THE PLASMA MEMBRANE

Cells are surrounded by the plasma membrane, which forms the boundary between their cytoplasm and the environment. The principal components of the plasma membrane and of all other cellular membranes are (glyco)lipids and (glyco)proteins.

In ultrathin sections, the plasma membrane appears quite simple in structure (panel A). It consists of two electron-dense leaflets and a lucent space in between, together reaching a thickness of about 75 nm. In the electron micrograph shown, the trilamellar plasma membranes of two adjacent enterocytes and the narrow intercellular space are visible. This fine structural monotony does not reflect the asymmetric and complex composition and the dynamic nature of the plasma membrane, which differs between cell types.

Freeze-fracture electron microscopy is highly suitable for the study of membranes and has provided proof of the presence of membrane-spanning proteins. The fracture plane preferentially passes through the hydrophobic membrane interior and produces two membrane halves: the P-face, which is the cytosolic membrane half, and the E-face, which corresponds to the external membrane half. In panel B, the E- and the P-faces of two neighboring red blood cells are shown. Both membrane faces are studded with intramembranous particles, which are related to fractured transmembrane proteins. The smooth parts of the fracture faces principally correspond to membrane lipids. As seen in panel B, the P-face usually contains a higher density of intramembranous particles. Variants of the freeze-fracture technique applied to cell cultures permitted the preparation of plasma membrane fracture faces of enormous size, as shown in panel C. In contrast to the uniform distribution of intramembranous particles in the erythrocyte plasma membrane, those of cultured hepatocytes are irregularly arranged. The clusters of intramembranous particles correspond to coated pits involved in receptor-mediated endocytosis (cf. Fig. 58). The numerous elevations correspond to plasma membrane processes (cf. Fig. 92).

The plasma membrane performs two basic functions. On the one side, the lipid bilayer constitutes an impermeable barrier for most water-soluble molecules. On the other, its membrane-spanning proteins make it porous for bidirectional transmembrane transport and diffusion, communication and signaling, and cell-cell and cell-matrix interactions. The lipid bilayer represents a two-dimensional fluid in which both lipids and proteins are relatively mobile in the plane of the membrane. However, lipids and proteins may be confined to specific membrane regions, the microdomains. Hence, the name "fluid mosaic model of membranes."

The lipid bilayer consists of phospholipids, cholesterol, and glycolipids, which are differentially distributed in the two membrane leaflets. The oligosaccharide side chains of glycolipids are exclusively found on the outer plasma membrane surface together with the oligosaccharides of glycoproteins and form the glycocalyx (cf. Fig. 94).

The plasma membrane asymmetry is not only confined to the two lipid layers. The apical and the basolateral plasma membrane in polarized cells can differ in their protein and lipid composition in relation to their specific functions.

References

Daley D (2008) The assembly of membrane proteins into complexes. Curr Opin Struct Biol 18:420

Fehon RG, McClatchey AI, Bretscher A (2010) Organizing the cortex: the role of ERM proteins. Nat Rev Mol Cell Biol 11:276

Frye L, Edidin M (1970) The rapid intermixing of cell surface antigens after formation of mouse-human heterokaryons. J Cell Sci 7:319

Ikenouchi J, Hirata M, Yonemura S, Umeda M (2013) Sphingomyelin clustering is essential for the formation of microvilli. J Cell Sci 126:3585

Kusumi A, Fujiwara TK, Chadda R, Xie M, Tsunoyama TA, Kalay Z, Kasai RS, Suzuki KGN (2014) Dynamic organization principles of the plasma membrane that regulate signal transduction: commemorating the fortieth anniversary of Singer and Nicolson's fluid-mosaic model. Annu Rev Cell Dev Biol 28:215

Lajoie P, Goetz JG, Dennis JW, Nabi IR (2009) Lattices, rafts, and scaffolds: domain regulation of receptor signaling at the plasma membrane. J Cell Biol 185:381

Lingwood D, Simons K (2010) Lipid rafts as a membrane-organizing principle. Science 327:46

Mukherjee S, Maxfield F (2004) Membrane domains. Annu Rev Cell Dev Biol 20:839

Nelson WJ (2009) Remodeling epithelial cell organization: transitions between front-rear and apical-basal polarity. Cold Spring Harb Perspect Biol 1:a000513

Shnyrova AV, Frolov VA, Zimmerberg J (2009) Domain-driven morphogenesis of cellular membranes. Curr Biol 19:R772

