 g for 2 min and resuspended in 300 mM sucrose containing either 10 μg/ml DiIOT(3) 5,3'-diododecylxloxacarbocyanine perchlorate (DiIOT), 5 μg/ml DiIC18(5) 1',1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiIC18(5)), or 10 μg/ml DiIC18(5) 1',1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiIC18(5)) (all fluorescent dyes were purchased from Molecular Probes, Eugene, OR) for 5 min. The cells were washed once with 300 mM sucrose, resuspended in 1× CMFM, and transferred to a 12-well dish coated with 1% agarose containing 1× CMFM (blastomeres from five animal caps per well). To initiate aggregation, CaCl2 was added to a final concentration of 2 mM, and the dish was rotated at 70 rpm on a horizontal rotating table. For antibody inhibition studies, the blastomeres were first transferred into 300 μg/ml mAb 6B6 or 300 μg/ml mouse nonimmune IgG.
in 1× CMFM. To obtain single blastomeres, dissociated blastomeres were kept in 1× CMFM during the aggregation period. At the indicated time, activin-induced, uninduced, and dissociated cells were layered together on a 25-cm column (6 ml) of 10% Ficoll (Sigma Immunochemicals) in 1× MMR to allow direct comparison of the samples. The cells were allowed to settle under unit gravity for 5 min. 1-ml fractions were collected by upward displacement and transferred to 1.5 ml centrifuge tubes. The cells were pelleted at 75 g for 2 min, and the supernatant was discarded. The cells were lysed in 500 μl of 1% NP-40 in solution A, and the fluorescence was determined with a fluorimeter. dI was read at 550 nm excitation/570 nm emission, clio at 484 nm excitation/505 nm emission, and diI(5) at 645 nm excitation/665 nm emission.

L-cell and LC-cell aggregation assays were performed as previously described (34), except the aggregation was performed in 1× MMR supplemented with 0.1% BSA, 0.1% glucose, and 25 μg/ml DNase at room temperature.

L Cell-Blastomere Binding Assays

Untransfected L cells and LC cells were labeled with 10 μg/ml dI for 5 h in DME supplemented with 10% calf serum as described (34). After labeling, the cells were rinsed once with PBS containing CaCl₂ and MgCl₂, and were subsequently harvested in 0.01% trypsin in PBS containing Ca²⁺, Mg²⁺ (PBS+), and 1 mg/ml glucose for 30 min. The cells were pelleted and rinsed once in PBS+ containing 1 mg/ml soybean trypsin inhibitor before being resuspended in Ca²⁺/Mg²⁺-free 1× MMR supplemented with 0.1% BSA, 0.1% glucose, and 25 μg/ml DNase (Sigma Immunochemicals). 1 ml of L-cell or LC-cell suspension containing 500,000 cells was added to activin-induced or uninduced blastomeres of five animal caps each. Each aggregation was initiated in a well of a 12-well dish coated with 1% agarose by the addition of CaCl₂ to a final concentration of 2 mM. The dish was rotated at 70 rpm on a horizontal rotating table for 2 h at room temperature. The blastomeres and L or LC cells were separated from the remaining L cells and aggregates of LC cells by centrifugation to their buoyant densities at 900 g for 10 min. The blastomeres and attached fibroblasts were collected by upward displacement, mixed with 2 vol of 1× MMR and pelleted at 200 g for 5 min. The pellet was lysed in 500 μl of 1% NP-40 solution A, and the number of fibroblasts bound was quantitated by determining the fluorescence in a fluorimeter.

Trypsin Accessibility of C-Cadherin on Blastomeres

Activin-induced or uninduced blastomere aggregates formed after a 90-min aggregation assay were rinsed once in 1× CMFM and transferred into 0.01% trypsin in 1× CMFM. Alternatively, after aggregation, the aggregates were transferred in 0.01% trypsin in 1× CMFM supplemented with 2 mM CaCl₂. Trypsinization was allowed to proceed for 60 min at room temperature before the reaction was stopped by two washes in 1 mg/ml soybean trypsin inhibitor in either 1× CMFM or 1× CMFM plus 2 mM CaCl₂. The blastomeres were lysed in 75 μl of 1% NP-40 in solution A, and C-cadherin was detected by immunoblotting as described above.

