Analysis of C-cadherin Regulation during Tissue Morphogenesis with an Activating Antibody

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Abstract. The regulation of cadherin-mediated adhesion at the cell surface underlies several morphogenetic processes. To investigate the role of cadherin regulation in morphogenesis and to begin to analyze the molecular mechanisms of cadherin regulation, we have screened for monoclonal antibodies (mAbs) that allow us to manipulate the adhesive state of the cadherin molecule.

_Xenopus_ C-cadherin is regulated during convergent extension movements of gastrulation. Treatment of animal pole tissue explants (animal caps) with the mesoderm-inducing factor activin induces tissue elongation and decreases the strength of C-cadherin–mediated adhesion between blastomeres (Brieher, W.M., and B.M. Gumbiner. 1994. _J. Cell Biol._ 126:519–527). We have generated a mAb to C-cadherin, AA5, that restores strong adhesion to activin-treated blastomeres. This C-cadherin activating antibody strongly inhibits the elongation of animal caps in response to activin without affecting mesodermal gene expression. Thus, the activin-induced decrease in C-cadherin adhesive activity appears to be required for animal cap elongation.

Regulation of C-cadherin and its activation by mAb AA5 involve changes in the state of C-cadherin that encompass more than changes in its homophilic binding site. Although mAb AA5 elicited a small enhancement in the functional activity of the soluble C-cadherin ectodomain (CEC1-5), it was not able to restore cell adhesion activity to mutant C-cadherin lacking its cytoplasmic tail. Furthermore, activin treatment regulates the adhesion of _Xenopus_ blastomeres to surfaces coated with two other anti–C-cadherin mAbs, even though these antibodies probably do not mediate adhesion through a normal homophilic binding mechanism. Moreover, mAb AA5 restores strong adhesion to these antibodies. mAb AA5 only activates adhesion of blastomeres to immobilized CEC1-5 when it binds to C-cadherin on the cell surface. It does not work when added to CEC1-5 on the substrate. Together these findings suggest that the regulation of C-cadherin by activin and its activation by mAb AA5 involve changes in its cellular organization or interactions with other cell components that are not intrinsic to the isolated protein.

Key words: adhesion • activin • C-cadherin • morphogenesis • _Xenopus_

The regulation of cadherin-mediated adhesion is important for many morphogenetic processes (Gumbiner, 1992, 1996). Most apparent is the loss or gain of cadherin expression, which is thought to control the extreme states of cell association in tissues (Takeichi, 1995; Gumbiner, 1996). However, there is also evidence that acute regulation of cadherin function at the cell surface is important for tissue morphogenesis. Examples include E-cadherin regulation during mouse embryo compaction (Fleming and Johnson, 1988), C-cadherin regulation during _Xenopus_ gastrulation (Brieher and Gumbiner, 1994; Lee and Gumbiner, 1995), and N-cadherin regulation during neurite outgrowth (Bixby and Zhang, 1990). Nevertheless, the role of cadherin functional regulation in various morphogenetic processes is still not well known, nor are the mechanisms underlying cadherin regulation well understood.

We have been studying the role of a _Xenopus_ cadherin, called C-cadherin, in the convergence and extension movements that drive morphogenesis during amphibian gastrulation (Brieher and Gumbiner, 1994). C-cadherin is the major cadherin expressed in the blastula and early gastrula and is largely responsible for holding the cells of the early embryo together (Heasman et al., 1994; Lee and Gumbiner, 1995). Both the expression of dominant negative forms of C-cadherin and the overexpression of wild-type...
C-cadherin perturb gastrulation movements, suggesting that appropriate levels of cell adhesion are required for normal morphogenesis (Lee and Gumbiner, 1995).

The adhesive activity of C-cadherin at the cell surface is decreased when animal cap tissue explants are induced by the growth factor activin to undergo tissue elongation (Briher and Gumbiner, 1994) which results from convergence extension movements (Symes and Smith, 1987). We hypothesized that this regulation of C-cadherin activity is important for the rearrangement of cells that underlies elongation of the tissue. Although perturbation by the dominant negative cadherins is consistent with this hypothesis, a direct test requires that we manipulate the regulation of C-cadherin.

