ORIGIN OF NECKLACE PARTICLES IN THYMIC CILIATING CELLS (1)

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ABSTRACT The formation of ciliary necklaces during ciliogenesis in a thymic cyst was observed in freeze-etched replicas. The necklaces first appear as clusters of particles arranged in concentric circles on a flat area of the cell membrane. As soon as the cilium begins to grow, the particles move to the periphery.

The necklace is characteristic of the ciliary membrane observed in freeze-fracture replicas (Satir and Gilula, '70). It is found in Protozoa (Dirksen et al., '71; Sattler and Staehelin, '74), Metazoa (Dirksen et al., '71; Gilula and Satir, '72; Tani et al., '74; Boisvieux-Ulrich et al., '77; Inoue and Hogg, '77) and in the flagella of the spermatozoa of certain species (Bergstrom and Henley, '73). In vertebrates, the necklaces are located at the base of the ciliary membrane. They are composed of chains of closely packed particles arranged in five or six parallel strands separated from one another by a distance of approximately 30 nm.

It is not yet known how ciliary necklaces are formed during ciliogenesis. This study shows that the particles of which they are composed are present on the cell membrane before the ciliary shaft is formed.

MATERIALS AND METHODS The thymus of young mice was fixed in 0.1 M phosphate-buffered 2% glutaraldehyde and then cryo-protected with phosphate-buffered 30% glycerol. Small pieces of tissue were mounted on gold specimen holders, frozen in super-cooled nitrogen, and fractured and shadowed in a
Balzers BAF 301 apparatus (Balzers A. G., Liechtenstein) according to the technique of Moor and Mühlethaler ('63). After thawing of the tissues, the replicas were cleaned in sodium hypochlorite, rinsed in distilled water, mounted on uncoated copper grids and examined in a Philips EM 301 electron microscope.

RESULTS Small epithelial cysts composed of mucous and ciliated cells were observed frequently in the thymus of the mice. At birth, these cysts contained poorly differentiated cells, some of which developed into ciliated cells (fig. 1).

The early stages of ciliogenesis were observed best in the replicas of the cell's apical face (fig. 2). Circular areas with a diameter comparable to the diameter of a ciliary shaft (200 - 250 nm) were found between the 75-nm wide crater-like structures corresponding to the fractured microvilli. Some of these circular areas were flat and comprised of aggregates of particles often arranged in concentric circles. Others formed dome-like structures, in which the particles were located on the periphery and absent at the apex of the dome. When a cilium developed from one of these dome-shaped areas (fig. 3), the particles formed 3 or 4 rings at the base of the cilium. These rings correspond to the strands of the ciliary necklace.

DISCUSSION Unicellular or pluricellular ciliated cysts are frequently observed in the thymus (Hoshino, '62; Sebuwufu, '68; Cordier, '74, '75). Their presence simply indicates the capacity of the embryonic foregut to produce ciliated cells (Kapa et al., '76).

The ciliary necklaces are located at the base of the cilium where the central tubules are absent and the peripheral tubules are joined to the ciliary membrane by Y-shaped filaments or radial linkers (Gilula and Satir, '72; Boisvieux-Ulrich et al., '77). Ciliary necklaces are particularly
numerous in the analogous region of the connecting cilia of the rods of the retina (Röhlich, '75). The region in which the necklaces are found appears rigid when examined in thin sections (Cordier, '75) and is bristling with beads arranged periodically like the strands of a necklace (Röhlich, '75; Boisvieux-Ulrich et al., '77).

The number of strands varies with the species: two to four in mollusks (Gilula and Satir, '72) and protozoa (Speth and Wunderlich, '72; Wunderlich and Speth, '72), six in the trachea of the rat (Gilula and Satir, '72) and the guinea pig (Inoue and Hogg, '77) and five to seven in the oviduct of the quail (Boisvieux-Ulrich et al., '77). The authors have observed only three to four necklace strands on the developing cilia of the mouse thymic cysts. This small number of parallel strands is probably specific either to the tissue used or to the mouse, since four parallel strands of particles were observed consistently on the completely formed cilia of the intracellular thymic cysts in the adult mouse (personal observation).

Several functions have been attributed to the ciliary necklaces (Gilula and Satir, '72; Boisvieux-Ulrich et al., '77). They do not appear to be involved in the mechanism of the ciliary beating movements, since they are also observed on non-vibratile sensory cilia (Kratzing, '72; Röhlich, '75). The hypothesis that they play a role in membrane permeability does not explain the regular arrangement of their particles even before the appearance of the ciliary shaft.

Sites with a high affinity for Ca\(^{++}\) have been observed in this area in paramecia (Fisher et al., '76), and the ciliary necklace particles may be equivalent to the spherical intra-membranous particles of the sarcoplasmic reticulum implicated in Ca\(^{++}\)-transport (MacLennan et al., '71; Tillack et al., '74). If the necklace particles are calcium pumps or sites of ionic
permeability and are able to control the direction of ion flow, the low concentration of intra-cellular Ca\(^{++}\) just beneath the circular areas with concentric particles may promote the formation of the microtubules and, thus, favor ciliogenesis. In vitro, the polymerization of tubulin is, in fact, prevented in the presence of Ca\(^{++}\) (Weisenberg, '72). The hypothesis that they serve to anchor the doublets is based on their relation with the radial linkers. According to this view, the necklaces would be involved in maintaining the architecture of the cilium (Boisvieux-Ulrich et al., '77). Their very early appearance and their immediate arrangement into regular strands suggests their involvement in positioning the doublets and in maintaining their particular arrangement during the development of the cilium.

The appearance of the necklaces is probably induced by the basal body. As soon as the basal corpuscle reaches the cell membrane, the membrane becomes thickened at this point and covered with spicules (Martinez Martinez and Daems, '68). This thickened area, visible in thin sections, no doubt corresponds to the circular areas observed in freeze-etched replicas.

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**FIGURE LEGENDS**

1 Freeze-fractured thymic epithelial cyst. Ciliogenesis is occurring in the two cells bordering the lumen (L) of the cyst. X 22,000.

2 P-face of apical cell membrane showing the early stages of ciliogenesis. (M.V. Microvilli) 1. Flat circular area, composed of a cluster of particles. Particles nearest the periphery are assembled like the beads of a necklace (arrow). 2. Circular area slightly protruding. The concentric arrangement of the particles is clearly visible. 3. Dome-shaped protrusions. Particles are totally absent at the apex of the dome. X 49,000.

3 Small cilia in the very first stages of growth. A ciliary necklace, composed of 2 or 3 parallel strands (arrows) is seen at the base of the cilia. X 50,000.
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REFERENCES

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