An Association between Mitochondria and the Endoplasmic Reticulum in Cells of the Pseudobranch Gland of a Teleost

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ABSTRACT

An elaborate and apparently unique specialization of the endoplasmic reticulum having the form of tubules and a precise orientation with respect to the mitochondria has been described for the specific cell of the pseudobranch gland. The tubules also are concentrated near the vascular border of the cell where they show continuity with the plasma membrane and open directly against the basement membrane. On the other side of the basement membrane, the endothelial cells of the sinusoid show openings or discontinuities characteristically associated with secretory cells. The pseudobranch gland is presumed to have carbonic anhydrase as one of its primary products, if not its only one, and the elaborate ultrastructure is thought to be associated with the special problems of secreting this enzyme.

INTRODUCTION

The structure of the gills in fishes is based on the cartilaginous visceral arches. Each arch bears finger-like filaments on the sides of which, in turn, are located the multitudinous and very thin respiratory platelets. The filaments are characteristically arranged in two rows along the radial edge of the arch. Each row is called a "hemibranch" and is arranged back-to-back with its neighbor in respect to anatomy and circulatory pattern.[1] The result is that water in passing on each side of the arch flows in a counter-current fashion past the circulating blood in the shelf-like platelets.

Most fish possess a half gill or hemibranch located anteriorly to the rest of the gill apparatus. In elasmobranchs it is found in the spiracular opening and in teleosts it is attached to the inside of the dorsal base of the operculum. These structures are called "pseudobranchs" because they receive oxygenated blood that has already passed through the next succeeding gill (1). They thus have no apparent respiratory function. The blood from the pseudobranch is added to the circulation of the eye and the brain. It has been suggested that under adverse oxygen tensions, the pseudobranch may increase the "purity" of the blood going to the eye and brain. In teleosts the pseudobranch is usually observed as a small but normal appearing hemibranch, but there are a series of intermediate species wherein the pseudobranch is covered in varying degree by a fold of epithelium. In a few species, including *Fundulus heteroclitus*, the pseudobranch is completely lost to sight, being buried in loose connective tissue. This condition constitutes the pseudobranch "gland."

The glandular pseudobranch has long been suspected of some obscure endocrine function. Probably the first experimental approach to the problem was made by Leiner (2) who postulated an "*Augenkeimdruse*" or "*Atmungsferment*" produced by the pseudobranch and essential to the respiratory transport mechanism in the thick retina of fish eyes. This is probably carbonic anhydrase, high levels of which have been described in the gland by Leiner (3), and Sobotka and Kann (4). The pseudobranch gland may also have a functional relationship to the swimbladder (5) via carbonic anhydrase (5, 6).

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[1] Diagrams and photograph of gill structure can be found in Fig. 1 of the following: D. E. Copeland, The cytological basis of chloride transfer in the gills of *Fundulus heteroclitus*, *J. Morphol.*, 1948, **82,** 201.

Materials and Methods

Pseudobranch glands of *Fundulus heteroclitus* were examined with particular regard to the mitochondria. For light microscopy the classical Regaud fixation followed by Altmann-fuchsin staining was used. For electron microscopy, the glands were fixed in Dalton's chrome-osmic mixture (7). A few attempts with Mann-Kopsch fixation failed to reveal any distinct localization of Golgi material and, therefore, this particular technique was not used further.

RESULTS AND DISCUSSION

The pseudobranch gland of *Fundulus* looks like a minute bunch of bananas buried in fatty tissue. The organ is composed predominantly of one type of glandular cell plus supportive connective tissue and it is richly vascularized. The pattern of the vascularization is a condensed version of that normal to the respiratory hemibranchs (Fig. 1).

The Regaud-Altmann preparations show the glandular cells to be heavily populated with mitochondria. The morphology of these cytoplasmic organelles suggests a cyclic behavior. In the same section, extremes from rod-shaped to granular-shaped forms can be seen. The greatest length of the cells parallels the blood supply and it is interesting to note that the rod-shaped mitochondria are oriented in the same direction. Thus, the long axes of the mitochondria are parallel to and not at right angles to the free surface of the cell. It is also interesting to note that the rod-shaped mitochondria tend to form packets or bands (Fig. 2).

