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Further observation on the early regenerates after fission in the planarian *Dugesia japonica*

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ABSTRACT. In addition to possessing remarkable regeneration powers, many species of planarians reproduce asexually by fission. Since fission takes place at the post-pharyngeal region in most cases, we amputated this body portion of intact animals and compared their regeneration process with that of fission fragments.

The fission fragments formed a well-defined blastema in the early regenerates, and the number of blastema-forming cells of the regenerates was larger than that of the amputated ones. When we compared the early regenerates from posterior fragments with those from anterior ones, it was clearly confirmed that posterior fragments invariably regenerated more rapidly. The present observations on the pre-fissioning planarians suggest that the rapid development of the early blastema is induced by fixed parenchyma cells at the preparatory parenchymal region for fissioning.

KEY WORDS: Platyhelminthes, planarian, fission, regeneration, differentiation.

INTRODUCTION

Triclads, especially the asexual strains of freshwater planarians, reproduce by fission (VOWINCKEL, 1970; GRASSO & BENAZZI, 1973; MORITA & BEST, 1984). Then each fission fragment regenerates the appropriate missing part to yield complete worms (BEST et al., 1969; LENDER, 1974; NENTWIG, 1978; BAGUÑÀ, 1998). By observing the fission fragments using light and electron microscopes, it is certainly possible to elucidate natural processes of regeneration more clearly. However, there is only little information about the regeneration of post-fissioned planarians (KENK, 1937; NENTWIG, 1978; MEAD, 1985; HORI & KISHIDA, 1998). It is uncertain if there is any similarity between the processes leading to formation of the missing body part in amputated animals and fission fragments. Observations on the fission fragments are expected to clarify details of cell behavior during regeneration because fissioning is never accompanied by mechanical tissue damage.

Our previous study has confirmed the occurrence of the preparatory stage before fissioning (HORI & KISHIDA, 1998). If any difference regarding the regeneration process is present between amputated animals and fission fragments, it is expected to appear as cell behavior within early regenerates. In this report, we offer information regarding some cytological changes occurring at the preparatory region, and then make comparison of early regenerates obtained from amputated animals and fission fragments.

MATERIAL AND METHODS

Asexual strains of the freshwater planarian *Dugesia japonica* were employed in this study. Worms were fed beef liver once a week. During all the experiments they were maintained in pond water at 18°C. Fissioning can be induced by increasing the level of some conditions such as temperature, population density, illumination, and by decapitation (CHILD, 1932; OKUGAWA & KAWAKATSU, 1956; BEST et al., 1969; VOWNICKEL, 1970; MORITA & BEST, 1984). To obtain a large number of fission fragments, well-fed animals (about 10 mm in length) were decapitated and placed in isolation (one animal/small dish). Then fissioning occurs most frequently at the post-pharyngeal region (BRØNDSTED, 1969; LENDER, 1974; NENTWIG & SCHAUBLE, 1974). For examining the preparatory zone of fissioning, we observed the post-pharyngeal portion of the animals three days after decapitation. Regenerates were obtained from two groups of the specimens; one was from fission fragments 20 and 24 hours

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after fissioning, and the other was from animals that had been amputated at their post-pharyngeal region 20 and 24 hours before. Pieces including the tail part are referred to as piece A, and those including the head part are referred to as piece P in this report.

All the specimens were fixed and prepared for light and electron microscopy as described in our previous report (HORI & KISHIDA, 1998).

RESULTS

Post-pharyngeal region

Comparisons were made between post-pharyngeal regions of the intact and decapitated animals. The intact region showed normal histological aspects in the epidermis and parenchymal tissues (Fig. 1a). Ultrastructurally, the parenchyma was occupied with various cells such as fixed parenchyma cells, regenerative or undifferentiated cells, gland cells, muscle fibers and protonephridial cells. These cells were arranged so intimately that the intercellular matrix was very small.

