Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 5763–5766, September 1984
Cell Biology

Lumen formation and redistribution of inframembranous proteins during differentiation of ducts in the rat mammary gland

(microvilli/desmosomes/end-buds/mammary development)

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Contributed by Renato Dulbecco, June 6, 1984

ABSTRACT **During the development of the rat mammary gland, ducts are formed from end-buds, which contain the stem cells. In this process a lumen is formed in the semisolid mass of the end-bud, and the cells acquire polarity. We have studied this process by following the localization of three inframembranous proteins present in the cells of both end-buds and ducts: microvillin, the microvillar protein p80, and the desmosomal plaque protein p205. We find that the development of ducts is accompanied by a redistribution of these proteins, which in immature parts of the end-buds are found together in the cell. Microvillin and p80 go together to the apical pole of the cells, in contact with the lumen, whereas p205 goes to the basal surface, in contact with cells of the myoepithelial lineage. The acquisition of polarity occurs at the same time as a lumen begins to form by local gaps between cells. It seems likely that the redistribution of the inframembraneous proteins is the consequence of the localization of surface glycoproteins that affect in opposite ways the adhesion between the cells.**

The rat mammary gland is made up of a system of branching ducts containing two main types of cells: lumenal—i.e., lining the lumen—and outer cells, related to myoepithelial cells. In young virgin animals in which the gland is incompletely developed the tips of the ducts are terminated by enlarged end-buds. At their distal end the buds are made up of loosely connected cells, without a lumen; at the proximal end where the duct begins, the cells are more tightly connected and form an irregular lumen. In the ducts the cells are held together by junctions, such as zonula occludens.

We have studied the differentiation of cells in the end-buds and ducts. The main tools have been immunological reagents, such as monoclonal antibodies to cultured mammary cells and to cytokeratins. We have shown that the end-buds contain the stem cells for the growth of the ducts as well as cells representing intermediate stages of differentiation both in lumenal and myoepithelial direction (1, 2). How the lumen is formed in the solid cell mass of the end-buds is not clear.

A monoclonal antibody (9B16-12) raised against rat mammary cells allows new insight into the process of lumen formation. This antibody recognizes a protein of 200 kDa, for which we have proposed the name microvillin (3). The protein is present in cultures of mammary cells, in which it has been characterized. In these cells microvillin is an inframembranous protein present, in association with other proteins, in microvilli. We show here that the distribution of microvillin in cells of end-buds and ducts of the rat mammary gland undergoes changes correlated with the formation of the lumen. The changes concern not only the cell types in which the protein is expressed but also the part of the cell in which it is localized. By using the monoclonal antibody it is possible to follow the redistribution of components of the outer layer of the cytoplasm that accompanies the development of cell polarity and lumen formation.

In this study we have also used antibodies to two other proteins with inframembranous localization: a protein of the brush border, which is also present in the microvilli of mammary cells (p80) (4), and a component of demosomal plaques (p205) (5). We show that they also change localization within cells as they differentiate. The pattern of redistribution of p80 is exactly the same as that of microvillin, whereas that of p205 differs, taking a complementary path.

MATERIALS AND METHODS

Animals. Sprague–Dawley rats, either 3 or 7 weeks old, were used. Cryostat sections were cut at 5 μm thickness from whole fourth and fifth glands frozen at −30°C.

Reagents. Rabbit antisera to the microvillar 80-kDa protein was generously provided by A. Bretscher (4), to the demosomal plaque 205-kDa protein by D. R. Garrod (6), and to collagen IV by L. A. Liotta. Monoclonal antibody 9B16-12 specific for microvillin has been described (3).

Immunofluorescence. Immunofluorescence was carried out by the sandwich technique as described (1).

RESULTS

In normal mammary glands of 3- or 7-week-old rats microvillin is restricted to the epithelial cells of both ducts and end-buds. In the ducts it is localized at the apical surface of the lumenal cells (Fig. 1). In the end-buds it has an irregular distribution (Fig. 2). No staining for microvillin is found in cells at the tips of the end-buds, which contain the presumptive mammary stem cells (1), nor in cells farthest from the lumen, which are at early stages of mammary differentiation (1). In intermediate parts of the end-buds closer to the lumen microvillin is distributed over large areas of the surface of many cells. The protein tends to be especially abundant on cells that are separated by small crevices probably related to lumen formation (Fig. 3).