Results

Activin Induction Decreases the Ca²⁺-dependent Adhesion between Blastomeres

As previously reported (37), activin induced an extensive elongation of isolated animal caps (Fig. 1 a). Uninduced animal caps never elongated, but they quickly healed into spheres of tissue (Fig. 1 b). Elongation of animal caps was used in all of the following experiments to confirm induction by activin. Since 5 ng/ml of human recombinant activin A consistently elicited dramatic morphogenetic movements in animal caps, this concentration was used in the experiments described below.

To determine if activin induces an alteration in adhesion between blastomeres, aggregation assays were performed on activin-induced and uninduced blastomeres. Animal pole cells were dissociated in CMFM and subsequently incubated in the presence or absence of activin. Calcium was then returned to the medium, and the cells were reaggregated against constant shear for 90 min. Activin-induced blastomeres formed smaller aggregates and had more single cells compared to uninduced blastomeres (Fig. 2).

The extent of aggregation was quantitated by determining the size distribution of the aggregates formed after an aggregation assay. Blastomeres were labeled with fluorescent dyes, treated with or without activin, and aggregated for 90 min. The resulting aggregates from activin-induced and uninduced blastomeres were fractionated together on a column of Ficoll to allow direct comparison of the size of the aggregates between the two conditions. Single, unaggregated blastomeres labeled with a third fluorescent dye were included on the column to serve as a marker for the position of single cells. Fractions were collected from the column, and the percentage of fluorescence in each fraction was determined. As shown in Fig. 3 α, uninduced blastomeres formed large aggregates that settled to the bottom of the column. In contrast, 80% of the single cells remained in the top fraction of the column, demonstrating the separation of aggregates and single cell populations by size. Activin-induced blastomeres formed smaller aggregates compared to uninduced blastomeres, resulting in a greater proportion of the activin-induced population to remain in the upper frac-
Figure 2. Aggregation of activin-induced and uninduced animal pole blastomeres. Dissociated blastomeres were treated with (a) or without (b) activin before Ca\textsuperscript{2+} was returned to the medium, and the blastomeres were reaggregated for 90 min. The entire sample is shown in each photograph. Arrows show examples of single blastomeres. Scale bar, 500 \(\mu\text{m}\) (applies to both micrographs).

Figure 3. Quantitation of blastomere aggregation. (a) Size distribution on a Ficoll column of aggregates of activin-induced blastomeres (---) or uninduced blastomeres (- - -) resulting from a 90-min aggregation assay. Unaggregated blastomeres (○ ○ ○) were included on the column as a marker for single cells. This experiment was performed 17 times, and a representative example is shown. (b) Experiment described in (a) performed as a function of time. At each time point, the fraction of the total fluorescence of each sample in the top fraction of the column is plotted. This experiment was performed four times with similar results.
seen at 5 ng/ml of activin. Concentrations of activin below 5 ng/ml produced adhesive differences but with less consistency than 5 ng/ml; 1 ng/ml activin failed to induce elongation of animal caps, and this concentration did not induce changes in aggregation.

**Blastomere Aggregation is C-Cadherin Dependent**

The calcium-dependent cell–cell adhesion of blastomeres is most likely mediated by C-cadherin, a 120-kD, maternally encoded cadherin that was originally identified by an antibody to a highly conserved region of the cadherin cytoplasmic domain (10, 32). The cDNA encoding C-cadherin has been cloned and sequenced (29) (Lee, C. H., and B. M. Gumbiner, GenBank Accession no. U04707), and it is almost identical to E/P cadherin, differing by only four amino acids (14).

To study C-cadherin function and to characterize anti-C-cadherin antibodies, the cDNA encoding C-cadherin was stably transfected into L cells establishing the LC cell line. Extracts of LC cells contain a 120-kD protein that is detected by the antipeptide antisera against the conserved cadherin cytoplasmic domain. No proteins are detected by this antiserum in untransfected L cells (Fig. 4 a). A bacterial fusion protein encoding the extracellular domain of C-cadherin was used to generate monoclonal antibodies against C-cadherin. Monoclonal antibody 6B6 recognizes a single polypeptide of 120 kD in LC cells, as well as in *Xenopus* eggs and in the *Xenopus* epithelial cell line A6 (Fig. 4 b). No proteins are detected with 6B6 in extracts from untransfected L cells. This antibody does not recognize *Xenopus* E-cadherin or N-cadherin as determined by blotting extracts of L cells stably expressing *Xenopus* E-cadherin or N-cadherin (Fig. 4 b).

