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Mouse antisera specific for desmosomal adhesion molecules of suprabasal skin cells, meninges, and meningioma

(desmosomes/keratinocytes/epidermis/epidermal differentiation/tumor markers)

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ABSTRACT **Mouse polyclonal antisera were raised to the M_r 130,000 and M_r 115,000 cell surface glycoproteins, desmocollins, of desmosomes from bovine nasal epithelium. Immunoblotting confirmed that the antisera were specific for the desmocollins. An immunofluorescence study showed that the antisera distinguished between the basal and suprabasal layers of bovine and human epidermis. The antibodies reacted with cultured keratinocytes only after calcium-induced stratification. In epidermis, therefore, there appears to be a difference between the desmocollins of basal and suprabasal cells that may be important in relation to epidermal differentiation. Previous work has shown that polyclonal antisera raised in other animals (guinea pigs and rabbits) against desmocollins, as well as against other desmosomal components, react with all desmosome-containing epithelia. In contrast, an immunofluorescence survey of bovine, rat, and human tissues showed that the present mouse antisera stained only suprabasal skin cells and the arachnoid layer of the meninges, demonstrating that these have common determinants that distinguished their desmocollins from those of all other tissues. The antibodies also stained 11 of 12 meningiomas and, therefore, may be useful as a marker not only for the diagnosis of these tumors but also for investigation of their histogenesis.**

Desmosomes are characteristic adhesive junctions of epithelial cells. Their protein and glycoprotein components and antigenic properties have been described in a series of recent publications (1–10). In desmosomes isolated from bovine nasal epithelium, two of the major components are a pair (sometimes a triplet) of glycoproteins having M_rs of 130,000 and 115,000 (4). These glycoproteins are immunologically related and have similar, but not identical, amino acid and carbohydrate compositions (9). We have suggested that these glycoproteins are directly involved in intercellular adhesion in the desmosome because (*i*) they are located on the surface of living Madin–Darby bovine kidney (MDBK) and canine kidney (MDCK) cells and normal and transformed human keratinocytes (11, 12) and (*ii*) Fab′ fragments of antibodies directed against them inhibit the formation of antibody-stainable desmosomal plaques in MDBK cells (11). To denote their adhesive role, we have termed them desmocollins I and II (Greek δεσμός = link; κόλλα = glue) (11). They also have been referred to as bands 4a and 4b (3) and as desmoglein IIa and IIb (5).

Desmosomal components are widely distributed and conserved between different tissues and animal species (6, 7). Immunoblotting studies have shown that the desmosomal glycoproteins are more variable in molecular weight, heterogeneity, and antigenic crossreactivity than are the desmosomal proteins (13, 14). This glycoprotein variability does not seem to be significant in relation to desmosomal adhesion, however, since mutual desmosome formation takes place in tissue culture between cells from human, bovine, canine, avian, and anuran amphibian sources (15), suggesting conservation of the adhesive recognition mechanism of desmosomes.

In seeking to study the mechanism of desmosomal adhesion, we have raised monoclonal antibodies to the desmocollins (to be described elsewhere). The sera derived from some of the mice immunized for hybridoma production showed novel properties, however, and these form the subject of our present paper. Unlike any of the other antibodies to desmosomal components so far described, these antisera appeared, on fluorescent staining of bovine nasal epithelium, to distinguish between the basal and suprabasal cells of epidermis, reacting specifically with the latter. Since this observation appeared to be significant in relation to epidermal differentiation, the properties of these sera were investigated further. In addition, the present antibodies stained human arachnoid and are used in this study to investigate the staining of intracranial meningiomas.

MATERIALS AND METHODS

Production of Antibodies. Mouse antibodies to the desmocollins were prepared essentially as described for guinea pigs (6). Mice were given 3 injections. (*i*) 100 μg of protein mixed 1:1 with Freund's complete adjuvant given intraperitoneally, followed 8 weeks later by (*ii*) 100 μg of protein mixed 1:1 with Freund's incomplete adjuvant given intraperitoneally, followed 4 weeks later by (*iii*) 50 μg of protein only given intraperitoneally. The animals were bled 3 days later. Guinea pig anti-desmosomal antibodies used here were as described and characterized previously (6, 13).

