Antibodies to the Retina N-Acetylgalactosaminylphosphotransferase Modulate N-Cadherin-mediated Adhesion and Uncouple the N-Cadherin Transferase Complex from the Actin-containing Cytoskeleton

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Abstract. Embryonic chick neural retina cells have at their surface an N-Acetylgalactosaminylphosphotransferase (GalNAcPTase) which is associated with, and glycosylates, the calcium-dependent cell–cell adhesion molecule, N-cadherin (Balsamo, J., and J. Lilien. 1990. J. Biol. Chem. 265:2923–2928). In this manuscript, we demonstrate that antibodies directed against the GalNAcPTase, as well as anti-N-cadherin antibodies, are able to inhibit adhesion of chick neural retina cells to a cell monolayer, to immobilized N-cadherin, or to immobilized anti-N-cadherin antibody. These results indicate that anti-GalNAcPTase antibodies modulate the function of N-cadherin, interfering with the formation of N-cadherin–mediated adhesions. We also demonstrate that actin is associated with the N-cadherin/GalNAcPTase complex and that binding of anti-GalNAcPTase antibodies to intact cells results in dissociation of actin from the complex. We suggest that the GalNAcPTase modulates N-cadherin function by altering its interaction with the cytoskeleton.

As a member of a family of calcium-dependent cell–cell adhesion molecules, N-cadherin appears to be important in a wide variety of morphogenetic processes (Takeichi, 1988). Antibodies directed against N-cadherin inhibit calcium-dependent, cell–cell adhesion (Crittenden et al., 1987; Hatta et al., 1985) and transfection of N-cadherin cDNA into cells lacking N-cadherin renders them adhesive competent (Hatta et al., 1988). Transfection experiments also suggest that adhesion depends on the interaction between N-cadherin molecules on adjacent cells. The amino-terminal region of the cadherin molecule appears to determine both its binding function and tissue-type specificity (Myiatani et al., 1989). Furthermore, the peptide sequence HAV, common to all cadherins, appears to be an important determinant of binding (Blaschuk et al., 1990, Nose et al. 1990).

Our previous work has established that N-cadherin is present at the surface of embryonic chick neural retina cells as part of a particulate complex containing an N-acetylgalactosaminyl-phosphotransferase. This enzyme catalyzes the transfer of N-acetylgalactosaminephosphate residues to an O-linked oligosaccharide chain on N-cadherin (Balsamo et al., 1986a; Balsamo and Lilien, 1990). The GalNAcPTase is present at the cell surface and has a denatured molecular mass of ~220,000 D and an isoelectric point of ~5.0. Like N-cadherin, the GalNAcPTase is protected from trypsin degradation in the presence of calcium (Balsamo, J., unpublished observations).

Based on its association with N-cadherin and its presence at the surface of adhesive competent cells, we have postulated that the GalNAcPTase is involved in the process of calcium-dependent cell–cell adhesion (Balsamo and Lilien, 1983, 1990). In this manuscript we report that antibodies directed against the cell surface GalNAcPTase inhibit calcium-dependent cell–cell adhesion. In addition, anti-GalNAcPTase antibodies inhibit N-cadherin–mediated adhesion to purified N-cadherin or to immobilized anti-N-cadherin antibody. The GalNAcPTase, thus, appears to act as a modulator of N-cadherin function. Coordinate with inhibition of adhesion, anti-GalNAcPTase antibodies cause a change in the association of the N-cadherin/GalNAcPTase complex with α-actin, with a concomitant change in the sedimentation characteristics of the complex. We suggest that the role of the GalNAcPTase in calcium-dependent cell adhesion is to modulate the association of N-cadherin with the cytoskeleton.

Materials and Methods

Antibodies

Polyclonal anti-GalNAcPTase antibody RA, affinity-purified RA, and mAbs IB11 and 7A2, both of the IgM class, were generated as described (Scott et al., 1990). These antibodies recognize a band of ~220,000 D on immunoblots of retina cell homogenates fractionated on 7% SDS-PAGE. When a 5–6% gel is used, this band resolves into two closely adjacent bands and in some cases, one or more lower molecular weight species, presumably