In these cells microvillin has a punctate appearance similar to that seen in cultures of mammary cells. This suggests that in end-buds, as in the cultures, the protein is localized in microvilli. Microvillin is not recognizable on the surface of alveolar cells in lactating glands. The distribution of the other microvillar protein, p80, is essentially identical to that of microvillin, both in ducts and end-buds. Images obtained in the same section with the two antibodies in double immunofluorescence completely overlap (data not shown).

The desmosome plaque protein, p205, has a different distribution. It is present on all cells throughout the end-bud from the presumptive stem cells to the cells lining the lumen. Often the plaques form double rows, suggestive of desmosomal formation. The fairly large separation of the plaques suggests that many of these desmosomes are imperfect (Fig. 4). In ducts of 7-week-old animals p205 dots are infrequent in lumenal cells. They are localized mostly at the border be-

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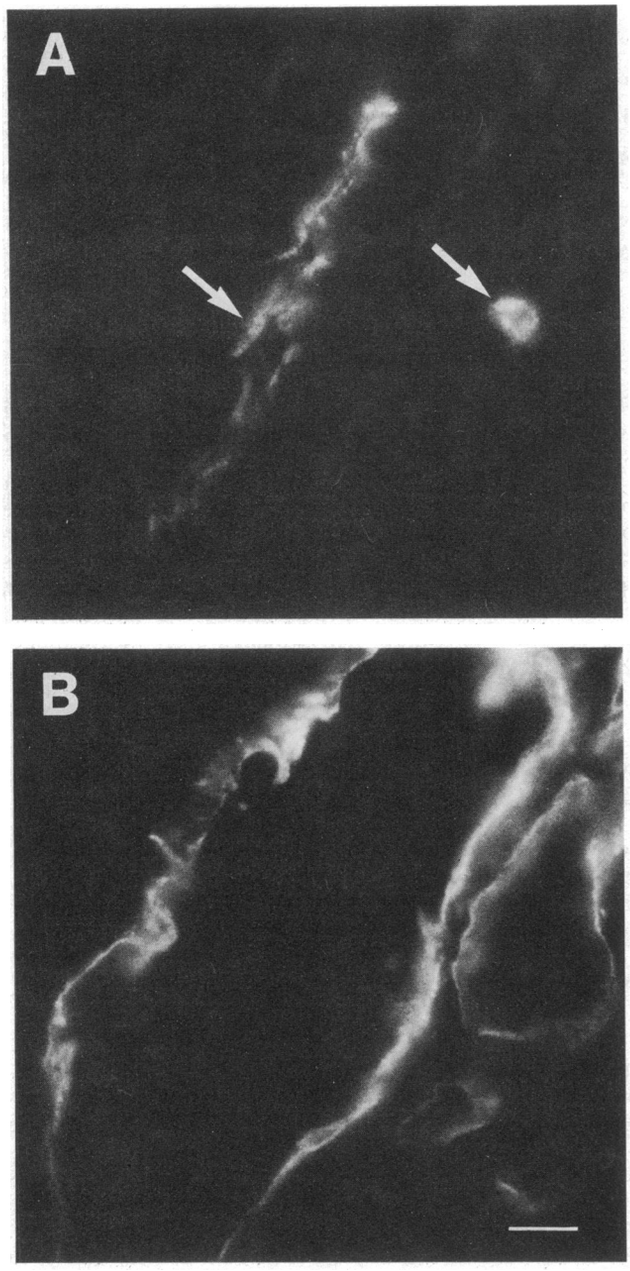

FIG. 1. Longitudinal section through a duct of the mammary gland of a 7-week-old female rat stained with both antibody 9B16-12 and rabbit anti-collagen IV serum, followed by a staining with a fluorescein-labeled goat anti-mouse Ig and a rhodamine-labeled goat anti-rabbit Ig serum. Photographed in double immunofluorescence. (*A*) Antibody 9B16-12 stains the apical surface of the cells lining the lumen of two ducts (arrow), one sectioned lengthwise and the other crosswise. (*B*) Collagen IV identifies the basement membrane that outlines the periphery of the duct. (Bar = 10 μm.)

tween the lumenal and the outer (myoepithelial lineage) cells and are usually single (Fig. 5). Plaques are also seen in lactating glands at the lumenal–myoepithelial border.

