

Electron Microscopy of the Intercellular Junction of Frog (*Rana temporaria*) Skin

J.S. Yoon, S.H. Chang, and K.D. Choi

Electron Microscope Laboratory, Yonsei University, College
of Medicine, Seoul, Korea

개구리 피부의 세포접착부에 관한 전자 현미경적 연구

윤 종 식 · 장 승 한 · 최 금 덕

(연세대학교 의과대학)

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초 록

개구리(*Rana temporaria*) 상피조직의 세포간 접촉부위를 전자현미경으로 관찰하였다. 새로운 절편방법을 시도하여 동 부위의 초미세구조를 밝혔으며, 세가지 세포간 접촉방법 중 하나인 데스모솜(Desmosome)은 상피세포 원형질막을 따라 절서있고 빈번하게 배열되어 있음을 처음으로 밝혀냈다.

본 연구 및 관찰결과와 관련하여 피부 암세포의 성장기 전 과의 관계를 논의하였다.

Introduction

Recent electron microscopic observations have revealed the nature of the contacts between cells and the mechanism of their cohesion. The unit membranes of adjoining cells are usually separated by a distance of 150~2000Å. The demension of this intercellular space remains extremely constant regardless of whether the cell boundaries are relatively straight or elaborately convoluted. The space appears to be a layer of thin mucopolysaccharide (Fawcett, 1964).

On the other hand, there are at least three local differentiations of opposing membranes between cells. Although from organ to another, the type differs in the exact arrangement, most epithelial cells show three local types of intercellular attachment(Farquhar and Palade, 1963).

In an effort to develop a consistant terminology for the various junctional complex, Farquhar and Palade (1963) proposed 1) *Zonular occludens* for the

close junctions or tight junctions which obliterate the intercellular space, 2) *Zonular adherens* for the band like attachments (intermediate junction), and 3) *Macular adherens* for the disc-shaped site of adhesion now commonly called desmosomes.

In this study we have again observed a number of frog epithelia, sectioned in many ways, such as perpendicularly and horizontally. Since recent technical developments in electron microscopy allow a more detailed visualization of the fine structure within the attachment sites, we are able to demonstrate the ultrastructural morphology of the area of the attachment of the cell membrane, and for the first time that the desmosomes are arranged regularly between all cell-to-cell attachments.

Materials and Methods

Frog skin of *Rana temporaria* was used for electron microscopic study of the details of the cellular attachment of epithelia. The tissues were fixed for 1~1 1/2 hours in 1 per cent osmium tetroxide (OsO₄)

buffered at pH 7.4 with phosphate buffer. Specimens were subsequently dehydrated in graded ethyl alcohols, and finally embedded in Epon 812.

Thin sections prepared from all the blocks were doubly stained first in uranyl acetate, then in lead citrate.

Cut sections were examined directly and immediately after staining, and micrographs were taken at original magnifications of 3,300 to 20,000 with a Hitachi HU-11E, operating at 75 Kv with a double condenser and a 50 μ objective aperture.

Observations

In general, four basic cell types can be distinguished in the frog epidermis (Fig. 1). As Farquhar and Palade (1964) reported, it is apparent that the frog epidermis consists of one or two outer layers of partially cornified squamous cells (*Stratum corneum*) about three layers of polyhedral cells (*S. granulosum* and/or *S. spinosum*), and a basal layer of cuboidal cells (*S. germinativum*) on a basement membrane which marks the epidermal-dermal border. The *S. germinativum* cells have the usual set of subcellular components, and well-developed tonofilaments grouped in bundles and attached to either desmosomal or basal plates (desmo-epidermal desmosome). As the cells differentiate flatten, the tonofilaments bundles become more voluminous and more tightly packed in the outer layers. Through each skin layer up to and including the *S. corneum*, only the cytoplasm retains its usual components, but the other cellular structures decrease.

