Endogenous Lectins from Cultured Cells: Subcellular Localization of Carbohydrate-binding Protein 35 in 3T3 Fibroblasts

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Abstract. In previous studies, a lectin designated as carbohydrate-binding protein 35 (CBP35) has been isolated from cultured 3T3 fibroblasts. In the present study, rabbit antibodies directed against CBP35 were used to analyze the subcellular distribution of CBP35 in 3T3 cells. Several lines of evidence indicate that CBP35 is found externally exposed at the cell surface: immunofluorescent staining of live 3T3 cells; agglutination of suspension of 3T3 fibroblasts by specific antibodies; and isolation, by immunoaffinity chromatography, of a Mr 35,000 component from cells surface-labeled with ^125I. In addition to the plasma membrane, CBP35 could also be found intracellularly, as revealed by immunofluorescence studies of fixed and permeabilized 3T3 cells. The staining pattern showed the presence of CBP35 on the nucleus and in the cytoplasm. These results are consistent with the finding that among several subcellular fractions, CBP35 can be found by immunoblotting procedures in the nuclear pellet, the soluble fraction, and the plasma membrane fraction of the postnuclear supernatant.

Materials and Methods

Cell Growth and Radiolabeling

The growth and maintenance of 3T3 fibroblasts has been previously described (33). Metabolic labeling of 3T3 cells with [35S]methionine (Amersham Corp., Arlington Heights, IL) was performed according to Roff and Wang (31).

Cell surface iodinations were performed using the iodogen method (16, 26). The 3T3 fibroblasts were grown to confluence in 100-mm tissue culture dishes in Dulbecco's modified Eagle's medium containing 10% (vol/vol) calf serum. Growth and maintenance of 3T3 fibroblasts has been previously described (33). Metabolic labeling of 3T3 cells with [35S]methionine (Amersham Corp., Arlington Heights, IL) was performed according to Roff and Wang (31).

Affinity Chromatography Procedures

Antiserum against CBP35 isolated from 3T3 cells was raised in New Zealand White female rabbits as previously described (31). This antiserum will be designated rabbit anti-CBP35. The immunoglobulin fraction of rabbit anti-CBP35 (15 mg), isolated by ammonium sulfate precipitation and DEAE-cellulose ion exchange chromatography, was dissolved in 4 ml of 0.1 M sodium bicarbonate buffer pH 8.0 (coupling buffer) and coupled to 1 ml Affigel 10 (Bio-Rad Laboratories, Richmond, CA). The column was then equilibrated with 2% (vol/vol) calf serum in coupling buffer to minimize nonspecific binding. Identical procedures were used to prepare affinity columns using the immunoglobulin fractions from rabbit anti-succinyl concanavalin A (19) and normal rabbit sera. Triton X-100 extracts of radioactively labeled cells were

Abbreviations used in this paper: CBP, carbohydrate-binding protein; LDH, lactate dehydrogenase; TK, 0.02 M Tris-HCl, 5 mM KCl buffer (pH 7.2) containing 105 mU/ml aprotinin.
Immunofluorescence

3T3 fibroblasts were removed from 100-mm tissue culture dishes by treatment with 5 mM EGTA in PBS on a rotary shaker for 1 h at 37°C. The cells were collected by centrifugation at 500 g for 3 min and resuspended in PBS to a final concentration of 2 × 10⁶ cells/ml. Serum samples (0.2 ml rabbit anti-CBP35 or preimmune) or concanavalin A (0.2 ml of a 50 μg/ml solution) was added to an equal volume of the cell suspension. After 10 min at room temperature, a drop of the solution was examined under the microscope for cell agglutination.

Agglutination Assay

3T3 fibroblasts were removed from 100-mm tissue culture dishes by treatment with 5 mM EGTA in PBS on a rotary shaker for 1 h at 37°C. The cells were collected by centrifugation at 500 g for 3 min and resuspended in PBS to a final concentration of 2 × 10⁶ cells/ml. Serum samples (0.2 ml rabbit anti-CBP35 or preimmune) or concanavalin A (0.2 ml of a 50 μg/ml solution) was added to an equal volume of the cell suspension. After 10 min at room temperature, a drop of the solution was examined under the microscope for cell agglutination.