Antibodies to laminin were prepared in the following way. Purified laminin (Bethesda Research Laboratories, GIBCO Limited, U.K.) was dissolved in phosphate-buffered saline (0.8% NaCl/0.02% KCl/0.02% KH_2PO_4/0.12% Na_2HPO_4, pH 7.4) at 4°C to a concentration of 0.4 mg/ml. Rabbits were injected intramuscularly with 0.2 mg of laminin mixed 1:1 with Freund's complete adjuvant. This was followed 8 weeks later by intramuscular injection of 0.2 mg of laminin mixed 1:1 with Freund's incomplete adjuvant. The rabbits were bled 4 weeks later. This antibody is used as a marker for the basal lamina in Fig. 2 *b* and *d*.

Immunoblotting. Antibodies were characterized by immunoblotting as described (13). Detection of bound mouse antibody was by ^{125}I-conjugated anti-mouse Ig and of bound guinea pig antibody was by ^{125}I-labeled protein A (Amersham International, U.K.).

Fluorescent Antibody Staining. A variety of bovine, rat, and human tissues were selected for staining (Table 1). Sections (5 μm) of frozen tissue were stained as described (6), with

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Table 1. Fluorescent staining of frozen tissues with mouse polyclonal anti-desmocollin antisera

	Tissues		
	Human	Bovine	Rat
Nonstaining	Bladder	Bladder	Bladder
	Kidney	Kidney	Kidney
	Liver	Liver	Liver
	S. intestine	S. intestine	S. intestine
	Colon	—	—
	Breast	—	—
	Esophagus	—	Esophagus
	—	Heart	Heart
	—	Cornea	Cornea
	—	—	Thyroid
	—	—	Ovary
	—	—	Uterus
	—	—	Tongue
	—	—	Cerebral cortex
	—	—	Spinal cord*
	—	—	Skeletal muscle*
Staining	Skin Meninges	Skin	†

S. intestine, small intestine.
*Controls—do not contain desmosomes.
†Rat skin was not tested.

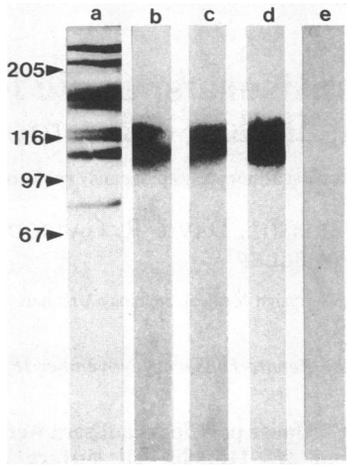

FIG. 1. Immunoblot showing specificity of mouse and guinea pig antisera. Lanes: a, Coomassie blue-stained gel of bovine desmosomal cores; b, autoradiograph of nitrocellulose strip of desmosomal cores after exposure to mouse anti-desmocollin antiserum at a dilution of 1:50, followed by ^{125}I-labeled anti-mouse Ig; c, as lane b but with serum from another mouse; d, as lane b but with guinea pig anti-desmocollin antiserum and ^{125}I-labeled protein A; e, pre-injection serum from mouse a. All three antisera react exclusively with the desmocollin bands and show no crossreactivity with other desmosomal proteins and glycoproteins. (A third mouse gave identical results—not shown.) Molecular weights of reference proteins given in kilodaltons.

fluorescein isothiocyanate-conjugated rabbit anti-guinea pig IgG (Wellcome) or fluorescein isothiocyanate-conjugated sheep anti-mouse Ig (Amersham International). In double-labeling experiments, sections were incubated with antiserum to laminin and antiserum to desmocollin together and then successively with fluorescein isothiocyanate-conjugated sheep anti-mouse Ig and Texas red-conjugated donkey anti-rabbit Ig (Amersham International).