Some of the decapitated animals showed histological and cytological changes suggestive of preparation for fissioning. They occurred at the post-pharyngeal region as a lower dense zone between intestinal tracts (Fig. 1b). In the middle area, numerous fixed parenchyma cells were observed extending their cytoplasmic processes. Regenerative cells were located at the subepidermal region. Occasionally the epidermis was invaded by the fixed parenchyma cells so that some of the epidermal cells became degenerative.

Regeneration after fissioning

It was common to observe the extensive blastema in piece A of 20 h regenerates (Fig. 2a). Migrating regenerative cells were easily identified. Two or three layers of fixed parenchyma cells were built up in the subepidermal region (Fig. 4). Their cytoplasm had a large number of

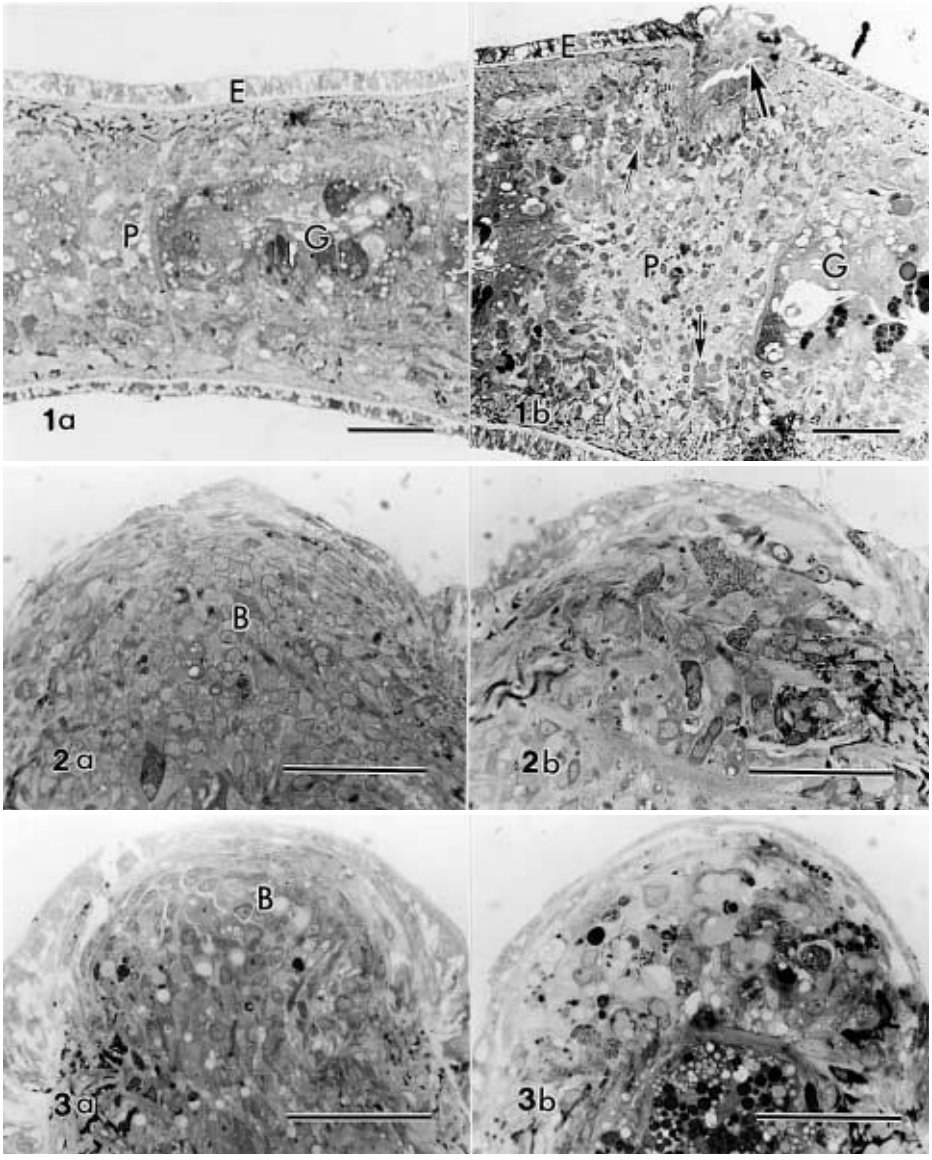

Fig. 1a. – Light micrograph of the post-pharyngeal region of the normal planarian. E; epidermis, P; parenchymal tissue, G; gastrodermis. Scale bar = 50 μm.