In end-buds, by using double immunofluorescence, microvillin and p205 are frequently seen within the same cell and often within the same part of the cell (Fig. 4). In the ducts the two proteins, when present in the same cells, are usually at separate locations. Microvillin is at the apical surface of lumenal cells, facing the lumen, whereas p205 is usually at the border between the lumenal cells and the outer cells, at the opposite end of the cell from microvillin (Fig. 5).

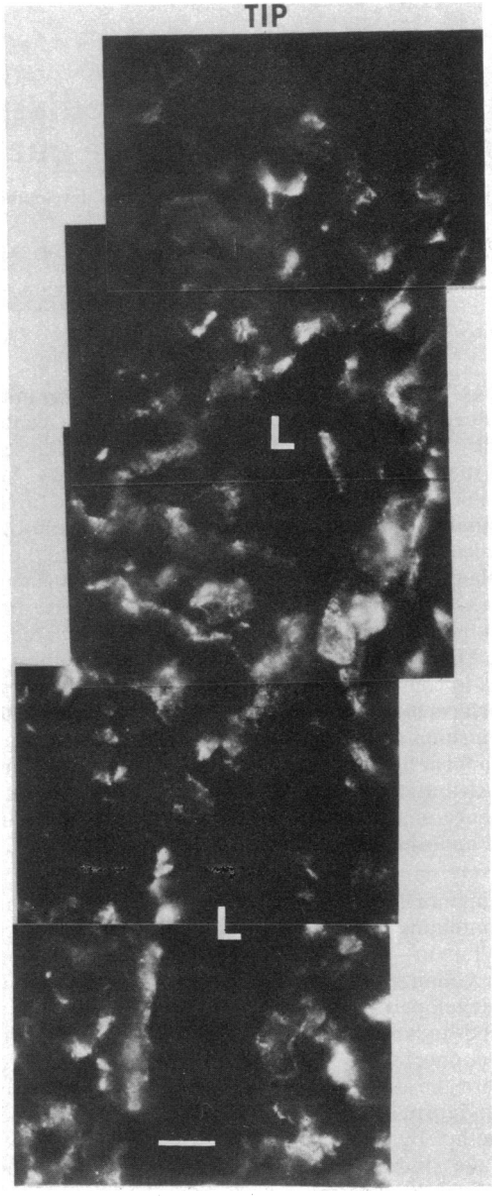

FIG. 2. Section through an end-bud from the tip to the base, where a fairly definite lumen begins to form (L). It is made up of five contiguous fluorescence photographs of a cryostat section of the mammary gland of a 3-week-old female rat stained with antibody 9B16-12 using a fluorescein-labeled goat anti-mouse Ig serum. The photographed area is all within the end-bud. (Bar = 20 μm.)

DISCUSSION

We have shown that three submembranous proteins located in the outer layer of the cytoplasm undergo a characteristic redistribution within the cells during the differentiation of presumptive mammary stem cells into the mature cells lining the ducts. In this process we see two events: one is the appearance of the proteins in cells formerly lacking them; the other is the subsequent redistribution of the proteins to specialized parts of the cells. During this differentiation two other phenomena take place: the cells develop polarity and a lumen is formed in the semisolid mass of end-buds. Two microvillar proteins (microvillin and p80) become segregated together, whereas a desmosome plaque protein (p205) undergoes a different redistribution.

In the immature cells of the end-buds the two types of proteins are often found close together in the same part of the cell, but as differentiation proceeds, they become segregated

FIG. 3. A part of the photo of Fig. 2 at higher magnification showing diffuse and punctate staining with antibody 9B16-12 and its localization where there are gaps between cells. (Bar = 10 μm.)

to opposite parts of the cells. The microvillar proteins become localized to the apical surface of the cells lining the lumen, whereas the plaque protein localizes mainly at the basal surface, in contact with the outer cells of the myoepithelial lineage. The lumen is formed by the accretion of small gaps between cells. The exposed surface of cells lining these gaps is rich in microvillar proteins. These parts of the cells at first also contain desmosomal plaque proteins, but, as differentiation proceeds, they lose them.

Redistribution of components of the cell surface are known to take place in many other situations—for instance, during differentiation of myoblasts and formation of myotubes (7–9) as well as during the cell cycle (10). In these and other cases (11) cell contacts are probably important for bringing about the redistribution.

