THE BASAL APPARATUS

Mass Isolation from the Molluscan Ciliated Gill Epithelium
and a Preliminary Characterization of Striated Rootlets

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ABSTRACT

The basal apparatus, consisting of an array of interconnected basal bodies bearing
bifurcating striated rootlets encompassing a nucleus, has been isolated from
hypertonically deciliated columnar gill epithelial cells of the bay scallop Aequi-
pecten irradians through gentle lysis with Triton X-100. The rootlets, 8–10 μm in
length, were not easily preserved with conventional electron microscope fixatives,
suggesting that the extent of their contribution to cellular architecture has been
somewhat underestimated, even though Engelmann described many of the
structural details of the basal apparatus in 1880. The striated rootlets were soluble
at high but not at low pH, in 2 M solutions of sodium azide and potassium
thiocyanate but not sodium or potassium chloride, in 1% deoxycholate but not
digitonin, and in the denaturing solvents 6 M guanidine-HCl, 8 M urea, and 1%
sodium dodecysulfate at 100°C. The protein found consistently when rootlets were
solubilized migrated on SDS-polyacrylamide gels as a closely spaced doublet with
apparent molecular weights of 230,000 and 250,000 daltons. This unique protein,
distinct from tropocollagen or various muscle components, has been named
ankyrin because of the rootlet’s anchor-like function in the cell.

Multiple cilia that occur on individual cells of
an epithelium are characteristically intercon-
ected beneath the cell membrane by amorphous
material bridging the adjacent basal bodies; these
in turn are anchored into the cytoplasm by means
of long, often bifurcating striated rootlets arising
from the proximal end of each basal body. This
complex of basal bodies, bridging material, and
rootlets is known as the basal apparatus (30) and
has been described in surprising detail by Engel-
mann (9).

More recent studies into related structures from
protozoans have culminated in the isolation of
basal body complexes from whole Tetrahymena (6,
22, 12, 2, 21, 17, 20), from Tetrahymena oral
apparatus (18, 31), from Paramecium pellicles
(14), from Oedogonium flagellar apparatus (13),
and from rhizoplasts of the ameba-flagellate
Naegleria (24). The purpose in many of these
studies was a search for either organellar DNA or
basal body proteins, but questions of contamina-
tion and postisolation changes have been major
complications in the interpretation of both struc-
tural and biochemical results (10, 20). Only the
studies by Hufnagel (14), Munn (17), and Simp-
son and Dingle (24) emphasized to any extent the
nature and properties of the striated rootlet, al-
though numerous authors have noted its variable
but somewhat collagen-like banding pattern and its relative chemical stability.

It has been demonstrated that cilia can be selectively removed from sea urchin embryos by hypertonic salt treatment (3, 15), causing breakage at a well-defined point at the basal plate (4). This basic methodology has been put to advantage for the mass isolation of cilia from the gills of the bay scallop *Aequipecten irradians* by simply treating the excised gills with hypertonic seawater to release the cilia and then removing gill fragments by low speed centrifugation (28, 27, 16). The extent of salt treatment was found to be critical in assuring optimal ciliary yield and minimal cellular contamination. The present study modifies this simple procedure by extending the time of treatment so as to obtain not only an optimum yield of isolated cilia but also to release and separate the deciliated epithelial cells and from them to isolate, under extremely mild conditions, the intact basal apparatus in sufficient amount and purity so as to be amenable to biochemical characterization of the elusive rootlet protein.

**MATERIALS AND METHODS**

**Cell Isolation Procedures**

Gills were excised from 12 medium-sized (3-in diameter; 2nd year size-class) bay scallops *Aequipecten irradians* and washed thoroughly by three or four transfers from 250-ml quantities of chilled, filtered seawater to remove silt and mucus. The gills, generally amounting to about 40 g wet wt, were placed in a volume of hypertonic seawater equal to 10 times their weight; the seawater contained 30 g/liter excess NaCl and was employed at 20°C. The gills were stirred moderately for 10 min to release the cilia, filtered onto cheesecloth, and the filtrate was spun for 5 min at 1,000 g to sediment any contaminating epithelial cells or cell debris. The resulting supernate was then spun for 10 min at 10,000 g to harvest the deciliated cilia. All steps up to this point are essentially those described previously for the mass production of gill cilia (28, 16). Historically, these steps were designed to minimize the removal of epithelial cells; in contrast, the following steps were designed to maximize cell release.

