A SYMMETRICAL, EXTRACELLULAR FIBRIL

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ABSTRACT

Symmetrical, extracellular fibrils, which are related to the "special fibrils" of the dermis described by Palade and Farquhar, have been found along the outer surface of the basement membrane covering the notochord in the tail of *Rana catesbeiana* (bullfrog) tadpoles. The fibrils are ~7,500 A long and occur singly or in clusters. The single fibrils are characterized by a symmetrical transverse band pattern and by attachment at both ends to the basement membrane. The clusters are various complex configurations which seemingly represent symmetrical fibrils in different states of aggregation. Symmetrical fibrils also occur in the skin of the tadpole tail and in the skin of the toad, *Bufo marinus*. It is proposed that a narrow, symmetrical fibril is the fundamental "special fibril."

INTRODUCTION

A new type of extracellular fibril, the "special fibril of the dermis," has been described in detail by Palade and Farquhar (1). The fibrils occur in the skin of the toad (*Bufo marinus*), frog (*Rana pipiens*), salamander (*Ambystoma punctatum*), and rat and are present along the dermal side of the epidermal basement membrane and beneath the basement membranes of several other epithelia. They consist of a *stem* (~2,800 A long) which has a characteristic asymmetrical transverse band pattern, short *branches* which arise from one end of the stem and enter the basement membrane, and long, *narrow extensions* which arise from the opposite end of the stem and mingle with similar elements from other special fibrils to form "knots." Together, the fibrils seem to form a network which anchors the basement membrane to the underlying connective tissue. Similar fibrils, described in more general terms, have been found along the basement membranes of human skin (2, 3), gingivae (4), nonmyelinated neurons (3), and ectocervix (5).

This paper describes a related "special fibril" having a symmetrical, rather than an asymmetrical, structure.

MATERIALS AND METHODS

Materials

The specimens examined include: (a) tissues at the periphery of the notochord in the tail of the bullfrog tadpole, (b) skin of the tadpole tail, and (c) abdominal skin of the adult toad.

TADPOLES: *Rana catesbeiana* larvae (65–80 mm long) were obtained from the Connecticut Valley Biological Supply, Co., Southampton, Mass. or from the Lemberger, Co., Madison, Wisconsin. In development the tadpoles appeared similar to *Rana pipiens* larvae at stages III to VII (6).

TOADS: Adult *Bufo marinus* (see reference 7 for remarks on classification), weighing ~400 g, were obtained from the Lemberger, Co. or from National Reagents, Bridgeport, Conn.

Methods

Notochord Specimens

FIXATION-EMBEDDING: Whole tails were fixed for ~4 hr in ice-cold glutaraldehyde fixative (8) (6.5% glutaraldehyde, Union Carbide Corp., Chemicals Div., New York, in 0.1 M phosphate buffer,

pH 7.5) and washed overnight, at ~4°, in the same buffer containing 10% sucrose. Transverse slices (~1 mm in thickness) then were made at eight regular intervals along the length of the tail. Blocks of tissue (2 × 3 mm or smaller), containing part of the peripheral region of the notochord,[1] were cut from the slices, fixed in buffered OsO_4 (1% OsO_4 in 0.1 M phosphate buffer, pH 7.5) for 1 hr at room temperature, dehydrated in graded ethanols, and embeded in Epon 812 (9). Several dehydrated specimens were kept overnight in 0.5% uranyl acetate in absolute ethanol before embedding.[2]

SECTIONS-MICROSCOPY: The specimens were oriented for sectioning so that the plane of section was either (a) normal to the longitudinal axis of the notochord,[1] i.e. in a transverse plane of the tail, (b) in the longitudinal axis of the notochord, or (c) grazing the notochord basement membrane. Sections ~1 μ thick, cut from such blocks, were examined by phase-contrast microscopy to determine the structure of the tissues and to select areas suitable for thin sectioning. Thus, in thin sections viewed by electron microscopy, the orientation of elongated structures (e.g. special fibrils, collagen fibrils) could be reliably related to the major axis and planes of the notochord and to adjacent structures.

Thin sections were estimated to be 500–900 A thick from first-order interference colors (16). They were mounted on grids covered with carbon-coated Formvar films (17), stained with aqueous (18) or alcoholic (19) uranyl acetate (1–10 min) followed by lead citrate (~5 min) (20), and examined in a Siemens Elmskop I (80 kv, 50 μ objective aperture) at magnifications of 20,000–40,000 or in an RCA EMU 3G microscope (50 kv, 50 μ objective aperture) at magnifications of up to 27,000.

Skin Specimens

TADPOLE TAIL: Pieces of skin from the muscular part of the tail were processed and examined as described above.

TOAD: Pieces of abdominal skin were fixed for 2 hr in ice-cold 2% OsO_4 in 0.1 M phosphate buffer, pH 7.6 (1, 21), dehydrated in graded ethanols, and embedded as described above.

OBSERVATIONS

The following observations deal primarily with the structure and the spatial relations of special fibrils[3] at the periphery of the notochord[1] in the tail of *Rana catesbeiana* tadpoles. The descriptions are based on an examination of the fibrils in the proximal half of the tail where they are especially well developed and favorable for study. Fibrils in the distal half of the tail are not considered here since their examination is usually hindered by the presence of the *elastica interna*.[1] Finally, brief descriptions of symmetrical special fibrils in the skin of the tadpole tail and in the skin of the toad, *Bufo marinus* are presented.

