Cell Tiss. Res. 190, 409–416 (1978)

Cell and Tissue Research

Ultrastructural Demonstration of Hormone-Induced Movement of Carotenoid Droplets and Endoplasmic Reticulum in Xanthophores of the Goldfish, *Carassius auratus* L.*

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Summary. The hormone-induced pigment dispersion in primary cultures of xanthophores of goldfish (*Carassius auratus* L.) has been shown to involve the dispersion of not only carotenoid droplets but also of smooth endoplasmic reticulum. The dispersion of these organelles is inhibited by cytochalasin B and is accompanied by thinning of the cell body, thickening of the processes, and also overall changes in cellular morphology (process extension) under certain conditions. Electron microscopic examination of heavy meromyosin treated glycerinated xanthophores in scales revealed the presence of actin filaments in these cells.

Key words: Endoplasmic reticulum – Actin filaments – Xanthophores – Pigment cells.

Introduction

Pigment cells of many cold-blooded vertebrates respond to hormonal stimuli by dispersing or aggregating their pigment-containing organelles (for review, see Taylor and Bagnara, 1972; Bagnara and Hadley, 1973) while little is known about movement of other organelles. We wish to present evidence indicating that in the goldfish xanthophore, movements of endoplasmic reticulum take place in concert with those of carotenoid droplets.

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* This work was supported, in part, by grants AM-5384 and AM-13724 from U.S.P.H.S.

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0302-766X/78/0190/0409/$01.60

Materials and Methods

Scales were plucked from the dorsal trunk region of xanthic goldfish (*Carassius auratus* L.) and the overlying epidermis removed manually after a brief treatment in collagenase-containing physiological saline (Obika, 1975). Heavy meromyosin binding was carried out according to the method of Ishikawa et al. (1969) with slight modification (Murray and Dubin, 1975). Scales were washed in standard saline solution (SSS) containing 0.05 M KCl, 5mM $MgCl_2$ and 6mM Na-phosphate buffer at pH 7.0, treated in a series of glycerol (5, 10, 20, 30 and 40 % in SSS) for 7 min each, followed by another series of glycerol (50, 20, and 5 % in SSS) for 2 h each at 0° C. Scales were then incubated in 5 % glycerol in SSS for 18 h at 4° C with or without heavy meromyosin (HMM, 4.5 mg/ml) which was prepared from rabbit skeletal muscle by trypsin digestion (Szent-Györgyi, 1953). After a brief rinsing in SSS, materials were fixed at room temperature with 3 % glutaraldehyde and 1 % OsO_4, both in 0.1 M cacodylate buffer at pH 7.4 for 1 h, respectively. Control scales and cultured cells were fixed without further treatment. Experimental and control tissues were embedded in Epon 812, and the sections stained with uranyl acetate and lead citrate were observed in a Philips 201 electron microscope at 80 kV.

Hormone-induced pigment translocations and the effect of cytochalasin B on this process were examined in primary cultures of xanthophores as previously described (Winchester et al., 1976) with the modification that the concentration of ACTH was lowered to 0.02 IU/ml. Light microscopic observations were made on a Leitz microscope with the Smith Interference Contrast System T.

Results

Physiological Studies. Interference microscopy showed that cultured cells with dispersed pigment have a relatively even thickness (Fig. 1 a). After treatment with epinephrine, there is a thickening of the cell body, thinning and partial retraction of cellular processes (Fig. 1 b). This process is reversed by removing the epinephrine and retreating the cells with ACTH (Fig. 1 c). Cytochalasin B also causes retraction of processes and inhibits the ability of ACTH to initiate process extension.