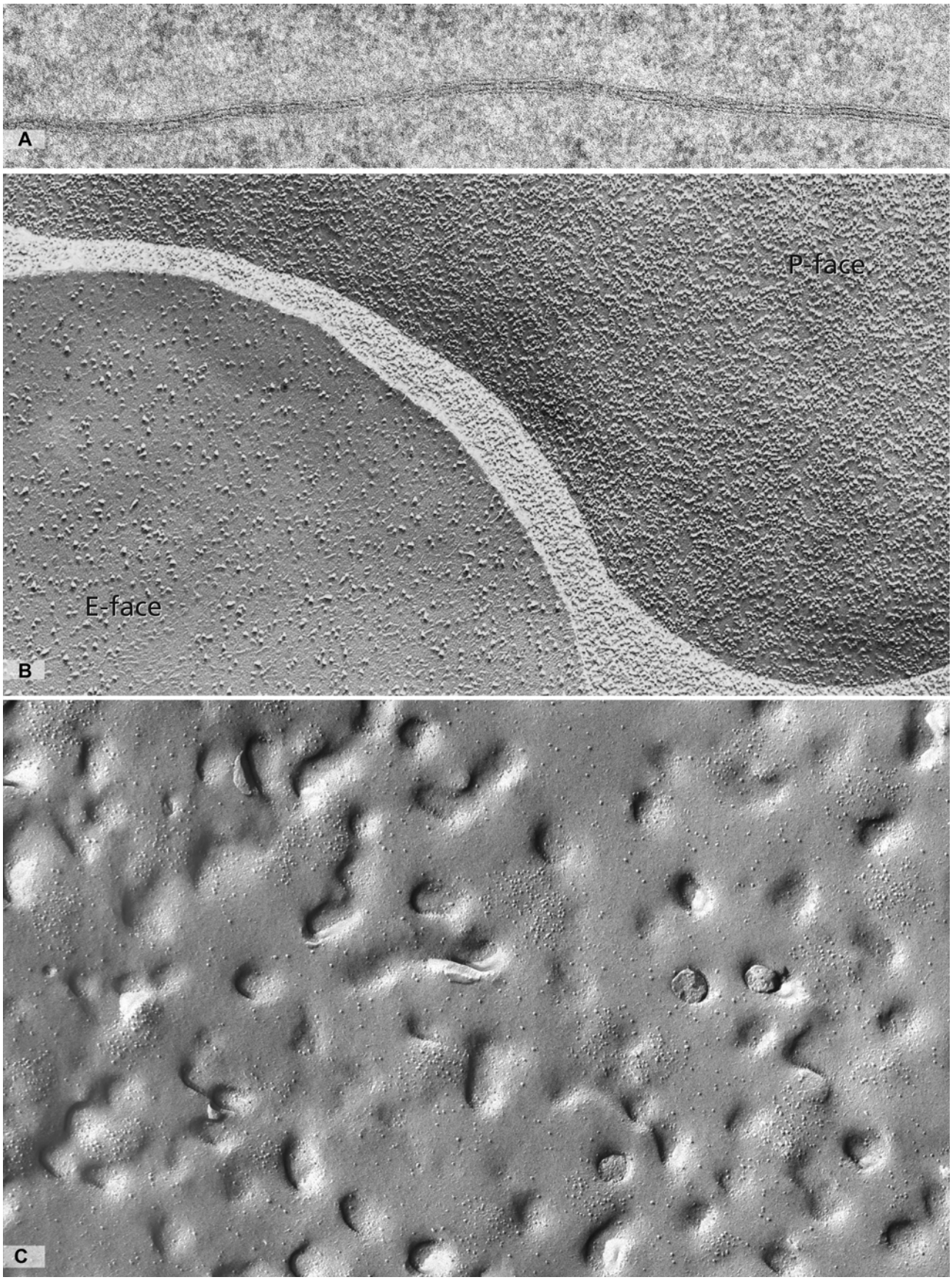
Simons K, Sampaio JL (2011) Membrane organization and lipid rafts. Cold Spring Harb Perspect Biol 3:a004697

Singer S, Nicolson G (1972) The fluid mosaic model of the structure of biological membranes. Science 175:720

van Meer G (2011) Dynamic transbilayer lipid asymmetry. Cold Spring Harb Perspect Biol 3:a004671

Magnification: ×75,000 (**A**); ×85,000 (**B**); ×54,000 (**C**)

M. Pavelka, J. Roth, *Functional Ultrastructure: Atlas of Tissue Biology and Pathology*, Third Edition,
DOI 10.1007/978-3-7091-1830-6_11, © Springer-Verlag Vienna 2015


A
P-face
E-face
B
C

CELLS IN CULTURE

Various cell types, including stem cells and epithelial, neuronal and mesenchymal cells, as well as different tumor cell types, can be grown *in vitro* as monolayers or in suspension. Routinely, plastic tissue culture dishes are used and cells can survive and multiply when supplied with appropriate culture medium, temperature, and atmosphere. Epithelial cells in tissues are polarized and monolayers of polarized epithelial cells can be obtained when grown on porous tissue culture membranes. Cell cultures provide excellent experimental tools because they can be studied microscopically or analyzed biochemically and can be used as host to synthesize and secrete foreign proteins such as monoclonal antibodies and recombinant proteins.

Observation of living cells by light microscopy and of fixed cells by scanning electron microscopy has provided a wealth of information on cell spreading and locomotion under culture conditions. Spreading of fibroblasts from a cell suspension occurs through thin, thread-like protrusions, the filopodia, which establish initial contacts with the substrate (arrowhead in panel A) that finally result in a well-attached, flattened cell. Attached fibroblasts and other cell types can crawl over the substratum. This represents a directional movement associated with the formation of lamellipodia, which are flat, two-dimensional protrusions formed at the leading edge of the cells. Thus, moving cells are distinctly polarized (arrows in panel A). Both types of cell protrusions contain actin: filopodia have long, bundled filaments and lamellipodia orthogonally cross-linked meshworks essentially arranged parallel to the substratum. The actin filaments of the lamellipodia in concert with myosin and microtubules and accessory cytoskeletal proteins are the active principle for the cell movement. The cytoskeleton is actively reorganized during cellular locomotion and includes the formation, contraction, and disassembly of actin networks in lamellipodia.

Panel B shows a group of rat hepatocytes attached to a plastic support, which form a coherent, monolayered sheet. Their surface is covered by microvilli-like membrane extensions that can be clearly seen in an ultrathin section cut perpendicularly to the plane of the cell monolayer (panel C). The ultrathin section shown in panel C reveals that the microvilli-like extensions are restricted to the free cell surface and that the basal cell surface is rather flat and focally attached to the plastic support. Epithelial cells grown on a solid plastic or glass support have a discoid shape, as seen in panels B and C, and form adherens junctions and desmosomes at sites of lateral cell-cell contacts. As mentioned, when grown on permeable, porous membranes, epithelial cells such as kidney epithelial cells form a highly polarized cell monolayer. This represents a most useful system to analyze aspects of polarity of cellular traffic and cytoarchitecture.

