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ROLE OF MICROTUBULES MOTORS TRANSDUCTION OF PIGMENT GRANULES IN FISH SPECIES OF PUNTIUS

Sita Agrawal and Arun Kumar*

Department of Zoology, Shri Baldeo P.G College, Baragaon, Varanasi, Uttar Pradesh, India

*Email: arunkumar@gmail.com

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Abstract

Microtubule motors (MTs) or actin dependent motors of the myosin family move organelles along microtubules or actin filaments in intracellular transport. When pigment granules move out of the cell center, the animal becomes more pigmented because melanophors are dispersed. On the other hand, when they gather in the cell center, the animal looks less pigmented. Isolated scales were first equilibrated in physiological saline, then immersed in colchicine at concentrations ranging from 10-6 to 10-4M, and then treated with epinephrine in order to study the function of microtubule motors (kinesin and dynein). The fact that colchicine (104M) successfully prevented the epinephrine-induced aggregation of melanosomes in melanophores on scale preparation suggested that microtubules play a function in the intracellular transport of melanosomes. To further support the idea that microtubules play a role in the intracellular transport of melanosomes, the medication colchicine (10-4M) successfully inhibited the epinephrine-induced aggregation of melanophores on scale preparation.

Keywords: Microtubule motors, dynein, kinesin, dispersion, aggregation, colchicine, melanophores, Fish.

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Introduction

As a means of communication or adaptation, certain animal groups have mastered the use of chromatophores, which are specialized pigment cells, to rapidly alter their color. Melanophores are pigment granules that contain tyrosin-derived melanin; chromatophores are chromatophores. Melanosomes can be either black or brown in color. In order to research intracellular transport and cytoplasmic organization, melanophores are suitable since they can either aggregate their melanosomes to the cell center or scatter them throughout the cytoplasm. Several hormones and neurotransmitters regulate the mobility of these melanosomes. Cytoplasmic microtubules play essential roles in spatial organization of the cytoplasm (Lane and Allan, 1998), intracellular transport (Vale, 2003; Welte, 2004; Caviston and Holzbour, 2006) and cell locomotion (Wittman and Waterman-Storer, 2001; Etienne-Manneville, 2013; Burakov *et al*., 2021). One common arrangement for microtubules is a polarized array, in which the plus ends stretch outward from the cell and the minus ends congregate at a microtubular organizing center, like the centrosome.

Although multicellular chromatophores are known to exist in certain lower forms of life, fingerman (1963) noted that in most higher forms, including teleost fishes, they tend to be unicellular. Though earlier it was thought that melanophores are amoeboid-like cell, which extend pseudopodia during pigment dispersion but Mathews (1931) realized that the concept was incorrect and proposed that melanophores had a rigid cell shape and that only the pigment granules within the cell moved during the two phases i.e., the dispersion or aggregation (Fig.1). Pigment granules can either collect in the cell center, making the animal look less colored, or they can migrate out of the cell center, making the animal appear more colored. Figure 1 also shows the aggregated and scattered stages of the identical melanophores from the fish scales that were separated for the investigation. The ultrastructure of chromatophores have revealed that a considerable change in cell shape occurs during pigment migration (Fujii, 1971; Schliwa and Bereiter-Hann, 1973). Oryzias latipes melanophores seemed somewhat flat when seen with a scanning electron microscope. There were no noticeable blebs or microvilli on the surface, which was slightly rough. Similar in length to their dispersed-state counterparts, the aggregated melanophores had nearly hemispherical dendrites (Obika, 1975). Organelles such as nuclei, centrioles, mitochondria, vesicular smooth-surfaced endoplasmic reticulum, micropinocytotic vesicles, ribosomes, and pigment granules (chromatosomes) are also found in chromatophores. Microtubules are another key cellular component that has been identified and is thought to play a major function (the mechanochemical basis) in the chromatosome's bidirectional migration within the chromatophores. (Bikle *et al*., 1966; Green, 1968) and actin filaments (Rodionov *et al*., 2003), which serve as “rails” for the movement of cargo, the organelles (i.e., the chromatosomes in the chromatophores). It is generally believed that microtubule motors (kinesins and dyneins) support long distance movement of organelles whereas actin filament-dependent motors, myosins are responsive for local transport (Kelleher and Titus, 1998, Hasegawa, *et al*., 2019). Now several lines of evidence indicate that microtubules and microfilaments are indispensable for rapid transport of pigment granules in fish chromatophores. Cytoplasmic dynein is a microtubule minus-end di-rected motor (Schroer *et al*., 1989; Vallee and Gee, 1998). Research has revealed that dynein may be involved in most microtubuleassociated