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due to degradation (see Fig. 10). Fab' fragments were prepared from purified RA IgG or normal rabbit serum IgG using the Fab' purification protocol (Pierce Chemical Co., Rockford, IL). mAb was precipitated from serum-free hybridoma tissue culture supernatant by 50% saturated ammonium sulfate, resuspended in 20 mM phosphate buffer, pH 7.2, containing 1.5 M NaCl, and dialyzed successively against the same buffer with 1.5 M NaCl, 0.5 M NaCl, and 0.15 M NaCl, and then against a buffer containing 20 mM Hepes, pH 7.2, 0.15 M NaCl, 2 mM glucose, 3 mM KCl, and 1 mM Ca (HBSGKCa). The final solution was clarified by centrifugation at 15,000 g, and concentrated in a centriprep (Amicon Corp., Grace Co., Danvers, MA) to ~10 mg protein/ml. The anti N-cadherin hybridoma cell line, NCD-2, was generously provided by M. Takeichi (Kyoto University, Kyoto, Japan) and maintained in our laboratory. This antibody was also precipitated from culture medium as described above, leaving out the high salt. As controls for the monoclonal IgGs, either a commercial mouse IgM was used (Sigma Chemical Co., St. Louis, MO) or IgM was prepared as described above from an unrelated hybridoma (AH6; Falkner et al., 1981). Commercially obtained rat IgG (Sigma Chemical Co.) was added to hybridoma culture medium and processed in parallel with NCD-2 for control purposes. Polyclonal anti-NCAM IgG was a generous gift from Urs Rutishauser (Case Western Reserve University, Cleveland, OH). Monoclonal anti-α actin, a mouse IgG, was purchased from Amer sham Corp. (Arlington Heights, IL) and used at a dilution of 1:1,000 for immunoblots and 1:50 for immunoprecipitation. Polyclonal anti-Cl is an antibody prepared against the carboxy terminus of the cadherin molecule (Crittenden, S. L., E. Napolitano, K. Ventstrom, L. Reichardt, and J. Lilien, unpublished observations).

**Adhesion Assays**

The cell adhesion assay used was based on that originally described by Wallner et al. (1973). Labeled probe cells were prepared by incubating intact 2-day-old chick neural retina tissue overnight (1 retina/ml), in leukine-free FI2 medium (Gibico Laboratories, Grand Island, NY) supplemented with 10 μCi of [3H-Leucine (53 Ci/mmol Dupont NEN, Boston, MA), at 37°C and 40 rpm, under an atmosphere of 10% CO2. Single cells were prepared from the labeled tissues by trypsin treatment in the presence of calcium (Grunwald et al., 1980). Target monolayer cultures were prepared by plating single cells prepared by trypsin dissociation in the absence of calcium (Grunwald et al., 1980) in 24-well tissue culture plates (106 cells/ml/well), in FI2 medium supplemented with 2 mg/ml glucose, 2 mM glutamine (Gibco Laboratories), 1× nonessential amino acids (Gibco Laboratories), and 50 μg/ml gentamicin (Sigma Chemical Co.). After incubation for 16 h under an atmosphere of 10% CO2, the cells formed a confluent monolayer, covering the bottom of the well.

To measure cell–cell adhesion, probe cells were resuspended in HBSGKCa containing the appropriate concentration of antibody (0.25 × 106 cells/200 μl of HBSGKCa/well) and incubated with target cells for 30 min at 37°C, under 10% CO2. Unbound cells were removed by aspiration and the wells were washed vigorously three times with 1 ml/well HBSGKCa containing 1% BSA. Bound cells were removed by solubilization in 1 M NaOH, and radioactivity was determined by liquid scintillation counting. Under these conditions, up to 105 cpm, or 50% of the added cells, remain bound to the monolayer. Each datum point represents the average of at least three measurements and standard errors are presented in the figures.

A similar protocol was used to measure adhesion of probe cells to proteins immobilized on tissue culture dishes. N-cadherin was purified from a crude plasma membrane preparation (Balsamo and Lilien, 1983). Membranes from ~400, 10-day embryonic chick retinas were extracted overnight in high salt buffer to dissociate N-cadherin from its associated compounds: 50 mM Tris, pH 8.0, containing 0.5 M KCl, 1% Emulgen (polyoxyethylene alkylaryl ether; Kato, Japan), protease inhibitors (50 μg/ml each antipain, aprotinin, leupeptin, chymostatin, and pepstatin, 1 mM PMSF, 3 mM 1,10-phenanthroline), and 50 μg/ml DNAse (all from Sigma Chemical Co.). The extract was clarified by centrifugation at 100,000 g, the KCl concentration reduced to minimize nonspecific associations, and the antigen-antibody complex was eluted in SDS sample buffer (3% SDS, 10% mercaptoethanol, 0.5% Tween 20 and one time with 1 M MgC12 in order to minimize nonspecific associations), and the antigen-antibody complex was eluted in SDS sample buffer (3% SDS, 1% β-mercaptoethanol, 0.125 M Tris, pH 6.8, and 10% glycerol). The nitrocellulose (Immobilon; Millipore Continental Water Systems, Bedford, MA) replicas of a 7.5% SDS-PAGE were prepared as described (Balsamo and Lilien, 1990) and reacted with a 1:1,000 dilution of antiactin antibody, a 1:100 dilution of 1BII, and a 1:100 dilution of antibodies for 30 min at room temperature. The wells were then washed 4× with 1% BSA in HBSGKCa and incubated with HRP-conjugated goat anti-mouse IgM (HRP-GAM-IgM; Cooper Biomedical, West Chester, PA) for another 30 min at room temperature. After extensive washing, the wells were incubated with HRP substrate (o-phenylenediamine) and binding was analyzed by measuring absorbance at 450 nm. Results represent the average of triplicate samples. Standard errors are included in the figure.