Cell crawling is a basic phenomenon in living organisms during embryogenesis and in adult organs. It is important for the function of cells involved in inflammation and immune defense, wound healing, and tissue remodeling as well as the spread of malignant cells.

References

Balcarova-Ständer J, Pfeiffer S, Fuller S, Simons K (1984) Development of cell surface polarity in the epithelial Madin-Darby canine kidney (MDCK) cell line. EMBO J 3:2687

Bretscher MS (2014) Asymmetry of single cells and where that leads. Annu Rev Biochem 83:275

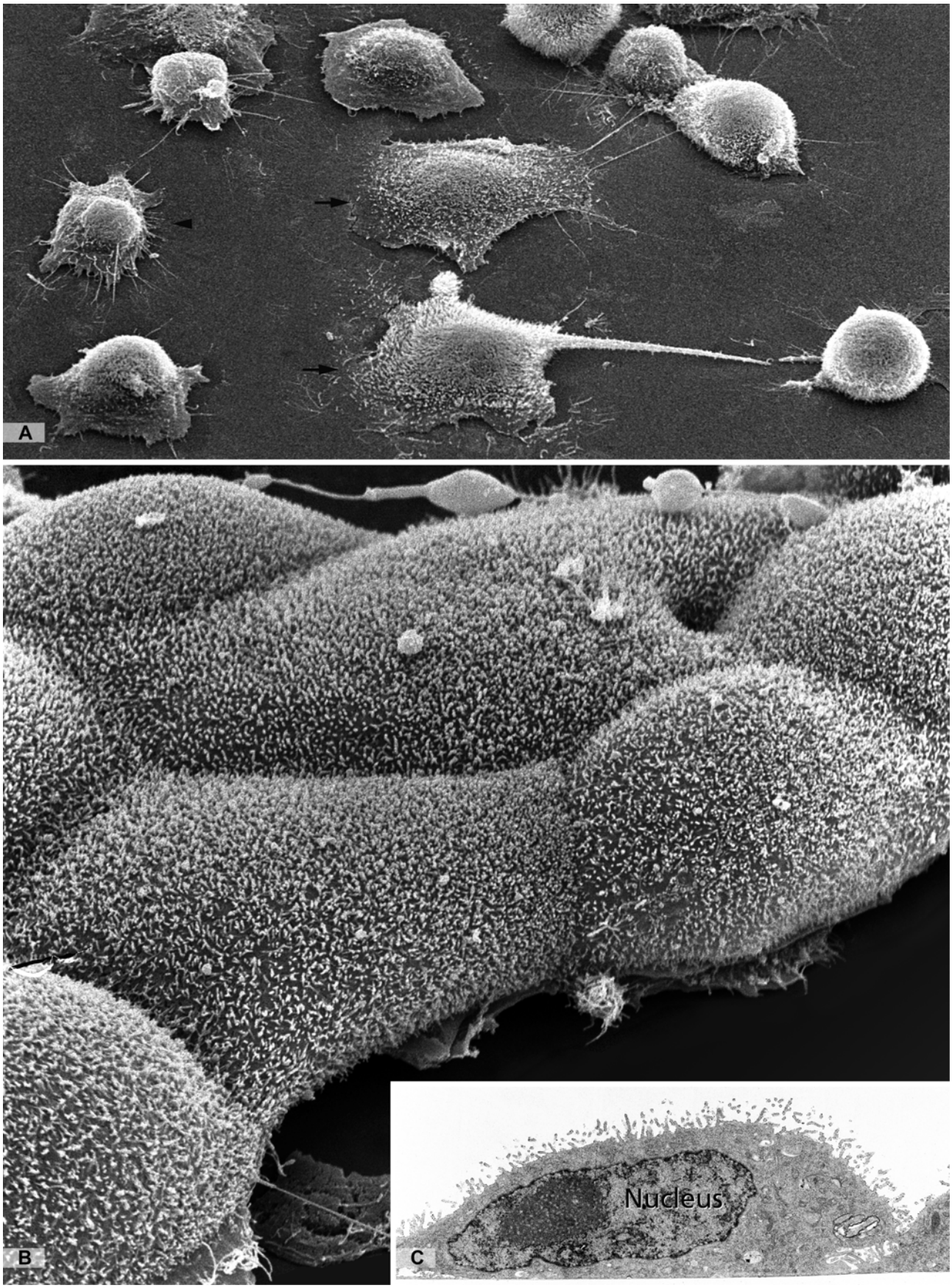
Cereijido M, Robbins E, Dolan W, Rotunna C, Sabatini D (1978) Polarized monolayers formed by epithelial cells on a permeable and translucent support. J Cell Biol 77:853

Condeelis J (1993) Life at the leading edge: the formation of cell protrusions. Annu Rev Cell Biol 9:411

Rodriguez-Boulan E, Macara IG (2014) Organization and execution of the epithelial polarity programme. Nat Rev Mol Cell Biol 15:225

Simons K, Fuller S (1985) Cell surface polarity in epithelia. Ann Rev Cell Biol 1:243

Magnification: ×500 (**A**); ×2,900 (**B**); ×3,500 (**C**)