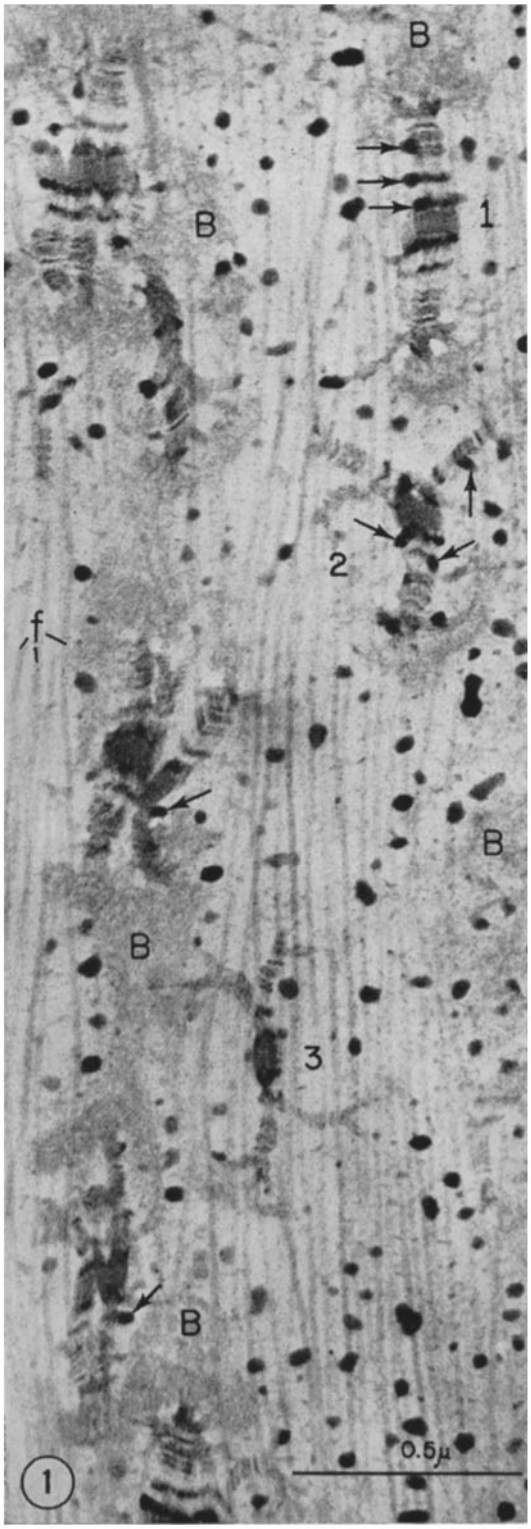
Special Fibrils at the Periphery of the Notochord

The fibrils are distributed over the outer surface of the notochord basement membrane in a layer ~1,200 A in thickness (Figs. 1, 3, 4). In general their longitudinal axies lie in transverse planes of the tail (see below). They occur most frequently in

[1] The notochord is a smooth-surfaced, tapered, cylindrical mass of cells which forms the longitudinal axis of the tadpole tail. In the specimens examined, the notochord in the proximal half of the tail was covered consecutively from its surface outward by four distinct layers: (1) a basement membrane, (2) the notochord sheath, (3) the *elastica externa*, and (4) dense, cellular connective tissue. The basement membrane is continuous, ~500 A thick, and identical in appearance with basement membranes found in many other tissues, e.g. at the dermal-epidermal junction in frog skin (10), in the walls of mammalian blood capillaries (11), and underlying the corneal epithelium (12). The acellular notochord sheath (~15 μ in thickness) consists mainly of collagen fibrils of ~150 A diameter and an amorphous matrix. In general the longitudinal axes of the collagen fibrils are parallel to the periphery of the notochord and lie in planes normal to its longitudinal axis. The *elastica externa* is an amorphous, perforated, dense layer ~1.5 μ in thickness. In the distal half of the tail toward the tip, the layers covering the notochord gradually become thinner and less well developed, and an *elastica interna* may exist, at certain stages of development, instead of the *elastica externa*. The *e. interna*, like the *e. externa*, is a dense, perforated, amorphous layer ~1.5 μ in thickness, but it occurs at the junction of the notochord basement membrane and the notochord sheath. (Unpublished observations) The terms *elastica externa*, *elastic interna*, and *notochord sheath* have appeared in descriptions of vertebrae (13) and vertebral development (14, 15).

[2] Claude, Albert. Personal communication.

[3] The term *special fibril* was originally introduced by Palade and Farquhar (1). For want of a better term, it will be used throughout this paper as a general name for symmetrical fibrils and the related fibrils in references 1–5. Descriptive terms such as *fibrillar complex* (4) and *tufted fibril* are not generally applicable to all special fibrils described at this time. The term *anchoring fibril* (1) connotes a function which has not been established and it is similar to *anchoring filaments* (22).



clusters containing 2–5 fibrils and occasionally as single fibrils. On the assumption that the clusters are uniformly distributed along the basement membrane, the frequency of clusters is estimated at $1.5/\mu^2$ of basement membrane front.

These characteristics (the regular distribution of numerous, well oriented clusters or single fibrils in a narrow zone along a smooth basement membrane) make it possible to study the special fibrils in full-face view, in side view, and in cross-section; and, therefore, to establish with confidence their three-dimensional structure and their spatial relations to nearby tissue components.

THE STRUCTURE OF SINGLE FIBRILS: Single symmetrical fibrils consist of a stem,[4] branches,[4] and tufts as shown in Fig. 2. They are ~7,500 A long, including the tufts, and 300–1,000 A

Figures 1–12 are electron micrographs of special fibrils that occur at the periphery of the notochord in the proximal half of the tadpole tail. Figures 13 and 14 depict special fibrils in the skin of the tadpole tail and in the skin of the toad, respectively. All specimens, unless otherwise indicated, were fixed in glutaraldehyde-OsO_4 and all sections were stained with uranyl acetate followed by lead citrate. The lines on all micrographs, except Fig. 1, represent 0.1 μ.

Abbreviations

C, notochord cell
B, notochord basement membrane
ns, notochord sheath
f, collagen fibrils of notochord sheath
g, extracellular, dense granule
t, tuft of special fibril
b, branch of special fibril
The characters designating specific transverse bands on the special fibrils are defined in the legend of Fig. 2.