Little is known about the mechanisms underlying cadherin regulation. There is some evidence that phosphorylation cascades by the v-src and receptor tyrosine kinases modulate cadherin-mediated adhesion (Behrens et al., 1993). Tyrosine phosphorylation of β-catenin has been observed (Matsuyoshi et al., 1992; Hamaguchi et al., 1993), but its physiological significance is uncertain (Takeda et al., 1995). Small rho-family GTPases may also affect cadherin-mediated adhesion (Hordijk et al., 1997; Tokman et al., 1997). A recent study suggests that IQGAP may alter cadherin–catenin interaction (Kuroda et al., 1998), but alteration of the complex is not observed in all cases of cadherin regulation (Behrens et al., 1995; Briher and Gumbiner, 1994). Even accepting that such intracellular signal transduction events are causally linked to regulation of cadherin-mediated adhesion, there is still a need to understand how structural modifications or rearrangements of cadherins at the cell surface regulate the state of cell–cell adhesion. By comparison, integrins are thought to be regulated either by affinity modulation or by changes in lateral clustering (Schwartz et al., 1995; Stuiver and O'Toole, 1995; Humphries, 1996).

One successful approach to the analysis of integrin regulation has been the use of specific mAbs that either activate the high adhesive state or bind selectively to activated integrins (Schwartz et al., 1995; Stuiver and O'Toole, 1995; Humphries, 1996). Therefore, we decided to search for C-cadherin activating antibodies and to use these antibodies to investigate the mechanism of cadherin regulation and to evaluate the role of C-cadherin regulation in the convergence extension–like elongation of the Xenopus animal cap.

**Materials and Methods**

**Protein Purification and Production of mAbs**

CEC1-5 protein was purified from conditioned media as reported previously (Briher et al., 1996). Production of monoclonal antibodies was based on methods previously described (Campbell, 1984). Briefly, BALB/c mice were immunized five times subcutaneously with 50 μg of purified CEC1-5 protein in the presence of adjuvant. 3 d before time of killing, mice were injected intraperitoneally with 100 μg of CEC1-5 protein in the absence of adjuvant. Spleens were removed and spleen cells were fused with Sp2 myeloma cells, and the resultant hybrid cells were transferred to 96-well plates. The supernatants were first screened by ELISA or immunoblots for reactivity against CEC1-5 protein. Positive anti-C-cadherin producers were then screened for functional activity as described below.

**Functional Screening Assay**

To assay for C-cadherin binding activity on the blastomere surface, Fluo-Spheres beads (Molecular Probes, Inc.) were coated with purified soluble C-cadherin ectodomain, CEC1-5 (Briher et al., 1996). FluoroSpheres beads were coated with CEC1-5 protein according to the manufacturer’s instructions. Briefly, FluoroSpheres were coupled with CEC1-5 at a ratio of 1 μg of protein per 1 μl of beads in 20 mM Hepes, 100 mM NaCl, 1 mM CaCl₂, pH 7.2, for 1 h, and then BSA and Tris were added to the medium at a final concentration of 2 mg/ml and 20 mM (pH 7.2), respectively. After incubation for 1 h, the coated FluoroSpheres were pelleted at 10,000 g for 10 min and then resuspended at 10 times their original volume in 20 mM Hepes, 100 mM NaCl, pH 7.2. The suspension was briefly sonicated to obtain single particles as determined by fluorescence microscopy before the addition of 1 mM CaCl₂.

**Cell Lines, mAbs, and Fab Fragments**

CHO K1 cells, CEC1-5 expressing CHO cells, full-length C-cadherin expression CHO cells (C-CHO), and tailless truncated C-cadherin expression CHO cells (CT-CHO) were described previously (Briher et al., 1996; Yap et al., 1997a).

To map the mAb AA5 binding epitope, cDNA encoding CEC1-5 and CEC1-4, which lacks the fifth repeat, were transfected transiently in CHO cells by lipofection as described previously (Briher et al., 1996; Yap et al., 1997b).