A
B
C
Nucleus

BRUSH CELL

Specialized surface differentiations exist in brush cells, alternatively called tuft cells, caveolated cells, or solitary chemosensory cells. Brush cells belong to a cell population that is widely distributed in the epithelial organs of the gastrointestinal and respiratory tracts, including the stomach, small and large intestine, bile duct, gallbladder and pancreatic duct, tracheal epithelium, and lung. The cells are so named for their brush-like or tuft-like apical surface specializations formed by long and thick microvilli, which differ from the microvilli of the brush borders of absorptive cells (cf. Figs. 88 and 128). A brush cell of the rat colon, which shows cytochemical labeling for demonstration of sialic acid residues, is on display. The apical microvilli forming the tuft (arrow) protrude into the gut's lumen and tower above the brush border microvilli of the neighboring absorptive cells (arrowhead). The differences in dimensions are clearly visible. Densely packed actin filaments build up the core of the tuft microvilli and extend into deep regions of the apical cytoplasm, where they still are bundled (open arrow) and accompanied by microtubules, intermediate filaments, and membrane vesicles.

Structural and functional characteristics point to the functional connection with chemoreceptive tasks and with the regulation of electrolyte concentrations in the secretory fluids of hollow organs. The apical ultrastructure resembles receptor cells in sensory epithelia, such as the sensory cells in taste buds. Brush cells of the stomach, intestine, and pancreatic duct system express a taste cell-specific GTP-binding protein, alpha-gustducin, which is particularly concentrated in the apical pole of the cells, similar to the taste cells, where it is associated with sweet and bitter gustatory functions. Brush cells also are particularly rich in enzymes involved in the production of nitric oxide (NO), such as NO synthase I. Brush cells may have a role as chemoreceptive cells and use NO as a paracrine gaseous messenger.

The microvilli plasma membrane contains a specialized composition of glycoconjugates and seems to be turned over rapidly. Both features are discussed in favor of a receptive cell function of brush cells, a concept that is supported further by the presence of intermediate filaments that are characteristic for mature neurons. Brush cells express two types of intermediate filaments, cytokeratin 18 filaments and neurofilaments, a combination that is not known to occur in other healthy cells. Cytokeratin 18 is densely concentrated in a network of intermediate filament bundles extending from the cell periphery to the perinuclear cytoplasm, whereas it is absent from the apical cytoplasmic regions, where actin filaments and microtubules are assembled with neurofilaments.

Results show that brush cells localized in the mucosal epithelium of the respiratory tract are cholinergic chemosensory cells. They are able to detect products of bacteria, such as quorum sensing molecules (QSM), in the fluid that lines the airways, leading to changes in respiration and influences on the regulation of mucociliary clearance.

References

Gebert A, Al-Samir K, Werner K, Fassbender S, Gebhard A (2000) The apical membrane of intestinal brush cells possesses a specialized, but species-specific, composition of glycoconjugates – on-section and in vivo lectin labelling in rats, guinea-pigs and mice. Histochem Cell Biol 113:389

Höfer D, Drenckhahn D (1996) Cytoskeleton marker allowing discrimination between brush cells and other epithelial cells of the gut including enteroendocrine cells. Histochem Cell Biol 105:405

Höfer D, Drenckhahn D (1998) Identification of the taste cell G-protein, alpha-gustducin, in brush cells of the rat pancreatic duct system. Histochem Cell Biol 110:303

Krasteva G, Kummer W (2012) "Tasting" the airway lining fluid. Histochem Cell Biol 138:365

Krasteva G, Canning BJ, Papadakis T, Kummer W (2012) Cholinergic brush cells in the trachea mediate respiratory responses to quorum sensing molecules. Life Sci 91:992

Luciano L, Groos S, Reale E (2003) Brush cells of rodent gallbladder and stomach epithelia express neurofilaments. J Hictochem Cytochem 51:187

Ogata T (2000) Mammalian tuft (brush) cells and chloride cells of other vertebrates share a similar structure and cytochemical reactivities. Acta Histochem Cytochem 33:439

Roth J, Lucocq JM, Charest PM (1984) Light and electron microscopic demonstration of sialic acid residues with the lectin from Limax flavus: a cytochemical affinity technique with the use of fetuin-gold complexes. J Histochem Cytochem 32:1167

Yamamoto K, Ishimaru Y (2013) Oral and extra-oral taste perception. Semin Cell Dev Biol 24:240

Magnification: ×29,500


Enterocyte
Enterocyte
Brush cell

GLYCOCALYX (CELL COAT)

The outer surface of all animal cells is covered by a glycocalyx composed of oligosaccharides (glycans) of glycoproteins and glycolipids and a layer of secreted mucus, particularly in the gastrointestinal, respiratory, and urogenital tracts. The biological roles of the glycocalyx are diverse. In general terms, it exerts stabilizing and protective functions. Specific functions are related to the glycan structure and cell type and to specific recognition and interaction of glycans with other molecules. Certain glycans are important for development and differentiation of organs through modulation of cell-cell and cell-matrix interactions and signaling. They also can function as receptors for certain pathogens or may represent ligands for various receptors and can be involved in turnover and trafficking of molecules.

