# Scanning Electron Microscopic Study of the Regeneration of Taste Buds and Dermal Papillae in the Barbels of *Corydoras aeneus*

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## INTRODUCTION

Taste cells are maintained in the epithelium under the influence of the nervous system[1-8]. Initial interactions between epithelial cells and nerve fibers are observed during the development of taste organs in mammals[9-11], chickens[12] and frogs[13,14]. Similar interactions are also observed during the regeneration of taste organs in fish[15-17]. However, it is not clear how nerve fibers penetrate into taste buds through the basal lamina during regeneration.

Recently, it became possible to observe the surface of the basal lamina by removal of the epithelium[18,19]. A wide area of the basal lamina can be observed under the scanning electron microscope after treatment of fixed samples with trypsin[20]. Thus, we attempted to examine in detail the penetration of nerve fibers and associated changes during the development of taste organs using this technique. Moreover, we anticipated that observations of the surface of basal lamina would reveal the process of formation of the dermal papillae.

An inner view of the tissue or cells also can be clearly visualized under the scanning electron microscope after preparation of samples by a freeze-cracking technique[21,22]. Observations of inner regions of the regenerating epithelium by scanning electron microscopy should also reveal the initial processes of regeneration of taste cells.

In this study, the initial stages of regeneration of the taste organs in the fish, Corydoras aeneus, were investigated by scanning electron microscopy with emphasis on an examination of the surface of basal lamina and the inner regions of the epithelium.

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## MATERIALS AND METHODS

The lower halves of the barbels of Corydoras aeneus were cut off under anesthesia with FA 100 (4-ally-2-methoxy-phenol; Tanabe Pharmaceutical Co., Osaka, Japan) and then the fish were reared at 22°C. Regenerating barbels were removed by cutting them off at the base of the barbels under anesthesia and the samples were fixed with a 10% solution of formalin. For freeze-cracking, the fixed barbels were frozen in liquid nitrogen and cracked along their longitudinal axes. For the observation of the surface of the basal lamina in the regenerating barbels, barbels that had been fixed for 30 min were treated at 4°C for 24 h with 0.25% solution of trypsin[20]. Gentle pipetting resulted in removal of epithelial cells. After refixation with 10% formalin, both sets of samples were dehydrated through a graded series of alcohols, dried with a critical-point dryer and coated with gold in vacuo for observation under a Hitachi S-430 Scanning Electron Microscope (Hitachi Ltd., Tokyo, Japan).

## RESULTS

*Corydoras aeneus* has numerous taste buds in its barbels. Taste buds are visible as protuberances of the epithelium (Fig. 1A). Three days after the end halves of the barbels had been removed, the cut surface of the barbels began to be covered with epithelial cells (Fig. 2A). The regenerating portion in the barbels initially was devoid of the pigmented cells that were usually present underneath the epithelium (Fig. 1B). The surface of the regenerating portion of the barbels was smooth 1 week after half of the barbel had been cut off (Fig. 2B), and the protuberances on the regenerating barbels were formed after 9 days (Fig. 2C). Numerous protrusions of cell processes of receptor cells were observed at the top of taste buds (Fig. 2D). The degree of regeneration dif-

