Myosin at the Apical Pole of Ciliated Epithelial Cells
As Revealed by a Monoclonal Antibody

Catherine Klotz, Nicole Bordes, Marie-Christine Laine, Daniel Sandoz, and Michel Bornens
Centre de Biologie Cellulaire, Centre National de la Recherche Scientifique, 94200 Ivry-Sur-Seine, France. Dr. Klotz's and Dr. Bornens' new address is Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, 91190 Gif-Sur-Yvette, France.

Abstract. A monoclonal antibody (CC-212), obtained in a fusion experiment in which basal bodies from quail oviduct were used as immunogen, has been shown to label the apical pole of ciliated cells and to react with a 200-kD protein. This monoclonal antibody was demonstrated to be an anti-myosin from smooth muscle or from nonmuscular cells using the following criteria: (a) On Western blots it reacted with the myosin heavy chains from gizzard and platelet extracts and from cultured cell line extracts, but did not react with striated muscle myosin heavy chains. (b) By immunofluorescence it decorated the stress fibers of well-spread cells with a characteristic striated pattern, while it did not react with myotubes containing organized myofibrils.

On native ciliated cells as well as on Triton-extracted ciliated cortices from quail oviduct, this monoclonal antibody decorated the apical pole with a stronger labeling of the periphery of the apical area. Ultrastructural localization was attempted using the immunogold technique on the same preparation. Myosin was associated with a filamentous material present between striated rootlets and the proximal extremities of the basal bodies. No labeling of the basal body itself or of axoneme was observed.

CONTRACTILE proteins have been reported to be ubiquitous in eukaryotic cells. Their presence is related to motile functions (7, 8, 11, 14, 18, 27, 34, 35, 37). In differentiated tissues, such as intestinal epithelium, the presence, localization, and role of actin and myosin and other contractile proteins are well documented (16, 20, 21). In pigmented epithelium of retina, the presence of actin bundles at the periphery of the apical pole of each cell, and the demonstration that these cells can contract in experimental conditions, has led Owaribe et al. (24) to hypothesize the presence of myosin in the apex of these cells.

On the contrary and despite numerous studies, the presence of contractile proteins in ciliated epithelia is not well documented. The first study of Reverdin et al. (30) established the presence of actin microfilaments in the cortical cytoplasm of tracheal cells. In a recent publication Reed et al. (29) present a detailed study of the cortical cytoskeleton of lateral cells from mussel gills. They propose a three-dimensional model in which the actin network and microtubules are organized from the basal feet. They further speculate that interactions between the different meshworks and basal bodies might allow the cell to determine the direction of an environmental stimulus and to respond to stimuli.

Interestingly, very few data have been reported on the association of the ubiquitous actin network with myosin. In the case of ciliated tracheal epithelial cells Gordon et al. (13) reported that myosin was tightly associated with basal bodies (as were tropomyosin and α-actinin), only a light and diffuse labeling being observed in the cytoplasm. Other results (31) pointed to the proximal part of the axonemes as the exclusive localization for myosin. These contradictory results are based on the use of polyclonal antibodies raised against chicken smooth muscle myosins in the first case and frog striated muscle myosins in the second.

In an attempt to re-examine the presence of myosin in the apical pole of ciliated cortices, and to avoid any problem of species or tissue specificity of the antibody used, we assumed that if myosin is a constitutive protein of these cortices, one might be able to raise a monoclonal antibody (MAb) against it, using a semi-purified basal body preparation as an immunogen. Indeed, several clones reacting with the apical pole of cortices were produced, among which one (CC-212) reacted with a 200-kD protein which was identified as myosin by several criteria. Its localization in the apical cortex from ciliated epithelial cells does not correspond to any of the descriptions reported in earlier studies (13, 31), but rather seems to co-localize with the actin microfilament meshwork described by Reed et al. (29).

Here we report the identification of the MAb CC-212 and the localization of myosin in ciliated epithelial cells from laying quail oviduct.