In the frog epithelia studied, three morphologically distinct types of surface modifications are seen along the sides of adjoining epithelial cells; a finding which has been already described in other amphibian epithelia (Fig. 2). Beginning adjacent to the epithelium and descending through the skin layers along the intercellular spaces, at first there is a tight junction, an intermediate junction, and finally typical desmosome.

The tight junction (*Zonula occludens*) which is the first element of the junctional complex, is located immediately below in the line of reflexion of the plasma membrane which extends from the outer edge (apex) to the inner (basal) area of the cell

body. This tight junction appears at an extremely narrow (-80\AA cross) area of the intercellular space. In general, there appears to be no visible fibrillar differentiation in the subjacent cytoplasmic matrix along this element of the junctional complex, although sometimes, a thin accompanying zone of diffuse densification is encountered.

Intermediate junction (*Zonula adherens*), which is the second element of the junctional complex, is located immediately below the tight junction, and above the desmosome. The intercellular space of the intermediate junction is occupied by a homogeneous, apparently amorphous material of moderate density. The zone of cytoplasmic densification along this junctional element has a tightly matted fine fibrillar texture with most of the fibrils running parallel to the cell membrane.

The desmosome (*Macular adherens*), the third element of the junctional complex, is formed by two plaques each one in the plasma membrane of the adjacent cell. They are located at a distance of 0.3 μ or more from the inner (basal) end of the intermediate junction. These circular (disk-shaped) dense intercellular plaques lay adjacent to each other on each inner side of the two facing plasma membranes, and their diameter is variable ($\sim 4,000\text{\AA}$). They are least numerous and complex in the *S. germinativum*, and reach their fullest development and highest frequency in *S. granulosum*.

It is very interesting to note that desmosomes are seen at frequent and regular intervals along the cell boundaries in both the cells of the *S. germinativum* and *S. granulosum*, but the structure of the desmosome is greatly altered on the portion of the membrane in the cells (Fig. 3). This change is characterized by the presence of laminar densities in the intercellular space, by local concentrations of dense amorphous and fibrillar material in the subjacent cytoplasmic matrix. The material linking the two plaques with the adjacent plasma membrane is not visible (Fig. 4).

In addition to the above three morphologically distinct types of modification of the intercellular surface, there is another method of intercellular attachment, namely that of finger-like projections (deep elaborate ridges) between cells. These pro-

junctions may contain a variety of cell organelles (Fig. 3). It is very interesting to note that while (mostly the attachment between cells is of one type desmosoms) there is a combination of more than one usually type depending on the mechanical and physiological requirements of the attached cells.

Numerous tonofilaments converge toward the desmosome. The arrangement of these is most clearly discerned in the micrograph (Fig. 4). It is apparent that most tonofilaments approach the disk-shaped plaque, then loop reversely in a wide arc and turn back going into the main tonofilament bundles of the cell. The majority of filament looping occur at a set distance (400-600Å) from the plaque.

It should be noted that the intercellular spaces of most of the epithelial cells vary considerably both in extent and configuration. The space are frequently distended in the *S. spinosum* and become particularly complex toward the base of the *S. germinativum* because of frequent cellular interdigitations (Fig. 3). Although intercellular spaces are rather variable in size, (most have the usual ~200Å gap) a few are closed by *zonular occludens*, or some are provided with desmosomes. In the epidermis, as a whole, the intercellular spaces form a complex and probably continuous network. The channels of this network acquire their largest dimensions in the *S. spinosum* and *S. germinativum*. Externally this intercellular space system is closed by *zonular occludens*. Internally the space system is separated from the interstitia of the dermis by a continuous basement membrane, and communicates with the dermis through relatively narrow but apparently patent gate.

Discussion

In the past there have been three concepts as to how cells were held together: First an adhesive intercellular cement, secondly by a specific attraction like that of antibody for antigen, or thirdly by the formation of chemical bonds between the membranes (see Fawcett 1964).

