# The Retina Cell-Surface *N*-Acetylgalactosaminylphosphotransferase Is Anchored by a Glycophosphatidylinositol†

Janne Balsamo and Jack Lilien*

*Department of Biological Sciences, Clemson University, Clemson, South Carolina 29631*

*Received April 14, 1993; Revised Manuscript Received May 28, 1993*

ABSTRACT: A polypeptide with *N*-acetylgalactosaminylphosphotransferase (GalNAcPTase) activity is present at the cell surface in stable association with adhesion molecules of the cadherin family. Antibodies directed against the GalNAcPTase inhibit homophilic cadherin-mediated adhesion and cadherin-mediated neurite outgrowth. We have determined that the GalNAcPTase is anchored at the cell surface by a glycophosphatidylinositol linkage. Treatment of intact cells with phosphatidylinositol-specific phospholipase C (PI-PLC) releases active GalNAcPTase and abolishes the ability of anti-GalNAcPTAse antibodies to modulate cadherin-mediated adhesion or neurite outgrowth. Furthermore, GalNAcPTase released from cells by PI-PLC is recognized by anti-CRD antiserum and is radiolabeled in cells incubated with [$^{14}$C]-ethanolamine.

We have characterized a cell-surface polypeptide with *N*-acetylgalactosaminylphosphotransferase (GalNacPTase)[1] activity (Balsamo & Lilien, 1982; Balsamo et al., 1986). It exists in stable, noncovalent association with the calcium-dependent cell–cell adhesion molecules N- and E-cadherin and cytoskeletal actin (Balsamo & Lilien, 1990; Balsamo et al., 1991; Bauer et al., 1992). Perturbation of the GalNAcPTase with appropriate antibodies results in complete inhibition of homophilic cadherin-mediated adhesion among neural retina cells (Balsamo et al., 1991) and pancreatic islet cells (Bauer et al., 1992). Concomitant with inhibition of adhesion, binding of inhibitory anti-GalNAcPTase antibodies to the cell surface results in release of cadherin from its association with the cytoskeleton (Balsamo et al., 1991). As the association of cadherin with the cytoskeleton is essential to its adhesion function (Nagafuchi & Takeichi, 1988; Kintner, 1992), this may be the basis for the inhibitory effect of anti-GalNAcPTase antibodies.

Perturbation of the GalNAcPTase on neuronal cells cultured on surfaces coated with adhesion receptors in three distinct families results in complete inhibition of neurite extension (Gaya-Gonzalez et al., 1991). This indicates that the GalNAcPTase modulates the activity of several distinct receptor types, possibly by modulating their association with the cytoskeleton (Balsamo et al., 1991).

In this paper, we report that the GalNAcPTase is anchored via a GPI tail and that the ability of anti-GalNAcPTase antibodies to modulate adhesion and neurite outgrowth is abolished on treatment of cells with phosphatidylinositol-specific phospholipase C.

## MATERIALS AND METHODS

*Materials.* *p*-(Chloromercuri)benzoate and protease inhibitors were purchased from Sigma Chemical Co. (St. Louis, MO). Purified phosphatidylinositol-specific PLC (PI-PLC) was a gift from Martin Low (College of Physicians and Surgeons, Columbia University, New York, NY). [$^{14}$C]-Ethanolamine (1 mCi/mmol, 0.5 mCi/mL), L-[$^{3}$H]methionine (15 Ci/mmol, 1 mCi/mL), and UDP-[$^{3}$H]GalNAc (8.3 Ci/mmol, 0.1 Ci/mL) were obtained from Du Pont—New England Nuclear. NHS-LC–biotin and HRP–streptavidin were products of Pierce Chemical Co.

