Mem. Fac. Sci. Shimane Univ.,
21, pp. 127–130 Dec. 20, 1987

Light Response of Severed Processes of the Melanophores in a Freshwater Teleost, *Zacco temmincki*

Hiroyuki NAORA and Tetsuro IGA

Department of Biology, Faculty of Science, Shimane University, Matsue 690, Japan
(Received September 5, 1987)

The melanophores of a freshwater teleost, *Zacco temmincki,* were photosenstitive: In darkness the melanophores induced a melanosome aggregation and in light they caused a melanosome dispersion. Processes surgically severed from the cells also responded to changes in illumination in the same manner as the cell bodies or the intact cells did. This result indicated that photoreceptive sites were distributed over the whole of the cell, and that the nucleus did not directly take part in the light response, in both dark-induced aggregation and light-induced dispersion.

Introduction

The melanophores of a freshwater teleost, *Zacco temmincki,* like those of other teleost fishes, are regulated through neurotransmitter and hormone receptors on the melanophore membrane. In addition, the melanophores of this fish species are photosensitive: In darkness the melanophores induce a pigment aggregation at the cell center and in light they cause a pigment dispersion. Although occurrence of photosensitive chromatophores has been reported in a variety of animals (Weber, 1983), mechanisms of photoreaction are quite unknown.

Recently, Iga and Takabatake (1986) showed that *Zacco* melanophores responded to light spot or local irradiation. They inferred that photosensitive sites were distributed over the whole of the cell.

The present experiment was to obtain additional evidence for the distribution of a photosensitive substance and to decide whether the cell nucleus took directly part in for inducing the light response. This possiblity was examined by the light response of processes surgically severed from the melanophores.

Materials and Methods

Scale melanophores of the freshwater teleost *Zacco temmincki* were used. Scales were pulled from the dark band running longitudinally on the fish body. Both innervated and denervated melanophores were used. The denervated melanophores were obtained from fishes injected intraperitoneally with 6–OH dompamine, as previously described (Iga and Takabatake, 1982). An isolated scale was fixed, dermal side

up, on the bottom of an experimental chamber filled with physiological saline solution which had the following composition: 128 mM NaCl; 2.6 mM KCl; 1.8 mM $CaCl_2$; 5 mM Tris-HCl buffer (pH 7.2). The chamber was placed on the stage of an inverted microscope.

Branched processes of the melanophores were microsurgically severed from the cell bodies with a glass microneedle that was controlled by a micromanipulator (MO-102, Narishige). After the operation, the preparation was kept under illumination for 10 min. The states and responses of the melanophores and the severed procersses were recorded by microphotograps.

Experiments were done in a dark room, at room temperature (20–30°C).

Results and Discussion

When subjected to the dark, the melanophores aggregated their pigment at the centrospheres, and under illumination the melanophores dispersed the melanosomes outward, recovering to their original dispersal. The severed melanophore processes responded to changes in illumination in the same manner as the mother cells or the intact melanophores did (Fig. 1). The velocities of aggregation and also of dispersion were almost the same as those in the mother cells. The severed processes could respond repeatedly to changes in illumination.

On the light response, there was no difference between innervated and denervated melanophores.

It has been well documented that pigment granules within a process severed from the centrosphere are still capable of aggregation and dispersion (Matthews, 1933; Kamada and Kinoshita, 1944; Nagahama and Adachi, 1969; MacNieven *et al.* 1984). These results indicate that informational receptors responsible for pigment movements are distributed on the whole membrane of the cell, and that the centrosphere at which the nucleus exists plays no part in the movement of pigment granules. Recent studies on local light stimulation of *Zacco* melanophors have shown that photoreceptive sites are distributed over the whole of the cells (Iga and Takabatake, 1986). The present result confirmed the previous conclusion. Furthermore, the present study showed that the nucleus was not directly involved in the light response of the melanophores.

Cycloheximide (100 μM/ml), an inhibitor of protein synthesis, blocked a dark-induced aggregation in *Zacco* melanophores, suggesting that the reaction was induced by an active substance synthesized in the dark (Iga and Takabatake, 1983). Regulation of specific protein syntehesis by light has been reported in various photoresponses (Hug, 1978). In the light response of the melanophores, a transcriptional regulation of specific protein synthesis by light is not involved, for severed processes are devoid of the nucleus. A possibility of the participation at a translational level is still remained. The translational regulation is well established in synthesis of ribosomal proteins of bacteria (Dean and Nomura, 1980).

