THE RELATIONSHIP BETWEEN CILIARY ROOTLETS AND OTHER CELL STRUCTURES

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The ciliary rootlets which, in single or paired condition, extend downward from the basal body toward the nucleus have been found in a great number of ciliated epithelia. It was early suggested (3) that the rootlets are contractile structures which produce the movements of the cilia. There are, however, many ciliated epithelia in which the rootlets are rudimentary or completely absent, and this fact indicates that they are not essential for ciliary function. Therefore, at present, it is generally considered that they play some secondary role only, such as being anchoring structures for the ciliary apparatus (5–7). A current investigation of the endostylar cells of lower chordates has disclosed some deviating conditions in rootlet position and relationship to other cell structures which may be of some interest in the discussion of the significance of the rootlets.

MATERIAL AND METHODS

Small pieces of the isolated endostylar grooves of the ascidian *Corella parallelogramma* and the lancelet *Amphioxus lanceolatus* were fixed in a mixture which was made up according to the traditional Michaelis-Palade formula. However, distilled water was in all instances substituted by filtered sea water of the same salinity as that in which the animals had been caught. A rinse in the same fluid but now without osmium tetroxide was followed by treatment with increasing concentrations of acetone in sea water. If a precipitation occurred in the higher concentrations of acetone, the sea water was substituted by tap water, optionally with a calculated concentration of sodium chloride. The tissue pieces were embedded in Vestopal W. Observations and micrographs were made with the aid of an Akashi TRS-50E electron microscope.

OBSERVATIONS

In the ciliated cell zones 5 and 8 (2) of the *Corella* endostyle the rootlets are characteristically situated almost perpendicular to the ciliary axoneme, paralleling the cell surface (Fig. 1). All the rootlets point in the same direction and terminate at the lateral cell wall close below the surface. The cell membrane in this region is thickened to form a terminal bar, and the rootlet fibrillae and the tiny filaments stemming at the terminal bars are inseparably intermingled.

Another type of association between ciliary rootlets and other cell structures is found in the cells which form one of the ciliated zones of the lancelet's endostyle, probably the ventral ciliated zone.

FIGURE 1

Portion of some epithelial cells from the endostyle of *Corella*, showing the horizontal rootlets which terminate at the terminal bars (*TB*) of the cellular membrane. × 25,000.

