# ANCHOR FILAMENT BUNDLES IN EMBRYONIC FEATHER GERMS AND SKIN

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## INTRODUCTION

During development of embryonic chick skin, fiber-like processes extend from the region of the epidermal-dermal interface deep into the dermis (1). As judged by phase contrast microscopy, the processes originate from epidermal basal cells that protrude downward into the dermis. Although occurring in younger skin, the extensions are most prominent at the time of feather placode formation, where they invariably extend downward from the centermost cells of the future feather epidermis. The processes are no longer apparent in the same region some 24 hr later, when the same group of cells participates in the initial elevation of epidermis that presages feather morphogenesis. The current report establishes that the extensions are extracellular fibers composed of fine filamentous subunits.

## MATERIALS AND METHODS

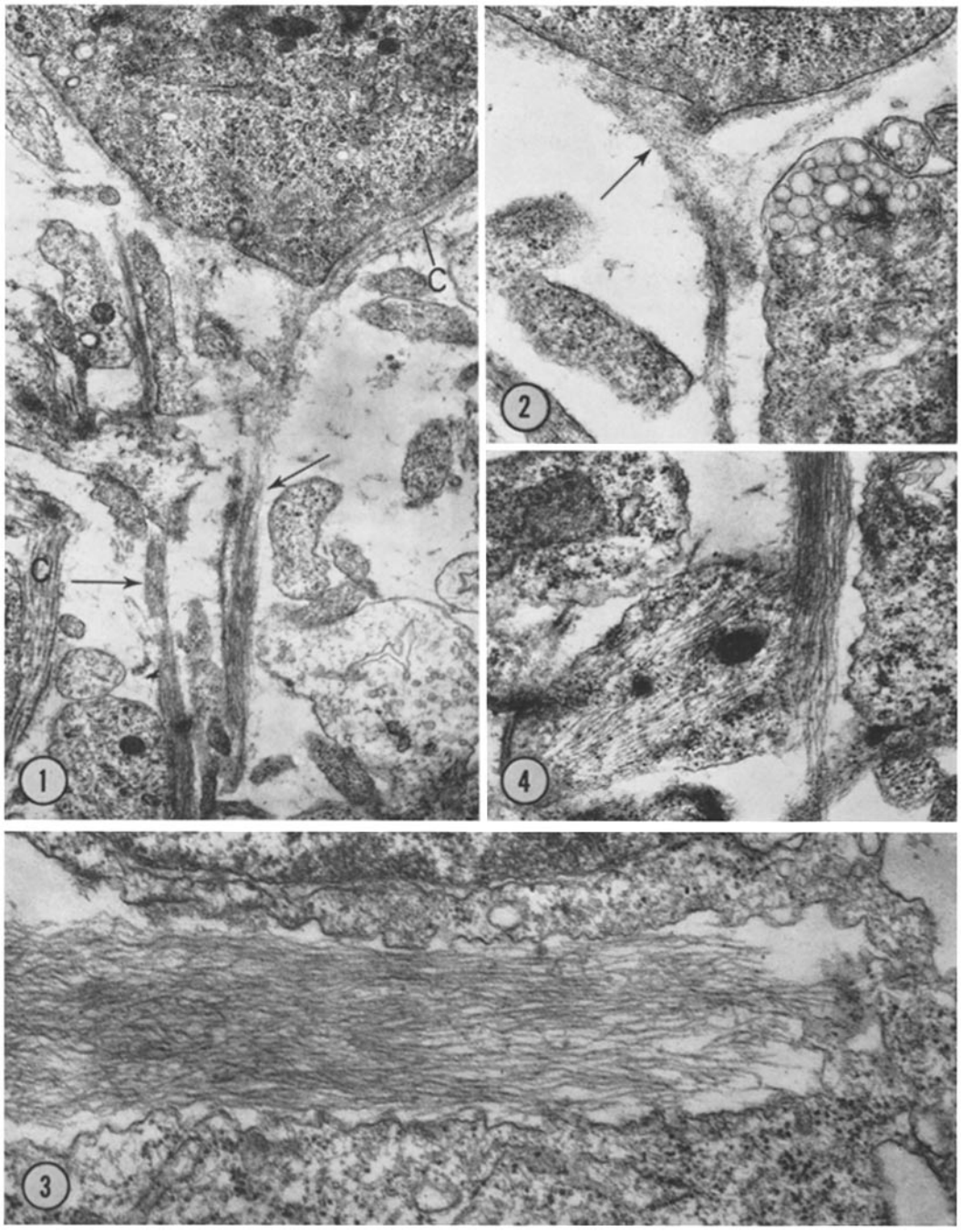
Back skin from stage 29–34 chicken embryos (2) was dissected in Tyrode's solution or a 1:1 mixture of Tyrode's and horse serum. After placement on the upper surface of a Millipore filter (to aid in orientation during sectioning), the tissues were fixed in ice-cold glutaraldehyde and postfixed in veronal-buffered osmium tetroxide (3). During the dehydration procedure, the tissues were stained with phosphotungstic acid (3).