The glycocalyx may be so well developed that it can be observed by ordinary transmission electron microscopy. The intestinal absorptive cells are an example. In panel A, the brush border of an absorptive enterocyte is shown. At the tips of the microvilli, the glycocalyx appears as prominently visible antennulae microvillares (arrows). Panel B represents cross-sectioned microvilli of an enterocyte and staining with ruthenium red, a cationic dye that binds electrostatically to ionized carboxylic acid groups of acid mucopolysaccharides. This staining results in a highly increased contrast of the glycocalyx. The drawbacks of this staining are the nondiscriminatory reaction with polyanions and the membrane impermeability of the dye, which limit its use for compact tissues and staining of intracellular glycans. These major limitations have been overcome with the use of lectins and monoclonal anti-carbohydrate antibodies of defined specificity and the use of tissue sections from Lowicryl K4M embedded tissue or ultrathin frozen tissue sections. In panel C, the sialic acid specific lectin from *Limax flavus* has been used to detect sialic acid residues in the glycocalyx of absorptive enterocytes. This resulted in gold particle labeling of the antennullae microvillares in the Lowicryl thin sections. In panel D, a monoclonal antibody against the blood group A substance has been applied to thin sections from Lowicryl-embedded human duodenum of a blood group A subject. The blood group A determinant, which is terminal nonreducing *N*-acetylgalactosamine, is present in the glycocalyx, and the adhering mucus as indicated by the gold particle labeling.

References

Blanquet P (1976) Ultrahistochemical study on the ruthenium red surface staining. I. Processes which give rise to electron dense marker. Histochemistry 47:63

Goldstein IJ, Poretz RD (1986) Isolation, physicochemical characterization, and carbohydrate-binding specificity of lectins. In: Liener IE, Sharon N, Goldstein IJ (eds) The lectins. Properties, functions and applications in biology and medicine. Academic, Orlando, p 35

Luft JH (1971) Ruthenium red and violet. I. Chemistry, purification methods of use for electron microscopy, and mechanism of action. Anat Rec 171:347

Rambourg A (1971) Morphological and histochemical aspects of glycoproteins at the surface of animal cells. Int Rev Cytol 31:57

Rillahan CD, Paulson JC (2011) Glycan microarrays for the decoding of the glycome. Annu Rev Biochem 80:797

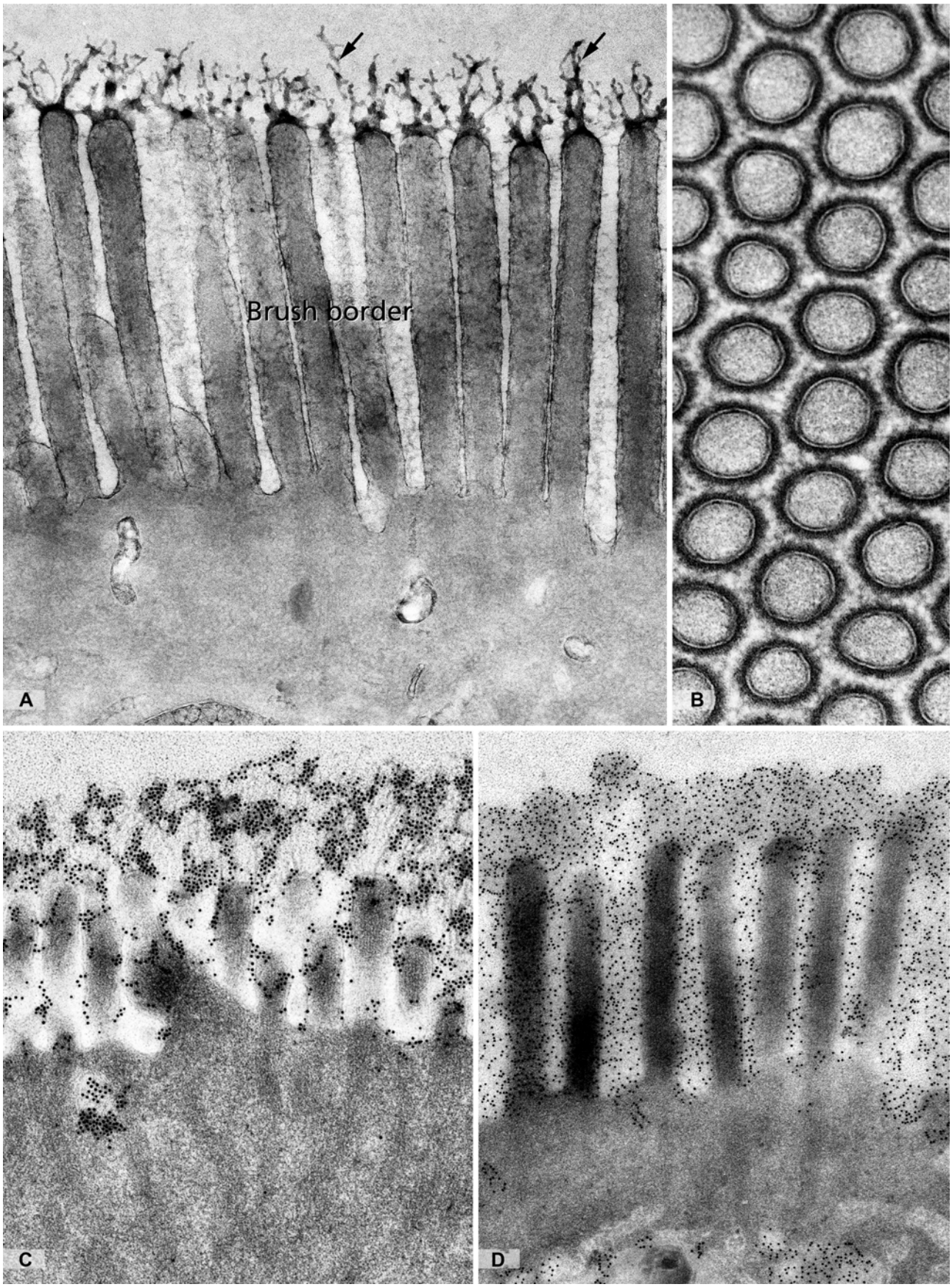
Roth J (2011) Lectins for histochemical demonstration of glycans. Histochem Cell Biol 136:117

