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Degenerating epidermal cells in *Xenoturbella bocki* (phylum uncertain), Nemertodermatida and Acoela (Platyhelminthes)

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ABSTRACT. Species of the Nemertodermatida and Acoela have a mode of withdrawing and resorbing worn ciliated epidermal cells, through the epidermis and into the gastrodermal tissue. The ultrastructure of these degenerating epidermal cells has been described from very few species, especially concerning the Nemertodermatida. New data are presented here from *Nemertoderma bathycola*. Studies of the body wall of the enigmatic *Xenoturbella bocki* revealed darkened, shrunken cells with epidermal-type cilia enclosed in a vacuole. These cells were found in basal parts of the epidermis and within gastrodermal cells. The cells, or remains of them, were more dissolved in structure the further into the body they were observed. The process of resorbing ciliated epidermal cells in *X. bocki* is essentially similar to that found in the Acoela and Nemertodermatida, thus supporting the hypothesis of a close relationship.

KEY WORDS: Ultrastructure, Metazoan phylogeny, *Xenoturbella*, Platyhelminthes, Acoela, Nemertodermatida, epidermis.

INTRODUCTION

A characteristic feature of members of the platyhelminth taxa Nemertodermatida and Acoela is the mode of internally withdrawing and digesting worn or damaged ciliated epidermal cells. Already in the late 19th century, researchers studying acoels noted peculiar rounded cells with cilia enclosed in a vacuole (e.g. GEDDES, 1879). The nature of these cells has been a matter of debate for a long time. It took over a century, and the advent of electron microscopical methods, until the issue was settled (TYLER et al., 1989; EHLERS, 1992). Putative degenerating epidermal cells have been reported from light microscopical studies of many acoel species (see references in EHLERS, 1992), and from one nemertodermatid, i.e. *Meara stichopi* Westblad, 1949 (see Westblad´s description of the species, plate I, fig. 4). From electron microscopical studies (TEM), degenerating epidermal cells have been reported from three acoel species; *Symsagittifera roscoffensis* (Graff, 1891) by DOREY (1965), *Convoluta pulchra* Smith and Bush, 1991 by TYLER et al. (1989) and *Anaperus tvaerminnensis* (Luther, 1912) by EHLERS (1992). SMITH et al. (1986) mentioned an observation of degenerating epidermal cells in a nemertodermatid specimen similar to the species *Sterreria psammophila* (STERRER, 1970). However, the only TEM micrographs of degenerating epidermal cells in a nemertodermatid published to date are from *Meara stichopi* (LUNDIN & HENDELBERG, 1996).

The degenerating epidermal cells in acoels and nemertodermatids are withdrawn straight through the body wall into the digestive tissue. The actual mechanism responsible for this movement is yet unknown. Autolysis of the cell body often begins already at the level of the epidermal surface, when the cells shrink or are being compressed. The basal portion of the cell with the nucleus becomes compressed at an early stage and is thus hard to recognize. The epidermal cilia and the rigid interconnected ciliary rootlet system withstand degeneration for a longer time (for nemertodermatids this also includes the thick terminal web) and are discernible even within the digestive tissue. When the degenerating cell sinks into the epidermis, the cilia become enclosed in a vacuole. Inside the vacuole the cilia retain some ability to move, at least in the acoels, hence the earlier designation “pulsatile bodies” of degenerating epidermal cells. The sunken degenerating cells most often attain a tilted position, more or less perpendicular to the vertical orientation of the ordinary ciliated cells in the epidermis. The degenerating cells

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always lie in between other cells, without anchoring structures to the membranes of the surrounding cells. When the degenerating cells reach the digestive tissue, the vacuole around the cilia eventually becomes fragmented and disappears, followed by an accelerated autolysis of the cilia.

Degenerating epidermal cells have been suggested as one of the few possible synapomorphic characters for the Nemertodermatida and Acoela (LUNDIN, 1997), supporting the hypothesis that the two groups are sistertaxa forming the Acoelomorpha (sensu EHLERS, 1985). Some of the other possible synapomorphies concern characters of the epidermal ciliary apparatus. However, several of the characters of the ciliary apparatus are also present in *Xenoturbella bocki* Westblad, 1949, an enigmatic vermiform animal whose systematic affinities are disputed (see LUNDIN, 1998, ISRAELSSON, 1999, and references therein). The present study describes degenerating epidermal cells in *Xenoturbella bocki*. Also presented here are the results of a search for degenerating epidermal cells in two species of the genus *Nemertoderma*.