Farquhar and Palade (1964 and 1965) reported that the amphibian skin consists of an epidermis and an underlying dermis containing blood vessels, glands and connective tissue elements. They pointed out that,

in larvae, the epidermis comprises only two-three layers, whereas, in adults, it consists of one or two outer layers of partially cornified squamous cells (*Stratum corneum*), three to five layers of cuboidal or polyhedral cells (*S. granulosum* and *Stratum spinosum*), and a basal layer of columnar cells (*Stratum germinativum*) on a basement membrane that marks the dermoepidermal boundary. Our findings in adult frog are well in agreement with theirs.

Moscona (1960) demonstrated that specific histotypic reaggregate capabilities for dissociated cells serve to emphasize recognitive adhesive potentials which many cells possess. Some experiments (Stainberg 1964) showed that the requisite selectivity is best explained on the basis of a general adhesive principle common to all or most cell surfaces. According to this view, some weakly adhesive sites available in a given cell population at a given time can be explained as some subtle differential in cellular aggregative behavior, so that it is still not possible to identify such general adhesive properties with discrete morphological units in or on the cell membrane. Using the electron micrograph, it is hard to detect the structure separating two cells of an epithelium within the electron-dense zone. However Kelly (1966) cited other evidence showing a trypsin-sensitive, calcium-dependent, and occasionally stainable cementing material occupying this intercellular spaces.

It appears probable that the quality, number and distribution of the firm attachment mechanisms at the boundaries of a differentiated cell are related to the proper maintenance of position, morphology, and function of that cell, even though their role in selective adhesion is not clear.

The participation of intercellular filaments in the formation of such strong attachment zone is probably not directly related to the adhesion per se, but rather to the structural support of the cell skeleton at the attachment site. (Kelly, 1966).

Desmosome, the strongest point of contact between epidermal cells, are seen at frequent regular intervals in both *S. spinosum* and *granulosum*, while irregular intervals in the basal body. The desmosome is seen to have an internal structure. Desmosomes of adult frog skin have been considered as a possible cell-to-

cell diffusion path for sodium ions (Ussing, 1965).

Current data on the involvement of separate desmosomes and tight junctions in cell-to-cell permeability, or on synaptic or tight junction adhesive mechanisms governing impulse transmission (Robertson, 1964; Dewey and Barr, 1964) also suggest that significant physiological events may be dependent upon specific attachment structures. Thus cells probably require proximity or attachment for a variety of reasons, both mechanical and physiological. Moreover, conditions of readjustment of intercellular attachment may prevail according to the mechanical and/or physiological demand, or according to the state of differentiation of the cells.

As mentioned above, tonofilaments are generally found singly as well as in bundles (Fig. 4). They enter the attachment disks of desmosome, as the structural units of tonofibrils. Recent studies of amphibian skin have shown that the tonofilaments, rather than ending in the attachment plaque, loop back into the cell interior often making contact with the desmosome (Kelly, 1966). However, this aspect of their organization remains conjectural.

It has been assumed that the tonofilaments course from one desmosome to another within the cell (Charles and Smiddy, 1957) although this has not been well demonstrated. Tonofibrils in *S. spinosum* maintain their attachments to desmosomes, but are distributed much more uniformly here than other cells. Recently some micrographs suggest the presence of filaments coursing parallel to the plaque (Kelly, 1966)

On the other hand, neither the plasma membrane nor the intercellular space show any particular specialization in relation to the invaginations. The space between plasma membrane is wider in relation to the desmosomes and narrower within the zone of the terminal bar (tight junction). The function of the invaginations may be to secure a firmer intercellular relationship, but in case of elaborate infolding it may reflect a means of increasing the cellular absorptive and exchanging surface.

It is worth while to discuss the matter of skin cancer from the view point of cellular adhesion, because the epidermis is entirely cellular, its cells

arise by division in a typical basal layer, and are known to synthesize a limited number of macromolecules (Mercer, 1961 and 1962).