Experiments designed to test whether evidence of a cyclic behavior exists at the ultrastructural level have not been completed. However, one consistent observation seems worth reporting at this time. That is the occurrence of a highly specialized tubular form of endoplasmic reticulum in association with the mitochondria and apparently in turn, with the vascular border of the cell. Fig. 3 is an electron micrograph of an oblique section through the midportion of a pseudobranch cell. Above and below, the cell is limited by a plasma membrane which, in turn, rests upon a distinct basement membrane separating the cell from the endothelium of associated blood vessels. The mitochondria in the upper half of the cell are for the most part obliquely cut, while those in the lower half are cut transversely and the small tubules in close proximity to them are obviously oriented in the same direction. Fig. 4 at higher magnification shows the detail in the peripheral portion of the cell and in an associated blood vessel. The small tubules in the border region do not show the same precise orientation of those associated with mitochondria but at the cell border they occasionally show direct continuity with the plasma membrane (arrows). The continuity of the adjacent endohelial cell shows many interruptions indicating that except for the presence of the basement memtrane there is direct communication between the bumen of the blood vessel and the lumina of the small tubules associated with the mitochondria. Fig. 5, also at higher magnification, suggests that branching of the tubules occurs in the peripheral portions of the cell but not in the mitochondrial region.

This elaborate organization clearly indicates the existence of a morphological adaptation related to a special function of the pseudobranch cell. To our knowledge no such complex differentiation of the endoplasmic reticulum exists in other cells of special function such as, for example, the proximal tubule cells of the kidney (8), principal cells of the small intestine (9), or the parietal cells of the gastric mucosa (10). Thus, it is reasonable to suggest that this specialization is related either to the production of carbonic anhydrase or to some activity of the pseudobranch cell at present unknown. (Grateful acknowledgement is made to Dr. Ross McCardle for the use of his laboratory in the preparation of the gross histology specimens.)

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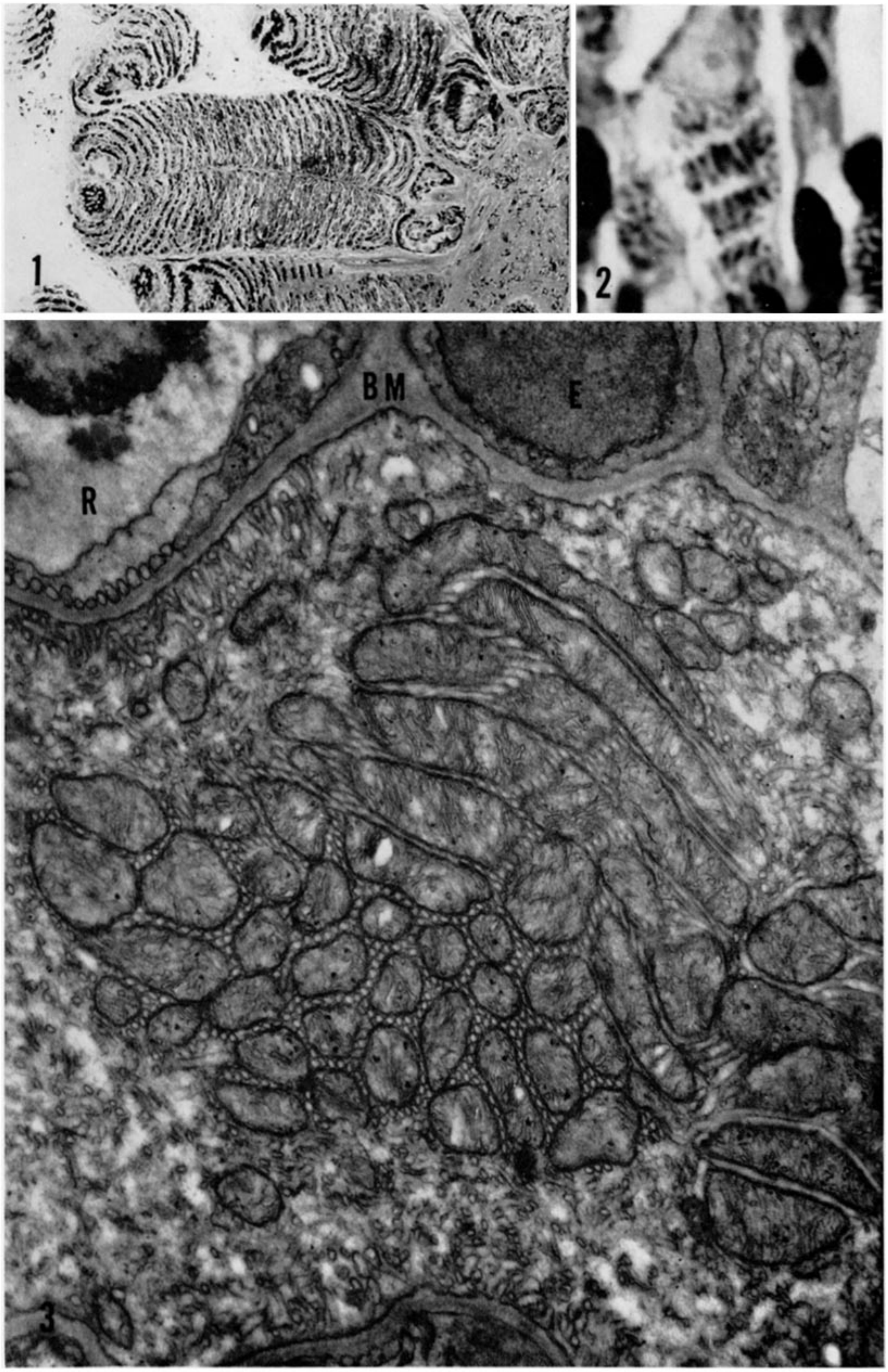
Explanation of Plates

