

# MICROTUBULAR APPARATUS OF MELANOPHORES

## Three-Dimensional Organization

MANFRED SCHLIWA

From the Arbeitsgruppe Kinematische Zellforschung, Fachbereich Biologie, Universität Frankfurt am Main, 6 Frankfurt am Main, Federal Republic of Germany

### ABSTRACT

Microtubular organization in the melanophores of the angelfish, *Pterophyllum scalare*, has been studied by serial thin sectioning. The course of microtubules has been followed in sets of transverse serial sections taken from the centrosphere and a segment of a cell process, respectively. Microtubules arise from a prominent zone in the cell center, the central apparatus, which is composed of numerous, small, electron-dense aggregates. The number of these loosely distributed densities is highest in the center of the centrosphere, but they may also be found at its periphery. Microtubules insert into, or become part of, the dense material, or at least start in its vicinity. Dense aggregates may be separated from centrioles by several micrometers rather than only being closely associated with these organelles. At some distance from the organizing zone, most of the microtubules gradually assume a cortical arrangement, i.e., take a course within about 100 nm of the limiting membrane. Serial sections were used to trace all microtubules within a 6- $\mu\text{m}$ -long segment of a cell process. 94% of the microtubules observed in this segment run its entire length; it is conceivable, therefore, that a considerable number of microtubules extend between the initiating site in the centrosphere and the outermost cell region. A three-dimensional model of the 6- $\mu\text{m}$ -long segment reveals that, despite changes in the cell process outline, microtubules maintain a strictly cortical arrangement which gives the impression of a microtubule "palisade" lining the cortex of the cell process. The features of the microtubular apparatus of angelfish melanophores are discussed in relation to factors controlling microtubule initiation and distribution.

**KEY WORDS** melanophores · microtubules · microtubule organizing centers · three-dimensional model

Microtubules were early noted to be present in vertebrate chromatophores (2, 7, 11), and they appear to be especially abundant in those cells displaying rapid movement of pigment granules. Among the chromatophores showing remarkably rapid granule movements are the melanophores

of angelfish *Pterophyllum scalare* (26) in which the inward motion of pigment (aggregation) may be completed within 20 s. Studies with antimicrotubular agents such as colchicine, vinblastine, cold, and hydrostatic pressure indicate an involvement of microtubules in pigment granule movement in different types of chromatophores (1, 15, 17, 18, 29, 36).

Particularly in the cell processes, it is possible to discern a set of microtubules associated with

the cortex (within about 100 nm of the cell membrane) and another set occupying central portions of the cytoplasm (18, 24, 28). In *Pterophyllum* melanophores, the cell type studied here, the cortical arrangement of microtubules within the cell processes is particularly striking (26). Beyond that more qualitative description, however, a number of basic questions concerning the microtubule system of chromatophores (and also other cells) are unanswered: What structure or principle governs the microtubule pattern? Do microtubules, in fact, as is generally believed, originate in, and extend from, the cell center, and what is their length? Is the relative position of neighboring microtubules maintained over long distances? A question of particular interest concerns the so-called cortical and central sets of microtubules. Detailed information about their topographical relationship and their site of origin should help to clarify their involvement during phases of pigment aggregation and dispersion.

A detailed study of the microtubular apparatus of a particular chromatophore, the melanophore of the angelfish, *Pterophyllum scalare*, therefore was undertaken. Much of the information, which helps to answer the above questions, has been obtained through the use of serial thin sections. The results of this investigation should contribute to an understanding of the organization of the microtubule system in animal cells.

## MATERIALS AND METHODS

Single scales from the lateral body regions of angelfishes (*Pterophyllum scalare*) were removed with fine forceps and processed for electron microscopy. Scales with melanophores in the desired state of dispersion were fixed according to methods described earlier (28). Serial thin sections or sections spaced at regular intervals were cut with a diamond knife on an LKB ultratome III (LKB Instruments, Inc., Rockville, Md.), and picked up on pioloform-coated grids (31). Sections were further stained with lead citrate and examined with a Hitachi H 500 electron microscope.

Most of the results presented here are based on an examination of transverse sections of melanophores. In horizontal sections, microtubules cannot be followed over their whole length since they may leave the plane of section. Individual microtubules were therefore traced in transverse serial sections. In a 6- $\mu\text{m}$ -long segment of a cell process, all microtubules were traced; for a three-dimensional model of the course of all microtubules in this segment, a template was made of every third section. Holes were drilled into the templates at locations of microtubule profiles. Individual microtubules were then represented by a firm cotton thread. The scale of the

model is 66,000:1. In another series of sections, all microtubules were traced but no model was built. In several other section series, microtubules were counted but not traced.

