Cell Surface Localization and Tissue Distribution of a Hepatocyte Cell-Cell Adhesion Glycoprotein (Cell-CAM 105)

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ABSTRACT We recently identified a 105,000-dalton plasma membrane glycoprotein, denoted cell-CAM 105 (CAM, cell adhesion molecule), that is involved in intercellular adhesion of reaggregating rat hepatocytes (Ocklind, C., and B. Öbrink, 1982, *J. Biol. Chem.*, 257:6788–6795). In this communication we used a monospecific rabbit antiserum against cell-CAM 105 to localize the antigen by indirect immunofluorescence on isolated rat cells and on frozen rat tissue sections. The antiserum stained the surface of freshly isolated hepatocytes. In liver sections, however, the fluorescence seemed to be located exclusively along the bile canaliculi. In addition, cell-CAM 105 showed a very specific tissue distribution. Thus a specific fluorescence was seen only in the epithelia of the stomach, the small intestine, the large intestine, the glandular epithelium of the parotid gland, and the tubules of the kidney. No specific fluorescence was found in a variety of other tissues, including cartilage, interstitial connective tissue, smooth muscle, skeletal muscle, heart muscle, eye, brain, skin, the epithelia of oesophagus, bladder, uterine mucosa, thyroid follicles, prostate gland, or collecting ducts of the kidney. In the simple epithelia of the intestine and the kidney tubules the fluorescence was confined to the apical, luminal portion. Thus, both in these epithelia and in liver, cell-CAM 105 was located where the typical junctional complexes between cells are found. These findings taken together with the fact that cell-CAM 105 is involved in intercellular adhesion between hepatocytes suggest that cell-CAM 105 is a member of the junctional complexes of hepatocytes and some simple epithelia.

Cell adhesion reflects a fundamental aspect of the physiology of multicellular organisms (1–6). It is generally believed that recognition and specific adhesion of cells governs morphogenesis in embryonic development (1–3). It is also obvious that the functional integrity and physiology of adult multicellular organisms depend on cell recognition and formation of specific adhesions (4). The regulation of basic biological phenomena like cellular motility and growth involve cell-to-cell contacts (1, 2, 4–6). Furthermore, altered recognition and adhesion properties of cells are likely to be important in various disease processes, such as invasion, metastasis, and abnormal growth in cancer (3, 6). For these reasons cell adhesion has been studied intensively since the beginning of this century, but in spite of these efforts our knowledge of the molecular mechanisms that are involved is still scanty.

In the tissues cells are held together by specialized structures termed intercellular junctions which are particularly well developed in epithelial linings (7, 8). In these tissues they are organized into a junctional complex consisting of three distinct morphological regions: (*a*) a tight junction (zonula occludens), (*b*) an intermediate junction (zonula or fascia adherens), and (*c*) a desmosome (macula adherens). Gap junctions are generally not found in these junctional complexes (8).

Junctions are defined at the ultrastructural level with electron microscopy, which does not allow studies on the dynamics involved in the formation, development, and turnover of these structures. Therefore we do not yet know if molecular components of junctions are involved in recognition and initial adhesion between cells. It should, however, now be possible to more directly address these questions since individual molecular components of some types of junctions have recently been described. This has been made possible by the isolation of junctions in partially purified forms. Isolated junctions include gap junctions from liver (9, 10) and similar junctions from eye lens (11, 12), desmosomes from bovine muzzle (13–15) and intercalated discs enriched in fascia adherentes from cardiac muscle (16). Using different approaches some components specifically associated with the cytoplasmic face of desmosomes (17) and intermediate junctions (17, 18) have been identified. An example of such a molecule is vinculin which has been

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ascribed a role in the linking of microfilaments to the membrane in the intermediate junction (18).

A more direct approach to analyses of mechanisms involved in cell adhesion has been to study reaggregating cells. Such investigations have during the last few years resulted in identification of cell surface molecules involved in adhesion in several cellular systems such as marine sponges (19), cellular slime molds (20, 21), teratocarcinoma cells (22), embryonic chicken cells from neural retina (23), brain (24), or liver (25, 26), hamster fibroblasts (27), and adult rat hepatocytes (28). It is not yet known if any of these adhesion molecules are related to the macromolecular components of cellular junctions.