The now deciliated gills were removed from the cheesecloth, suspended in cold 0.5 M NaCl containing 1 mM EDTA, and 10 mM Tris-HCl, pH 8, and stirred for 5 min at 20°C to release the epithelial cells. The resulting suspension was filtered through cheesecloth to remove the bulk of the gill material, while the free epithelial cells were recovered by sedimentation for 5 min at 1,000 g.

Alternatively, during the initial deciliation the gills were simply left in the hypertonic medium for an additional 10 min, the suspension was filtered through cheesecloth, and the epithelial cells were pelleted by a 5-min centrifugation at 1,000 g. Cilia were then harvested by a 10-min centrifugation at 10,000 g. To free the epithelial cells of contaminating cilia, the cells were resuspended in cold seawater and recentrifuged for 5 min at 1,000 g. The basic difference between these two approaches was that the first removed the epithelium completely, regardless of cell type, while the second, giving less pure cilia and a lower overall cell yield, produced a cell population rich in the desired ciliated columnar epithelial type.

An interesting variation on the above procedures was to suspend the gills immediately in the 0.5 M NaCl-EDTA-Tris medium without first removing the cilia with hypertonic salt. The gills were stirred moderately for 10 min at 20°C, the suspension was filtered through cheesecloth, and the free epithelial cells were harvested by centrifugation for 5 min at 1,000 g. Cells with beating cilia still attached were thus obtained, although the yield was low and the degree of contamination with cellular debris was high.

**Basal Apparatus Preparation**

Deciliated columnar epithelial cells derived from 24 gills were suspended in 40 ml of cold 1% Triton X-100 detergent containing 30 mM Tris-HCl, pH 8, and 3 mM MgCl₂ (25) to remove the cell membrane and disperse the bulk of the cell contents. Brief, gentle homogenization was occasionally employed to disperse any contaminating mucus. After extraction for a minimum of 10 min, the suspension was spun for 5 min at 2,000 g to sediment the basal apparatuses. Preparations were typically washed twice further by centrifugation from fresh Triton-Tris-Mg medium to ensure maximum extraction and then once more with 10 mM Tris-HCl, pH 8, to reduce the magnesium ion concentration. To further reduce extraneous protein content, some preparations of Triton- and Tris-washed basal apparatuses were suspended in 10 ml of a solution of 2 M NaCl, 10 mM Tris-HCl, pH 8, and 1 mM EDTA for 10 min at 0°C, sedimented for 5 min at 2,000 g, and washed once with 10 mM Tris-HCl, pH 8, by centrifugation for 5 min at 2,000 g. In either case, the extracted basal apparatus preparations were stored for 1-2 days as a pellet on ice, or indefinitely at −20°C as a suspension in 10 mM Tris-HCl, pH 8, and 50% glycerol.

**Extraction and Solubility Procedure**

Centrifuged aliquots of the basal apparatus preparations were uniformly suspended in 5 vol of the solvent under investigation. The course of extraction was periodically monitored by phase-contrast microscopy. After appropriate extraction, the suspended material was centrifuged for 15 min at 45,000 g, the pellet discarded, and
the resulting clear supernate was regarded as protein-
"soluble" under the conditions employed.

Light Microscopy
Preparations were observed and photographed with a
Zeiss RA microscope equipped with Neofluar phase-
contrast and Nomarski interference-contrast optics, and
a Nikon MFA automatic 35-mm camera unit. Kodak
Panatomic X or Plus X film was used; development was
with Kodak Microdol X (Eastman Kodak Co., Roch-
ester, N.Y.).