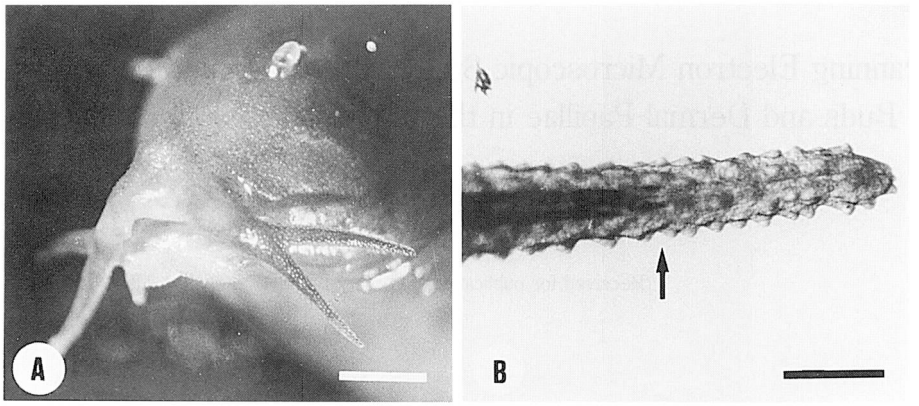


**Fig. 1** Light micrographs of the barbels of *Corydoras aeneus*. A: Low-magnification view of two pairs of maxillary barbels extending laterally from the corners of the mouth. Bar=2 mm. B: High-magnification view of regenerating barbels 10 days after cutting of the barbels. The top of the barbels was the region that regenerated, and the taste buds were observed as numerous protuberances similar to those on the remaining region, but no pigment cells were found in the regenerating portion of the barbels. The boundary between the non-regenerating and regenerating regions is indicated by an arrow. Bar=0.5 mm.

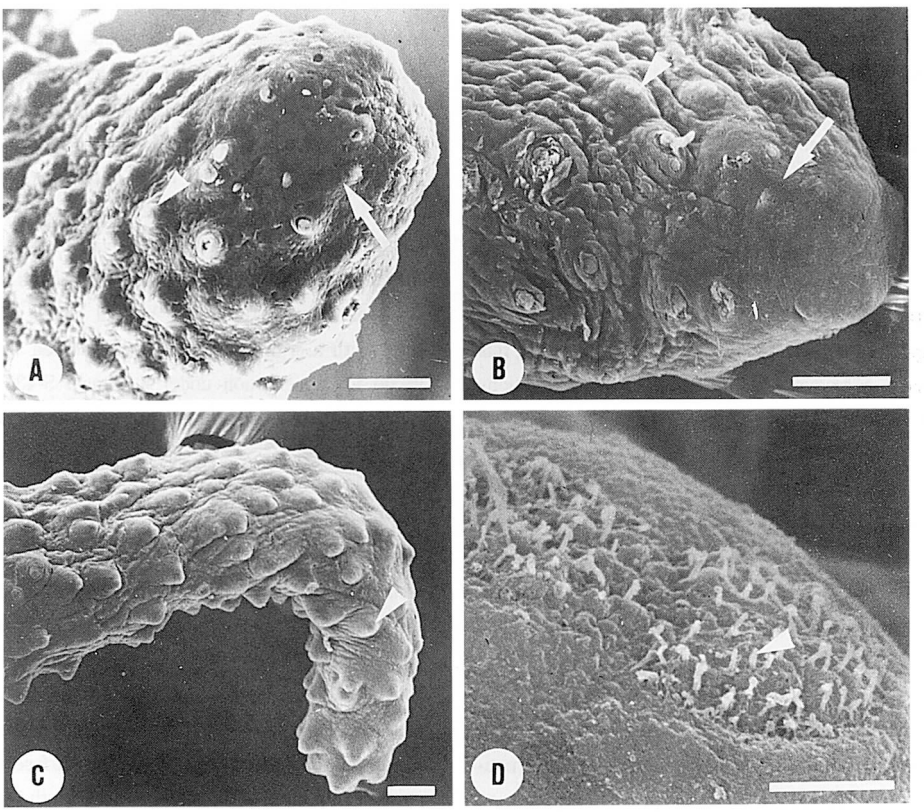


**Fig. 2** Scanning electron micrographs of the surface of regenerating barbels. A: The distal tip of the barbel was covered with epithelial cells 3 days after cutting of the barbels. The boundary of regenerated region is indicated by an arrow. In the remaining region, numerous taste buds (arrow head) were observed. Bar=50 $\mu$m. B: The distal tip of the barbel regenerated and elongated, but it exhibited a smooth surface 7 days after cutting of the barbels. Bar=50 $\mu$m. C: The distal tip of the barbel regenerated to form taste buds (arrow head) 21 days after cutting. Bar=50 $\mu$m. D: High-magnification view of taste buds in regenerating barbels 10 days after cutting. Cell processes of the receptor cells (arrow head) were observed. Bar=5 $\mu$m.

