Development of Junctions During Differentiation of Lens Fibers

(intramembranous particles/membrane differentiation/low resistance junctions/eye lens)

E. LUCIO BENEDETTI*†, IRENE DUNIA*, AND HANS BLOEMENDAL‡

* Institut de Biologie Moléculaire du CNRS et de l'Université Paris VII, 2 Place Jussieu, Paris VII, France; and † Department of Biochemistry, University of Nijmegen, Geert Grooteplein Noord 21, Nijmegen, The Netherlands

Communicated by F. Jacob, October 7, 1974

ABSTRACT Throughout the differentiation of eye lens epithelium into fibers, an extensive system of intercellular junctions develops. The junctional assembly is initially characterized by the accumulation of 9.0-nm intramembranous particles, forming linear rows in the matching plasma membranes of adjoining fibers. At the final stage of the fiber differentiation, the junctional particles are assembled in geometrically packed arrays. The formation of linear rows and bidimensional lattices of intramembranous particles probably favors reciprocal recognition of cell surfaces and specific cell-to-cell interlocking. Moreover, the existence of a rather rigid lipid core of the plasma membrane of eye lens fiber may promote the clustered distribution of intramembranous particles and facilitate the junctional assembly.

In almost all animal tissues, low-resistance passageways and metabolic cooperation correlate with the presence of "gap" junctions (1-3). Freeze-fracturing has revealed that this type of junction is characterized by arrays of repeating particles associated with the inner hydrophobic matrix of the junctional membranes. Moreover, the arrays of particles are interlocked by a hexagonal lattice of pitted facets, likely originating from the merging of both outer membrane leaflets (4, 5). Although many membrane-mediated functions, such as fusion (6) and interaction of cell surface receptors with specific ligands (7), have been tentatively correlated with the spatial rearrangement and perturbation of the inner membrane particles made visible by freeze-fracturing, circumstantial evidence illustrating the differentiation of plasma membranes during cell contact and assembly of low-resistance junctions is still incomplete.

Some data have been already presented illustrating the junctional features in embryonic and developing tissues (8, 9). From these interesting studies it is, however, not easily apparent whether some plasma membrane aspects identified in freeze-fracturing experiments represent the assembly of "gap" junctions or stages of its breakdown and dilution during organogenesis and tissue differentiation. In the present study, the development of intercellular junctions in the eye lens is investigated. The vertebrate lens has several unique features that make this avascular tissue particularly suitable for the analysis of differentiation processes. In contrast to many other developing tissues, the lens is composed of one type of epithelium which during the entire life of the animal will continuously, and in one direction, differentiate into lens fibers (10). Furthermore, the latter cells are extensively connected by a rather unique type of junction (11, 12).

We present some evidence that in the region of cellular elongation, where the epithelium loses the ability to divide

and differentiates into cortical fibers, the assembly of newly formed junctions is characterized by the progressive accumulation of particles. Initially these inner membrane particles form linear rows. Later they assemble in geometrically packed arrays.

MATERIALS AND METHODS

For freeze-fracturing, calf lenses were fixed for 30-60 min in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde (v/v) in 0.2 M cacodylate buffer.

Occasionally, after fixation the tissue was infiltrated for 1 hr with 25% buffered glycerol at room temperature. Very small fragments, carefully dissected from the zone of cellular elongation and from the lens cortex, were mounted on gold or copper discs, rapidly frozen in Freon 22, and stored in liquid nitrogen.

Balzers apparatuses 360 and 300 were used for freeze-fracturing and platinum-carbon shadowing. During the whole operation, the specimen temperature was maintained at -140° . The cleaned replicas were studied with a Philips EM300 electron microscope.

RESULTS

In replicas of freeze-etched zones of cellular elongation the epithelial cells can be easily recognized because they have a relatively small cuboidal shape with complex interdigitations. Moreover, they contain cytoplasmic vesicular organelles and large elliptical nuclei. The differentiating fibers appear as cellular elements several times more elongated than the epithelium and displaying a rather polygonal contour in cross-fracture. The fibers are arranged in a honeycomb pattern, and the cell surfaces either run for a large distance parallel to each other in close proximity or form local complex convolutions and fingerlike processes.

