# CILIOGENESIS IN THE MOUSE OVIDUCT

## A Scanning Electron Microscope Study

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## INTRODUCTION

The mouse oviduct is a complex, coiled tube which can be conveniently divided into three distinct regions: fimbria, ampulla, and isthmus. The funnel-like fimbriated tip, through which the ovum is transported after ovulation, is the most heavily ciliated portion of the oviduct. In the adult the cilia function to transport ova into the ostium of the fimbriated tip, and from there into the ampullary region, where fertilization occurs (4). Interspersed among the ciliated cells are a few nonciliated cells whose function appears to be secretory (15).

During the development of the oviduct, ciliated cell differentiation is manifested by the production of large numbers of cilia on the surface of each cell. Although extensive formation of cilia on the mouse oviduct begins only after birth and continues for approximately 12 days, or until most of the cells of the epithelium are fully ciliated; a few ciliated cells are already present at birth. The formation of ciliated cells is random over the surface of the oviduct both in space and in time. However, cilia emerge in a somewhat ordered pattern on the surface of each individual cell.

These results, obtained by scanning electron microscopy (SEM), add additional details to the description of the complex sequence of events which occur during ciliated cell differentiation and ciliary morphogenesis which could not have been predicted from previous studies by transmission electron microscopy (5, 6, 7, 13, 16, 21, 23). The limitations imposed by thin-sectioning techniques can be avoided in scanning electron microscopy, where large surface areas can be viewed simultaneously.

## MATERIALS AND METHODS

Suckling Swiss-Webster female mice ranging in age from 1 through 12 days were sacrificed and their oviducts removed. The tissues were prepared for scanning electron microscopy by methods which have been described in detail elsewhere (8, 10). After fixation with 3% glutaraldehyde and 1% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), these tissues were treated in a special manner, however. They were placed in dialysis casing, which was necessary in order to prevent the loss of these extremely small specimens through the wire mesh of the specimen holder during critical point drying. The procedure consisted of dehydration through a graded series of alcohols and final exchange with amyl acetate for liquid $CO_2$ (2). During dehydration, the tissues with their dialysis casing were placed in a beaker with the various solutions and were continuously agitated by means of a magnetic stirrer in order to accelerate fluid exchange. After critical point drying, the fimbriated tips were dissected from the rest of the oviducts, mounted on SEM specimen stubs, and coated with gold. The specimens were examined and photographed in a Cambridge Stereoscan Model S-4 SEM at 20 KV and the magnifications read directly from the calibrated meter.

## RESULTS AND DISCUSSION

Extensive ciliated folds or fimbriae characterize the adult mouse oviduct (8). Instead of this complex surface topology, the fimbriated tip of the newborn consists of a simple tube on whose surface cilium formation occurs in random patches (Fig. 1 and 5). It is possible to find cells in every stage of development at every age, but, as the oviduct develops, cells with immature cilia become fewer in number, while with each succeeding day the number of ciliated cells increases. These events continue until all presumptive ciliated cells acquire their full complement of cilia. At 7 days, for example, although a considerable number of the cells are ciliated, there will still be present a few cells with immature cilia. Finally, by 12 days, very few cells can be found with immature cilia.

For each cell, ciliary growth proceeds in a characteristic pattern. Cilia first appear as stubby cylinders interspersed among the narrower, more tapering microvilli (Fig. 2, 3 and 4). They are first generated circularly at the periphery of the cell and later appear in the center, producing daisy-like structures (Fig. 3, 5 and 6). This pattern of cilium formation is surprisingly similar in most cells observed. Although a future ciliated cell may have no cilia on its surface, it can be differentiated from a future nonciliated cell by its large number of microvilli (Fig. 1, 2, 4 and 5). It is upon the surface