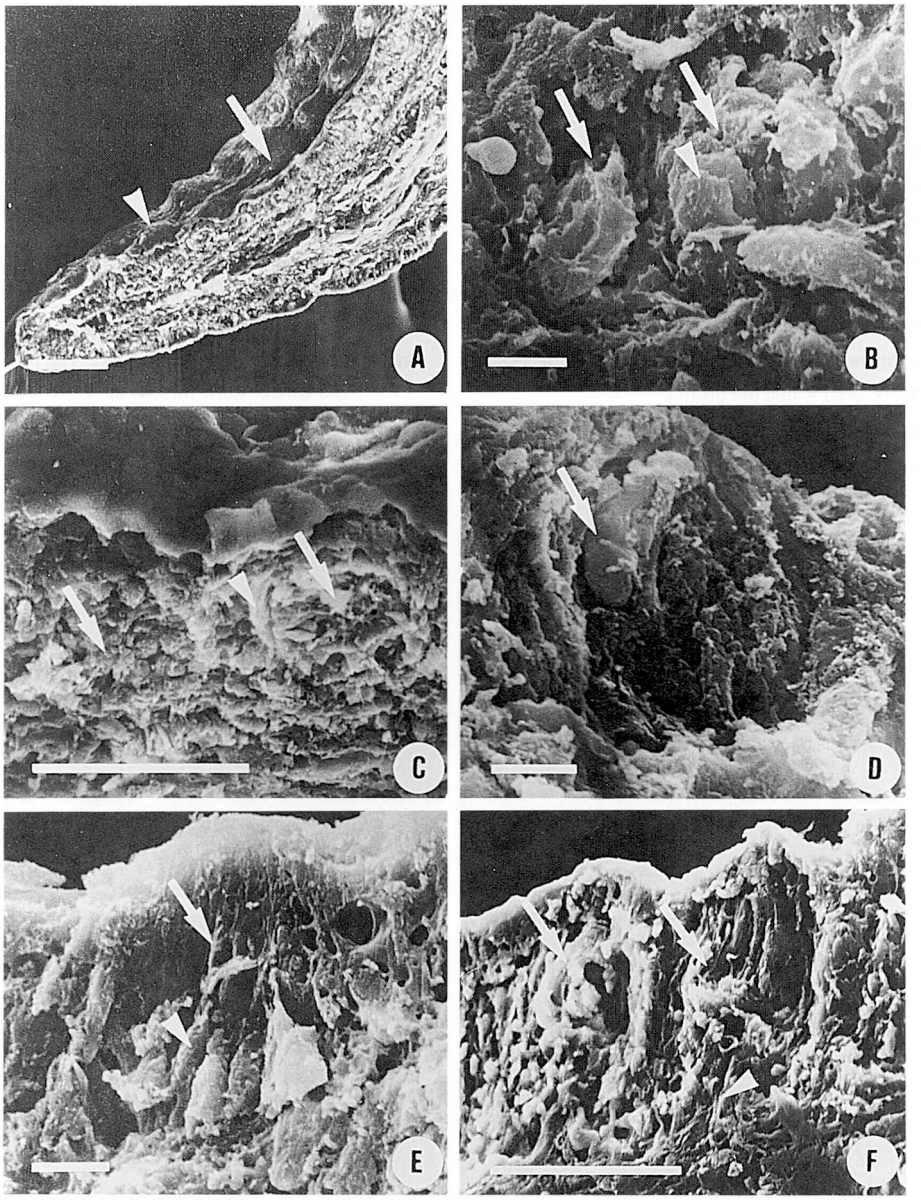


**Fig. 3** Scanning electron micrographs of the inner view of the regenerating barbels 9 days after cutting of the barbels. A: Low-magnification view of the entire regenerating barbel. An arrow indicates the region between the regenerating and original regions. Numerous protuberances (arrow head) were formed in the regenerating regions. Bar=100 μm. B: Two small aggregates of cells in the regenerating epithelium. The aggregates (arrow) exhibited an onion-like shape and the outer surface of aggregates was covered with thin cells (arrow head). Bar=5 μm. C: Two aggregates that had enlarged in size. Aggregates of spherical cells (arrow) were covered with thin cells (arrow head). Bar=50 μm. D: Elongation of cells in aggregates. The cells (arrow) within the aggregates became elongated upwards. Bar=5 μm. E: Formation of long, thin cellular process (arrow) within the aggregates of cells. The cell bodies (arrow head) were present in the basal portion of aggregates and long cellular processes had elongated upwards. Bar=5 μum. F: Formation of pear-shaped taste buds (arrow) and dermal papillae (arrow head) in regenerated barbels. Taste buds exhibited a pear-like shape and the dermal papillae became higher. Bar=50 μm.

fered in proportion to the distance from the distal tip of the regenerating barbels; regeneration progressed more markedly near the basal region of the regenerating barbels.