intracellular transport and also in organisation of the cytoplasm (Koonce *et al*., 1999), making dynein one of the most ubiquitous motors. There were early indications for a role of dynein in melanosome aggregation (Clark and Rosenbaum, 1982), and it was proven by injection of inhibitory antibodies against dynein (Nilsson and Wal- lin, 1997). Dynein associates with the dynactin complex, and inhibition of organelle transport can be seen either by effecting either dynein or dynactin (Holleran *et al*., 1998). Dynactin seems to be required for dynein-dependent melanosomes transport in fish melanophores (Nilsson *et al*., 2001; Vorobjev *et al*., 2001), human melanocytes (Byers *et al*., 2000; Vancoille *et al*., 2000a), and probably in frog melanophores too (Reese and Haimo, 2000). Current researches are diverted on the elucidation of the role of microtubular motors transduction of pigment granules in fish species of Puntius.

Materials and Methods

The experiment was conducted using Puntius species freshwater teleosts, regardless of sex. We brought the fish in from Tighra reservoir, which is 23 km from Gwalior (M.P.), and we acclimated them by keeping them in fresh water aquariums (90 X 45 X 45 cm) at our facility for a week. Everything was done at room temperature, which is between 240 and 280 degrees Celsius. Using delicate forceps, the scale slips were delicately removed off the animal's dorsal trunk surface. Physiological saline solution with the following milligram concentrations: 128.3 M NaCl, 2.8 M KCl, 5.6 M glucose, 1.8 M CaCl2, and 0.5 M Hepes-NaOH with a pH of 7.4 was immediately added to the separated scale. The effect of drug on the response of certain groups of melanophores were studied with light microscope and were evaluate according to Hogben and Slome (1931) in amphibian melanophores where 1, representing the maximum aggregation and 5, representing maximum dispersion and 2,3,4 as intermediate stage of aggregation dispersion (Fig-2).

Results

Colchicine is a toxic natural product and secondary metabolite, originally extracted from plants of the genus colchicum (Autumn crocus, Colchicum autumnale) also known as “Meadow saffron”. Pedanius Dioscorides, writing in the first century CE in De Materia Medica, initially described colchicum extract as a remedy for gout. In 1820, French chemists P.S. Pelletier and J. Caventon were the first to isolate colchicine, an alkaloid. The alkaloid's capacity to bond with tubulin was later connected to its pain-relieving and anti-inflammatory properties for gout. It was later determined that the compound was a tricyclic alkaloid. By attaching to tubulin, a primary component of microtubules, colchicine prevents their polymerization. Because colchicine blocks axoplasmic transport in neurons, it influences melanosome translocations within melanophores. The dispersed state of the isolated scale preparations was achieved by equilibrating them in physiological saline for 15-20 minutes. Melanophores with distributed melanosomes are visible when they are equilibrated in PS. After five minutes of being exposed to E (10-6M), the melanosomes have fully clumped together (Fig 3). After being dissolved in physiological saline for 15-20 minutes, the isolated scale preparations were submerged in a colchicine solution of different concentrations (10-6-10-4M) for 30 minutes. In their scattered form, the melanophores persisted. Epinephrine, at a concentration of 10-6M, was subsequently administered to these melanophores for duration of 10 minutes. Regardless of the situation, epinephrine led to pigment aggregation. However, this reaction was less severe than in the control group, where melanophores that had been equilibrated in PS were left in PS for an additional 30 minutes before being treated with the same dosage of epinephrine. Which caused nearly complete aggregation of pigment (M.I.=1). The epinephrine induced melanosome aggregation was almost completely inhibited by 10-4 M colchicine, and was partially inhibited by 10-5M to 10-6M concentrations (Fig 4). Colchicines significantly inhibited the response to epinephrine in a concentration dependent manner over the effective concentration range that was studied (10-6 to 10-4M). This indicates that the drug affects the microtubules intracellularily (Fig 5).