Plate 174

Fig. 1. Longitudinal section (slightly oblique) of a glandular pseudobranch filament. Basal area to the right, distal to the left. The pattern is such that all of the specific cells have at least one longitudinal border adherent to the vascular supply. Approximately × 115.

Fig. 2. Light microscopic detail of a pseudobranch cell with a nucleus at the top and vascular supply on the right. Note mitochondria aligned parallel to the free border of the cell and in turn organized into packets. Approximately × 2350.

Fig. 3. An electron micrograph of a section through part of a pseudobranch cell. Mitochondria with numerous well developed cristae are grouped near the center of the cell and between them are numerous, small, precisely oriented tubules. Above and below, the plasma membrane of the cell is in intimate contact with a prominent basement membrane (*BM*). At top center is part of the nucleus and cytoplasm of an endothelial cell (*E*) forming the border of a blood vessel. At the extreme upper left is part of the nucleus and cytoplasm (*R*) of an erythrocyte. Chrome-osmic fixation (pH 7.4). Approximately × 32,000.



(Copeland and Dalton: Association between mitochondria and ER)

Plate 175

Fig. 4. Higher magnification of the upper part of the cell shown in Fig. 3. Numerous small tubules are present between the mitochondria. They also extend outward toward the cell periphery. They are precisely oriented when in association with the mitochondria but do not show this degree of organization near the cell border. The tubules are considered to be a highly specialized form of the endoplasmic reticulum. Their outside diameter averages approximately 460 A, their lumen 350 A, and their walls approximately 60 A. The space between the tubule wall and the outer limiting membrane of the mitochondrion is frequently less than 30 A. At several points the tubules show continuity with the plasma membrane (small arrows), while on the opposite side of the basement membrane (*BM*) the cytoplasm and plasma membrane of an endothelial cell (*E*) show numerous discontinuities (large arrows) so that there is direct communication between the basement membrane and the lumen of the blood vessel (*L*). Approximately × 56,800.

Fig. 5. Higher magnification of the lower part of the cell shown in Fig. 3. The tubules near the cell periphery show considerably greater variation in size than do those associated with the mitochondria. There is some evidence of branching in the former area (arrows) but not in the latter. Approximately × 56,800.