Rutishauser U (2008) Polysialic acid in the plasticity of the developing and adult vertebrate nervous system. Nat Rev Neurosci 9:26

Schachter H (2010) *Mgat1*-dependent N-glycans are essential for the normal development of both vertebrate and invertebrate metazoans. Semin Cell Dev Biol 21:609

Tabak LA (2010) The role of mucin-type O-glycans in eukaryotic development. Semin Cell Dev Biol 21:616

Magnification: ×87,500 (**A**); ×120,000 (**B**); ×55,500 (**C**); ×52,000 (**D**)


Brush border
A
B
C
D

GLYCOCALYX: CELL TYPE SPECIFICITY AND DOMAINS

The analysis of the expression of various glycosyltransferases by Northern blot analysis and specific enzyme assays has shown their differential tissue expression. On this basis, the view is held that specific glycan structures in general reflect the expression of the respective glycosyltransferases. Cytochemical *in situ* studies provide distinct advantages over Northern blot analyses and specific enzyme assays because particular glycans can be localized to specific cell types in organs with complex cellular composition. With the application of lectins and monoclonal antibodies, histochemistry therefore represents an important tool in studies of cell type-specific glycosylation and may provide a clue about their possible functions.

Panel A shows the apical portions of a dark and a light cell of normal human breast duct epithelium and provides an example of cell type-specific and plasma membrane domain-related expression of a glycan. This Lowicryl thin section was incubated with a monoclonal antibody reactive with an *O*-glycan in normal human breast and breast carcinoma. The plasma membrane of the dark cell type is labeled (filled arrowheads), and that of the adjacent light cells is unlabeled (open arrowheads). A further detail is that the labeling of the dark cell is restricted to the apical plasma membrane domain, which is separated from the lateral plasma membrane by junctions (arrows).

In panel B, part of a capillary loop from rat renal glomerulus is seen and exemplifies another grade of glycocalyx domain formation in the podocyte plasma membrane. The labeling in the base of the podocyte foot processes (arrowheads) is the result of binding of gold-labeled *Helix pomatia* lectin, which has a nominal specificity for terminal, nonreducing *N*-acetylgalactosamine residues. Lectin binding did not occur to the rest of the podocyte plasma membrane as no labeling was detectable in the capillary endothelial cells (asterisk) and glomerular basement membrane. This showed that the podocyte foot process plasma membrane is highly specialized in terms of the composition of their glycocalyx. In contrast, the wheat germ lectin, which has a nominal specificity for *N*-acetylglucosamine residues, did label all regions of the podocyte plasma membrane, the glomerular basement membrane, and capillary endothelia (panel C). RBC: red blood cell.

References

Brown D, Roth J, Orci L (1985) Lectin-gold cytochemistry reveals intercalated cell heterogeneity along rat kidney collecting ducts. Am J Physiol 248:C348

Gersten KM, Natsuka S, Trinchera M, Petryniak B, Kelly RJ, Hiraiwa N, Jenkins NA, Gilbert DJ, Copeland NG, Lowe JB (1995) Molecular cloning, expression, chromosomal assignment, and tissue-specific expression of a murine alpha-(1,3)-fucosyltransferase locus corresponding to the human ELAM-1 ligand fucosyl transferase. J Biol Chem 270:25047

Kerjaschki D, Noronha-Blob L, Sacktor B, Farquhar MG (1984) Microdomains of distinctive glycoprotein composition in the kidney proximal tubule brush border. J Cell Biol 98:1505

Kitagawa H, Paulson JC (1994) Differential expression of five sialyltransferase genes in human tissues. J Biol Chem 269:17872

Lairson LL, Henrissat B, Davies GJ, Withers SG (2008) Glycosyltransferases: structures, functions, and mechanisms. Annu Rev Biochem 77:521

