# Peculiarities of Proliferation of Epidermal Cambial Cells in Mouse Skin

**T. M. Yavisheva, S. D. Shcherbakov, and I. V. Dubinkin**

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The long axis of epidermal cambial cells determining the direction of their division is determined before the beginning of division and is oriented perpendicularly, but not parallel, to the basal membrane, as in other basal cells. As a result, only one of the two newly formed cells adheres to the basal membrane and at the expense of traction forces detaching one cell from the other and elastic force of the basal membrane is formed as a maternal cell and stays in the focus of multiplication. The other cell, turning around under the effect of traction forces perpendicularly to the maternal cell, cardinally changes the direction of its long axis and is polarized parallel to the basal membrane like other cells. This cell becomes the daughter cell, its shape with the "head" and "tail" allows its rapid migration into other rosettes for differentiation.

**Key Words:** *epidermal cambial cell; division axis; formation of maternal and daughter cells*

The study of the viability of stem cells, mechanisms of their proliferation is important for medicine, because impairment of cell homeostasis processes can induce a tumor process. The capacity to retain their population at a certain level is a fundamental characteristics of stem cells. C. Leblond [7] noted that only basal layer cells undergo division in stratified epithelium of rat esophagus and the daughter cells have no definite function. They can both move towards the surface and differentiate into cells of the external layers or remain in the basal layer and form new stem cells, or one cell can move upward and differentiate, while the other stays to perform the role of a stem cell. It is unknown whether the daughter cell will differentiate or remain a stem cell; any of them has equal potentialities for developing by this or that route. Some authors [4,5,8] believe that stem cells in mouse small intestinal epithelium more often divide asymmetrically, as a result of which one stem cell remains in the focus of multiplication, while the other daughter cell differentiates. This division of a stem cell is due to accidental distribution of the stem cell-specific gene into maternal cell. In symmetrical division (both cells are stem cells) the number of stem cells should increase. Presumably, stem cells under different conditions can switch over from one type of division to the other, thus maintain their number in small intestinal crypts. One "extra" stem cell can produce 64-128 cells. A mechanism for stabilization of stem cell number is spontaneous apoptosis, which was discovered by the authors in the area of stem cells location.

Asymmetry or polarity of daughter cells, resultant from division of the stem cell, is due to factors determined by organization (architecture) of this cell [1].

Hence, there is no consensus about the peculiarities of stem cell proliferation. We studied the morphology and function of epidermal cambial cells during their division.

## MATERIALS AND METHODS

Growth factor neupogen (Hoffmann-La Roche) was used for stimulation of cambial elements of the skin epidermal basal layer. The study was carried out on 14 male intact BALB/cj mice (20 g). Experimental

N. N. Blokhin National Center for Cancer Research, Russian Academy of Medical Sciences, Moscow

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animals ($n$=10) received a single subcutaneous injection of 120 μg/kg neupogen. Neupogen was diluted to a required concentration in 5% glucose solution and subcutaneously injected to animals at 10.00. Controls ($n$=4) received no agent.

Ear tissue specimens were daily collected in animals during 5 days. Tissue fragments were put in EDTA solution (pH 7.4) and incubated at 37°C for 3.5 h. After this treatment the epidermis was completely detached from the derma. Total epidermal preparation was stained with iron hematoxylin by Heidenhein's method. Plane preparations were examined under an optic Opton microscope at ×1000.

## RESULTS

It was previously shown [3] that neupogen (growth factor) stimulated the proliferation of cambial cells situated in the center of the structural morphofunctional unit of the epidermal basal layer — in the rosette consisting of 6-8 peripheral cells and 2-3 central cells.

In our study one small round cambial cell with a large round dark nucleus surrounded by a narrow rim of the cytoplasm was detected in the center of rosettes in the basal layer of skin epidermis in all experimental mice on days 2 and 3 after neupogen injection. Cambial cells were rarely seen in experimental animals on days 4-5 and in the controls throughout the entire observation period (0-1 per visual field).

Analysis of the morphology and functions of these cells in all samples showed a dynamic picture of their functioning. Proliferation of epidermal cambial cells differs from that of other basal cells. Examination of plane preparations of the epidermis showed that peripheral cells in rosettes and their nuclei were elongated. When these cells divide, two daughter nuclei lie in the same plane parallel to the basal membrane during the telophase (according to a known Hertwig's rule, an egg and any cell in general usually divides perpendicularly to their long axis, along which the division spindle is positioned [2]). Hence, the long axis of these cells and the division spindle, determining the direction of their division, are parallel to the basal membrane.