Methods. After incubation of 3T3 cells seeded on a microscope slide coverslip were rinsed with ice-cold Dulbecco's modified Eagle's medium containing 0.5% (wt/vol) bovine serum albumin and buffered with 20 mM Hepes to pH 7.5 (buffer A). In some experiments 0.1 M lactose was included in buffer A to inhibit possible lectin binding to carbohydrate structures. The coverslip was then incoated on 200 ml of a 1:10 dilution of antisera in buffer A and incubated at 4°C for 2 h. Unbound primary antibody was removed by three 5-min incubations with buffer A at 4°C, and the cells were then incubated with 1:30 dilution of rhodamine conjugated goat anti-rabbit immunoglobulin (Miles Laboratories Inc., Elkhart, IN) in buffer A for 30 min at 4°C. Unbound fluorescent antibody was removed with three 10-min washes in buffer A, and the cells were immediately observed on a Leitz epiphase fluorescence microscope using a 50x water immersion objective. Pictures were taken using Kodak Tri-X Pan film.

Results

Characterization of Antibodies Against CBP35

The initial characterization of the rabbit anti-CBP35 involved the specific precipitation of CBP35 from a partially purified preparation of lectins (CBP35, CBP16, and CBP13.5) from 3T3 cells. This antisera was also shown to recognize CBP35 in Triton X-100 extracts of 3T3 cells and mouse lung tissue by immunoblotting techniques (13). When 3T3 cells were extracted with SDS (4% wt/vol) and the extracts were electrophoresed and immunoblotted with rabbit anti-CBP35, only a single polypeptide band (M, 35,000) was observed (Fig. 1, lane a). Parallel analysis with preimmune rabbit serum failed to yield this band (Fig. 1, lane b).

To complete the characterization of this antibody, it remained to be ascertained whether the monoclonal antisera would specifically recognize only CBP35 from a more complex, nonpuriﬁed, protein mixture. The immunoglobulin fraction of rabbit anti-CBP35 was coupled to Affigel 10 beads. A Triton X-100 extract of 3T3 cells labeled with 125I-methionine was prepared in a hypotonic buffer (5 mM phosphate buffer, 0.5% [vol/vol] Triton X-100, pH 8.0). This extract was then fractionated on the Affigel column containing rabbit anti-CBP35. In parallel, the extract was also fractionated on Affigel columns containing rabbit anti-succinyl concanavalin A or normal rabbit immunoglobulin. The polypeptides bound to the respective columns were examined by PAGE. The material bound and eluted from the rabbit anti-CBP35 col-
umn yielded three major components (Mr’s 35,000, 48,000 and 57,000) (Fig. 2, lane b). Two of these components (Mr’s 48,000 and 57,000) were also found in the material from the control columns containing anti-succinyl concanavalin A (Fig. 2, lane c) or normal rabbit immunoglobulin (Fig. 2, lane d). In contrast, the Mr 35,000 band, which co-migrated with an authentic sample of CBP35 (Fig. 2, lane a), was found only in the material bound by the column containing rabbit anti-CBP35. Therefore, under these conditions of extraction and fractionation, the anti-CBP35 column recognizes and binds specifically CBP35 out of a complex mixture of proteins present in the cell extract.

**Evidence for CBP35 at the Cell Surface**

Live 3T3 fibroblasts were stained with rabbit anti-CBP35 using indirect immunofluorescence. The staining and washings were carried out at 4°C to minimize internalization of the antibody by endocytosis. Weak fluorescent staining of the cell surface was observed. At different focal planes, the periphery of the cell was outlined by numerous fluorescent patches (Fig. 3, A and C), a rather characteristic pattern of cell surface staining in live cells. Staining the cells with normal rabbit serum did not result in any significant amount of labeling (Fig. 3 B). Similar results were obtained when these experiments were carried out in the presence of lactose (0.1 M) (Fig. 3, C and D). Therefore, the antigenic target detected by immunofluorescence staining with rabbit anti-CBP35 was probably not due to lectin, released by dead cells, that bound to cell surface glycoconjugates. These observations suggest the possibility that CBP35 can be detected on the external surface of 3T3 fibroblasts.