FIGURE 1 A field showing the distribution of special fibrils along the outer surface of the notochord basement membrane (*B*). A single symmetrical fibril is evident at position 1; a Y-shaped figure appears at position 2; and an X-shaped one, at 3. Granules (diameter 200–800 A) of various shapes and densities are scattered among the special fibrils or are associated with their transverse bands (arrows). This field (3.9 μ^2) demonstrates the relative "smoothness" of the notochord basement membrane (*B*) over a relatively large area and the more or less parallel relation between the longitudinal axes of the special fibrils and of collagen fibrils[5] (*f*) in the notochord sheath. × 60,000.

[4] Terms introduced in reference 1.

[5] These fibrils are designated *collagen fibrils* although it is recognized that their chemical nature is unknown.

wide. Their stems are straight (Fig. 2) or curved (Fig. 11) and seem to consist of numerous, laterally packed, thin filaments. An identical symmetrical transverse band pattern is apparent in both full-face (Fig. 2) and side-views (Fig. 3) of the stem; consequently the transverse bands must extend through the thickness of the stem. The branches at the ends of the stem are ~200 A wide and up to 900 A long. Ordinarily they are homogeneous, but two faint transverse bands are sometimes evident. Except for a few instances where the branches appear to enter directly into the basement membrane, they are covered with a "cap" of material resembling basement membrane in texture (Figs. 3, 5). Such material will be named a *tuft* and it will be considered as part of the fibril because its density sometimes differs from the density of the basement membrane (Fig. 13) and because it accompanies fibrils separated from the basement membrane (Fig. 12). Admittedly, the origin of the tuft is unknown. The tuft may represent basement membrane adhering to the branches, the frayed ends of the branches, or both of these.

CLUSTERS OF SPECIAL FIBRILS: As indicated above, most special fibrils at the periphery of the notochord occur in groups of 2–5 fibrils. In full-face view such clusters appear as complex configurations which are designated, for convenience of description and discussion, as *symmetrical* or *polarized.*

A symmetrical cluster is defined as a group of laterally aligned special fibrils showing identical, but reciprocal transverse band patterns (d_1–d_5 and d_5'–d_1') on opposite sides of band d_6 (Figs. 6, 7). The fibrils are fused near the center of the stem with their transverse bands in register; the lateral parts of the stems are either separated (Fig. 7) or close together (Fig. 6). In the latter case it is not always possible to distinguish between a single broad fibril and several narrow contiguous ones.

A polarized cluster is considered as an aggregate of special fibrils characterized by a prominent, broad, transverse band pattern (d_1–d_5) on one side of the d_6 band and by several narrow, unbanded or faintly banded elements on the opposite side of d_6 (Figs. 8, 9).

In addition to these two major types of clusters, triangular- and Y-configurations (Figs. 10 and 11, respectively) or clusters of disordered fibrillar fragments occasionally are found in sections grazing the notochord basement membrane.

In side-view the symmetrical fibrils are attached at both ends to the basement membrane as shown in Fig. 3. They usually appear as single fibrils or as two fused fibrils. Separate special fibrils overlying one another have not been observed in the specimens examined.

When the special fibrils are examined in cross-section, the stems appear as circular (Fig. 4) or irregular dense figures up to ~1,000 A in diameter. They are distributed unevenly along the outer surface of the basement membrane and either lie against it or are separated slightly from it by a few collagen fibrils of the notochord sheath. Small (200–300 A diameter), dense figures found near the basement membrane may represent cross-sections of slender stems or branches.

When the full-face views, side-views, and cross-sections of the fibrils are considered together, it becomes apparent that the special fibrils are spread over the outer surface of the notochord basement membrane in a layer only ~1,200 A in thickness.

SPATIAL RELATIONS OF SPECIAL FIBRILS: The longitudinal axes of the special fibrils and of the collagen fibrils in the notochord sheath lie in transverse planes of the tadpole tail. These relations are known because the plane of section through the notochord was established when the blocks of tissue were oriented for thin sectioning (see Methods).

Although most special fibrils occur on the outer surface of the notochord basement membrane as stated above, a few of them are seen occasionally in other locations. They have been found between the basement membrane and the peripheral notochord cells, and at various positions within the notochord sheath, distinctly separated from the basement membrane (Fig. 12). In one instance fragments of special fibrils were found about 15μ from the basement membrane on the inner surface of the *elastica externa.*

Dense granules, which are scattered among the special fibrils or associated with their transverse bands (Fig. 1), are numerous along the outer surface of the basement membrane at the proximal end of the *elastica interna.*[1] (In the cases studied, this is about midway between the base and the tip of the tail.) Such granules have not been seen in the base of the tail and are either obscured or replaced by the *elastica interna* in the distal part. Their function and chemical nature are unknown, but they may represent pieces of degenerating *elastica interna.* They do not resemble the adepidermal globules (granules) which occur between the basal

epidermal cells and the underlying basement membrane in the skin of frog tadpoles (23–25) and newts (26).

Symmetrical Fibrils in Skin

TADPOLE TAIL: Symmetrical special fibrils also occur along the dermal side of the epidermal basement membrane in the skin of the bullfrog tadpole tail (Fig. 13). They are oriented at approximately right angles to the basement membrane: the tuft at one end of the fibril connects to the basement membrane while the other end lies free among the collagen fibrils of the basement lamella or curves around a collagen fibril in a manner already shown by Susi, Belt, and Kelly (4). The fibrils usually follow a wavy course; therefore, complete longitudinal views are rare in single thin sections. Fragments of the fibrils are frequently seen deep in the basement lamella.

