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Selective resorption in nutritive phagocytes of the sea urchin *Anthocidaris crassispina*

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Summary

Phagocytic resorption during spermatogenesis was studied in the sea urchin *Anthocidaris crassispina*. Nutritive phagocytes in gonad absorbed both waste sperm cells and residual bodies discarded from maturing spermatids, and these materials were subsequently compartmented in heterophagosomes. Based on 180 heterophagosomes examined by transmission electron microscopy, over 99% of heterophagosomes contained either residual bodies or sperm cells only. Simultaneous resorption of sperm cells and residual bodies in a heterophagosome was uncommon, with only ~0.56% occurrence, suggesting that heterophagosomes have a selective resorption ability in nutritive phagocytes.

Keywords: Sea urchin, Sperm, Residual body, Resorption, Ultrastructure

Introduction

Resorption of waste sperm and residual bodies discarded from spermatids are very common in gametogenesis of metazoan animals (Kasyanov *et al*., 1980; Grier, 1981; Walker, 1982; Buckland-Nicks & Chia, 1986; Jørgensen & Lütsen, 1997; Sutovsky *et al*., 2001). Both the sperm cells and the residual bodies are taken up and resorbed by accessory cells (Roosen-Runge, 1977; Grier, 1981; Buckland-Nicks & Chia, 1986), which is thought to be important for gonad nutrition and gamete quality control (Kasyanov *et al*., 1980; Walker, 1982; Sutovsky *et al*., 2001). In marine invertebrates, ultrastructural information on phagocytic resorption of waste sperm and residual bodies is very limited. It has only been described for three representative prosobranch molluscs (Buckland-Nicks & Chia, 1986), and the heterophagosomes enclosed both waste sperm and residual bodies in the same vacuole. Our preliminary ultrastructural examination of sea urchin *Anthocidaris crassispina*, however, revealed that the sperm cells and residual bodies in nutritive phagocytes (i.e. accessory cells) were compartmented in separated heterophagic vacuoles. This might imply the presence of a selective resorption ability in nutritive phagocytes of *A. crassispina*. Thus, the aim of this work is to study more sperm- and residual-body-containing heterophagosomes to infer the presence of selective resorption phenomenon in the sea urchin *A. crassispina*.

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Materials and methods

Sea urchins, *A. crassispina* (Echinodermata, Echinoida), were collected from subtidal water at Kat O, Hong Kong. Upon arrival at the laboratory, specimens were dissected for their gonads. Testes were removed, cut into small pieces and fixed for 2 h in primary fixative (containing 1% tannic acid and 2.5% glutaraldehyde in 0.1 M cacodylate buffer with 8.55% sucrose, pH 7.5). Fixed tissues were washed (in decreasing concentrations of sucrose–buffer solutions and buffer), postfixed in 2% buffered OsO_4 for 2 h, rinsed in buffer and distilled water, dehydrated in an ethanol series and acetone, infiltrated and embedded in Spurr's resin. Ultrathin sections were stained with 2% alcoholic uranyl acetate and aqueous lead citrate before being examined with a JEOL 100SX transmission electron microscope (TEM) at 80 kV.

Gonads of three male individuals were studied. Three blocks from each individual were randomly chosen for sectioning. One technically perfect section from each block was examined at the TEM level. A total of nine sections from three individuals were studied. For each section, 20 heterophagosomes were randomly selected from nutritive phagocytes to study the occurrence and distribution of waste sperm and residual body inside the organelle. A total of 90 sperm-containing heterophagosomes and 90 residual-body-containing heterophagosomes were examined. Heterophagosomes containing both sperm cells and residual bodies were recorded.

Results

Similarly to other invertebrates, the prespawning testis of *A. crassispina* contained sperm cells and residual bodies discarded from spermatids (Au *et al.*, 1998). In sea urchins, the 'nutritive phagocytes' function as somatic accessory cells to phagocytose these cellular materials (Cavey & Märkel, 1994). In *A. crassispina*, nutritive phagocytes contained both waste sperms and residual bodies in the cytoplasm (Fig. 1A). Subsequent absorption of these materials by heterophagosomes appeared to be selective. All of the 90 sperm-digesting heterophagosomes (Fig. 1B) examined at the TEM level contained sperm materials only. Remnants of residual bodies have never been encountered inside any of these sperm-digesting heterophagosome. Similarly, the residual-body-containing heterophagosomes resorbed only residual bodies, except one that was found with a sperm nucleus inside (Fig. 1C). This equivalent to an occurrence of 0.56% (1/180), implying that the simultaneous resorption of these waste materials in heterophagosome is not significant.