*Antibodies.* Anti-CRD is a polyclonal rabbit IgG raised against the cross-reacting determinant from VSG (variant surface glycoprotein of *Trypanosoma brucei*) and was purchased from Oxford Glycosystems (Rosedale, NY). Anti-$\alpha$-actin is a mouse monoclonal IgM from Amersham Corp. 1B11 is a mouse monoclonal IgM specific to the *N*-acetylgalactosaminylphosphotransferase prepared in our laboratory (Balsamo et al., 1991; Scott et al., 1989). NCD-2 is a rat monoclonal IgG specific for N-cadherin and was originally a gift from M. Takeichi (Kyoto University, Japan). Goat anti-mouse IgM and goat anti-rat IgG conjugated to magnaspheres were products from Advanced Magnetics, Inc. (Cambridge, MA). HRP-conjugated second antibodies used in immunoblots were from Cappel Research Products (Durham, NC).

*Preparation of Single Cells and Culture Conditions.* Neural retina cells were prepared by trypsin dissociation of embryonic tissues (embryonic age 10 days) in the presence (CaT cells) or absence (Trp cells) of 1 mM calcium, as described (Grunwald et al., 1980). Cell monolayers were prepared by plating Trp cells on 24-well plates, at a concentration of $5 \times 10^5$/well in F12 medium supplemented with 2 mM glutamine and 50 $\mu$g/mL gentamicin, and incubating overnight at 37 °C and 5% $CO_2$.

*Assay for GalNAcPTase Activity.* CaT cells at a concentration of $\sim 10^7$/mL were incubated in 100 $\mu$L of HEPES buffered saline (HBSGKCa: 20 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM KCl, 2 mM glucose, and 1 mM $CaCl_2$) with added protease inhibitors (10 $\mu$g/mL each aprotinin, leupeptin, chymostatin, and pepstatin) and with increasing concentrations of PI-PLC for 30 min at 37 °C. To the 100000*g* supernatants was added 10 $\mu$L of a mixture of 100 mM HEPES, pH 7.6, 500 $\mu$g/mL asialoagalactofetuin, 20 $\mu$L/mL UDP-[$^{3}$H]-GalNAc, and 50 mM $MnCl_2$, and the assay mixtures were incubated for 1 h at 37 °C. Incorporation of radioactivity into macromolecular material was determined as described previously (Balsamo & Lilien, 1982; Balsamo et al., 1986).

† Supported by Grants EY 05860 and EY 08888 from the NIH to J.L.

[1] Abbreviations: GalNAcPTase, *N*-acetylgalactosaminylphosphotransferase; GPI, glycophosphatidylinositol; PI-PLC, phosphatidylinositol-specific phospholipase C; CRD, cross-reactive determinant; HRP, horseradish peroxidase; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; BSA, bovine serum albumin.

0006-2960/93/0432-8246$04.00/0 

Assay for the transfer of [$^3$H]GalNAc from [$^3$H]UDP-GalNAc to endogenous acceptors in cell extracts was carried out before and after treatment of cells with PI-PLC. The cells were homogenized in HST buffer (20 mM HEPES, pH 7.2, 150 mM NaCl, and 1% Triton X-100) and protease inhibitors as above. The homogenates were centrifuged at 13000*g* for 5 min, and aliquots of the supernatant solution were mixed with an equal volume of a solution containing HST, 4 mM $MnCl_2$, and 2 μL of UDP-[$^3$H]GalNAc. The mixture was incubated for 30 min at 37 °C, and the radioactivity incorporated into macromolecules was determined.

*Cell Labeling.* For surface labeling with biotin, Trp cell monolayers were washed in ice-cold HBSGKCa and incubated with 0.1 mg/mL freshly prepared NHS-LC–biotin, in HBSGKCa, for 30 min, on ice. Cell layers were washed 3 times with ice-cold HBSGKCa before digestion with phospholipases.

For adhesion assays, labeled CaT cells were prepared from whole retinas incubated overnight in methionine-free Eagle's basal medium with added [$^3$H]methionine.

For biosynthetic labeling of glycophospholipids, Trp cells were plated at a density of $10^8$/10 cm$^2$ petri plate, in 5 mL of F12 medium containing 20 μCi/mL [$^{14}$C]ethanolamine. After about 24 h, the cells were harvested with a Pasteur pipet, washed, and prepared for immunoprecipitation.

