THE RELATIONSHIP OF CILIA WITH CELL DIVISION AND DIFFERENTIATION

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INTRODUCTION

The existence of a reciprocal relationship between the presence of cilia on cells and mitotic activity was originally proposed by Henneguy (1898) and by Lenhossék (1898). Evidence has been presented recently that lends support to this hypothesis. Dingemans (1969) has determined the frequency of cilia on cells in the rat adenohypophysis before and after stimulation of mitotic activity, and has found that "most of the cells contain a cilium, but that cells which can be considered to have a high mitotic activity contain one less frequently." Rash et al. (1969) have determined that cilia are to be found during cardiac differentiation in the embryonic chick, and concluded that "the abrupt transformation from mitotic replicative tissue to nonmitotic structuring tissue is correlated with the disappearance of centrioles and the formation of cilia." It may be concluded from these papers, and from many earlier papers, that in some cell types there is a negative correlation between the presence of cilia and mitotic activity. However, it has not been demonstrated that a negative

correlation of this kind need be found in all cell types.

We have been examining ultrastructural changes that occur in the mesenchyme during morphogenesis of the embryonic chick limb. In these studies we have observed that cells in the mesenchyme of the limb bud of a stage 19 chick embryo (Hamburger and Hamilton, 1951) often possess cilia. Previous studies of changes in rates of cell division in the limb bud have demonstrated that 100% of the cells in the limb mesenchyme of a stage 19 embryo are dividing (Janners and Searls, 1970; Searls and Janners, 1971). We conclude that cells in the division cycle (in G_1, S, or G_2) may have cilia.

MATERIALS AND METHODS

Limb buds from stage 19 White Leghorn embryos were fixed in glutaraldehyde buffered with phosphate to pH 7.8 (Coleman et al., 1969) for 1 hr at 20°C. After a brief rinse in the same buffer, they were postfixed for an additional hour in 1% osmium tetroxide in the same buffer at pH 7.8 and embedded in Araldite (R. P. Cargille, Laboratories, Inc., Cedar Grove, N.J.). Sections approximately 0.1 μ thick were stained with saturated uranyl acetate in 50% ethanol and lead citrate (Venable and Coggeshall, 1965). Photographs were taken with either a Zeiss EM9A or a Philips EM 300 electron microscope.

RESULTS

When mesenchyme cells in the limb bud of a stage 19 embryo were examined with the electron microscope, cilia were frequently seen. All of these cilia were found to be stereocilia of the diplosomal type similar to those observed in cartilage (Scherft and Daems, 1967), heart (Rash et al., 1969), and the very early somite (Trelstad et al., 1967). Cross-sections (Fig. 1) contained the nine outer doublets but no inner core of tubules. Longitudinal sections (Fig. 2) always showed one centriole at the base of the cilium and usually a portion of the second centriole. The shaft was irregular in width and the internal organization was disrupted as compared with the motile cilium. The tubules, which were continuous with the distal centriole (basal body), entered the outgrowth in a parallel fashion. However, they began to diverge only a short distance distally, and not all of them were as long as the cilium. When the tip was seen, it was bulbous. In many cases the cilium emerged from a pouch in the cytoplasm (Fig. 3). Other cilia appeared to be enclosed within a pouch of cytoplasm; but since

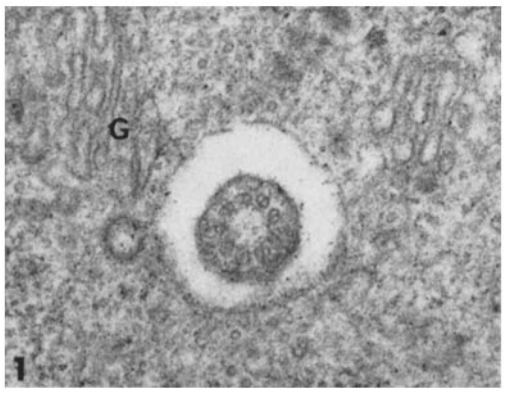

FIGURE 1 Cross-section of a cilium in a stage 19 limb bud mesenchyme cell. All nine outer doublets can be identified but there are no central tubules. The cilium lies close to the Golgi region (G) of the cell. × 88,290.

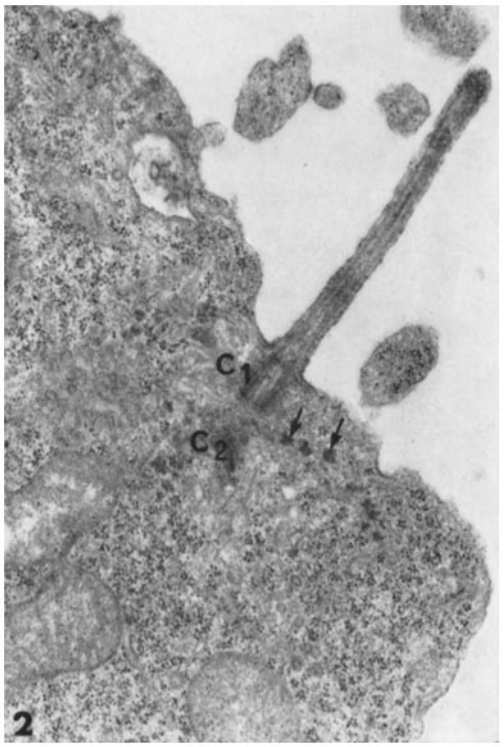

FIGURE 2 Longitudinal section of a typical stereocilium on the surface of a mesenchyme cell. Tubules extend from the distal centriole (C_1) almost to the tip of the cilium. Satellites (arrows) lie between the distal and tangentially cut proximal (C_2) centrioles. × 27,720.

they were not followed in serial sections, it is not clear whether they represent developmental stages or tangential sections through longer cilia.

