SEPTATE AND GAP JUNCTIONS IN MOLLUSCAN GILL EPITHELIUM

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INTRODUCTION

Previous studies on the electrically-coupled gill epithelium of the mussel Elliptio complanatus failed to reveal the presence of gap junctions. Therefore, the septate junction, which is present as a prominent belt between all the epithelial cells in this tissue, seemed to be an attractive candidate for providing the low-resistance pathway necessary for electrical coupling (7, 19), as also was the case in invertebrate salivary gland epithelium (20). Recently, there have been several reports that macular gap junctions can be found along with septate junctions in various invertebrate epithelia (8, 9, 10, 13, 16). Since the gap junction is the membrane specialization found in the electrotonic synapse of the crayfish giant septate axon (14) and has been implicated in low-resistance coupling of vertebrate tissues (1, 5, 6, 17, 18), we thought it necessary to reexamine mussel gill material more extensively for gap junctions as part of our attempt to define the role of junctions in intercellular communication. In this paper, utilizing conventional thin-section and freeze-etch electron microscopy, we report the coexistence of septate and gap junctions in mussel gill epithelium.

MATERIALS AND METHODS

For thin sections, small pieces of gill tissue were excised from freshwater mussels, usually Elliptio complanatus (Carolina Biological Supply Co., Gladstone, Oregon), and fixed for 2-4 hr at room temperature in a solution of 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), followed by treatment with 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 hr at room temperature. The tissue was then rapidly dehydrated in a graded series of ethanol and embedded in Epon 812. Thin gray sections (less than 600 A thick) were cut on a Porter-Blum MT-2 ultramicrotome and stained with uranyl magnesium acetate and lead citrate before viewing in a Siemens Elmiskop IA at 80 kv.

For freeze-etching, small pieces of the gill were fixed for 5-10 min in 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2), and then treated with a 25% glycerol solution for 2-4 hr before routine freezing. Fracturing was performed with a Balzers apparatus utilizing -115° C since no etching was desired (2, 11).

RESULTS AND DISCUSSION

In the mussel gill, the septate junction forms a 2–3 µm wide continuous belt or zonula around the apical region of the ciliated columnar epithelial cells. The septate junction comprises that region where two adjacent cell membranes are joined periodically by 40–50 A thick septa that extend across a 150 A intercellular space (Fig. 1) (19). Immediately below the septate junction, a non-junctional region of cell membrane is always present where the intercellular space slightly increases in size. This region is occasionally followed by an arrangement of one or more regions where the cell membranes are approximated to within about 20–40 A of each other. Each of these regions is a macula or plaque that has been descriptively termed the "gap junction" (17). Below this region of gap junction, the cell membranes extend to the
basal lamina without further junctional modification.

Freeze-fracture replicas facilitate the study of the relationship of these gap elements to the septate junction. Membrane fracture faces from regions similar to the one in Fig. 1 demonstrate the disposition of the two junctional membrane specializations and their exact sizes and distribution. The fracture face associated with the junctional membrane particles (face A', Fig. 2) contains both the characteristic septate junctional membrane particle rows and the gap junctional membrane particle plaques. The complementary fracture face (face B', Fig. 3) contains both the complement of the septate particle rows (rows of depressions) and the complement of the gap particle plaques (aggregates of depressions or pits).

Gap junctions are difficult to detect in this tissue due to their small size (from 35–400 nm), their sparse distribution, and their macular character. Since many of the gap junctions present are only about 400 A in diameter, their detection in thin sections is extremely difficult. In normal 500–600 A thick sections, they are likely to be obscured by material above or below, so as to give the appearance of a tilted membrane region. From the freeze-fracture faces containing gap junctions, one would expect to detect a maximum of three gap junctions in series in a 400 A thick section. A rather puzzling observation is the inconstancy of the distribution of gap junctions. Most of the fracture faces we have seen are completely void of the gap arrays, while some fracture faces of this region contain several of the gap junctions in a small area. Obvious explanations for this result could be that (a) the material is variable in terms of developmental or physiological state, (b) gap junctions are not present between all of the cells in this tissue, or (c) that gap junctions, are not necessarily present on all lateral borders of each cell in this tissue. At the present time, there is little evidence to allow us to decide between these possibilities.

The gap junctions in this tissue are quite similar to those described in vertebrate epithelia (3, 17), with a few minor exceptions. In general, these gap junctions are smaller than those typically found in vertebrate epithelia, while the particle arrangements are generally oblong or irregular, rather than circular. In some cases the 85 A particles are polygonally packed, while in other instances they seem more loosely packed. The small 25 A dots that have been described on gap junctional membrane particles (12) can also be seen on these particles. The gap particle arrays are associated with the cytoplasmic side of the membrane fracture as is the case with the gap junction of vertebrate tissues (4). Particles from fractures of septate and gap junctions in this tissue are always found on the same fracture face (face A), while depressions or pits from both junctions are found on face B. It is interesting to note that models of the gap junction that placed the freeze-etch particles at the membrane surface would be ruled out by this observation, since models of the gap and septate junction would have to have compatible fracture faces. The most current proposals for gap junction structure are in fact compatible with our earlier model for the septate junction (7). In this tissue, the two junctional membrane specializations are completely segregated, whereas in some invertebrate epithelia the gap junctions appear to be intercalated within the septate junction region (B. Filshie and D. Smith, Personal Communication). The two different arrangements may have some functional or developmental significance. In the mussel gill the 20–40 A gap is apparent, whereas

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3 The convention used differs from that of Gilula et al. (7).

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**Figure 1** Thin-section appearance of septate-gap junctional arrangement between two lateral epithelial cells in the mussel gill. The septate junction (s) lies apical to the gap junctions (g). The junctions are separated by regions of nonjunctional plasma membrane (2). × 200,000.

**Figure 2** Fracture face A of the plasma membrane contains the septate junctional freeze-etch differentiation (s) and the gap junctional particle plaques (g) embedded in regions of nonjunctional plasma membrane (2). Some of the gap junctional particles have 25 A dots present on them (arrow). × 80,000.

**Figure 3** The complementary fracture face (B) contains the depressed rows of the septate junction (s), aggregates of depressions at the gap junctions (g), and very few particles in the nonjunctional plasma membrane regions (2). × 100,000.
in other invertebrate epithelia the gap has not always been apparent, and this has been responsible for many erroneous descriptions of tight junctions in invertebrate epithelia. It now seems that the gap junction, along with the septate junction, is a common feature of invertebrate epithelia, and that the existence of tight junctions in invertebrate epithelia is doubtful.

The coexistence of gap and septate junctions may either clarify or complicate our understanding of intercellular coupling. On the one hand, the presence of the gap junction in the mussel gill may substantiate the hypothesis that the gap junction is universally responsible for electrical coupling. On the other hand, one cannot discount the extremely important membrane modification associated with the septate junction and the morphological match across a wide intercellular space of adjacent modified membranes. The possibility exists that one type of cell junction may not be versatile or efficient enough to account for the multiple functions that have already been ascribed to intercellular communication. At any rate, the coexistence of two junctional elements definitely increases the difficulties of defining the function of either of the junctions in this tissue. At least three important questions may be raised: (a) are all gap junctions functionally identical; (b) is the gap junction the only membrane differentiation which is responsible for electrical coupling, i.e., the flow of ions; and (c) are structures other than the gap junction involved in the passage of molecules, other than ions, from cell to cell?

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