Anti-C-cadherin mAbs (6B6 and 5G5) were described previously (Briher and Gumbiner, 1994). Large amounts of mAb AA5 were produced with a bioreactor by the mAb facility of Memorial Sloan-Kettering Cancer Center. AA5, 6B6, and nonimmune mouse IgG were purified from the supernatants using affinity chromatography on protein G–Sepharose column. Fab fragments of AA5, 6B6, and nonimmune mouse IgG were prepared using papain digestion and purification by protein A–Sepharose as described previously (Campbell, 1984).

**SDS-PAGE and Western Blots**

Cells or blastomeres were extracted in 1% NP-40 extraction buffer (1% NP-40, 10 mM Hepes, 150 mM NaCl, 1.5 mM EDTA, pH 7.4), supplemented with protease inhibitors as described previously (Choi and Gumbiner, 1986). Volumes were adjusted to make all samples equal in protein concentration by addition of SDS sample buffer, and proteins were separated by SDS-PAGE. Proteins were transferred to nitrocellulose membrane and probed with the mAbs AA5, 6B6, or nonimmune mouse IgG. Immunolabeling was detected by the ECL detection system according to the manufacturer’s instructions (Amersham Corp.).

**Embryos and Explants**

Xenopus eggs and embryos were obtained by standard techniques (Kay and Peng, 1991). 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 (1967).

To analyze antibody effects on the elongation of activin-induced animal caps, Xenopus animal caps were isolated from stage 8 embryos and incubated in 1× MMR with or without activin (5 ng/ml) and either mAb AA5 Fab (1 μg/ml) or mouse IgG Fab (1 μg/ml) overnight at 15°C. The animal caps were analyzed by photographing and scoring for presence or absence of elongation.

**RNA Injection**

To construct the IL-2β receptor–C-cadherin cytoplasmic tail fusion protein chimera, the IL-2β receptor cytoplasmic tail was subcloned into the C-terminus of C-cadherin. The C-cadherin construct was linearized with XbaI and was electroporated into CHO cells. CHO cells expressing the C-cadherin chimera were isolated by selection on G418. The C-cadherin chimera was then confirmed by immunoblotting.
cDNA, PCR was used to attach 5′ HindIII and 3′ XbaI sites on the cytoplasmic tail of C-cadherin. The PCR product was fused in frame to the IL-2 receptor using the IL-2 receptor-specific mAb BB 10 was used for the experiments (Brieher and Gumbiner, 1994). Anti–C-cadherin mAbs 6B6 and 5G5 have been described previously (Brieher et al., 1996; Yap et al., 1997b, 1998). 1-mm internal diameter glass capillaries were used to measure C-cadherin regulation, which entailed the binding of fluorescently labeled C-cadherin expressing fibroblasts to the blastomeres (Brieher and Gumbiner, 1994). In the modified assay, we used fluorescent beads coated with purified C-cadherin ectodomain (CEC1-5) as the binding probe in place of the fibroblasts (Fig. 1 A). Numerous CEC1-5–coated beads bound to the surface of untreated blastomeres (left), and treatment of blastomeres with activin greatly reduced the number of beads bound (center).

mAbs were raised to CEC1-5 and screened first for reactivity to CEC1-5 by ELISA or Western blotting. The positive hybridoma supernatants were then screened in the functional assay described above for C-cadherin activating activity. Greater than 600 supernatants from 6 fusions were positive for reactivity with C-cadherin in ELISA. Of these, only one exhibited clear reproducible activating activity. This mAb, designated AA5, stimulated the binding of CEC1-5–coated beads to activin-treated blastomeres (Fig. 1 A, right). mAb AA5 was therefore subcloned and characterized further.

mAb AA5 binds to purified CEC1-5 in Western blots (Fig. 1 B) and specifically recognizes C-cadherin expressed in CHO cells (Fig. 1 C). We were also able to map the AA5 binding epitope to the fifth extracellular cadherin repeat that is predicted to lie nearest to the membrane (Fig. 1 D). CEC1-5 and CEC1-4, which lacks the fifth repeat, were expressed transiently in CHO cells and immunoblotted with either mAb AA5 or another anti–C-cadherin mAb (6B6) described previously. Although mAb 6B6 recognizes both CEC1-5 and CEC1-4, mAb AA5 recognizes only the full-length ectodomain.