Electron Microscopy
Centrifuged pellets of basal apparatus preparations, 1
mm or less in thickness, were fixed for from 15 min to 1 h
with cold 3% glutaraldehyde in 0.1 M phosphate buffer,
pH 7, washed three times for 20 min with cold phosphate
buffer, and postfixed for 1 h with 1% OsO₄ in either 0.1
M phosphate or Veronal-acetate buffers. Some prepara-
tions were fixed with osmium tetroxide alone. The
material was dehydrated in a graded ethanol series,
embedded in Araldite, and sectioned with a DuPont
diamond knife (E. I. DuPont de Nemours & Co.,
Wilmington, Del.). Sections were stained with uranyl
acetate and lead citrate and then viewed and photo-
graphed with either an RCA 3G or a Philips 300 electron
microscope.

SDS-Polyacrylamide Gel Electrophoresis
For analysis of soluble protein fractions, extracts were
dialyzed against 10 mM sodium phosphate buffer, pH 7,
in order to reduce the concentration of extracting salts or
detergents, adjust pH, or eliminate potassium ions before
electrophoresis. The 5% polyacrylamide-SDS gel system
of Shapiro et al. (1967) was used without modification.
Gels were stained with Coomassie Blue in accord with the
methods of Weber and Osborn (29).

Relative subunit molecular weights were estimated on
a 3% polyacrylamide-SDS gel system, modified from
Shapiro et al. by simply reducing the gel concentra-
tion. Rabbit myosin, human y-globulin, bovine serum al-
bumin, and scallop ciliary tubulin served as molecular
weight standards.

For quantitation, gels were scanned on a Joyce-Loebl
MK III double beam recording microdensitometer, with
a red filter.

RESULTS
Cilia, Cell, and Basal Apparatus Isolation
Brief treatment of well-washed scallop gills with
hypertonic seawater results first in the selective
release of cilia, followed by general disintegration
of the epithelium. Conditions described previously
for the isolation of cilia are still considered op-
timum in terms of a compromise between yield and
degree of cellular contamination (28, 16). A ran-
don field of such cilia is shown in Fig. 1 to
illustrate purity.

After removal of cilia, further exposure of the
gill to a buffered isotonic medium containing
EDTA results in nearly complete removal of all
epithelial cell types, some dispersal of endothelial
cells and connective tissue, and occasional cell
lysis. Such a mixed cell population is shown in Fig.
2. This condition represents maximal cell yield and
minimal ciliary contamination but the heterogene-
ity of the cells and their apparent fragility present
major disadvantages.

By sacrificing both the purity of cilia and the
overall cell yield, one can obtain a remarkably
good, selective release of most frontal cells by
simply extending the initial hypertonic seawater
treatment an additional 10 min. After two succes-
sive sedimentations out of isotonic seawater, the
cells are satisfactorily free of extraneous cilia (Fig.
3). The population consists primarily of the desired
previously ciliated columnar epithelial cells
(chiefly the frontals), plus some squamous and
mucus-secreting cells which occur along the gill
filament.

Extended treatment of the gill initially with
isotonic NaCl containing EDTA results in the
general removal of the epithelium and disintegra-
tion of other tissues, just as was the case after
similar treatment of the deciliated gill fragments,
but here the ciliated cells remain ciliated (Fig. 4),
and in fact, the cilia often continue to beat quite
actively and do so in a co-ordinated fashion.
However, a marked degree of swelling takes place
in this divalent cation-free medium, often resulting
in loss of cilia or in cell lysis, as evidenced by free
cilia, nuclei, and cell debris in such preparations.
The ciliated cells literally beat themselves to death.

When all factors are considered, the single,
extended hypertonic seawater treatment of gill
tissue, initially to release cilia and later selectively
to free the frontal columnar epithelial cells, ap-
ppears to be an optimal situation. Repeated differ-
ential centrifugation quite adequately frees the
cells of entrapped cilia while the ciliary fraction
can be freed of cells and cell debris by low-speed
centrifugation, particularly when accompanied by
Triton X-100 extraction to produce the somewhat
less dense axonemes (28, 16). Cells produced by
this simple hypertonic saltwater method were the
starting material for essentially all of the following
procedures.
FIGURE 1 Cilia isolated by 10 min of hypertonic salt treatment of *Aequipecten* gills. Zeiss-Nomarski optics. ×1,850. Scale = 10 µm.