It has long been held (Coman, 1947 and 1954) that cancer cells are less adhesive than normal cells, and there is evidence (Abercrombie and Heaysman, 1953 and 1954) that the surface of cancer cells differs from that of normal with respect to their tendency to cling together on making contact. The present view is that, in differentiating tumours, delay in the development of intercellular adhesion on account of the failure to synthesize adequate amounts of surface adhesive layers, is significant because of its influence on the tissue constructive properties of cells.

The tissue construction power arises mainly from intercellular surface adhesion. The aggregative property being investigated is very complicated. From genetic point of view the inability to produce normal tissue is thought to arise from a failure of the interlocking cellular control mechanisms. These are responsible for ensuring that each constructional component required is present at the necessary time and place.

Summary

Electron microscopy on the skin of young frogs, *Rana temporaria*, has been carried out with particular reference to cellular attachment sites. For the first time now several technical developments allow a more detailed visualization of the fine structure within the cellular attachment sites as well as making it possible to show the ultra-structural morphology of the junctional complexes, and to demonstrate that the desmosomes are regularly distributed around each skin cell, especially in the *S. granulorum*.

The relations of these findings to those of previous investigations concerning the functional organization of the junctional complexes and to the findings in skin cancer from a cellular adhesion view point have been briefly discussed.

References

1. Abercrombie, M., and Heaysman, J.E.M. (1953). Speed of movement of chick heart fibroblasts in

- relation to their mutual contacts. *Exp. Cell Res.*, 5, 111.
2. Abercrombie, M., and Heaysman, J.E.M. (1954). Monolayering of fibroblasts. *Exp. Cell Res.*, 6, 293.
 3. Coman, D.R. (1947). Mechanism of the invasiveness of cancer. *Science*, 105, 347.
 4. Coman, D.R. (1954). Cellular adhesiveness in relation to the invasiveness of cancer: Electron Microscopy of Liver Perfused with a Chelating Agent. *Cancer Res.*, 14, 519.
 5. Charles, A., and Smiddy, F.G. (1957). The tonofibrils of the human epidermis. *J. Invest. Dermat.*, 29, 327.
 6. Dewey, M.M., and Barr, L. J. (1964). A study of the structure and distribution of nexus. *Cell Biol.*, 23, 553.
 7. Farquhar, M.G., and Palade, G.E. (1963). Junctional complex in various epithelia. *J. Cell Biol.*, 17, 375.
 8. Farquhar, M.G., and Palade, G.E. (1964). Functional organization of amphibian skin. *Proc. Nat. Acad. Sci.*, 51, 569.
 9. Farquhar, M.G., and Palade, G.E. (1965). Cell junctions in amphibian skin. *J. Cell. Biol.*, 26, 263.
 10. Fawcett, D.W. (1964). In histology and cytology, *Modern Developments in Electron Microscopy*, Academic Press. N.Y. 257-332.
 11. Kelly, D.E. (1966). Fine structure of desmosomes, hemidesmosomes, and an adepidermal globular layer in developing newt epidermis. *J. Cell Biology*, 28, 51-72.
 12. Mercer, E.H. (1961). In *Keratin and keratinization*, p. 80. Pergamon Press, Oxford.
 13. Mercer, E.H. (1962). The cancer cell, in *Electron Microscopy*, British Medical Bulletin, 187-192.
 14. Moscona, A.A. (1960). In *Developing Cell Systems and their Control*, 18th Symposium of the Society for the Study of Development and Growth, (D. Rudnick, editor), New York, Ronald Press, 45.
 15. Robertson, J.D. (1964). In *Cellular Membranes in Development*, 22nd Symposium of the Society for the Study of Development and Growth (M. Locke, editor), New York, Academic Press, Inc., 1.
 16. Steinberg, M. (1964). In *Cellular Membranes Development*, 22nd Symposium of the Society for the Study of Development and Growth, (M. Locke, editor), New York, Academic Press, Inc., 321.
 17. Ussing, H.H. (1965). *Transport of electrolytes and water cross epithelia*, Harvey lectures, N.Y. and London, Academic Press, Inc., 1.