The protuberances of the epithelium were observed in the regenerating barbels 9 days after cutting of the barbels (Fig. 3A). Small groups of cells in contact were observed in the epithelium (Fig. 3B). The number of cells in contact with each other increased with the progression of regeneration and the outer cells looked as if they were covering the inner mass of cells. Large groups of cells in contact were seen within the protuberances of the epithelium (Fig. 3C). The cells in groups differentiated to form cellular processes that elongated upwards (Fig. 3D). Then the cells in aggregates became slender with long, thin processes (Fig. 3E) and dermal papillae were completed under the regenerated taste buds (Fig. 3F).

Removal of regenerating epithelial cells revealed the regenerating basal lamina. The surface of the basal lamina was smooth about 5–7 days after cutting of the barbels (Fig. 4A). There were numerous pores in the basal lamina and numerous filamentous rod structures were extruded through the pores (Fig. 5A). The surface of the basal lamina became rugged, and this structure was similar in appearance to the controls. The protuberances in the basal lamina were formed after 10 days (Fig. 4B). The protuberances became higher with the progression of regeneration (Fig. 4C). The basal lamina near each pore

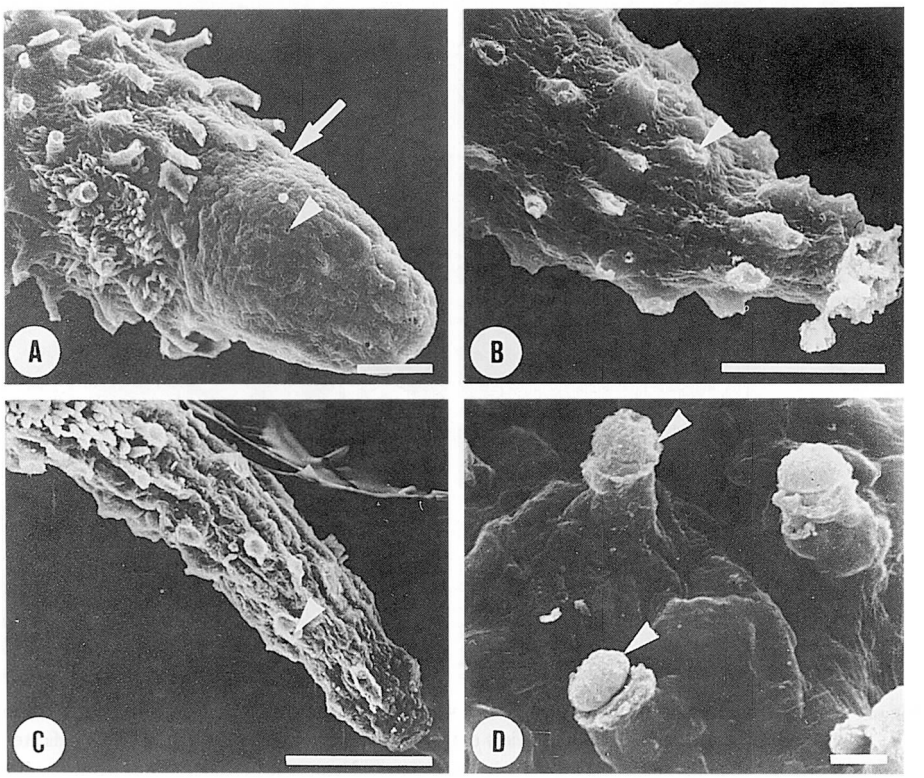


**Fig. 4** Scanning electron micrographs of the surface of the basal lamina in regenerating barbels. A: The distal tip of the barbel in the process of regeneration, 7 days after cutting of the barbels. The surface of basal lamina in the regenerating region was smooth, but no dermal papillae were observed. The boundary between the non-regenerating and regenerating regions is indicated by an arrow. There were numerous pores (arrow head) in the regenerating basal lamina. Bar=50 $\mu$m. B: The surface of the basal lamina, 10 days after cutting of the barbels, exhibited numerous protuberances. The tops of the protuberances (arrow head) were sunken. Bar=50 $\mu$m. C: The surface of regenerating dermal papilla 14 days after cutting of the barbel. There was a pore (arrow head) in the top of each dermal papilla. Bar=50 $\mu$m. D: Regenerated dermal papilla 21 days after cutting of the barbels. Aggregates of taste cells (arrow head) were observed on some dermal papilla. Bar=5 $\mu$m.