Fig. 1b. – Light micrograph of the post-pharyngeal region of the pre-fissioning planarian. Large arrow indicates the disorganization of the epidermis. Small arrows indicate an aggregation of regenerative cells. E; epidermis, P; parenchymal tissue, G; gastrodermis. Scale bar = 50 μm.

Fig. 2a. – Light micrograph of the regenerate, 20 h after fission; piece A. B; blastema. Scale bar = 50 μm.

Fig. 2b. – Light micrograph of the regenerates, 20 h after fission; piece P. Scale bar = 50 μm.

Fig. 3a. – Light micrograph of the regenerate, 20 h after amputation; piece A. B; blastema. Scale bar = 50 μm.

Fig. 3b. – Light micrograph of the regenerate, 20 h after amputation; piece P. Scale bar = 50 μm.

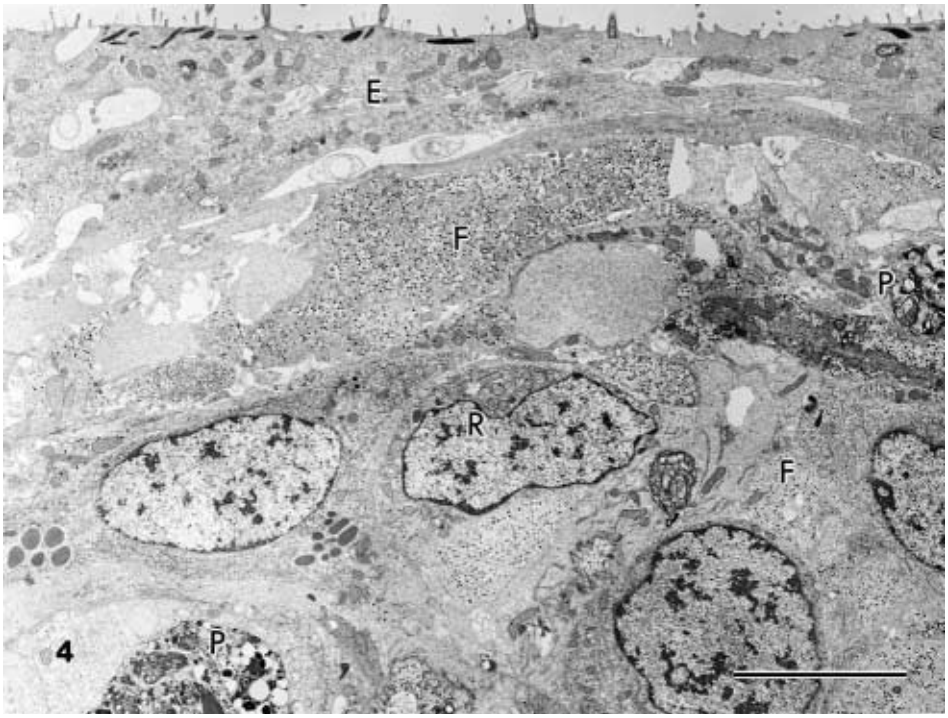

Fig. 4. – Electron micrograph of the regenerate, 24 h after fission. E; epidermal cell, F; fixed parenchymal cells, R; regenerative cell, P; phagosomes. Scale bar = 5 μm.

glycogen granules. Large phagosomes were occasionally observed containing cell debris. Regenerative cells appeared in the middle blastemal area in greater quantity than in the subepidermal region. In piece P, the blastema was not clearly formed (Fig. 2b). The wound zone consisted of several regenerative cells and differentiated cells from the uninjured tissue area. Occasionally amorphous substances were observed at the subepidermal extra cellular space.