Another picture is observed during division of the central cambial cells. Two daughter cells formed as a result of mitosis lie one under the other and so the second cell can be seen only due to an accidental turn of the epidermal tissue in a fold (Fig. 1, *a*). Hence, the cambial cell division axis is directed perpendicularly to the basal membrane, but not parallel to it, as is the case with other basal cells, and is determined before the beginning of division. As a result, only one of the two formed cells remains on the basal membrane. The cytokinesis of these cells is also special. Two formed cells remain connected by a bridge for some time, this bridge, in contrast to other basal cells, being situated not in the centers of the touching surfaces of these cells, but at the distant periphery.

Cytokinesis is an intricate process involving the entire cellular cortex [6,9-11]. Global cortical contraction and equatorial collapse of the cell take place during cytokinesis. As a result, oppositely directed traction forces, generated beyond the equatorial area, tear the cell into two parts along thinned equator [6,11].

In our study oppositely directed traction forces, dividing the two cells carry away weakened equatorial areas of these cells inside each of them causing their invagination (Fig. 1, *c*). Later the basal membrane becomes more important for the formation of divided cells. As one of the cells lies on the basal membrane, it is exposed to the elastic force of the membrane as an elastic base. Therefore, the cell is more and more tightly pressed to the basal membrane and is flattened when coming in contact with it, which increases the radius of its curvature, and the cell acquires a canoe-like shape (Fig. 1, *d*). The cells with this very shape can generate endogenous electricity [3]. Chromatin density in them sharply increases due to invagination, and the nuclei become darker. This cell remains in the center of the rosette as maternal cell, with only its shape deformed; its initial spatial disposition can be restored. For more tight connection of this cell to the basal membrane, a finger-like protrusion forms at one of its ends with a compact cytoplasm crown, touching the membrane (Fig. 2, *a*).

The formation of the other cell, not contacting with the basal membrane, is different. The radius of its curvature is less than that of maternal cell, as this cell, when detached from maternal cell, meets no such a mechanical barrier as the basal membrane. Later the traction forces separating one cell from the other turn over this cell perpendicularly to the maternal cell at the expense of compact connection between the cells, left at the far periphery. As a result, the cell with the lesser radius of curvature is compactly inserted in the semispherical shape of the maternal cell, thus blocking the polarization of this latter one along its main axis (Fig. 1, *b*, *c*). The second cell is polarized parallel to the basal membrane, as the direction of its long axis changes cardinally during its turn (Fig. 1, *c*, *d*). Invagination remains at a small area, as a result of which a compact "head" and narrow body ("tail") form, that is, the cell acquires an elongated shape (Fig. 2, *b*). This shape permits the cells move rapidly; cells separated from maternal cells move to the centers of other rosettes for differentiation (become daughter cells).

Thus, cells different by their morphology and functions develop from two morphologically similar cells,

