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Histological Differences between the Color Patterns of Two Strains of the Compound Ascidian *Polyandrocarpa misakiensis*

TERUHISA ISHII[1], YASUNORI SAITO[1]
and YASUHO TANEDA[2]

[1]*Shimoda Marine Research Center, University of Tsukuba, Shimoda 5-10-1, Shizuoka 415, and* [2]*Department of Biology, Faculty of Education, Yokohama National University, Tokiwadai 156, Hodogaya-ku, Yokohama 240, Japan*

ABSTRACT—We examined histological differences between the color patterns of two strains of the compound styelid ascidian *Polyandrocarpa misakiensis*. Two strains in this species show different color patterns. In the white-spot strain, each zooid has a large white spot on its dorsal side between the branchial and atrial siphons. In the spotless strain, each zooid has a fine white band, instead of the circular spot, between the siphons. Thin-layer chromatography on their pigments showed that there is no qualitative difference between the two strains. Observation of cryostat sections revealed that the differences between the color patterns of the two strains were mainly based on the distribution of red pigments in the epidermal layer. The area that lacks red pigments forms a circular white spot in the white-spot strain or a fine white band in the spotless strain on the dorsal side. In this area, white pigment cells (a type of blood cell), which are distributed in the mesenchymal space, can be seen through the epidermal layer.

INTRODUCTION

In 1970, a new species of compound styelid ascidian was collected in Misaki, Kanagawa Prefecture, Japan, and it was named *Polyandrocarpa misakiensis* [12]. The zooids of this colony were red, and each zooid had a fine white band in the area between its branchial and atrial siphons. Later, another colony of the same species was collected in Shimoda, Shizuoka Prefecture. Each zooid of this colony had a large white spot, rather than a fine band, on its dorsal area between the branchial and atrial siphons. Based on these color patterns, the latter was named the white-spot strain (WSS), and the former was named the spotless strain (SLS; see Fig. 1; [2]).

Descendant clones of this species have arisen by asexual reproduction from the first zooids that were collected (unpublished data). Their phenotypes are very stable through asexual reproduction. In this species, a functional zooid can be regenerated from a small fragment of a zooid [9]. Therefore, much work has been done on morphogenesis during asexual reproduction and regeneration in this species [1–4, 7–11]. Furthermore, in this species, *situs inversus* zooids are easily induced experimentally from the normal zooids [7], and chimeric zooids can be produced by the fusion of body fragments derived from zooids of different strains [11].

Chimeras between two different strains have been used for the study on the regulation of morphogenesis, and in that study colormorph is used as a marker for identifying the origin of each component in a chimera [2, 3, 11]. Although the two colormorphs of *P. misakiensis* are very useful for such morphogentic studies, the basic knowledge about their color patterns is very little. For example, it is not known whether pigments are deposited in the free coelomocytes or epithelial cells, nor what causes the difference in color pattern between the two strains. In this work, we studied histologically of the color patterns of the

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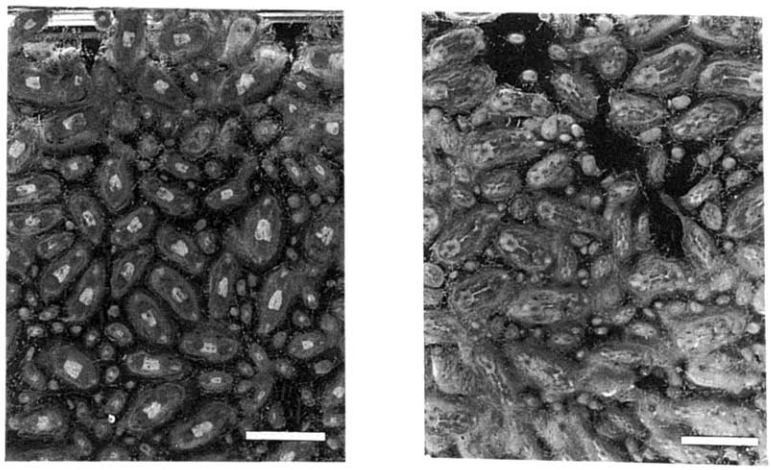


FIG. 1. Two phenotypes with different color patterns in *Polyandrocarpa misakiensis*. A: Blastozooids of the white-spot strain. Each zooid has a large, circular white spot on the dorsal side between the branchial and atrial siphons. B: Blastozooids of the spotless strain. Each zooid has a fine white band on the dorsal side between the branchial and atrial siphons. Scale bar, 10 mm.

