Identification of Lysosome-like Structures in a Unicellular Red Alga *Cyanidioschyzon merolae*

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Received August 12, 2005; accepted September 10, 2005

Summary *Cyanidioschyzon merolae* is considered to be a suitable model system for cytological studies of organelle proliferation and partitioning because these unicellular cells contain each organelle singly. However, lysosomes of *C. merolae* have yet to be identified. Polyphosphate have been known to be accumulated in the vacuoles of many microorganisms including alga. The cells stained with Neisser staining method, which visualizes polyphosphate bodies, showed the lysosome-like structures. They were about 500 nm in diameter and usually found as four copies in a single cell. The structures changed their localization dynamically during the cell cycle. During interphase, they were observed in the cytosol. At the beginning of mitosis, they moved over the mitochondria. During cytokinesis, they were inherited to the daughter cells almost evenly, suggesting the existence of mechanisms for the ordered partitioning.

Key words *Cyanidioschyzon merolae*, Lysosomes, Polyphosphate, Neisser staining method.

The unicellular red alga *Cyanidioschyzon merolae* is a small organism (2 μ m in diameter) that lives in sulfate-rich acidic hot springs (pH 1.5, 45°C). This alga has been used for studies of organelles, especially for proliferation and partitioning of organelles, because the cells contain only one nucleus, mitochondrion, chloroplast, microbody, and Golgi body (Kuroiwa *et al.* 1994, 1998). It has also tremendous advantages that divisions of organelles and cytokinesis can be highly synchronized by light/dark cycles (Suzuki *et al.* 1994) and the complete genome sequence (16.5 Mb) is also available (Matsuzaki *et al.* 2004).

Lysosomes are single membrane-bound, acidic organelles that contain dozens of acid hydrolases. In this meaning, including vacuoles, lysosomes have been found in all eukaryotes (Holtzman 1989). Various materials are digested in lysosomes and this activity is indispensable for cell maintenance, with more than 40 diseases of human known to be caused by deficiency of lysosomal enzymes (Winchester *et al.* 2000). Lysosomes, however, have not been identified in *C. merolae*.

The presence of polyphosphate granules in vacuoles was reported in *Chlorella pyrenoidosa* (Peverly and Adamec 1978), *Dunaliella salina* (Pick and Weiss 1991), and *Saccharomyces cerevisiae* (Vorisek *et al.* 1982) and *Neurospora crassa* (Cramer *et al.* 1980). The most extensively used method for determination of polyphosphate is based on the staining of cells by certain basic dyes such as toluidine blue, neutral red and methylene blue (Kulaev *et al.* 2004). Neisser staining method which uses methylene blue together with crystal violet and chrysoidine is one of effective technique because of its higher contrast between the granule and the cell (Bartholomew 1981, Serafim *et al.* 2002). In this study, in order to identify lysosomes, we stained cells with this method and found

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lysosome-like structures.

Materials and methods

C. merolae 10D-14 strain (Toda *et al.* 1998) was used in this study. Culture and synchronization were performed as described previously (Suzuki *et al.* 1994). Cells were maintained in swirling conical flasks in 2×Allen's media (pH 2.5) at 42°C under continuous light. For synchronization, cells were diluted to an OD₄₄₄ of 1.0 and cultured under 12 h light/12 h dark cycles with vigorous aeration.

Cells were dried on the slide glass and stained with the reagent containing 2% (v/v) ethanol, 2% (v/v) acetic acid, 0.04% (w/v) methylen blue and 0.04% (w/v) crystal violet for 10 sec, then washed with distilled water and stained with 0.3% (w/v) for 10 min and washed with distilled water. Images were viewed using an optical microscope (BX51; Olympus, Tokyo, Japan) with a 3CCD digital camera (C7780; Hamamatsu photonics, Hamamatsu, Japan)