## RESULTS

The general fine structure of *Pterophyllum* melanophores has been described earlier (6, 26, 28). Therefore, only those features pertinent to the present study are briefly recalled. Melanophores in the dispersed state are flat, disk-shaped cells with numerous cell processes radiating from the central cell body or centrosphere (for nomenclature, see Fig. 1). In the cell processes, which are of relatively uniform size and length, microtubules are found to be arranged predominantly in the cortical region and to run parallel to the long axis of the cell processes.

In order to obtain more detailed information about the structure of the microtubule system, the course of individual microtubules was followed in serial thin sections of melanophores in the dispersed state. Two of the cell areas studied in detail are depicted in Fig. 1. The series near the cell center will be considered first.

### *Microtubular Array in the Centrosphere*

Fig. 2 shows three representative sections of series no. 1. The cell process whose base is included in this series contains 225 microtubules, all of which were followed in their course towards the cell center. It can easily be inferred that, near the cell center, the microtubules associated with this cell process cluster in the mid-region of the cell, i.e., halfway between the upper and lower membrane. Melanosomes are expelled from this region. In some instances, more than 100 microtubules populate an area of less than 1  $\mu\text{m}^2$ , the mean intermicrotubule distance thereby being less than 30 nm. Even the cortical microtubules of the cell process are observed to cluster in the mid-region of the cell. Of 142 microtubules found in the cortical region of the cell process base, only 14 are still associated with the cell membrane in the cell center.

Microtubules were counted in different sections of this series spaced at regular intervals in order to follow variations in microtubule number. Fig. 3 shows a constant decrease in the number of microtubules associated with the cell process, while the total number of microtubules within this transection increases rapidly and reaches a