MATERIAL AND METHODS

Specimens of *Nemertoderma bathycola* Steinböck, 1931 and *Nemertoderma westbladi* Steinböck, 1938 were collected in 1997 and 1998 near Kristineberg Marine Research Station on the Swedish west coast. The specimens were fixed with 3% glutaraldehyde in 0.2 M sodium cacodylate buffer, followed by postfixation in 1% osmium tetroxide and further preparation with standard methods for transmission electron microscopy. Embedded material of *Xenoturbella bocki* Westblad, 1949, collected in 1976 near Kristineberg Marine Research Station, was kindly provided by Dr. Jan Hendelberg. For the examinations a Zeiss CEM 902 was used, located at the EM Department of the Anatomical Institution, Göteborg University.

ABBREVIATIONS

c	cilia
cr	ciliary rootlets
f	fibrillar extracellular matrix
m	mitochondria
n	nucleus
t	terminal web

RESULTS

In the specimens of *Nemertoderma bathycola* sunken, dark ciliated epidermal cells were very scarce in the epidermis and gastrodermis (Fig. 1 A). These cells correspond well to degenerating epidermal cells reported from other species of the Nemertodermatida and Acoela. No degenerating epidermal cells were found in the studied specimens of *Nemertoderma westbladi.* The degenerating cells of *N. bathycola* were tilted and situated in between other cells, without any visible attachment structures to the lateral cell membranes of the surrounding cells. The basal parts (with the nucleus) of the degenerating cells were greatly reduced in size. The ciliary rootlets of these cells were embedded in a compressed, electron-dense terminal web closely associated with an aggregation of mitochondria beneath it (Fig. 1 A). In the epidermis, the cilia of the degenerating cells were enclosed in a large vacuole. In degenerating cells observed within the gastrodermis, the vacuole around the cilia appeared fragmented (Fig. 1 A, arrowheads), and the cilia showed indications of autolysis.

In *Xenoturbella bocki,* a few sunken, tilted ciliated epidermal cells were observed, occurring in the basal part of the epidermis and of the gastrodermis (Fig. 1 B-D). These cells had cilia and ciliary rootlets of the same type as those found on the epidermal surface. The cilia were enclosed in a large vacuole. In such degenerating cells located basiepidermally (Fig. 1 B, C) there was a central electron-dense region, with what appeared like a compressed cytoplasm and a nucleus. Surrounding the central region was a peripheral area, with less dense cytoplasm and scattered mitochondria. The degenerating cells were situated in between other epidermal cells, without visible cell junctions to the surrounding cells. The intercellular space between the cells was filled with the fibrillar components of the extracellular matrix. In the gastrodermis, remnants of the degenerating cells were found only within large gastrodermal cells (Fig. 1 D). The engulfed degenerating cells showed a high degree of autolysis, and the former vacuole around the ciliary axonemes had vanished. The axonemes were electron-dense, and numbers and patterns of the ciliary axonemes varied greatly as a result of the breakdown of ciliary structure. In the most advanced stages of degeneration observed, the bulk of the degenerating cells began to take on a granulated appearance (as partly visible in Fig. 1 D, arrowheads).

DISCUSSION

The structure of degenerating epidermal cells in specimens of *Nemertoderma bathycola* is similar to that of the nemertodermatid *Meara stichopi* (cf. LUNDIN & HENDELBERG, 1996), with a thick terminal web underlined by an aggregation of mitochondria. Note that in *M. stichopi* the cilia are often, but not always, cast off at the epidermal surface before the cell begins to be withdrawn (LUNDIN & HENDELBERG, 1996). Where the cilia are retained, the degenerating epidermal cells of *M. stichopi* appear almost identical to those of *N. bathycola*. Concerning the apparent absence of degenerating epidermal cells in *N. westbladi*, the process of replacing epidermal cells is not a permanent one, and when present the degenerating cells may nevertheless be scarce. They are often absent from many individuals of acoel and nemertodermatid species from which degenerating epidermal cells have been reported (cf. EHLERS, 1992; own observations of *M. stichopi*). Degenerating cells would probably have