Results

C. merolae cells contain one nucleus, one mitochondrion, and one chloroplast; the mitochondrion is localized just over the chloroplast. During mitosis, the chloroplast, the mitochondrion, the nucleus and microbody divide in this order (Suzuki *et al.* 1994, Miyagishima *et al.* 1999). The mitochondrion becomes V-shape before division (Fig. 1). Neisser staining showed brown-stained lysosome-like structures. They were about 500 nm in diameter and found as four copies in a single cell on average. During the cell cycle, they changed their location (Fig. 1). In the interphase, they were observed in the cytosol. In cells with the chloroplasts elongating laterally, they were seen in the cytosol and over the chloroplasts, *i.e.* over the mitochondria, suggesting that the structures changed their location during this stage. In cells with dividing chloroplasts, they were located over the mitochondria. In cells with divided chloroplasts, they were arranged in a V-shape, suggesting the association of granules with the mitochondria. They were often observed close to the dividing plane in cells at cytokinesis, consequently distributing to each daughter cell. They returned to the cytosol after cytokinesis. These results suggest the existence of mechanisms for ordered partitioning.

Discussion

Polyphosphate granules are known to exist in vacuoles of many microorganisms (Peeverly and Adamec 1978, Pick and Weiss 1991, Vorisek *et al.* 1982, Cramer *et al.* 1980). The function of polyphosphate has not fully determined but many functions, such as phosphate reserve, cation sequestration and storage, regulation of cytoplasmic pH, and regulation of osmolarity have been suggested (Pick and Weiss 1991, Weiss *et al.* 1991, Shirahama *et al.* 1996, reviewed in Kulaev *et al.* 2004). In *C. merole*, polyphosphate may also have similar functions.

It is suggested that the lysosome-like structures were partitioned by an ordered mechanism. In mammalian Madin-Darby canine kidney (MDCK) cells, ordered rather than precise partitioning of lysosomes has been suggested since a change in location was observed during the cell cycle: they were dispersed throughout the cytosol in the early prophase, but just after cytokinesis they were observed in a juxtanuclear position (Bergeland *et al.* 2001). Ordered partitioning has also been suggested in another cell type, Hep-2 cells (human larynx carcinoma cells) (Dunster *et al.* 2002). The underlying mechanisms for the partitioning have not revealed yet, but it seemed difficult to reveal since the animal cells contain hundreds of lysosomes. In contrast, the cell of *C. merolae* is considered to provide a simple model system for studying the partitioning mechanisms of lysosomes since the number of lysosome-like structures per cell was small and the cell division can be highly syn-

Fig. 1. Visualization of the lysosome-like structures. (a) Phase contrast-fluorescent microscopy images showing a mitotic cells after DAPI staining. *C. merolae* cells contain one nucleus, one mitochondrion, and one chloroplast; the mitochondrion is localized just over the chloroplast and becomes V-shape before division. (b–h) Cells stained with Neisser staining method. Brown lysosome-like structures were observed in every cells. (c) A cell in interphase. The lysosome-like structures were seen in the cytosol. (d) A cell with the elongating chloroplast. The lysosome-like structures were seen in the cytosol. (e) A cell with the elongating chloroplast at early stage of chloroplast division. The structures were seen over chloroplasts, *i.e.* over the mitochondria. (f) A cell with constricting chloroplast at middle stage of chloroplast division. The structures were seen over chloroplasts, *i.e.* over the mitochondria. (g) A cell with the divided chloroplasts. The lysosome-like structures arranged in a V-shape. (h) A cell at cytokinesis. The lysosome-like structures were observed close to the division plane of the cell. nu, nucleus; mt, mitochondrion; cp, chloroplast. Bars: 2 μ m.

chronized (Suzuki *et al.* 1994).

Acknowledgements

This work was supported by a Grant-in-Aid from the Japan Society for the Promotion of Science Fellows 11033 (to F. Y.) from the Ministry of Education Culture, Sports, Science and Technology of Japan; grants-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (nos. 17051029 and 14204078 to T. K.); a grant-in-aid from the Promotion of Basic Research Activities for Innovative Biosciences (ProBRAIN to T. K.).

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