Epon-embedded material was cut into thin sections (about 1200 A) using a Porter-Blum MT-2 Ultramicrotome. The sections were stained for 30 min in a saturated solution of uranyl acetate in 50% ethyl alcohol and then in lead citrate (4) for 5 min. Micrographs were taken using an RCA EMU-3F electron microscope. Both feather germ and surface epidermis regions were examined at times before and during formation of epidermal feather placodes (1).

## RESULTS

The fiber-like processes of chick skin are composed of extracellular bundles of filaments. The bundles invariably take origin from epidermal basal cells whose proximal region juts downward into the dermis (see 1 for complete description). In feather placodes, sets of two or three bundles are frequently seen in the central cell region (Fig. 1), whereas single bundles are more common in non-feather regions.

Bundles of anchor filaments vary in thickness, being ca. 0.7–0.95 $\mu$ in diameter distally and ca. 0.5 $\mu$ or less proximally in the dermis. Bundles extend into the dermis in straight lines, in most circumstances (Fig. 5). On occasion, they appear to alter direction and bend around the edge of fibroblasts; alternatively, they may appear to pass over or under cytoplasmic regions of the cells. Frequently, fibroblasts seem to be oriented in relation to the fibers; their long axes may even parallel the fibers, whereas neighboring cells invariably are aligned parallel to the epidermal surface. Surface regions of mesoderm cells near the fibers are often




FIGURES 1 and 2 Survey views showing anchor filament bundles extending toward the lowermost projections of epidermal basal cells. Note the suggestion (arrows) that the filaments turn laterally and parallel the lower cell surface. Collagen (*C*) with 620-A repeat periodicity is seen in Fig. 1. × 14,000.

FIGURE 3 A bundle of anchor filaments in close apposition to the surfaces of dermal condensation fibroblasts. × 31,500.

FIGURE 4 Fine filaments extending at an oblique angle to an anchor filament bundle. It is unclear whether these filaments are within or on the surface of the mesenchymal cell. Note that, to the left, two collagen fibers are passing over the end of the same cell. × 14,000.

in intimate association with the filaments (Figs. 3, 6). At various points along the bundles, lateral filaments or groups of filaments extend laterally into the intercellular spaces (Figs. 4–6). Unfortunately, despite much searching, unequivocal identification of the proximal end of a fiber bundle has not been achieved.

Individual filaments that make up the bundles extend to the region of the basement membrane complex, and there, as judged by a few favorable observations, seem to turn laterally to parallel the lower basal cell surface for an undefined distance (Fig. 2).

Each anchor filament bundle is composed of large numbers of filaments that average 140–150 A in lateral dimension. The filaments twist in three dimensions as they extend downward (Fig. 3), so that it has been impossible to determine the length of an individual filament. The longest linear dimension measured with surety was 0.9 $\mu$. Normally, no repeat unit periodicity is associated with the fibers. In Figs. 6 and 7, the most extreme cases of periodicity are noted: in the former, beadlike repeat units are seen with approximately 300-A periodicity in individual filaments that lack lateral registry; and in the latter, lateral registry occurs, with a repeat distance of ca. 680 A. Collagen with 620-A repeat periodicity is often seen in the same thin sections as the usually aperiodic anchor filaments.

## DISCUSSION

Palade and Farquhar (5) have recently reviewed the types of anchoring fibers found in vertebrate skin. The filaments reported herein for embryonic chick skin would seem to be grossly similar to the 100–200-A diameter fibers which have been seen in, or in association with basement membranes, collagen fibers, or elastic fibers (5–12). The unique aspect of the chick filaments is, however, their aggregation into thick bundles that apparently take origin from the basement membrane and extend for up to 60 $\mu$ into the dermis (1). The chemical nature of the filaments is unknown, although since Karrer (8), most authors have considered extracellular filaments of similar dimension to be related to the collagen-reticulin system. Our single good observation of an anchor filament bundle possessing ca. 680-A periodicity is suggestive that, under some circumstances, the beaded (ca. 300-A repeat distance) filaments can aggregate in lateral registry in a manner similar to collagen subunits (13).

Developmentally, the bundles are of most interest because of their presence at the center of the early feather germ just at the time when inductive tissue interactions may be occurring (1). Basal cells attached to such fibers are elongated and extend deeper into the dermis than any other epidermal cells of the skin. Whether this results from action of the fibers is unknown. Perhaps significant is the unusual alignment of dermal fibroblasts with relatively long expanses of plasma membrane in close apposition to the filament bundles. Finally, the fate of the bundles is of interest, because a few hours after the stages treated in this paper, the elongated basal cells at the center of each feather placode shorten, and take part in initial outgrowth of the feather germ. Despite much searching, to date, the bundles have not been found in such regions, although laterally around each feather germ they remain intact.