Keratinocytes. Human foreskin keratinocytes were cultured, stained whilst alive, and then fixed in 3.5% formaldehyde as described (12). Involucrin staining was carried out on cells that had been permeabilized with cold methanol. Specific anti-involucrin antibody was kindly provided by F. Watt.

RESULTS

Specificity of Antisera. The specificities of mouse antisera raised against desmocollins were determined by immunoblotting on desmosomal cores (2) from bovine nasal epithelium. Antisera from three mice were used in this study. Each reacted specifically with the two desmocollin bands (Fig. 1) and showed no crossreactivity with other desmosomal components. Furthermore, none of the antisera showed reactivity with cytokeratin. However, as a precautionary measure, all antisera were absorbed with cytokeratin before fluorescent staining (3). The guinea pig anti-desmocollin antiserum reacted with the same desmosomal components as did the mouse antisera (Fig. 1, lane d).

Fluorescent Antibody Staining of Epidermis. Frozen sections of bovine nasal epithelium and human skin were stained by indirect immunofluorescence. Specific antisera against desmocollins, as well as antisera against other desmosomal components, raised in guinea pigs and rabbits stained the entire epidermis, including the basal layer (stratum ger-

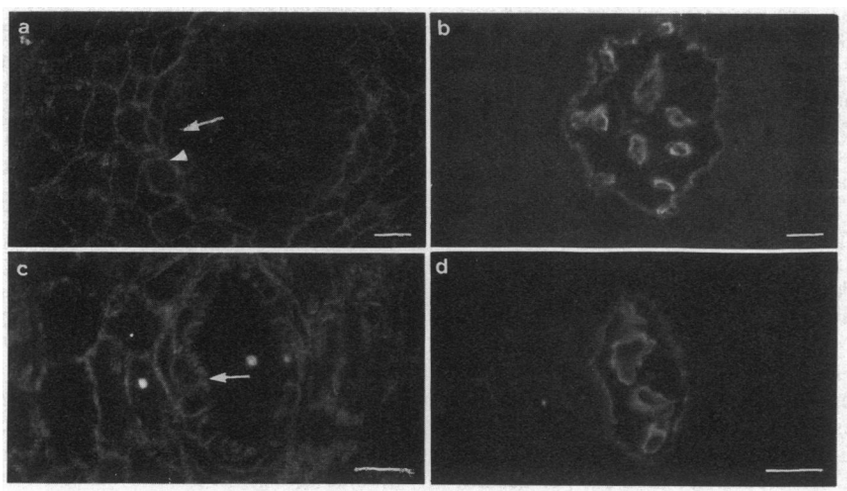

FIG. 2. Frozen sections of bovine nasal epithelium stained with anti-desmocollin antisera (*a* and *c*) and anti-laminin antiserum (*b* and *d*). (*a*) Antiserum from mouse a (see lane b, Fig. 1). Arrow, basal lamina; arrowhead, junction between basal and suprabasal cells. (Fluorescein.) (*b*) Same field as *a* showing basal lamina. (Texas red.) (*c*) Guinea pig anti-desmocollin antiserum. Arrow, basal lamina. (Fluorescein.) (*d*) Same field as *c*. (Texas red.) Identical results were obtained with the other two mouse sera. (Bar = 20 μm.)

minativum), with the staining becoming weak in the cornified layers (refs. 3, 6, 7, 13; unpublished observations) (Fig. 2 *c* and *d* and Fig. 3*b*). However, the three mouse antisera stained only the suprabasal layers, leaving the basal layer unstained (Fig. 2 *a* and *b* and Fig. 3*a*). The interface between the first layer of suprabasal cells and the basal cells was also stained by these sera. Antisera obtained from other mice immunized against the same antigen stained all layers.