FIGURE 2

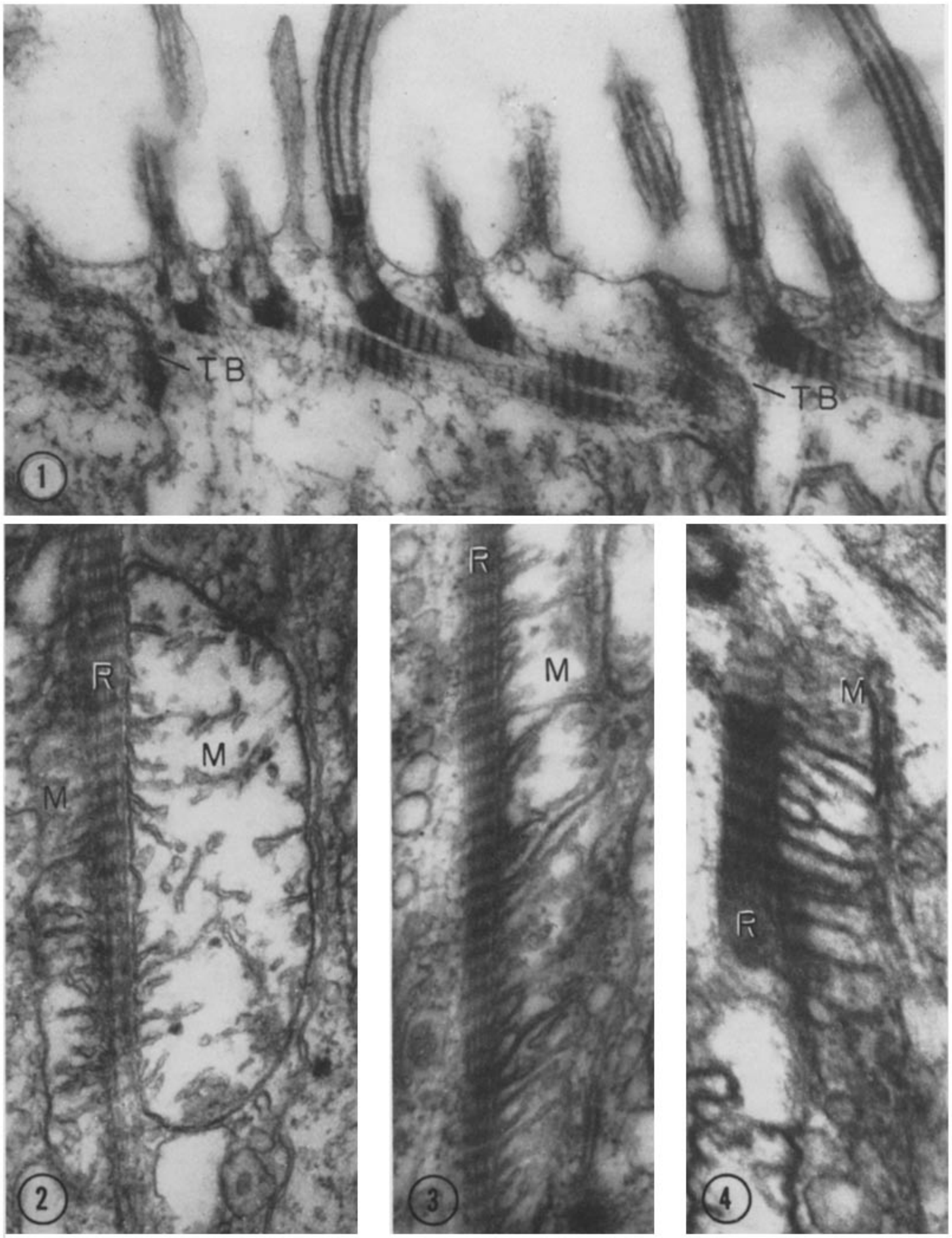
Two mitochondria (*M*) (or probably a single mitochondrion) surrounding a rootlet (*R*) in the lancelet endostyle. The mitochondrion has poorly developed cristae except at the surface which borders the rootlet. × 42,000.

FIGURE 3

Same material and symbols as in the preceding micrograph. The well developed cristae always appear as direct continuations from the rootlet dark periods. × 39,000.

FIGURE 4

Oblique section through rootlet (*R*) and mitochondrion (*M*) of the lancelet. Note the complete coincidence in position and inclination between dark periods and cristae. × 55,000.


TB
TB
1
R
M
M
2
R
M
3
M
R
4

A pair of rootlets extends downward from the basal body, its members diverging toward the nuclear zone, where rootlets and nuclei can often be seen in close contact with each other. A peculiar feature of this epithelium is the pronounced association between the rootlets and some mitochondria (Fig. 2). In oblique sections the tall mitochondria appear to envelop the rootlets, which thus might be situated in a mitochondrial furrow almost completely surrounded by mitochondrial membranes.

A very striking feature of the structures described here is not only the close association between mitochondria and rootlets, but also a pronounced relationship between the dark periods of the rootlets and mitochondrial cristae (Figs. 3 and 4). The outer membrane of the mitochondrion follows the surface of the rootlet without complication while the inner membrane of the mitochondrion usually forms a crista at the level of every dark period of the rootlet.

The rootlets of this epithelium have an average diameter of 140 mμ and they are composed of longitudinal fibrils which, in the median section, number about 9. The dark periods appear as local thickenings of these fibrils for a distance of about 45 mμ. The dark periods may have a maximum inclination of 23 degrees against the rootlet perpendicular. The tiny rootlet filaments, which have a mean diameter of 6 mμ, show a discrete periodicity of their own in the most successful micrographs. This periodicity is about 9 mμ.

DISCUSSION

The very intimate association between rootlets and mitochondria which is observed in the lancelet material suggests that the rootlets are not only supporting elements of the ciliary apparatus. It is well known that mitochondria may be firmly associated with certain intracellular areas with a specialized activity, an arrangement which indicates a high rate of energy requirement in these areas. Such more or less well defined relationships between mitochondria and other cell structures are known in several cases, for example, in muscle cells and in the ellipsoid of retinal cells. A very close association between ciliary structures and mitochondria is known in the case of the kinetoplast or kinetonucleus of flagellate protozoans and sperm cells (1, 4, 8–10). The mitochondrial structures here are often in intimate contact with the proximal parts of the flagella. A very high concentration of cristae indicates a high rate of energy production by the mitochondria.