We recently identified a cell surface molecule that is involved in the initial cell-to-cell adhesion of freshly isolated rat hepatocytes (28). This molecule, which was denoted cell-CAM 105 (CAM, cell adhesion molecule), is a plasma membrane integral glycoprotein with an apparent molecular weight of 105,000. In the present communication we have used indirect immunofluorescence to analyze the cell surface localization and tissue distribution of cell-CAM 105. The results show that cell-CAM 105 has a high degree of tissue specificity and indicate that it is possibly a member of the junctional complexes in liver and in simple epithelia. This allows us to now ask more specific questions about possible relations between initial adhesion and junction formation.

MATERIALS AND METHODS

Hepatocytes: Hepatocytes were isolated from young, male Sprague-Dawley rats by a collagenase perfusion procedure as described previously (29), and were seeded on coverslips coated with bovine plasma fibronectin (29). The attached cells were then investigated by immunofluorescence either directly or after fixation for 10 min either with paraformaldehyde (2.5% in a balanced salt solution [buffer 3 in reference 29]) or with acetone.

Cryostat Sections: Tissues were taken from young male Sprague-Dawley rats that had been starved over-night. In some experiments small intestine from guinea pigs or from human biopsies were also used. The tissues were placed in Histocon (Histo-lab, Göteborg, Sweden) at 4°C, frozen in liquid isopenthane (−70°C) and were stored at this temperature until sectioned. Sections (4 μm) were cut on a cryostat, fixed for 10 min in acetone, air-dried for 1–2 h, and stored at −70°C until used.

Antiserum: A rabbit antiserum denoted anti-cell CAM_2 (28) was used. In a previous communication (28) we showed by immunoprecipitation and immunoblotting techniques that this antiserum reacted monospecifically with the hepatocyte cell adhesion molecule cell-CAM 105. Pre-immune serum from the same animal was used.

Indirect Immunofluorescence: The frozen tissue sections were thawed for 20 min and were then covered with antiserum or pre-immune serum diluted 1:10 or 1:20 with PBS (0.13 M NaCl, 0.01 M sodium phosphate, pH 7.4). The sections were incubated in a humid chamber for 45 min and were then washed four times with PBS. They were then covered with FITC-conjugated goat anti-rabbit IgG (Nordic Immunological Laboratories, Tilburg, The Netherlands, or DAKO-Immunoglobulins A/S, Copenhagen, Denmark) (0.25 mg/ml). After incubation for 45 min the sections were washed as before and mounted under coverslips in Kaisers Glyceringelatine (Merck, Darmstadt, W. Germany). Isolated hepatocytes attached to fibronectin-coated coverslips were prepared for immunofluorescence by the same procedure. A Leithz Orthoplan microscope equipped with epi-illumination was used. Photographs were taken with an automatic Orthomat camera using Kodak Tri-X film.

In all experiments parallel samples were stained either with anti-cell-CAM_2 or with pre-immune serum, respectively, to ensure the specificity of the observed reactions.

RESULTS

Indirect immunofluorescence staining of freshly isolated hepatocytes with anti-cell-CAM_2 showed the antigen to be present on the cell surface (Fig. 1 *a*). In some cells it seemed to be nonrandomly organized. This staining pattern was observed both on nonfixed, viable cells and on paraformaldehyde-fixed, nonpermeabilized cells (Fig. 1 *a*) which demonstrated that the antigen was accessible on the external surface of the cells. A similar picture was observed in acetone-fixed, permeabilized cells (not shown), showing that there is no large intracellular pool of this protein.

A different staining pattern was observed when frozen sections of liver were examined (Fig. 1 *b*). The fluorescence in liver was localized between the hepatocytes but was not found all around them or on their sinusoidal surfaces. This staining pattern is identical to that obtained when the bile canaliculi are visualized either by classical silver staining methods or by histochemical staining for ATPase activity (see references 30 and 31 for pictures). In many areas in Fig. 1 *b* it can also clearly be seen that the fluorescence was found between the hepatocytes exactly at the location of the bile canaliculi. We conclude from these data that cell-CAM 105 is localized along the bile canaliculi in the liver.