*Assays for Adhesion and Neurite Extension.* $^3$H-Labeled CaT cells (~$10^6$/100 μL) were suspended in HBSGKCa containing 100 μg of BSA/mL and 10 μg/mL aprotinin and leupeptin, and incubated with or without PI-PLC (5 units/mL) for 3 min at 30 °C. Cells were then washed in HBSGKCa/BSA and incubated in the appropriate antibody dilution for 15 min at 4 °C. Binding of labeled cells to NCD-2-coated wells was assayed as described previously (Balsamo et al., 1991). To analyze the effect of PI-PLC on neurite outgrowth, unlabeled CaT cells were plated on NCD-2- or laminin-coated 96-well plates (100 μg/well) after incubation with 5 units/mL PI-PLC or buffer control for 30 min at 30 °C. Approximately 2 h after being plated, medium containing the appropriate antibody was added and neurite extension observed after 24 h. Culture medium was F12 containing 1% ITS (insulin, transferrin, and selenium) and 50 μg/mL gentamycin.

*Immunoprecipitation, Electrophoresis, and Immunoblotting.* Cells were lysed in HST buffer containing 10 μg/mL each of aprotinin, leupeptin, chymostatin, and pepstatin, 100 μg/mL DNase, and 15 mM 1,10-orthophenanthroline (HSTI). Lysates were centrifuged at 14000*g* for 5 min, to remove insoluble material. Possible protein complexes were disrupted by making the lysate 1% with 20% SDS and heating in a boiling water bath for 5 min; the SDS concentration was then brought down to 0.1% with HST. GalNAcPTase was immunoprecipitated from cell lysates by incubation with ~10 μg/mL 1B11 for 4 h at 4 °C under gentle agitation, followed by 1-h incubation with a slurry of goat anti-mouse IgM covalently linked to magnetic spheres. The precipitate was washed 3 times with immunomix (1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% SDS in 20 mM Tris, pH 8.0), 3 times in 0.1× immunomix, once in 1 M $MgCl_2$, and once in 20 mM Tris, pH 8.0. Immunoprecipitated material was eluted from the magnaspheres by boiling in SDS sample buffer and submitted to SDS–PAGE. Gels containing $^{14}$C-labeled proteins were treated with Amplify (Amersham Corp.), dried, and fluorographed. Gels containing biotinylated or unlabeled proteins were transferred to Immobilon (Millipore Corp.) and reacted with HRP–streptavidin or with the desired antibody

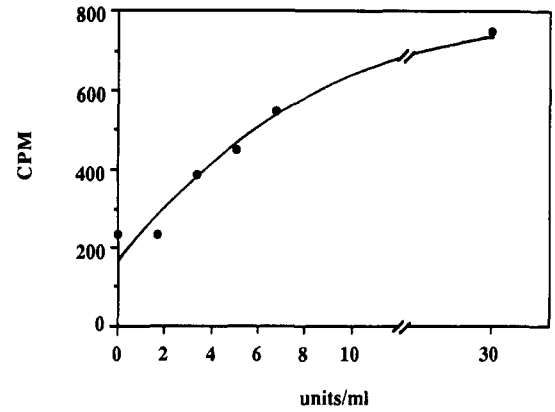


FIGURE 1: Activity of GalNAcPTase released by PI-PLC. Equal numbers of CaT cells were incubated with increasing concentrations of PI-PLC. After 30 min at 30 °C, the reaction mixtures were centrifuged at 100000*g* and the supernatants assayed for GalNAcPTase activity as described under Materials and Methods.

followed by the appropriate HRP-conjugated second antibody. The blots were developed with the ECL chemiluminescent system from Amersham.

To assay for coprecipitation of GalNAcPTase with N-cadherin and α-actin, crude membrane fractions were prepared from 10-day embryonic retinas. Approximately 10 retinas were homogenized in 2 mL of 0.1 M HEPES, pH 7.6, containing 10 μg/mL each aprotinin, leupeptin, chymostatin, and pepstatin and 100 μg/mL DNase. The postnuclear supernatant was centrifuged at 100000*g*; the membrane pellet was resuspended in the same buffer/inhibitors and incubated with or without PI-PLC (5 units/mL). After 30 min at 37 °C, the membranes were recovered by centrifugation at 100000*g* for 30 min, solubilized in HSTI, and immunoprecipitated with anti-α-actin antibody or NCD-2. The immunoprecipitates were recovered and analyzed as described above and immunoblotted with 1B11.

