Freeze-Fracture Images of the Zonula Occludens in the Mouse Oviduct Epithelium*

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Summary. Freeze-fracture images of zonulae occludentes of the oviduct epithelium of adult mice were observed in the electron microscope.

The structure of zonulae occludentes varies depending on the types of oviduct epithelial cells. Those between two secretory cells consist of 5-14 (9.2±2.0) strands interposed between the luminal and the lateral plasma membrane. The strands run irregularly making well developed anastomoses with one another.

The tight junction between two ciliated cells consists of 4-14 (8.5±2.3) strands. Among them the upper 3-9 strands are unique in shape. The strands run regularly, compactly and parallel to the luminal surface and to each other with a few anastomoses. The distance between the adjacent strands is about 40 nm. Under this characteristic part several strands are very loosely distributed and run irregularly. The pattern of the strands in the zonula occludens between a secretory cell and a ciliated one resembles that between adjacent ciliated cells. The junction consists of 4-13 (8.3±2.0) strands. The characteristically dense and parallel arrangement of the strands demonstrated in this study is presumed to be related to the ciliary movement.

All the zonulae occludentes in the mouse oviduct epithelium are "very tight" in type. No large gap junctions are seen in the mouse oviduct epithelium.

Materials and Methods

Materials used were CF-1 female mice in various phases of estrous cycle, aged 4-6 months. The oviducts were removed and fixed in 3.0% glutaraldehyde buffered with 0.2 M Millonig's phosphate at pH 7.4 for several hours. After washing with the buffer, the tissues were treated with 20-30% glycerol for 12 hrs in a cold room and frozen in liquid Freon cooled with liquid nitrogen. The specimens were processed in a JEE-4C freeze-etching apparatus and shadowed with platinum-palladium. The replicas were examined in a Hitachi H-500 electron microscope. For conventional ultrathin sections, small pieces of the tissue were fixed for 2 hrs in the same fixative as mentioned above, and postfixed in 1% OsO₄ buffered with Millonig's phosphate for 1 hr. They were embedded in Epon and sections cut were examined

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in a Hitachi H-500 electron microscope.

**Observations**

It is well known that the oviduct epithelium consists of at least two kinds of cells in the adult mouse: the secretory cells and ciliated cells (Reinius, 1970; Dirksen, 1971; Komatsu, 1977; Komatsu and Fujita, 1978). The zonula occludens between these cells consists of strands which correspond to the fused portion of the cell membranes. No large gap junctions are found in the mouse oviduct epithelium.

**Zonula occludens between secretory cells**

The overall junctional depth is 0.3–1.1 μm (0.68 ± 0.16 μm) and there are 5–14 (9.2 ± 2.0) strands interposed between the luminal and the lateral plasma membranes. The strands run irregularly, making well developed anastomoses with one another. Large or small polygonal compartments are made up by the anastomoses of the strands (Fig. 1, 2).

**Zonula occludens between ciliated cells**

The overall junctional depth is 0.3–1.1 μm (0.60 ± 0.16 μm) and there are 4–14 (8.5...

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**Fig. 1.** Zonula occludens between secretory cells of the mouse oviduct epithelium. Strands of the zonula occludens run irregularly, forming polygonal compartments of various sizes by their anastomoses. L oviduct lumen, M microvilli. ×35,000
Fig. 2. Zonula occludens between secretory cells, in closer view. ×81,000

Fig. 3. Zonula occludens between the adjacent ciliated cells. Upper four to eight strands are arranged compactly, parallel to the luminal surface and to each other. Anastomoses are very few among them. Below these strands are some irregular strands. Notice cilia (C) with necklaces (N). M microvilli. ×36,000
Zonula occludens between the luminal and the lateral plasma membranes. Among them the upper 3–9 (5.6 ± 1.7) strands near the luminal surface run compactly and almost parallel to the luminal surface and to one another, with a relatively regular distance of 40 nm. A few anastomoses are recognized among these parallel strands. Below this region, there are some strands running irregularly and distributed very loosely. Some of these are not parallel but oblique or even perpendicular to the luminal surface forming irregular waves. Anastomoses among them are sometimes recognized (Fig. 3). At the place where three epithelial cells abut, a relatively wide strand which is the extension of the most apical element of the zonula occludens runs vertically accompanying several strands that arise as extensions of the upper part of the zonula occludens. They are also compactly arranged and almost parallel with one another (Fig. 4).

