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Autonomous fluorescence of ascidian blood cells with special reference to identification of vanadocytes

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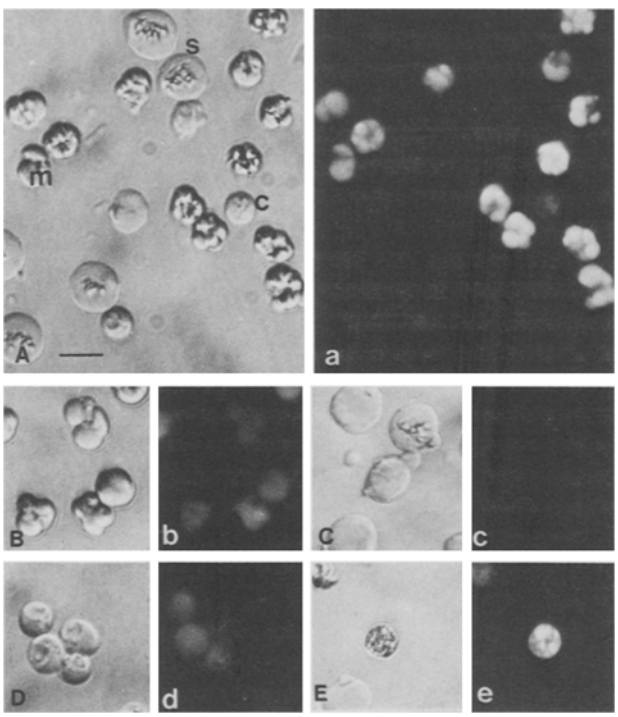
Summary. A tunichrome that has been suggested to be involved in the accumulation of vanadium ions in ascidian blood cells produces an autonomous fluorescence upon excitation with blue-violet light. However, we have found that signet ring cells, which contain large amounts of vanadium, do not fluoresce upon such excitation. The strongest fluorescence due to the tunichrome was observed in morula cells, which do not contain vanadium.
Key words. Ascidian; tunicate; vanadium; vanadocyte; blood cell, fluorescence; vanadium accumulation.

Ascidians belong to the family Ascidiidae and are known to concentrate vanadium ions from seawater to levels as high as one million times those in seawater[1,2]. They have between six and nine different types of blood cells. Among these various cells, the morula cell has been proposed to be the vanadocyte that contains a high level of vanadium ions[3-5]. A tunichrome with a possible involvement in the accumulation of vanadium has been isolated from ascidian blood cells[6]. This substance has been reported to emit a specific autonomous fluorescence after excitation with blue light[7]. The strongest fluorescence in blood cells that could be ascribed to the tunichrome was observed in the morula cells[8]. However, we have recently verified that the morula cell contains no vanadium, whereas the signet ring cell contains a very large amount of vanadium. Therefore, the actual vanadocyte is most likely the signet ring cell[9]. In this study, we examined the fluorescence emitted from the various blood cells in order to ascertain whether the fluorescent tunichrome takes part in the accumulation of vanadium.

Materials and methods. Ascidia ahodori were collected from the seawater tank of the Ushimado Marine Biological Station, Okayama University, Ushimado, Okayama, Japan. They were maintained in a temperature-controlled aquarium in our laboratory. Blood, drawn by making an incision through the lower part of the tunic and puncturing the mantle, was suspended in Ca^{2+}- and Mg^{2+}-free artificial seawater to avoid clotting. Living specimens were observed by multi-microspectrophotometry (MMSP) in an Olympus system which was equipped with a mercury lamp and a fluorescence optics unit.

Results and discussion. The tunichrome, which can be extracted from the ascidian blood cells of *A. nigra* and which has been proposed to be involved in the accumulation of vanadium ions[1,6,8], is known to fluoresce at 532 nm and 581 nm after excitation at 488 nm[7]. Blood cells that fluoresce under these conditions are, therefore, considered to be the sites of the tunichrome, as a result of studies of cellular fluorescence properties[7,8].

In the present experiment, the morula cells, the compartment cells, and the orange cells emitted autonomous fluorescence after excitation with blue-violet light composed of line spectra at 405 nm and 435 nm and wide spectrum at 490 nm (using a BG-12 filter). The fluorescence emitted from the morula cell was cut by O515 barrier filter, indicating that its wavelength was longer than 515 nm. The compartment cell



Blood cells of *Ascidia ahodori* were observed with a light (A) and a fluorescence microscope (a). The morula cell (m), signet ring cell (s) and compartment cell (c) were shown. The detailed observation was made in clusters of each blood celltype. The morula cells fluoresced strongly upon excitation with blue light (B and b) but signet ring cells did not fluoresce (C and c). The fluorescence was also observed in the compartment cells (D and d) and orange cell (E and e). Amoebocyte, granular amoebocyte and lymphocyte did not emit fluorescence (not shown). Photographs marked with (A–E) and (a–e) are those observed with a light and a fluorescence microscope, respectively. Scale bar indicates 10 μm.

and orange cell emitted a fluorescence longer than 475 nm and 530 nm, respectively. However, no fluorescence was detected from the signet ring cell, amoebocyte, granular amoebocyte or lymphocyte (fig.).

In contrast to earlier results[3-5], we have verified that the signet ring cell is the vanadocyte in *A. ahodori* after fractionation of cells on a Ficoll density gradient, ESR spectrometry and neutron activation analysis of vanadium[9]. Our conclusions are supported by evidence obtained by X-ray microanalysis[10-12].

If the tunichrome is involved in the accumulation of vanadium ions in ascidian blood cells, it would seem necessary that the vanadocyte (the signet ring cell) contain the tunichrome. However, no fluorescence due to the tunichrome was detected in the vanadocytes from *A. ahodori*.

We have extracted a vanadium-binding substance called vanadobin from the blood cells of *A. sydneiensis samea*. This substance is colorless and can maintain the vanadium ion in the vanadyl form (VO (IV)) even under aerobic conditions. Moreover, this substance has an affinity for exogenous vanadium ions (V) and contains a reducing sugar[13]. Taking all the above data into account, we suggest that it is not the tunichrome but rather the vanadobin that is the substance involved in the accumulation of vanadium ions from seawater in ascidian blood cells.

de Vincentiis and his co-worker first noted the emission of fluorescence from ascidian blood cells and the follicle and test cells of ascidian eggs[14,15]. Recently, it has been reconfirmed, in a detailed study, that ascidian eggs emit fluorescence from the myoplasmic region of the cytoplasm[16]. It will be of interest to determine the substance(s) from which such autonomous fluorescence is derived.

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