## RESULTS

*Active GalNAcPTase Is Released from Intact Cells or Cell Membranes by PI-PLC.* Single cells prepared by trypsin dissociation of tissues in the presence of calcium (CaT cells) retain the GalNAcPTase at their surface (Balsamo et al., 1991). Treatment of such cells with PI-PLC results in accumulation of GalNAcPTase activity in the supernatant solution and loss from the cell surface. The amount of activity accumulating in the supernatant solution is dependent on the concentration of PI-PLC as measured by the transfer of [$^3$H]-GalNAc from UDP-[$^3$H]GalNAc to an exogenous acceptor (Figure 1). Similar effects are observed when cell membranes are treated with PI-PLC (not shown). The total amount of GalNAcPTase activity was measured before and after PI-PLC treatment, and the released GalNAcPTase represents approximately 40% of the total activity (not shown). This agrees with our previous estimates of the amount of enzyme activity localized to the cell surface (Balsamo & Lilien, 1982).

The major form of the GalNAcPTase has an apparent molecular mass of approximately 224 000 Da. Immunoblots of material released from CaT cells by PI-PLC with anti-GalNAcPTase antibody reveal an immunoreactive species with the same molecular mass as that seen in intact tissue (Figure 2, compare lanes a and c). Release does not occur in the absence of PI-PLC (Figure 2, lane d) and is inhibited by *p*-(chloromercuri)benzoate (Figure 2, lane b), an inhibitor of *Bacillus thuringiensis* PI-PLC (Ikezawa & Taguchi, 1981; Low, 1989).

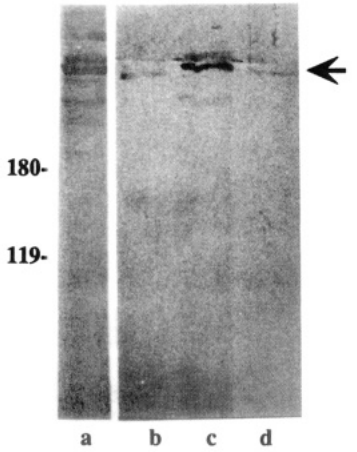


FIGURE 2: Release of GalNAcPTase by phosphatidylinositol-specific phospholipase C. Retina cell monolayers prepared in 24-well plates were incubated for 30 min, at 30 °C, with or without PI-PLC. Supernatants of three cultures were pooled, centrifuged at 100000*g*, concentrated, separated by SDS–PAGE, and transferred to Immobilon. Blots were probed with 1B11. Lane a, control immunoblot of homogenate of whole retina; lane b, PI-PLC plus *p*-(chloromercuri)-benzoate; lane c, 5 units/mL PI-PLC; lane d, no PI-PLC. Numbers to the left of the figure represent the migration of molecular weight markers ($\times 10^{-3}$). The boldface arrow indicates the position of the GalNAcPTase.

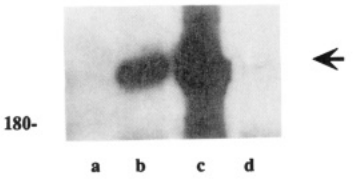


FIGURE 3: PI-PLC removes GalNAcPTase from the cell surface. Biotinylated CaT cells were incubated with or without PI-PLC for 30 min at 30 °C. The high-speed supernatants and the cell lysates were immunoprecipitated with 1B11, separated by SDS–PAGE, and transferred to Immobilon. Blots were probed with HRP–avidin and developed with the Amersham ECL system. Lane a, control supernatant; lane b, PI-PLC supernatant; lane c, control cells; lane d, PI-PLC cells. The boldface arrow indicates the position of the GalNAcPTase.