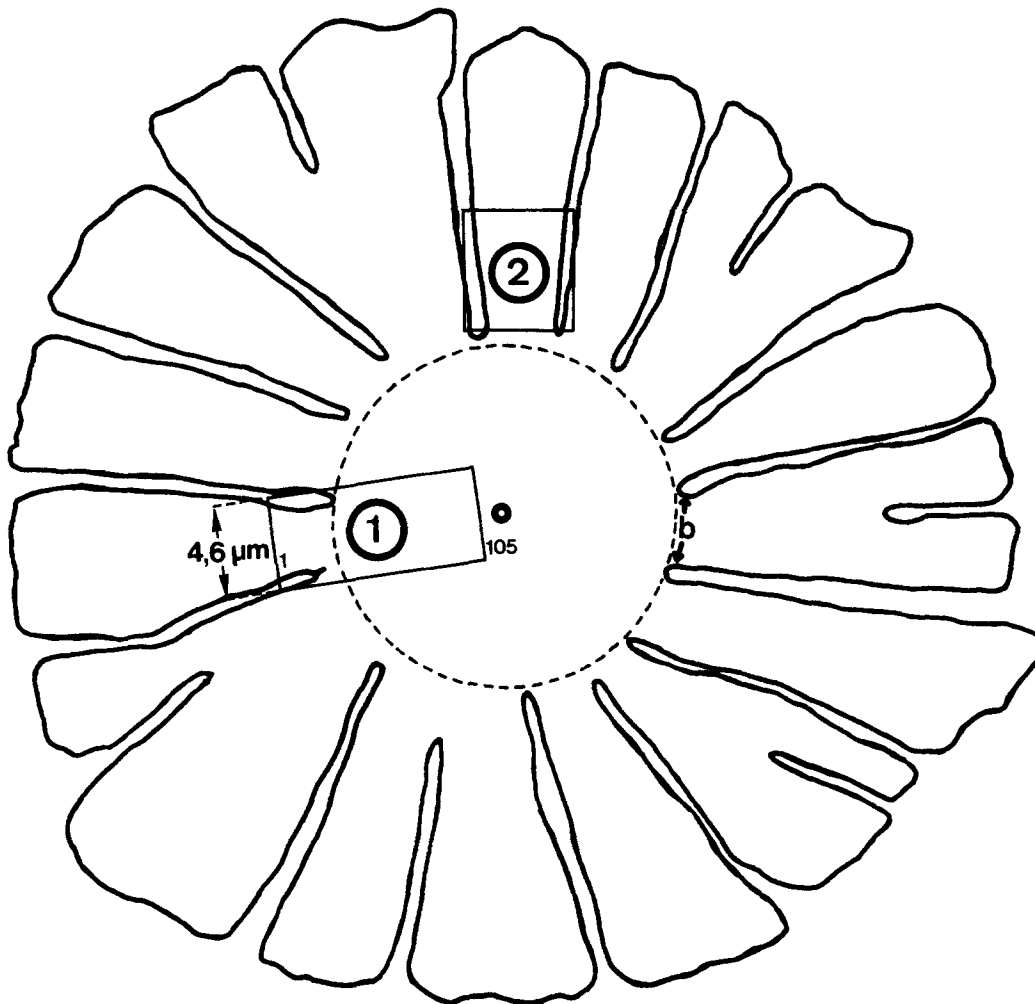
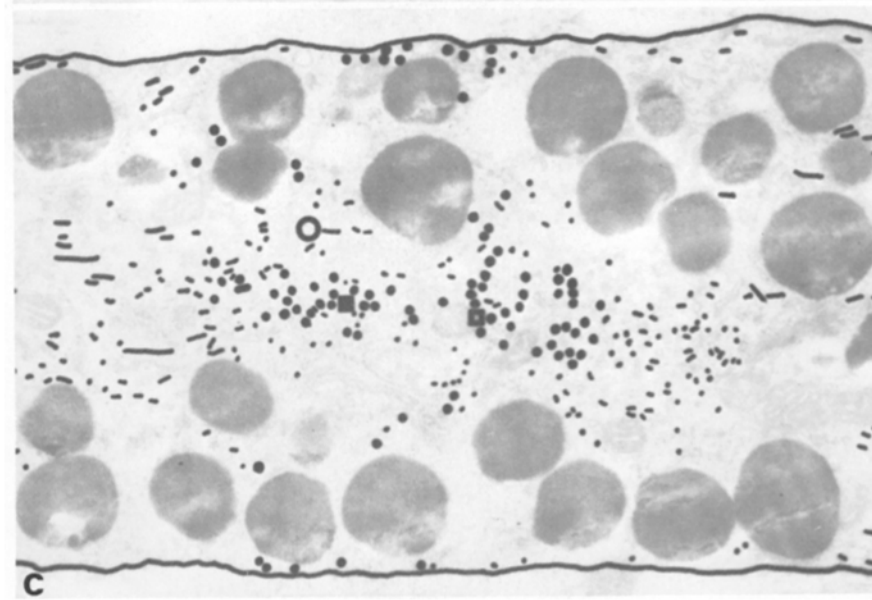
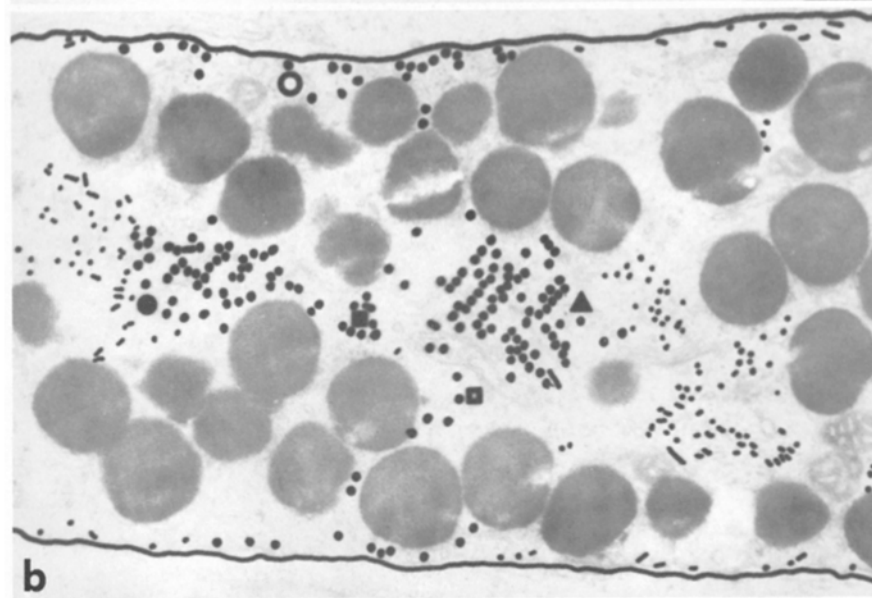
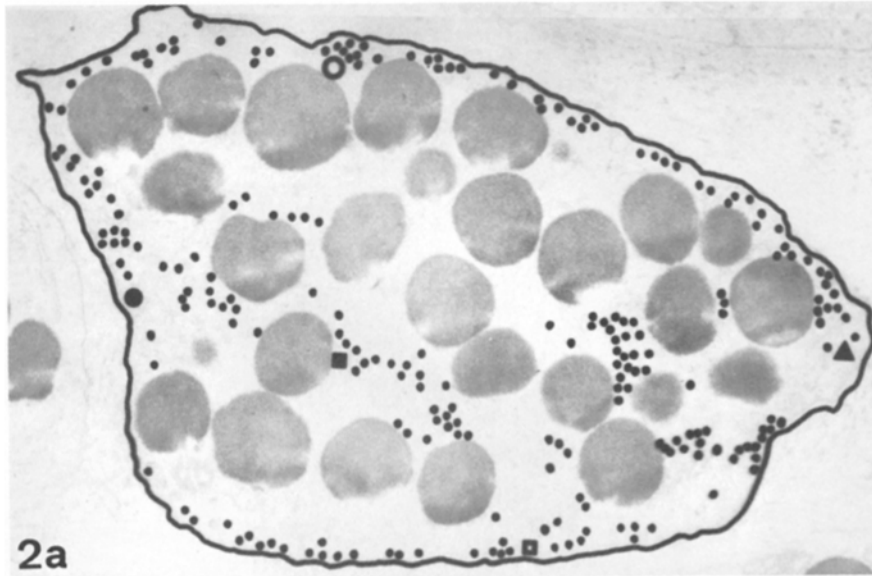