Staining of Cultured Keratinocytes. Human foreskin keratinocytes maintained in medium containing low calcium (0.1 mM) remain as a monolayer and fail to differentiate. When the calcium concentration is raised to physiological levels (1.8–2 mM), rapid desmosome formation occurs, followed by stratification and cell differentiation (16–18). Guinea pig anti-desmocollin antibodies stain the surface of living keratinocytes in both low- and normal-calcium media (12). Furthermore, cells which are about to stratify can be identified in the monolayer by staining for the cytoplasmic protein, involucrin (19). Mouse anti-desmocollin antisera specific for suprabasal epidermal cells gave no staining of keratinocytes in low-calcium medium, nor did they stain cells up to 6 hr after increase in calcium concentration, by which time the first signs of stratification were evident. Furthermore, they did not recognize involucrin-positive cells within the monolayer. However, after stratification had been allowed to proceed for 48 hr, the antisera stained the living keratinocytes in the same pattern as did guinea pig anti-desmocollin antisera (Fig. 4).

Survey of Fluorescent Antibody Staining of Different Tissues. Guinea pig polyclonal antibodies against all desmosomal components stain a wide variety of tissues from different vertebrate animal species (7). Mouse anti-desmocollin antisera specific for subrabasal epidermis are much more selective. A survey of many epithelial tissues of human, bovine, and rat origin showed these antisera to be specific for suprabasal epidermal cells and the arachnoid layer of the meninges (Fig. 5*a*). No reactivity with any other tissue was encountered (Table 1).

Meningiomas. Since there is no specific marker for meningiomas, we tested the ability of the antisera to stain these tumors. In common with other desmosomal antibodies, they were found to stain frozen sections of meningiomas in a punctate fashion (Fig. 5*b*). Of the 12 meningiomas, 3 were

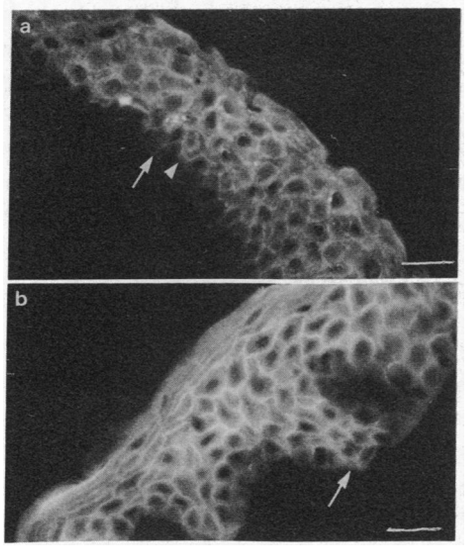

FIG. 3. Frozen sections of human thigh skin stained with anti-desmocollin antibodies from mouse a (see lane b, Fig. 1) (*a*) and from guinea pig (*b*). Arrows, basal lamina (position confirmed by anti-laminin staining—not shown); arrowhead, junction between basal and suprabasal cells. Identical results were obtained with the other two mouse sera. (Bar = 20 μm.)

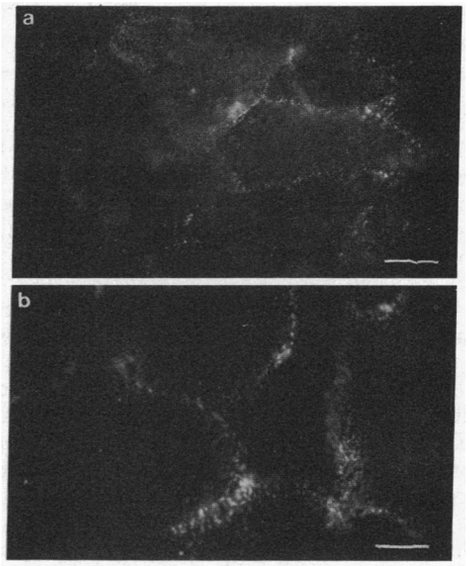

FIG. 4. Cultured human foreskin keratinocytes (after 48 hr in medium with physiological calcium concentration) stained whilst alive with anti-desmocollin antisera from mouse a (see lane b, Fig. 1) (*a*) and from guinea pig (*b*). Identical results were obtained with the other two mouse sera. (Bar = 20 μm.)

meningothelial, 3 transitional, 4 fibroblastic, 1 papillary, and 1 angioblastic (hemangiopericytic) in their histological pattern (20). Eleven of the 12 meningiomas showed punctate staining. No staining of gliomas was obtained (Fig. 5*c*).