Discussion

Our quantitative TEM data suggest that heterophagosomes in nutritive phagocytes of *A. crassispina* can distinguish and selectively absorb waste sperm or residual bodies for further digestion. In prosobranch molluscs, however, the heterophagosomes could not distinguish waste sperm from residual bodies, and resorbed them non-selectively in a heterophagosome (Buckland-Nicks & Chia, 1986). For the first time, we have reported selective resorption of waste materials in heterophagosomes of phagocytes. The category 'selective' have been previously applied to the recognition and elimination of defective spermatozoa in mammalian epididymis only (Roussel *et al.*, 1967; Sutovsky *et al.*, 2001).

The present report therefore reveals the existence of two patterns of resorption in phagocytes of invertebrates. Although selective resorption might suggest

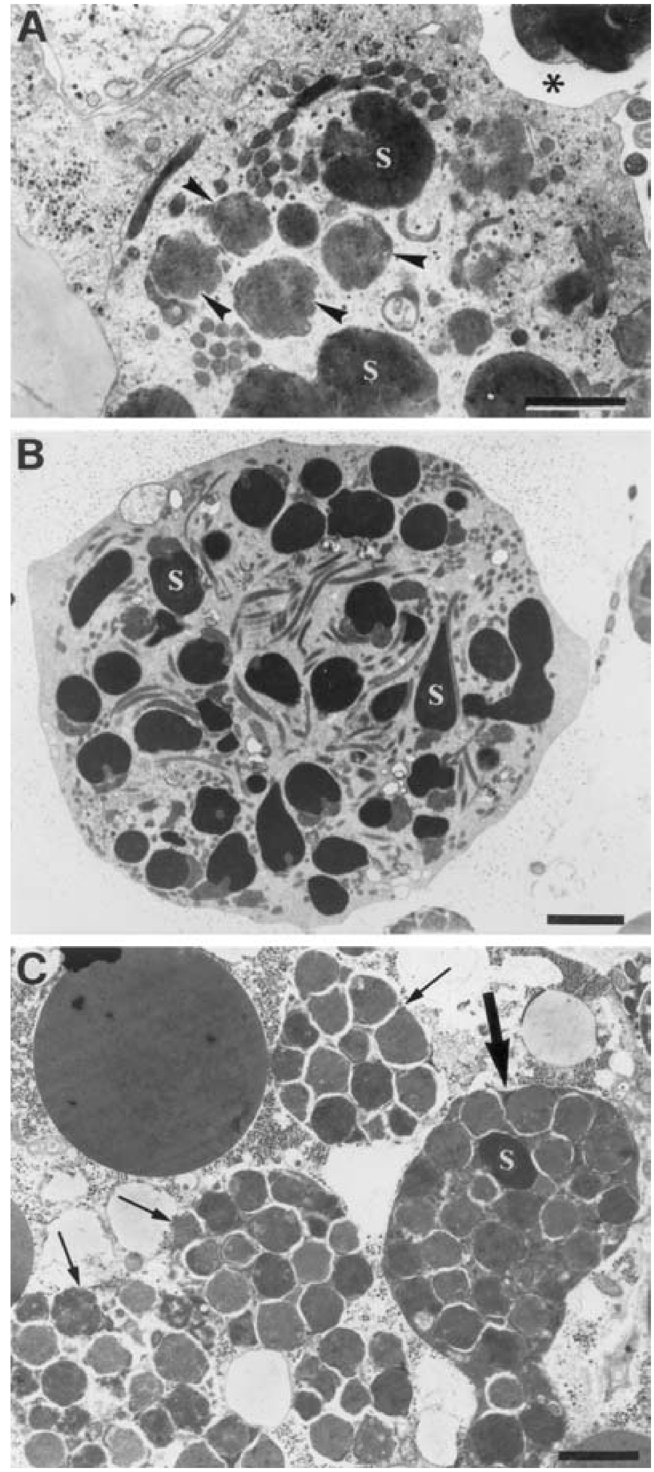

Figure 1 Resorption of sperm cells and residual bodies in nutritive phagocytes of sea urchin *Anthocidaris crassispina*. (A) Sperm (s) and residual bodies (arrowheads) in the cytoplasm of nutritive phagocyte; asterisks show the gonad cavity. (B) A heterophagosome containing sperm (s). (C) Residual-body-containing heterophagosomes (small arrows); big arrow indicates rare occurrence (0.56% chance) of sperm materials (s) inside a residual-body-containing heterophagosome. Scale bars represent 1 µm.

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a more effective way to process waste materials, it still remains uncertain whether selective resorption of heterophagosomes is evolutionarily more 'advanced' than non-selective resorption. Ultrastructural studies of more metazoan representatives are therefore required for verification.

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