The Presence of Spontaneous and Induced Filaments in the Melanophores of Three Species of Teleosts*

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Summary. Electron microscope study of the melanophores in three species of teleosts shows the presence of 11 nm filaments in their cytoplasm. Three sympathomimetic substances (adrenalin, 6-hydroxydopamine and 5-hydroxydopamine) act in vivo and in vitro to induce a considerable increase in the number of these filaments, mainly in the cell branches.

Since the initial description of microtubules in the melanophores of the teleost Fundulus heteroclitus (BIKLE, TILNEY and PORTER, 1966), these structures have received much attention as a possible site responsible for pigment granule migration in pigment cells. This idea received support mainly from the fact that microtubule depolymerizing agents, such as colchicine, vinblastine and cold, inhibit pigment migration (JUNQUEIRA and Porter, 1969; JUNQUEIRA, 1972; JUNQUEIRA, RAKER and PORTER, 1974; SCHLIWA and BEREITER-HAHN, 1973b; WIKSWO and NOVALES, 1969, 1972; MURPHY and TILNEY, 1974).

Recent evidence, however, suggests that microtubules may not be solely responsible for pigment migration. Thus the disassembly of microtubules during pigment aggregation in erythrophores was described recently (PORTER, 1973). Furthermore evidence was presented favoring the presence of a microtubule-independent contractile system in melanophores of the teleost Pterophyllum scalare (SCHLIWA and BEREITER-HAHN, 1975).

Comparative studies in the chromatophores of 14 species of teleosts (JUNQUEIRA and FARIAS, 1976) show a relatively scarce population of microtubules in erythrophores and xanthophores, despite the fact that these cells also transport their pigment droplets. These cells, however, contain a large number of filaments in their cytoplasm. Furthermore unpublished results from this laboratory show that iridophores (cells that transport guanine crystals) contain a great amount of filaments and few microtubules.

Based on the observation that Fundulus melanophores treated by adrenalin (a compound that promotes pigment aggregation in teleost melanophores) presented microfilaments in their cytoplasm while these structures were absent in control cells (JUNQUEIRA, RAKER and PORTER, 1974), it was thought of interest to study the effect of sympathomimetic compounds on the ultrastructure of these cells. The desirability of this approach is reinforced by the observation that compounds that stimulate pigment migration in amphibian pigment cells induce the appearance of great quantities

* This study was supported by the Milnel Foundation, Muscular Dystrophy Association and Fundação de Amparo à Pesquisa do Estado de São Paulo.
of filaments in their cytoplasm (Moellman, McGUIRE and Lerner, 1973). We report here the presence of a scarce amount of filaments in the cytoplasm of control melanophores of three species of teleost and show that sympathomimetic compounds induce the appearance of great quantities of these structures in these cells.

**Materials and Methods**

The following species of marine teleosts were studied: *Fundulus heteroclitus* (killifish), *Opsanus tau* (toad fish) and *Paralichthys dentatus* (summer flounder). Normal fish were obtained from the Supply Department of the Woods Hole Marine Biological Laboratory and maintained in aquaria with running sea water.

The following substances that are known to have sympathomimetic activity (Kostrewa and Jacobowitz, 1974; Tanzer and Thoenen, 1967) were used: Adrenalin (Parke-Davies Co.), 6-hydroxydopamine (6-HODA) and 5-hydroxydopamine (5-HODA) (both from Sigma Chemical Co.). 5-HODA and 6-HODA solutions were freshly prepared in $10^{-3}$ N HCl.

The effect of the sympathomimetic compounds was always to aggregate pigment in the melanophores. This was ascertained by paling of the injected animals or by direct observation of isolated scales under the microscope.