FIGURE 2 Epithelial cells released from hypertonically deciliated gills by isotonic NaCl and 1 mM EDTA. Phase-contrast. ×740.

FIGURE 3 Epithelial cells released by extended treatment of gills with hypertonic deciliation medium and washed once with isotonic seawater. Phase-contrast. ×740.

FIGURE 4 Ciliated epithelial cells released by treatment of gills with isotonic NaCl and 1 mM EDTA. Phase-contrast. ×740. Scale = 50 µm.
Suspension of the cells in at least 10 vol of the Triton-Tris-Mg medium results in immediate cell lysis, but the basal apparatus, consisting of a nucleus surrounded by a "basket" of long rootlets originating from a field of basal bodies, remains quite stable. At this stage of purification, the rootlets and basal bodies appear to have fine cytoplasmic material associated with them and some cell debris is evident (Fig. 5). Most of this material is removed by washing the preparation twice with fresh Triton-Tris-Mg medium, followed by a low ionic strength Tris wash to lower the magnesium concentration and prevent clumping of the purified basal apparatuses.

**Basal Apparatus Structure**

The basal apparatus, here derived principally from the frontal columnar epithelial cells, consists of a field of 75-200 basal bodies occurring in two or three rows, with each basal body bearing two rootlets 8-10 μm in length and extending well below the nucleus. The original cells are generally not more than 8 μm wide but the isolated basal apparatus ranges from 15 to nearly 30 μm in width. The apparatus is basically sheetlike and appears to be able to extend considerably upon flattening. Three examples of varying states of such extension are illustrated in Fig. 6. An end-on view of an extended basal apparatus is given in Fig. 6 c. Considering the almost hexagonal packing of the basal bodies and the sheetlike nature of the basal apparatus, it must wrap around the nucleus in a jelly roll fashion in order to fit the dimensions of the intact cell. The analogy of a basketball caught in its net seems appropriate for the arrangement of the nucleus within the basal apparatus (Fig. 6 a).

Attempts to fix purified basal apparatus preparations (such as those illustrated in Fig. 6) for electron microscopy with glutaraldehyde and osmium tetroxide or osmium tetroxide alone met with only limited success. Nuclear remnants and basal bodies were reasonably well preserved but rootlets were only rarely observed and when seen they appeared to be only 1-2 μm in length. The bulk of the region known from phase- and interference-contrast observations to contain an extensive rootlet system showed only amorphous or fine filamentous material.

However, when the initially-released basal apparatuses (such as those illustrated in Fig. 5) were fixed under identical conditions, the rootlet system so prominent in light microscope observations became quite evident in thin section (Fig. 7). In the purified preparations, the extensive array of rootlets is apparently grossly disrupted by common electron microscope fixatives, except for an initial 1-2 μm immediately adjacent to the basal bodies. In the crude preparations, on the other hand, there is evidently sufficient cytoplasmic contamination surrounding the rootlets so that they are physically unable to fray apart and hence remain intact during fixation.

The fine structure of the striated rootlet is essentially the same as that described previously by Gibbons (1961) for the gill of the freshwater mussel *Anadonta*. A prominent 655 ± 15 Å major periodicity is consistently seen, along with considerable intraband fine structure (Figs. 7, 8). Even in relatively well-preserved material, there is evidence for marked lateral fraying of the rootlets into fine filaments 40-50 Å in diameter (Fig. 8, arrow). Essentially identical results are obtained with uranyl acetate negative staining, but because the stain considerably disrupts the rootlets and because of the extreme thickness of such whole mount preparations, this technique was regarded as neither reliable nor very practical. Regardless of whether thin-sectioned or negatively stained material was observed, noticeable shortening of the rootlets took place in the electron beam, a fact that casts some doubt upon the reality of the measured periodicities.

**Identification of the Rootlet Structural Protein**

The ready availability of milligram quantities of basal apparatuses, coupled with the relative ease with which rootlets may be observed in phase- or interference-contrast, led to a series of solubility studies, the aim of which was the selective extraction of rootlet protein.