SLS and WSS strains of *P. misakiensis*.

MATERIALS AND METHODS

Animals

Blastozooids of two strains, spotless strain (SLS) and white-spot strain (WSS) of *Polyandrocarpa misakiensis* were used. SLS and WSS were derived from colonies collected in Misaki and Shimoda, respectively. Both strains were reared on glass slides in culture boxes immersed in Nabeta Bay near Shimoda Marine Research Center, University of Tsukuba.

Thin-layer chromatography

For thin-layer chromatography, pigments were extracted with methanol from living animals of the SLS and WSS strains. The majority of the pigments was extracted with methanol immediately, and thereafter animals of the both strains became indistinguishable from each other. The extract was mixed with approximately an equal volume of diethylether. The pigments were transferred to the ether layer by shaking with a 10% NaCl solution. After 3 to 4 washing with the NaCl solution, two layers appeared. The bottom layer methanol and NaCl solution was removed, and the upper layer ether which contained the pigments, was used (see Yokohama [13]). The pigments of the SLS and WSS strains were compared by thin-layer chromatography on Kieselgel 60 (Merck) by the ascending method. The solvent was petroleum ether : acetone (7 : 3).

Histological preparation

Animals were fixed in 10% formalin in seawater. Thereafter, they were rinsed, dehydrated with alcohol, and embedded in paraffin. All specimens were cut transversally into sections of 10 μm thick and stained with Mayer's hematoxylin and 1% eosin. For observation of cryostat sections, fresh specimens were embedded in O.C.T. compound and were cut transversally into sections of 12 μm thick at -30°C to -25°C. The paraffin and cryostat sections were examined by light microscope.

Observation of live blood cells

The tunic and epidermis were surgically removed from animals and blood cells (or coelomocytes) were collected from an open wound with

Pasteur capillary pipettes. These were examined using a differential interference contrast microscope, a darkfield microscope, and a fluorescence microscope.

RESULTS

Thin-layer chromatography

Color pigments of the SLS and WSS strains were analyzed by thin-layer chromatography. As shown in each lane in Figure 2, two clear spots are found, with Rf values 0.49 (orange color, arrowheads) and 0.73 (red color, arrows). There are also some faint spots of pigments. However, there were no qualitative differences among the pigments of these two strains.

Histological observation

In *P. misakiensis,* a zooid consists of two envelopes of cell layers: the outer one is the epidermal layer and the inner one is the peribranchial wall (Fig. 3). The tunic matrix covers the epidermal layer, between the epidermal layer and the peribranchial wall is the mesenchymal space, where blood cells, muscles and the ganglion exist. The branchial sac, stomach, intestine, and endostyle are surrounded by the peribranchial wall. No morphological differences were found between WSS and SLS based on observations of paraffin sections because the pigments in the specimens were extracted by alcohol during the dehydration process. On the other hand, a clear difference was observed between them in cryostat sections (Figs. 4A, and 4B). In a WSS zooid, the red pigments in the epidermal layer are not present in the area of the large white circular spot between the branchial and atrial siphons. In contrast in a SLS zooid, red pigments are distributed throughout the entire epidermal layer except for the fine white band