The lancelet mitochondria which are associated with rootlets have, however, poorly developed cristae, and only those mitochondrial surfaces which are in direct contact with rootlets give rise to cristae in an amount which could be expected for a very active mitochondrion. (Fig. 5).

It is known that the mitochondrial cristae are the site of the respiratory chain enzymes as well as the phosphorylating enzymes which produce ATP during electron transport. It is likely that the rootlets need something which is produced by the mitochondria, and a close association between the cristae and the rootlet dark periods increases the efficiency of the system. This would mean that the rootlet is the trapping system for the mitochondrion, and receives its energy. This process apparently takes place at the dark periods of the rootlet, which in its turn may induce the formation of well developed cristae at these places.

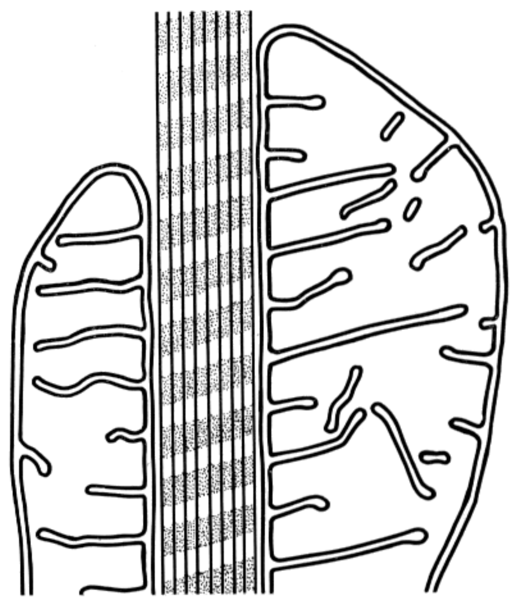


FIGURE 5

Schematic diagram showing the relationship between the rootlet dark periods and the cristae of a mitochondrion which surrounds the rootlet.

The horizontally situated rootlets of *Corella* are not associated with mitochondria in this manner, and the significance of this specialization must remain an open question. It may be noted, however, that fine tubular fibrillae extending from the basal body have been observed to take the same course in other ciliated cells (7).

The great variation already observed in occurrence and appearance of rootlets in ciliated epithelia makes it clear that it is impossible to postulate the general significance of these structures for ciliary function. It is, however, also clear that rootlets, when they occur, may have some function other than that of merely acting as footholds for the distal ciliary portion.

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REFERENCES

1. André J., Étude au microscope électronique de l'évolution du chondriome pendant la spermatogénèse du papillon du chou *Pieris brassicae*, *Ann. Sc. Nat.: Zool.*, 1959, **1,** 283.
2. Barrington, E. J. W., The distribution and significance of organically bound iodine in the ascidian *Ciona intestinalis*, *J. Marine Biol. Assoc. U. K.*, 1957, **36,** 1.
3. Benda, C., Weitere Mittheilungen über die Mitochondria, *Arch. Anat. u. Physiol.*, 1899, 376.
4. Clark, T. B., and Wallace, F. G., A comparative study of kinetoplast ultrastructure in the Trypanosomatidae, *J. Protozool.*, 1960, **7,** 115.
5. Fawcett, D. W., Structural specializations of the cell surface, *in* Frontiers in Cytology, (S. L. Palay, editor), New Haven, Yale University Press, 1958, 19.
6. Fawcett, D. W., Cilia and flagella, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, 1961, **2,** 217.
7. Gibbons, I. R., The relationship between the fine structure and direction of beat in gill cilia of a lamellibranch mollusc, *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 179.
8. Grassé, P.-P., Carasso, N., and Favard, P., Les ultrastructures cellulaires au cours de la spermiogénèse de l'escargot (*Helix pomatia*): Évolution des chromosomes, du chondriome, de l'appareil de Golgi, etc., *Ann. Sc. Nat.: Zool. et Biol. Animale*, 1956, **18,** 339.
9. Kaye, J., Changes in the fine structure of mitochondria during spermatogenesis, *J. Morphol.*, 1958, **102,** 347.
10. Steinert, M., Mitochondria associated with the kinetonucleus of *Trypanosoma mega*, *J. Biophysic. and Biochem. Cytol.*, 1960, **8,** 542.