These fibrils are similar to the symmetrical fibrils at the periphery of the notochord (cf. Figs. 2 and 13). The fibrils at both sites have stems, branches, tufts, an overall length of ~7,500 A, and identical transverse band patterns. They differ, however, in their relation to adjacent structures. In the skin of the tail the longitudinal axes of the special fibrils

[6] The designation of transverse bands introduced in reference 1 will be used here, except for the following modifications: (1) Only dark bands will be marked. (2) The designation d_6 for the broad central band will be retained here to facilitate the description and the subsequent discussion of the fibrils. Sub-bands within this band will be marked d_6 plus distinguishing superscripts. (3) Since the transverse band pattern of the fibril is symmetrical, each band on one half of the stem and its correspondent on the other half will be marked with the same character; bands on one half will be distinguished from those on the other half by a prime mark. A prime mark will also distinguish branches and tufts at opposite ends of the stem.

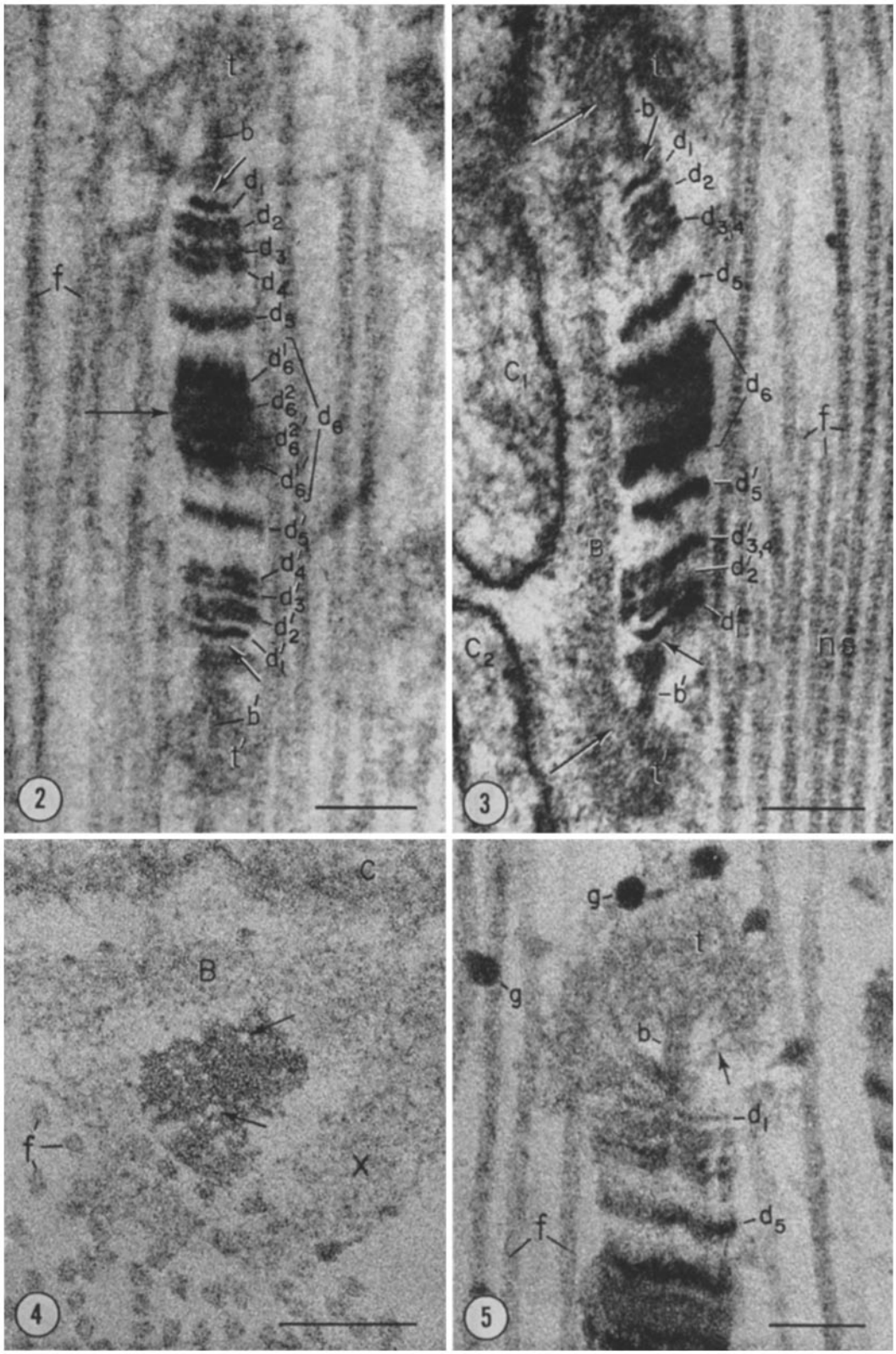
FIGURES 2–4 show special fibrils sectioned in 3 mutually perpendicular planes, i.e. Fig. 2 shows a fibril in full-face view; Fig. 3 shows one in side view; and Fig. 4, in cross-section.

FIGURE 2 Full-face view of a single symmetrical fibril showing its banded stem (between short arrows), short branches (*b*, *b'*), and tufts (*t*, *t'*).[6] The stem (~4,500 A long) has the following dense, transverse bands: d_1, d_1', ~65 A; d_2, d_2', ~200 A (5 bands, 3 dark separated by 2 light, are occasionally resolved within this band); d_3, d_3', ~65 A; d_4, d_4', ~65 A; d_5, d_5', ~130 A and d_6, ~1160 A. Band d_6 includes 4 dense sub-bands: d_6^1, $d_6^{1'}$, ~140 A; and d_6^2, $d_6^{2'}$, ~100 A. Note the symmetry of the transverse band pattern about a line bisecting the fibril at the long arrow. Collagen fibrils of the notochord sheath are marked *f*. This section passes near the outer front of the notochord basement membrane, the region containing the special fibrils. × 159,000.