Ultrastructural Studies. The ultrastructure of cells with aggregated pigment shows a dense perinuclear mass of almost exclusively carotenoid droplets interspersed among smooth endoplasmic reticulum (Fig. 2 b). Other regions of the cytoplasm contain pterinosomes, and mitochondria but little smooth endoplasmic reticulum. In cells with dispersed pigment, organelles are found throughout the cytoplasm (Fig. 2 a). The ultrastructure of a xanthophore process in the scale (Fig. 3) contains smooth endoplasmic reticulum, small carotenoid droplets without typical limiting trilaminar membranes, microtubules which usually emanate radially from the center of the cell, and 5–7 nm diameter microfilaments which occasionally appear as bundles. Both glycerinated and non-glycerinated xanthophores exhibit microfilaments. In glycerinated cells, the microfilaments in the cortical regions of the xanthophores are decorated with heavy meromyosin as a fuzzy mesh (Fig. 4 a). In the smaller dendritic processes, decorated filaments are sometimes found in parallel alignment (Fig. 4 b). Decorated filaments (fuzzy) between pterinosomes, found in random orientation, are also common in thicker portions of processes, but no appreciable association between the filaments and other cytoplasmic organelles is observed. Fuzzy decorated filaments are absent where actin-heavy meromyosin interaction is absent (Fig. 4 c). Most of the microfilaments in the perinuclear region and some in the cortical regions are not decorated by heavy meromyosin. Glycerinated xanthophores, recognized by their characteristic pterinosomes and carotenoid droplets, no longer possess microtubules. In those cases where the