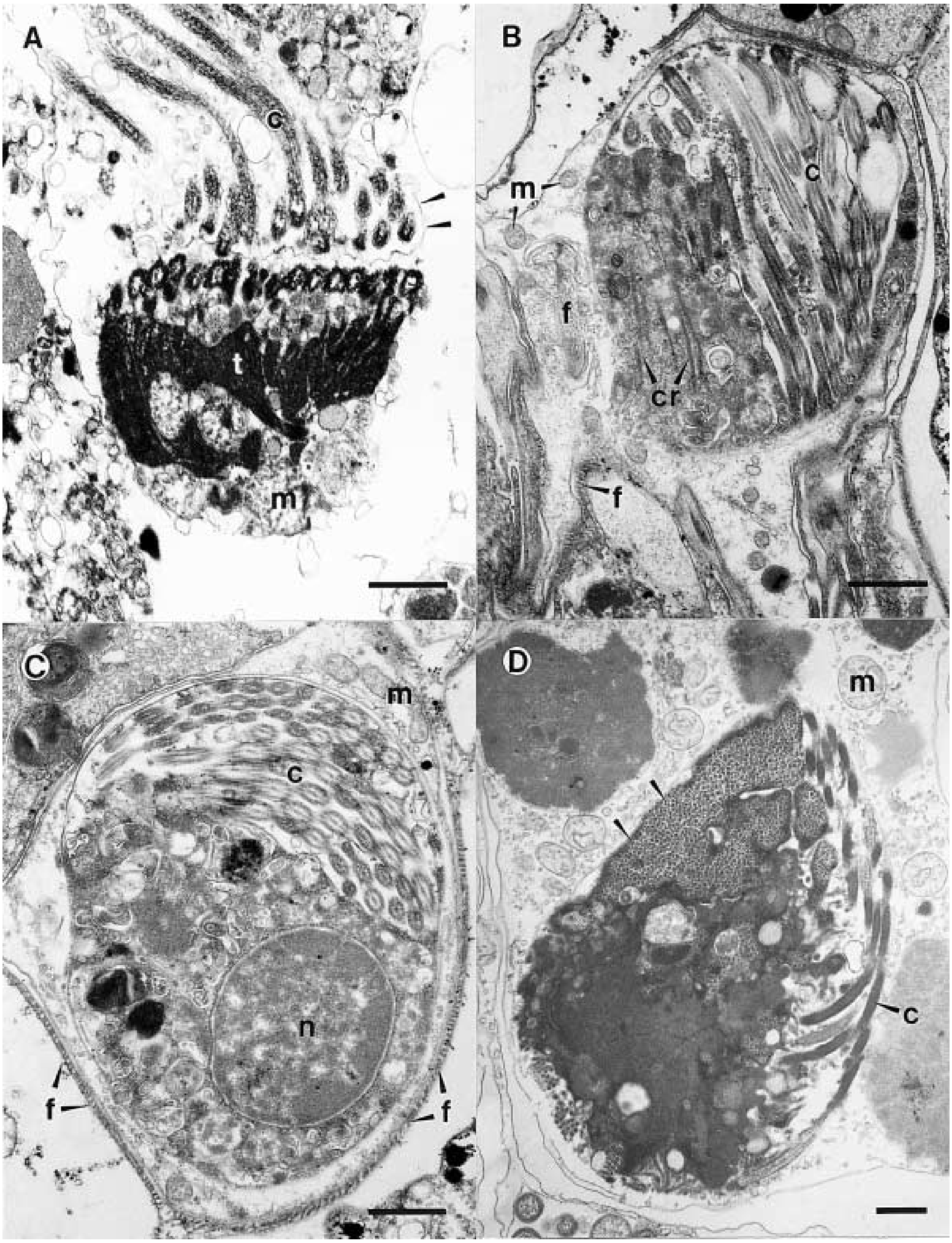

Fig. 1. – Degenerating ciliated epidermal cells of *Nemertoderma bathycola* (A) and *Xenoturbella bocki* (B-D). – A. *N. bathycola*. Degenerating epidermal cell located in the gastrodermal tissue, at 100 µm from the epidermal surface. The cell lies between the gastrodermal cells. The vacuole enclosing the cilia has been fragmented (arrowheads) and the cilia are in a late stage of autolysis. The terminal web (*t*) is underlain by an aggregation of mitochondria (*m*). – B. *X. bocki*. Degenerating epidermal cell located in the basal part of the epidermis, about 150 µm from the epidermal surface. The cilia are enclosed in a vacuole. Note ciliary rootlets (*cr*). Possibly the degenerating cell is enclosed in another cell, as indicated by surrounding unaltered cytoplasmic space with mitochondria (*m*). Fibrillar components of the basal matrix can be seen at the periphery (*f*). – C. *X. bocki*. Basiepidermally located degenerating epidermal cell. Note nucleus (*n*). Fibrillar components of the basal matrix surround most of the cell. – D. *X. bocki.* Degenerating epidermal cells found within a large gastrodermal cell in the gastrodermal tissue. The vacuole around the cilia has vanished and the cilia are darkened from extensive autolysis. The bulk of the degenerating cell has begun to attain a granular appearance (arrowheads) as a final step of the autolysis. TEM. All scale bars: 1 µm.

been found in *N. westbladi* if a larger number of specimens had been investigated. The presence of degenerating epidermal cells in specimens of *N. westbladi* has been confirmed by Ulrich Ehlers (personal communication).

The electron-lucent peripheral areas of the cytoplasm (Fig. 1 B, C) could perhaps imply that the degenerating cells are engulfed by other cells already in the epidermis, but no other structures indicating this were observed. So far, no explanation can be given for the mechanism behind the inward migration of the degenerating cells, or how they pass through the basal lamina in *X. bocki*. In the nemertodermatids and acoels, the extracellular matrix is strongly reduced or absent. EHLERS (1992) discussed the possibility that the mode of internal withdrawal and digestion of epidermal cells could occur in other flatworm groups (rhabditophorans and