FIGURE 1 Schematic illustration of the outline of a melanophore. Area circumscribed by the dotted line, central cell region or centrosphere. Small circle, geometric cell center. *b*, base of a cell process. Large numbers and rectangles, section series 1 and 2. Small numbers in series 1, location of sections 1 and 105, respectively. The width of section series 1 in which counts of microtubules are made is also indicated.

plateau level near the cell center. Obviously, microtubules associated with cell processes in the immediate vicinity of the selected cell process (small dots in Fig. 2 b and c) contribute to this increase. The maintenance of a plateau level, on the other hand, can only be explained by the assumption that neighboring cell processes all exhibit a microtubule profile similar to that shown in the lower curve of Fig. 3. If all the microtubules of neighboring cell processes should reach the cell center, the total number of microtubules should increase steadily.

Associated with the microtubule clusters in the mid-region of the cell are small, spherical aggregates of finely granular electron-dense material, as well as elements of, presumably, the smooth endoplasmic reticulum (Fig. 5). The number of dense aggregates steadily increases from the periphery of the centrosphere towards the cell center (Fig. 4) and thereby parallels the decrease in microtubule number (Fig. 3, lower curve). An analysis of appropriate sections reveals that microtubules may insert into, or become part of, this dense material (not shown). The whole of the



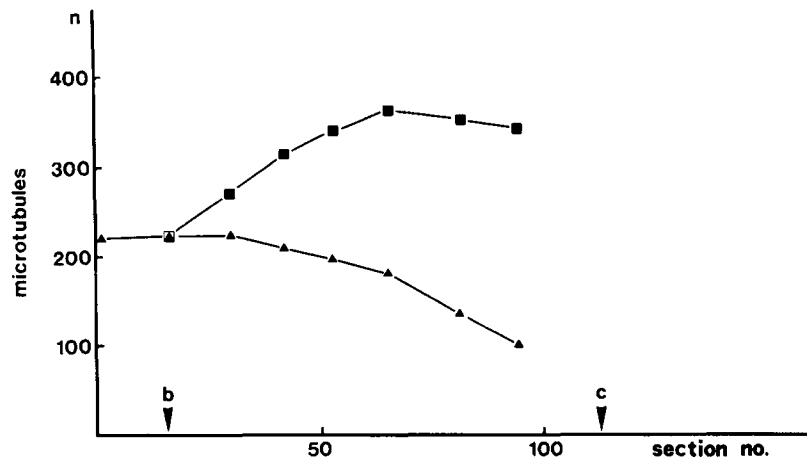


FIGURE 3 Counts of microtubules in different regions of section series 1. *b*, Base of the cell process. *c*, Cell center. (▲) Microtubules associated with the cell process. (■) Total number of microtubules in a 4.6- $\mu\text{m}$  wide transection (= width of the cell process base).