Although mAb AA5 stimulates CEC1-5–coated bead binding to blastomeres, we wished to determine whether it stimulated blastomere adhesion using more standard types of adhesion assays. Furthermore, to rule out artifactual adhesion due to crosslinking by the bivalent antibody, we also tested monovalent Fab fragments of mAb AA5. Fab fragments of mAb AA5 also stimulated CEC1-5–coated beads binding to blastomeres (data not shown). In all further experiments described, purified Fab fragments of mAb AA5 were used (although still referred to as mAb AA5). Activin treatment reduces the rate of Ca2+-depen-
dent aggregation of animal cap blastomeres that had been dissociated by Ca\(^{2+}\) removal (Brieher and Gumbiner, 1994) (Fig. 2 A, left and center). Inclusion of mAb AA5 in the aggregation assay stimulated the rate of blastomere aggregation of activin-treated blastomeres, producing larger aggregates after the same time interval (Fig. 2 A, right). We also examined the activity of mAb AA5 in a cell attachment assay (Fig. 2 B). Attachment of dissociated blastomeres to surfaces coated with CEC1-5 was assayed. No attachment to BSA-coated surfaces was observed (data not shown). Activin treatment reduced the number of blastomeres that adhered to CEC1-5 under gentle shaking conditions, and mAb AA5 significantly increased the number of activin-treated blastomeres that adhered to CEC1-5. Thus, monovalent Fab fragments of mAb AA5 stimulated C-cadherin–mediated adhesion of activin-treated blastomeres to near the level of adhesion of untreated blastomeres.

**Figure 1.** Detection of anti–C-cadherin activating mAb AA5 in a functional screen. (A) mAb AA5 restores high C-cadherin–mediated adhesion to activin-treated *Xenopus* blastomeres. Dissociated *Xenopus* animal cap blastomeres were treated with or without activin (5 ng/ml). The activin-induced blastomeres were treated with either mAb AA5 hybridoma supernatant or nonimmune mouse IgG. Blastomere samples were then incubated with CEC1-5–coated FluoSpheres in the presence of calcium for 60 min. (B, C, and D) Western blot analysis demonstrating mAb AA5 binds to domain 5 of C-cadherin. CEC1-5 protein (B), CEC1-5 expressing CHO cell extracts (C), and CEC1-4 expressing CHO cells extracts (D) were probed with anti–C-cadherin 6B6 mAb, AA5 mAb, or nonimmune mouse IgG.

**Figure 2.** mAb AA5 enhances the C-cadherin–mediated adhesion of activin-induced animal cap blastomeres. (A) Blastomere aggregation assay. Dissociated blastomeres were treated with or without activin (5 ng/ml). Activin-treated blastomeres were incubated either with mAb AA5 Fab (1 μg/ml) or nonimmune mouse IgG Fab (1 μg/ml) in the presence of calcium, and then allowed to aggregate with constant shaking for 15 min. (B) Blastomere adhesion to CEC1-5. Dissociated blastomeres were treated with or without activin. Activin-treated blastomeres were incubated with either mAb AA5 Fab or nonimmune mouse IgG Fab, and then allowed to attach to spots of CEC1-5 protein coated on a tissue culture plate in the presence of calcium for 30 min. The plates were then shaken continuously for 2 min on a horizontal shaker and the numbers of blastomeres per unit area remaining attached were counted. The graph shows the results of three different experiments, each performed in duplicate.