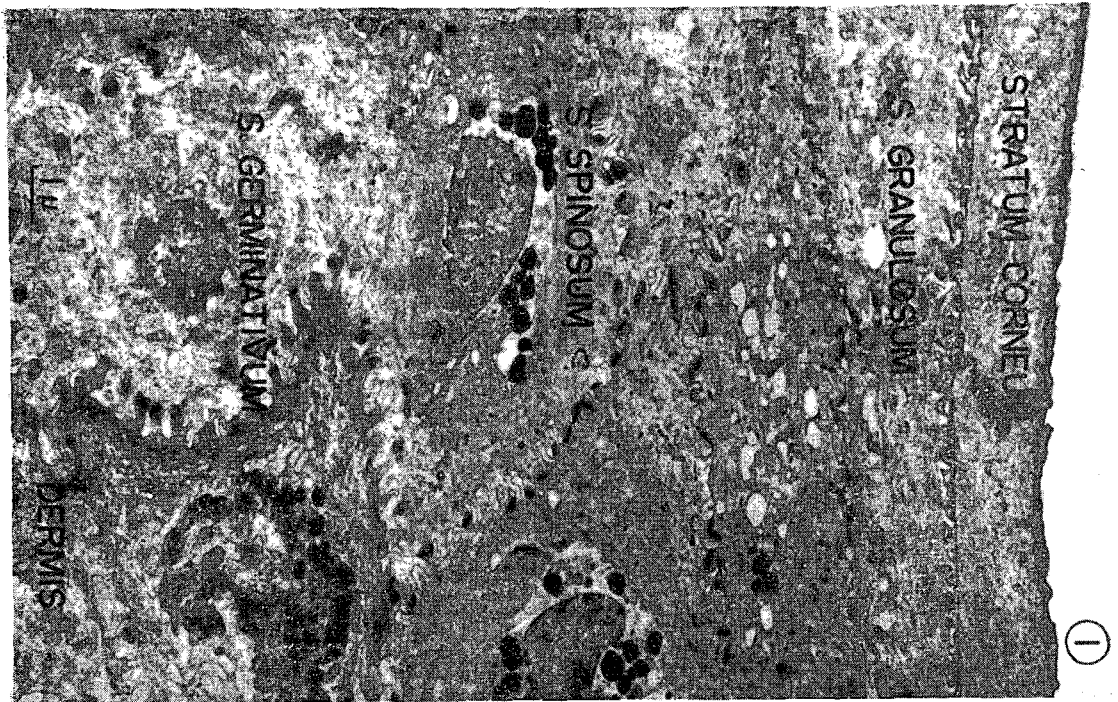


Fig. 1. Electron micrograph of the full-thickness of the epidermis constructed for orientation purpose. $\times 7,500$.



Fig. 2. Adhesive components include a tight junction (T), an intermediate junction (I), and a small desmosome (D). $\times 32,000$