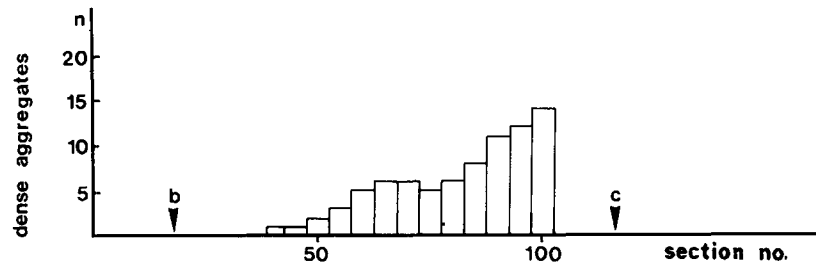


FIGURE 4 The distribution of dense aggregates in different regions of section series 1. For further explanations, see Fig. 3.

aggregates comprises the central apparatus (24) which occupies, in the case of the angelfish melanophores, a considerable portion of the centrosphere. Its diameter may reach 10  $\mu\text{m}$  with a thickness of up to 1  $\mu\text{m}$ .

Centrioles were not included in the section series analyzed here. In general, dense aggregates may be separated from centrioles by several micrometers rather than only being closely associated with these organelles.

Proximal ends of microtubules have been observed almost exclusively at or near the electron-dense aggregates, i.e., in the central apparatus. Only two microtubules with a cortical position in the cell process are observed also to start in the cortex of the centrosphere. The microtubules that assume a cortical position at some distance from the cell center are slightly curved, but never sharply bent, before maintaining a course parallel to the cell membrane.

FIGURE 2 Sections no. 14 (*a*), 65 (*b*), and 96 (*c*) of section series 1. This series consists of 105 sections and represents a segment approx. 10  $\mu\text{m}$  in length. Distally, it includes the base of a cell process and, proximally, very closely approaches the geometric cell center. Microtubules associated with the cell process are represented by large black dots. Five microtubules, represented by five different symbols (○, ▲, □, ●, ■), have been selected to show their individual course; in some respects, they are representative for the behavior of the majority of microtubules associated with this cell process. Two of them (▲, ●) end between section 65 (*b*) and section 96 (*c*). The positions of other microtubules in *b* and *c* (not associated with the cell process) are depicted by small black dots or thin lines. Together with the limiting membrane outlined in black, they are redrawn into a faint photo print of the respective section showing the location of melanosomes and other cell organelles.

*Microtubule Distribution within the Cell Processes*

In order to follow the course of individual microtubules in more peripheral regions of the melanophore, a 6- $\mu\text{m}$ -long segment of a cell process was studied in serial sections (Fig. 1, series no. 2). Near the base, 120 microtubules were counted (Fig. 6*a*), while their number at the other end of the segment was 115 (Fig. 6*b*). Two microtubules started near the cell process

base, and seven ended, predominantly in the peripheral region. This means that 113 microtubules (94%) ran the entire length of the 6- $\mu\text{m}$ -long segment. Counts of microtubules in other section series of cell processes also revealed a remarkable constancy of microtubule number. According to the low number of ending microtubules, it is conceivable that microtubules of angel-fish melanophores are quite long; many of them can be expected to extend into the peripheral cell regions.

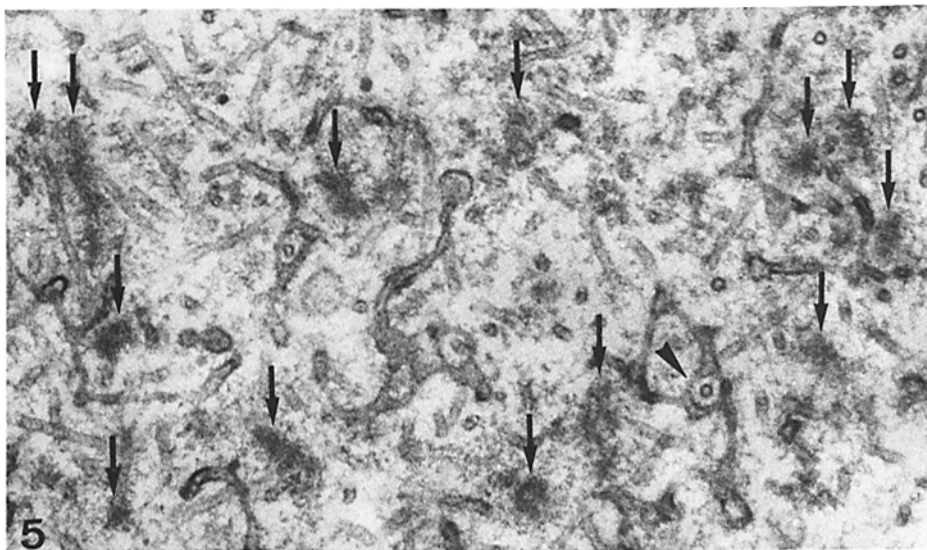


FIGURE 5 Part of the central apparatus of a melanophore with pigment dispersed in transverse section. Numerous electron-dense aggregates (arrows) appear in association with microtubules and elements of the endomembrane system. Arrowhead denotes a microtubule encircled by a membrane cisterna.  $\times 74,000$ .