catenulids) and metazoans as well, but have not yet been detected because the epidermal cells become too strongly diminished when they pass through the basiepidermal layer of extracellular matrix. The presence of degenerating cells in the gastrodermis of *X. bocki,* despite a well-developed basal lamina, speaks against this notion. Based on available ultrastructural data, the mode of withdrawal and digestion of epidermal cells probably represents a unique, homologous character for species of the Nemertodermatida, Acoela and *X. bocki*.

Apart from the degenerating epidermal cells, acoels, nemertodermatids and *X. bocki* also share several distinct characters of the complex ciliary apparatus, suggesting a close relationship. On the other hand, there are molecular and structural indications that *X. bocki* is a strongly modified protobranch bivalve (NORÉN & JONDELIUS, 1997; ISRAELSSON, 1997, 1999). No specific similarities with the ciliary apparatus of *X. bocki* have, however, been found in the Protobranchia, nor in any other of the major molluscan taxa (LUNDIN & SCHANDER, 1999, in press a, b, unpublished studies). A recent study of Bock´s and Westblad´s old microscope slides revealed no morphological trace that would support a bivalvian nature or the existence of a pericalymma-like larva of *X. bocki* (Gerhard Hazsprunar, personal communication).

The apparent incongruence between different character sets concerning the phylogenetic affinities of *Xenoturbella* could be resolved by the hypothesis given recent support by Hox-gene data (BALAVOINE, 1998), that the flatworms arose by progenesis from an interstitial larva of a coelomate with a biphasic life-cycle (RIEGER, 1994; TYLER, 2000). This ancestral coelomate could possibly have been related to *X. bocki*. A problem with this comparison is that the assumed larva of *X. bocki* is relatively advanced in structure. If *X. bocki* really undergoes a far-reaching structural reduction during its development, the evolutionary events leading to this state are nevertheless enigmatic. Usually such a reduction is caused by small size or parasitic life-style, neither of which applies to *X. bocki*. Maybe there were intermediate interstitial (i.e. flatworm-like) larval stages that now are lost.

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