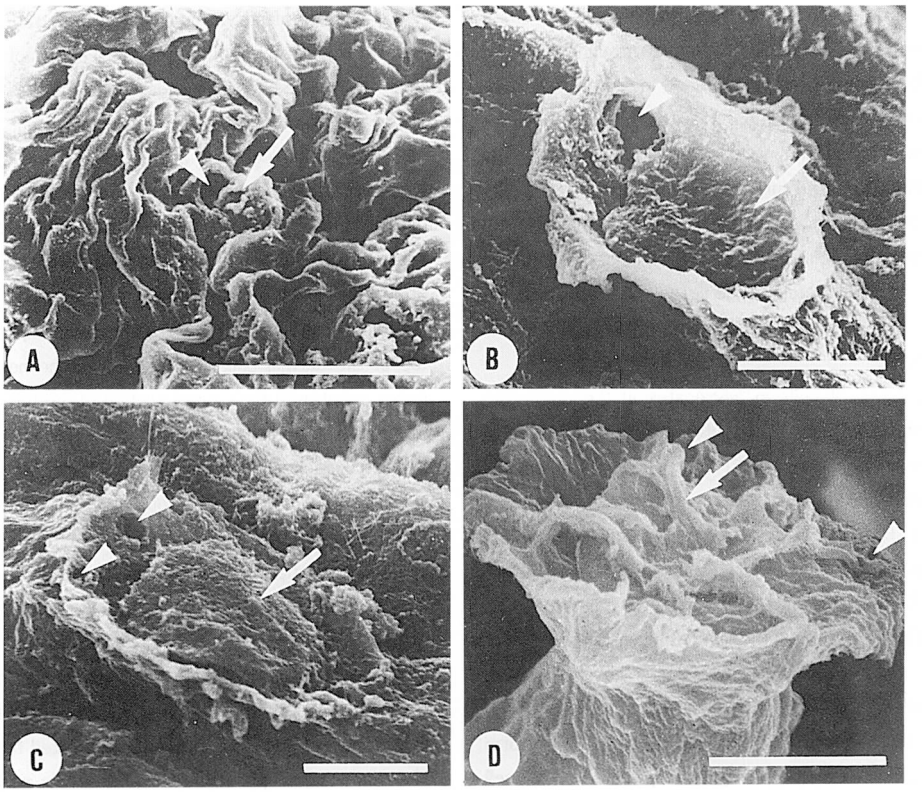


**Fig. 5** Scanning electron micrographs of the surface of regenerating dermal papillae. A: High-magnification view of the basal lamina in regenerating barbels 5 days after cutting of the barbels. Numerous filamentous extrusions (arrow) were observed in the pore (arrow head) of the basal lamina. Bar=5 μm. B: High-magnification view of regenerating dermal papillae 14 days after cutting of the barbels. There was a large pore (arrow head) at the periphery of the top of each dermal papilla. The top of each dermal papilla was smooth and sunken, and was covered with a large flat cell (arrow). Bar=5 μm. C: The surface of regenerating dermal papillae 14 days after cutting of the barbels. There were several pores (arrow head) in the peripheral region of the top of each dermal papilla. However, the dermal papillae were rather short. Bar=5 μm. D: The regenerated dermal papillae grew taller, the top of each was covered with a large flat cell, and numerous filamentous fibers (arrow) were extruded from several pores (arrow head) on the periphery 21 days later. Bar=5 μm.

was smooth and formed a circular depression (Fig. 5B). The periphery of the basal lamina in a circular depression protruded outwards as a thin membrane. The surface of each depression in the basal lamina was covered with a large flat cell (Fig. 5C). In regenerated taste buds, some dermal papillae retained a group of cells on the top (Fig. 4D). A large flat cell was also present on the top of each dermal papilla and a few, fine bundles of nerve fibers were extruded through the basal lamina on the periphery of the top of the dermal papillae (Fig. 5D).