**Inhibition of Activin-induced Morphogenetic Movements by mAb AA5**

Activin induces *Xenopus* animal cap explants to elongate as a result of convergent extension movements (Symes and Smith, 1987; Brieher and Gumbiner, 1994) (Fig. 3). We proposed that the activin-induced decrease in C-cadherin–mediated adhesion is necessary for tissue elongation (Brieher and Gumbiner, 1994). To test this hypothesis, we asked whether mAb AA5, which maintains high levels of C-cadherin–mediated adhesion even after activin treatment, would affect animal cap elongation (Fig. 3 A and B). As described previously, activin induced ~95% of the animal caps to elongate significantly, forming asymmetric structures. In the absence of activin, all of the animal caps form nearly spherical structures. mAb AA5 strongly inhibited animal cap elongation in response to activin (Fig. 3 A, far right). Only ~30% of the animal caps formed asymmetric structures that could be scored as elongated (Fig. 3 B), and even these were relatively stunted compared to the nonimmune IgG control. Although the majority of the mAb AA5-treated caps did not elongate significantly, they appeared “lumpier” than the smooth spherical structures formed in the absence of activin, suggesting that mAb
AA5 did not completely block all morphogenetic movement. A different mAb that inhibits C-cadherin–mediated adhesion, 6B6, did not block elongation to the same extent as mAb AA5, even though it did affect the morphology of the elongated caps. Thus, preventing the activin-induced decrease in C-cadherin–mediated adhesion significantly inhibited the morphogenetic elongation of the tissue, indicating that the change in C-cadherin–mediated adhesion is required for the process.

mAb AA5 could affect morphogenesis of the animal caps in two ways: either directly by affecting the physical adhesion between blastomeres in the tissue, or indirectly by somehow inhibiting the whole process of mesoderm induction by activin. The latter possibility was tested by determining whether mAb AA5 inhibited the induction of mesoderm specific genes using RT-PCR analysis (Fig. 3 C). Two mesodermal specific markers were analyzed. Goosecoid is a marker for dorsal mesoderm, and its expression is associated with convergent extension morphogenesis (Smith et al., 1991). Brachyury is a marker for ventral–lateral mesoderm, and while its expression can be induced by activin, it is often expressed in untreated animal caps when they are large and/or taken from later stage blastulae (Cho et al., 1991). Similar to previous publications (Smith et al., 1991), we find that activin induces goosecoid expression in animal caps, yet mAb AA5 did not inhibit goosecoid induction. Also, mAb AA5 did not inhibit the expression of brachyury, which, in this experiment, was expressed even without activin treatment. Thus, mAb AA5 does not inhibit general mesodermal gene expression in response to activin. Its effects on morphogenesis are more likely due to its direct effects on C-cadherin–mediated adhesion.

Analysis of Mode of Action of mAb AA5

Experiments were undertaken to determine how mAb AA5 activates C-cadherin. For integrins, there are two general classes of activating mAbs. One class, exemplified by anti-ß3 mAbs including P41 (O’Toole et al., 1990), Ab 62 (O’Toole et al., 1990), and D3GP3 (Kouns et al., 1990), binds to and stabilizes a high-affinity conformation of the isolated integrin protein. The second class is more complex and requires cellular processes to activate the integrin (Shattil et al., 1985; Dransfield and Hogg, 1990). To determine whether mAb AA5 influences the homophilic binding function of the purified CEC1-5 ectodomain, its effect on the aggregation of CEC1-5–coated beads was assayed. Previous experiments showed that bead aggregation depended on calcium and the dimerization of CEC1-5 and was strongly inhibited by inhibitory anti–C-cadherin mAbs 6B6 and 5G5 (Brieher et al., 1996). In contrast, mAb AA5 weakly, but reproducibly enhanced the rate of CEC1-5–coated bead aggregation (Fig. 4 C). This suggests that mAb AA5 can affect to some extent the intrinsic homophilic binding function of the CEC1-5 ectodomain independent of cellular context. To try to analyze the effect of mAb AA5 on C-cadherin in a simple cellular context, its effect on the strength of C-cadherin–mediated adhesion when expressed in CHO cells was assayed (Fig. 4). The strength of adhesion to purified CEC1-5 was measured by a laminar flow detachment assay described previously (Brieher et al., 1996; Yap et al., 1997a, 1998). mAb AA5 has no significant effect on the strength of attachment of CHO cells expressing wild-type C-cadherin (Fig. 4 A). This suggests that in the context of CHO cells C-cadherin either exists in a completely activated state (like blastomeres before treatment with
activin) or is somehow refractory to activation by mAb AA5.