**Preparation of N-Cadherin/GalNAcPTase Complexes from Neural Retina Tissues and Immunoprecipitation with NCD-2**

Neutral retina cell homogenates enriched for N-cadherin/GalNAcPTase complexes were prepared as described (Balsamo et al., 1986b; Balsamo and Lilien, 1990). Aliquots of equal volume were incubated with control rat IgG or NCD-2 overnight at 4°C. Protein G coupled to agarose beads (Pharmacia-LKB, Piscataway, NJ) was used to precipitate the immunocomplexes. The pellets were washed three times with TBS (20 mM Tris, pH 8, 150 mM NaCl), containing 0.5% Tween 20 and one time with 1 M MgC12 in order to minimize nonspecific associations, and the antigen-antibody complex was eluted in SDS sample buffer (3% SDS, 1% β-mercaptoethanol, 0.125 M Tris, pH 6.8, and 10% glycerol). The nitrocellulose (Immobilon; Millipore Continental Water Systems, Bedford, MA) replicas of a 7.5% SDS-PAGE were prepared as described (Balsamo and Lilien, 1990) and reacted with a 1:1,000 dilution of antiactin antibody, a 1:100 dilution of 1BII, and a 1:100 dilution of antibodies for 30 min at room temperature. The wells were washed 4× with 0.1% BSA in HBSGKCa and incubated with HRP-conjugated goat anti-mouse IgM (HRP-GAM-IgM; Cooper Biomedical, West Chester, PA) for another 30 min at room temperature. After extensive washing, the wells were incubated with HRP substrate (o-phenylenediamine) and binding was analyzed by measuring absorbance at 450 nm. Results represent the average of triplicate samples. Standard errors are included in the figure.
Figure 2. Adhesion of probe cells to cell layers. The assay was performed as described in Materials and Methods in the presence of the indicated additives. Adhesion was calculated as percent of control values. Approximately 50% of the cells added attached to the monolayer under control conditions. The data are representative of eight experiments. Each value represents the average of three measurements. (a) Effect of added EGTA (10 mM), NCD-2, or anti-GalNAcPTase antibodies (1 mg/ml). (Co) Control, no addition; (IgM) control mouse IgM at 1 mg/ml; (IgG) control rat IgG at 1 mg/ml. (b) Adhesion in the presence of increasing concentrations of RA Fab' fragments.

Treatment of Intact Cells with Anti-GalNAcPTase Antibodies and Cytochalasin B

To test the effect of the anti-GalNAcPTase antibodies on the association between the N-cadherin/transferase complex and actin, we compared the effect of preincubating intact cells with 1B11, 7A2, the control IgM, AH6, and cytochalasin B. Cells prepared by trypsin dissociation in the presence of calcium (Grunwald et al., 1980) were incubated in HBSGKCa containing 1 mg/ml antibody or 1 μg/ml cytochalasin B for 30 min at 37°C (×10⁶ cells/ml in a 10-ml erlenmeyer rotated at 120 rpm). The cells were collected by centrifugation, washed once in HBSGKCa, and homogenized in 20 mM Hepes buffer, pH 7.2, containing 150 mM NaCl, 1% Triton X-100 (HST), DNAse (50 μg/ml), and the protease inhibitors named above. The homogenates were kept on ice for 30 min, centrifuged at 50,000 g and the supernatant was incubated with antibacterial agent for 4 h at room temperature. Immunocomplexes were recovered by incubating the reaction mixture with 50 μl of goat anti-mouse IgM attached to Agarose (Sigma Chemical Co.), washed, and eluted as described above. The solubilized samples were fractionated by SDS-PAGE, the proteins transferred to Immobilon and reacted with either 1B11 or NCD-2 as described (Balsamo and Lilien, 1990).