In an attempt to discover the frequency of cilia, the number of cilia and the number of nuclei were determined in six random grid areas (cf. the procedure of Dingemans, 1969). In these six areas, we found 478 sections through nuclei and 39 sections through cilia with a basal body, or a ratio of one cilium with basal body to 12.3 nuclei. Since the basal bodies are approximately 0.3 μ in diameter and the nuclei approximately 4.0 μ in diameter, we would expect to find in 0.1 μ sections that each basal body was in three sections and each nucleus in 40 sections. If each cell had one cilium with basal body, we should expect to find one cilium with basal body for each 13 nuclei. These calculations are liable to many sources of error; it cannot be concluded that every cell in the mesenchyme of a stage 19 chick limb bud has a cilium, but it can be concluded that the majority of the cells have cilia. However, cilia were not observed in cells that were in mitosis.

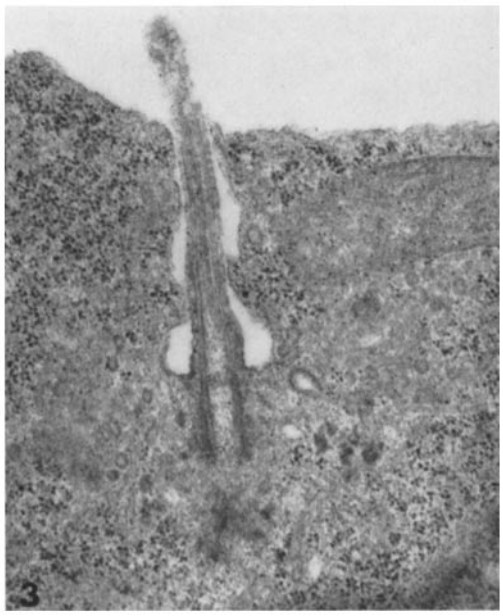

FIGURE 3 Longitudinal section of a cilium within a pouch, cut through the center of the distal centriole. The proximal centriole was just grazed by the section. Note the lack of uniformity in the width of the shaft. × 31, 140.

CONCLUSIONS

It has been proposed that a reciprocal relationship exists between the presence of a cilium on a cell and cell division (originally by Henneguy [1898] and by Lenhossék [1898] and more recently by Dingemans [1969] and by Rash et al. [1969]). We have observed that better than half of the cells in the mesenchyme of the limb of a stage 19 embryo have a cilium. In previous experiments we have observed that all of the cells in the mesenchyme of the limb at stage 19 become labeled with tritiated thymidine (Janners and Searls, 1970; Searls and Janners, 1971). We have calculated that all of the cells in the limb mesenchyme of a stage 19 White Leghorn embryo are dividing with a generation time of 10.1 hr (Searls and Janners, 1971). Within 1 hr after an injection of tritiated thymidine, 50–55% of the cells in the limb mesenchyme become labeled. This was the shortest period of time that reliably produced labeling of the limb mesenchyme cells, and indicates that approximately 50% of the cells in the limb mesenchyme are in the S phase of the division cycle (Searls and Janners, 1971). Since more than half of the cells in the mesenchyme have cilia, and more than half of the cells in the mesenchyme are in the S phase of the division cycle, it is clear that cells in the S phase of the division cycle have cilia. Cells in other phases of the division cycle (other than M) may also have cilia.

Cartilage and muscle first appear in the limb approximately 48 hr after stage 19. The mesenchyme cells of the stage 19 limb are completely regulative; they become cartilage or muscle, depending upon their position in the limb (Searls, 1967). We have demonstrated that many of the limb mesenchyme cells from a stage 19 embryo have cilia. The cells of the early limb mesenchyme are not the only cells in the very early embryo that have cilia. Cilia have been observed in the sclerotome of the stage 11 somite (Trelstad et al., 1967), in the epiblast and hypoblast of the preprimitive streak stage, and in the mesodermal layer during the primitive streak stage (Rash et al., 1969). The rate of cell division in these tissues is not known, but all of the cells in the sclerotome of the somite of the stage 19 embryo become labeled after injection of tritiated thymidine (Searls and Janners, unpublished observations). Thus all of the cells in the sclerotome of a stage 19 embryo are in the division cycle. It

would be expected that all of the cells in the somite of a stage 11 embryo would also be dividing. The presence of cilia on cells that are somewhere in the division cycle and have not yet attained an "adult" state of differentiation is not limited to the limb bud.

It is clear from the preceding discussion that cells that are undergoing rapid cell division and that have not yet attained an "adult" state of differentiation frequently have cilia. On the other hand, cells that are postmitotic and that *have* attained an "adult" state of differentiation have frequently been found to have cilia (i.e. thyroid [Fujita, 1963; Hilfer and Hilfer, 1966], neural retina [Allen, 1965]). Evidently the presence of cilia may not be taken to indicate either the frequency of cell division or the state of differentiation of the ciliated cells.

Supported by Research Grant 1R01 HD04669-01 from the United States Public Health Service.

Received for publication 2 September 1970, and in revised form 28 October 1970.

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