## DISCUSSION

The regeneration and development of the taste organs in the fish have been investigated morphologically. The fine structure of taste cells as they develop has been revealed by transmission electron microscopy and the surface morphology has been revealed by scanning electron microscopy. Inner views of tissue and of the surface of basal lamina can be studied under the scanning electron microscope after freeze-cracking or removal of the epithelium[18,21,22]. These techniques make it possible to observe a wide area of the inner structure of the regenerating taste organs at high magnification.

Removal of epithelium from fixed barbels by trypsin revealed the surface structure of dermal papillae under the taste cells in the fish. The basal lamina rapidly covered

the regenerating portion of the fish barbels, and there were many pores in the basal lamina through which the nerve fibers seemed to be extruded into the epithelium. At this stage of regeneration, small aggregates of cells in contact with each other were formed and could be clearly distinguished from the surrounding epithelial cells. In the rat, the nerve fibers enter the epithelium and initiate the development of the special characteristics of the epithelial cells[9]. The innervation of the nerve fibers is also necessary for the maintenance of taste organs in fish and mammals[2,5].

The number of cells in aggregates in the epithelium increased with the duration of regeneration. Cells in aggregates differentiated into two types of cells: the inner, spherical cells; and the surrounding, thin cells that covered the inner, spherical cells. The basic structure of the taste organs is similar to that of such aggregates; taste cells in the taste organs are covered with numerous thin cells. The separation of the taste organs from the epithelial cells might be required during the initial stages of regeneration.

Some cells in the aggregates differentiated to form cellular processes that elongated upwards. The cell bodies were located in the basal region. The formation of slender processes is accompanied by the formation of microtubules and cytoplasmic tubules[23]. These structures may function as the cytoskeleton necessary for this process.

The regeneration of taste organs was accompanied by another morphogenetic process. The regeneration of dermal papillae occurred concomitantly with regeneration of taste cells. The initial stages of regeneration of taste organs were not accompanied by the regeneration of dermal papillae. The regeneration of dermal papillae required much more time than the regeneration of taste cells. Regeneration of dermal papillae was completed during the later stages. The exact mechanism of the formation of dermal papillae is not clear, but it must be a complex phenomenon associated with the synthesis of collagens, blood vessels and the entrance of the nerve fibers.

There were numerous pores in the surface of basal lamina during the regeneration, but the penetration of nerve fibers through pores was hardly observed. However, it is likely that nerve fibers penetrate through pores into the epithelium. Nerve fibers also penetrate through pores of the basal lamina into mouse taste organs[19]. Thick bundles of nerve fibers might penetrate the basal lamina through pores during the initial stages of regeneration of fish taste buds. Subsequently, the area near each pore became smooth with a circular depression, as if these structures received small aggregates of cells as saucer-like structures. These latter structures then protruded outwards and formed dermal papillae. The nerve fibers seem to be extruded as a bundle through pores at the top of each regenerating dermal papilla. Finally, the nerve fibers penetrated as fine bundles in numerous places at the top of the dermal papillae. However, the nerve fibers never penetrated the central portion of the top of dermal papillae, which was covered with the basal cells.

The regeneration of taste buds in the fish was studied by scanning electron microscopy and it was evident that the regeneration involved complex phenomena associated with the formation of dermal papillae.

## SUMMARY

The regeneration of taste buds and dermal papillae in barbels of *Corydoras aeneus*, after removal of approximately half of each barbel, was investigated by scanning electron microscopy. Basal lamina in the regenerating portion of the barbels exhibited numerous pores about 5 days after cutting of the barbels. Small groups of cells began to aggregate in the regenerating epithelium about 1 week after cutting. The number of cells in aggregates increased with time and the aggregates were covered with numerous thin cells. The periphery of the basal lamina under the large aggregates of cells protruded outward and several large pores were observed on the periphery. These structures then protruded outwards and formed dermal papillae. Thereafter, some cells in aggregates began to form cellular processes that elongated upwards. In mature taste buds, a few fine bundles of nerve fibers penetrated through the basal lamina on the periphery of the top of the dermal papillae. These results suggest that regeneration of taste buds is initiated with the formation of aggregates of cells, possibly with the penetration of nerve fibers into the epithelium, and is then associated with the formation of dermal papillae.

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