Regeneration after amputation

A fairly extensive regeneration blastema was formed in piece A (Fig. 3a). It was mainly composed of regenerative cells. Fixed parenchyma cells extended their cytoplasmic processes among these regenerative cells and participated in phagocytosing cell debris. The regeneration blastema was poorly formed in piece P (Fig. 3b). Only a few regenerative cells were observed in the regenerate. Intercellular space at the wound region was dilated and filled with amorphous substances.

Quantitative data

In order to compare development of the blastema from the regenerates, the cell number per 100 μm^2 of the blastema was calculated on the light micrographs. The data are summarized in Table 1. It is of interest to note that cell density of the blastemal zones was much higher in piece A than piece P. It was also obvious that the blastema developed more rapidly in the fission fragments than in the amputated animals.

TABLE 1

Number of cells per 100 μm^2 of the blastema

REGENERATES		20hr	24hr
FISSION	Piece A	1.14	1.52
	Piece P	0.63	0.87
AMPUTATION	Piece A	1.01	1.20
	Piece P	0.40	0.80

DISCUSSION

Preparation for fissioning

No morphologically differentiated plane of fracture is evident before the onset of fission (Best et al., 1969; Moraczewski, 1977). However, histologically the first recognizable indication of the actual fission is evident at the post-pharyngeal region (Pedersen, 1958; Peter, 1995; Hori & Kishida, 1998). In *Microstomum lineare*, it appears as an occurrence of nerve fibers, muscle fibers and activation of cell reproduction (Reuter & Palmberg, 1983). The present observation shows the portion having low histological staining intensity at the post-pharyngeal region of the decapitated animals. The observation suggests that preparation for fissioning occurs both in the epidermis and the parenchymal tissue. Aggregation of the regenerative cells in the subepidermal region seems to provide for the forthcoming regeneration.

Regeneration after fissioning

Some authors have reported that fission fragments form no blastema because the regeneration after fissioning could be carried out not by epimorphosis but by morphallaxis (KENK, 1937; NENTWIG, 1978). In the present study, however, each fission fragment formed a well-defined blastema though the spatial patterns of cell distribution are not identical between early regenerates of fission fragments and amputated animals. In particular, development of the blastema is crucial in piece A.

The organization of structurally unique fixed parenchyma cells in the pre-fissioning zone is required for a rapid formation of the blastema. At the onset of fissioning, these cells are first organized into functional cell layers beneath the degenerating epidermis. Glycogen granules in their cytoplasm are possibly used for energetic support of regeneration (MORITA, 1995). In the case of regeneration after artificial amputation, it takes a length of time to recover from the mechanical damage. MORITA (1991; 1995) has observed that the fixed parenchyma cells or reticular cells arrive at the wound surface very rapidly, and then phagocytose cell debris of degenerative cells in the decapitated planarians. The present study suggests another role of these cells in the regeneration of fission fragments.

During epitheliogenesis of the wound epidermis, we have observed some extent of amorphous substances at the subepidermal extracellular space (HORI, 1979). These substances seem to be the precursor of the basement membrane. Localized deposit of amorphous substances at the extracellular space indicates rapid reorganization of the epidermal basement membrane in the fission fragments.

The difference in cell density within the blastema between pieces A and P is due to some factors such as the difference of tissue organization with and without a pharynx, cell numbers of pre-existing undifferentiated cells, and positional pattern of nerve cords.

Nerve cords are often observed penetrating the blastemal area during regeneration (NENTWIG, 1978). It is believed that the nervous system of turbellarians could play a regulatory role in cell behavior and proliferation (BEST et al., 1969; LENDER, 1974; PIGON et al., 1974; REUTER and PALMBERG, 1983; MORITA & BEST, 1984; BAUTZ & SCHILT, 1986; BAGUÑÀ et al., 1989). However, it is unknown how the nervous system relates to the positional difference in cellular activity for the regeneration. Understanding of the control mechanism of the nervous system is expected to elucidate the difference of regeneration between pieces A and P.

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