To demonstrate that the released GalNAcPTase activity originated at the cell surface, intact cells were labeled with NHS-LC–biotin, a reagent that does not permeate the cell membrane (Sargiacomo et al., 1989). The biotinylated cells were then treated with PI-PLC, and the GalNAcPTase released into the supernatant or remaining associated with the cells was precipitated with anti-GalNAcPTase antibody 1B11. All of the labeled GalNAcPTase is released into the supernatant following PI-PLC treatment. In control, untreated cells, all of the labeled GalNAcPTase remains at the cell surface; there is no immunoprecipitable GalNAcPTase in the supernatant (Figure 3).

*The Glycophospholipid Is Covalently Attached to the GalNAcPTase.* To demonstrate that the GalNAcPTase itself contains covalently bound phospholipid, retina cells were metabolically labeled with [$^{14}$C]ethanolamine. Cells were then lysed, and the GalNAcPTase was immunoprecipitated with anti-GalNAcPTase antibody 1B11. After separation of the immunoprecipitated material by SDS–PAGE and fluorography, a labeled polypeptide of approximately 220 000 Da is seen, indicating the presence of lipid covalently bound to the GalNAcPTase (Figure 4).

To further demonstrate the presence of a glycophospholipid tail associated with the GalNAcPTase, we made use of an antibody specific to the glycan moiety of the GPI anchor. This antibody, called anti-CRD for cross-reactive determinant,

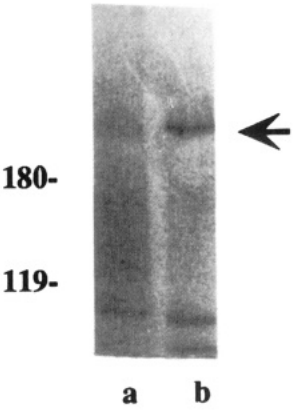


FIGURE 4: Labeling of GalNAcPTase with [$^{14}$C]ethanolamine. Retina cells were incubated overnight in culture medium containing labeled precursor. Labeled cells were homogenized and immunoprecipitated with 1B11, and the immunoprecipitates were separated by SDS–PAGE. Radioactive bands were detected by fluorography. Lane a, immunoprecipitation with control IgG; lane b, immunoprecipitation with 1B11. The boldface arrow indicates the position of the GalNAcPTase.

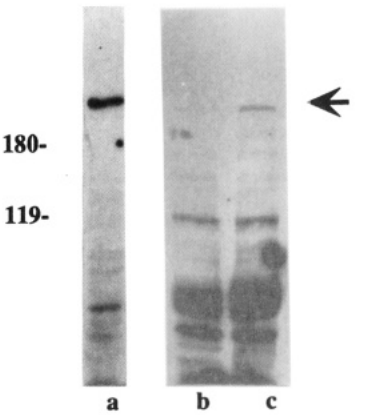


FIGURE 5: Presence of the "cross-reactive determinant" in GalNAcPTase released by PI-PLC. CaT cells were treated with PI-PLC; the released material was immunoprecipitated with 1B11, separated by SDS–PAGE, and immunoblotted with anti-CRD antibody. Lane a, control immunoblot of whole cell homogenate; lane b, immunoprecipitation with control IgG; lane c, immunoprecipitation with 1B11. The boldface arrow indicates the position of the GalNAcPTase. Lower molecular weight ($\times 10^{-3}$) bands seen in lanes b and c may be due to precipitating antibody which is cross-reactive with the secondary antibody.

is a polyclonal IgG raised against the soluble form of VSG (variable surface glycoprotein from *Trypanosoma brucei*) and has been shown to react with the glycan moiety of a number of GPI-anchored proteins (Low, 1989; Cross, 1990; Ferguson, 1988). Retina cells were treated with PI-PLC, the 100000*g* supernatant was immunoprecipitated with anti-GalNAcPTase antibody 1B11 (Figure 5, lane c) or control IgM (Figure 5, lane b), and the precipitate was fractionated by SDS–PAGE and immunoblotted with the anti-CRD antibody. The cross-reactive determinant is precipitable with anti-GalNAcPTase antibody (Figure 5, lane c, indicated with an arrow) but not by control IgM (figure 5, lane b). Anti-CRD antibody does not react with intact, untreated tissue (not shown). For comparison, Figure 5 lane a shows an immunoblot of intact tissue reacted with anti-GalNAcPTase antibody.