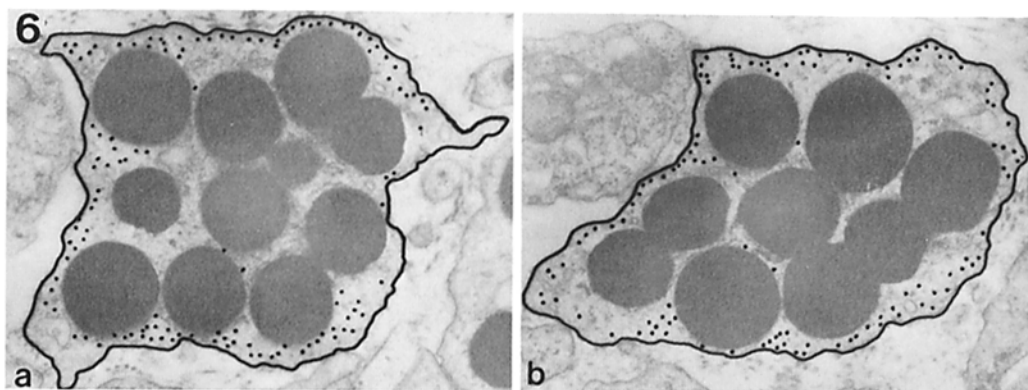


FIGURE 6 Distribution of the microtubules and outline of the cell process from which a model has been constructed. (*a*) section 8; and (*b*) section 46. Despite changes in the cell process outline, microtubules remain arranged around the periphery of the cell process.

The cross-sectional area of the cell process segment studied here changes gradually from a roughly square-shaped (Fig. 6a) to a more rhomboidal (Fig. 6b) outline. Despite this alteration, microtubules maintain a strictly cortical arrangement. This aspect is particularly well illustrated by the three-dimensional model that has been constructed from this set of serial sections (Fig. 7). Microtubules seem to form a "palisade" around the cortex of the cell process. Their center-to-center distances thereby range from 60 to 90 nm. Despite slight changes in their position relative to one another, groups of microtubules can be followed along the length of the segment. Alignment and grouping of microtubules near the cell membrane suggest the presence of intermicrotubule and microtubule-membrane connections which, however, were observed in only a few instances.

The basic features of the microtubule system of melanophores are schematically summarized in Fig. 8.

## DISCUSSION

The analysis of serial thin sections of angelfish melanophores demonstrate an elaborate system of microtubules with a precise radial arrangement. Several interesting features not evident in single thin sections were observed: (a) Almost all microtubules originate in a prominent zone in the cell center, the central apparatus, at or near electron-dense aggregates; (b) they gradually assume a cortical arrangement; with respect to their origin, a "cortical" and "central" set of microtubules cannot be distinguished; (c) nearly all microtubules seem to extend considerable distances, most of them presumably from the center to the periphery of the cell; and (d) their arrangement parallel to the long axis of the cell processes as well as their association with neighboring microtubules or the cell membrane are maintained over long distances.

Since these ultrastructural characteristics concern two important aspects of the biology of cytoplasmic microtubules, namely, initiating and distribution, both these factors will be considered in more detail.

### *Control of Microtubule Initiation*

A common pattern of microtubule distribution is a roughly radial arrangement relative to a central cell region near the nucleus. This feature is well illustrated in tissue culture cells by immu-

nofluorescence microscopy with the use of tubulin antibodies (4, 22, 34). Often, this central cell region contains a pair of centrioles; the idea that centrioles and/or the pericentriolar zone can function as an organizing center for microtubules has often been discussed (reviews in references 23 and 32). However, there are other instances where microtubules make contact with material unrelated to centrioles, such as electron-dense bodies in the cytoplasm (e.g., reference 3) or membranes and/or membrane-associated material (20, 25).