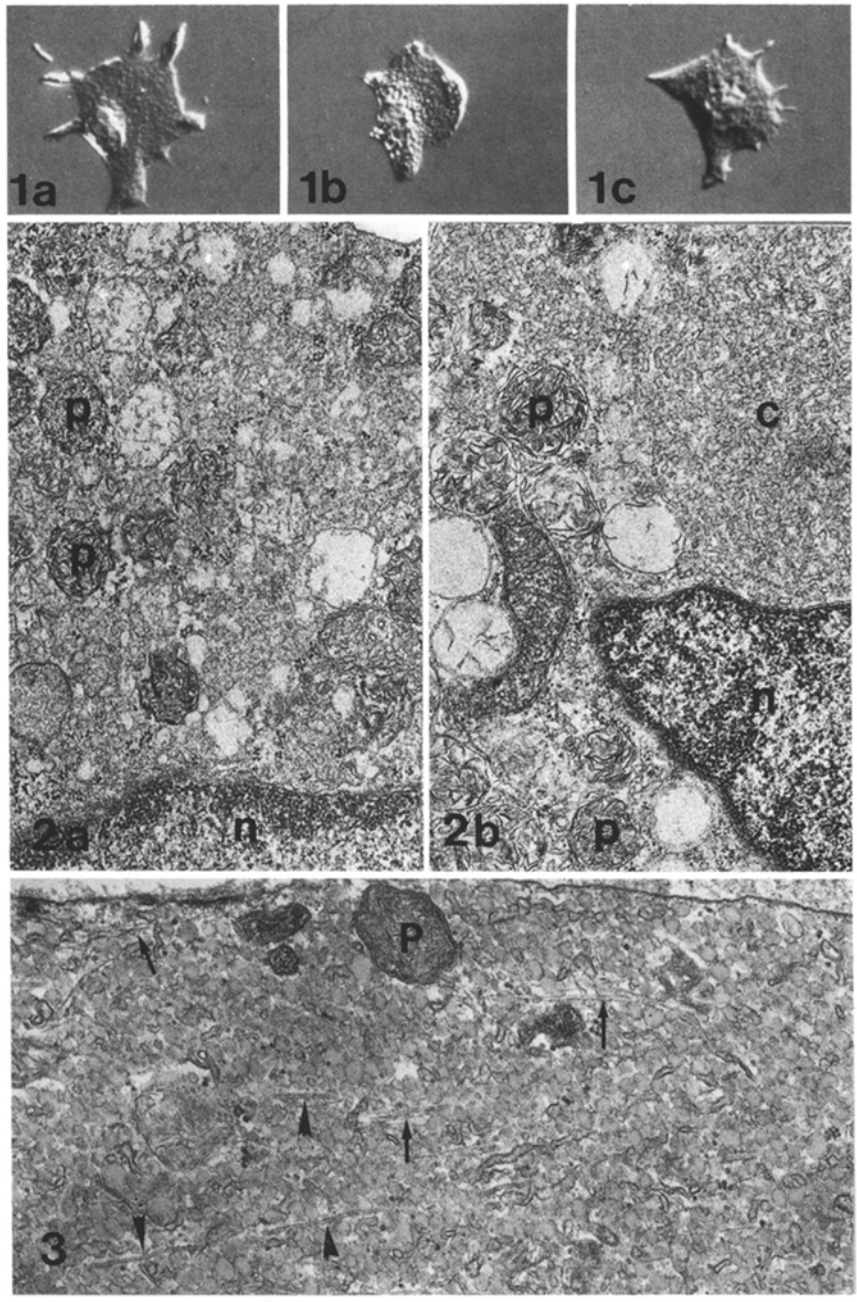

Fig. 1a–c. Interference photomicrographs of xanthophores in primary culture; reproductions made from color transparencies. Retraction and re-establishment of cellular processes in an isolated xanthophore; a xanthophore with dispersed pigments (**a**); 3 h after epinephrine perfusion (**b**); and 1.5 h after administration of ACTH (**c**). Note lack of processes and thickening near nucleus in (**b**). ×1000

Fig. 2a and b. Electron micrographs of portions of xanthophores in culture. **a** Xanthophore with dispersed pigments. *n* nucleus; *p* pterinosome. Carotenoid droplets and ER dispersed throughout cell. ×17,000. **b** Xanthophore with aggregated pigments. Aggregated mass of carotenoid droplets and endoplasmic reticulum (*c*) on upper right. Note scarcity of these organelles elsewhere. ×21,100

Fig. 3. Electron micrograph of scale xanthophore process. Portion of xanthophore process with dispersed pigments, containing numerous carotenoid droplets, smooth endoplasmic reticulum, some pterinosomes (*P*), microfilaments (arrows) and microtubules (arrowheads). ×28,000