To determine if C-cadherin is necessary for the aggregation of inner animal pole blastomeres, dissociated blastomeres labeled with diI or diO were aggregated in the presence of calcium and either mAb 6B6 or nonimmune antibodies. After 90 min, the resulting aggregates were loaded along with single cells labeled with diI(5) on a Ficoll column and separated by size as described above (Fig. 5). Blastomeres aggregated in the presence of nonimmune antibodies formed large aggregates that settled to the bottom of the column. Blastomeres incubated with mAb 6B6 settled in a pattern similar to dissociated cells or small aggregates. To determine the average effect of the antibodies, we analyzed the top and bottom fractions as described above for activin induction. The mean of the ratios ± SEM of cells incubated in mAb 6B6 to cells incubated in nonimmune antibodies was 2.35 ± 0.04, n = 6, for the first fraction, and 0.44 ± 0.04, n = 6, for fraction 6. These results demonstrate that C-cadherin is necessary for the aggregation of blastomeres.

**Activin Induction Reduces the Binding of C-Cadherin-expressing L Cells to Blastomeres**

Although C-cadherin is necessary for the aggregation of blastomeres, changes in cell–cell adhesion after activin induction could occur either through C-cadherin or another protein. To examine the effect of activin induction on C-cadherin specifically, the ability of LC cells to adhere to blastomeres was examined. The adhesive capacity of L cells and LC cells by themselves was first examined in aggregation assays. L cells and LC cells were trypsinized and dissociated in a way that leaves the cadherins intact (34). The cells were then aggregated in the presence of calcium for 1 h against a constant shear (Fig. 6). LC cells formed aggregates while L cells not expressing the cadherin remained as single cells, demonstrating that the C-cadherin protein expressed in LC cells is a functional adhesion molecule.

To determine the number of L cells or LC cells that bind to blastomeres, L cells and LC cells were labeled with diI and harvested in a manner that leaves the cadherins intact. After dissociation in calcium-free medium, the L cells or LC cells were added to dissociated blastomeres that had been
treated with or without activin. Calcium was returned to the media and the blastomeres and L cells allowed to aggregate. After 90 min, the blastomeres and L cells were layered on a preformed gradient of Percoll, and the blastomeres were separated from the unattached L cells or LC cells by centrifugation to their buoyant densities. Fractions were collected from the gradient and inspected by fluorescence microscopy (data not shown). The L cells and aggregates of LC cells were well separated from the blastomeres. The first fraction contained exclusively L cells or aggregated LC cells, while the middle fraction was free of any cells or blastomeres. The bottom fraction contained blastomeres with LC cells adhering to them.

The number of L or LC cells attached to the blastomeres was quantified by fluorimetric determination of dil (Fig. 7). A significant proportion of the LC cells were found attached to the blastomeres. The binding of LC cells to blastomeres was specific for C-cadherin because very few untransfected L cells adhered to blastomeres. Treating the blastomeres with activin resulted in a substantial decrease in the number of LC cells bound by these blastomeres. This demonstrates that activin induction results in a specific decrease in the adhesive function of C-cadherin.

Expression of Cadherins and Catenins after Activin Induction
Activin induction of animal caps results in a variety of responses, including numerous changes in gene expression (23). Animal caps treated with or without activin were analyzed for changes in the expression of cadherins by Western blotting. Animal caps were analyzed for C-cadherin at times around the time course of the adhesion assay (Fig. 8 a), as well as at later time points during which elongation of the animal cap occurs (Fig. 8 b). The steady-state levels of C-cadherin were unaffected by activin induction. E-cadherin is normally synthesized in the ectoderm near the onset of gastrulation (9, 28). Western blots for E-cadherin were performed to see if changes in its expression could explain the changes in aggregation after activin induction. E-cadherin, however, was not present during the adhesion assay or at the time adhesive differences between induced and uninduced blastomeres were seen (Fig. 8 c). Although E-cadherin expression was suppressed by activin induction, E-cadherin was not detected in uninduced blastomeres until 90 min after the adhesive differences between induced and uninduced blastomeres were demonstrated, excluding E-cadherin expression as an explanation for the adhesive differences in this assay.