1. Abbreviations used in this paper: MAb, monoclonal antibody; TBS-Tw, 10 mM Tris buffer, pH 7.4, 0.15 M NaCl containing 0.1% Tween 20.
Materials and Methods

Immunogen and Fusion Experiments

The preparation of basal bodies as immunogen and the fusion protocol have previously been described (17). Briefly, basal bodies from laying quail oviduct (Coturnix coturnix japonica) were prepared according to Anderson (1) with an additional treatment of the cortices with heparin (10) or with RNase and DNase, to solubilize the chromatin. Immunization was performed on BP mice (3), and fusion was achieved according to Gallré and Miletin (22) with 363 Ag8-653 myeloma cells.

The MAb CC-212 used in this study was an IgM as determined by Ouchterlony’s double diffusion test (data not shown).

Cell Cultures

A Potoroo kidney epithelial cell line (PtK2), mouse fibroblasts (3T3), baby hamster kidney (BHK21), and human epithelial cells (HeLa) were grown on coverslips to be used for subsequent immunofluorescence or in 150-cm² Falcon tissue culture dishes (Falcon Labware, Becton, Dickinson & Co., Oxnard, CA) to be used for protein sample preparation for electrophoretic analysis.

PtK2 were grown in Eagle’s minimum essential medium, 3T3 in RPMI 1640, and BHK21 in Glasgow minimum essential medium. All media were supplemented with 2 mM glutamine, 10% fetal calf serum, 10 μg/ml penicillin, and 20 μg/ml streptomycin (Flow Laboratories, Irvine, Ayrshire, Scotland). Trypsin phosphate broth was also added to the culture medium of the BHK21 cell line (60 ml/500 ml). All the reagents were from Flow Laboratories.

Human myogenic cell cultures containing both myoblasts and myotubes are described elsewhere (33).

Preparations of Cortices

Ciliated or deciliated cortices were prepared either from one oviduct of laying quail or from musk gills according to Anderson (1) with one modification; an antiprotease mixture (9) was added to all solutions. The suspensions of cortices were used immediately for immunofluorescence or ultrastructural studies, and for electrophoretic analysis.

Indirect Immunofluorescence Study

Immunofluorescence studies on ciliated cells were done on fragments of cortices as described previously (17). The coverslips were then incubated with 50 μl of culture supernatant undiluted for 20 min at room temperature and washed twice in 10 mM Tris buffer, pH 7.4, 0.15 M NaCl containing 0.1% Tween 20 (TBS-Tw). Fluorescein isothiocyanate-labeled goat IgG anti-mouse whole immunoglobulins, pre-absorbed with a suspension of ciliated cortices, were then applied for 20 min and washed twice in TBS-Tw. For double labeling experiments a mixture of both antibodies CC-212 and a rabbit anti-actin was applied in the incubation medium.

Western Blot Analysis. Western blots were performed from SDS PAGE (5–15%) of total extracts from quail ciliated or deciliated cortices, quail gizzard, quail striated myofibrils, PtK2, and human platelets. As shown in Fig. 1, CC-212 labeled a band of 200 kD in every case except in the case of the quail striated myofibril sample, which was unlabeled. It was verified that this protein was not present in the Triton-soluble fraction obtained during the preparation of cortices. The monoclonal antibody CC-212 was selected for its strong labeling of ciliated cortices from quail oviduct and for its interaction with a 200-kD protein on Western blots of proteins extracted from the deciliated cortices (see below). Before describing the results on ciliated oviducts we will present the cytochemical and histochemical data that allowed us to demonstrate the specificity of this MAb for smooth muscle and nonmuscular myosins.

Characterization of the MAb CC-212

Western Blot Analysis. Western blots were performed from SDS PAGE (5–15%) of total extracts from quail ciliated or deciliated cortices, quail gizzard, quail striated myofibrils, PtK2, and human platelets. As shown in Fig. 1, CC-212 labeled a band of 200 kD in every case except in the case of the quail striated myofibril sample, which was unlabeled. It was verified that this protein was not present in the Triton-soluble fraction obtained during the preparation of cortices.