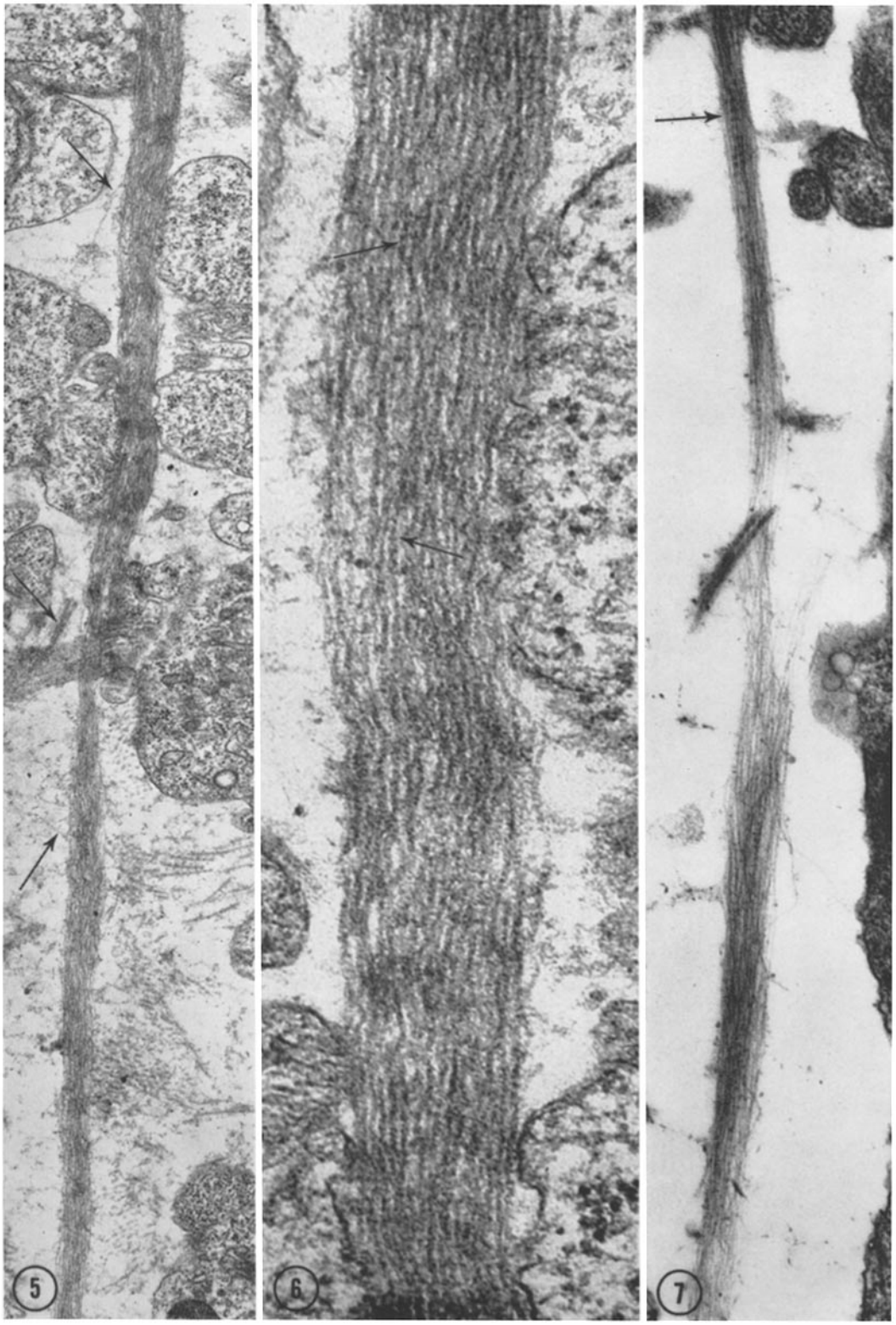
## SUMMARY

The fibrous processes of embryonic chick feather germs and skin are described as being extracellular bundles of filaments (each filament ca. 150 A width) that extend from the basement membrane deep into the dermis.

FIGURE 5 A long stretch of anchor filaments passing through the dermis and showing suggestions of lateral filaments joining the main bundle (arrows). Collagen is seen at the lower right. × 14,000.

FIGURE 6 Higher magnification of part of the bundle seen in Fig. 5. Individual filaments average 150 A in width, and in places give hint of staining periodicity (arrows). × 66,500.

FIGURE 7 The most extreme case (arrows) of periodicity due to lateral registry of filaments that has been seen in an anchor filament bundle. Repeat distance averages 680 A. × 21,000.



The initial observations of this study were made by Dr. Frances Kallman prior to her death in 1966. The work has been completed and manuscript prepared by Mrs. Evans and Dr. Wessells. Research was supported by United States Public Health Service Grants GM-10060 and GM-08719.

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