If this is also true for the mammary cells, the polar distribution of the proteins in the end-buds and ducts might be induced by the formation of the zonula occludens between the cells. One would expect that this mechanism might bring about a polar distribution of all proteins present at the surface of the cells lining the lumen of ducts. This, however, is not the case. In the course of our work we have studied many monoclonal antibodies recognizing antigens exposed at the surface of cells in mammary ducts. None of them except antibody 9B16-12 shows a polar distribution: they tend to be distributed uniformly over all sides of the cells. Formation of a zonula occludens, therefore, does not cause a polar distribution of cell surface components.

A more likely explanation is that the distribution of he inframembranous proteins is secondary to the polar localization of surface glycoproteins with which they interact. A glycoprotein interacting with the cytoskeleton of microvilli has been isolated from the intestinal brush border (12), and a polar localization of glycoproteins has been observed in various organs (13–17). Especially relevant to this point ar studies on the budding of enveloped viruses from various surfaces of polarized epithelial cells in culture. Viral glycoproteins can assume a polarized distribution that is different for certain viruses, such as influenza and vesicular stomatitis (18, 19). The polarized distribution is a property of the viral glycoproteins themselves, rather than of the submembranous matrix proteins with which they associate in the virions (19). The primary polar distribution of the viral glycoproteins may be a function of the transport mechanism by which they reach the surface membrane (20). The matrix proteins, in all likelihood, find their location according to the previous localization of the glycoproteins, unless they use an independent but equally polarized mechanism.

The possibility that the proteins we have studied find their localization by establishing connections with previously localized transmembrane proteins is in accord with several observations. In end-buds microvillin becomes localized at sites where there are gaps between cells. The formation of these gaps must depend on properties of the surfaces of the two neighboring cells. Microvillin could not fulfill this func-

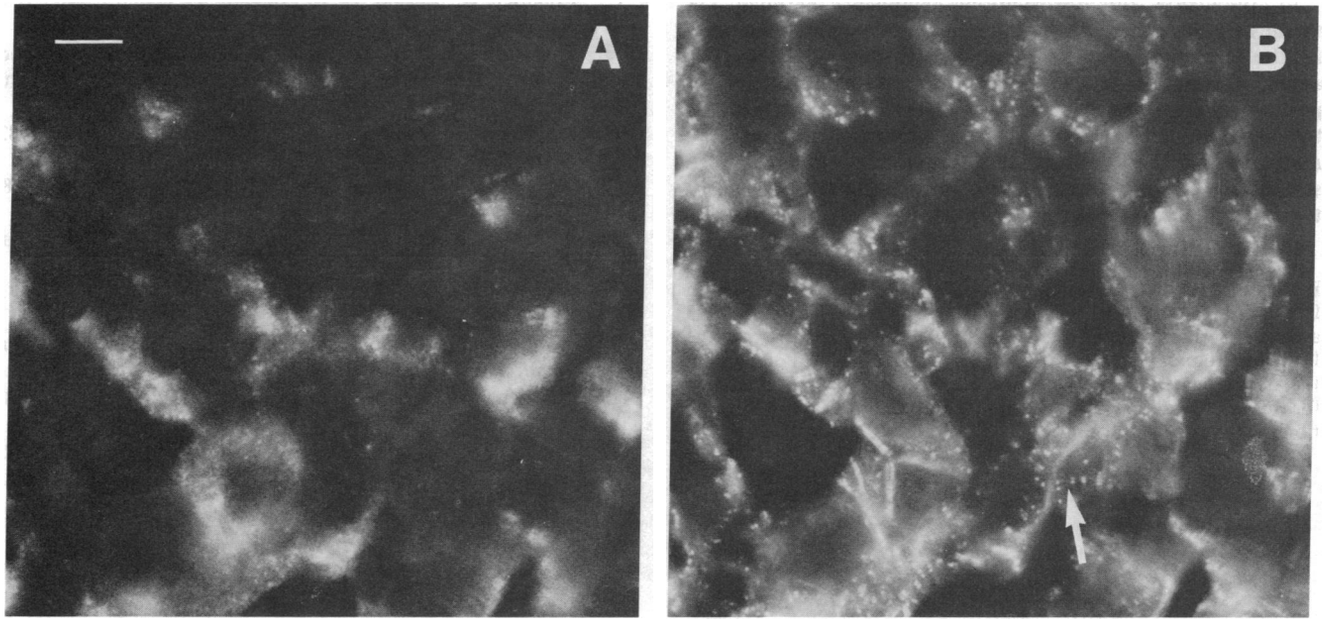

FIG. 4. Double immunofluorescence with antibody 9B16-12 (*A*) or guinea pig antiserum to demosomal plaque 205-kDa protein (*B*). Section through an end-bud of a 3-week-old female rat. An arrow points to plaques in register but with large separation. (Bar = 10 μm.)