Our results are based on the study of the following material:

a) Two control *Fundulus* injected with $10^{-3}$ N HCl and 10 non-injected animals.

b) Four *Fundulus* injected intraperitoneally with 5-HODA (100mg/kg) and sacrificed 4 hrs later.

c) Four *Fundulus* injected intraperitoneally with 6-HODA (80mg/kg) and sacrificed after various intervals from 1 to 24 hrs.

d) Four normal *Fundulus* whose scales were isolated and immersed in a solution of $5 \times 10^{-4}$ M adrenalin in teleost Ringer’s solution (Joung, 1933) for 30 min.

e) Three control *Paralichthys* injected with $10^{-3}$ N HCl.

f) Two *Paralichthys* injected 1 hr. previously with 6-HODA (80mg/kg).

g) Three normal *Paralichthys* whose scales were immersed for 30 min in $5 \times 10^{-4}$ M adrenalin in Ringer’s solution.

h) Six control *Opsanus*.

i) Four *Opsanus* injected 1 hr previously with adrenalin (3mg/kg).

The skin that covers the scales (*Fundulus* and *Paralichthys*) and fins (*Opsanus*) was fixed in 2% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) at room temperature for 2 hrs. The material was then post-fixed in 1% osmium tetroxide, block-stained with 1% uranyl acetate, and embedded in an Epon-araldite mixture, sectioned in a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Jeol 100 C electron microscope. The diameter of the filaments was measured by comparing them with the diameter of the microtubules present in the same picture. Microtubules are known to measure 24 nm in fish melanophores (Bikle, Tilney and Porter, 1966; Junqueira, Raker and Porter, 1974; Schliwa and Bereiter-Hahn, 1973).
Results

Control animals

Filaments are found in the cytoplasm of the melanophores of all three species studied. These structures appear mainly in the branches of these cells and usually align themselves parallel to the long axis of the branches. They are scarce in these cells, but sometimes associate into small bundles of loosely knit filaments. They are most common between the melanin granules and the plasma membrane (Fig. 1, 3).

Occasionally aggregates of filaments can also be seen at the base of the cell branches where they insert on the cell body (Fig. 2).

Filaments are also frequently observed surrounding the nuclear envelope. Here they do not have a uniform direction but rather form a loose perinuclear network. Since this type of filament has already been described and can be found frequently in a variety of cell types (Blose and Chacko, 1976; Wikswo and Novales, 1972) it will not be further dealt with in this paper. It is worth mentioning, however, that these perinuclear filaments appear the same in control and sympathomimetic treated animals.

Filaments are more common in Paralichthys and Opsanus than in Fundulus, where their presence was rarely registered.

The filaments measure an average of 11 nm in diameter, regardless of the species studied.

Fig. 1. Section through the branch of a melanophore from a control Opsanus. Observe the presence of filaments mainly below the cell membrane. To the right is a segment of an iridophore whose cytoplasm contains many filaments. ×41,250
Fig. 2. Control *Opsanus* melanophore in the region of insertion of a branch of the cell body. 
$\times 57,500$

Fig. 3. Tangential section just below the membrane of the branch of a control *Opsanus*
showing a network of filaments located between the pynocytotic vesicles. 
$\times 60,000$
Fig. 4. Bundles of filament in the branch of a melanophore from a 5-HODA treated *Fundulus*. ×32,500

Fig. 5. Thick bundles of filaments in a melanophore of a *Fundulus* injected 24 hrs beforehand with 6-HODA. ×67,400
Experimental animals

In all of the species studied, treatment with sympathomimetics promoted the appearance of frequent bundles of closely packed filaments, mainly in the cell branches (Fig. 4, 5).

Occasionally these bundles were so thick and dense that they completely filled up the cell branch. Tangential sections of the branches disclosed the presence of a net of fibers oriented parallel to the long axis of the branch and running just beneath the plasma membrane between the micropinocytotic vesicles (Fig. 5). These vesicles have been long known to be abundant in the fish melanophores, although their function is obscure (Bikle, Tilney and Porter, 1966; Junqueira, Raker and Porter, 1974; Schliwa and Bereiter-Hahn, 1973a).

The filaments measured an average of 11 nm in diameter, irrespective of the species studied. Although abundant and clearly visible in all three species, they are more frequent in Paralichthys and Opsanus than in Fundulus, which is precisely the situation found in the control animals.

Each of the three drugs used was effective in inducing the appearance of filaments. No definite statement can be made as to their relative potency, for different dosages were used in each case. However, it is our distinct impression that with the doses used, 6-HODA is the most effective. Further controlled experiments will have to be done to quantitate this more accurately.