Although the total amount of C-cadherin was not affected by activin induction, it is possible that the amount of C-cadherin present on the surface of the cell could account for the changes in adhesion. The amount of C-cadherin on the surface was analyzed by determining the amount of C-cadherin accessible to trypsin digestion on the cell surface of intact, activin-induced, and uninduced blastomeres (Fig. 9). As described above, activin-induced and uninduced blastomeres expressed equivalent amounts of total C-cadherin. Treatment of the aggregates with trypsin in the absence of calcium resulted in a substantial loss of C-cadherin. Similar amounts of C-cadherin were susceptible to trypsin digestion in activin-induced and uninduced blastomeres. Degradation of C-cadherin was not caused by internalization of trypsin or degradation after lysis in detergent. Intact blastomeres treated with trypsin in the presence of calcium, a procedure that protects cadherins from trypsin digestion (22), had the same amount of C-cadherin present as blastomeres incubated in the ab-

Figure 6. Ca+-dependent aggregation properties of L cells (a) and LC cells (b). Cells were dissociated and subsequently reaggregated for 60 min, and representative fields of each were photographed.

Figure 7. Binding of L cells and LC cells to blastomeres. Blastomeres were separated from L or LC cells by centrifugation to their buoyant densities on a Percoll gradient. The number of fluorescently labeled L cells or LC cells bound to activin-induced or uninduced blastomeres after a 90-min aggregation was determined. Two examples are shown representing the range of LC cell binding in response to activin seen in four experiments performed in triplicate. Values are mean ± SEM, n = 3.
Figure 8. Time course of expression of cadherins in blastomeres after activin induction. Animal caps were isolated and treated with or without activin at stage 8, and extracts of these explants were made when control embryos reached the stage indicated. (a) Western blots for C-cadherin from aggregates of activin-induced or uninduced blastomeres. t = 0 min corresponds to the initiation of the aggregation assay by the addition of Ca²⁺. (b) Experiment performed as in a at later stages of development. (c) Experiment performed as in a but blotted for E-cadherin.

Figure 9. Accessibility of C-cadherin on intact blastomeres to trypsin. (a) Activin-induced and uninduced blastomeres were treated with or without trypsin after a 90-min aggregation assay in the presence or absence of calcium, and the amount of C-cadherin remaining was determined by Western blotting. (b) Differential accessibility of C-cadherin and its precursor. Activin-induced and uninduced blastomeres were trypsinized as in a.

Discussion

The current results demonstrate that cell-cell adhesion and C-cadherin function are regulated in animal pole blastomeres after activin induction. Activin-treated blastomeres formed smaller aggregates compared to untreated blastomeres in aggregation assays, demonstrating that activin decreases the calcium-dependent adhesion between these cells. The decrease in cell-cell adhesion is very likely caused by a change in C-cadherin function. First, C-cadherin, but not E-cadherin, was present in the blastomeres at the time that the adhesive difference was evident. Second, an antibody against C-cadherin inhibited the aggregation of blastomeres, demonstrating that C-cadherin is necessary for the calcium dependent adhesion of blastomeres at this time. Finally, when C-cadherin-expressing L cells were used in blastomere-binding assays as a probe for C-cadherin activity, activin-induced blastomeres bound fewer LC cells than...
uninduced blastomeres. This assay demonstrated a decrease in C-cadherin activity after activin induction. Since the steady-state levels of C-cadherin and the amount of C-cadherin expressed on the cell surface remain the same in activin-induced and uninduced blastomeres, the observed change in C-cadherin activity results from regulation of C-cadherin function at the cell surface.

C-cadherin is maternally expressed and is the major, and possibly only, cadherin expressed during the pregastrula stages. U-cadherin (1) and XB-cadherin (21) have also been described in Xenopus eggs and embryos, but the identity of these proteins and their relationship to C-cadherin is not certain. A cadherin expressed at this time could account for the highly calcium sensitive association of blastomeres in the early embryo. C-cadherin is localized at cell–cell contacts throughout the early embryo (27), which suggests that C-cadherin could be an important component of the cell–cell adhesion machinery throughout the entire pregastrula embryo.

The mechanism through which activin induction results in C-cadherin regulation is not known. Decreased adhesion could be indicative of a global decrease in adhesion, possibly through large changes in the actin cytoskeleton or by expression of an inhibitor of cell adhesion on the cell surface, such as the polysialic acid residue periodically present on neural cell adhesion molecule (36). Such global mechanisms for decreasing adhesion are unlikely, however, because activin induction also causes an increased adhesion of blastomeres to fibronectin (37). In our experiments in which activin caused a decrease in C-cadherin–mediated adhesion, we also found that blastomere adhesion to fibronectin was increased (unpublished data). Consequently, the regulation of C-cadherin function does not result from mechanisms so general that they compromise all adhesion systems.