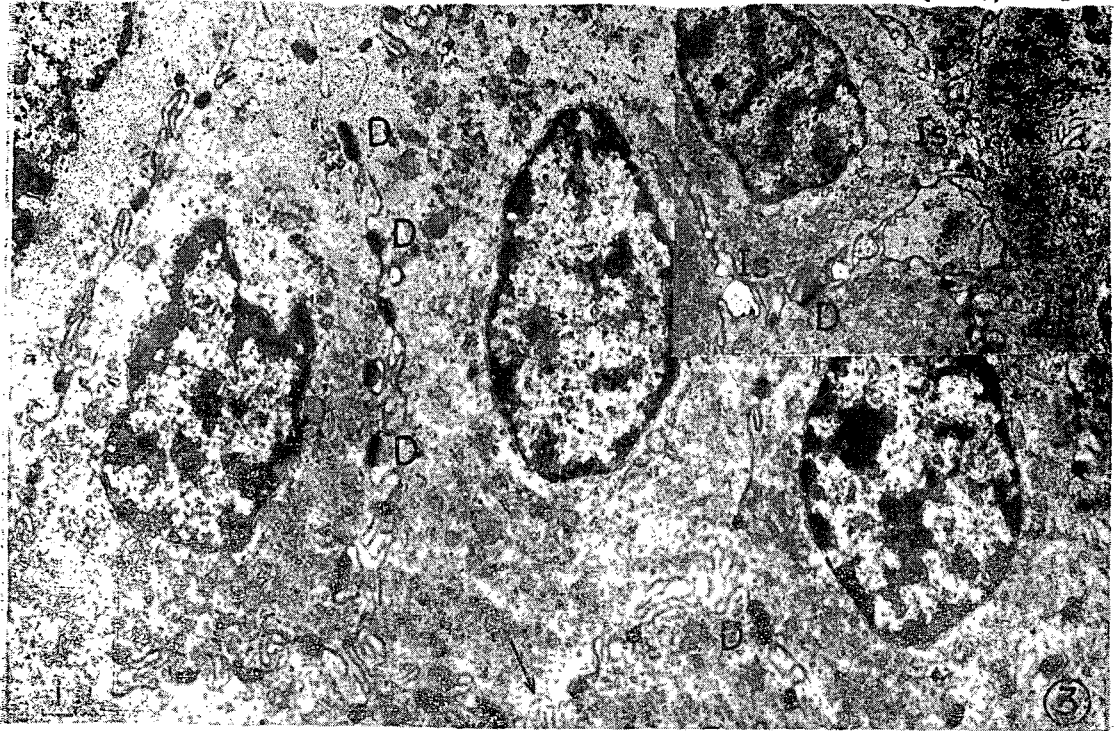


Fig. 3. Group of cells in the *S. spinosum* of a frog skin. Note that desmosomes (D) are arranged in regular intervals along plasma membrane of the cells. The intercellular spaces (Is) and cell invaginations (Ic) are visible (arrows). $\times 8,000$

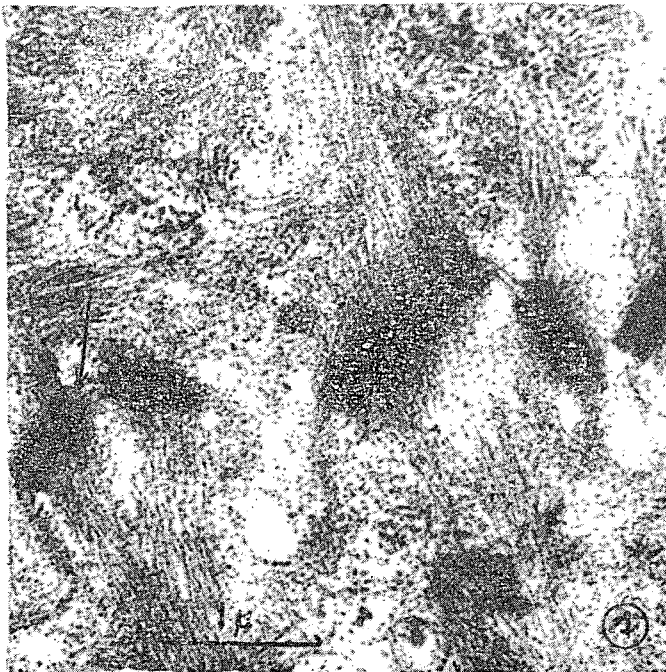


Fig. 4. Desmosomes between adjacent epidermal cells seen in perpendicular to the plane of the desmosome plaques. The cell membranes can be distinguished as single dense lines. The intercellular gap of the desmosome is occupied by moderately dense material (arrow). Tonofilaments appear in cytoplasm of the adjacent cells. $\times 19,500$