In angelfish melanophores, microtubules apparently arise from electron-dense aggregates in the cell center. Microtubule reformation after cold treatment starts in their vicinity, thus corroborating the view that the aggregates function as microtubule-initiating sites (Schliwa, M., unpublished observations). Centrioles were not found within the set of sections analyzed here, arguing against the view that the dense material is firmly centriole associated. Rather, the findings suggest that the microtubule-organizing structure does not necessarily need centrioles although they are, of course, present in the melanophores. This view is supported by recent studies on the nature of organizing centers in surf clam eggs (35) and Chinese hamster ovary cells (10).

A relatively close structural relationship exists between the microtubule-initiating complex and elements of the endomembrane system. Since a possible role of calcium ions in the regulation of microtubule assembly is still discussed (8, 12, 13, 16, 21, 27), it seems reasonable to assume that the initiating sites may be associated with structures which, at least locally, reduce the concentration of cytoplasmic calcium to allow for polymerization. The membrane system observed here may serve such a function. In this regard, it is worth mentioning that in other cell systems a relationship between intracellular membranous elements and microtubules has been observed. Examples include the vesicular system of dividing sea urchin eggs (13) and the endoplasmic reticulum associated with the poles of the spindle apparatus in plants (5, 14, 37).

### *Control of Microtubule Distribution*

The regular cortical arrangement of microtubules is gradually assumed at some distance from the organizing structure and is unlikely to be related to intrinsic factors in the initiating zone. Microtubules approaching the cell membrane at