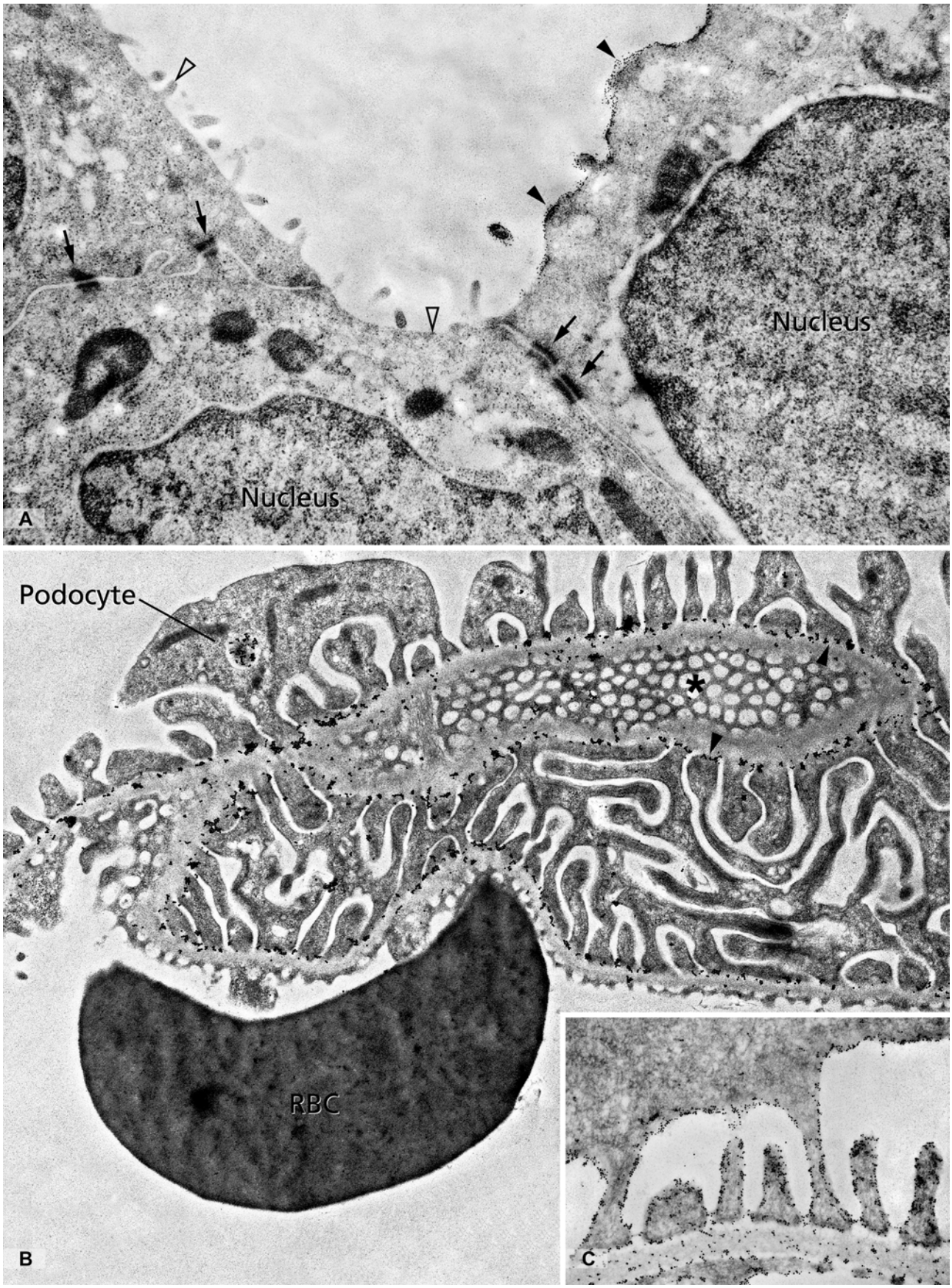
LeHir M, Kaissling B, Koeppen BM, Wade JB (1982) Binding of peanut lectin to specific epithelial cell types in the kidney. Am J Physiol 242:C117

Paulson JC, Rademacher C (2009) Glycan terminator. Nat Struct Mol Biol 16:1121

Roth J, Brown D, Orci L (1983) Regional distribution of *N*-acetyl-*D*-galactosamine residues in the glycocalyx of glomerular podocytes. J Cell Biol 96:1189

Yoshida Y, Kurosawa N, Kanematsu T, Taguchi A, Arita M, Kojima N, Tsuji S (1996) Unique genomic structure and expression of the mouse alpha 2,8-sialyltransferase (ST8Sia III) gene. Glycobiology 6:573

Magnification: ×20,000 (**A**); ×35,500 (**B**, **C**)


Nucleus
Nucleus
A
Podocyte
RBC
B
C

GLYCOCALYX CHANGES IN TUMORS

Particular glycans exhibit spatiotemporal expression patterns during embryonic development and may become reexpressed in malignant human tumors. Carcinoma-associated cell surface glycans can be involved in invasive and metastatic growth or of clinical importance as predictive markers. A commonly observed change is the increased synthesis of β 1,6-branched tri- and tetra-antennary glycans. This correlates with the metastatic potential of certain tumors and is an independent predictive marker in colon carcinoma. Sialylated glycans terminated in α2,6-linked sialic acid or the sialosyl-Tn antigen are associated with colon carcinoma progression and of predictive value. These findings, however, cannot be generalized.

Sialic acids also exist as homopolymers in α2,8-ketosidic linkages and such a polysialic acid is present on the neural cell adhesion molecule NCAM. This unique glycan modulates cell-cell and cell-substratum interactions during brain development and neuronal functions in the adult. Unexpectedly, polysialylated NCAM was detected in embryonic kidney and found to be reexpressed in the Wilms tumor, a highly malignant kidney tumor. In panel A, the presence of an electron dense surface coat of variable thickness (arrowheads) in a Wilms tumor is demonstrated. Small lumina (asterisks in panels A and C) are formed at sites of high surface coat thickness because of reduced cell adhesion. This surface coat has also been interpreted as a basement membrane, but, as shown in panels B and C, consists of polysialic acid as revealed by immunogold labeling with a monoclonal antibody. Polysialic acid exists in malignant neuroendocrine tumors and is of diagnostic importance. Experimental studies with small cell lung carcinoma cells have directly demonstrated the role of polysialic acid for invasive and metastatic growth properties. Clinical studies revealed the importance of measuring serum levels of polysialic acid in patients with neuroendocrine tumors as an indicator of tumor stage and progression.