Several other tissues were sectioned and analyzed by indirect immunofluorscence with anti-cell-CAM_2. We observed also a specific fluorescence staining in the epithelia of the small intestine (Fig. 1 *c*), the large intestine, the stomach, and the tubules of the kidney (Fig. 1 *d*), and the glandular epithelium of the parotid gland (Fig. 1 *e*). We observed no specific fluorescence in interstitial connective tissue of parenchymal organs, cartilage, smooth muscle of uterus, bladder or vessel walls, skeletal muscle, heart muscle, eye, brain, skin, the epithelia of oesophagus, bladder, uterine mucosa, thyroid follicles, prostate gland, or collecting ducts of the kidney. Thus, in addition to liver, only simple epithelia—with the exceptions of those of the uterine mucosa, the thyroid follicles and the collecting ducts of the kidney—were stained for cell-CAM 105. In no case were stratified epithelia stained with our antiserum.

In the simple epithelia stained by anti-cell-CAM_2 the staining was again found in a very specific pattern, which could be best seen in the small intestine (Fig. 1 *c*) and in the kidney tubules (Fig. 1 *d*). Here the staining was localized exclusively to the apical, luminal parts of the cells.

Sections of the small intestine from guinea pigs and humans were also analyzed by indirect immunofluorescence with anti-cell-CAM_2. No specific staining was found demonstrating that the antibodies at least for small intestine seem to be species specific.

DISCUSSION

Analysis of the tissue distribution of cell-CAM 105, which has been shown to be involved in cell-cell adhesion of freshly isolated rat hepatocytes (28), demonstrated that this cell adhesion molecule has a high degree of tissue specificity. In addition to the liver we found it only in the simple epithelia of the gastrointestinal tract, the tubules of the kidney and the glandular epithelium of the parotid gland. This is in good agreement with the specificity of cell-cell adhesion observed for rat hepatocytes by Albanese et al. (32) and suggests that cell-CAM 105 is involved in cell- and tissue-specific adhesion.

The immunofluorescence staining of freshly isolated hepatocytes clearly demonstrated that cell-CAM 105 was found on the cell surface, as was expected since anti-cell-CAM 105 antibodies have been shown to inhibit the reaggregation of viable cells (28). On the isolated cells the antigen was distributed around the cells, which was in contrast to its confinement in the intact liver to the regions of the bile canaliculi. Thus a dispersion of cell-CAM 105 seemed to occur as a consequence of the dissociation of the cells. Preliminary data suggested that