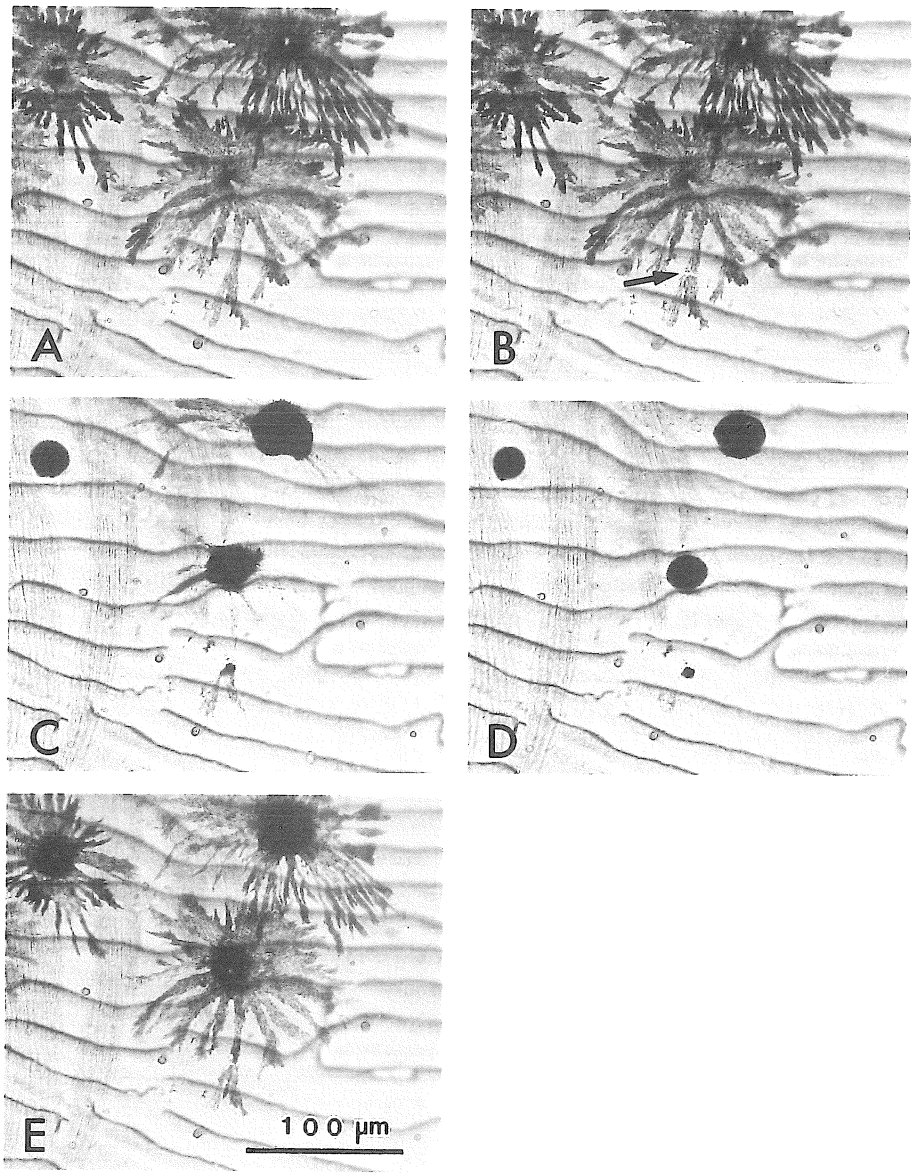

Fig. 1. The light response of the melanophores and severed processes. A, intact melanophores under illumination; B, a melanophore process was severed from a melanophore. An arrow indicates the position of the cut; C, D, dark-induced aggregation. 30 min and 70 min after the dark, respectively. E, light-induced dispersion. 5 min after the light.

References

Dean, D. and Nomura, M. (1980). Feedback regulation of ribosomal protein gene expression in *Escherichia coli.* Proc. Natl. Acad. Sci. USA, **77**: 3590–3594.

Fujii, R. and Oshima, N. (1986). Control of chromatophore movement in teleost fishes. Zool. Sci., **3**: 13–47.

Hug, D. H. (1978). The activation of enzymes with light. In: Photochemical and photobiological reviews, Vol. 3, Smith, K. C. ed. pp. 1–33, Plenum Press, New York.

Iga, T. and Takabatake, I. (1982). Denervated melanophores of the dark chub *Zacco temmincki*: Method of denervation and the evaluation of preparations for physiological experiments. Annot. zool. Jpn., **55**: 61–69.

Iga, T. and Takabatake, I. (1983). Melanophores of *Zacco temmincki* (Teleostei) are light sensitive. J. Exp. Zool., **227**: 9–14.

Iga, T. and Takabatake, I. (1983). Effect of some chemicals on the dark-induced melanosome aggregation of *Zacco* melanophores. Zool. Mag., **92**: 544.

Iga, T. and Takabatake, I. (1986). Local light stimulation of melanophores of a teleost, *Zacco temmincki.* J. Exp. Zool., **238**: 385–391.

Kamada, T. and Kinosita, H. (1944). Movement of granules in fish melanophores. Proc. Imp. Acad. Tokyo, **20**: 488–492.

Matthews, S. A. (1931). Observations on pigment migration within the fish melanophores. J. Exp. Zool., **58**: 471–486.

Nagahama, H. and Adachi, S. (1969). Movement of granules in isolated processes of fish melanophores. J. Sci. Hiroshima Univ., B (1), **22**: 1–8.

Weber, W. (1983). Photosensitivity of chromatophores. Am. Zool., **23**: 495–506.