This conclusion was corroborated by the observation that 3T3 cells can be agglutinated by rabbit anti-CBP35. The 3T3

![Figure 2. SDS PAGE of 35S-labeled polypeptides bound on a column (1.5 x 2 cm) of Affigel 10 covalently derivatized with the immunoglobulin fraction of rabbit anti-CBP35. Triton X-100 extracts of 3T3 cells labeled with [35S]methionine (1.2 x 10^7 total cpm per column) were fractionated as detailed in Materials and Methods. The radioactive components bound to the column were eluted and subjected to PAGE. Lane a, authentic 35S-labeled CBP35 from 3T3 cells; lane b, polypeptides bound on rabbit anti-CBP35 column; lane c, polypeptides bound on rabbit anti-succinyl concanavalin A column; lane d, polypeptides bound on normal rabbit immunoglobulin column. Approximately 15,000 cpm were electrophoresed in lanes b–d, and the fluorogram was exposed for 2 d. The arrowhead on the left indicates the position of migration of CBP35. Molecular weight markers are indicated on the right.](image)

![Figure 3. Indirect immunofluorescence detection of the binding of rabbit anti-CBP35 to the surface of live 3T3 fibroblasts. The binding of the rabbit antibody (4°C, 2 h) was detected by rhodamine-labeled goat anti-rabbit immunoglobulin (4°C, 30 min). The figure shows the pattern of fluorescence labeling when the cells are stained with rabbit anti-CBP35 in the absence (A) or presence of 0.1 M lactose (C). B and D show staining with normal rabbit serum in the absence (B) or presence of 0.1 M lactose (D). Bar, 50 μm.](image)
fibroblasts were removed from their substratum with EGTA in order to preserve the integrity of the cell surface components. Rabbit anti-CBP35 serum, normal rabbit serum, and PBS were tested for their ability to agglutinate the cells. Concanavalin A, a lectin known to agglutinate 3T3 cells by binding to cell surface carbohydrates, was also used as a positive indicator of agglutination. The results indicated that the rabbit anti-CBP35 serum agglutinated the cells strongly (Fig. 4A), as did concanavalin A (Fig. 4C). Normal rabbit serum (Fig. 4B) and PBS (Fig. 4D), however, failed to agglutinate the cells. These results also indicate that an immunoreactive component (presumably CBP35) exists at the cell surface of 3T3 fibroblasts.

**Molecular Identification of the Cell Surface Component Reactive with Rabbit Anti-CBP35**

Proteins externally exposed on the surface of 3T3 fibroblasts were labeled with 125I using the insoluble chloramide, iodogen (16, 26). A Triton X-100 extract of the labeled surface components was then chromatographed on an Affigel column conjugated with rabbit anti-CBP35, and the bound proteins were examined by PAGE. The autoradiogram of the SDS gel showed that a protein of Mr 35,000 was bound on the anti-CBP35 column (Fig. 5, lane a) but not on the control column (Fig. 5, lane b), which contained covalently coupled rabbit anti-succinyl concanavalin A. Both columns bound identically two other polypeptides of higher molecular weight; these were assumed to represent nonspecific binding. These results parallel that obtained when 35S-labeled extracts of whole 3T3 cells were subjected to affinity chromatography on columns containing rabbit anti-CBP35 or rabbit anti-succinyl concanavalin A (Fig. 2, lanes b and c). Therefore, the present data indicate that the immunoreactive component on the cell surface of 3T3 fibroblasts that was initially implicated by immunofluorescence and cell agglutination is in fact CBP35.

Several control experiments were carried out to ascertain that the CBP35 detected on the cell surface was not actually derived from internal proteins (released by a low percentage of lysed cells) that become adsorbed to the cell surface. First, medium conditioned by exposure to 3T3 fibroblasts, analyzed by PAGE and immunoblotting, failed to yield an Mr 35,000 component cross-reactive with rabbit anti-CBP35. In addition, immunoblotting analysis of the medium after incubation with lactose (0.1 M) for 30 min did not show any CBP35. Finally, 3T3 cells were labeled with either [35S]methionine or with 125I and then chased in unlabeled medium. There was no evidence of release of the lectin from the labeled cells. Together with the immunofluorescence staining obtained in the presence and absence of lactose and with the agglutination results, the present data strongly suggest that CBP35 is externally exposed at the cell surface.