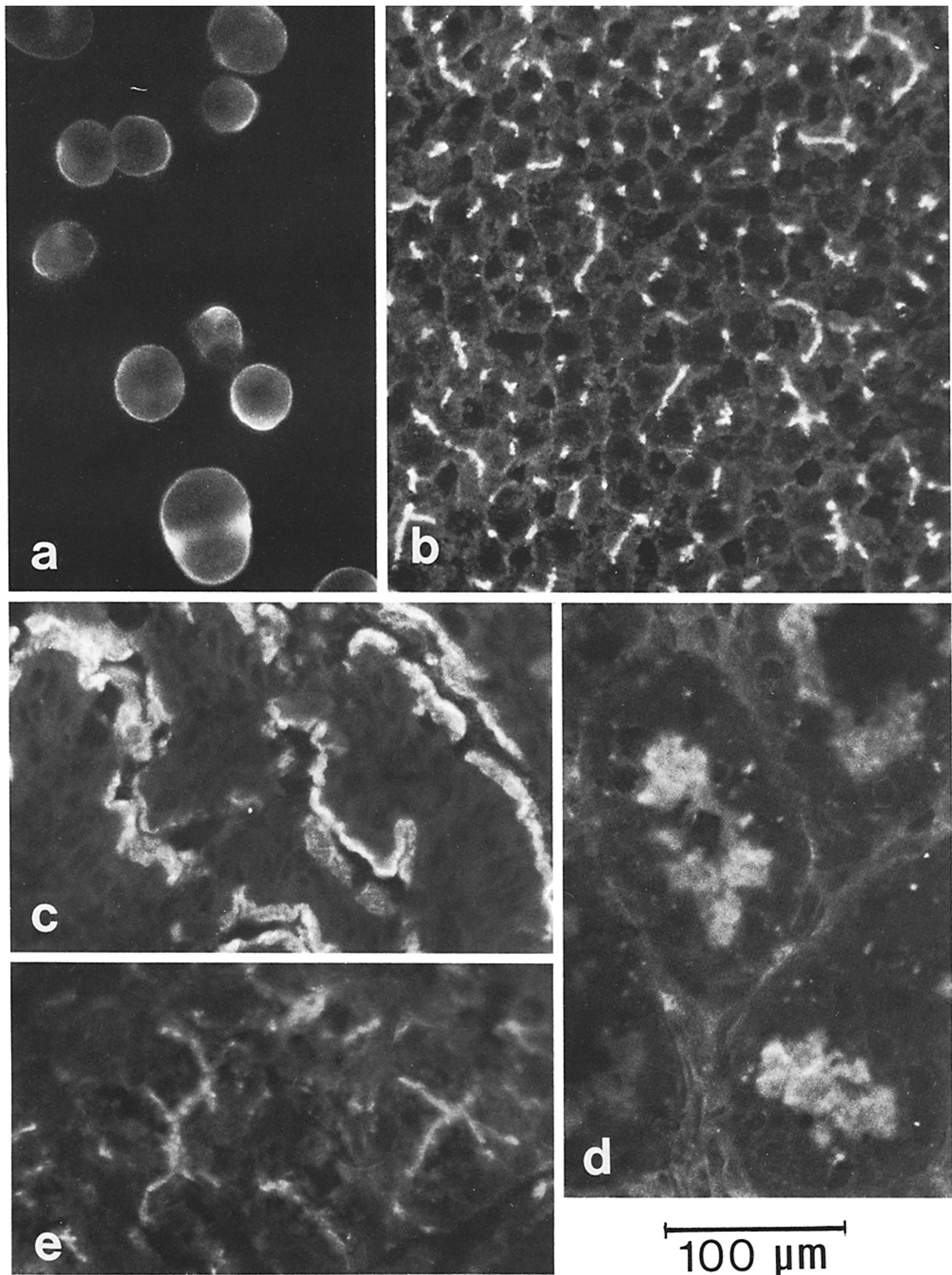

FIGURE 1 Indirect immunofluorescence obtained with anti-cell-CAM_2 antiserum. (*a*) Freshly isolated hepatocytes were seeded on a fibronectin-coated coverslip. 30 min after seeding the cells were fixed with paraformaldehyde and prepared for indirect immunofluorescence. (*b*) A frozen section of liver tissue. Especially where the specific fluorescence appears as dots it can clearly be seen that it is located between the hepatocytes at the location of the bile canaliculi. (*c*) A frozen section of the small intestine (jejunum). The nuclei of the columnar epithelial cells of the mucosa can be seen as oval black holes. The specific fluorescence is found at the very apical portion of the cells. (*d*) A frozen section of the cortex of the kidney. In the typical tubular structures the specific fluorescence is located at the apical portion of the cells, close to the lumen. (*e*) A frozen section of the parotid gland.

in cells making contacts with each other in vitro, cell-CAM 105 again redistributes and becomes localized to areas of cell-to-cell contact.

The bile canaliculi are formed by the plasma membranes of adjoining hepatocytes held together by typical epithelial junctional complexes (7). Thus, since it is involved in cell-cell adhesion of hepatocytes it seems highly likely that cell-CAM 105 is a member of the junctional complex of hepatocytes. This conclusion is strongly supported by the apical localization of cell-CAM 105 in the epithelia of the small intestine and of the kidney tubules. In these epithelia well developed junctional complexes are found at the apical portion of the cells, which is facing the lumen (7).

The resolution of the light microscope is not enough to give information about in which specific type of junction cell-CAM 105 is located. However, the tissue distribution of cell-CAM 105 showed some very interesting features in comparison with the tissue distribution of the various types of junctions reported by Farquhar and Palade (7, 33). Tissues like epidermis and other stratified epithelia that contain an abundancy of desmosomes and well developed tight junctions were not stained for cell-CAM 105. Neither were simple epithelia like those of the collecting ducts of the kidney and the uterine mucosa that also contain well developed tight junctions. Accordingly, if we assume that tight junctions in different tissues and desmosomes in different tissues respectively contain the same kind of molecules (which only is an assumption with as yet no experimental data to support it), it seems less likely that cell-CAM 105 is a member of desmosomes or tight junctions. The gap junction also seems to be a less likely candidate since (*a*) it is not a member of the junctional complexes and therefore might show a somewhat different distribution in the liver and in the intestinal mucosa than that observed for cell-CAM 105 and (*b*) no such large protein as cell-CAM 105 has been observed in gap junctions. Liver gap junctions seem to contain only one protein with a molecular weight around 28,000 (10).