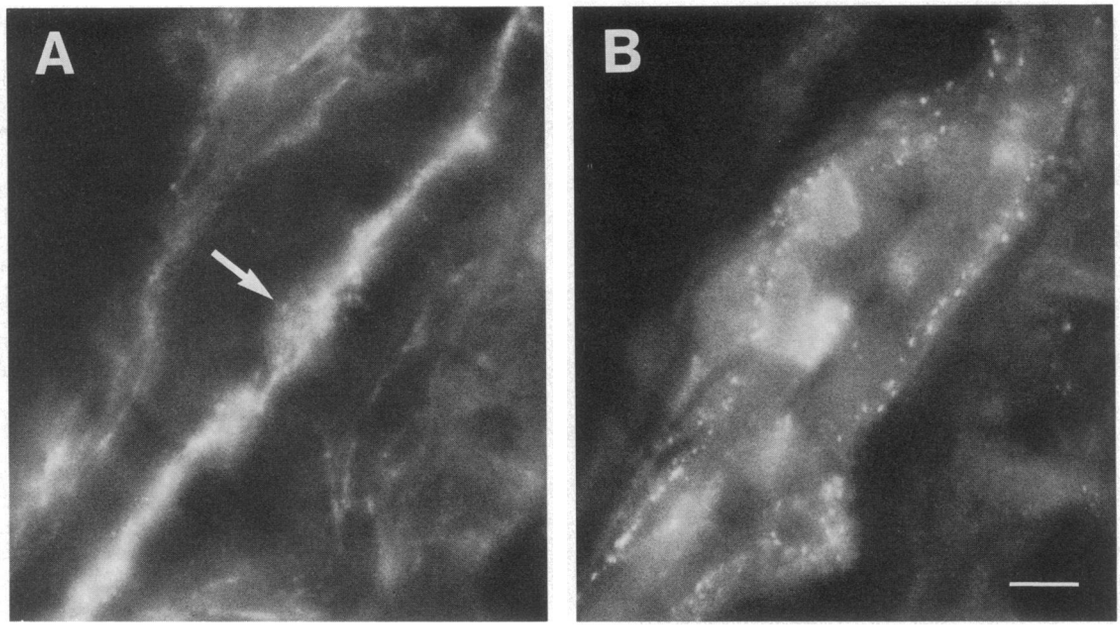

FIG. 5. Double immunofluorescence of a longitudinal section of a duct stained with antibody 9B16-12 (arrow) (*A*) or guinea pig antiserum to desmosomal plaque 205-kDa protein (*B*). (Bar = 10 μm.)

tion because it is not exposed at the cell surface. Even stronger is the case for the localization of the desmosmal plaque protein, which is always found in pairs of plaques in register on two adjacent cells. Often the cells are not in intimate contact and the separation of the plaques is unusually large. Presumably they are imperfect or incomplete desmosomes. The localization of the plaques is likely to be determined by bridges of transmembrane proteins and intercellular proteins spanning the space between the cells (6). Formation of desmosomes, more or less complete, may be important for the development of polarity and lumen formation. Desmosomes appear to have a transient function in the end-buds because their number becomes much reduced in mature ducts, where the lumenal cells are held together mainly by the zonula occludens. Most of the plaques originally present in the end-buds probably disappear.

In conclusion, our observations establish a pathway for the development of polarity in mammary epithelial cells and for the formation of a lumen in the solid end-buds. The first event is probably a polar redistribution of glycoproteins exposed at the cell surface, which causes the adhesion of the two adjacent cell surfaces. Where adhesion fails, lumen formation begins. Localization of microvillar proteins at these sites and exclusion from them of desmosomal plaque proteins are likely to be secondary parallel events.

We gratefully acknowledge the gift of antisera by Drs. A. Bretscher (p80), D. R. Garrod (p205), and L. A. Liotta (collagen IV). This investigation was supported by Grant 1-R01CA21993 awarded by the National Cancer Institute and by grants from the Hammer Foundation and the Samuel Roberts Noble Foundation, Inc. This research was conducted in part by the Clayton Foundation for Research, California Division. R.D. is a Senior Clayton Foundation Investigator.

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