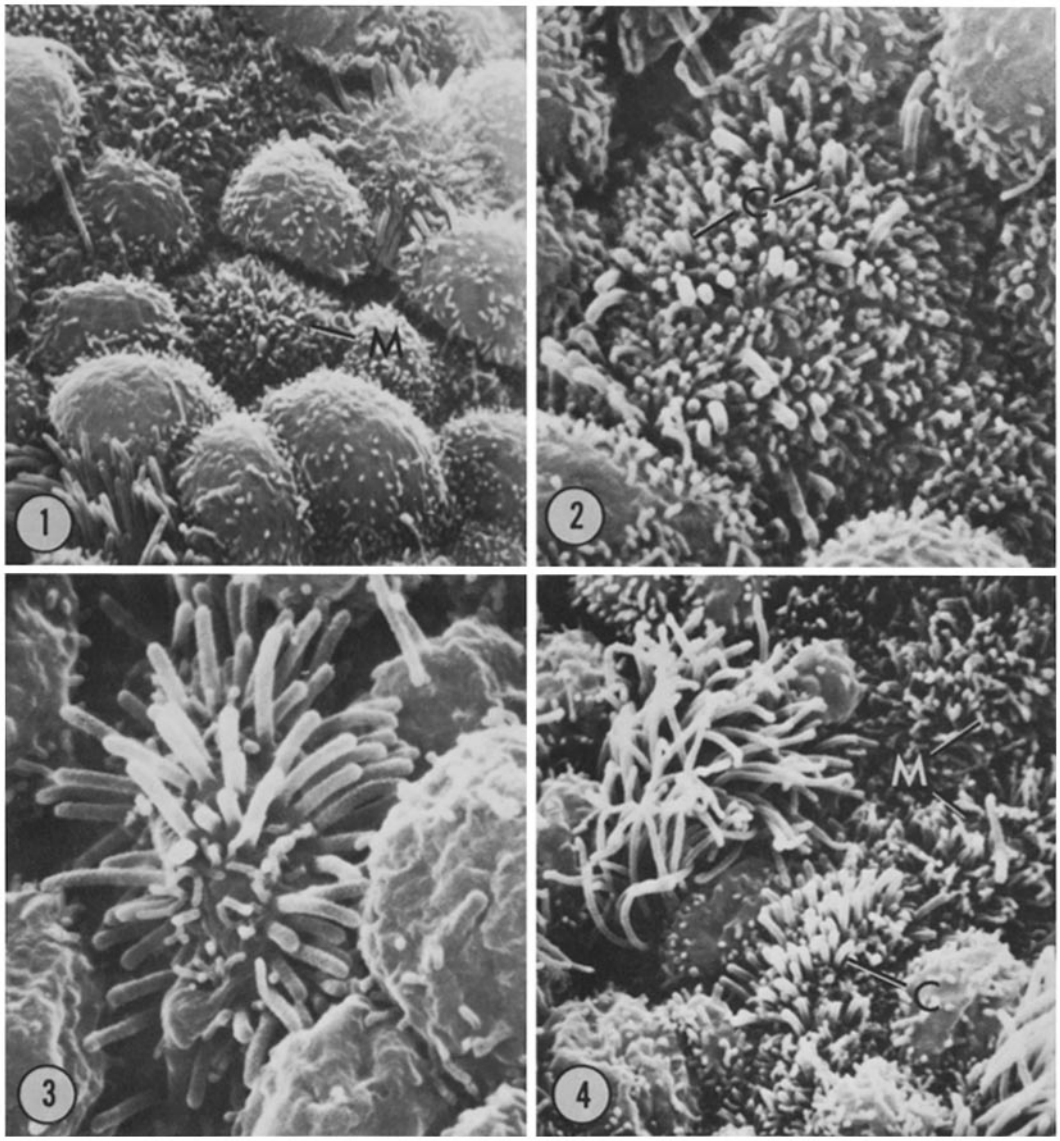


FIGURE 1 Surface of the fimbria from an oviduct of a 1-day old mouse demonstrating that only a few of the cells are ciliated. The surface of some of these cells are covered with short microvilli (*M*), evidence that these are the first cells destined to become ciliated. Peripheral cilia can be seen on the cell at the upper, right corner and more mature cilia at the lower, left. × 6,000.

FIGURE 2 A cell of the fimbria from a 1-day old mouse with the first stubby projections which will develop into cilia (*C*). This cell is also extensively covered with microvilli, as are two of the adjoining cells. × 12,000.

FIGURE 3 Immature cilia, varying in length, are found on a cell of the fimbria of a 3-day old mouse. At the periphery of the cell the cilia appear to be longer and are more abundant than in the center. × 12,000.

FIGURE 4 Cells in various stages of ciliogenesis on the surface of the fimbria of a 5-day old mouse. Presumptive ciliated cells covered with microvilli (*M*) are seen, as well as cells with mature and developing cilia (*C*). × 5,800.

of these microvilli-filled cells that the first short stubby cilia appear (Fig. 2 and 4). Once cilia achieve their full length of approximately 5 $\mu$m they taper slightly at the tip (Fig. 5), due to the loss of one of the microtubules from the peripheral doublets at the terminating tip.

It is of considerable interest to note that both ciliated and nonciliated cells of the adult mouse oviduct (8) have less microvilli on their surface than the presumptive ciliated cells of the newborn. Once cilia cover the cell surface, microvilli are no longer visible by SEM, but thin-section studies demonstrate that the number of microvilli has decreased in the mature ciliated cell (8). The presence of large numbers of microvilli on the surface of cells which are in the process of becoming ciliated raises the possibility that the surface membrane necessary to sheath the ciliary axonemes could be derived from the microvilli, which could act as storage organelles for membrane components. Follett and Goldman (11) have suggested that membrane is conserved in the form of microvilli which could then act as a reservoir for large surface area changes such as spreading of tissue culture cells, pinocytosis, cell division, or as, in this case, ciliogenesis.


FIGURE 5 On the fimbria of a 5-day old mouse, presumptive ciliated cells can be recognized by their microvilli-coated surfaces (*M*). A few cells are fully ciliated while others have the daisy-like appearance produced when cilia are first generated at the cell periphery (e.g., at arrow). × 5,000.