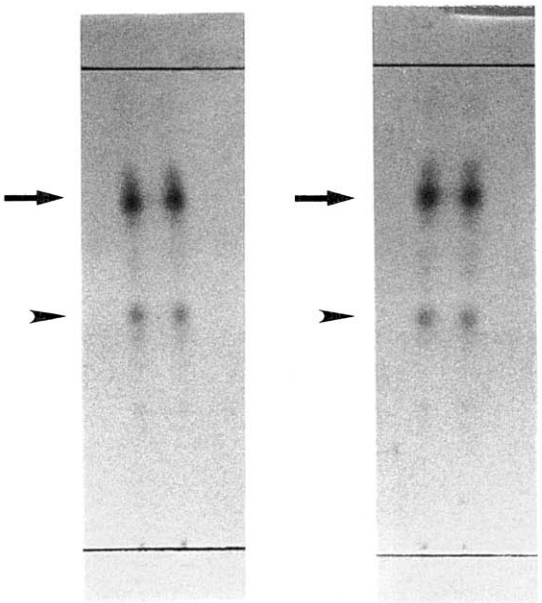


FIG. 2. Kieselgel thin-layer chromatograms of the pigments from two strains of *P. misakiensis.* The developing solvent is petroleum ether and acetone (7 : 3, v/v). A: White-spot strain. B: Spotless strain. Arrows: 0.73 Rf value (red color). Arrowheads: 0.49 Rf value (orange color).

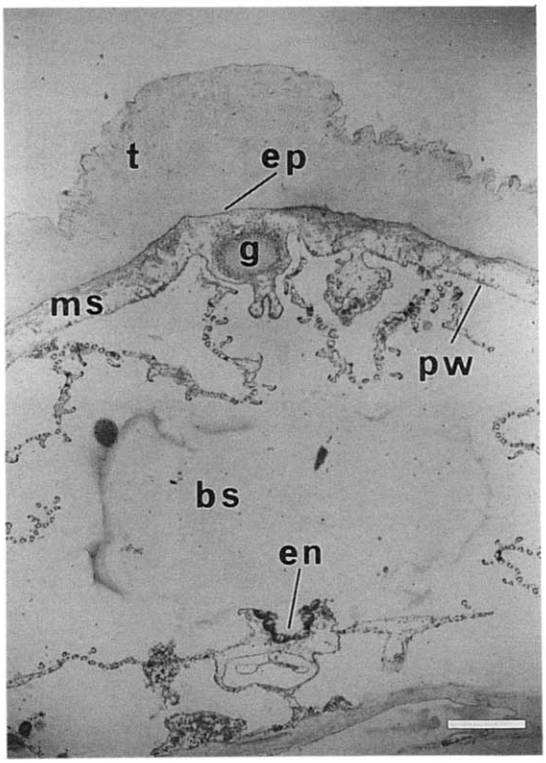
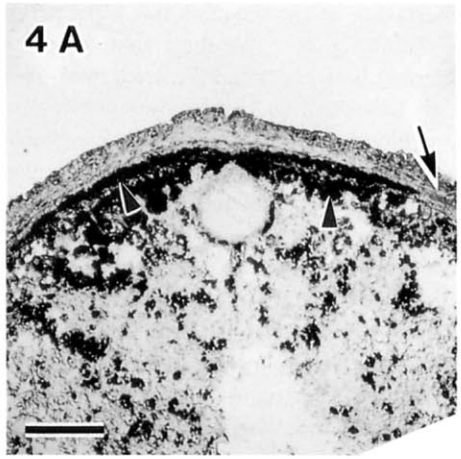
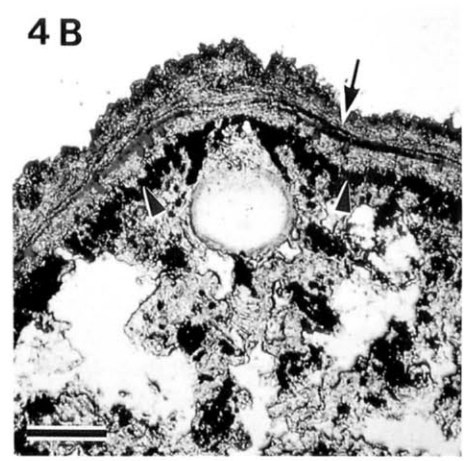
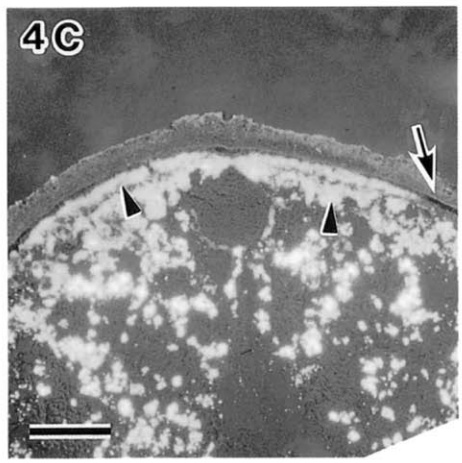
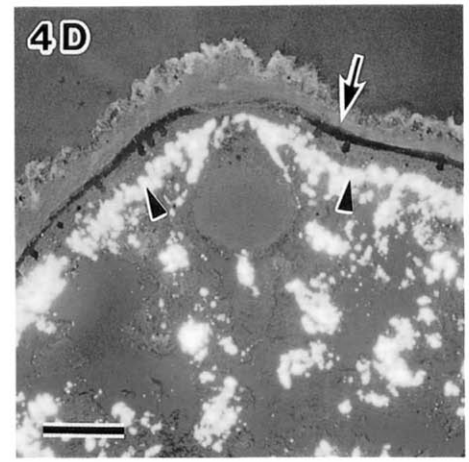