Since the extractability of rootlet material decreased with the age of the preparation, at least in the absence of sulphydryl compounds, these studies were conducted on material less than 48 h old and with 1 mM dithiothreitol present. Extractions were performed at room temperature (20-25°C) and were generally no longer than 30 min in duration.

Pepsin and trypsin, at their pH optima and at concentrations of 10 μg/ml, readily digest the rootlets within minutes, attacking other structures in the basal apparatus less readily. Collagenase
Figure 5. Initial basal apparatus preparation resulting from lysis of cells such as those shown in Fig. 3 with 1% Triton X-100 in 30 mM Tris-HCl, pH 8, 3 mM MgCl₂; 10 min. Zeiss-Nomarski interference contrast. x3,100. Scale = 10 µm.
FIGURE 6 Basal apparatuses from preparations washed further with Triton X-100 medium. (a) Undistorted basal apparatus, phase-contrast. (b) Partially extended basal apparatus, Zeiss-Nomarski optics. (c) Top view of a basal apparatus such as that shown in (b), Zeiss-Nomarski optics. (d) Maximally extended basal apparatus, phase-contrast. All x 3,400. Scale = 10 μm.

under the same conditions also causes dissolution of the rootlets, but the action is slow and hence the action of contaminating proteases cannot be strictly excluded. However, basal bodies are apparently unaffected by collagenase, even after the rootlets are completely dissolved (several hours), so there is clearly a differential effect. In any event, the rootlets are unquestionably protein.

The rootlets are readily soluble at high but not at low pH. Sodium carbonate at 1 M readily solubilizes the rootlets but also dissolves the basal bodies and considerable nuclear material. Acetic acid at 1 M, a common collagen solvent, had little effect upon the rootlets, other than causing considerable swelling.

Urea at 8 M, guanidine-HCl at 6 M, or hot 1% sodium dodecylsulfate (SDS) at 1% brings about almost immediate solubilization of the basal bodies but only slow solubilization of the rootlets, leaving in all cases a "ghost" in the rootlet region.
Common salts at high ionic strength have little immediate effect upon the rootlets. Sodium, potassium, magnesium, and calcium chlorides were employed at 2 M concentrations; the first two salts markedly reduced the refractility of the nucleus and to some extent the basal bodies. All salts caused considerable swelling of the rootlets but none brought about any obvious solubilization.

**Figure 7** Fixed and embedded freshly released basal apparatus preparation. High magnification view: $\times 60,000$, scale $= 0.5 \mu m$. *Inset*, $\times 24,000$, scale $= 0.5 \mu m$. 
Sodium deoxycholate at 1%, and sodium azide or potassium thiocyanate at 2 M are equally effective in solubilizing the rootlets almost completely within 30 min. All, however, extract material from the basal bodies, as evidenced by their near disappearance in phase-contrast. Deoxycholate was effective down to a concentration of 0.1%, but digitonin at 0.5% had no effect whatsoever on the rootlets.

Thus no single agent could be found which could be said to extract selectively only the rootlet components from the complex basal apparatus structure. However, the identity of the rootlet constituents can be inferred from circumstantial evidence obtained from a combination of the last two sets of observations, with SDS-polyacrylamide gel analysis of solubilized proteins.

When agents such as deoxycholate, azide, thiocyanate, or high pH are used to extract the basal apparatus, rootlets are dissolved but so also are basal bodies and apparently much nuclear material. Total extracts from the first two of these agents are illustrated in Fig. 9 a, b. Both extracts show the presence of a high molecular weight doublet (arrow), a pair of proteins which comigrate with tubulin (T) and actin (A), and two prominent low molecular weight classes (X). However, when the basal apparatus preparation is first extracted briefly with 2 M NaCl (rendering the nucleus less refractile, apparently removing material from around the basal bodies, and causing swelling but not dissolution of the rootlets) and then re-extracted with 2 M sodium azide (which dissolves the rootlets), the high molecular weight doublet is found in essentially the same amount as in the whole extract (Figs. 9 c and 10). One low molecular weight component, in much reduced amount, is also prominent. Similarly, an initial NaCl pre-extraction of the basal apparatus, followed by a 2 M potassium thiocyanate extraction of the rootlet material, yields this same high

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**Figure 8** High magnification view of a basal rootlet in a freshly released basal apparatus preparation, illustrating segment banding and protofibrillar substructure (arrow). x 100,000. Scale = 0.25 μm.