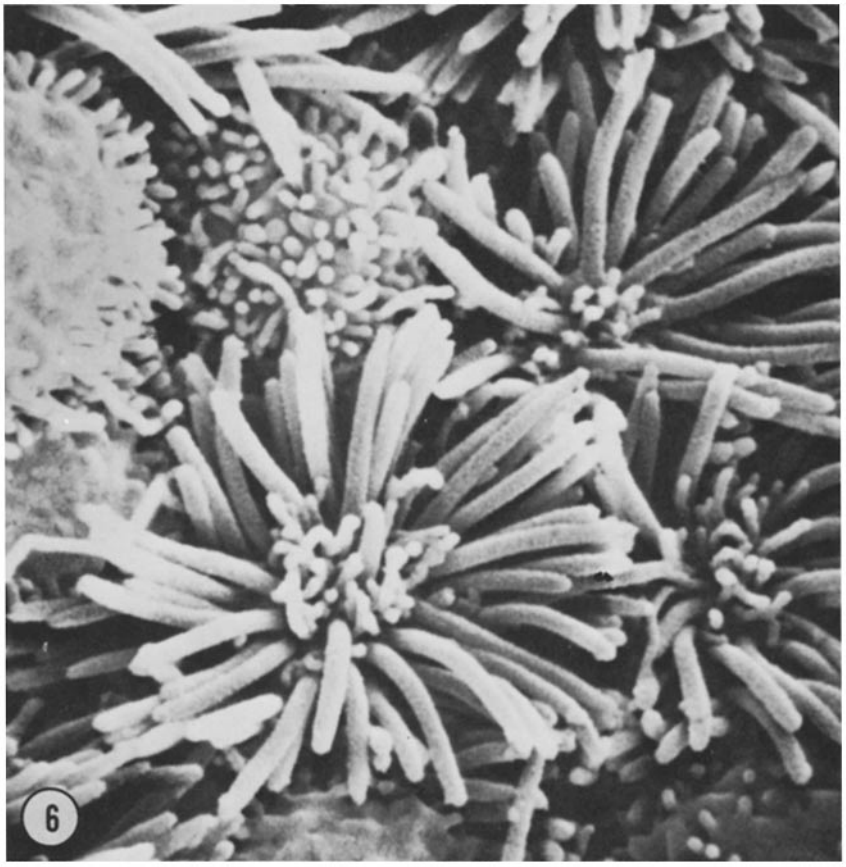


FIGURE 6 A higher magnification of an area in Fig. 5, arrow. The daisy-like appearance is produced by the initial emergence of cilia at the periphery of the cell, the shorter, less mature cilia being found at the center. Here, many slender microvilli will also be present. × 14,500.

From the present scanning electron microscope observations it appears that although the sequence of appearance of differentiating ciliated cells is random over the surface of the epithelium, cilia are generated in each individual cell in a more orderly pattern first at the periphery of the cell and then at its center. This manner of ciliary emergence and elongation in the mouse oviduct as observed by SEM is consistent with the observations obtained by transmission electron microscopy of centriole formation before ciliary development. In the suckling mouse oviduct, centrioles, which give rise to the necessary number of basal bodies for the final induction of cilia, begin to develop in the cell shortly after birth by a complex morphogenetic process involving precursor structures which do not resemble the mature organelle but may be packaged microtubule protein (7, 9, 22). All the centrioles required by the cell are not formed at one time but are generated in waves. As a wave of centrioles is produced, these migrate to the cell surface where they attach to the membrane, form the recently described ciliary necklace (19, 20), grow axonemes, and function as basal bodies. While this movement is taking place anoher replicative cycle is occurring in the cytoplasm. What was not always apparent from thin-section analyses and transmission electron microscopy, due to sampling limitations, was that the first replicative-cycle of basal bodies oriented themselves at the periphery of the cell, thus determining the nucleating sites for the polymerization of the ciliary microtubules, and that subsequent groups moved progressively toward the center. It is the microtubular elements of the basal bodies that elongate to produce ciliary growth. This occurs at the

same time that the cell membrane grows to accommodate the ciliary axonemes. Similar patterns of centriole and ciliary development have been reported in other vertebrate ciliated epithelia (1, 6, 12, 21, 23). With scanning electron microscopy we now see more clearly that the process is not synchronous in neighboring cells, nor is it synchronous within one cell, since waves of emerging cilia overlap during development.

Regardless of the length of the cilium, as observed in the SEM preparations of developing mouse oviduct, the relationship of cilia to the cell membrane at the base does not vary. No bulging areas, for example, have been seen at the base of growing cilia (e.g. Fig. 3 and 4), which implies that whatever axonemal material is being synthesized, it is being transported immediately to the distal tip and does not accumulate at the base. This suggests, although it is not proof, that cilium growth occurs by the assembly of microtubular precursors at the distal tip of the organelle (3, 17, 18, 25). The microtubule protein required for ciliary growth can be synthesized very rapidly and is assumed to be present in a soluble pool in the cytoplasm (14, 18, 24).

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