Immunofluorescence Study. When assayed on established cell lines 3T3, BHK21, and PtK2, a striated staining was observed along stress fibers and at the cell periphery of well-spread cells; on spreading cells, a bright staining of microspikes was observed (Fig. 2). On myogenic cell cultures, where myoblasts and myotubes coexisted, CC-212 stained mononucleated cells, either fibroblasts or myoblasts, with the striated aspect of stress fibers observed in other cell lines. The striated myofibrils in myotubes were not labeled by CC-212 but were labeled by anti-actin and showed the usual striated pattern (Fig. 2). From these and the above data, we concluded that the pro-
tein target of CC-212 was the heavy chains of myosin present in smooth muscle and in nonmuscular cells, with no detectable cross-reaction with the striated muscle isoform.

Localization of the Antigen CC-212 on Ciliated Epithelium

Indirect Immunofluorescence. The staining of native ciliated cells obtained after methanol fixation constitutes the more native localization of the antigen. Top views of epithelial fragments (Fig. 3 a) showed a heavy staining of a belt at the periphery of the ciliated cells as well as a punctuated labeling of the apical area. This CC-212 labeling co-localized with the peripheric staining observed with a polyclonal antibody against actin (Fig. 3 b), except for the lower part of cilia which is labeled by anti-actin (as observed by others [31]), but was not by CC-212. Depending on the localization of the fragment in the oviduct epithelium, the ciliated cells were either adjacent to or separated by cells unstained by CC-212 probably unciliated mucus cells. Apparently, the antigen localization was not modified by a treatment with 0.1% Triton X-100 before the fixation by methanol (data not shown). Moreover, ciliated or deciliated cortices gave the same staining (Fig. 3, c–e): a very thin fluorescent line with a punctuated aspect at the apical region and a brighter staining at the lateral parts. No labeling of cilia was observed.

It is noteworthy that similar staining was observed on ciliated epithelia from several origins (mussel gills, frog palate, and human bronchial biopsies). An example is shown in Fig. 3 f.

Electron Microscopy. Ultrastructural localization (Fig. 4) supported the observations described above. CC-212 strongly labeled a fibrillar material which seemed to join together the proximal parts of the basal bodies as well as filaments connected to the basal feet. In addition, some dispersed gold granules were found in a deeper region between the cell apex and the nucleus. A peripheral material close to the zonula oc-
Figure 2. CC-212 labeling of cells in cultures. (A) Indirect immunofluorescence of PtK2 labeled by CC-212 showing one well-spread cell with striated stress fibers. Striations are in register particularly in Y-shape fibers. Spreading cells only show a staining of microspikes. Bar, 5 μm. (B and C) Double immunofluorescence labeling of PtK2 by anti-actin (B) and CC-212 (C). Bars, 2 μm. (D) Indirect immunofluorescence staining of human myogenic cells labeled by CC-212. Mononucleated cells show a striated staining of stress fibers. Myotubes exhibit a diffuse unlocalized fluorescence. Myofibrils are not labeled. Bar, 10 μm. (E) Phase-contrast of D. (F and G) Double immunofluorescent labeling of human myotubes with CC-212 (F) and anti-actin (G) corresponding phase-contrast (H). Bar, 10 μm.