To test the solubility of the GalNAcPTase after treatment of intact cells...
Anti-GalNAcPTase Antibodies Inhibit Both EGTA and the anti-N-cadherin antibody NCD-2 inhibit the attachment of retina probe cells to a retina cell monolayer. This indicates that the assay reflects calcium-dependent, N-cadherin-mediated adhesion (Fig. 2 a). Anti-GalNAcPTase antibodies are also potent inhibitors of adhesion in this assay. Three such antibodies were tested: one polyclonal (RA) used as Fab fragments or affinity-purified IgG, and two monoclonal mouse IgMs (IB11 and 7A2). Inhibition by RA Fab fragments is dose dependent with a plateau at 2 mg/ml (Fig. 2 b). Of the two mAbs tested, only one, IB11, is inhibitory (Fig. 2 a). IB11 and NCD-2 are inhibitory when reacted with just probe cells or just target cells (Fig. 3), indicating that the two cell populations are equivalent in their adhesive properties.

The lack of effect of 7A2 on adhesion is not due to a lack of binding; both 7A2 and IB11 show equivalent binding to cell surfaces (Fig. 4 a). This indicates that bound antibody alone has no effect and that 7A2 recognizes an epitope distinct from that recognized by IB11. To further eliminate the possibility that binding of IB11, but not 7A2, masks a site on N-cadherin necessary for adhesion, we measured the binding of NCD-2 to the cell surface in the presence of saturating amounts of bound IB11. As NCD-2 is an inhibitory antibody, we presumed that it recognizes an active site of N-cadherin (Hatta et al., 1988). Binding of saturating amounts of IB11 to probe cells does not interfere with binding of NCD-2, and vice versa, thus, IB11 does not mask the adhesive site on N-cadherin (Fig. 4 b).

**Inhibition of Adhesion by Anti-GalNAcPTase Antibodies Is through an Effect on N-Cadherin**

To determine whether the GalNAcPTase is directly involved in the formation of adhesive bonds or exerts its effect via N-cadherin, we tested the effect of IB11 on attachment of adhesive competent cells to affinity-purified N-cadherin or the anti-N-cadherin antibody, NCD-2, immobilized on tissue culture dishes. Adhesion of cells to immobilized N-cadherin is inhibited by NCD-2 (Fig. 5). This is true whether the antibody is included in the assay or bound to the cells before the assay (not shown), indicating that cell surface N-cadherin is essential for adhesion to immobilized N-cadherin. IB11 (but not 7A2) and RA (used as purified IgG or affinity purified) also inhibit the adhesion of cells to N-cadherin. Similar results are obtained when NCD-2 is used as a substrate instead of N-cadherin (Fig. 5). These results indicate that the anti-GalNAcPTase antibody disrupts N-cadherin-mediated adhesion. On the other hand, adhesion of cells to substrates unrelated to N-cadherin, such as untreated tissue culture plastic or plastic coated with antibodies against the calcium-independent cell adhesion molecule NCAM, is not affected by IB11 or RA (Fig. 5).

The effect of IB11 and RA and N-cadherin-mediated adhesion is not due to crossreactivity of the anti-GalNAcPTase antibodies with N-cadherin. When these antibodies at 1 mg/ml are reacted with western transfers of purified N-cadherin, no reaction is seen (Fig. 1).

N-cadherin-mediated adhesion is not only dependent on N-cadherin interactions, but also on an intact actin network. As shown in Fig. 6, cell adhesion to immobilized N-cadherin or NCD-2 is inhibited by cytochalasin B. This is also true of adhesion of cells to immobilized anti-NCAM and clearly distinguishes the effect of IB11 from cytochalasin B (Figs. 5 and 6). In contrast, adhesion to Poly-L-lysine-coated substrates is minimally affected by cytochalasin B (Fig. 6). These data suggest that recognition via a specific receptor system is only one part of the process of forming a measurable (i.e., stable) adhesion (see Lotz et al., 1989; Jaffe et al., 1990).