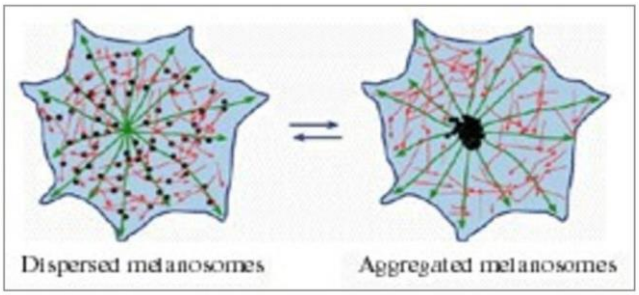

Figure-1: A diagram of a motile light-absorbing or light-reflecting chromatophore with chromatosome aggregated (right) and dispersed (left) throughout the cell.

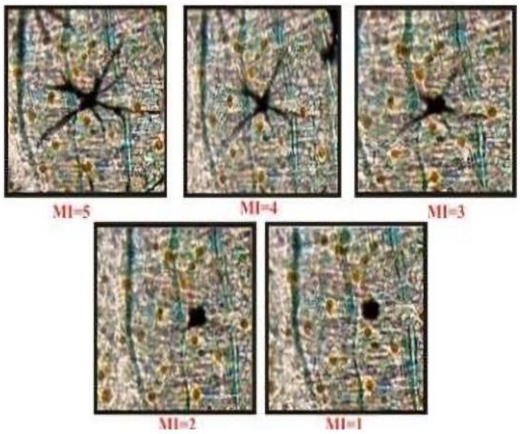

Figure-2: Melanophore indices (5-1) as were used for measurement of melanophore responses in the study.

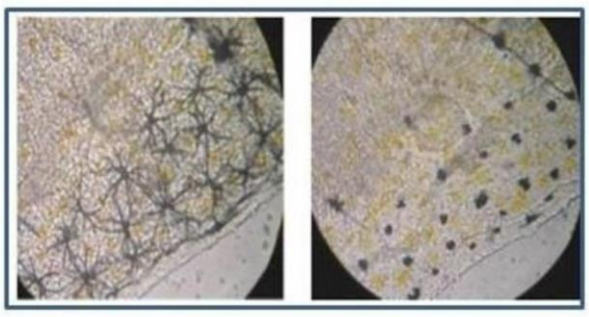

Figure-3 Photomicrograph (A and B) of the same field showing motile responses of melanophores on the isolated scale of the fish, Puntius, viewed from the dermal side x100. A. Equilibrated in PS melanophores with dispersed melanosomes are visible. B. Five min after the application of epinephrine (10-6M), melanosomes are completely aggregated

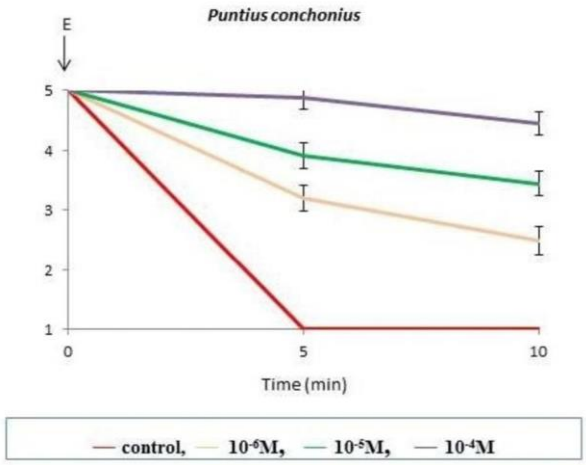

Figure-4 The response of melanophores to epinephrine (10-6M) for 10 min after pretreatment with varying concentration of colchicine for 30 min. Control represent preincubation in PS for 30 min.