**Immunofluorescence Staining of CBP35 in Permeabilized Cells**

The intracellular localization of CBP35 was examined by indirect immunofluorescence staining of formaldehyde fixed and Triton X-100-permeabilized 3T3 fibroblasts using rabbit anti-CBP35. For comparison, we carried out the identical staining protocol with a rabbit antiserum raised against pig muscle LDH. Immunoblotting analysis of SDS extracts of 3T3 cells showed that this antiserum reacted with a single polypeptide (Fig. 1, lane c), migrating at a position that corresponded to the molecular weight of mouse LDH (Mr ~37,000). Since LDH is a generally accepted marker enzyme for the cytosol fraction, this staining provided a reference.

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Figure 4. Agglutination of 3T3 fibroblasts in suspension by rabbit anti-CBP35. The final concentration of the cells in the assay was 1 x 10⁶ cells/ml. (A) Rabbit anti-CBP35 serum; (B) normal rabbit serum; (C) concanavalin A (25 μg/ml); (D) PBS. Bar, 10 μm.

Figure 5. SDS PAGE of 125I-labeled polypeptides bound on a column (1-ml bed volume) of Affigel 10 covalently derivatized with the immunoglobulin fraction of rabbit anti-CBP35. Cells were surface-labeled with 125I and iodogen; Triton X-100 extracts were fractionated as detailed in Materials and Methods. The radioactive components bound and eluted were subjected to PAGE. Lane a, polypeptides bound on rabbit anti-CBP35 column; lane b, polypeptides bound on rabbit anti-succinyl concanavalin A column. Approximately 2,000 and 1,000 cpm were electrophoresed in lanes a and b, respectively; the fluorogram was exposed for 26 d. Molecular weight markers are indicated on the right.
pattern expected for a cytoplasmic protein in cells with a prominent cytoskeleton.

Fixed and permeabilized 3T3 fibroblasts stained with rabbit anti-CBP35 showed intracellular labeling in all cells (Fig. 6 A). There was prominent labeling of the nucleus and variable staining of the cytoplasm. Cytoplasmic areas devoid of phase-dense intracellular vesicles stained diffusely in a uniform manner, whereas areas rich in vesicular bodies stained in a highly reticular manner. We suspect this is due to the exclusion of the fluorescent stain from the vesicles themselves (Fig. 6 A). In contrast, staining with normal rabbit serum resulted in insignificant labeling (Fig. 6 B).

In parallel experiments, 3T3 fibroblasts stained with rabbit anti-LDH showed a diffuse distribution of fluorescence within the cytoplasm of the cells (Fig. 6 C). Cytoplasmic staining was again variable and gave a reticular pattern in areas rich in vesicular bodies as previously observed for anti-CBP35 staining. In general, the cytoplasmic staining of the two antibodies was very similar, but more important for our present study, there was only weak diffuse staining of nuclei, probably resulting from overlying or underlying cytoplasm, when the anti-LDH antibody was used for staining. This is in direct contrast to the results obtained with rabbit anti-CBP35, which showed prominent labeling of the nucleus (compare Fig. 6, A with C).

Quantitation of CBP35 in Subcellular Fractions

Homogenates were prepared from 3T3 cells swollen in hypotonic 0.02 M Tris-HCl, 5 mM KCl buffer (pH 7.2) containing 105 mU/ml aprotinin. After low speed centrifugation to remove nuclei and remaining intact cells, the postnuclear supernatant was separated into a high speed P150 pellet and a soluble S150 fraction (Table I). The subcellular fractions were then subjected to PAGE and immunoblotting with rabbit anti-CBP35 and 125I-labeled goat anti-rabbit immunoglobulin (Table I and Fig. 7). After autoradiography, the intensity of the band corresponding to CBP35 was quantitated by densitometric scanning.