A mutant form of C-cadherin lacking its cytoplasmic tail exhibits low levels of adhesion when expressed in CHO cells (CT-CHO), reflecting its basic homophilic binding activity (Brieher et al., 1996; Yap et al., 1998). We wished to determine whether mAb AA5 could induce a change in the C-cadherin ectodomain that is normally provided by the cytoplasmic tail and associated cytoskeletal proteins. mAb AA5 only weakly enhances the attachment strength of CT-CHO cells (Fig. 4 B). This is consistent with the bead aggregation assay and suggests that the mAb AA5 can affect to some extent the homophilic binding activity of the protein in the absence of cytoplasmic interactions.

Note, however, that mAb AA5 was not able to restore full cell adhesion activity to the tailless mutant C-cadherin compared to wild-type C-cadherin, demonstrating that the changes elicited by the antibody cannot substitute for the normal cytoplasmic interactions of the cadherin.

Regulation of cell adhesion may involve complex changes in the state of the adhesion molecule other than modulation of binding affinity. Therefore, we investigated whether activin and mAb AA5 could regulate C-cadherin–mediated adhesion independent of its homophilic binding site. To do so, we assayed blastomere attachment to two anti-C-cadherin mAbs raised against portions of C-cadherin expressed in bacteria, 6B6, and 5G5 (Brieher and Gumbiner, 1994), which are not expected to interact with C-cadherin via its homophilic binding site (Fig. 5). *Xenopus* blastomeres do attach to surfaces coated with these mAbs, and like attachment to CEC1-5, activin decreases the number of blastomeres that attach to the antibodies (Fig. 5 A). This effect is specific to C-cadherin, because activin did not decrease attachment to other substrates. Activin did not affect the attachment of blastomeres to mAbs raised against another cell surface protein, the IL-2β receptor ectodomain fused to the C-cadherin cytoplasmic tail (Fig. 5 C). Moreover, as reported previously (Smith et al., 1990), activin stimulates, instead of decreasing, attachment of blastomeres to fibronectin (Fig. 5 B). Therefore, activin specifically regulates the adhesion of blastomeres to substrates coated with CEC1-5, anti–C-cadherin mAbs.
blastomeres to two different anti-C-cadherin mAbs as well as adhesion by the normal C-cadherin homophilic interaction. Blastomere adhesion to mAbs 6B6 and 5G5 is also activated by mAb AA5 (Fig. 5 A). Addition of mAb AA5 stimulated the adhesion of activin-induced blastomeres to the mAbs to the same extent as it stimulated adhesion to CEC1-5, the homophilic binding substrate. Since mAbs 6B6 and 5G5 are highly unlikely to interact with C-cadherin through its homophilic binding site, these findings provide evidence that regulation of C-cadherin-mediated adhesion by activin and its activation by mAb AA5 occur by mechanisms other than modulation of homophilic binding affinity.

These findings raise an important question about the observations in Fig. 4, B and C. The intrinsic homophilic binding properties of the cadherin ectodomain, CEC1-5, are enhanced by mAb AA5. To what extent can the effects of mAb AA5 on the homophilic binding activity of CEC1-5 account for the reversal of activin-induced regulation of C-cadherin on the blastomere surface? To try to address this question, we asked whether the activation of blastomere adhesion by mAb AA5 occurs primarily through direct effects on the purified CEC1-5 ectodomain or through effects on the normal C-cadherin expressed on the cell surface (Fig. 6). Blastomere adhesion to CEC1-5 provides C-cadherin in both contexts, purified ectodomain on an inert surface and normal C-cadherin and its associated cytoplasmic proteins at the blastomere surface. mAb AA5 was added either to the surface-bound CEC1-5 or to the activin-treated blastomeres separately. mAb AA5 had little or no effect when added to the surface-bound CEC1-5, but exerted its full effect when added to the blastomeres. This finding suggests that the enhanced homophilic binding activity of the cadherin ectodomain does not account significantly for the activation of adhesion by mAb AA5. Activation by mAb AA5 probably occurs primarily by altering C-cadherin function in the context of the cell surface.