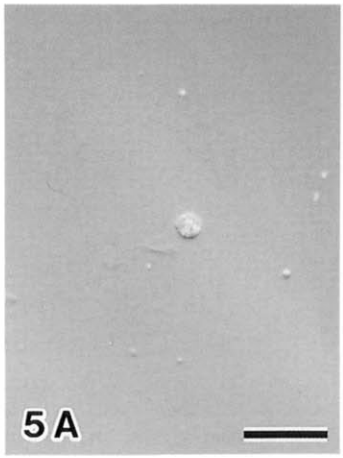
FIG. 3. Transverse section of a zooid of the spotless strain, stained with Mayer's hematoxylin and eosin. t:tunic, ep: epidermal layer, ms: mesenchymal space, pw: peribranchial wall, bs: branchial sac, en: endostyle. g: ganglion. Scale bar, 200 μm.

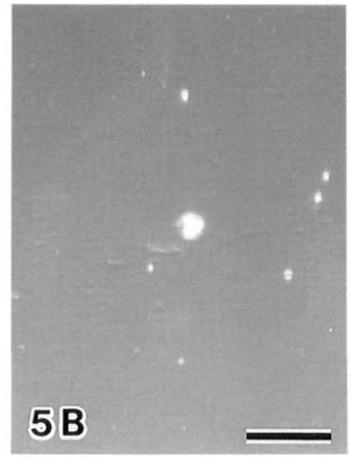












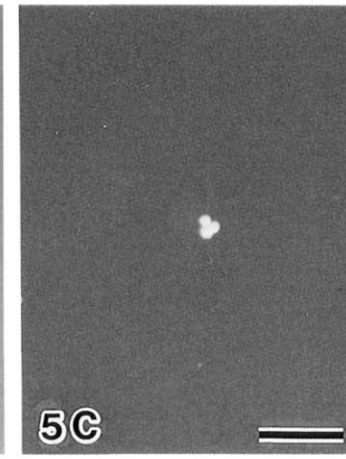


FIG. 5.

between the branchial and atrial siphons. The orange-colored pigments were distributed near the tunic surface of both strains (see Figs. 4A and 4B).