One important point should be noted in interpreting the immunoblot shown in Fig. 7. Equal amounts (100 µg) of total protein from each subcellular fraction were electrophoresed in the individual lanes. Therefore, the intensity of the band corresponding to CBP35 reflects the proportion of the lectin relative to the total protein content of the subcellular fractions, and comparisons of the relative amount of the lectin from one subcellular fraction to another must take into account of the total protein contents of the fractions.

CBP35 was found predominantly in the S150 fraction, along with most of the total cellular proteins and with 98% of the total LDH activity, a marker for soluble proteins of the

Figure 6. Immunofluorescence staining of 3T3 fibroblasts after fixation with formaldehyde (3.7%) and permeabilization with Triton X-100 (0.2%). (A) Rabbit anti-CBP35 (1:10 dilution of antiserum); (B) normal rabbit serum (1:10 dilution). (C) Rabbit anti-LDH (1:10 dilution); (D) preimmune serum control for anti-LDH staining. The binding of the primary immunoglobulin was detected by rhodamine-labeled goat anti-rabbit immunoglobulin. Incubations with both the primary antibody and the secondary antibody were carried out at room temperature for 1 h. Bar, 50 µm.
The presence of CBP35 in the various subcellular fractions is qualitatively consistent with the patterns obtained from immunofluorescence. However, an apparently much higher level of CBP35 is detected in the nucleus by indirect immunofluorescence than by analysis of nuclei obtained after subcellular fractionation. The nuclear staining with rabbit anti-CBP35 was striking as compared with parallel staining with rabbit anti-LDH, an enzyme marker of cytosolic proteins. It is possible that the conditions employed for the subcellular fractionation, low ionic strength buffers and absence of metal ions, do not favor the association of CBP35 with the nucleus, thus releasing it into the cytoplasm in a soluble form. Nevertheless, even under the buffer conditions used for subcellular fractionation, the presence of CBP35 in the nuclear pellet was significant, particularly in view of the fact that 5% of the total lectin recovered was found in a fraction accounting for only 1% of the total protein. It is possible that the CBP35 found here is due to the presence of intact cells in the nuclear pellet but the amount of LDH activity in the pellet argues against this notion.

The detection of CBP35 in a nuclear fraction should be compared with the findings of Feizi and co-workers, who have localized a lectin corresponding to CBP13.5 at the nucleus using a polyclonal (11) and a monoclonal (9) antibody. Moreover, observations consistent with the nuclear localization of β-galactoside-binding lectins in epithelial tissues have also been reported by Beyer and Barondes (6). Glycosylated proteins have been localized in the nucleus both at the ultrastructural (17) and light microscopic (15) levels, and monoclonal antibodies prepared against the major nuclear matrix-pore complex-lamina glycoprotein bind specifically to the nuclear envelope in situ.

CBP is also localized in the cytoplasm, as detected by immunofluorescence. The staining pattern of the cytoplasm obtained with rabbit anti-CBP35 is similar to that observed with rabbit anti-LDH, representative of cytosolic proteins. Consistent with these results, 90% of the total CBP35 behaves as a soluble, cytoplasmic component under our subcellular fractionation conditions. Several other lectins of bovine and avian origin have also been predominantly localized in the cytoplasm (3, 27).

Finally, a small amount of CBP35 is found at the cell surface. The finding that a lectin is simultaneously surface exposed and localized within the cytoplasm of the cell is strikingly similar to the recent report of Raz et al. (30). Using monoclonal antibodies directed against tumor cell lectins (M, 34,000 and 68,000), they have shown that the lectin(s) is exposed at the cell surface. Staining of viable B16-F1 melanoma cells was in the form of microclusters distributed randomly at the cell circumference, a result mimicked by the staining of rabbit anti-CBP35 on 3T3 cells. Moreover, Raz et
al. (30) also found that most of the lectin(s) is inside the cell, as revealed by immunofluorescence after fixation and permeabilization. The relationship between CBP35 from 3T3 cells and the tumor cell lectin(s) remains to be elucidated.