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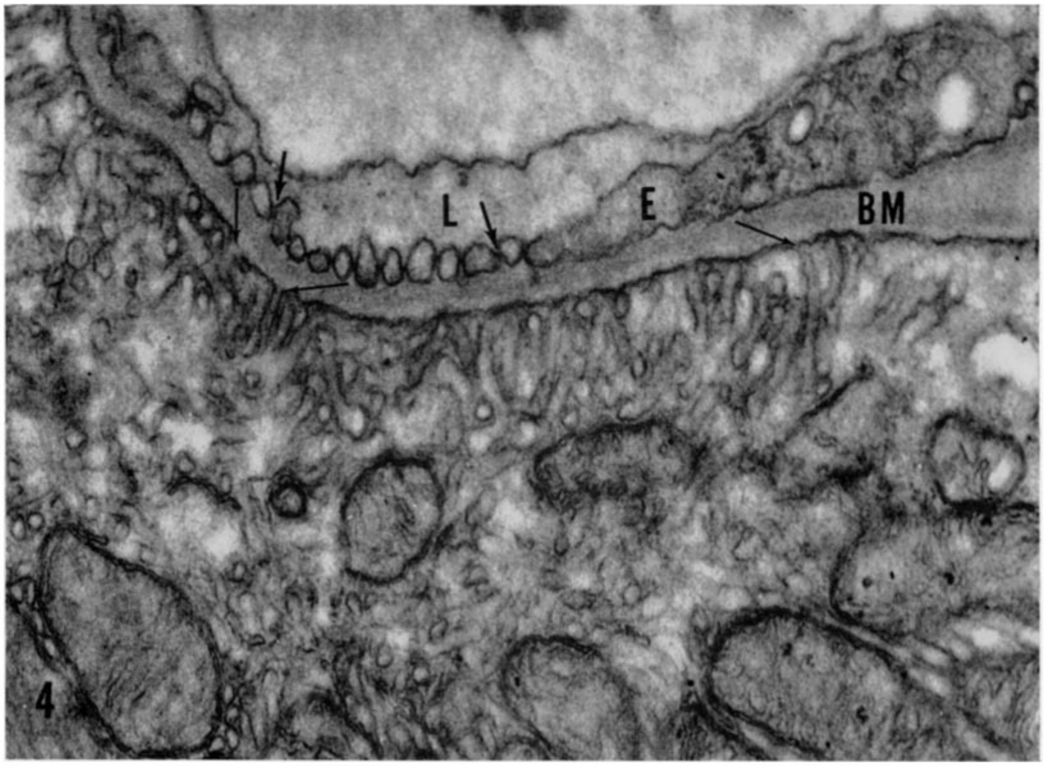

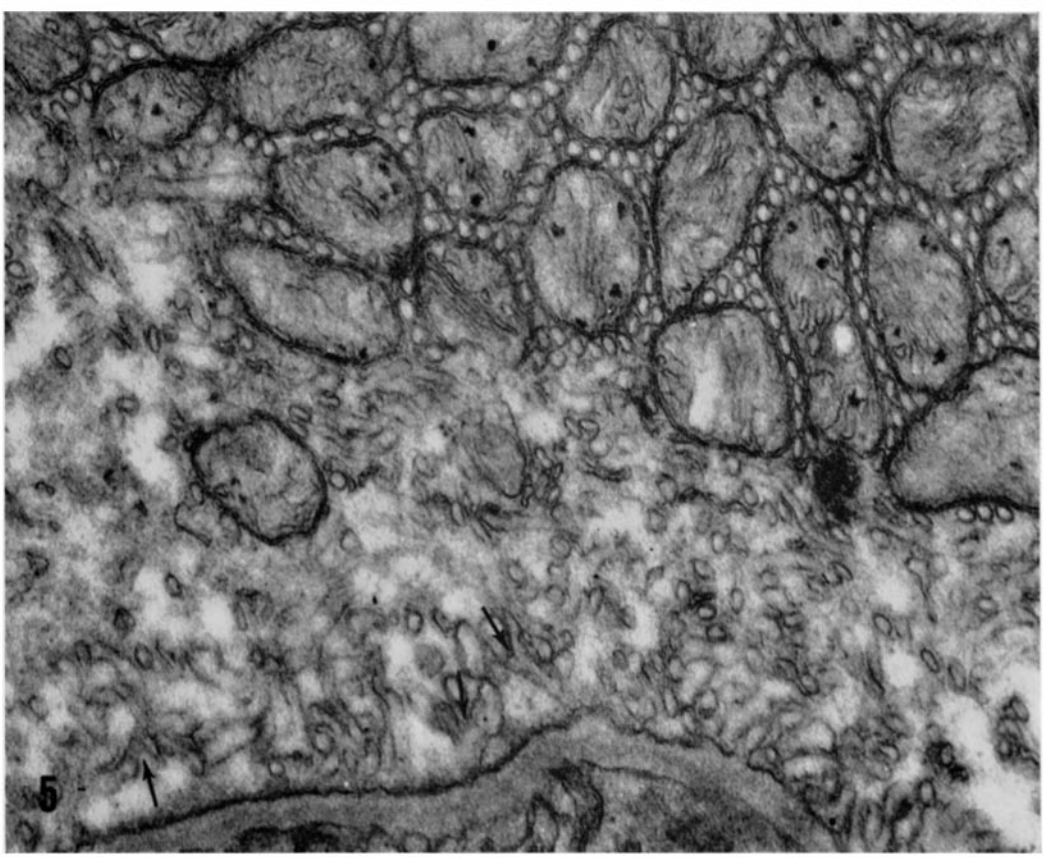

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