FIGURE 3 Side view of a symmetrical fibril showing its relation to: two cells at the periphery of the notochord (C_1, C_2), the notochord basement membrane (*B*), and the notochord sheath (*ns*). The fibril shows a banded stem (between short arrows), branches (*b*, *b'*), and tufts (*t*, *t'*) which fuse with the basement membrane (at long arrows). Note that the transverse band pattern of the stem is similar to the band pattern shown in Fig. 2. The plane of this section lies in a transverse plane of the notochord. The tissue block of this specimen was impregnated with uranyl acetate in absolute ethanol prior to embedding. × 159,000.

FIGURE 4 Cross-section of the stem of a special fibril. Part of a cell at the periphery of the notochord is marked *C*; the notochord basement membrane, *B*; and transverse sections of collagen fibrils in the notochord sheath, *f*. Since the material at *x* appears to be part of a tuft, it is likely that this fibril is sectioned near one end of the stem. Note the spots of low density (arrows) within the contour of the stem. The plane of this section lies in the longitudinal axis of the notochord. × 212,000.

FIGURE 5 Details of a branch and tuft at one end of a special fibril (full-face view). The branch (*b*) extends from the blunt end of the stem into the tuft (*t*) which seems to consist of thin, branching filaments (arrow). × 149,000.


t
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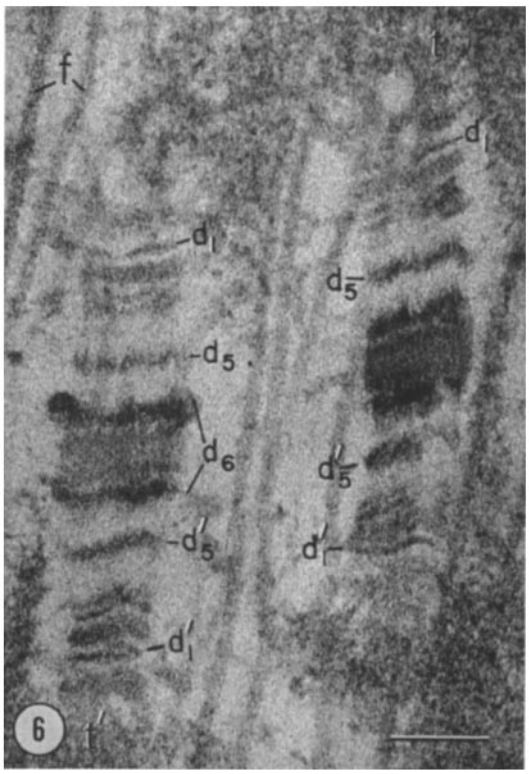

FIGURES 6–12 show, in full-face view, symmetrical, polarized, and complex clusters of special fibrils.

FIGURE 6 Two broad, symmetrical fibrils. The fibril at right of field appears to consist of 2 fused fibrils. × 129,000.

are oriented at right angles to the dermal side of the basement membrane and to the longitudinal axes of the orthogonal collagen fibrils in the basement lamella. But at the periphery of the notochord, the longitudinal axes of the fibrils are parallel to the outer surface of the basement membrane and to the longitudinal axes of the adjacent collagen fibrils in the notochord sheath (Figs. 1–3).

TOAD: The discovery of symmetrical fibrils in the tadpole tail prompted a search for similar fibrils in the abdominal skin of the toad, an organ studied by Palade and Farquhar (1) for their original report on the structure of special fibrils.

Our observations on this organ are similar to those reported by Palade and Farquhar. In addition, they disclose the presence of symmetrical special fibrils having the same general characteristics of symmetrical fibrils as in the tadpole tail, namely a central d_6 band ~1,000 A long and complementary band patterns on opposite sides of the d_6 segment (cf. Figs. 2, 13, 14).

DISCUSSION

Symmetrical Special Fibrils

The symmetrical fibrils described in this report are, to our knowledge, the first reliable examples of naturally occurring, extracellular fibrils having a finite length and a symmetrical, banded structure.

These elements clearly are related to the special fibrils of the dermis described by Palade and Farquhar (1). Both types have (a) the same transverse band sequence, d_1–d_6, (b) similar short branches arising from the stem, and (c) a position along the connective tissue side of morphologically similar basement membranes.

Although similar in these respects, the two types of fibrils are not identical. Those in the dermis have

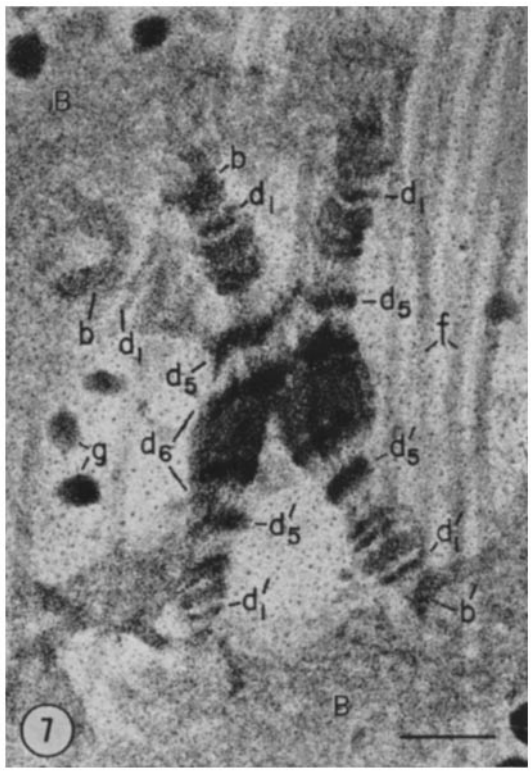

FIGURE 7 A symmetrical cluster. Several fibrils joined in the central region of the stem. Observe the reciprocal transverse band patterns, d_1 to d_5 and d_5' to d_1', on opposite sides of the d_6 region. × 124,000.