DISCUSSION

Some polyclonal mouse antisera that are specific for desmocollins, surface adhesion molecules of desmosomes,

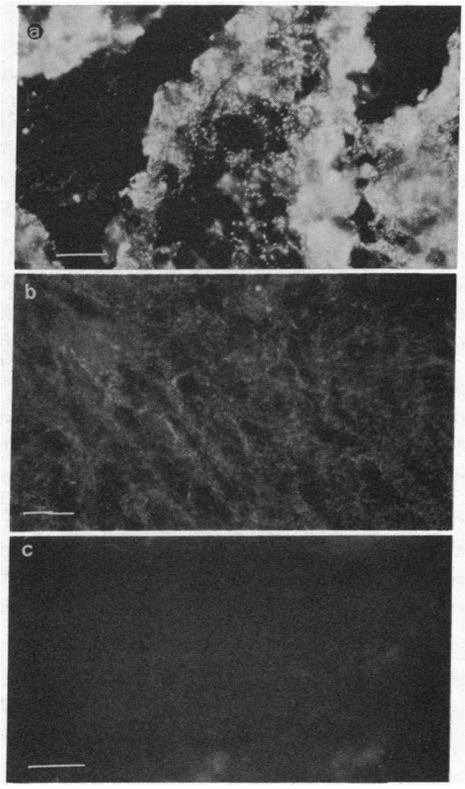

FIG. 5. Frozen sections of normal arachnoid (*a*), meningioma (*b*), and glioma (*c*) stained with anti-desmocollin antiserum from mouse a (see lane b, Fig. 1). Identical results were obtained with the other two mouse sera. (Bar = 20 μm.)

show restricted tissue crossreactivity. They distinguish between basal and suprabasal layers of epidermis and stain no other tissues except meninges. Cowin *et al.* (7) showed that guinea pig anti-desmocollin antisera stained the epidermis of vertebrate species from man to frog with equal intensity but gave much weaker staining of nonepidermal epithelia. This suggested that there are antigenic differences between desmocollins of epidermal and nonepidermal epithelia. Our present results demonstrate such a difference conclusively. Furthermore, they demonstrate that there is also an antigenic difference between the basal and suprabasal layers of epidermis. Whether this difference is due to the absence of a determinant or masking of a determinant in the basal layer remains to be elucidated.

In 1964, Listgarten (21) reported slight differences in ultrastructure between the desmosomes of basal and suprabasal skin cells. More recent studies have concluded, however, that the most striking difference between desmosomes in the basal, spinous, and granular layers of epithelia is quantitative rather than qualitative: in the basal layer, desmosomes are fewer than in the more superficial strata (22–30). In fact, desmosome morphology appears to become markedly altered only as granular cells change into keratinized squame cells (29). Thus, the antigenic difference that we have discovered does not correlate with any obvious variation in desmosomal ultrastructure.

There are desmosomes at the interface between basal and suprabasal cells, and staining for suprabasal desmocollins was found at this interface. Since the two halves of a desmosome are contributed by the two adhering cells, this means either that basal cells possess antigenically different desmocollins on their apical and lateral surfaces or that desmosomal adhesion between basal and suprabasal cells involves antigenically distinct molecules. If we assume that the molecular difference is a consequence of cellular differentiation in the suprabasal cells, the latter of these two possibilities seems more likely. This interpretation is supported by the finding that cultured keratinocytes acquire reactivity with the antibody only after stratification. This would mean that, whatever the nature of the chemical difference between the molecules, it is not related to the function of adhesive recognition: basal and suprabasal cells contribute chemically distinct desmocollin molecules to opposite halves of their mutual desmosomes. This is not unreasonable because it has been shown that mutual desmosome formation can take place between cells of different tissues and species (15, 31), even though the desmosomal glycoproteins differ in molecular weight, heterogeneity, and antigenic crossreactivity (13). Giudice *et al.* (14) have also reported antigenic differences between desmosomal glycoproteins from bovine nasal, corneal, and esophageal epithelia.