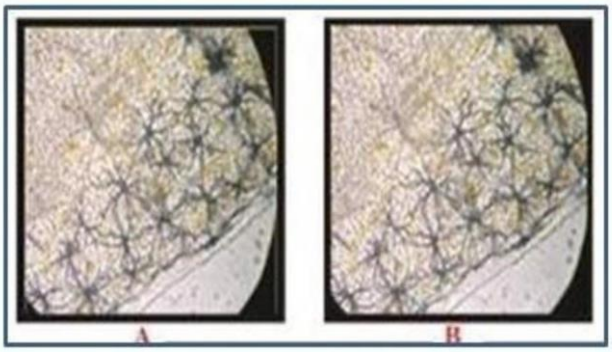

Fig ure-5 Photomicrograph (A, and B) of the same field showing motile responses of melanophores on the isolated scale of the fish, Puntius, viewed from the dermal side x100. The response of melanophores to epinephrine (10-4M) for 10 min after pretreatment with colchicine (10-6M) for 30 min. Colchicine effectively blocked the epinephrine induced melanosome aggregation in melanophores.

Discussion

The observations pertaining to inhibitory effect of colchicine on pigment aggregation responses in Puntius melanophores due to a highly potent drug epinephrine, are in accordance with the results presented by earlier workers (Wikswo and Novales, 1969, Murphy and Tylney, 1974; Janqueira *et al*., 1974, Patil and Jain, 1996, Yadav and Jain, 2017). However, the effect of drugs such as colchicine, is not always related to the disruption of cytoplasmic microtubules (Obika, 1986), as in medaka melanophores, colchicine at 5mM produces a retardation in pigment aggregation although a substantial number of microtubules survive even after a prolonged exposure to the drug. That colchicine may act at a site unrelated to microtubules and still can influence the pigmentary response is suggested by the action of lumicolchicine, which has little binding activity to tubulin but has been found to be capable of preventing pigmentary response in the same manner as colchicine does (Obika *et al*., 1978). Drugs like vinblastin, nocodazole, colcemid and taxol having different mechanism of action on the microtubules may be tested for their effects on melanosome movements to elucidate the mechanochemical basis of pigment translocation in fish melanophores. In addition to this as actin filaments have also been implicated to have a definite role, it would be interesting to include the studies pertaining with drugs that are known to disrupt the cytoplasmic filaments. It is worth mentioning here that Obika and Mayer-Rochow (1986) working with the Antarctic teleost, Pagothenia borchgrevinki could report a cold-resistant microtubule system on which melanosome movements depend in sharp contrast to melanophores from Fundulus heteroclitus, Gymnocorymbus ternetzii, Oryzias latipes and Pterophyllum scalare, inhibitants of temperate and tropical zones, in which exposure of melanophores to low temperatures (0 to 50C) for 30 to 60 min causes a complete disassembly of microtubules and the cells, then devoid of microtubules, are not able to respond to aggregative stimuli with a rapid pigment aggregation (Murphy and Tilney, 1974; Obika *et al*., 1978; Schliwa and Enteneuer, 1978). The aggregation of melanosomes transported by dynein in zebrafish melanophores by bright-field microscopy at a high recording rate i,e 800 fps (Hesagawa *et al*., 2019). Microfilaments have also been observed in melanophores of many higher teleosts and they, in association with microtubules have also been implicated in melanosome movements. Filamentous actin, like microtubules have a built-in polarity and short microfilaments (2-3µm in length) have a certain distribution in the melanophores, (their density was greater at cell periphery than at the centre) with no preferential orientation. Thus Rodinov *et al*., (1998) could suggest that microtubules serve as routes for transport over long distances, while actin filaments support movement to local sites; microtubules provide tracks for initial fast motion toward the periphery, but the final uniform distribution of pigment granules is achieved by a 2-D ‘random walk’ on actin filaments. Colchicine (10-4M) effectively blocked the epinephrine induced melanosomes aggregation in melanophores on scale preparation implicating a role for microtubule motors in transport of melanosomes in the cell.

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