**Binding of Anti-GalNAcPTase Antibodies to Intact Cells Alters the Association of N-Cadherin/GalNAcPTase Complexes with Actin**

The results presented above indicate that the anti-GalNAcPT-
Figure 5. Adhesion of probe cells to immobilized proteins: effect of anti-GalNAcPTase antibodies. Attachment of probe cells to protein-coated tissue culture plastic. Assays were performed in the presence of the indicated additions at 1 mg/ml. Data are representative of six experiments and represent the average of three measurements. (Co) Control, no additions.

ase antibody, 1B11, inhibits calcium-dependent, N-Cadherin-mediated adhesion via an indirect mechanism and that cytoskeletal integrity is essential to the formation of those adhesions. Prior evidence also points to the importance of the cytoskeleton in N-cadherin function (Hirano et al., 1987; Nagafuchi and Takeichi, 1988). This suggested to us the possibility that binding of 1B11 affects N-cadherin function by altering its association with the cytoskeleton. As a first approach, we assayed for the presence of actin in the N-cadherin/GalNAcPTase complexes. Neural retina cell homogenates enriched for N-cadherin/GalNAcPTase complexes (Balsamo et al., 1986; Balsamo and Lilien, 1990) were immunoprecipitated with anti-N-cadherin antibody, the resulting precipitate was fractionated by SDS-PAGE, and western transfers were reacted with antiactin antibody. Actin is indeed present in the complex (Fig. 7, lane e). This result is not due to a nonspecific association between actin and NCD-2, as nonimmune rat IgG used as control does not immunoprecipitate actin (Fig. 7, lane f). Immunoblots with 1B11 (Fig. 7, lanes a and b) and anti-C1 (Fig. 7, lanes c and d) confirm the presence of the GalNAcPTase and N-cadherin in the NCD-2 immunoprecipitate. The anti-C1 antisera also recognizes two other molecules at ~100,000 and 112,000 kD (Crittenden, S. L., and E. Napolitano, K. Venstrom, L. Reichardt, and J. Lilien, unpublished results).

We next examined whether disruption of the actin net-

Figure 6. Effect of cytochalasin B on the attachment of probe cells to cell layers and coated tissue culture plastic. Tissue culture wells were coated with a target cell monolayer, NCD-2, purified N-cadherin, anti-NCAM IgG, or poly-L-lysine (PLL). Adhesion was measured as percent of control values. (Co) No addition; (CB) adhesion in the presence of 1 μg/ml cytochalasin B.
work by cytochalasin B altered the association between the N-cadherin/Transferase complex and actin. Intact cells were incubated in the presence of cytochalasin B for 30 min. The cells were then washed, homogenized, and the 50,000-g supernatant fraction was immunoprecipitated with antiactin antibody. The immunoprecipitates were fractionated on SDS-PAGE, transferred to nitrocellulose, and reacted with IB11, anti-C1, or anti-α-actin antibody. Numbers to the left of the figure represent the migration of molecular weight standards (×10^3). (lanes a, c, and e) Immunoprecipitation with NCD-2; (lanes b, d, and f) immunoprecipitation with control rat IgG.

To investigate whether IB11 has an effect comparable to that of cytochalasin B on the association between N-cadherin/Transferase and actin, an experiment similar to the one described above was performed. Intact cells were incubated for 30 min in the presence of IB11, 7A2, or control IgM. The cells were then washed free of unbound antibody, homogenized, and the 50,000 g supernatant fraction was incubated with antiactin antibody. (The anti-actin antibody is in large excess over surface-bound IB11 and 7A2; thus, the anti-IgM-conjugated Agarose will preferentially react with antiactin-antigen complexes.) The immunoprecipitates were fractionated on SDS-PAGE, transferred to nitrocellulose, and reacted with IB11 or NCD-2. The results are illustrated in Fig. 9; after preincubation of cells with IB11, but not 7A2 (which is also bound to the cell surface; Fig. 4) or control IgM, neither N-cadherin nor GalNAcPTase coprecipitates with actin (compare lanes c and f with a, b, d, and e). However, the association between N-cadherin and GalNAcPTase is not disrupted by either cytochalasin B or IB11. The GalNAcPTase and N-cadherin coprecipitate when cells preincubated with cytochalasin B or IB11 and homogenized as described for Figs. 8 and 9 are immunoprecipitated with NCD-2 (results not shown).