The mode of anchorage of CBP35 on the plasma membrane is not known. The observations that the presence of lactose did not affect surface staining by rabbit anti-CBP35 and that lactose cannot wash the lectin off the membrane in quantities detectable by immunoblotting procedures (1–5 ng [13]) argue against the notion that it is bound to cell surface glycoconjugates. It is possible that the lectin is anchored through interactions other than carbohydrate binding. This in turn implies that the surface-exposed CBP35 has carbohydrate-binding sites free to interact with extracellular matrix components or other cells. Barondes and co-workers have reported surface localization and externalization of the chicken lactose lectin 1 (5) and have postulated that the function of this lectin may be to organize glycoconjugate networks (4).

We thank Dr. John E. Wilson for the gift of rabbit antiserum directed against purified pig muscle lactate dehydrogenase and for many helpful suggestions throughout the course of this work.

This work was supported by grants GM-27203 and GM-32310 from the National Institutes of Health and grant 83-CRCR-1-1288 from the U.S. Department of Agriculture. J. L. Wang was supported by Faculty Research Award FRA-221 from the American Cancer Society.

Received for publication 10 May 1985, and in revised form 14 October 1985.

References

1. Avruch, J., and D. F. H. Wallach. 1971. Preparation and properties of plasma membrane and endoplasmic reticulum fragments from isolated rat fat cells. Biochim. Biophys. Acta. 233:334–347.
2. Bajers, E. M., A. C. Newby, K. Siddle, and J. P. Luzio. 1982. Solubilization and purification of rat liver S-nucleotide by use of a Zwitterionic detergent and a monoclonal antibody immunoadsorbent. Biochem. J. 203:245–251.
3. Barak-Briles, E. B., W. Gregory, P. Fletcher, and S. Kornfeld. 1979. Vertebrate lectins: comparison of properties of β-galactoside-binding lectins from tissues of calf and chicken. J. Cell Biol. 81:528–537.
4. Barondes, S. H. 1981. Lectins: their multiple endogeneous cellular functions. Annu. Rev. Biochem. 50:207–231.
5. Barondes, S. H., and P. L. Haywood-Reid. 1981. Externalization of an endogenous chicken muscle lectin with in vivo development. J. Cell Biol. 91:568–572.
6. Beyer, E. C., and S. H. Barondes. 1980. Chicken tissue binding sites for thymus-specific lectin. J. Supramol. Struct. 13:219–227.
7. Beyer, E. C., S. S. Zweig, and S. H. Barondes. 1980. Two lactose binding lectins from chicken tissues: purified lectin from intestine is different from those in liver and muscle. J. Biol. Chem. 255:4236–4239.
8. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72:248–254.
9. Carding, S. R., S. J. Thorpe, R. Thorpe, and T. Feizi. 1985. Transformation and growth related changes in levels of nuclear and cytoplasmic proteins antigenically related to mammalian β-galactoside-binding lectin. Biochim. Biophys. Res. Commun. 127:680–686.
10. Childs, R. A., and T. Feizi. 1979. β-Galactoside-binding muscle lectins of man and monkey show antigenic cross-reactions with those of bovine origin. Biochem. J. 183:255–268.
11. Childs, R. A., and T. Feizi. 1980. β-Galactoside-binding lectin of human and bovine tissues. Cell Biol. Int. Rep. 4:755.
12. Courtneidge, S. A., A. D. Levinson, and J. M. Bishop. 1980. The protein encoded by the transforming gene of avian sarcoma virus (pp60csrc) and a homologous protein in normal cells (pp60c-src) are associated with the plasma membrane. Proc. Natl. Acad. Sci. USA. 77:3783–3787.
13. Crittenden, S. L., C. F. Roff, and J. L. Wang. 1984. Carbohydrate-binding protein 35: identification of the galactose-specific lectin in various tissues of mice. Mol. Cell. Biol. 4:1252–1259.