According to Farquhar and Palade (7) the intermediate junction is well developed in simple epithelia with exception of the epithelia of the thyroid follicles, the uterine mucosa, the distal tubules, and the collecting ducts of the kidney. Furthermore, this junction was not seen in the stratified epithelium of skin (33). The tissue distribution of the intermediate junction thus correlates excellently with that of cell-CAM 105. Taken together these observations suggest that cell-CAM 105 could be a member of intermediate junctions. However, it is quite clear that methods with better resolution, like immunoelectron microscopy, must be used to elucidate the exact location of cell-CAM 105 at the ultrastructural level. Such work is now in progress in our laboratory and until we have these results we do not exclude the possibility that cell-CAM 105 is contained in some other structure than the intermediate junction.

Our results suggest that a cell surface molecule, used by the hepatocytes early in their intercellular adhesion and aggregation, later may become organized into a mature cell-to-cell junction. In this context it is interesting to note that the first type of morphologically recognizable junction that appears both in reaggregating hepatocytes (34) and between kidney epithelial cells (17) in vitro is the intermediate junction. The intermediate junction is the type of epithelial junction which on its cytoplasmic face is associated with actin-containing microfilaments (18). This raises the possibility that cell-CAM 105—if it is a member of this junction—might be involved in the organization of microfilaments with possible consequences for motility, growth, and polarization of epithelial cells.