Observations of live blood cells

A piece of tissue containing the white pigmentation was removed from live animals of both strains and examined using differential interference contrast microscope, darkfield microscope, and fluorescence microscope. The white material was found in a type of blood cell in both strains (Fig. 5). These cells were about 10 μm in diameter, and the white material inside them was autofluorescent under blue-light excitation. The autofluorescence of this material was also observed in cryostat sections, and the cells containing the white material are present in the mesenchymal space (Figs. 4C and 4D). These blood cells are distributed in the mesenchymal space of zooids of each strain. In Figures 4A and 4B, these blood cells were observed as black because of observations under penetrating light. It seems that there are more of these cells in WSS than in SLS.

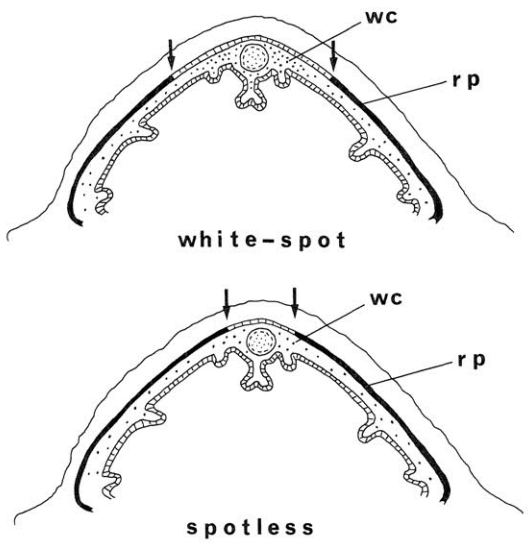


FIG. 6. Illustrations of transverse sections of WSS and SLS zooids. Top: In WSS red pigments in the epidermal layer are absent in the area between the two arrows. Bottom: In SLS red pigments occur up to the vicinity of the ganglion. wc: white cells, rp: red pigments.

DISCUSSION

Observation of paraffin sections could not reveal the origin of the difference between the color patterns. Observation of cryostat sections revealed that the red pigments were distributed in the epidermal layer. Kawamura [1] reported that in a palleal bud of this species orange-colored pigments were deposited in cells of the epidermal layer and the peribranchial wall. He examined cryostat sections of fixed palleal buds. We tried to use fixed adult zooids as well as living ones, but it was too difficult to get a good section in which the peribranchial wall appeared as a distinct cell layer because that wall is so thin in an adult zooid. If the peribranchial wall showed red and/or orange colors strongly in an adult zooid, we could detect that pigments as a layer. Therefore, the peribranchial wall in a adult zooid may have less pigments than that in a bud, although we can not comment obviously whether the peribranchial wall has orange-colored pigments in adult zooids. As the red pigments were clearly observed in cells of the epidermal layer, which lies outside the peribranchial wall, we concluded that the peribranchial wall did not contribute to the differences in color patterns.

In the tunic, there were the orange-colored pigments of both strains. The orange-colored pigments were present in the whole tunic even above the nonpigmented epidermal layer. However there were no differences between the two strains with respect to distribution of orange-colored pigments. Therefore the orange-colored pigments in the tunic surface did not contribute to

FIG. 4. Transverse cryostat sections of zooids of the two strains. A, C: White-spot strain. B, D: Spotless strain. A,B:Images under light microscope. C, D: Images under fluorescence microscope under blue-light excitation. Arrows indicate the red pigments in the epidermal layer. Arrowheads indicate white pigment cells in the mesenchymal space. Scale bars, 200 μm.

FIG. 5. A white pigment cell of *P. misakiensis*. A: Image under Nomarski optics using a differential interference contrast microscope. B: Image under darkfield microscope. C: Image under fluorescence microscope. Scale bars, 20 μm.

the differences in color patterns.