The cleavage of the nonjunctional plasma membranes produces, as in several other types of cells, two distinct fracture faces which, although they may be in close proximity, are always separated by an intercellular space (Fig. 1). The inner aspect of the membrane leaflet adjacent to the cytoplasm (fracture face A) is covered by a number of randomly dispersed particles with heterogeneous sizes ranging from 4.0 to 9.0 nm in diameter. Occasionally, even in the nonjunctional fracture face A, a few identical 9.0-nm particles exhibit cluster distribution or form linear rows interspersed with the other randomly distributed particle population (Fig. 2). Conversely, in the inwardly directed fracture face of the outer membrane leaflet (fracture face B), only few particles are visible, but numerous small pits and depressions are found (compare Fig. 1). These depressions likely correspond to the em-

† To whom requests for reprints should be addressed.

FIG. 1. Lens epithelial cell in the zone of cellular elongation. The fracture has exposed the A and the B faces of the membrane, and in some regions these two fracture faces are separated by the intercellular space (IS). The latter is obliterated in the junctional regions (J). Note the linear array ($\blacktriangle$) of 9.0-nm particles on fracture face A and the complementary pitted images (Δ) on the B face.

FIG. 2. Epithelial cell in the zone of cellular elongation. The fracture reveals the inner aspect of the leaflet close to the cytoplasm (face A), where two classes of particles are visible. One type consists of particles of heterogeneous sizes (4.0–8.0 nm). The other 9.0-nm particles form linear arrays or polygonal clusters ($\uparrow$). Note a point of junction (J).

FIG. 3. Epithelial cell in the zone of cellular elongation. The fracture reveals a macular "gap" junction characterized by a hexagonal array of pitted images on the B face and geometrical packing of 9.0-nm particles on the A face.

placement of spanning particulate entities, which during the cleavage remain associated with the complementary fracture face A. In differentiating epithelium and in fibers of the zone

of cellular elongation the junctional areas are easily identified. At their level the two adjacent plasma membranes are in close proximity and the intercellular space is occupied by the inter-

locking layer. The fracture, in a step-like fashion, splits the two plasma membranes and simultaneously exposes the outer fracture face A of the membrane leaflet adjacent to the cytoplasm and the fracture face B of the outer membrane leaflet. The latter face exposes the inner aspect of the interlocking device (J, Fig. 1).

Morphologically the junctional domains may vary according to shape, size, and extension of the particle assembly in the junctional membranes and to the proper feature of the interlocking structure. In the elongation zone, close neighbor epithelial cells are connected by typical "gap" junctions. As in other tissues, they are characterized by geometrically packed arrays of particles associated with both fracture faces A of the junctional membranes and by an interlocking hexagonal lattice of pitted facets (fracture face B; Fig. 3).

The most special and elaborated junctional feature that is frequently found connecting epithelium and differentiating lens fibers consists of 9.0-nm particle streams of various length. These linear arrays of particles intersect or closely approach one another, but they are never organized into continuous belts around the cell contour. In some areas small polygonal clusters of 9.0-nm particles are encased by the branching linear arrays (compare Fig. 1).

As the elongation of the lens fiber proceeds, the plasma membrane is characterized by even longer and more elaborated streams of particles scattered over large areas of fracture face A. Furthermore, the latter surface is covered by an impressive number of polygonal assemblies of particles displaying a great variety of size and shape Fig. 4. Many linear arrays of particles and smaller polygonal clusters clearly merge with the periphery of the larger aggregates of particles.

On fracture face B of elongating lens fibers, pitted images and small depressions form linear rows, clusters, and large assemblies. This pattern shows a strict complementarity with the topographic distribution of the particulate entities associated with the junctional fracture face A.

In the lens cortex, where the fibers have elongated and reached a total cell surface that is more than hundred times larger than that of the epithelial cells, the intercellular junctions are extremely developed and characterized by very extensive polygonal particle aggregates (Fig. 5). The junctional particles, although closely packed, very seldom form hexagonal arrays. Consistently, the pitted images coplanar with fracture face B are not arrayed in a regular geometric lattice. Occasionally small macular "gap" junctions are scattered within the large junctional areas.

DISCUSSION

The results of this study provide some evidence that significant changes of the plasma membrane organization develop during the process of cellular elongation in the lens. An essential morphological feature of the plasma membrane during the differentiation of the epithelium into fibers concerns the presence and the progressive accumulation of particles within the hydrophobic core of the membrane leaflets in potential areas for cell-to-cell contact.

The accumulation of particles, in fact, seems to be the prominent membrane structural event leading to the formation of an extensive system of junctions that will connect lens fibers at the stage of their terminal differentiation. The process of junctional assembly is characterized morphologically by the convergence of 9.0-nm particles, forming linear rows

or streams in the two matching plasma membranes. The linear rows either anastomose, forming an irregular network or interconnected aggregates of geometrically packed particles.