To further demonstrate that treatment of cells with IB11 results in a change in the properties of the GalNAcPTase/N-cadherin complexes, the 50,000-g supernatant fractions from homogenates of cells preincubated in the presence of IB11, 7A2, or AH6 were centrifuged at 100,000 g. Under control conditions the GalNAcPTase present in the 50,000-g supernatant is totally pelletable (Fig. 10 a, b, d, and e). However, after treatment of cells with IB11 the GalNAcPTase remains in the 100,000-g supernatant (Fig. 10, c and f). In the SDS-PAGE system used in this experiment (6% polyacrylamide), a doublet is seen at the 220,000 position. Two additional, lower molecular weight bands are seen in the
We have shown that antibodies to the embryonic chick neural retina GalNAcPTase perturb calcium-dependent cell-cell adhesion. Two alternative types of interactions could account for this inhibition: first, inhibition due to a direct interference with the formation of adhesive bonds by masking of adhesive sites at the cell surface, and second, inhibition due to the GalNAcPTase antibodies on the sedimentation properties of the GalNAcPTase. Cells incubated in the presence of Ab6 (a and d), 7A2 (b and e), or IB11 (c and f) were homogenized in buffer containing Triton-X-100, and the postnuclear supernatant fluid was centrifuged sequentially at 50,000 and 100,000 g. The 100,000 g pelletable and soluble fractions were fractionated on a 6% SDS polyacrylamide gel, transferred to Immobilon, and reacted with IB11. A doublet is seen at 220,000 under these conditions. (a-c) Soluble fraction; (d-f) pelletable fraction.

100,000-g precipitate, probably the result of protein degradation.

**Discussion**

We have shown that antibodies to the embryonic chick neural retina GalNAcPTase perturb calcium-dependent cell-cell adhesion. Two alternative types of interactions could account for this inhibition: first, inhibition due to a direct interference with the formation of adhesive bonds by masking of adhesive sites at the cell surface, and second, inhibition due to the modulation of the activity of the calcium-dependent cell-cell adhesion molecule, N-cadherin. We are able to eliminate the first alternative; under conditions where adhesion is clearly mediated by N-cadherin/N-cadherin interactions or by N-cadherin/NCD-2 interactions, anti-GalNAcPTase antibodies are inhibitory. Furthermore, inhibition is not due to masking the adhesive domain of N-cadherin, as demonstrated by the binding of NCD-2 in the presence of anti-GalNAcPTase antibodies.

Anti-GalNAcPTase antibodies appear to inhibit calcium-dependent adhesion by modulating the activity of N-cadherin. It is conceivable that modulation results from glycosylation by the GalNAcPTase. However, glycosylation is not affected by the antibodies used in this study (our unpublished observations) and inclusion in the adhesion assay of UDP at concentrations that inhibit catalysis or UDP-GalNAc at concentrations that stimulate catalysis has no effect on adhesion (results not shown). We conclude that glycosylation at the cell surface is not involved. It is, however, possible that a glycosylation step by the GalNAcPTase prior to expression of N-cadherin at the cell surface is crucial to its function.

These results distinguish the role that the GalNAcPTase plays in cell-cell adhesion from that played by the Galactosyltransferase. In all cases where the role of the Galactosyltransferase has been investigated, it is directly involved in the formation of adhesive bonds and sugar transfer to the receptor (acceptor) inhibits or reverses the interaction (reviewed by Shur, 1989).

Our data suggest that anti-GalNAcPTase antibodies may interfere with the association of N-cadherin with the cytoskeleton and thus modulate activity. We found that the N-cadherin/GalNAcPTase complexes contain actin and, on binding of IB11, but not 7A2, to intact cells, actin is dissociated from the complex. Coincident with the loss of actin there is a change in the sedimentation properties of the complexes and a loss of adhesion. These data are consistent with the observation that N- and E-cadherin codistribute with the actin-containing cytoskeleton (Hirano et al., 1987) and that the cytoplasmic carboxy terminus of E-cadherin is essential to function, although its deletion does not alter exposure at the cell surface (Nagafuchi and Takeichi, 1988). E-cadherin has also recently been shown to be tightly associated with the submembranous fodrin network in MDCK cells, providing further evidence of direct association of cadherins with the cytoskeleton (Nelson et al., 1990).

Anti-GalNAcPTase antibodies and cytochalasin B both alter the state of the cytoskeleton, but they clearly have distinct effects. IB11 does not inhibit adhesion mediated by NCAM, yet cytochalasin B does. While cytochalasin B affects the state of actin polymerization resulting in absence of intact filaments, the anti-GalNAcPTase antibodies most likely function by uncoupling intact filaments from the cytoplasmic domain of N-cadherin. Such an uncoupling may be a result of changes at the carboxy terminus of N-cadherin induced by interaction with transferase, or it may be the result of signals that alter the state of the cytoskeleton, resulting in release of a select population of cell surface receptors. We are at present addressing the molecular mechanics of this process.

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