More specific regulation of C-cadherin could occur by controlling the clustering of C-cadherin in adhesive junctions (18). Cadherins are frequently localized in specialized regions of cell–cell contact, the adherens junction, and clustering cadherins in such structures has been proposed to increase the avidity of cadherins for like cadherins. For example, during mouse embryo compaction, E-cadherin becomes concentrated at cell–cell borders in the zona adherens (40). Activin induction could lead to the disassembly of clusters of C-cadherin, which would lower the avidity of C-cadherin and decrease cell–cell adhesion.

Alternatively, activin induction could lower the affinity of C-cadherin for other C-cadherin molecules by inducing a conformational change in C-cadherin. Although there is no evidence for such regulation of cadherins, well-documented examples exist for integrins. For example, the platelet integrin GPIbβIIa undergoes a conformational change upon platelet activation, which results in increased affinity for ligands (15).

A decrease in C-cadherin–mediated adhesion may be a prerequisite for activin-induced cell movements to occur. This is consistent with findings from tumor cells. Migratory, invasive carcinomas usually do not express functional cadherins (39). Suppression of E-cadherin function by antibodies or antisense RNA imparts an invasive phenotype to cadherin-expressing cells (3, 41), while transfection of E-cadherin cDNA into invasive cells suppresses their invasive potential (8, 13, 41). Similarly, neural crest cells lose N-cadherin before migration (19). While C-cadherin does not disappear from the moving cells of the activin-induced animal cap, its adhesive function is clearly decreased. Unlike the migratory cells described in the previous examples, blastomeres of the activin-induced animal cap do not dissociate during elongation of the animal cap, but they remain closely associated with one another (38). The continued presence of C-cadherin, despite its decreased function, may be necessary for the adhesion between these cells. Consistent with this idea, our findings suggest that, while C-cadherin activity is diminished, some functional activity remains.

Although the role of decreased C-cadherin function after activin induction may be limited to decreasing cell–cell adhesion to allow cell movements, a more active role for C-cadherin in these movements is also possible. Xenopus gastrulation is largely driven by the process of convergent-extension (26). During convergent-extension, a sheet of cells elongates by local cell rearrangements and intercalations. Unlike the locomotion of individual cells on a substratum, convergent-extension requires cell movement on the surface of another cell. Perhaps C-cadherin acts as the adhesive molecule with which a moving cell generates traction. Cadherin-based cell motility is possible because neurite outgrowth can occur on purified N-cadherin (5).

A decrease in C-cadherin–mediated adhesion might be expected during cell migration. For comparison, decreased integrin-mediated adhesion is associated with cell migration on extracellular matrix molecules. For example, neurons of the olfactory epithelium migrate maximally on a less adhesive substrate (6). Furthermore, careful measurements of adhesive strength vs cell migration in smooth muscle cells demonstrate that maximal migration occurs at intermediate attachment strengths (11). It is possible, therefore, that the decreased yet persistent C-cadherin–mediated adhesion might reflect a dynamic population of C-cadherin in which some cadherin molecules have released adhesive contacts while others are forming as the cells move.

The activin-induced animal cap provides a system that should allow for the assessment of the role of cadherins in such morphogenetic movements. Additionally, the animal cap and the adhesion assays described provide an experimentally accessible system that will assist in identifying mechanisms through which cadherin function is regulated.

We are very grateful to Ms. Dale Appapira for the development of the LC cell line and the anti-C-cadherin monoclonal antibodies. We thank Elena Levine and Pierre McCrea for many valuable discussions, as well as Francois Fagotto and Marylin Munson for helpful suggestions on adhesion assays. We also thank Francois Fagotto and Kris Vlemincek for their helpful comments on the manuscript.

This work was supported by National Institutes of Health grant no. GM37432 awarded to B. M. Gumbiner and by the Cancer Center Support grant NCI-P30-CA-08748.

Received for publication 25 January 1994 and in revised form 6 April 1994.

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Briehl and Gumbiner Activin-induced Regulation of C-Cadherin Activity 527