*PI-PLC Treatment of Retina Cells Affects N-Cadherin-Mediated Adhesion and Neurite Extension.* Retina cells prepared by trypsin dissociation in the presence of calcium are able to adhere to a substrate coated with anti-N-cadherin antibody NCD-2. Under these conditions, adhesion is dependent on the presence of calcium and is inhibited both by

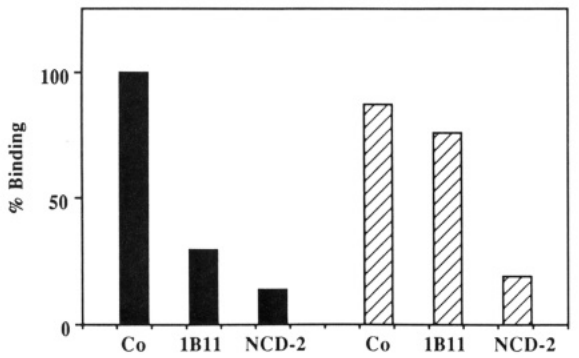


FIGURE 6: Effect of PI-PLC on N-cadherin-mediated adhesion. Control or PI-PLC-treated $^3$H-labeled CaT cells were assayed for adhesion to NCD-2-coated tissue culture dishes in the presence or absence of NCD-2 or 1B11. Binding was measured as the ratio of bound cpm to total cpm/well and expressed as the percent of control (untreated cells in the absence of perturbing antibodies). Control binding was around 80%. Results represent the average of three measurements. Solid bars, untreated cells; hatched bars, PI-PLC-treated cells. Co, adhesion in the presence of control IgG; 1B11, adhesion in the presence of 100 $\mu$g/mL anti-GalNAcPTase antibody; NCD-2, adhesion in the presence of 100 $\mu$g/mL anti-N-cadherin antibody.

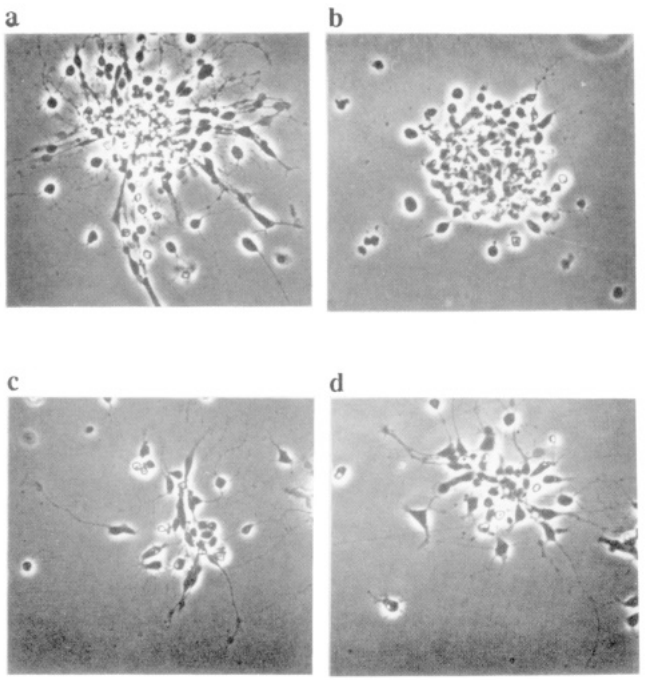


FIGURE 7: Effect of PI-PLC on neurite extension. Control or PI-PLC-treated CaT cells were cultured on NCD-2-coated 24-well plates and allowed to adhere for 2 h. Culture medium containing the indicated antibody was then added, and the cells were observed after 24 h. (a) Control cells, no antibody; (b) control cells incubated with 1B11; (c) PI-PLC-treated cells, no antibody; (d) PI-PLC-treated cells, incubated with 1B11.