**Zonula occludens between a secretory cell and a ciliated cell**

The pattern of the strands in this cell connection is principally similar to that between adjacent ciliated cells. The overall depth is 0.3–1.2 μm (0.64 ± 0.19 μm) and there are 4–13 (8.3 ± 2.0) strands. The strands near the luminal surface are almost parallel to one another as those between the adjacent ciliated cells, though the distance between adjacent strands in this case is somewhat less regular. In the deeper region the strands are loose and anastomosed somewhat frequently with one another.
Discussion

Claude and Goodenough (1973) classified the junctional complex of epithelia into 5 types; very leaky, leaky, intermediate, intermediate to tight, and very tight. According to them, the "very tight" junction has 4–11 strands and measures 0.3–1.1 μm in depth. In the present study of mouse oviduct epithelium, the strands count 5–14 (9.2 ± 2.0) between two secretory cells, 4–14 (8.5 ± 2.3) between two ciliated cells, and 4–13 (8.3 ± 2.0) between the secretory and ciliated cell, while the depth of the junction measures 0.3–1.1 μm (0.68 ± 0.16 μm) between two secretory cells, 0.3–1.1 μm (0.60 ± 0.16 μm) between two ciliated cells and 0.3–1.2 μm (0.64 ± 0.19 μm) between the secretory and ciliated cells. This result indicates that the zonulae occludentes formed by different combinations of cells in the mouse oviduct epithelium all belong to the category of "very tight."

The tracheal epithelium consists also of ciliated and goblet cells. The freeze-fracture image of the tracheal epithelium of the normal guinea pig has been published by Inoue and Hogg (1977). They reported that the strands numbered 5–6 between two ciliated cells, while conspicuously variable between the goblet cell and the ciliated cell; when the goblet cell appeared to be actually secreting, the junction was of leaky type. The zonula occludens in the mouse oviduct epithelium thus much differs in arrangement from that in the guinea pig tracheal epithelium examined in this study.

The unusually compact arrangement of parallel strands evidenced between adjacent ciliated cells as well as between the ciliated and secretory cell in the oviduct epithelium deserves special attention. The strands in this case are gathered densely and parallel to each other. It is considered that the apical part of the oviduct ciliated cell is vibrated by a strong ciliary movement, and the well developed and characteristically arranged strands in the zonulae occludentes might support the cell bodies from the violent ciliary movement.

マウス卵管上皮の閉鎖帯の凍結断面像

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マウスの卵管上皮の細胞間結合を，おもに凍結断面法を用いて観察した。
成熟マウスの卵管上皮では、少なくとも分泌細胞と線毛細胞の2種の細胞から成っている。細胞間結合のうち閉鎖帯は、凍結切断像では、稜（P面）もしくは溝（E面）のつくる線条の網目構造として観察され、これは超薄切片像で見られる細胞膜の癒合部に対応する。マウス卵管上皮の閉鎖帯の構造は細胞の種類によって異なる。すなわち、相接する分泌細胞間の閉鎖帯は、5～14（9.2±2.0）本の線条で構成されており、各線条の走行は不規則で、分枝や交又も多く、全体としてよく発達した網目を形成している。隣り合う線毛細胞間の閉鎖帯は、4～14（8.5±2.3）本の線条で構成され、このうちの管腔側の3～9本の線条が管腔表面に平行して、また互いにもほぼ平行に、かつきわめて密に走っているのが大きな特徴である。これらの平行に走る線条の間隔は約40nmで、ここでは分枝や吻合はきわめて少ない。平行線条の下方には数本の不規則に走るややまばらな線条があり、ここでは枝分かれや吻合も認められる。3個の線毛細胞が接する部分では、最上端の線条の延長が基底側に向かって深く伸び、これに伴って数本の線条がややほぼ平行に、かつ密に、基底側に向かって走っている。分泌細胞と線毛細胞の間の閉鎖帯は、線毛細胞間のそれと類似しており、これを構成する線条の数は4～13（8.3±2.0）本である。

マウス卵管上皮の閉鎖帯はすべて"very tight"の型に相当するが、線毛細胞間の閉鎖帯はとくに緊密であると考えられる。また線毛細胞間の閉鎖帯の特異な構造は、この細胞が行なう強い線毛運動に対応するものとも考えられる。

卵管上皮には、大きいギャップ結合は認められない。

References