**Discussion**

Activating mAbs have provided powerful tools to analyze the regulation of integrin-mediated adhesion (Schwartz et al., 1995; Stuiver and O'Toole, 1995; Humphries, 1996). Until now, this approach had not been used for other types of adhesion molecules. Since cadherins are thought to be regulated at the cell surface like the integrins, it seemed likely that they would be good targets for a similar approach. We have been able to generate such a cadherin-activating antibody by taking advantage of our ability to detect two adhesive states of C-cadherin on the surface of *Xenopus* blastomeres, a high adhesive state and a lower adhesive state that is induced by treatment with the mesoderm-inducing factor activin (Brieher and Gumbiner, 1994). The mAb AA5 activates C-cadherin from the low to the higher adhesive state, effectively reversing the response of C-cadherin to activin treatment.

mAb AA5 provided a tool to test the requirement for C-cadherin regulation in the elongation of the *Xenopus* animal cap. Previously our laboratory had established a correlation between C-cadherin regulation and elongation (Brieher and Gumbiner, 1994). It has been shown that interference with normal C-cadherin function by overexpression or by dominant negative mutations perturbs morphogenesis, but interpretation of these findings is limited because of the requirement of C-cadherin in overall tissue integrity (Heasman et al., 1994; Lee and Gumbiner, 1995). What is needed instead is a selective inhibition of the activin-induced regulation of adhesion in *Xenopus* blastomeres. mAb AA5 achieves this selective inhibition, since it blocks the response of C-cadherin to activin treatment, but has no effect on C-cadherin–mediated adhesion in uninduced blastomeres, or in CHO cells expressing wild-type C-cadherin. mAb AA5 strongly inhibits activin-induced elongation of *Xenopus* animal caps without affecting induction of mesoderm by activin. These findings provide direct evidence that the downregulation in C-cadherin adhesive activity is required for the morphogenetic movements underlying tissue elongation.

Different roles for C-cadherin regulation in tissue elongation can be envisioned (Brieher and Gumbiner, 1994). Elongation of tissues in the early *Xenopus* embryo is due to convergence and extension, a morphogenetic process that involves local cell rearrangements (Keller, 1987; Gumbiner, 1992). To rearrange, cells must exchange neighbors despite being held together by C-cadherin. Downregulation of C-cadherin activity might play a passive role in morphogenesis, by simply allowing cells to move relative to each other, with motility driven by another mechanism. In this regard, it is interesting that the activation state of integrin in the *Xenopus* embryo and animal caps is also regulated by activin (Ramos and DeSimone, 1996). Alternatively, a more active role for C-cadherin in these movements is also possible, similar to the roles of integrins in the locomotion of individual cells on a substratum. Convergent extension requires cell movement on the surface of another cell (Keller, 1987; Gumbiner, 1992), and C-cadherin could act as the adhesive molecule with which a moving cell generates traction. Indeed, cadherin-based cell motility seems plausible, because neurite outgrowth can occur on purified N-cadherin (Bixby and Zhang, 1990). In an active role, the global downregulation of C-cadherin activity detected in these experiments may reflect a more complex spatial and temporal regulation...
needed to produce net movement, as is thought to occur with integrins in locomoting cells (Lauffenburger and Horwitz, 1996).