**Figure 9** SDS-polyacrylamide gel electrophoresis of extracts containing rootlet components. (a) 1% deoxycholate extract of total basal apparatus. (b) 2 M sodium azide extract of same. (c) 2 M sodium azide extract of a NaCl pre-extracted preparation. (d) 2 M potassium thiocyanate extract of a NaCl pre-extracted preparation. (e) 2 M potassium thiocyanate extract of an epithelial cell preparation. (f) Extract as in (d), run on a 3% gel for molecular weight determination. All other gels are 5% polyacrylamide. Coomassie Blue staining. Arrow indicates basal rootlet protein; T and A are proteins which comigrate with tubulin and actin, respectively.

Monovalent cations in the presence of EDTA or EGTA were no more effectual on the rootlets than the salts used alone, but did appear to be more effective in extracting the nucleus and basal bodies.
Subunit Molecular Weight Determination

Rootlet protein, extracted selectively with 2 M potassium thiocyanate from 2 M NaCl pre-extracted basal apparatus preparations, migrates as a closely spaced doublet of equally dense bands when subjected to SDS-polyacrylamide gel electrophoresis on 3% gels (Fig. 9f). This system is linear with log molecular weight over a range of about 50,000–500,000 daltons. Taking the molecular weights of the relevant standards as absolute, the apparent molecular weights of the two rootlet protein bands are 230,000 and 250,000 ± 15,000 daltons (Fig. 11). These same values are obtained whether the protein and standards are run separately or in various combinations; both protein bands migrate separate from and clearly higher than the single-handed heavy chain of myosin, assumed here to have a molecular weight of 200,000 daltons. Identical results are also obtained when 25 mM Tris-glycine, pH 8.3, is substituted for the 0.1 M sodium phosphate, pH 7, as buffer in the gel system, a substitution that appears to enhance anomalies in SDS binding (5). This precaution was taken when it was noted that the rootlet proteins migrate in the same region as certain membrane proteins characteristic of molluscan and echinoderm flagella, but not cilia, and...
having molecular weights of about 240,000 daltons (26). With this buffer modification, such membrane proteins migrate anomalously and are rendered separable from either myosin or the rootlet doublet bands. The rootlet protein doublet is clearly a unique and separate entity.

DISCUSSION

The "faserapparat" of Engelmann was described as a complex of interconnected basal bodies bearing long rootlets in close association with and often surrounding and extending beyond the nucleus. The rootlets were even illustrated as being periodically blebbed or striated, although it is not at all clear how this observed striation might relate to the periodicities seen in more recent electron microscope work. Engelmann discovered that high ionic strength salt treatment preferentially removed cilia from cells by breakage immediately above the basal body, a report thus predating by nearly 90 years those of Auclair and Siegel (3), Iwakawa (15), and Stephens and Linck (28), and also the interpretation thereof by Blum (4).

Ciliated epithelial cells were found to be dissociated from tissues by treatment with borate or salicylate, this perhaps being the first report of selective cell dissociation by divalent cation-binding agents. Engelmann then went on to isolate the basal apparatus by both microdissection and dissolution of the remainder of the cell. He also noted the remarkable insolubility of the rootlets and basal bodies, an observation bearing some importance to the discussion which follows.

It is difficult to imagine a better anchoring mechanism for intensively active, co-ordinated cilia than a field of interconnected basal bodies bearing an array of bifurcating rootlets which, in turn, encompass the nucleus and extend nearly to the opposite side of the cell. That the architecture of the basal apparatus has not been emphasized in recent years is partly due to the clarity of arrangement which only becomes apparent after isolation, and further, to the relative lability of lateral bonding between the protofibrils of the rootlets when subjected to standard fixatives for electron microscopy.