Discussion

In an attempt to re-examine the presence and the ultrastructural localization of myosin in the apical region of ciliated epithelial cells, we have raised MAb against a crude preparation of basal bodies extracted from the ciliated epithelia of laying quail oviduct. Among secreting clones, CC-212 has been identified as anti-myosin from smooth muscle and non-muscular cells using the following criteria: (a) Immunodetection on blots of myosin heavy chains from human platelets, quail gizzard, and PtK2. No reaction was observed with myosin from striated muscle. (b) Immunocytochemistry on established cell lines showed the typical striated pattern of stress fibers described for several contractile proteins (α-
Figure 3. Immunofluorescence study of ciliated epithelia. (a and b) Top views of ciliated fragments of quail oviduct fixed by methanol (−20°C) without detergent treatment. (a) Immunofluorescence and phase-contrast images of cells treated by CC-212. Myosin is located at the periphery of ciliated cells and on the ciliated surface with a punctate localization. Nonciliated cells, probably mucus cells, are not labeled. Bar, 4 μm. (b) Immunofluorescence and phase-contrast images of cells treated by anti-actin antibody. A peripheric localization of actin similar to that of myosin is observed. The punctate aspect of the surface cell is obliterated by the labeling of the lower part of cilia. Bar, 4 μm. (c–e) Triton-treated cortices from quail oviduct stained by CC-212 with the paired phase-contrast images. (c) A field of ciliated cortices viewed side on and showing a brighter staining of the edges (arrows) of the basal body region. Bar, 4 μm. (d) Higher magnification of a ciliated cortex showing the labeling of the basal body region and the absence of labeling of cilia. Bar, 2 μm. (e) Deciliated cortices showing the same bright staining of the basal body region. Bar, 2 μm. (f) Mussel gill cortices stained by CC-212 and the corresponding phase-contrast. A thin line with a punctated aspect is stained at the apical pole. Cilia are not labeled. Bar, 2 μm.

actinin, tropomyosin, myosin, or caldesmon (4, 11, 14, 34). (c) Immunocytochemistry on cultured myoblasts and myotubes showed that the striated staining of stress fibers observed in mononucleated cells switched off after cell fusion. No labeling of myofibrils was observed. This qualitative change of contractile protein isoforms from a nonmuscle to a muscle type is well documented (5).

Using this tool to label native ciliated cells or isolated cortices, we demonstrate the exclusive presence of myosin at the apical pole of the ciliated cells. Nonciliated cells in the same epithelium clearly did not show such a staining.

The ultrastructural localization of myosin reported in this study rested on the indirect immunogold labeling technique applied to Triton-extracted cortices. Myosin was found at the apical region of the cell between basal bodies and was more abundant at the edge of the apical pole. This localization resembles the organization of actin bundles and microfilaments described by Reed et al. (29) in ciliated cells from mussel gills where the actin microfilaments form a square meshwork between basal bodies. In nonciliated epithelia such as intestinal brush border (20, 21) and pigmented epithelia (24), an actin belt has also been reported.

This localization of myosin differs from two earlier reports. In the first study Gordon et al. (13) described a very close association of myosin with the basal bodies of ciliated tracheal epithelial cells with a diffuse, slight labeling of the cytoplasm. However, Sandoz et al. (31) reported that myosin was localized in axonemes above the ciliary neck region and was absent from the apical cytoplasm. We have not observed any labeling of axonemes. This could be due to the absence of the MAb target epitope on an eventual ciliary isoform of myosin. However, we have no explanation for the reported absence of myosin in the cortical cytoplasm from ciliated cells, except that the specificity of the antiserum used in this
Figure 4. Immunogold labeling of demembranated ciliated cells of quail oviduct. (A) Oblique section through the apical pole showing the distribution of gold granules between basal bodies. The fibrillar material associated to the apical junctional complex (arrow) is also strongly labeled. Scattered gold granules decorate the striated rootlets (r) and the terminal web (tw). Axonemes and basal body cores are not labeled. (B) Control on a transverse section through the apical pole of ciliated cortices. Only few scattered gold granules are observed. To appreciate the structural preservation of cortices, higher contrast was achieved. Note the good preservation of the basal body organization at the basal foot level (large arrows) or at the alar sheet level (large arrowheads). In some cases the microtubule triplets are easily observed. Transverse
early study—raised against the muscular myosin of amphibians—could have prevented the detection of a bird cytoplasmic myosin.

The presence of myosin and actin in epithelial cells poses the question of the role of this contractile machinery in a highly differentiated nonmuscular tissue. Further studies are required to elucidate this problem. But yet, two points argue in favor of the functional presence of these contractile proteins: (a) the existence of an ATPase activity associated with basal bodies (1), and (b) the occurrence of an ATP-dependent structural event in basal body preparations (2).

Microinjections of the monoclonal antibody CC-212 could give further information on the eventual function of this actomyosin at the apical pole of ciliated cells.