We have begun to use mAb AA5 to try to elucidate the molecular mechanisms underlying C-cadherin regulation. Activating mAbs have been helpful in clarifying the mechanisms of integrin regulation, and provide valuable examples for comparison (Schwartz et al., 1995; Stuiver and O'Toole, 1995; Humphries, 1996). There are two classes of mAbs that activate the adhesive function of the platelet integrin, gpIIbIIa (Stuiver and O'Toole, 1995; Humphries, 1996). One class acts by binding to and stabilizing the high-affinity conformation of the isolated protein, directly enhancing its binding to its ligand, fibrinogen (Stuiver and O'Toole, 1995; Humphries, 1996). Another class of mAbs acts in a more complex and less understood manner by activating adhesion only in the context of the cell, suggesting a role for other plasma membranes or cytoplasmic components (Stuiver and O'Toole, 1995; Humphries, 1996). For C-cadherin and mAb AA5 it is more difficult to make the distinction between these two modes of regulation, since we do not have a method for measuring homophilic binding affinity. Nevertheless, knowledge about integrin-activating mAbs provides a conceptual framework for the analysis of cadherin-activating mAbs.

C-cadherin downregulation by activin and activation by mAb AA5 most likely occurs as a result of altering the state of the protein in the cell membrane beyond simple changes in the affinity of the homophilic binding site. Regulation of C-cadherin function by activin is apparent even when blastomeres attach to two different anti-C-cadherin mAbs. mAb AA5 activates attachment of activin-induced blastomeres to these antibodies, providing evidence that this regulation occurs by a mechanism similar to the normal regulation of adhesion to C-cadherin. Because C-cadherin is unlikely to attach to these mAbs (6B6 and 5G5) via its usual homophilic binding mechanism, another more global property of C-cadherin is probably altered during regulation. Examples of such a property include the state of oligomerization or clustering of the cadherin, its attachment to the actin cytoskeleton, or a global change in the conformation of the protein (Gumbiner, 1996; Yap et al., 1997a, b).

mAb AA5 is able to enhance somewhat the rate of bead aggregation mediated by the soluble C-cadherin ectodomain (CEC1-5), suggesting that mAb AA5 causes changes intrinsic to the protein. However, the effect of mAb AA5 on the isolated ectodomain does not seem to contribute quantitatively to activation of blastomere adhesion. Using an adhesion assay involving both the immobilized ectodomain and cell-associated C-cadherin, we found that mAb AA5 could not activate adhesion when added to the immobilized CEC1-5, but it was able to fully activate adhesion when added to the cells alone. Therefore, mAb AA5 is able to activate C-cadherin only when it is in a cellular context and similar to the second class of integrin-activating mAb. Since mAb AA5 must initiate its effects through binding to the extracellular region, it may be possible to reconcile an intrinsic change in CEC1-5 with a requirement for cellular context. For example, mAb AA5 could induce a conformational change in the extracellular region that would both exert a very small effect on its homophilic binding activity (detectable with sufficient sensitivity), and transduce a signal across the membrane to effect a change in cytoplasmic function or interactions. There is evidence that the cadherin ectodomain can transmit a signal across the membrane that influences its organization. C-cadherin clustering is ligand-dependent, occurring only when cells specifically attach to C-cadherin (Yap et al., 1997a). It is possible that mAb AA5 binding mimics an initial homophilic binding event that leads to a change in C-cadherin organization.

Determination of the molecular effect of mAb AA5 on the C-cadherin adhesive complex would provide interesting insights into the mechanism of cadherin regulation. mAb AA5 binds to the fifth extracellular cadherin repeat (EC5), which is adjacent to the membrane. This domain differs somewhat in sequence from the other four cadherin repeats in the classical cadherins, but its specific function is not yet known (Takeichi, 1995; Gumbiner, 1996). Lateral dimerization seems to be required for cadherin adhesive function (Brieher et al., 1996), and it would be interesting to know whether mAb AA5 binds preferentially to dimers or induces dimerization. Despite several attempts, we have been unable to detect any differences in binding to monomeric or dimeric species of soluble CEC1-5, or any changes in their relative distributions (our unpublished observations). Also, we have not yet observed any changes in the amounts of b-catenin or a-catenin interacting with C-cadherin in Xenopus in response to activin treatment (Brieher and Gumbiner, 1994). Changes in other protein interactions, or more subtle or transient changes that we have not yet been able to measure, may underlie regulation. Nonetheless, mAb AA5 provides us with an additional tool that we can use to help understand the molecular mechanisms underlying the regulation of C-cadherin-mediated adhesion by activin signaling.

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