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REFERENCES

1. Trinkaus, J. P. 1969. Cells into Organs. Prentice-Hall Inc., Englewood Cliffs, NJ.
2. Frazier, W., and L. Glaser. 1979. Surface components and cell recognition. *Annu. Rev. Biochem.* 48:491–523.
3. Marchase, R. B., K. Vosbeck, and S. Roth. 1976. Intercellular adhesive specificity. *Biochim. Biophys. Acta.* 457:385–416.
4. Öbrink, B., C. Ocklind, K. Rubin, M. Höök, and S. Johansson. 1980. Cell-cell and cell-collagen adhesion. *In* Biochemistry of Normal and Pathological Connective Tissues. Colloques Internationaux du C. N. R. S., A. M. Robert and L. Robert, editors. Editions du Centre National Recherche Scientifique, Paris. 287:239–247.
5. Heaysman, J. E. M., and S. M. Pegrum. 1973. Early contacts between fibroblasts. An ultrastructural study. *Exp. Cell Res.* 78:71–78.
6. Lieberman, M. A., D. Raben, and L. Glaser. 1981. Cell surface-associated growth inhibitory proteins. Evidence for conservation between mouse and human cell lines. *Exp. Cell Res.* 133:413–419.
7. Farquhar, M. G., and G. E. Palade. 1963. Junctional complexes in various epithelia. *J. Cell Biol.* 17:375–411.
8. McNutt, N. S., and R. S. Weinstein. 1973. Membrane ultrastructure at mammalian intercellular junctions. *Prog. Biophys. Mol. Biol.* 26:45–101.
9. Goodenough, D. A. 1975. Methods for the isolation and structural characterization of hepatocyte gap junctions. *Methods Membr. Biol.* 3:51–80.
10. Nicholson, B. J., M. W. Hunkapiller, L. B. Grim, L. E. Hood, and J. P. Revel. 1981. Rat liver gap junction protein: properties and partial sequence. *Proc. Natl. Acad. Sci. USA* 78:7594–7598.
11. Hertzberg, E. L., D. J. Anderson, M. Friedlander, and N. B. Gilula. 1982. Comparative analysis of the major polypeptides from liver gap junctions and lens fiber junctions. *J. Cell Biol.* 92:53–59.
12. Bok, D., J. Dockstader, and J. Horwitz. 1982. Immunocytochemical localization of the lens main intrinsic polypeptide (MIP26) in communicating junctions. *J. Cell Biol.* 92:213–220.
13. Skerrow, C. J., and A. G. Matoltsy. 1974. Isolation of epidermal desmosomes. *J. Cell Biol.* 63:515–523.
14. Skerrow, C. J., and A. G. Matoltsy. 1974. Chemical characterization of isolated epidermal desmosomes. *J. Cell Biol.* 63:524–530.
15. Gorbsky, G., and M. S. Steinberg. 1981. Isolation of the intercellular glycoproteins of desmosomes. *J. Cell Biol.* 90:243–248.
16. Colaco, C. A. L. S., and W. H. Evans. 1981. A biochemical dissection of the cardiac intercalated disk: isolation of subcellular fractions containing Fascia adherentes and gap junctions. *J. Cell Sci.* 52:313–325.
17. Kartenbeck, J., E. Schmid, W. W. Franke, and B. Geiger. 1982. Different modes of proteins associated with adhaerens junctions and desmosomes: experimental separation of lateral contacts induces endocytosis of desmosomal plaque material. *EMBO (Eur. Mol. Biol. Organ. J.) J.* 1:725–732.
18. Geiger, B., A. H. Dutton, K. T. Tokuyasu, and S. J. Singer. 1981. Immunoelectron microscope studies of membrane-microfilament interactions: distributions of α-actinin, tropomyosin, and vinculin in intestinal epithelial brush border and chicken gizzard smooth muscle cells. *J. Cell Biol.* 91:614–628.
19. Burger, M. M., W. Burkart, G. Weinbaum, and J. Jumblatt. 1978. Cell-cell recognition: molecular aspects. Recognition and its relation to morphogenetic processes in general. *In* Cell-Cell Recognition. A. S. G. Curtis, editor. Cambridge University Press, Cambridge. 1–24.
20. Müller, K., and G. Gerisch. 1978. A specific glycoprotein as the target site of adhesion blocking Fab in aggregating Dictyostelium cells. *Nature (Lond.).* 274:445–449.
21. Geltosky, J. E., J. Weseman, A. Bakke, and R. A. Lerner. 1979. Identification of a cell surface glycoprotein involved in cell aggregation in D. discoideum. *Cell.* 18:391–398.
22. Yoshida, C., and M. Takeichi. 1982. Teratocarcinoma cell adhesion: identification of a cell-surface protein involved in calcium-dependent aggregation. *Cell.* 28:217–224.
23. Thiery, J. P., R. Brackenbury, U. Rutishauser, and G. M. Edelman. 1977. Adhesion among neural cells of the chick embryo. II. Purification and characterization of a cell adhesion molecule from neural retina. *J. Biol. Chem.* 252:6841–6845.
24. Hoffman, S., B. C. Sorkin, P. C. White, R. Brackenbury, R. Mailhammer, U. Rutishauser, B. A. Cunningham, and G. M. Edelman. 1982. Chemical characterization of a neural cell adhesion molecule purified from embryonic brain membranes. *J. Biol. Chem.* 257:7720–7729.
25. Bertolotti, R., U. Rutishauser, and G. M. Edelman. 1980. A cell surface molecule involved in aggregation of embryonic liver cells. *Proc. Natl. Acad. Sci. USA.* 77:4831–4835.
26. Nielsen, L. D., M. Pitts, S. R. Grady, and E. J. McGuire. 1981. Cell-cell adhesion in the embryonic chick: partial purification of liver adhesion molecules from liver membranes. *Dev. Biol.* 86:315–326.
27. Urushihara, H., and M. Takeichi. 1980. Cell-cell adhesion molecule: identification of a glycoprotein relevant to the Ca^{2+}-independent aggregation of Chinese hamster fibroblasts. *Cell.* 20:363–371.
28. Ocklind, C., and B. Öbrink. 1982. Intercellular adhesion of rat hepatocytes. Identification of a cell surface glycoprotein involved in the initial adhesion process. *J. Biol. Chem.* 257:6788–6795.
29. Öbrink, B. 1982. Hepatocyte-collagen adhesion. *Methods Enzymol.* 82:513–529.
30. Einarson, L., G. Glimstedt, and J. Jansen. 1958. Lärobok i Histologi. Svenska Bokförlaget, Bonniers, Stockholm. 237.
31. Bloom, W., and D. W. Fawcett. 1975. A Textbook of Histology. W. B. Saunders Company, Philadelphia. 713.
32. Albanese, J., M. S. Kuhlenschmidt, E. Schmell, C. W. Slife, and S. Roseman. 1982. Studies on the intercellular adhesion of rat and chicken hepatocytes. Tissue-specific adhesion in mixtures of hepatocytes and heart myocytes. *J. Biol. Chem.* 257:3165–3170.
33. Farquhar, M. G., and G. E. Palade. 1965. Cell junctions in amphibian skin. *J. Cell Biol.* 26:263–291.
34. Miettinen, A., I. Virtanen, and E. Linder. 1978. Cellular actin and junction formation during reaggregation of adult rat hepatocytes into epithelial cell sheets. *J. Cell Sci.* 31:341–353.