We are grateful to Dr. K. Schwartz for helpful discussions and the gift of an anti–myosin antibody, Dr. Pho for the gift of platelet extract, Dr. A. M. Tassin for providing us with myogenic cell culture, and Dr. E. Puchelle for providing us with frog palastr ciliated cells. We thank M. Louette for photographic work and N. Gaussia for typing the manuscript.

This work was supported by grant No. 508031 from Institut National de la santé et de la Recherche Médicale.

Received for publication 6 August 1985, and in revised form 3 March 1986.

References

1. Anderson, R. G. W. 1977. Biochemical and cytochemical evidence for ATPase activity in basal bodies isolated from oviduct. J. Cell Biol. 74:547-560.
2. Anderson, R. G. W., and A. K. Floyd. 1978. Adenosine triphosphate induced change in light scattering of isolated basal bodies. Cell BioL Int. Rep. 2:487-494.
3. Biozzi, G., D. Mouton, Y. Bouthillier, and J. C. Mevel. 1984. Amplification des caractères "bonne production" ou "mauvaise production" d'animaux-constructeurs, produits par sélection génétique chez la souris. Correlation entre l'immunodéficience et l'incidence de lymphomes. J. Cell Biol. 99:1045-1125.
4. Bretscher, A., and W. Lynch. 1985. Identification and localization of immunoreactive forms of caldesmon in smooth and nonmuscle cells: a comparison with the distributions of tropomyosin and α-actinin. J. Cell Biol. 100:1656-1663.
5. Buckingham, M. E., and A. J. Minty. 1983. Contractile protein genes. In Eukaryotic Genes. N. Mac Lean, S. P. Gregory, and R. A. Flavell, editors. Chapt. 21. Butterworth, London. 365-397.
6. Burnett, W. N. 1981. "Western blotting": electrophoresis transfer of proteins from SDS-polyacrylamide gels to unmodified nitrocellulose and radiographic detection of radioiodinated protein. Anal. Biochem. 121:195-205.
7. Chaitin, M. H., B. G. Schneider, M. G. Hall, and D. S. Papernasaster. 1984. Actin in the photoreceptor connecting cilium: immunocytochemical localization to the site of outer segment disk formation. J. Cell Biol. 99:239-247.
8. Clarke, M., and J. A. Spudich. 1977. Non-muscle contractile proteins: the role of actin and myosin in cell motility and shape determination. Annu. Rev. Biochem. 46:797-822.
9. Collins, J. H., and C. W. Borysenko. 1984. The 110,000-dalton actin and calmodulin-binding protein from intestinal brush border is a myosin-like ATPase. J. Biol. Chem. 259:14128-14135.
10. COURVALIN, J. C., M. DUMONTIER, AND M. BORNENS. 1982. Solubilization of nuclear structures by the polyanion heparin. J. Biol. Chem. 257:456-463.
11. Fujiwara, K., and T. Pollard. 1976. Fluorescent antibody localization of myosin in the cytoplasm, cleavage furrow and mitotic spindle of human cells. J. Cell Biol. 71:848-875.
12. Galfre, G., and C. Milstein. 1981. Preparation of monoclonal antibodies: strategies and procedures. Methods Enzymol. 73:3-46.
13. Gordon, R. E., B. P. Lane, and F. Miller. 1980. Identification of contractile proteins in basal bodies of ciliated tracheal epithelial cells. J. Histochem. Cytochem. 28:1189-1197.
14. Herman, J. M., N. J. Cris image, and T. D. Pollard. 1981. Relation between cell activity and the distribution of cytoplasmic actin and myosin. J. Cell Biol. 90:84-91.
15. Karsenti, E., P. Goumon, and M. Bornens. 1978. Immunocytochemical study of lampbrush chromosomes: presence of tubulin and actin. Biol. Cell. 31:219-224.