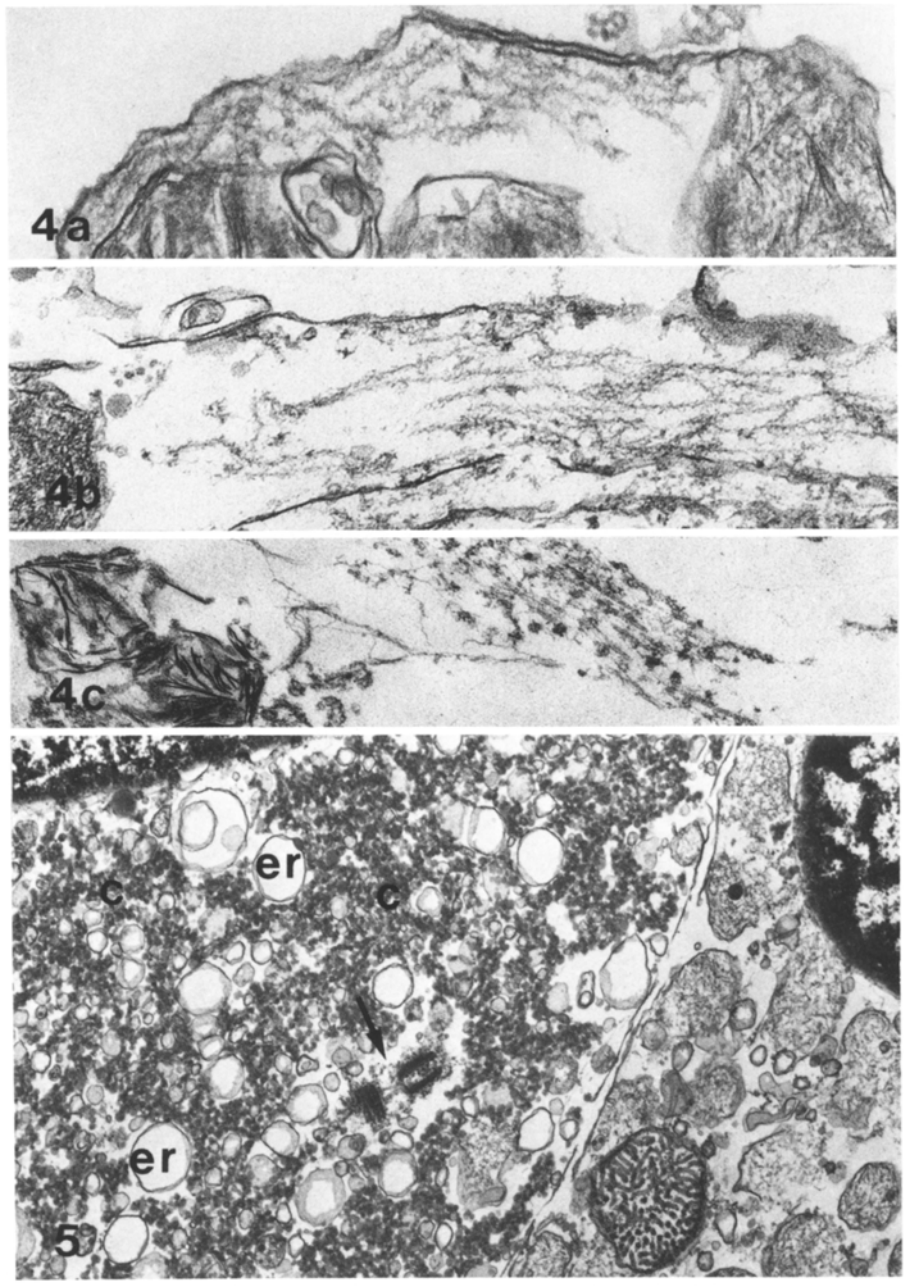

Fig. 4a and b. Xanthophores treated with heavy meromyosin (HMM). **a** Microfilaments decorated with HMM near periphery of cell. ×96,500. **b** HMM decorated microfilaments in processes. ×50,000. **c** Glycerinated controls with no decoration of filaments. ×30,000

Fig. 5. Xanthophore with aggregated carotenoid droplets (*c*) and endoplasmic reticulum (*er*) following glycerination. ER swollen and vesicular within mass of carotenoid droplets. Note centriole (arrow) and relatively sparse ER outside of aggregate (upper left corner and cell on right). ×14,000

carotenoid droplets and smooth ER are aggregated prior to glycerination they remain aggregated after glycerination although the organelles are packed less densely (Fig. 5). The swelling of the endoplasmic reticulum vesicles renders them more distinct than in non-glycerinated cells. The density of such vesicles is at least four times higher in the aggregate of carotenoid droplets than elsewhere in the cytoplasm.

Discussion

In animal cells, many organelles undergo non-random movement. Among the most dramatic examples are the hormone-induced pigment granule migrations which, in extreme cases, lead to complete dispersion throughout the cell versus aggregation in the perinuclear region within a few minutes (for review, see Taylor and Bagnara, 1972; Bagnara and Hadley, 1973). Although other organelles also may undergo directional movement, it is never of such rapidity. An example of the latter kind (for review, see Bloom and Fawcett, 1975) is the movement of some endoplasmic reticulum-related vesicles, such as secretory granules of endocrine or exocrine glands, lipid droplets of intestinal epithelial cells and synaptic vesicles. Aside from being relatively slow, the movements of these vesicles differ from those of pigment organelles in two other respects. First, they occur in one direction and are not reversible. Second, although hormones often regulate the secretion of the content of secretory vesicles, this effect appears to be primarily the induction of fusion of the organelle membrane with plasma membrane, thus leading to the emptying of the contents of the secretory vesicles to the outside of the cell (for review, see Turner and Bagnara, 1976). To our knowledge, there is no hormonal regulation of the intracellular movement of these or of endoplasmic reticulum. In this respect, the hormone-induced endoplasmic reticulum movement reported here is unique.

Previous reports from our laboratory (Winchester et al., 1976) and by Ozato (1977) have shown that the xanthophore pigment movements are affected by colchicine and cytochalasin B, implicating the involvement of microtubules and actin filaments in this process. The presence of microtubules and actin filaments in these xanthophores was verified by electron microscopy. In addition, electron microscopic examinations of xanthophores treated by chromatophorotropic and/or inhibitory agents indicated that the smooth ER and carotenoid droplets always move in concert. However, as is the case for pigment organelle movement in general, the exact role of microtubules and microfilaments in this process remains uncertain. Rather than speculating on the role of these structures, we shall present below the complicating factors which underlie this uncertainty.