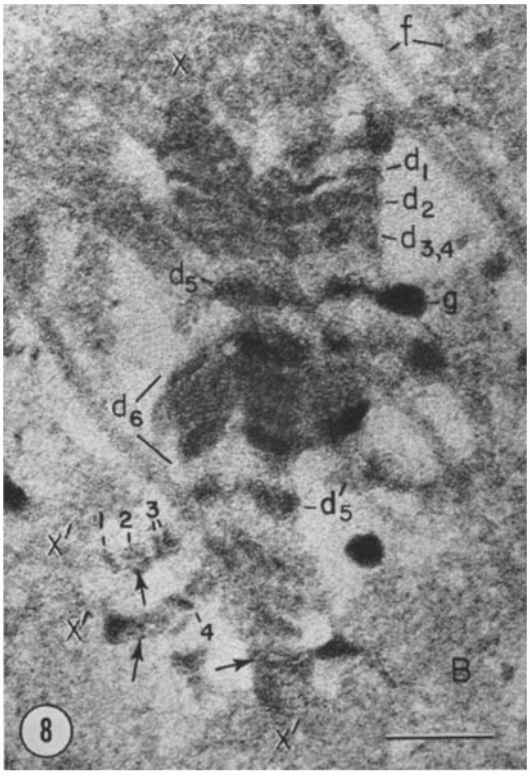

FIGURE 8 A polarized cluster. A broad, banded segment (d_1 to d_5) lies on one side of the d_6 region, and several indistinct elements lie on the opposite side (arrows). Notice band d_5' and the faint transverse bands (1, 2, 3) which appear to be reciprocals of bands d_1–d_4. Another band, probably a d_1', is visible at 4. This cluster of fibrils may be interpreted as a group of narrow, symmetrical special fibrils fused in the d_1 to d_6 region of the stem and separated over the remaining part of the stem, i.e., between d_6, or perhaps d_5' and x'. The material marked x and x' represents tufts or basement membrane. × 137,000.

an asymmetrical band pattern ~2,800 A long, whereas those around the notochord have a symmetrical band pattern ~4,500 A long. Furthermore, the special fibrils of the dermis have, at one end of the stem, short branches which enter the basement membrane, and at the other end, long narrow elements which mingle with similar elements from other special fibrils to form "knots." In contrast, the notochord fibrils have short branches covered with tufts at both ends of the stem (Figs. 2, 3).

Generalization of the Observations

On the basis of the morphological information presented here and in other reports, it is proposed that a narrow, symmetrical fibril is the fundamental special fibril. In other words, it seems likely that the special fibrils described here and in the literature (refs. 1–5) are symmetrical fibrils or aggregates of symmetrical fibrils. This generalization seems to be correct for the following reasons:

(a) Symmetrical fibrils exist at the periphery of the notochord (Figs. 2, 3, 6, 7), in the skin of the tadpole tail (Fig. 13), and in the abdominal skin of the toad (Fig. 14).

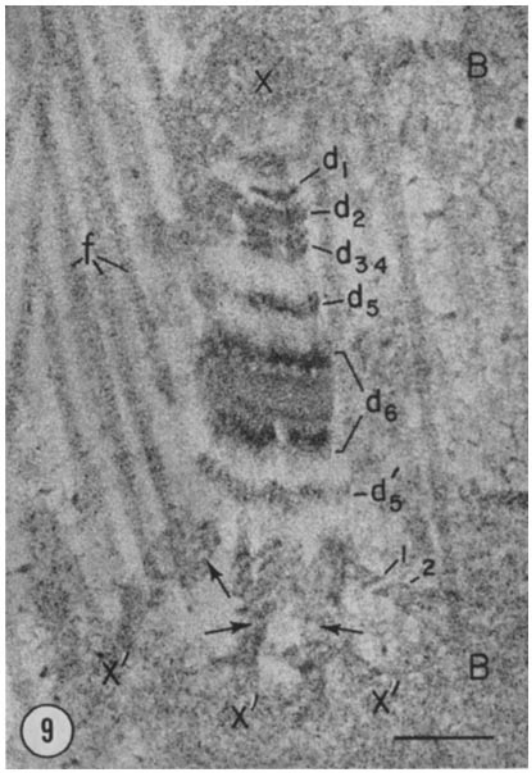

FIGURE 9 Another polarized configuration. The usual transverse band pattern, d_1–d_5, is evident on one side of band d_6 and several indistinct elements (arrows) appear on the opposite side. One element shows a faint transverse band (*1*) and perhaps a branch (*2*). The x and x' mark either tufts or notochord basement membrane; hence, this section grazes the outer front of the basement membrane (see side view of fibril in Fig. 3). Note that the indistinct elements (arrows) occupy a position (between d_5' and x') which would be occupied by bands d_4'–d_1' in a symmetrical fibril. × 130,000.

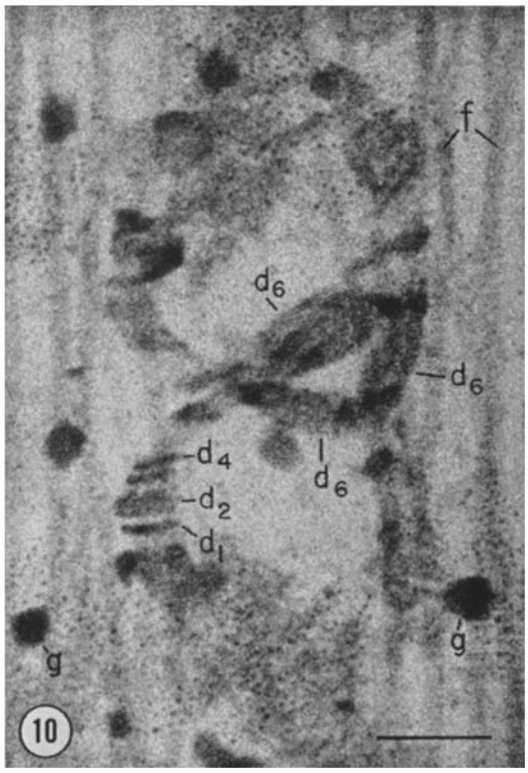

FIGURE 10 An aggregate apparently comprising several disordered special fibrils. Observe the triangle formed by what seems to be three d_6 bands. × 150,000.