16. Keller, III, T. C. S., K. A. Conzelman, R. Chases, and M. S. Mosesk. 1985. Role of myosin in terminal web contraction in isolated intestinal epithelial brush borders. J. Cell Biol. 100:1647-1655.
17. Klotz, C., N. Bondes, M. C. Laine, D. Sandoz, and M. Bornens. 1986. A 175,000-mol. wt. protein associated with striated rootlets in ciliated epithelia, as revealed by a monoclonal antibody. Cell Motility and the Cytoskeleton. 6:56-67.
18. Korn, E. D. 1978. Biochemistry of actinomycin-dependent cell motility (a review). Proc. Natl. Acad. Sci. USA. 75:588-599.
19. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.). 227:800-805.
20. Madden, P. S. 1983. Actin binding proteins of the brush border (a review). Cell. 35:11-13.
21. Moserok, M. S., E. M. Bonder, K. A. Conzelman, D. J. Fishkind, C. L. Howe, and T. C. S. Keller III. 1984. Brush border cytoskeleton and integration of cellular functions. J. Cell Biol. 99:1045-1125.
22. O'Farrell, P. H. 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. 250:4007-4021.
23. Osborn, M. 1981. Localization of proteins by immunofluorescence techniques. In Techniques in Cellular Physiology. Vol. 107. 1-28. Elsevier/North-Holland Scientific Publishers Ltd., County Clare, Ireland.
24. O’waribe, K., R. Kodama, and G. Eguchi. 1981. Demonstration of contractility of circumferential actin bundles and its morphogenetic significance in pigmented epithelium in vitro and in vivo. J. Cell Biol. 90:507-514.
25. Perry, S. V. 1952. The bound nucleosides of isolated myofibrils. Biochem. J. 51:495-499.
26. Phillips, D. R., L. K. Jennings, and H. H. Edwards. 1980. Identification of membrane proteins mediating the interaction of human platelets. J. Cell Biol. 86:77-86.
27. Pollard, T. D., and R. R. Weinberg. 1974. Actin and myosin and cell movement. CRC Crit. Rev. Biochem. 1:1-65.
28. Porzio, M. A., and A. M. Pearson. 1977. Improved resolution of myofilament proteins with sodium dodecyl-sulfate-polyacrylamide gel electrophoresis. Biochim. Biophys. Acta. 490:27-34.
29. Reed, W., J. A. Avolio, and P. Satir. 1984. The cytoskeleton of the apical border of the lateral cells of fresh water mussel gill: structural integration of microfilaments and actin-filament-based organelles. J. Cell Sci. 68:1-33.
30. Reverdin, N., G. Gabbiani, and Y. Kapanec. 1976. Actin in tracheobronchial ciliated epithelial cells. Experientia. 31:1348.
31. Sandoz, D., P. Goumon, E. Karsenti, and M. E. Sauron. 1982. Immunocytochemical localization of tubulin, actin and myosin in xenomes of ciliated cells from quail oviduct. Proc. Natl. Acad. Sci. USA. 79:3198-3202.
32. Sobieszek, A., and R. D. Brenmmel. 1975. Preparation and properties of vertebrate smooth muscle myofibrils and acto-myosin. Exr. J. Biochem. 55:49-60.
33. Tassin, A. M., B. Maro, and M. Bornens. 1985. Fate of microtubule-organizing centers during myogenesis in vitro. J. Cell Biol. 100:35-46.
34. Weber, K., and U. Grosemann-Stewart. 1974. Antibody to myosin: the specific visualization of myosin-containing filaments in non-muscle cells. Proc. Natl. Acad. Sci. USA. 71:4561.
35. Weeds, A. 1982. Actin-binding proteins-regulators of cell architecture and motility. Nature (Lond.). 296:811-816.
36. Wray, W. T., Boulikas, T. Wray, and R. Hancock. 1981. Silver staining of proteins in polyacrylamide gels. Anal. Biochem. 118:197-203.
37. Yerna, M. J., R. Kabrowska, D. J. Hartshorne, and R. Goldman. 1979. Calcium-sensitive regulation of actin-myosin interactions in baby hamster kidney (BHK-21) cells. Proc. Natl. Acad. Sci. USA. 76:184-188.