14. DeWaard, A., S. Hickman, and S. Kornfeld. 1976. Isolation and properties of β-galactoside binding lectins of calf heart and lung. J. Biol. Chem. 251:7581–7587.
15. Filson, A. J., A. Lewis, G. Blobel, and P. A. Fisher. 1985. Monoclonal antibodies prepared against the major Drosophila nuclear matrix-pore complex lamina glycoprotein bind specifically to the nuclear envelope in situ. J. Biol. Chem. 260:3164–3172.
16. Fraker, P. J., and J. C. Sperck. 1978. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. Biochem. Biophys. Res. Commun. 80:849–857.
17. Gerace, L., Y. Otsuwanai, and C. Kondor-Koch. 1982. Identification of a major polypeptide of the nuclear pore complex. J. Cell Biol. 95:863–873.
18. Greenberg, M. E., and G. M. Edelman. 1983. The 34 kd pp60 src substrate is located at the inner face of the plasma membrane. Cell. 33:767–779.
19. Gunscher, G. R., J. L. Wang, J. Yahara, B. A. Cunningham, and G. M. Edelman. 1973. Concanavalin A derivatives with altered biological activities. Proc. Natl. Acad. Sci. USA. 70:1012–1016.
20. Hancock, K., and V. C. W. Tsang. 1983. India ink staining of proteins on nitrocellulose paper. Anal. Biochem. 133:157–162.
21. Kaplan, N. O., and R. D. Cahn. 1962. Lactic dehydrogenases and muscular dystrophy in the chicken. Proc. Natl. Acad. Sci. USA. 48:2123–2130.
22. Krishna, G., B. Weiss, and B. B. Brodie. 1968. A simple, sensitive method for the assay of adenylyl cyclase. J. Pharmacol. Exp. Ther. 163:379–385.
23. Krzyzek, R. A., R. L. Mitchell, A. F. Lau, and A. J. Faras. 1980. Association of pp60src and src protein kinase activity with the plasma membrane of nonpermissive and permissive avian sarcoma virus-infected cells. J. Virol. 36:805–815.
24. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature ( Lond.). 227:680–685.
25. Levi, G., and V. I. Teichberg. 1981. Isolation and physicochemical characterization of electrocutein, a β-thio-galactoside binding lectin from the electric organ of Electrophorus electricus. J. Biol. Chem. 256:5735–5740.
26. Moutsatsos, I. K., and S. Coc. 1985. A “hanging” coverslip method for cell surface iodination of monolayer cultures. Anal. Biochem. 148:408–412.
27. Nowak, T. M., D. Kobiler, L. E. Roel, and S. H. Barondes. 1977. Developmentally regulated lectin from embryonic chick pectoral muscle: purification by affinity chromatography. J. Biol. Chem. 252:6026–6030.
28. Powell, J. T. 1980. Purification and properties of lung lectin. Rat lung and human lung β-galactoside-binding proteins. Biochem. J. 187:123–129.
29. Radke, K., V. C. Carter, P. Moss, P. Dehazy, M. Schlwa, and G. S. Martin. 1983. Membrane association of a 36,000-dalton substrate for tyrosine phosphorylation in chicken embryonic fibroblasts transformed by avian sarcoma viruses. J. Cell Biol. 97:1601–1611.
30. Raz, A. N., L. Meromsky, P. Carmi, R. Karakash, D. Lotan, and R. Lotan. 1984. Monoclonal antibodies to endogenous galactose-specific tumor cell lectins. EMBO J. (Eur. Mol. Biol. Organ.) 3:2979–2983.
31. Roff, C. F., and J. L. Wang. 1983. Endogenous lectins from cultured cells. Isolation and characterization of carbohydrate-binding proteins from 3T3 fibroblasts. J. Biol. Chem. 258:10657–10663.
32. Roff, C. F., P. R. Rosevear, J. L. Wang, and R. Barker. 1983. Identification of carbohydrate-binding proteins from mouse and human fibroblasts. Biochem. J. 211:625–629.
33. Steck, P. A., P. G. Voss, and J. L. Wang. 1979. Growth control in cultured 3T3 fibroblasts: assays of cell proliferation and demonstration of a growth inhibitory activity. J. Cell Biol. 83:562–575.
34. Towbin, H., T. Staehlin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA. 76:4430–4435.