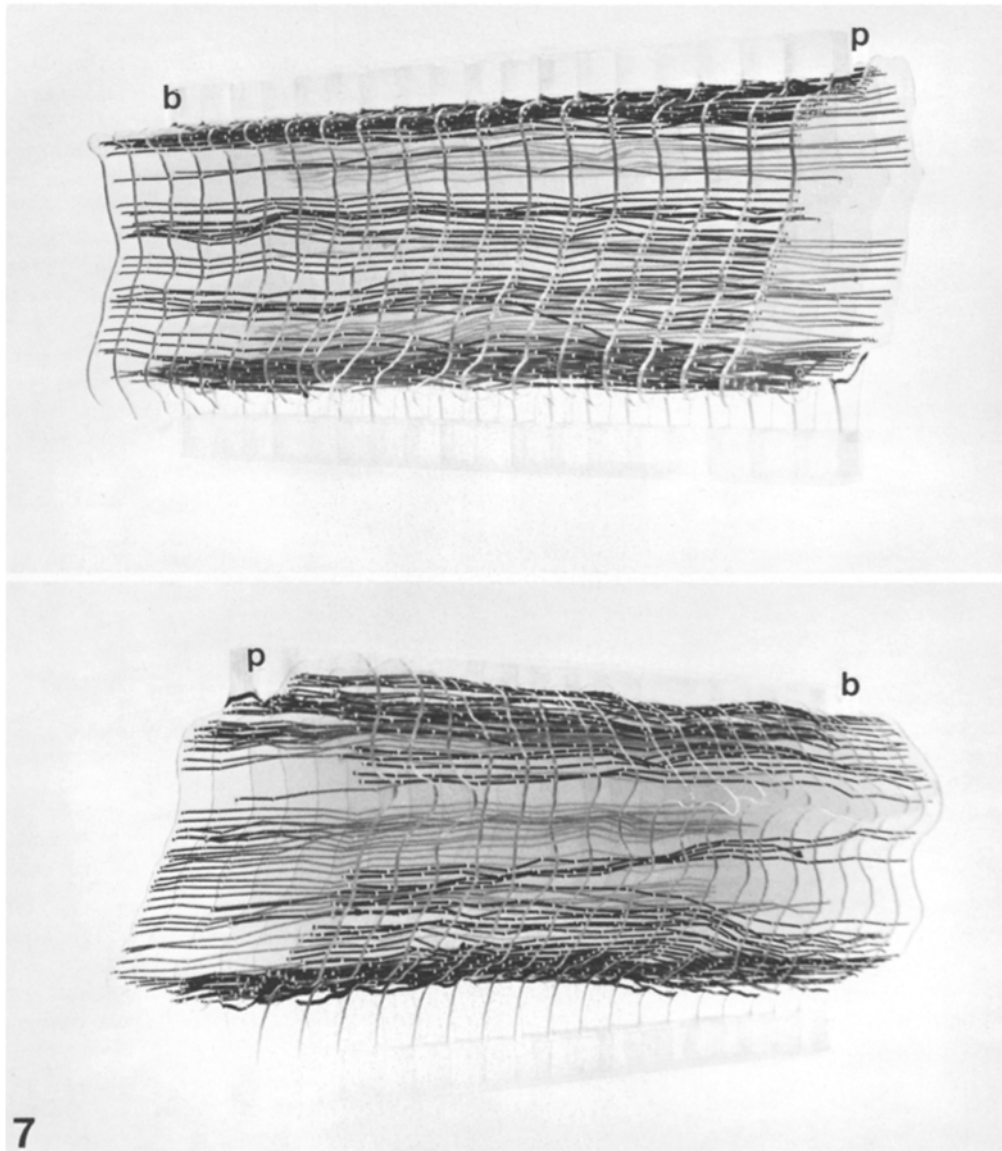


FIGURE 7 Two aspects of the three-dimensional model, scale, 66,000:1, of the microtubule system within a 6- $\mu\text{m}$  long segment of a cell process. Note the strictly cortical and parallel arrangement of microtubules. (*b*, and *p*) basal and peripheral regions of the cell process, respectively.

some angle bend slightly until they take their course roughly parallel to the plasmalemma. They thereby maintain a relatively constant arrangement which suggests the presence of a stabilizing factor. Although cross-bridging between neighboring microtubules or microtubules and the cell membrane has only rarely been observed, the ordered arrangement of microtubules strongly suggests such a stabilizing element. Possibly, the

material of the bridges is not adequately fixed by the methods presently available or does not appear in a clearly identifiable form.

The microtubule tracings also provide information about the length of microtubules in this particular cell type. The vast majority of microtubules within a 6- $\mu\text{m}$ -long segment of a cell process whose total length is about 30  $\mu\text{m}$  extend the entire length. Taking into account the analysis of



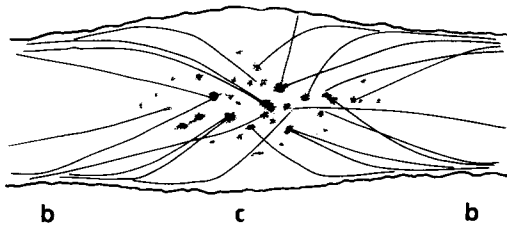


FIGURE 8 Schematic summary of the basic features of the microtubular apparatus in angelfish melanophores. Microtubules radiate from the central apparatus which is characterized by a large number of electron-dense aggregates occupying a considerable portion of the cell center (c). They gradually assume an ordered cortical arrangement until, at the cell process bases (b), many microtubules have associated with the plasma membrane. This position is then maintained over long distances.

microtubule distribution near the cell center which shows that microtubules are continuous between the initiating site and the cell process base, it is conceivable that most of the microtubules extend between the cell center and the cell periphery. This is in marked contrast to the situation in myogenic cells in the regenerating tails of *Rana pipiens* tadpoles (33). In tracings of microtubules within an 8- $\mu\text{m}$ -long segment of a myogenic cell whose total length is more than 100  $\mu\text{m}$ , as many as 50% of all microtubules were observed to terminate, indicating that only relatively short segments of microtubules are present in this cell.

#### Relationship to Pigment Movements

The aggregation of pigment granules in the cell center and the concomitant dramatic change in cell shape can be expected to markedly influence the microtubule framework in the cell processes. Earlier observations (26, 28) have shown that melanophores with aggregated pigment change their shape from a disk to a sphere with collapsed cell processes. Similar changes have been observed in several other chromatophores *in situ* and *in vitro* (9, 19, 24, 38), so that they appear to be a characteristic accompaniment of pigment movements. There is, however, some controversy about the behavior of microtubules during pigment movements. Murphy and Tilney (18) state that in *Fundulus* melanophores the shape of the cell processes and the number and location of microtubules are unchanged in the dispersed and aggregated states. In collapsed cell processes of angelfish melanophores in the aggregated state, the number of microtubules is reduced, so some

microtubules must have depolymerized. According to the present study, cell processes of angelfish melanophores in the dispersed state appear as tubes stiffened by a cortical palisade of microtubules. It may simply be that, after withdrawal of melanosomes during aggregation, this cortical framework is no longer needed; it is partly broken down and redevelops with pigment dispersion. This does not mean that pigment movements are based upon a push-pull mechanism depending on assembly and disassembly of microtubules. Possibly, forces that are unrelated to microtubules but depend on their presence to produce ordered movements participate in the processes of pigment aggregation and dispersion. Evidence for such forces has been presented for the maintenance of the aggregated state (30), and they may also operate in the production of mass movements.

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