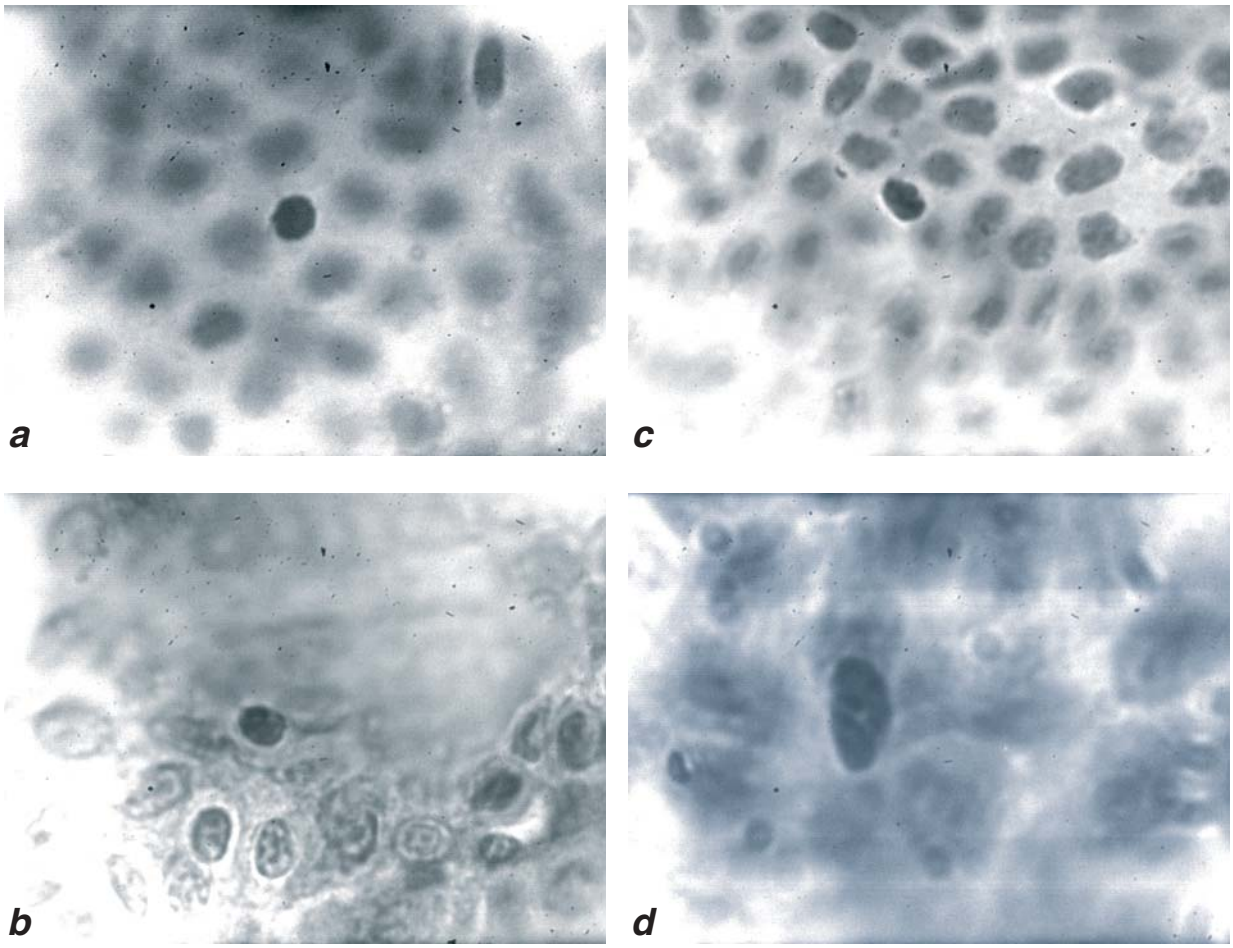


**Fig. 1.** Stages of transformation of maternal and daughter cells, resultant from division of cambial cell. Hematoxylin staining, ×1000. *a*) one cell under the other, part of underlying cell is partially seen; *b*) daughter cell turns around perpendicularly to maternal cell; *c*) polarization of daughter cell parallel to the basal membrane, blockade of maternal cell polarization along its long axis; *d*) maternal cell of a canoe shape with flattened surface turned to the basal membrane and polarized daughter cells are parting.

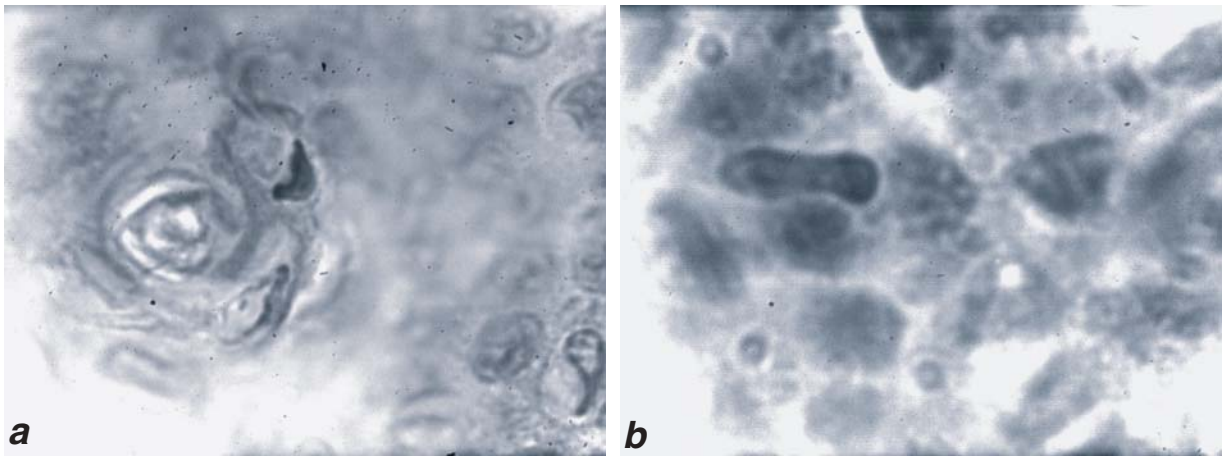


**Fig. 2.** Separated maternal and daughter cell. Hematoxylin staining, ×1000. *a*) canoe-shaped maternal cell forms finger-like protrusion with flattened crown of the cytoplasm adhering to the basal membrane; *b*) daughter cell with formed compact "head" and narrow body ("tail").

emerging as a result of cambial cell division. One of these cells forms as the maternal and stays in the focus of multiplication, the other is a daughter cell and differentiates. Determination of the cambial cell axis to the basal membrane before division and specific features of its cytokinesis promote this process.

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