Whether in the intact gills or in well-washed, isolated preparations, striated rootlets are typically apparent for only the first micrometer or two below the basal body array when fixed with glutaraldehyde-osmium tetroxide or with osmium tetroxide alone. On the other hand, thin sections of fixed, freshly isolated basal apparatuses or images of negatively stained whole mounts indicate bifurcating, tapering rootlets extending for a full 8–10 μm, as light microscope observations would predict. Consequently, much of the fibrillar material surrounding the nucleus, as typically seen in thin sections of intact cells or of purified basal apparatus preparations, must correspond to laterally dissociated protofibrils of the rootlets. Other than in Engelmann's original report and in Wilson's (30) citation and discussion of it, the full extent to which the basal apparatus contributes to ciliated epithelial cell structural integrity has been greatly underestimated. Thus it would seem that modern electron microscope fixation techniques are not adequate for the preservation of a rather major intracellular super-structure.

The evidence that the rootlet structural protein is the 230,000- and 250,000-dalton equally staining doublet band detected on SDS-polyacrylamide gels is, to some extent, circumstantial. In terms of percentage of total cell protein, it is enhanced quite markedly when the basal apparatus is freed from the cell proper, and it is further enhanced in rootlet preparations from which extraneous proteins have been extracted by high salt treatment, leaving the rootlets morphologically unchanged. Furthermore, these are the only components uniquely present when the rootlets are dissolved by high pH, deoxycholate, azide, or thiocyanate. However, the only unequivocal proof of identity would be in vitro reconstitution of striated rootlets from the purified pair of components, a goal as yet unrealized.

Strengthening this circumstantial identity is the important fact that the rhizoplast isolated from the flagellate form of *Naegleria gruberi* yields principally a single protein component with a molecular weight of 240,000 when the organelle is either dissolved directly in an SDS medium or selectively solubilized with deoxycholate (A. D. Dingle, manuscript in preparation). This protozoan flagellar rootlet protein comigrates (A. D. Dingle, manuscript in preparation) with the two molluscan ciliary rootlet components described here.

Rubin and Cunningham (20) very tentatively identify a 21,000 dalton protein as the subunit of *Tetrahymena* kinetodesmal fibers, a rootlet-like structure with 320 Å periodicity. Their argument was based on dissolution of a heterogeneous basal body preparation with 1% phosphotungstate. Similar techniques, when applied here to *Aequipecten* rootlets, indicate dispersal into protofibrils but no true solubilization when 1% neutral phosphotung-
ular weight in flagellar rhizoplasts (A. D. Dingle, 1974a)
dent apparent molecular weight in ciliary rootlets and only one component of an intermediate molecular weight in flagellar rhizoplasts (A. D. Dingle, manuscript in preparation) raises the question of whether the "native" protein exists as a coiled-coil dimer of two different polypeptide chains, separable on SDS-polyacrylamide gels in the case of rootlets but not in the case of rhizoplasts. The observation of 40 Å protofibrils would indicate that such a quaternary structure could be involved. Present data do not permit any decision as to whether two like chains or two dissimilar chains may dimerize to form a coiled coil which, in turn, forms the rootlet paracrystalline array, or whether two completely separate proteins copolymerize to form the rootlet.

On the basis of an assumed mean residue weight of 115 g/mol, a 240,000 dalton single polypeptide chain would contain about 2,087 amino acids. A simple α-helix of 3.6 residues per 5.4 Å would thus be 3,130 Å in length, while a coiled-coil structure with 3.6 residues per 5.1 Å would be 2,957 Å in length. If the rootlet protein assembles in the same manner as, for example, tropocollagen (8), or paramyosin (7), the commonly reported periodicities of 350 and 700 Å could be generated by 1/8 or 1/4 displacement, respectively, of the laterally associating molecules. The wide variety and variability of periodicities reported in other rootlet-related systems (24) would require a far more complex numeration, beyond the scope of this Discussion.

By the various criteria outlined above, the structural protein of the striated rootlet is apparently a unique entity. Because of the bifurcating nature and anchor-like function of ciliary rootlets, the generic name ankyrin, from the Greek ankyra (anchor) is proposed for these characteristic high molecular weight structural proteins of rootlets and rootlet-related organelles.

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