(b) The polarized clusters (Figs. 8, 9) may be interpreted as aggregates of symmetrical special fibrils. They show a dense band (d_5') which is a reciprocal of band d_5, and several narrow, curved elements which appear to extend from the d_5' band. The latter elements seem to be narrow stems because (*1*) some of them show faint transverse bands[7] which are complementary to bands d_1–d_4 on the opposite side of band d_6 (Fig. 8), and (*2*) they lie in the exact position where we would expect to find the d_4'–d_1' part of the stem in a symmetrical fibril or in a symmetrical cluster. These two characteristics suggest that the polarized clusters are aggregates of narrow symmetrical fibrils.

A possible objection to this interpretation is that the polarized clusters represent superimposed separate fibrils, but this seems unlikely because the narrow elements are complementary to banded segments on the opposite side of the d_6 band. The complementarity suggests that the narrow elements are extensions of the d_6 band instead of being merely narrow stems superimposed randomly on the d_6 band.

The organization of special fibrils in complex configurations (Figs. 10, 11) cannot be determined from the information now available. It is interesting that none of their structural features contradict the idea of a fundamental symmetrical fibril. The only figure introducing any question is the Y-configuration (Fig. 11) because each of its two d_6 bands are about the same width as the d_1–d_4 segment of the stem. The significance of this configuration cannot be determined, however, until its three-dimensional structure is known.

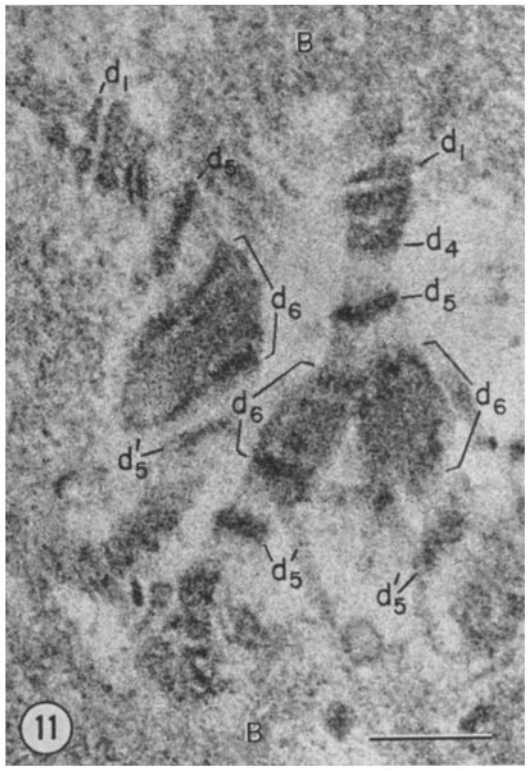

FIGURE 11 A curved special fibril at left of field and an inverted Y-configuration at right. In the latter case two confluent d_6 bands appear to form a single d_1–d_5 segment. Notice that each d_6 band is approximately the same width as the d_1–d_5 part of the stem. × 160,000.

[7] Transverse bands are likely to be faint or unresolved on stems only ~200 A wide and curved within the section.

(c) The polarized special fibril of the dermis (Fig. 2 in reference 1) has characteristics suggesting that it, too, contains symmetrical components. It shows a d_5' band and a narrow element (between *cl* and *k*, marked *af*) having transverse bands which are complementary to bands d_1–d_4 on the opposite side of d_6. Hence, this configuration resembles the polarized type of cluster found on the notochord basement membrane (Fig. 9). For the reasons discussed above, such configurations may be interpreted as aggregates of symmetrical special fibrils.

In addition to these arguments, the existence of a fundamental, symmetrical fibril would clarify the intricate character of the "knot" associated with the "special fibril of the dermis" (1) and it would explain the nature of fibrils which appear to have both ends inserted into the basement membrane (Fig. 4 in reference 3).

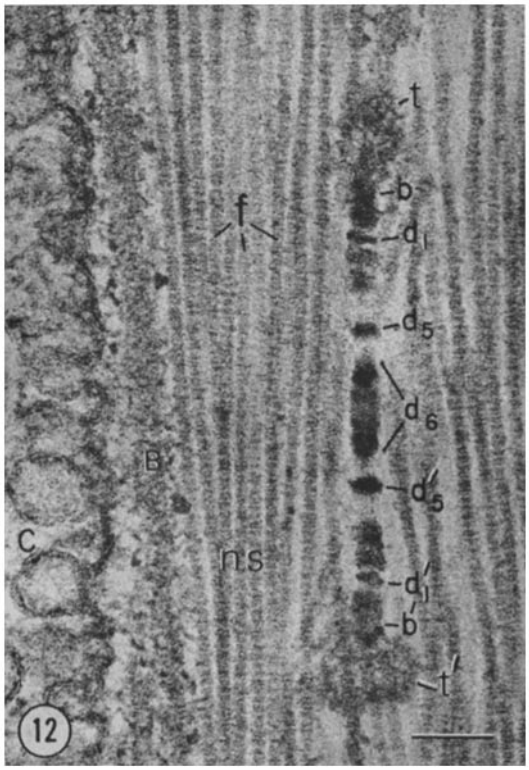

FIGURE 12 A symmetrical special fibril separated from the notochord basement membrane (*B*) by collagen fibrils (*f*) of the notochord sheath (*ns*). The plane of this section and staining as for Fig. 2. × 107,000.