In mature animal tissue (ref. 13; compare ref. 2) some low-resistance passageways have been found associated with very similar junctional features to those observed by us at the early stage of cellular elongation in the lens. In particular, Raviola and Gilula (14) have described the presence of "gap" junctions coupling cones to retinal rods that were characterized primarily by linear rows of particles rather than by hexagonally packed membrane subunits. This similarity may therefore imply that identical plasma membrane features characterize either fully developed intercellular junctions or dynamic stages of their assembly.

It can be postulated that linear arrays of particulate inner membrane entities could provide a strong structural frame, triggering further particle assembly. Moreover, the intersecting rows developing in adjacent plasma membranes could also account for a local tangential polarity coding for reciprocal recognition of the two cell surfaces and for specific cell-to-cell interlocking.

It is noteworthy that streams of inner membrane particles are found either during the formation or as a consequence of the breakdown of "tight" junctions (8, 9) (and see ref. 15).

Our observations do not establish a clear correlation between the preexisting "gap" junctions coupling lens epithelial cells (manuscript in preparation) and the developmental process of the junctions connecting fibers. The preexisting "gaps" apparently do not act as nucleation sites for the apposition of new junctional material; it is more likely that remnant "gaps" are diluted during the elongation process within the frame of the largest junctional structures connecting the fibers.

Evidence derived from rapidly growing cells *in vitro* or from reaggregation experiments indicates that the formation of low-resistance pathways, revealed by appropriate electrophysiological measurements, may occur in a short period of the order of a few minutes (1, 16-18). It is thus unlikely that this rapid event at the plasma membrane level should be associated with a program involving new biosynthesis and insertion of junctional constituents.

It is, therefore, tempting to conclude that at least in some cellular systems the rapid assembly of junctions correlates with the lateral movement of a preexisting pool of junctional particles. However, the same morphogenetic principle is not necessarily valid for the differentiation of fiber junctions. In the lens there is a unique pattern of growth resulting in a slow apposition, layer upon layer, of fiber cells originating from cuboidal epithelium (10).

Since, during elongation the amount of surface membranes of the fibers as compared to the epithelial cells increases almost a thousand times, one has to conclude that the differentiation of the epithelium into fibers must involve the biosynthesis of new plasma membrane material (19).

Although a sound biochemical analysis of this process is still lacking, incorporation studies have in fact shown that the phospholipid metabolism is mainly active in the epithelium and in the elongation zone. There, a characteristic type of plasma membrane is built up, displaying a molar ratio of cholesterol to phospholipids higher than in many other membranes and rich in fatty acids either fully saturated or at most

FIG. 4. Lens fibers in the zone of cellular elongation. The fracture has exposed large areas of the inner aspects of the A and B faces. On the A face, most of the 9.0-nm particles form elaborated streams and linear rows. A large assembly of 9.0-nm particles is also visible (J). The junctional domain is interrupted in some areas and the intercellular space (IS) is visible.

FIG. 5. Cortical lens fibers. Developed intercellular junction characterized by a polygonal assembly of particles exposed on the A face. The B face exposes the inner aspect of the interlocking structure, consisting of a rather irregular packing of pitted images.

monounsaturated (11). These data suggest the existence of a rather rigid lipid matrix in the lens plasma membranes.

From tissue culture experiments (20, 21) there is growing evidence that clustered distribution of inner membrane particles, which seems to be a membrane structural feature essential for cell-to-cell interaction, preferentially correlates with a rather rigid lipid core of the plasma membrane.

Thus, the proper lipid composition of the lens fiber plasma membranes *per se* could be a condition promoting the assembly of junctional particles that likely consist of identical or very few closely related hydrophobic proteins. As a consequence, the inner membranous particles will tend to aggregate, forming a bidimensional array of repeating subunits within a rather rigid lipid domain probably other than a bilayer.

This assumption is probably consistent with the morphological features of fiber junctions and with our recent observation that those structural complexes, isolated by detergent solubilization, display a rather simple protein pattern characterized by a major constituent of 34,000 daltons (12).

The functional role of the fully developed lens fiber junctions remains to be established. Electrophysiological studies on the lens have not provided conclusive evidence of the existence of an electrical coupling between fibers (22). The fact that the proper architecture of fiber junction deviates from that of a typical "gap" cannot be taken as a proof that the fibers are uncoupled. Actually, morphological variants of "gap" junctions between electrically coupled cells have repeatedly been described (see ref. 2).

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