Filament induction was observed as early as 30 min after adrenalin treatment and seems to persist for up to 24 hrs after the administration of 6-HODA.

The observation of sympathomimetic treated melanophores of Opsanus tau disclosed a drastic reduction in the diameter of the branches of cells in which the pigment had aggregated (Fig. 6), confirming the results obtained in the teleost Pterophyllum (Schliwa and Bereiter-Hahn, 1973a).

A fortuitous observation that we made was the presence of hemidesmosomes in the branches of the Opsanus melanophores (Fig. 6). There has been speculation as to how melanophore arms remain extended from the cell body after pigment migration to the cell interior. Recently the presence of desmosomes was described (Schliwa and Bereiter-Hahn, 1973a) between adjacent cell branches in melanophores of Pterophyllum and it was postulated that these structures contribute to keep the branches extended. The hemidesmosomes we found may serve an analogous function in cells whose branches are not in direct contact.

Discussion

Our results show the presence of filaments with approximately 11 nm of diameter in the cytoplasm of melanophores of normal and sympathomimetic treated animals. Their presence has already been noted by other authors (Fujii and Novalet, 1969; Schliwa and Bereiter-Hahn, 1973a; Murphy and Tilney, 1974; Wikswor and Novalet, 1969; Imaki and Chavin, 1975), but they have hitherto received no great attention. Consequently, the possibility that they participate in pigment transport has up to now had no experimental support.

The capacity of sympathomimetic compounds to induce pigment migration coinciding with the appearance of abundant filaments in fish melanophores suggests
Fig. 6. Section of a melanophore from a adrenalin injected Opsanus. Observe the constriction of the cell branches (arrows) with aggregation of the pigment granules into the cell body. $\times 7,500$. The upper insert shows, in larger magnification ($\times 35,550$), a branch containing filaments. The arrowheads show the location of hemidesmosomes, one of which is seen at higher power ($\times 100,000$) in the lower insert.
that this cellular component should be considered in the analysis of the possible mechanism(s) responsible for pigment migration in teleost. The fact that they can be induced in a relatively short period of time (30 min) suggests that this process is independent of protein synthesis and is probably due to the polymerization of pre-existing monomers.

These results coincide with those obtained in amphibian melanophores (Moellman, McGuire and Lerner, 1973) where it was shown that other substances that promote granule migration such as melanophore stimulating hormone and cyclic AMP also induce the appearance of bundles of filaments. Recent observations in human skin melanocytes suggests that the presence of filaments seems to be related to movement and transport of melanosomes (Jimbow and Fitzpatrick, 1975).

Three lines of evidence suggest that microtubules are not the main site of the force-generating mechanism responsible for pigment migration. One is the observation that microtubules depolimerize during pigment aggregation in the erythrophores of the teleost Holocentrus (Porter, 1973). The second is that the pressure exerted by the cell body of aggregated melanophores from Pterophyllum is maintained despite microtubular depolymerization (Schliwa and Bereiter-Hahn, 1975).

The third line of evidence is a recent study (Junqueira and Farias, 1976) of the erythrophores and xantophores in 14 species of teleosts which shows the relative abundance of filaments and scarcity of microtubules in these cell types despite the fact that they are capable of migrating their carotenoid-containing pigment droplets. Since these pigment droplets are not membrane bounded, it is unlikely that all types of pigment migration can be explained by the hypothesis whereby microtubules are thought to interact with protein present on a membrane surrounding the pigment granule.

Recently we had the opportunity to study the iridophores of the skin of Opsanus. These cells contain and transport crystals of guanine. We observed very few microtubules but a remarkable number of filaments in the cytoplasm of these cells (Fig. 1). This corroborates the observations mentioned above for the other pigment cell types.

Our results suggest the advisability of further studies regarding the possible role of filaments in the processes of pigment migration in chromatophores.

Acknowledgement. The authors are indebted to Prof. K. R. Porter for working facilities at the Woods Hole Marine Biological Laboratory and helpful discussion.

References


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