References

Brockhausen I (1999) Pathways of *O*-glycan biosynthesis in cancer cells. Biochim Biophys Acta 1473:67

Dennis JW, Granovsky M, Warren CE (1999) Glycoprotein glycosylation and cancer progression. Biochim Biophys Acta 1473:21

Gluer S, Schelp C, Madry N, von Schweinitz D, Eckhardt M, Gerardy-Schahn R (1998) Serum polysialylated neural cell adhesion molecule in childhood neuroblastoma. Br J Cancer 78:106

Hakomori S-I (1996) Tumor-associated carbohydrate antigens and modified blood group antigens. In: Montreuil J, Vliegenthart J, Schachter H (eds) Glycoproteins and disease. Elsevier, Amsterdam/Lausanne/New York/Oxford/Shannon/Tokyo, p 243

Kobata A (1996) Cancer cells and metastasis. The Warren-Glick phenomenon – a molecular basis of tumorigenesis and metastasis. In: Montreuil J, Vliegenthart J F G, Schachter H (eds) Glycoproteins and disease, Elsevier, Amsterdam/Lausanne/New York/Oxford/Shannon/Tokyo, p 211

Roth J, Rutishauser U, Troy FA (1993) Polysialic acid. From microbes to man. Birkhäuser Verlag, Basel/Boston/Berlin

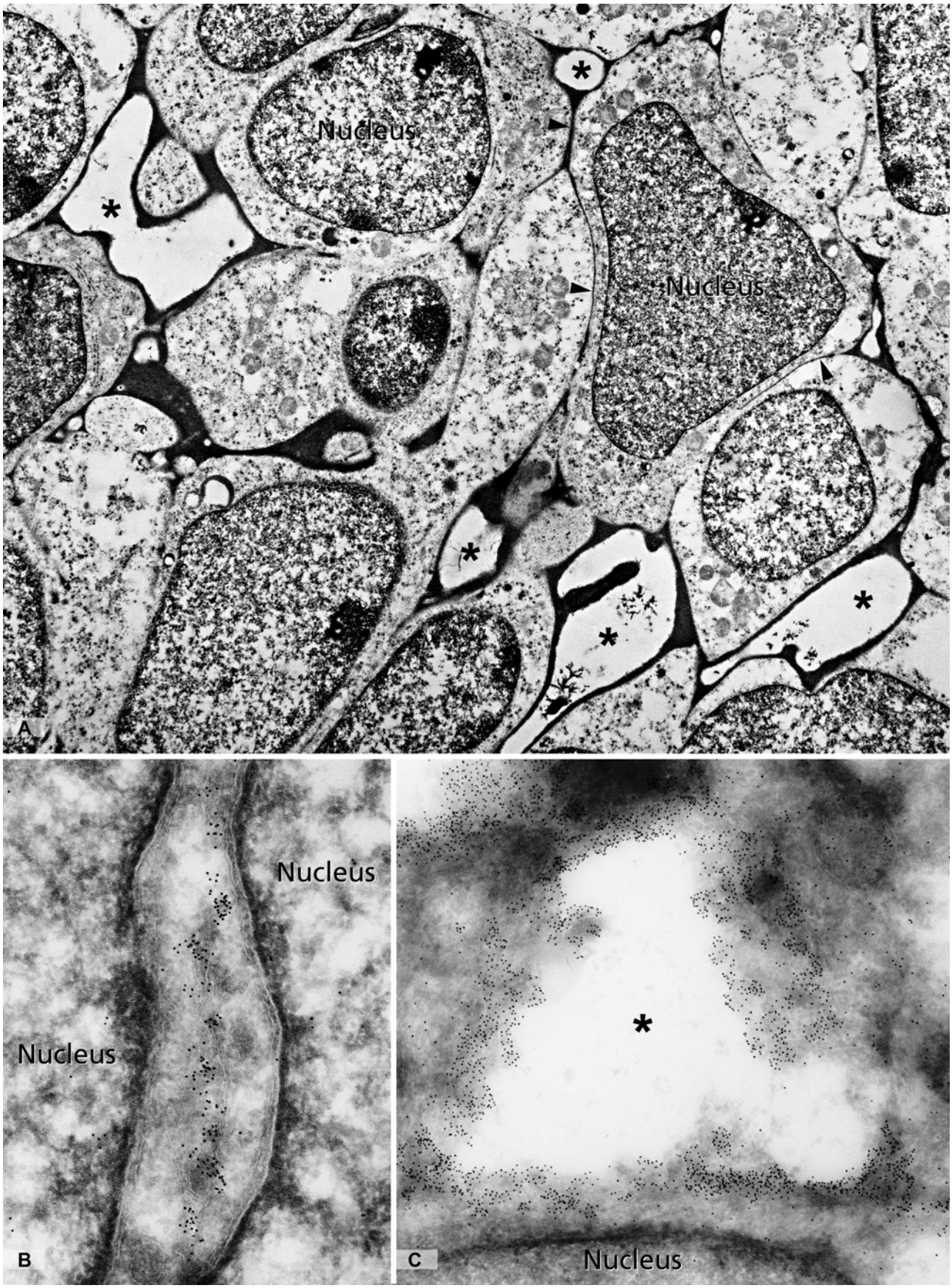
Roth J, Taatjes DJ, Wagner P, Weisgerber C, Heitz PU, Goridis C, Bitter-Suermann D (1988) Reexpression of poly(sialic acid) units of the neural cell adhesion molecule in Wilms tumor. Proc Natl Acad Sci USA 85:2999

Rutishauser U (1996) Polysialic acid and the regulation of cell interactions. Curr Opin Cell Biol 8:679

Seelentag WKF, Li WP, Schmitz SFH, Metzger U, Aeberhard P, Heitz PU, Roth J (1998) Prognostic value of beta 1,6-branched oligosaccharides in human colorectal carcinoma. Cancer Res 58:5559

Tanaka F, Otake Y, Nakagawa T, Kawano Y, Miyahara R, Li M, Yanagihara K, Inui K, Oyanagi H, Yamada T (2001) Prognostic significance of polysialic acid expression in resected non-small cell lung cancer. Cancer Res 61:1666

Magnification: 8,500 (**A**); ×58,000 (**B**); ×55,000 (**C**)


Nucleus
Nucleus
A
Nucleus
Nucleus
B
C
Nucleus