Microtubules and microfilaments, including actin filaments, have been postulated to serve both as cytoskeletons and as essential components of the motile apparatus. In pigment cells, these structures serve an additional function, namely, the aggregation and dispersion of pigment organelles. In this regard several questions may be raised. First, with two sets of structures, microtubules and microfilaments, is it possible that one is used for pigment dispersion and another for pigment aggregation? The answer is no, as microtubules have been implicated in both pigment aggregation and dispersion (Bikle et al., 1966; Green, 1968; Fujii and

Novales, 1969; Wikswo and Novales, 1972; Schliwa and Bereiter-Hahn, 1973a; 1973b; Murphy and Tilney, 1974; Murphy, 1975) and microfilaments generally seem to be involved in pigment dispersion in amphibians (Malawista, 1971; McGuire and Moellmann, 1972; McGuire et al., 1972; Novales and Novales, 1972) and in pigment aggregation in fishes (Obika, 1975; Schliwa, 1975; Schliwa and Bereiter-Hahn, 1975). Second, is there firm evidence that microfilaments are involved in pigment movement? Unfortunately, the evidence consists largely of the effects of cytochalasin B and, as reviewed by Pollard and Weihing (1974) and Pollard and Rifkin (1976), the exact mode of action of this agent is not clear. Third, are there different microfilaments with different function(s)? It is well recognized that microfilaments were originally considered to include filaments of a range of sizes, with diameters of approximately 5–10 nm. It is generally accepted that, whereas actin filaments are probably present in most, if not all, higher animal cells, many cell types contain additional specialized filaments, such as neurofilaments, gliafilaments and tonofilaments. In pigment cells, there may be present not only actin filaments (Murray and Dubin, 1975), but perhaps also non-actin filaments (Jimbow et al., 1976) with unknown function. Even in the case of non-muscle actin filaments, there appears to be structural and functional divergence. Based on the recent reports of Pollard (1976) and Lazarides (1976), one may postulate two, perhaps more, families of non-muscle actin filaments. The stress fibers are structural elements of larger diameter, associated with tropomyosin, relatively resistent towards osmium tetroxide, and decorated by heavy meromyosin to form "arrowheads". The actin filaments that are situated beneath the plasma membrane and involved in movements such as ruffling are of smaller diameter, not associated with tropomyosin, relatively more labile towards osmium tetroxide, and decorated by heavy meromyosin to form a "meshy network". The xanthophores in Fig. 4a appear to contain the latter type of actin filaments but it is unknown whether all the microfilaments in the xanthophores are indeed actin filaments and if so, whether there are two types of actin filaments. Fourth, what is the relationship between the hormonal effects on pigment and on the overall cell morphology? Classical work on the process of pigment movement under in vivo conditions showed that the pigment organelles move within stable dendritic processes. From this, it was concluded that hormones do not cause the retraction and/or extension of dendrites. Is should, however, be pointed out that these results do not tell us whether there is any change in cell morphology in the third dimension, namely, thickening or thinning of the cell body or the dendrites. The results presented in this paper, show clearly that, in cultured xanthophores, hormones do regulate the overall cell morphology. ACTH induces not only carotenoid droplet-endoplasmic reticulum dispersion, but also thinning of the cell body and thickening of dendritic processes (centrifugal cytoplasmic flow) and, in some instances, extension of dendrites. In contrast, epinephrine accelerates carotenoid droplet-endoplasmic reticulum aggregation, thickening of the cell body and thinning of dendritic processes (centripedal cytoplasmic flow) and, in some instances, retraction of dendrites. Similar results have been reported recently for other pigment cells (Obika, 1975; Butman et al., 1977). The exact interrelationship between pigment movement and cytoplasmic flow is, however, unknown. Finally, Porter (Porter, 1976; Byers and Porter, 1977) has proposed that all cytoplasmic structures are

connected by a network of microtrabeculae which plays an essential role in the movement of these structures. This hypothesis is based on results of high voltage electron microscopy and is both novel and intriguing. It is too early to judge the validity of this hypothesis. However, it is clear that some additional features must be added to it to explain the differential movement of different organelles, for example, the failure of pterinosomes in xanthophores to undergo hormone induced aggregation or dispersion.

This discussion points out some, but not all, of the uncertainties regarding the role of microtubules and microfilaments in the movement of organelles. We shall, therefore, not speculate on the possible mode of action of these structural elements but simply conclude that much more work is needed in this area.

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Accepted March 14, 1978