Many cells containing white pigments were found in the mesenchymal space of zooids of both strains. These pigments had a kind of crystal structure and showed Brownian movement. There were more white pigment cells in WSS than in SLS. In both WSS and SLS, many white pigment cells seemed to be trapped by fibrous substances in the mesenchymal space, especially in the area under the nonpigmented epidermal layer. That area was seen as a fine white band in SLS or as a large, circular white spot in WSS. As shown in Figure 6, the color of the white pigment cells in the mesenchymal space can be seen through the nonpigmented epidermal layer. Mukai [6] reported that an accumulation of pigment cells in the buds and zooids of *Botryllus primigenus* occurred in an area illuminated at a light intensity above a particular threshold. The accumulation of white pigment cells in *P. misakiensis* may also involve the intensity of light penetrating through the tunic and epidermal layer. Based on morphological features, these white pigment cells may be the same cells that Milanesi and Burighel [5] called nephrocytes. They reported that the nephrocyte was a large cell with numerous "granules" exhibiting Brownian movement in large vacuoles. If the white pigment cell of *P. misakiensis* is a nephrocyte, it seems reasonable that the white pigment cells increase in number as the animals become older, because it is thought that the nephrocyte stores metabolic products.

In summary, we have found that the differences between the color patterns of two strains of *P. misakiensis* depend on the distribution of red pigments in the epidermal layer. Thus, these two colormorphs can be used for chimera experiments, employing the epidermal cell marker.

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REFERENCES

1 Kawamura K (1982) Localized morphogenetic activity in the palleal bud of a polystyelid ascidian, *Polyandrocarpa misakiensis*. I. A histological study. Mem Fac Sci Kouchi Univ 3: 55–69

2 Kawamura K (1984) The mechanism of anteroposterior cell determination in ascidian palleal buds: A gap of positional values triggers posterior formation. Roux's Arch Dev Biol 193: 24–35

3 Kawamura K (1984) Morphogenetic tissue interactions during posterior commitment in palleal buds of the polystyelid ascidian, *Polyandrocarpa misakiensis*. Dev Biol 106: 379–388

4 Kawamura K, Watanabe H (1982) Pattern development in palleal buds of the polystyelid ascidian, *Polyandrocarpa misakiensis*: Bud grafting induces bilateral asymmetry conversion through polarity reversal. J Exp Zool 224: 145–156

5 Milanesi C, Burighel P (1978) Blood cell ultrastructure of the ascidian *Botryllus schlosseri*. I. Hemoblast, granulocyte, macrophage, morula cell and nephrocyte. Acta Zool (Stockh) 59: 135–147

6 Mukai H (1974) Photo-induced accumulation of pigment cells in a compound ascidian, *Botryllus primigenus*. Annot Zool Japonenses 47: 43–47

7 Oda T, Watanabe H (1982) Induction of malformed zooids and determination of polarity in palleal buds of the polystyelid ascidian, *Polyandrocarpa misakiensis*. J Exp Zool 220: 21–31

8 Oda T, Watanabe H (1986) Developmental pattern of colonies in the polystyelid ascidian, *Polyandrocarpa misakiensis*. Inter J Invert Rep and Dev 10: 187–199

9 Taneda Y (1985) Size regulation of regenerated organs in the compound ascidian, *Polyandrocarpa misakiensis*. J Exp Zool 233: 331–334

10 Taneda Y (1986) Double-zooid monsters derived from the fusion of two anterior halves in the compound ascidian, *Polyandrocarpa misakiensis*. Rep Manazuru Mar Lab for Sci Edu Fac Edu, Yokohama National Univ 3: 11–17

11 Taneda Y (1987) Possible role of atrial epithelium as a carrier of the positional information in the compound ascidian, *Polyandrocarpa misakiensis*. Rep Manazuru Mar Lab for Sci Edu Fac Edu, Yokohama National Univ 4: 1–9

12 Watanabe H, Tokioka T (1972) Two new species and one possibly new race of social styelids from Sagami Bay, with remarks on their life history, especially the mode of budding. Publ Seto Mar Lab 19: 327–345

13 Yokohama Y (1983) A xanthophyll characteristic of deep-water green algae lacking siphonaxanthin. Botanica Marina 26: 45–48