Epidermis is a dynamic tissue in which there is a rapid loss and renewal of cells. Changes in cell–cell contact would seem to be required in order to allow migration of cells upwards from the basal layer. We have suggested that desmosomal breakdown and reformation may play a role in this (12), and it is possible that differences between basal and suprabasal desmocollins are significant in this regard: the desmosomes of basal cells may be more labile than those of suprabasal cells.

Several other proteins have been shown to vary during epidermal differentiation. The expression of different keratins depends on the stage of differentiation of epidermal cells. Basal epidermal cells express keratins of M_rs 50,000, and 58,000 (32, 33). The M_r 56,000 and M_r 65,000–67,000 keratins are expressed only by the suprabasal cells and, thus, are characteristic of terminal differentiation (32, 33). Involucrin synthesis also provides a differentiation marker; in frozen sections of skin, it is not seen in the basal layer but begins several layers above it (34–36). The distribution of keratinocyte-specific transglutaminase corresponds with that of involucrin (37). In contrast, the liver cell adhesion molecule (L-CAM) is present only in the basal layer of epidermis (38).

The lectin peanut agglutinin stains the suprabasal layers of skin, but not the basal layer, in a pattern identical to that of our antisera. Treatment of skin sections with neuraminidase abolishes this differential staining by peanut agglutinin so that it stains all layers (39). Neuraminidase treatment did not cause loss of differential staining with our antisera, suggesting that the staining pattern is not due to the masking of a carbohydrate determinant in the basal layer by sialic acid. It remains possible, however, that the differential staining pattern is due to the presence of carbohydrate differences between the basal and suprabasal layers.

The arachnoid layer of the meninges possess desmosomes that are associated with vimentin, rather than keratin, tonofilaments (40). These cells also stain with the mouse antisera, demonstrating that their desmocollins possess in common with suprabasal skin cells an antigenic determinant(s) that appears to be exclusive to these two cell types. The biological significance of this observation is obscure, but it could be important in relation to the histogenesis of the meninges, which is a controversial area (41). The antisera also stained 11 of 12 meningiomas of various histological types, demonstrating that the antigenic determinant(s) is not obscured or altered by neoplastic transformation. This observation may be of considerable clinical importance because there is no reliable marker for meningiomas. This specific property of meningiomal desmosomes should make it possible to raise monoclonal anti-desmocollin antibodies that will identify specifically most intracranial and spinal tumors of arachnoid origin.

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1. Skerrow, C. J. & Matoltsy, A. G. (1974) *J. Cell Biol.* **63,** 515–523.
2. Gorbsky, G. & Steinberg, M. S. (1981) *J. Cell Biol.* **90,** 243–248.
3. Franke, W. W., Schmid, E., Grund, C., Mueller, H., Engelbrecht, I., Moll, R., Stadler, J. & Jarasch, E.-D. (1981) *Differentiation* **20,** 217–241.
4. Franke, W. W., Moll, R., Schiller, D. L., Kartenbeck, J. & Mueller, H. (1982) *Differentiation* **23,** 115–127.
5. Cohen, S. M., Gorbsky, G. & Steinberg, M. S. (1983) *J. Biol. Chem.* **258,** 2621–2627.
6. Cowin, P. & Garrod, D. R. (1983) *Nature (London)* **302,** 148–180.
7. Cowin, P., Mattey, D. L. & Garrod, D. R. (1984) *J. Cell Sci.* **66,** 119–132.
8. Gorbsky, G., Cohen, S. M., Shida, M., Guidice, G. J. & Steinberg, M. S. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 810–814.
9. Kapprell, H.-P., Cowin, P., Franke, W. W. & Ponstingl, H. (1985) *Eur. J. Cell Biol.* **36,** 217–229.
10. Garrod, D. R. & Cowin, P. (1985) in *Receptors in Tumour Biology*, ed. Chadwick, C. M. (Cambridge Univ. Press, Cambridge, England), in press.
11. Cowin, P., Mattey, D. L. & Garrod, D. R. (1984) *J. Cell Sci.* **70,** 41–60.
12. Watt, F. M., Mattey, D. L. & Garrod, D. R. (1984) *J. Cell Biol.* **99,** 2211–2215.
13. Suhrbier, A. & Garrod, D. R. (1985) *J. Cell Sci.* **81,** 223–242.
14. Guidice, G. J., Cohen, S. M., Patel, N. H. & Steinberg, M. S. (1984) *J. Cell. Biochem.* **26,** 35–45.
15. Mattey, D. L. & Garrod, D. R. (1985) *J. Cell Sci.* **75,** 377–399.
16. Garrod, D. R. (1985) in *Cellular and Molecular Control of Direct Cell Interactions in Developing Systems, Series A. Life Sciences*, NATO Advanced Studies Institute, ed. Marthy, H.-J. (Plenum, New York), pp. 43–83.
17. Hennings, H., Michael, D., Cheng, C., Steinert, P., Holbrook, K. & Yuspa, S. H. (1980) *Cell* **19,** 245–254.
18. Jones, J. C. R., Goldman, A. E., Steinert, P. M., Yuspa,