Relations to Other Structures

Although the symmetrical fibrils described here differ morphologically from other banded figures reported in the literature (e.g. 27–47), their similarity to certain banded structures described by Olsen (48) should be mentioned. In negatively stained preparations of vitreous bodies, he found symmetrical structures ~4,800 A long and of various widths. These structures show a prominent central band ~1,250 A long and a complex, symmetrical transverse band pattern. Olsen concludes that these structures consist of laterally packed tropocollagen molecules[8] arranged so that their "A" ends point in opposite directions and overlap ~1,250 A.

In terms of two-fold symmetry, overall length, and the presence of a long central band, these structures resemble the stems of the symmetrical fibrils described in this report. The two structures differ, however, in other characteristics. The structures from vitreous bodies lack branches and tufts, and the two types of fibrils do not have identical transverse band patterns. Moreover, the fibrils described here were found in situ while the "vitreous structures," as noted by Olsen, may represent unusual aggregates of tropocollagen formed during preparation of the specimen.

Addendum

Symmetrical extracellular fibrils in uterine mucosa have been described recently by Rowatt (56). They apparently belong to the class of "special fibrils" and, hence, lend further support to the idea that the basic special fibril is a symmetrical fibril.

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[8] Tropocollagen molecules are ~3,000 A long (50, 51).

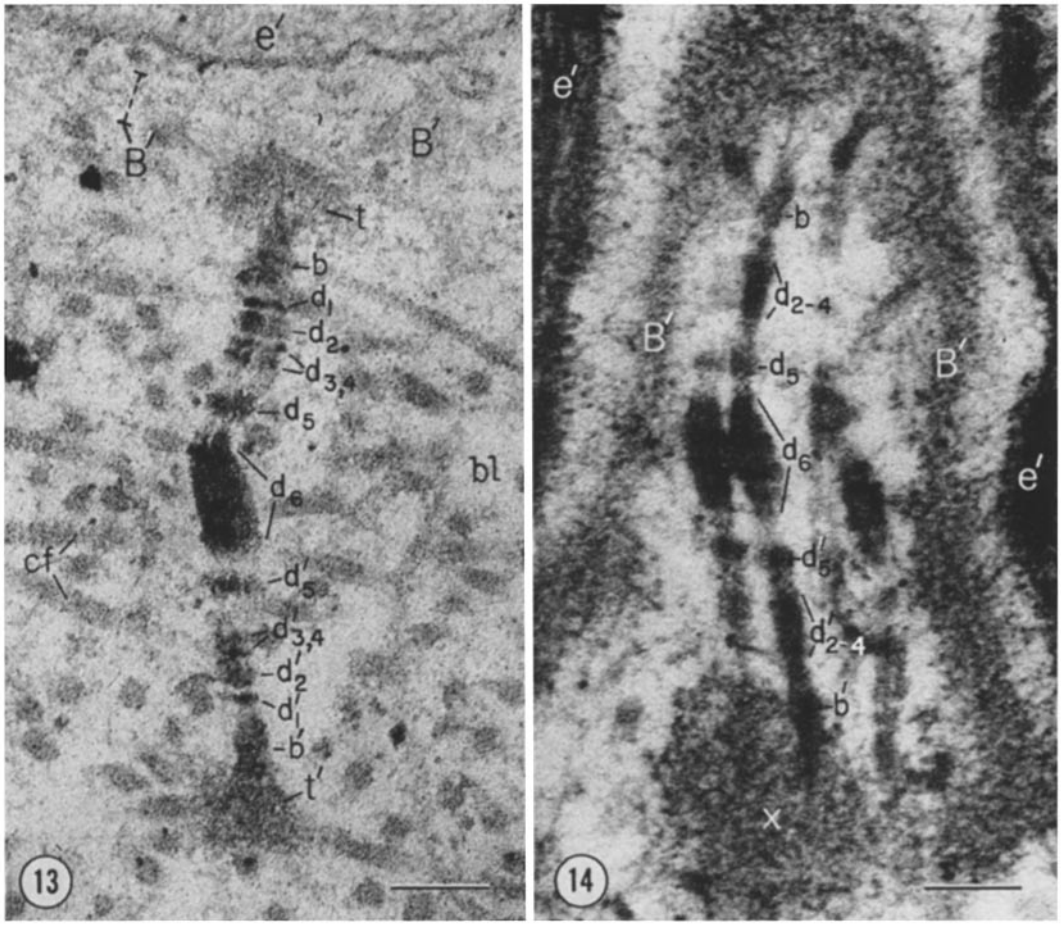

FIGURE 13 A symmetrical special fibril in the skin near the tip of the tadpole tail. The fibril extends from the epidermal basement membrane (B') into the basement lamella (bl). Part of a cell at the base of the epidermis is marked e'. This fibril displays the same type of banded stem (d_1–d_1'), branches (b, b'), and tufts (t, t') found in symmetrical fibrils at the periphery of the notochord (cf., Fig. 2). Note that the density of the tufts (t, t') is greater than the density of the basement membrane (B'). At this level in the tadpole tail, the basement membrane is rudimentary, adepidermal globules (granules) are absent, and the basement lamella consists of poorly organized collagen fibrils (cf) of small diameter (~250 A). (Compare this basement lamella with developing (25, 52) and well developed (53, 54) basement lamellae in frog tadpoles and in other species (26, 55)). The plane of this section is normal to the surface of the skin. × 127,000.

FIGURE 14 A symmetrical special fibril in the abdominal skin of the toad, *Bufo marinus*. A basal cell of the epidermis is marked e' and the epidermal basement membrane, B'. The material at x appears to be part of the basement membrane. Fixation—OsO_4. × 127,000.

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