NCD-2 and by anti-GalNAcPTase antibody 1B11 (Balsamo et al., 1991). After release of cell-surface GalNAcPTase by PI-PLC, there is no apparent loss of the ability of CaT cells to adhere to the NCD-2-coated substrate (Figure 6); however, following treatment with PI-PLC, anti-GalNAcPTase antibodies are no longer effective in blocking cadherin-mediated adhesion (Figure 6). Similarly, neurite outgrowth on a substrate prepared with the anti-N-cadherin antibody is unaffected by PI-PLC treatment, but anti-GalNAcPTase antibodies no longer inhibit neurite outgrowth after treatment of cells with PI-PLC (Figure 7). A similar result is observed when cells are plated on a laminin-coated surface; treatment of cells with PI-PLC does not abolish the ability of cells to

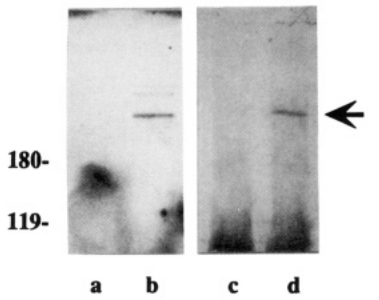


FIGURE 8: Effect of PI-PLC on the association between GalNAcPTase and N-cadherin and actin. Cell membrane preparations treated with PI-PLC or control buffer were immunoprecipitated with anti-N-cadherin antibody (lanes a and b) and with anti-$\alpha$-actin antibody (lanes c and d). The immunoprecipitates were fractionated by SDS–PAGE, transferred to Immobilon, and reacted with 1B11. Lanes b and c, control membranes; lanes a and d, PI-PLC treated. The boldface arrow indicates the position of the GalNAcPTase.

grow neurites, but anti-GalNAcPTase antibodies lose their inhibitory activity (not shown).

Consistent with the results of the experiments described above, after PI-PLC treatment of CaT cells the association between N-cadherin and the cytoskeleton is not affected, but the N-cadherin/cytoskeletal complex no longer contains GalNAcPTase. CaT cells or membranes were incubated with PI-PLC or control buffer, lysed in Triton X-100, and immunoprecipitated with anti-N-cadherin or anti-$\alpha$-actin antibodies; the immunoprecipitates were separated by SDS–PAGE, transferred to Immobilon, and reacted with anti-GalNAcPTase antibody 1B11. After treatment with PI-PLC, GalNAcPTase no longer coprecipitates with either-N-cadherin (Figure 8a,b) or $\alpha$-actin (Figure 8c,d).

## DISCUSSION

The results presented here indicate that the embryonic chick neural retina cell-surface GalNAcPTase is anchored in the membrane by a covalently linked glycophospholipid. Three criteria were used to establish that the GalNAcPTase is anchored via a GPI tail: (1) GalNAcPTase is released from intact cells or cell membranes by phosphatidylinositol-specific phospholipase C. This enzyme was isolated by Low et al. (Low, 1988; Henner et al., 1988) from *Bacillus thuringiensis* and has been used to solubilize GPI-anchored proteins in many different systems (Low, 1989). Release of GalNAcPTase does not appear to be due to contaminating proteases as released GalNAcPTase is not degraded and retains transferase activity, and release is not affected by the presence of a cocktail of protease inhibitors of different specificites. Furthermore, release is prevented by *p*-(chloromercuri)benzoate, an inhibitor of *B. thuringiensis* PI-PLC (Ikezawa & Taguchi, 1981; Cross, 1990). (2) [$^{14}$C]Ethanolamine is incorporated into the GalNAcPTase, demonstrating the presence of covalently bound lipid. (3) Released GalNAcPTase is recognized by an antibody directed against the cross-reactive determinant (CRD) of *Trypanosoma brucei*. This antibody is described as reacting with the carbohydrate epitope containing an inositol cyclic 1,2-phosphate moiety (Zamze et al., 1988) and has been used to confirm the presence of a GPI anchor in a number of proteins (Low, 1989; Cross, 1990; Ferguson, 1988).