S. H. & Goldman, R. D. (1982) *Cell Motil.* **2**, 197–213.
19. Watt, F. M. & Green, H. (1982) *Nature (London)* **295**, 434–436.
20. Kepes, J. J. (1982) *Meningiomas: Biology, Pathology and Differential Diagnosis* (Masson, New York).
21. Listgarten, M. A. (1963) *Am. J. Anat.* **14**, 49–69.
22. Farquhar, M. G. & Palade, G. E. (1963) *J. Cell Biol.* **17**, 375–412.
23. Farquhar, M. G. & Palade, G. E. (1965) *J. Cell Biol.* **26**, 263–291.
24. Kelly, D. E. (1966) *J. Cell Biol.* **28**, 57–72.
25. Breathnach, A. S. (1975) *J. Invest. Dermatol.* **65**, 2–15.
26. Allen, T. D. & Potten, L. S. (1975) *J. Ultrastruct. Res.* **51**, 94–105.
27. Shimono, M. & Clementi, F. (1976) *J. Ultrastruct. Res.* **56**, 121–136.
28. Skerrow, C. J. (1978) *Invest. Cell Pathol.* **1**, 23–37.
29. White, F. H. & Gohari, K. (1984) *J. Submicrosc. Cytol.* **16**, 407–422.
30. White, F. H. & Gohari, K. (1984) *J. Cell Sci.* **66**, 411–429.
31. Overton, J. (1977) *Dev. Biol.* **55**, 103–116.
32. Woodcock-Mitchell, J., Eichner, R., Nelson, W. G. & Sun, T.-T. (1982) *J. Cell Biol.* **95**, 580–588.
33. Sun, T.-T., Eichner, R., Nelson, W. G., Tseng, S. C. G., Weiss, R. A., Jarvinen, M. & Woodcock-Mitchell, J. (1983) *J. Invest. Dermatol.* **81**, 109s–115s.
34. Rice, R. H. & Green, H. (1979) *Cell* **18**, 681–694.
35. Banks-Schlegel, S. & Green, H. (1981) *J. Cell Biol.* **90**, 732–737.
36. Watt, F. M. & Green, H. (1981) *J. Cell Biol.* **90**, 738–742.
37. Thacher, S. M. & Rice, R. H. (1985) *Cell* **40**, 685–695.
38. Thiery, J.-P., Delouvée, A., Gallin, J., Cunningham, B. A. & Edelman, G. M. (1984) *Dev. Biol.* **102**, 61–78.
39. Watt, F. M. (1983) *J. Invest. Dermatol.* **81**, 100s–103s.
40. Kartenbeck, J., Schwechheimer, K., Moll, R. & Franke, W. W. (1984) *J. Cell Biol.* **98**, 1072–1081.
41. Le Douarin, N. (1982) *The Neural Crest* (Cambridge Univ. Press, Cambridge, England).