In embryonic neural retina, the GalNAcPTase is associated with N-cadherin and the actin-containing cytoskeleton; the integrity of this association appears to be crucial for the formation and/or maintenance of stable adhesions (Balsamo et al., 1991). Perturbation of cells with the anti-GalNAcPTase antibody 1B11 releases N-cadherin from its association with

α-actin but does not interfere with the association between GalNAcPTase and N-cadherin (Balsamo et al., 1991). Unlike the antibody effect, treatment of intact cells with PI-PLC releases GalNAcPTase but does not affect the association of N-cadherin with α-actin. This is consistent with the fact that N-cadherin-mediated adhesion among PI-PLC-treated cells is only marginally affected but inhibition by 1B11 is abolished. Similarly, extension of neural processes on surfaces coated with anti-N-cadherin antibodies or laminin is minimally affected by removal of GalNAcPTase by PI-PLC, but inhibition of neurite extension of 1B11 is abolished.

The GalNAcPTase may regulate cell adhesion and neurite extension via a direct interaction with cadherin or through a "second party". GPI-anchored glycoproteins have been reported to be associated with tyrosine kinases, providing a potential mechanism for the transduction of a signal (Štefanorà et al., 1991). Kinase activity is stimulated by binding of antibody to GPI-anchored glycoproteins (Štefanorà et al., 1991). Furthermore, kinase activity is known to be involved in neurite extension mediated by N-cadherin (Doherty et al., 1991). We are at present pursuing the mechanism through which GalNAcPTase regulation of adhesion and neurite outgrowth occurs.

## REFERENCES


Balsamo, J., & Lilien, J. (1982) *J. Biol. Chem. 257*, 349–354.

Balsamo, J., & Lilien, J. (1990) *J. Biol. Chem. 265*, 2923–2928.

Balsamo, J., Pratt, R. S., & Lilien, J. (1986) *Biochemistry 25*, 5402–5407.

Balsamo, J., Thiboldeaux, R., Swaminathan, N., & Lilien, J. (1991) *J. Cell Biol. 113*, 429–436.

Bauer, E., Balsamo, J., & Lilien, J. (1992) *J. Cell Sci. 103*, 1235–1241.

Cross, G. A. M. (1990) *Annu. Rev. Cell Biol. 6*, 1–39.

Doherty, P., Rowett, L. H., Moore, S. E., Mann, D. E., & Walsh, F. S. (1991) *Neuron 6*, 247–258.

Ferguson, M. A. J. (1988) *Annu. Rev. Biochem. 57*, 285–320.

Gaya-Gonzalez, L., Balsamo, J., Swaminathan, N., & Lilien, J. (1991) *J. Neurosci. Res. 29*, 474–480.

Grunwald, G. B., Geller, R. L., & Lilien, J. (1980) *J. Cell Biol. 85*, 766–776.

Henner, D. J., Yang, M., Chen, E., Helmiss, R., Rodriguez, H., & Low, M. G. (1988) *Nucleic Acids Res. 16*, 10383–10389.

Ikezawa, H., & Taguchi, R. (1981) *Methods Enzymol. 71*, 731–741.

Kintner, C. (1992) *Cell 69*, 225–236.

Low, M. G. (1988) *J. Immunol. Methods 113*, 101–111.

Low, M. G. (1989) *Biochim. Biophys. Acta 988*, 427–454.

Nagafuchi, A., & Takeichi, M. (1988) *EMBO J. 7*(12), 3679–3684.

Sargiacomo, M., Lisanti, M., Graeve, L., Bivic, A. L., & Rodriguez-Boulan, E. (1989) *J. Membr. Biol. 107*, 277–286.

Scott, L. J. C., Balsamo, J., Sanes, J. R., & Lilien, J. (1989) *J. Neurosci. 10*, 346–350.

Štefanorà, I., Hořejší, V., Ansotegui, I. J., Knapp, W., & Stockinger, H. (1991) *Science 254*, 1061–1018.

Zamze, S. E., Ferguson, M. A. J., Collins, R., Dwek, R. A., & Rademacher, T. W. (1988) *Eur. J. Biochem. 176*, 527–534.